Catnip (*Nepeta cataria* L.) Essential Oil: Analysis of Chemical Constituents, Bacteriostatic and Fungistatic Properties

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ABSTRACT: The composition of the essential oil of flowering catnip (*Nepeta cataria* L., Lamiaceae) was analyzed by means of GC/MS. Besides the already known nepetalactones 4aα, 7α, 7aα-nepetalactone; 3,4β-dihydro-4aα, 7α, 7aα-nepetalactone; 4aα, 7α, 7aβ-nepetalactone and β-caryophyllene, five new constituents were identified: dimethyl-3,7 oxa-1 bicyclo [3,3,0] oct-2-ene, piperitone, thymol methyl ether, hexenyl benzoate and humulene oxide. The essential oil of two samples of the plant, collected at two different stages of development, was compared as to their nepetalactone content. The oil samples and a hexane extract were subjected to microbiological tests (five bacteria and seven fungi) and compared to natural compounds known for their antimicrobiological activities.

KEY WORD INDEX: *Nepeta cataria*, Labiatae, catnip, essential oil composition, nepetalactones, bacteriostatic activity, fungistatic activity.

INTRODUCTION: Among the 250 species of the *Nepeta* (1) (Lamiaceae) genus reported, our study concentrated on *N. cataria*, a species which is commonly known as catnip. This plant is found from Central Europe down to the Iranian plateaus and in Central Asia. The harvest is carried out before (June) or during flowering of the plant (September). Even though catnip oil has been the subject of previous study (1-5), the species is not well known as an aromatic plant. In 1967 Regnier et al. elucidated the

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chemical structure of the main components of the essential oil; the so-called nepetalactones (2); which were the first cyclopentanoid monoterpenes identified (3). They seem to be responsible for the use of this plant in the 17th century as a tonic or a disinfectant for rhinitis. The most claimed original property of catnip is its attractive potency on the feline race (1,6,7) but no proof has been obtained. Moreover, in 1978 Saxena (8) reviewed the study of Eisner (9) in which 17 insect species were driven away by nepetalactone vapors. Because of the biological activity attributed to catnip and the nepetalactones, the plant captured our attention. We also wanted to carry out a more complete chemical analysis of the oil together with a study of its antibacterial and fungistatic properties and the component sesquiterpene lactones.

EXPERIMENTAL: ***Plant Material***—Two samples originating from the Province Center of France were investigated: the first sample (50 kg) which was collected before flowering was air-dried and ground. The second sample (5 kg) was collected during flowering, air-dried and ground.

Methodology—*Hydrodistillation*: Distillation was carried out in a 10 L apparatus approved by the French Pharmacopoeia (10); the operating conditions were: load/nominal volume ratio: 57.5 to 75%; load/water ratio: 8 to 13%; hydrodistillation time: 2.5-12 h; stirring: 0 to 1000 revolutions/min; hydrodistillation flow: 12 to 14.5 mL/min. The distillation water was subjected to two successive diethyl ether extractions. The ethereal phase was dried over anhydrous Na_2SO_4, filtered and concentrated at room temperature under atmospheric pressure.

Solvent extraction of flowering catnip: The extraction of plant material (80 g, 1st sample) by organic solvent (1400 cm^3) was carried out in a Soxhlet apparatus for 8 h. Solvents of increasing polarity were used: hexane, trichloro-1,1,2-trifluoro-1,2,2-ethane (T.T.E.), dichloromethane, chloroform and ethanol. After extraction and concentration under reduced pressure we obtained the resinoid.

Analytical Techniques—*Analysis*: GC/MS analyses were performed on a Hewlett-Packard instrument (HP 5970B GC/MSD system) equipped with a nonpolar OV 101 capillary column, 25 m x 0.22 mm (film thickness: 0.25 μm). The operating conditions were: injector and detector temp 250°C; oven temp programming: 60°C (2 min), 60-250°C (5°C/min) with helium, 40 mL/min as the carrier gas.

GC analysis and quantifications were performed on a 5890 A Hewlett-Packard gas chromatograph equipped with a 50 m x 0.22 mm (film thickness 0.25 μm) OV 101 nonpolar capillary column and a similarly configurated Carbowax 20 M column. The operating conditions were: injector and detector temp: 200°C and 220°C respectively; oven temp: 60°C (1 min), 60-220°C (3°C/min); detector: FID; carrier gas: helium, 40 mL/min. Components were identified by comparison of their mass spectra and retention indices with those of reference substances, isolated from or identified in essential oils of known composition.

Microbiological Techniques—*Bacteria, yeast and mold culture*: Test bacteria, corresponding to the NF-T 72-150 standard (11) as well as *Aspergillus niger* and *Candida albicans* were supplied by the Institut Pasteur (Paris, France). The fungal phytopathogenic clones originated from the Centraal Bureau voor Schimmelcultures (Baarn, the Netherlands).

The cultures were plated out in appropriate media: Trypcase soja for the bacteria; Sabouraud for *Aspergillus niger* and *Candida albicans*. The same agar media were used for techniques in solid phase. These two media were supplied by the Bio-Merieux Co. (Paris, France). Moreover, potato dextrose agar (Serva, Heidelberg, Germany) was used for the phytopathogenic clones.

Techniques in liquid phase (microplates): The essential oil was emulsified in water with acetone and Tween 80, with amounts not higher than 10%, so these compounds had no effect on the microorganism growth. A 1 µL inoculum (10^8 microorganism/L) was sowed in a medium (100 µL) made equally of emulsion and liquid agar.

Techniques in solid phase (petri boxes): The emulsion (essential oil, Tween 80, water and acetone) was mixed with liquefied agar in a bath at 50°C after which it was poured into petri dishes. After cooling, the microorganisms were plated on the surface.

Dilutions: In a beaker, a Co emulsion containing 8,000 µg/mL of the active principle was prepared. For the microplate cultures, 100 µL of this emulsion was mixed with 100 µL of liquefied agar medium, appropriate for the plated microorganisms. Sample of 100 µL taken from this M_1 mixture was diluted with 100 µL of agar (M_2). This operation was repeated until M_{10}, in which the active principle concentration was $M_1/2^9$ (7.8 µg/mL).

For the petri dish cultures, the liquefied agar (7 mL) was mixed to 1 mL of the stock solution (4 Co concentration) in hemolysis tubes and solidified at room temperature in petri dishes. The initial medium was afterwards diluted two, four and eight times respectively.

RESULTS AND DISCUSSION: We can see that the oil yield depends on the distillation time but is not a exponential function as shown in Figure 1. This phenomenon can be explained (Figure 2) by the change of chemical composition of fractions of the oil collected over several hours, which showed a relative increase of the lactone content of the fraction when the distillation time was longer.

Organic solvent extraction (Table I) was used to obtain the maximum amount of volatile compounds. Except for T.T.E., it appeared that more polar the solvent, the better the yields,

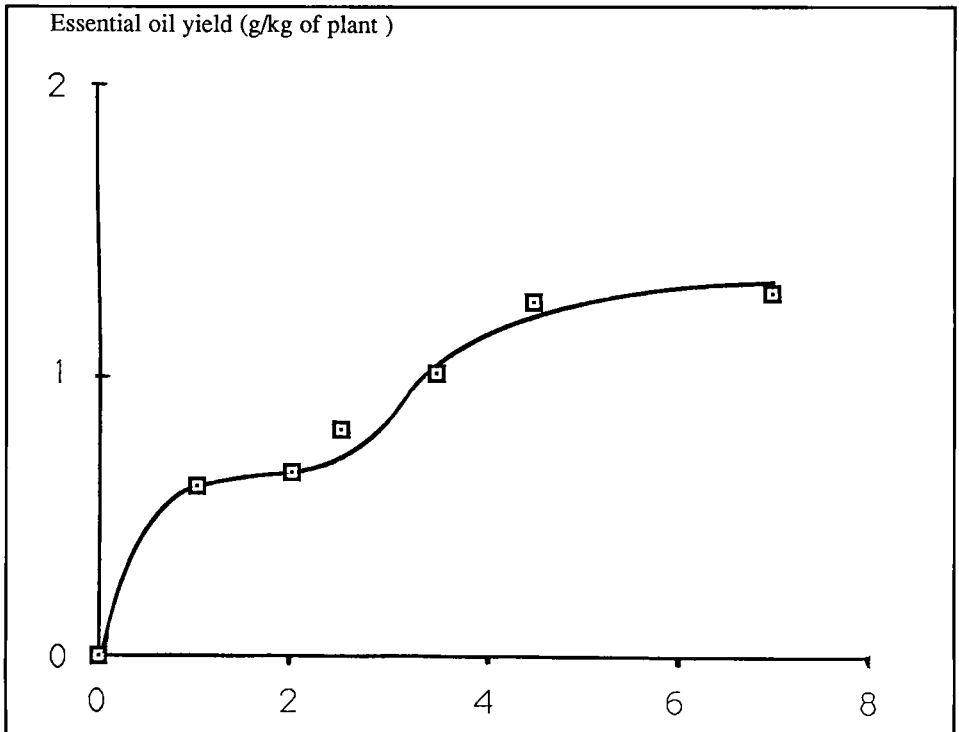

Figure 1. Hydrodistillation of flowering catnip

Table I. Extraction of flowering catnip plants by various solvents

	Solvent				
	Hexane	T.T.E.	CH_2Cl_2	$CHCl_3$	EtOH
resinoid yield (%)	2.36	1.28	3.60	4.23	21.97
absolute yield (g/kg of plant)	19.50	9.80	33.70	37.40	206.10
lactones in absolute (%)	21.10	40.00	54.50	51.08	27.80
lactone yield (g/kg of plant)	4.11	3.92	18.36	19.10	57.24

T.T.E. = trichloro-1,1,2-trifluoro-1,2,2-ethane

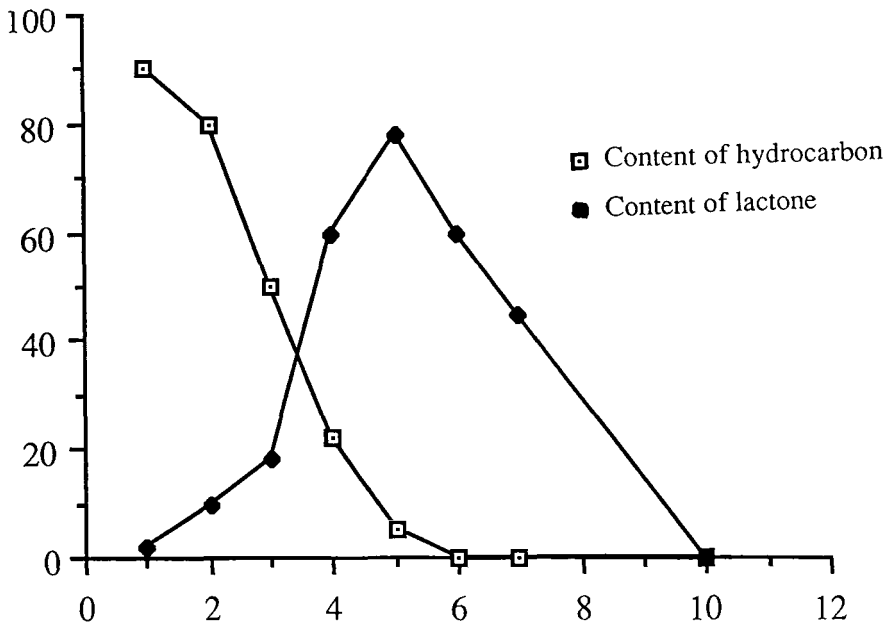

Figure 2. Changes of the chemical composition of the essential oil during hydrodistillation of flowering catnip plants

especially the lactones. A 57.24 g/kg nepetalactones yield was obtained using ethanol.

The results of our analysis complement those previously published (1,3,4) (Table II). Five new compounds were identified: dimethyl-3,7 oxa-1 bicyclo [3,3,0] oct-2-ene, piperitone, thymol methyl ether, 3-hexenyl benzoate and humulene oxide I. These compounds have not been described before in any *Nepeta* species. The 13 compounds identified (Table II) represented 91.6% of the oil (Figure 3). The lactone fraction varied from 15% to 64%. Caryophyllene oxide reached up to 18%. Analyses of the two samples showed the influence of maturity of the plant on the chemical composition of the oil, especially the increase of lactone formation during flowering.

Table II. Percentage composition of the essential oil and hexane extracts of catnip plants*

Peak no.	Retention indices on OV-101	Constituents	A essential oil	A hexane extract	B essential oil
1	1082	dimethyl-3,7-oxa-1 bicyclo[3,3,0]oct-2-ene	0.30	4.30	0.7
2	1228	piperitone	1.50	1.85	0.8
3	1306	nepetalactone N_1	1.30	9.40	2.8
4	1311	M = 168 } isomers	0.03	-	0.5
5	1313	M = 168 }		-	0.4
6	1317	nepetalactone N_2	11.40	9.40	56.9
7	1365	β-elemene	1.20	-	0.2
8	1370	3-hexenyl ester	1.20	1.30	0.9
9	1393	dihydronepetalactone N_3	2.00	2.30	1.7
10	1428	β-caryophyllene	24.60	26.70	6.2
12	1434	α-humulene	1.30	0.08	0.9
13	1448	(E)-β-farnesene	2.00	0.90	0.8
14	1457	M = 166	0.25	0.30	0.8
15	1470	thymol methyl ether	0.90	0.20	0.4
16	1510	M = 166	10.68	0.64	3.5
18	1553	3-hexenyl benzoate	0.30	0.17	0.2
19	1577	caryophyllene oxide	14.30	5.30	18.2
20	1595	humulene oxide I	1.10	-	1.6

* = collected before (A) and during (B) flowering

The nepetalactone structures N_1, N_2 and N_3 were deduced from Kovats indices calculated for different isomers (1); the stereochemistry of the three nepetalactones are: N_1=4aα, 7α, 7aα-nepetalactone; N_2=4aα, 7α, 7αβ-nepetalactone; and N_3=3,4β-dihydro-4aα, 7α, 7aα-nepetalactone.

The test bacteria corresponded to those of the AFNOR NF-T72-150 standard: *Escherichia coli* CNCM 54127; *Mycobacterium smegmatis* CNCM 7326; *Staphylococcus aureus* CNCM 53154; *Pseudomonas aeruginosa* CNCM A22; *Streptococcus faecalis* CNCM 5855.

Yeast and mold were *Candida albicans* and *Aspergillus niger* for human pathogens, *Sclerotinia sclerotiorum*, *Stereum purpureum*, *Botrytis cinerea* and *Cylindrocarpon mali* for phytopathogens.

The petri dish technique indicated no bacteriostatic activity of the essential oil (Table III), even though one microplate showed a low activity (about 1,000 µg/mL). Nevertheless, the results of the petri dish technique revealed a bacteriostatic activity against three microorganisms (Table IV) for the tested resinoid. The test phytopathogen fungi could not grow under the conditions of the microplate technique, so they were screened using the petri dish technique. However, fungistatic activities were observed for *Candida albicans* and *Aspergillus niger* with the microplates technique (Minimal Inhibitory Concentration (MIC) = 1,000 µg/mL, 2,000 µg/mL respectively).

The same difference of activity level as observed for the phytopathogen fungi was obtained for the bacteria, using the petri dish technique. The results showed that the catnip oils had higher fungistatic activities on phytopathogens (*Cylindrocarpon mali*, *Botrytis cinerea* and

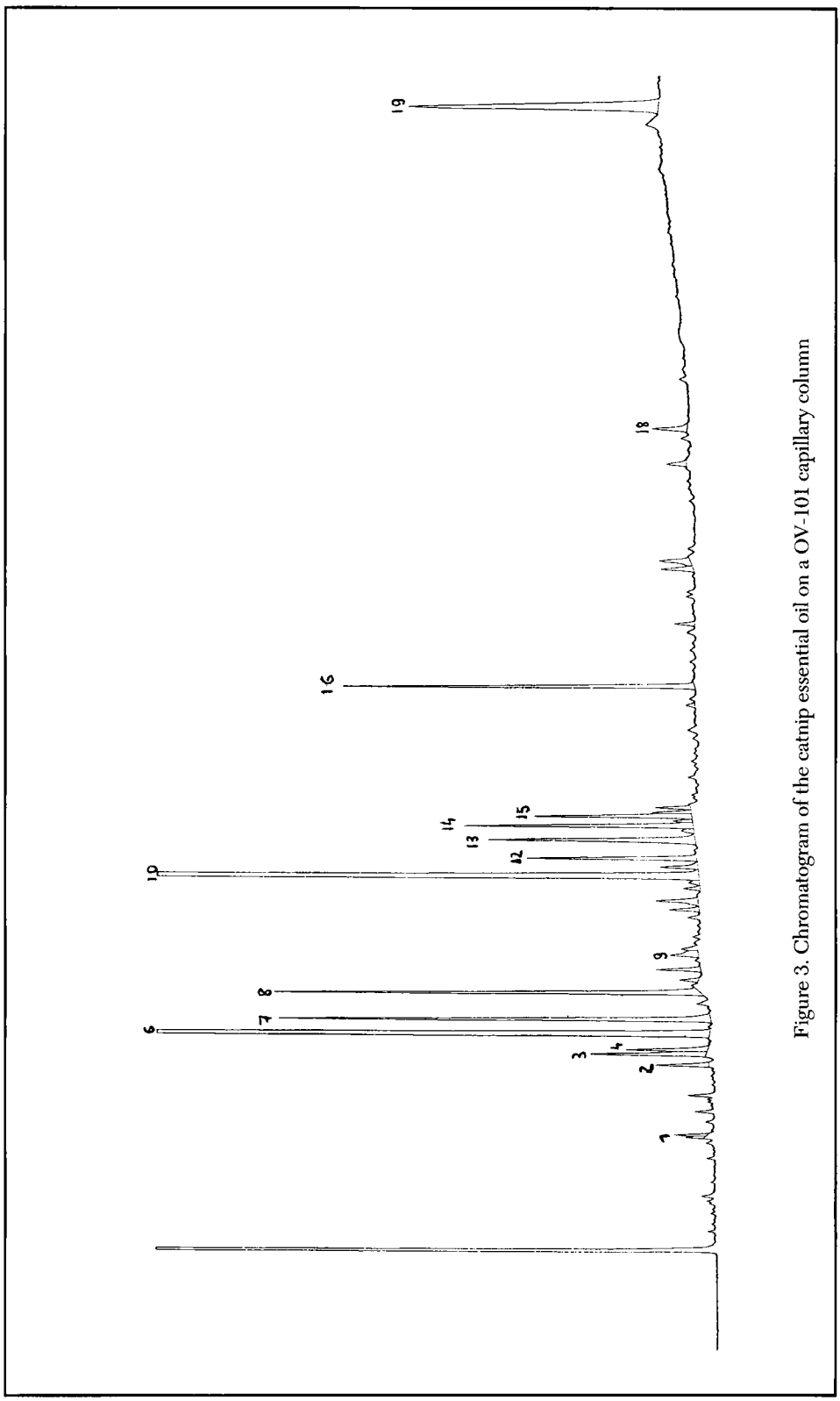

Figure 3. Chromatogram of the catnip essential oil on a OV-101 capillary column

Table III. Quantification of the bacteriostatic potency of catnip oils and a hexane extract

Method	Microorganism				
	Escherichia coli	*Streptococcus faecalis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Mycobacterium smegmatis*
Microplates					
MIC (μg/mL) oil from flowering plants	4,000	4,000	1,000	4,000	4,000
Petri dish					
MIC (μg/mL) oil from plants before flowering	>4,000	>4,000	>4,000	>4,000	>4,000
MIC (μg/mL) oil from flowering plants	>4,000	>4,000	>4,000	>4,000	>4,000
MIC (μg/mL) resinoid (21.1% lactone)	>4,000	>4,000	4,000	4,000	4,000

MIC = minimal inhibitory concentration

Table IV. Quantification of fungistatic potency of catnip oils and a hexane extract

Method	Fungi					
	Human pathogen		Phytopathogen			
	Candida albicans	*Aspergillus niger*	*Sclerotinia sclerotiorum*	*Stereum purpureum*	*Botrytis cinerea*	*Cylindrocarpon mali*
Microplates						
MIC (μg/mL) oil from flowering plants	2,000	1,000	Ci	Ci	Ci	Ci
Petri dish						
MIC (μg/mL) oil from plants before flowering	4,000	>4,000	4,000	4,000	500	500
MIC (μg/mL) oil from flowering plants	2,000	4,000	2,000	500	1,000	1,000
MIC (μg/mL) resinoid (50% lactones)	>4,000	>4,000	2,000	500	2,000	4,000

Ci = impossible culture in our experimental conditions
MIC = minimal inhibitory concentration

Table V. Bacteriostatic and fungistatic activities of pure compounds

	Microorganisms*								
	Bacteria				Fungi				
Pure compounds	1	2	3	4	5	6	7	8	9
eugenol (MIC µg/mL)	1,000	500	>4,000	>4,000	500	500	500	500	500
citral (MIC µg/mL)	1,000	250	500	2,000	500	500	500	500	500
p-cymene (MIC µg/mL)	>2,000	>2,000	>2,000	>2,000	>4,000	4,000	>4,000	2,000	1,000
1,8-cineole (MIC µg/mL)	>2,000	>2,000	>2,000	>2,000	>2,000	1,000	>2,000	>2,000	>2,000
α-pinene (MIC µg/mL)	>2,000	>2,000	>2,000	>2,000	>2,000	250	>2,000	>2,000	>2,000
camphor (MIC µg/mL)	2,000	>2,000	>2,000	>2,000	>2,000	>2,000	>2,000	>2,000	>2,000

*1=*Escherichia coli*, 2= *Staphylococcus aureus*, 3=*Streptoococcus faecalis*, 4=*Pseudomonas aeruginosa*, 5=*Aspergillus niger*, 6=*Sclerotinia sclerotiorum*, 7=*Stereum purpureum*, 8=*Botrytis cinerea*, 9=*Cylindrocarpon mali*
MIC = minimal inhibitory concentration

Sclerotinia sclerotiorum) than on human pathogens. Finally, the catnip extract only revealed weak activities, when compared to the oils, against *Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Cylindrocarpon mali*.

The results indicated that the oil obtained from flowering plants of *Nepeta cataria* was rich in lactones in contrast to the oil obtained from plants harvested before flowering.

It seems that an increase of the antifungal activity against *Candida* is due to the lactones, as a lactone-rich sample (70%) gave a 2,000 µg/mL activity, whereas a lactone-poor sample (10%) had to be concentrated (to 4,000 µg/mL) to give the same activity.

Catnip oil was found to be fungistatic against *Aspergillus niger* using the microplate technique. The fungistatic as well as the bacteriostatic activity must be related to the fact that microorganisms come more directly in contact with the oil with this technique than with the petri dish technique. As for the phytopathogenic fungi, the activity cannot only be related to the lactones since the oil that was richest in lactones, was less fungistatic against *Cylindrocarpon mali*, *Botrytis cinerea*, and *Stereum purpureum* compared to the lactone deficient oil. The opposite was the case against *Sclerotinia sclerotiorum*.

Generally, oils seem to be more active than resinoid, especially against *Botrytis cinerea* and *Cylindrocarpon mali*.

In order to put the anti-microbiological activity of catnip oil into perspective, its activity was compared with natural products of known activity, such as eugenol, citral, p-cymene, 1,8-cineole, α-pinene and camphor (Table V). It was determined that catnip oil had an activity comparable to eugenol and citral against *Cylindrocarpon mali* and *Botrytis cinerea*, while it was more comparable to 1,8-cineole and p-cymene against *Sclerotinia sclerotiorum*.

Finally, it was found that catnip oil was less active than eugenol and citral against bacteria and *Aspergillus niger*.

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