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SHORT NOTES

Antifungal effect of essential oils from some Turkish herbs against *Rhizoctonia solani* Kühn

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Summary. The antifungal properties of essential oils of *Origanum minutiflorum* (native to Turkey), *O. onites, Thymbra spicata* and *Satureja cuneifolia* were tested against six *Rhizoctonia solani* isolates obtained from infected seedlings from various forest nurseries in Turkey. Of the two methods to test the essential oils, the volatile assay was slightly more effective than the contact assay. While all *Rhizoctonia* isolates were strongly affected (>84.7) in the contact assay, the fungal isolates were completely inhibited by all the essential oils in the volatile assay. *R. solani* isolate Rs6 was the most sensitive, being suppressed by *O. minutiflorum* (100%), *S. cuneifolia* (100%) and *T. spicata* (99.6%). *R. solani* isolate Rs3 was also strongly inhibited by *O. onites* (99.1%).

Key words: carvacrol, damping off, forest nursery.

Introduction

Damping-off is one of the most important problems in forest nurseries. Serious damage is caused primarily by a variety of damping-off pathogens, including *Alternaria alternata* (Fr.), *Botrytis cinerea* Pers., *Cladosporium* spp., *Fusarium* spp., *Macrophomina phaseolina* Tassi Goid, *Phytophthora* spp., *Pythium* spp. and *Rhizoctonia solani* Kühn (Viljoen *et al*., 1994; Chin, 1995; James *et al*., 1995; Lilja *et al*., 1997; Camporota and Perrin, 1998). These pathogens cause considerable economic losses (Bloomberg, 1973, Paige *et al*., 1995) both before and after planting out (Ozdamar and Turhan, 1999).

Soil sterilisation with methyl bromide and other fumigants has traditionally been used to control fungal pathogens in forest nurseries, especially in developing countries. This method is effective but has disadvantages. To reduce reliance on methyl bromide and other fumigants, which are a hazard to the environment and to human health, it is necessary to develop an integrated, biologically-based method of disease control (Zher, 1982; Sidhu and Chakravarty, 1990).

Several plant families, especially the *Lamiaceae* (*Labiatae*) contain high amounts of essential oil (>2%) (Baser, 1993, 1994), and Turkey is regarded as an important gene-centre for this family. *Origanum minutiflorum* O. Schwarz and P.H. Davis, *O. onites* L., *Thymbra spicata* L. and *Satureja cunei-*

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folia Ten. commonly grow wild in the Isparta region, almost 1000 of these plants are exported every year (Baydar *et al*., 2004). Baydar *et al*. (2004) reported the major constituents of the essential oils from these plants as the following: for *O. onites*, carvacrol (86.9%), γ-terpinene (3.9%) and *p*-cymene (2.9%); for *T. spicata*, carvacrol (75.5%), γ-terpinene (11.6%) and *p*-cymene (9.2%); for *S. cuneifolia*, carvacrol (57.2%), γ-terpinene (28.0%) and *p*-cymene (6.7%); and for *O. minutiflor*um, carvacrol (84.6%), *p*-cymene (4.2%) and γ-terpinene (3.3%) (Table 1).

The antimicrobial properties of essential oils have been known for a long time and many essential oils from different plants have been investigated for their effectiveness against damping off fungi in agriculture and forestry, including *R. solani,* (Kishoren and Mishra, 1991; Pandey and Dubey, 1992, 1994; Mulleriebau *et al*., 1995; Zambonelli *et al*., 1996; Pitarokili *et al*., 2002, 2003; Curini *et al*., 2003; Dhingra *et al*., 2004; Zambonelli *et al*., 2004). Essential oil of *Cinnamomum osmophloeum* Kaneh leaves has also recently been investigated for its effectiveness against *R. solani* from a forest nursery (Lee *et al*., 2005). However these essential plant oils were not studied for their effects against damping-off pathogens of forest tree seedlings before Mullerriebau *et al*. (1995), who reported that *T. spicata* essential oil had a strong antifungal effect against soilborne pathogens, including *R. solani* from tomato plants (*Lycopersicon esculentum* L.). In this work, the antifungal activity of essential oils from *T. spicata*, *O. onites*, *O. minutiflorum* and *S. cuneifolia* was studied against pathogenic isolates of *R. solani*.

Materials and methods

Fungal strains

Five *R. solani* isolates collected from diseased Anatolian black pine (*Pinus nigra* Arn. subsp. *pallasiana* [Lamb.] Holmboe) nursery seedlings were used (Rs1 and Rs2: from Seydisehir; Rs3 and Rs4: from Egirdir; Rs5: from Golhisar; and Rs6: from Torbali).

The *R. solani* isolates were initially identified and tested for pathogenicity by Tugba Dogmus, Department of Botany, Faculty of Forestry, University of Süleyman Demirel, as part of work on her PhD thesis. Cultures of isolates were maintained on potato dextrose agar (PDA) slants stored at 4°C.

Essential oils

Essential oils of *O. minutiflorum*, *O. onites, S. cuneifolia*, and *T. spicata* were taken from Gülcan Ozkan in the Department of Food Engineering, Faculty of Agriculture, University of Süleyman Demirel, Isparta. The essential oils were maintained at -18°C.

Determination of antifungal activity

Antifungal activity was determined using the contact assay and the volatile assay (Alvarez-Castellanos *et al*., 2001).

Contact assay

PDA plates were prepared using 9-cm glass Petri dishes containing 20 ml of PDA. A 5-mm disc of agar was removed from the centre of the dishes using a sterile cork borer. Ten µl of undiluted oil

Table 1. Chemical composition of the essential oils (% total peak area) of *Origanum onites, Thymbra spicata, Satureja cuneifolia* and *O. minutiflorum*.

Essential oil component	Essential oil sources (%)			
	Origanum onites	*Thymbra spicata*	*Satureja cuneifolia*	*O. minutiflorum*
p-myrcene	1.3	1.3	1.8	1.5
α-terpinene	0.9	1.1	2.1	0.8
γ-terpinene	3.9	11.6	28.0	3.3
p-cymene	2.9	9.2	6.7	4.2
Bornylacetate	0.4	0.3	0.1	0.8
Borneol	0.6	0.1	0.1	0.5
Thymol	0.2	0.3	0.1	1.7
Carvacrol	86.9	75.5	57.2	84.6

was pipetted into each well. Two 5-mm diameter discs of each test species were cut from the edge of less than 1-week-old cultures on PDA dishes and placed upside down on opposite edges of the test dishes against the sides of the dishes. The dishes were sealed with parafilm and incubated in the dark at 23°C. Three days after inoculation, colony growth of the test fungi towards the central well was measured as the distance between the edge of the inoculum disc and the colony margin at the point nearest the edge of the well. Mean growth measurements were calculated from four replicates of each fungal strain.

Volatile assay

PDA dishes were prepared using 9-cm glass Petri dishes containing 20 ml of PDA. A 5-mm diameter disc of each test species was cut from the edge of less than 1-week-old cultures on PDA dishes using a sterile cork borer and placed with the mycelial surface down on the centre of the dish, which were placed upside down. A 10 µl aliquot of undiluted oil was pipetted on the lid without agar. Control dishes with no oil were also prepared. The dishes were sealed with parafilm and incubated in the dark at 23°C. Three days after inoculation, colony growth was measured (mm). Mean growth measurements were calculated from four replicates of each fungal strain.

Statistical analysis

Growth inhibition of the treatment as compared with the control was quantified according to the following equation: percent inhibition = (control population - treated population / control population) × 100. Results were tested for statistical significance by one-way ANOVA. Differences were considered statistically significant at the $P \leq 0.05$ level (Ozdamar, 1999).

Results

Rhizoctonia solani isolates failed to exhibit any growth after three days of incubation in the dishes containing 10 µl of essential oil. Growth of fungal isolate was completely inhibited by all essential oils in the volatile assay; in the contact assay (Table 2), essential oils were also found highly effective (>84.7) against all pathogenic isolates but less so than in the volatile assay. Statistically significant differences were found between the fungal isolates from the different nurseries at the $P \leq 0.05$ level. Rs6 was completely inhibited by *O. minutiflorum* and *S. cuneifolia* and 99.5% inhibited by *T. spicata,* while *O. onites* inhibited 99.1% of Rs3. The oils of *O. minutiflorum*, *O. onites*, *T. spicata* and *S. cuneifolia* inhibited almost all the *R. solani* isolates. *O. minutiflorum*, *O. onites* and *T. spicata* oils were similar in effectiveness and were more effective than *S. cuneifolia* oil.

Discussion

Although both the contact and the volatile assays significantly reduced the fungi in comparison with the control, the volatile assay was slightly more effective than the contact assay. With the volatile assay all fungal isolates were completely inhibited by all essential oils at 10 µl by the third day. Alvarez-Castellanos *et al*. (2001) reported

Table 2. Antifungal activity of essential oils of some Turkish herbs (*Origanum minutiflorum, O. onites, Thymbra spicata* and *Satureja cuneifolia*) tested by the contact assay method.

Fungal strain	Inhibition (%)[a]			
	Origanum minutiflorum	*O. onites*	*Thymbra spicata*	*Satureja cuneifolia*
R1	99.59±0.82 a	97.95±4.10 a	97.95±2.46 ab	91.39±1.57 bc
R2	94.76±4.03 ab	87.50±8.37 b	89.92±3.05 cd	84.68±3.84 d
R3	96.61±2.40 ab	99.15±1.69 a	95.76±2.19 abc	98.73±1.62 a
R4	91.25±4.59 b	92.50±8.77 ab	93.75±6.44 abcd	96.67±4.71 ab
R5	89.91±7.63 b	96.93±4.15 a	92.54±5.04 bcd	85.09±7.65 cd
R6	100.00±0.00 a	97.95±2.46 a	99.59±0.82 a	100.00±0.00 a

[a] Differences between means indicated by the same letters are not statistically significant (Duncan's multiple range test, $P \leq 0.05$).

that volatile oil of *Chrysanthemum coronerium* L. was less persistent for fast growing species such as *R. solani*. They recorded a 66.7% inhibition of *R. solani,* also at a concentration of 10 μl.

The fungitoxic activity of the essential oils was probably due to volatile phenolic compounds such as the monoterpenes (Farag *et al*., 1989; Ruiz *et al*., 1993; Tzakou *et al*., 1998). According to Faid *et al*. (1996) the oil components with the greatest antimicrobial activity are, in order of their effectiveness, phenols > alcohols > aldehydes > ketones > ethers > hydrocarbons. Baydar *et al*. (2004) reported that the antimicrobial activity of these oils could be attributed to the occurrence of high amounts of carvacrol. Carvacrol is widely reported at high levels to possess antimicrobial activity (Panizi *et al*., 1993; Mullerriebau *et al*., 1995; Sivropoulou *et al*., 1996; Aligiannis *et al*., 2001). Mullerriebau *et al*. (1995) also studied the antifungal effect of the essential oil of *T. spicata*. They found that *R. solani* was strongly affected by this oil, and especially by the components thymol and/or carvacrol. This finding was corroborated by our study. In contrast, Zambonelli *et al*. (2004) found that the fungicidal activity of commercial *T. vulgaris* oil against *R. solani* was due to high thymol levels in that oil (22–38%) rather than to the carvacrol level, which was low in all the oils they tested, ranging between 1 and 2%.

It is concluded that the four essential oils, which are especially rich in carvacrol can be used as natural fungicides and fumigants against *R. solani*. After this basic study on the antifungal activity of essential oils against *R. solani* under laboratory condition, further work is necessary to determine how these oils can best find an application in forest nurseries.

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