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In vitro Antifungal Activity of Essential Oils and Their Compounds on Mycelial Growth of *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder and Hansen

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Abstract: The increasing recognition and importance of phytopathogenic fungi, the difficulties encountered in their control and the increase in resistance to antifungal have stimulated the search for natural alternatives. The antifungal effects of essential oils and their compounds were investigated on mycelial growth inhibition bioassays of *Fusarium oxysporum* f. sp. *gladioli*. The essential oils have been used empirically. In general, a significant antifungal effect was observed with *Cinnamomum zeylanicum*, *Thymus vulgaris* and *Syzygium aromaticum* oils which had total inhibition at 100, 150, 200, 250 and 300 ppm. *Teloxys ambrosioides*, *Mentha piperita* and *Citrus aurantifolia* oils exhibited a dose dependent inhibition on mycelial growth to increase the dose of 100 at 300 ppm. While *Allium sativum*, *Capsicum* sp., *Ruta chalepensis* and *Eucalyptus globulus* oils had no antifungal activity at different concentration tested. All compounds with the exception of cineole had a fungicide or fungistatic effect.

Key words: Antifungal activity, gladiolus, fungistatic effect, carvacrol, citral, citronellol

INTRODUCTION

Fusarium corm rot, or yellows, is caused by the fungus *Fusarium oxysporum* f. sp. *gladioli*. This is the most common and serious disease of gladiolus. In México rots caused by *Fusarium oxysporum* can account for more than 60% during storage, in favorable conditions can reach 100% causing extensive financial loss to the growers. To control this disease, the corms should be treated with systemic fungicides before planting and/or after harvesting. Despite many attempts to control this disease, the problem is still prevalent (Roebroeck and Mes, 1992). Consequently, the development of appropriate alternatives to chemical fungicides for the management of this disease would be useful in reducing the undesirable environmental effects, soil contamination with chemicals and public exposure to pesticides (Lewis *et al*., 1998). Therefore, use of plant natural products could be an attractive alternative for the management of this disease.

Essential oils are complex volatile compounds and their constituents have been used as biological agents for their therapeutic activity and toxicity against insects and plant pathogenic fungi (Delespaul *et al*., 2000). They are needed for reduction in the use of chemical in agriculture increases interest in the possibility of the application of essential oils to control plant pathogens. The essentials oils of *Thymus vulgaris* has been reported to inhibit fungal growth. Their fungistatic activity has been attributed to the presence of thymol, at 50.06% in the oil tested (Zambonelli *et al*., 1996). The oil of *Chenopodium ambrosioides* completely inhibited the mycelial growth at 100 ppm of *Aspergillus flavus* Link and *Fusarium oxysporum* (Kumar *et al*., 2007). Fungal toxicity of the essential oil of *Mentha piperita* was evaluated against *Rhizopus stolonifer*, *Botrytis cinerea* and *Aspergillus niger*, the different concentrations of oil have antifungal activity against these fungi. Menthanol (36.24%) and menthone (32.42%) were the major compounds of the *M. piperita* essential oil (Behnam *et al*., 2006). The specific objectives of the present work were to determine *in vitro* the antifungal activity of the essential oils and their purified active compounds on growth of *Fusarium oxysporum* f. sp. *gladioli*.

MATERIALS AND METHODS

This study was carried out in the Biotic Products Development Center in Yautepec, State of Morelos, México in January-June of 2008.

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Essential oils and their compounds: Ten commercial essential oils (Aceites y Esencias S.A., México, D.F.) were evaluated for their fungicidal activity: herb epazote (*Teloxys ambrosioides*), peppermint (*Mentha piperita*), fringed rude (*Ruta chalepensis*), thyme (*Thymus vulgaris*), cinnamon (*Cinnamomum zeylanicum*), clove (*Syzygium aromaticum*), garlic (*Allium sativum*), Mexican lime (*Citrus aurantifolia*), peppers (*Capsicum* sp.) and eucalyptus (*Eucalyptus globules*) against *Fusarium.* Ten commercial essential oils compounds (Sigma Aldrich) were evaluated: carvacrol, cineole, citral, citronellol, geraniol, linalool, menthol, thymol, trans-cinnamaldehyde and trans-cinnamic acid. These compounds have been reported in cinnamon, eucalyptus, fringed rude, Mexican lime, peppermint and thyme.

Test microorganism: *Fusarium oxysporum* f. sp. *gladioli* was isolated from gladiolus corm rots of Cuautla, Morelos, México and the isolates was maintained on PDA. To maintain pathogenicity of the fungus, periodic inoculations and reisolations from infected corms were carried out.

After sterilization of media of Potato-Dextrose-Agar (PDA) essential oils and their compounds were added at concentrations of 100, 150, 200, 250 and 300 ppm and poured in Petri plates (60×15 mm). A five mm agar disc containing the pathogen of nine days of culture was placed at the center of each plate and incubated at 25°C for seven to nine days. Mycelial growth (colony diameter) was measured with vernier every day. Six replications were considered simultaneously for each concentration of essential oils. Control petri plates contained only PDA.

The growing mycelial colony was recorded for each treatment until control plates were completely colonized with mycelium. The experiment was repeated twice.

Statistical analysis: The mycelial growth data was subjected to ANOVA statistical analysis using Sigma Stat 2.0 software and when the effect revealed to be significant, a Tukey's test was applied for mean separation at $p \leq 0.05$. Growth rate was determined by linear regression.

RESULTS AND DISCUSSION

There were significantly differences between treatments, *C. zeylanicum*, *S. aromaticum* and *T. vulgaris* oils had total inhibition at 100, 150, 200, 250 and 300 ppm (Table 1). The growth rate of *Fusarium oxysporum* f. sp. *gladioli* for this oils were 0.0 compared with control 5.6 mm day^{-1}. A dose dependent inhibition of *Fusarium oxysporum* f. sp. *gladioli* mycelial growth was caused by *M. piperita*, *T. ambrosioides* and *C. aurantifolia*. The lowest mycelial growth (8.6 mm) was obtained with *M. piperita* at 250 ppm in comparison with control (50.8 mm). The growth rate decrease of 5.4 at 0.6 mm day^{-1} when the dose increase from 100 to 300 ppm. However, a no significant activity was observed with *A. sativum*, *Capsicum* sp., *R. chalepensis* and *E. globulus* oils. The obtained results are in agreement with those of Montes and Carvajal (1998), who reported that *A. flavus* was totally inhibited with *C. zeylanicum, T. ambrosioides* and *M. piperita.* Wilson *et al.* (1997) found that among the 49 essential oils tested, *C. zeylanicum* demonstrated a greatest antifungal activity against *Botrytis cinerea*. Iscan *et al.* (2002) reported that essential oils of *M. piperita* strongly inhibited plant diseases pathogens. *M. piperita* essential oils inhibited aflatoxin B_1 production by 85-90% in *Aspergillus flavus* and *Aspergillus parasiticus* (Bluma *et al.*, 2008). *Cinnamomum zeylanicum* and *S. aromaticum* were fungistatic and fungicidal against *Colletotrichum musae*, *Lasiodiplodia theobromae* and *Fusarium proliferatum* pathogens isolated from banana (Ranasinghe *et al*., 2002). Antifungal properties of essential oils belonging to six populations of *Thymus zygis* against *Rhizoctonia solani, Fusarium oxysporum* and *Colletotrichum acutatum* has been reported previously by Pérez-Sánchez *et al.* (2007). The essential oil volatiles from two clonal types of *T. vulgaris* exhibited antifungal activity against *Botrytis cinerea* and *Rhizopus stolonijer*, two common postharvest pathogens of strawberries (*Fragaria ananassa*) (Bhaskara *et al*., 1998). The oil of *T. numidicus*, endemic species of East Algeria, exhibited the strongest

Table 1: Effect of plant essential oils on mycelial growth of *Fusarium oxysporum* f. sp. *gladioli* at nine days after incubation

	Oil concentration (ppm)					
Essential oil	Control	100	150	200	250	300
Allium sativum	51.6 (5.6)a	50.9 (5.8)a	49.9 (5.7)a	51.8 (5.8)a	48.0 (5.6)a	41.1 (4.7)b
Capsicum sp.	51.6 (5.6)a	52.0 (6.1)a	50.5 (5.6)a	49.2 (5.6)a	47.4 (5.4)a	45.6 (5.3)b
Cinnamomum zeylanicum	51.6 (5.6)a	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b
Citrus aurantifolia	50.3 (5.0)a	49.6 (4.9)a	48.3 (4.7)a	46.8 (4.7)a	41.0 (4.0)b	35.5 (3.4)c
Eucalyptus globulus	50.3 (5.0)a	52.0 (5.1)a	52.0 (5.0)a	52.0 (4.9)a	52.0 (4.9)a	51.6 (4.8)a
Menta piperita	50.8 (5.4)a	41.1 (5.9)b	27.1 (3.8)c	16.4 (2.0)d	8.6 (0.6)e	8.9 (0.7)e
Ruta chalepensis	48.3 (5.0)a	47.3 (5.6)a	50.0 (5.7)a	50.3 (5.6)a	50.3 (5.6)a	48.9 (5.6)a
Syzygium aromaticum	51.6 (5.6)a	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b
Teloxis ambrosiosides	48.3 (5.6)a	47.9 (5.5)a	44.3 (4.9)b	40.0 (4.5)b	39.1 (4.4)b	35.0 (3.8)c
Thymus vulgaris	51.6 (5.6)a	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b

Means followed by different letter(s) in each row are significantly different by to Tukey's Test (p = 0.05), Values in parenthesis indicate growth rate in mm day^{-1}

Table 2: Effect of commercial essential oils compounds on mycelial growth of *Fusarium oxysporum* f. sp. *gladioli* at nine days after incubation

	Oil concentration (ppm)					
Compounds	Control	100	150	200	250	300
Carvacrol	49.5 (5.4)a	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b
Cineole	50.3 (5.0)a	52.0 (5.2)a	51.6 (5.1)a	51.5 (5.1)a	51.3 (5.1)a	51.0 (5.0)a
Citral	50.8 (7.0)a	37.3 (5.8)b	25.1 (3.3)c	7.8 (0.4)d	14.1 (1.4)de	4.9 (0.0)e
Citronellol	49.6 (5.9)a	37.6 (5.0)b	20.1 (2.1)c	8.6 (0.5)d	0.0 (0.0)e	0.0 (0.0)e
Geraniol	49.5 (5.4)a	46.8 (5.3)a	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b	0.0 (0.0)b
Linalool	50.8 (7.0)a	47.1 (6.9)a	44.3 (6.6)a	33.3 (4.8)b	28.8 (4.1)bc	21.1 (2.8)c
Menthol	49.6 (5.9)a	46.3 (5.7)a	46.8 (5.8)a	41.9 (5.4)b	42.4 (5.4)b	39.9 (5.0)b
Thymol	50.1 (6.3)a	35.0 (4.3)b	38.0 (4.8)b	18.2 (1.9)c	13.1 (1.2)d	6.9 (0.3)e
Trans-cinnamaldehyde	49.6 (5.2)a	11.6 (2.4)b	0.0 (0.0)c	0.0 (0.0)c	0.0 (0.0)c	0.0 (0.0)c
Trans-cinnamic acid	50.1 (6.3)a	46.6 (5.8)a	45.7 (5.7)a	40.3 (5.2)b	39.6 (4.9)b	38.8 (4.9)b

Means followed by different letters in each row are significantly different by to Tukey's Test ($p = 0.05$), Values in parenthesis indicate growth rate in mm day^{-1}

antifungal activity. Its antifungal effect was 31 fold more important that *T. vulgaris* thymol chemotype. It was evident that there was a positive correlation between antifungal activity and phenols (thymol and carvacrol) content (Giordani *et al*., 2008). *Cinnamomum zeylanicum* and *S. aromaticum* oils had strong inhibition of conidial germination of *C. gloeosporioides* at 50 ppm and a dose dependent inhibition mycelial growth was caused by these oils (Barrera-Necha *et al*., 2008). *S. aromaticum* and *T. vulgaris* oils presented inhibitory effects on growth of *Aspergillus niger* and *Aspergillus flavus*. Clove essential oil was a stronger inhibitor against *A. niger* than against *A. flavus* (Viuda-Martos *et al*., 2007).

Carvacrol, geraniol and trans-cinnamaldehyde had total inhibition (fungicide) at 150, 200, 250 and 300 ppm (Table 2). These compounds have been reported in cinnamon and thyme, which also had total inhibition of mycelial growth. Growth rate for this compounds were 0.0 compared with control 5.4 mm day^{-1}. A dose dependent inhibition of *Fusarium oxysporum* f. sp. *gladioli* mycelial growth was caused by trans-cinnamic acid, citral, citronellol, linalool, menthol and thymol. The lowest mycelial growth (4.9 mm) was obtained with citral at 300 ppm in comparison with control (50.8 mm).The growth rate decrease of 7.0 at 0.0 mm day^{-1} to increase the dose of 100 at 300 ppm. Citronellol had total inhibition mycelial growth a 250 and 300 ppm. Cineole presented null activity. This compound of eucalyptus and fringed rude also had null activity on mycelial growth. There was observed correlation between the antifungal activity with essential oils and their compounds. Plant essential oils are usually mixtures of several components. The oils with high levels eugenol (clove bud and cinnamon leaf), cinnamic aldehyde (cinnamon bark, cassia oil) are usually strong antimicrobials. The volatile terpenes carvacrol, p-cymene and thymol are probably responsible for the antimicrobial activity of oregano, thyme and savory (Davidson and Naidu, 2000). In the present research were evaluated the effectiveness of these compounds on mycelial growth of *F. oxysporum* f. sp. *gladioli*

Carvacrol, thymol and citral showed complete growth inhibition of *Botrytis cinerea*, *Alternaria arborescens* and *Rhizopus stolonifer* (Plotto *et al*., 2003). The major component of essential oils from *Thymus eriocalyx* and *Thymus X-porlock* was thymol. Antifungal activities of the oils were studied on the inhibition of *Aspergillus parasiticus* growth and aflatoxin production, both were inhibited at 250 ppm for two oils. Transmission electron microscopy of *A. parasiticus* showed irreversible damage to cell wall, cell membrane and cellular organelles (Rasooli and Owlia, 2005). *Listeria monocytogenes* treated with essential oils from the two thyme species exhibited a thickened or disrupted cell wall with increased roughness and lack of cytoplasm (Rasooli *et al*., 2006). *Thymus pulegioides* essential oil exhibited a significant activity against clinically relevant fungi mainly due to lesion formation in the cytoplasmic membrane and a considerable reduction of the ergosterol content (Pinto *et al*., 2006). Substantial changes were observed on the long chain unsaturated fatty acids when the *Escherichia coli* and *Salmonella enterica* strains grew in the presence of limonene and cinnamaldehyde and carvacrol and eugenol, respectively (Di Pasqua *et al*., 2006). Dominant constituent in *Cinnamomum osmophloeum* was cinnamaldehyde and Wang *et al*. (2005) have proven that this compound is a strong antifungal agent against *Coriolus versicolor* and *Laetiporus sulphureus*. Cinnamaldehyde may be a potential lead compound for the development of antifungal drugs through the control B-(1-3)-glucan and chitin synthesis in yeast and molds (Bang *et al*., 2000).

CONCLUSION

The essential oils of *C. zeylanicum*, *S. aromaticum* and *T. vulgaris* total inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *gladioli*. The compounds carvacrol, geraniol and trans-cinnamaldehyde presented a high antifungal activity against this fungus. Based on the present results the oils and components could be suggested as alternative fungicides.

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