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IN VITRO EVALUATION OF SELECTED ESSENTIAL OILS AS FUNGICIDES AGAINST *PENICILLIUM DIGITATUM SACC.*

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Abstract. **Several essential oils were evaluated for their efficacy as potential fungicides against *Penicillium digitatum*. Potato dextrose agar plates were streaked with a suspension of the spores of *P. digitatum*, then 10 µL of various essential oils were placed into a well in the center. After a period of growth the zone of inhibition was measured. Oils of cinnamon leaf, clove leaf, cumin and spearmint displayed larger than average zones of inhibition while the oils of *Citrus* sp. and wintergreen displayed minimal inhibition. While most plates displayed strong mycelial growth and sporulation outside the zone of inhibition, on the plates treated with cumin and wintergreen mycelial growth was weak and no spores developed.**

In the last few years there has been and increasing interest in essential oils as substitutes for conventional synthetic pesticides. This has been due, in part, to concerns over pollution, the development of resistance to conventional pesticides (Holmes and Eckert, 1999), and to the needs of producers of organic agricultural products.

There are several essential oil based products currently available commercially. Formulations of rosemary oil are offered as insecticide/miticides on fruit, nut and vegetable crops, another formulation containing rosemary oil is offered as a broad-spectrum fungicide for agricultural crops. Products containing clove oil are available for use as herbicides, potato sprout inhibitors and fungicides.

The literature gives several examples of previous trials that have tested the fungicidal properties of various essential oils. A solvent extract of clove flower buds has demonstrated antifungal activity against *Alternaria* sp., *Fusarium* sp., *Botrytis* sp. and *Septoria* sp. (Soatthiamroong et al., 2003). The oils of citronella and cinnamon have demonstrated antifungal activity against *Fusarium moniliforme* (Baruah et al., 1996), sage oil has been demonstrated to have efficacy against *Botrytis cinerea* (Carta et al., 1996) and the oils of oregano, thyme, lemongrass and cilantro have been identified as having fungicidal activity against the postharvest diseases of tomato (Plotto et al., 2003).

Essential oils may be defined as volatile oils that may be obtained from plant materials by steam distillation (Guenther, 1948). Other methods may be used such as cold pressing, enzyme digestion before centrifuging or distilling, or solvent extraction. Unfortunately these methods may change the composition of the final oil. Also, since they are from natural sources it follows that there would be variations in the composition of essential oils from the same species grown in different areas or at different times. This is also true of different species within the same genus grown under similar conditions (Zhu et al., 1994). Oils from different parts of the same plant may also vary considerably in composition. For example, the principle constituent of cinnamon oil from the leaves of the plant is eugenol with cinnamaldehyde as a secondary component, while in oil from the bark quantities these constituents are nearly reversed (Lawless, 1992).

With the limited availability of citrus postharvest fungicides in the US, alternatives to conventional treatments are becoming more attractive. Green mold of citrus, caused by *Penicillium digitatum,* is a cause of postharvest loss to fresh citrus in Florida. Identifying which essential oils may have fungicidal effects against this organism is an important first step in developing a possible postharvest treatment.

Materials and Method

Sterile unmodified potato dextrose agar (PDA) was poured into 87 mm petri dishes. When cool, a 4 mm well was cut in the center and the plate was streaked with a suspension

Table 1. Essential Oils Tested.

Scientific Name[Z]	Common Name	Principle Ingredients[Y]	Reference
Cinnamonium zeylancium	Cinnamon Leaf	eugenol, cinnamaldehyde	Lawrence 1994b Lawless 1992
Sygium aromaticum	Clove Leaf	eugenol	Lawless 1992
Cumin cyminium	Cumin	p-mentha-1,4dien-7-al, cuminaldehyde	Lawless 1992
Anethum graveolens	Dill Seed	carvone	Lawless 1992
Pelargonium graveolens	Geranium	citronellol, citronellol formate, gerinol	Lawrence 1994a Lawless 1992
Citrus paradisi	Grapefruit	limonene	Guenther 1949 Lawrence 1994c
Citrus limon	Lemon	limonene, beta-pinene	Guenther 1949 Lawrence 1994c
Cymbopogon citratus	Lemongrass	citral, myrcene	Lawless 1992
Citrus reticulata	Mandarin	limonene	Guenther 1949
Citrus sinesis	Orange	limonene	Guenther 1949
Mentha piperata	Peppermint	menthol, menthone	Lawless 1992
Rosemarinus officinalis	Rosemary	borneol, bornyl acetate	Guenther 1949
Mentha spicata	Spearmint	carvone	Lawless 1992
Gaultheria procumbins	Wintergreen	methyl salicylate gaultherrilene	Lawless 1992

[Z]Source plant for oil.
[Y]Often comprising more than 10% of oil.

of *Penicillium digitatum* spores. Then 10 µL of selected essential oils from various commercial sources (Table 1) were pipetted into the wells.

After 7 d incubation at ambient conditions, the plates were examined for growth and sporulation. A zone of inhibition, if present, surrounding the point of oil introduction, was measured. Mycelial growth vigor was rated on a scale of 0-3, 0 indicating no growth and 3 very heavy growth. Spore production on the mycelia was also rated on a scale of 0-3, 0 indicating no spores and 3 heavy sporulation (Table 2).

Results and Discussion

Of the oils tested, clove leaf produced the largest zone of inhibition (Fig. 1), followed closely by cinnamon leaf and spearmint. Since eugenol is a principal component of cinnamon leaf and clove leaf oil (Table 1), it is likely that this component is responsible for the bulk of their activity. Some inhibition of growth was seen with cumin, dill seed, geranium and peppermint. None of the others displayed a zone of inhibition or area where mycelia did not grow. Citrus oils with *d*-limonene as the principle component demonstrated no activity agains *P. digitatum.*

The mycelial vigor was strong outside the zone of inhibition in all oils except for cumin, peppermint and wintergreen. All oils allowed strong sporulation development where the mycelia grew, except for cumin, peppermint, spearmint, and wintergreen. The mycelia on the cinnamon leaf plates displayed slightly reduced spore production.

The oils cumin, peppermint, spearmint and wintergreen, possibly had a vapor effect in suppressing sporulation. The mycelial growth outside the zone of suppression was very weak with little or no spores produced. In the case of Wintergreen, even though there was no zone of inhibition around

Table 2. Summary of Results.

Common Name	Average Zone of Inhibition mm.	Mycelial Vigor[Z]	Sporulation[Y]
Cinnamon Leaf	45	3	2
Clove Leaf	52	3	3
Cumin	26	1	0
Dill Seed	11	3	3
Geranium	14	3	3
Grapefruit	0	3	3
Lemon	0	3	3
Lemongrass	12	3	3
Mandarin	0	3	3
Orange	0	3	3
Peppermint	16	1	0
Rosemary	0	3	3
Spearmint	40	3	1
Wintergreen	0	1	0

[z]0 = No growth; 1 = Weak growth; 2 = Moderate growth; 3 = Heavy growth.
[Y]0 = No spores visible; 1 = Some; 2 = light sporulation; 3 = Heavy sporulation.

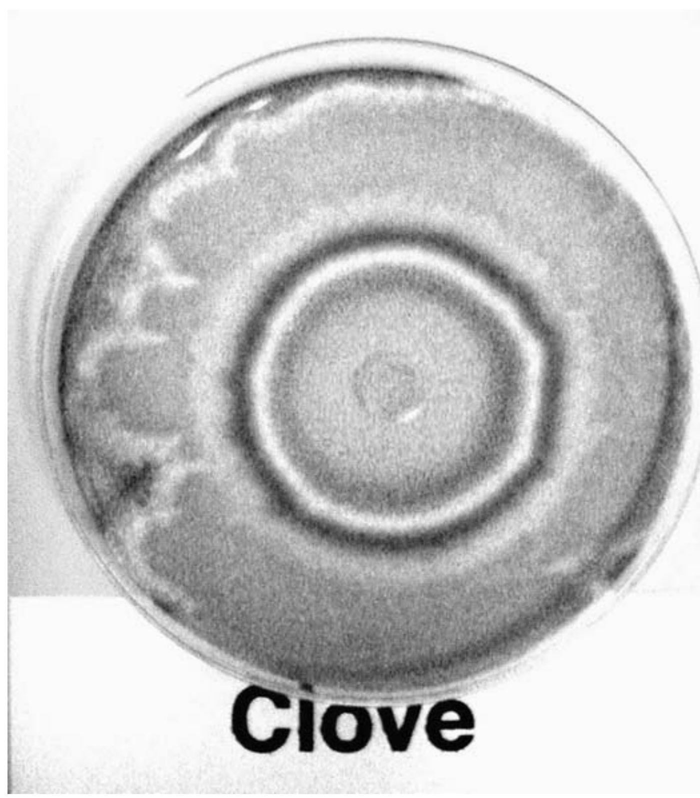

Fig. 1. Effect of clove oil.

the point of application, the mycelial growth was so weak that it was not noticeable from a distance (Fig. 2). Cumin oil displayed similar characteristics.

If essential oils are to be used as pesticides there are considerations to be taken into account. The first of these is the effect of the oil on the commodity to be treated. In trials in California, eugenol, the principal component of clove leaf and cinnamon leaf oils, was found to be phytotoxic to lemons (Smilanick, 2004). In tests on tomatoes the oils of oregano and thyme caused phytotoxicity to the extent that disease in treated tomatoes increased with oil concentration (Plotto et al., 2003). Such phytotoxicity would eliminate a compound from consideration.

A second concern is the legality of the use of essential oils as pesticides. Some of these oils qualify for exemption from the requirements of registration under Section 25(c) of the Federal Insecticide and Rodenticide Act, however, there are strict guidelines laid down by the US Environmental Protection Agency (EPA) regarding claims, labeling and other ingredients for these applications (Anomyous, 2000). Under that regulation the oils of cinnamon, clove, geranium, lemon grass, mint and rosemary are specifically exempted when used as active ingredients.

A third is the issue of safety. In high concentrations many of the essential oil ingredients have been identified as human health hazards (Tisserand and Balacs, 1995).

In developing a useful method of application many difficulties are yet to be overcome. The optimum method of application needs to be determined. Compatibility with other chemicals needs to be explored as well as the legality and safety of the method used. Plotto et al. (2003) found that combining some oils with certain emulsifiers apparently inhibited their antifungal properties.

Since oils of the same kind vary in composition with growing areas and seasons, the principle active components need to be identified, as well as the methods of ensuring consistency that will be used in the final application. Much is yet to be done to determine the commercial viability of essential oils as citrus postharvest fungicides.

Fig. 2. Effect of wintergreen oil.

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