IOCAG 2025 Conference
The 3rd International Online Conference on Agriculture
22–24 October 2025 | Online
SCHOOL OF AGRICULTURE
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In Vitro Evaluation of the Antifungal Effect of Carvacrol-based Essential Oils on *Alternaria* and *Fusarium* Fungi

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INTRODUCTION

Phytopathogenic fungi are the main source of important economic losses in fruits and vegetables after harvest, during their storage, transportation, and marketing. Due to the high toxicity of the synthetic fungicides, their long degradation period, and residuality, their use has been placed under a regime of restrictive measures on postharvest disease control [1]. The use of essential oils (EOs) from some aromatic plants is an alternative and environmentally friendly method for crop protection from post-harvest diseases. In this work was studied *in vitro* the antifungal effect of three Lamiaceae family EOs; *Satureja horvatii* ssp. *macrophylla*, *Coridothymus capitatus*, and *Origanum vulgare* ssp. *hirtum* on the phytopathogenic fungi *Fusarium* and *Alternaria* sp.

METHODS

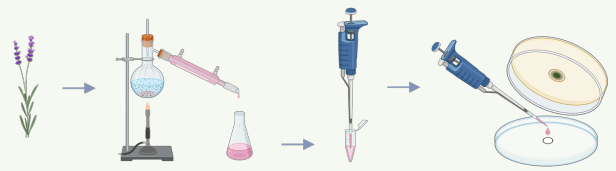

Figure 1. Schematic presentation of the EOs preparation, and fumigant assay method, used in the experiment.

The EOs used in the experiments were obtained by hydro-distillation (Fig. 1) and their analysis (Table 1) was performed by GC-MS (Master GC- TOF MS, Dani) equipped with an autosampler. Infected potato tubers and tomato fruits with symptoms of dry rot (Fig 2A) and black spot (Fig 2B), respectively, were transported to the laboratory and isolated. The Fumigant effect of the EOs *S. horvatii* ssp. *macrophylla* (**S.h.**), *O. vulgare* ssp. *hirtum* (**O.v.**), *C. capitatus* (**C.c.**), and Carvacrol (**Carv.**) was evaluated in multiple quantities (**1-2-3-4-5 μL EO plate^{-1}**) on the radical growth of *Fusarium* sp. (Fig 3) and *Alternaria* sp. (Fig 4) after 8 days of exposure on PDA petri plates, and calculated as a percentage (%±SE) compared to the control (**C=0 μL EO plate^{-1}**).

RESULTS & DISCUSSION

After 8 days of incubation, the *Fusarium* sp. growth was completely inhibited in the presence of carvacrol, and the EOs of *C. capitatus*, and *O. vulgare* ssp. *hirtum* at the amount of 2 μL plate^{-1} with a statistically significant difference with C (100%) (p<0.001). In the case of *S. horvatii* ssp. *macrophylla*, the mycelial growth was completely inhibited at 3 μL plate^{-1} with a statistically significant difference with the C (p≤0.001).

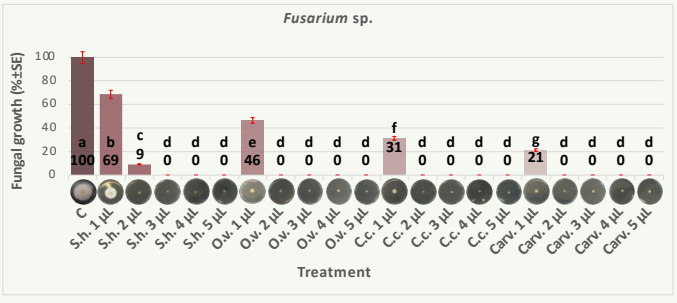

Figure 3. The fumigant effect of the EOs on the radical growth (%±SE) of *Fusarium* sp. on PDA petri plates after 8 days of incubation. Different letters between treatments represent a statistically significant difference (LSD p≤0.05).

CONCLUSIONS

With the proper standardization and formulation, these EOs could be used for post-harvest applications and for the disinfection of storage warehouses from *Fusarium* sp. and *Alternaria* sp. as opposed to synthetic agrochemicals the EO utilization has the advantages of low toxicity for humans and the environment.

After 8 days of incubation, the *Alternaria* sp. growth was completely inhibited by carvacrol, and the EOs of *C. capitatus* and *O. vulgare* ssp. *hirtum* at the amount of 3 μL plate^{-1}, presenting a statistically significant difference with C (p<0.001), according to Figure 2. In the case of *S. horvatii* ssp. macrophylla, the mycelial growth was completely inhibited at the amount of 5 μL plate^{-1} with a statistically significant difference with the control (p<0.001).

Table 1. Chemical composition (GC-MS) of the three EOs used in the experiment

No	RT	RI	Compounds	Area (%)		
				O. vulgare ssp. *hirtum*	*C. capitatus*	*S. horvatii* ssp. *macrophylla*
1	6.09	928	α-Thujene	2.3	0.9	1.3
2	6.36	937	α-Pinene	1.1	0.7	1.4
3	6.89	955	Camphene			1.8
4	7.76	986	1-Octen-3-ol	1.2	0.8	1.7
5	7.98	993	α-Myrcene	3.0	1.9	1.1
6	8.68	1012	α-Phellandrene	0.6	0.4	0.2
7	9.03	1023	α-Terpinene	2.9	2.4	1.6
8	9.40	1033	p-Cymene	10.2	6.2	12.9
9	9.53	1035	Limonene	0.4	0.4	0.6
10	6.19	1038	b-Phellandrene	0.6	0.5	
11	9.76	1039	1.8-cineole			1.4
12	10.59	1065	γ-Terpinene	9.2	5.7	5.5
13	11.13	1180	cis sabinene hydrate	1.4		1.1
14	12.25	1107	Linalool		1.4	1.6
15	15.55	1185	Borneol	0.8	1.8	5.0
16	15.84	1192	Terpinene-4-ol	0.8	1.1	1.3
17	16.24	1201	p-Cymen-8-ol			0.3
18	16.55	1207	α-Terpineol			0.4
19	18.41	1242	Thymol methyl ether			3.2
20	21.86	1304	Thymol	18.9		6.2
21	22.68	1313	Carvacrol	42.5	70.0	41.4
22	31.417	1424	Caryophyllene	2.7	4.9	4.5
23	32.72	1444	Aromadendrene			0.6
24	33.85	1461	α-Humulene			0.3
25	36.06	1494	Viridiflorene			0.3
26	36.347	1498	γ-Elemene			1.5
27	37.10	1514	β-Bisabolene	1.0	0.3	
28	40.63	1591	Spathulenol			0.9
29	40.75	1594	Caryophyllene oxide	0.4	0.8	2.0
			Monoterpene hydrocarbons	30.3	19.0	26.5
			Oxygenated monoterpens	62.9	74.2	57.6
			Sesquiterpene hydrocarbons	3.8	5.2	7.0
			Oxygenated sesquiterpenes	0.4	0.8	2.9
			Others	2.6	0.8	5.9

RI = Retention Index relative to n-alkanes on BPX-5 capillary column; RT = Retention Time.

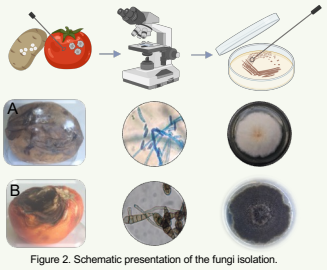

Figure 2. Schematic presentation of the fungi isolation.

The EOs *C. capitatus* and *O. vulgare* ssp. *hirtum* showed a mycostatic effect on *Fusarium* sp. at 3-5 μL plate^{-1}. *S. horvatii* ssp. *macrophylla* EO presented a mycostatic effect on *Fusarium* sp. at 5 μL plate^{-1}. The carvacrol demonstrated a mycostatic effect on *Fusarium* sp. at 2-4 μL plate^{-1} and mycotoxic at 5 μL plate^{-1}. The effect of carvacrol on *Alternaria* sp. was mycostatic at 3-5 μL plate^{-1}.

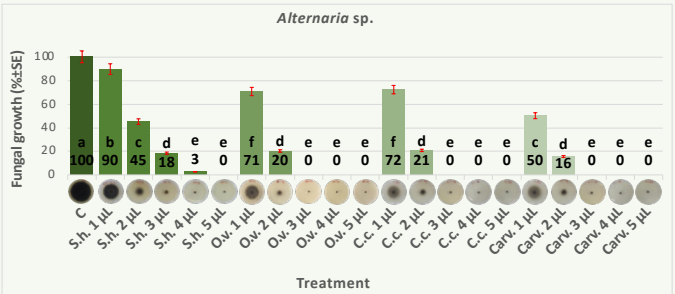

Figure 4. The fumigant effect of the EOs on the radical growth (%±SE) of *Alternaria* sp. on PDA petri plates after 8 days of incubation. Different letters between treatments represent a statistically significant difference (LSD p≤0.05).

The inhibitory effect of EOs on the mycelial growth of *Fusarium* sp. was greater compared to *Alternaria* sp. The EOs were effective, and this may be attributed to their high content of the terpenic phenols carvacrol and thymol. Carvacrol can inhibit ergosterol synthesis in a dose-dependent manner to *Rhizopus stolonifer* that causes postharvest rot in peaches [2]. Zhao et al. 2023 [3] suggest that carvacrol inhibits membrane-associated poly-carbohydrates such as chitinase and β-1.3-glucanase and Wang et al. 2024 [4] observed the inhibitory effect of carvacrol on *Alternaria* sp. The antifungal impact of carvacrol [5,6] comes in agreement with this study on *Fusarium* sp. and *Alternaria* sp. A similar mode of carvacrol has been reported on *Fusarium moniliforme*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum,* and *Phytophthora capsici* [7]. As the carvacrol content or the terpene phenol content increases, the minimum EO amount that inhibits fungal growth decreases, as shown in Figures 3 and 4 following the EOs composition in Table 1.

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