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THYMUS CAPITATUS ESSENTIAL OIL REDUCING CITRUS FRUIT DECAY

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Abstract. Thymus capitatus essential oil was employed as vapour in postharvest to reduce Botrytis cinerea grey mould in mandarin fruits. The latter were sprayed with 10⁵ spores/ml suspension, placed in 15-litre desiccators at 25°C and fumigated under vacuum conditions at varying concentrations of thyme oil. The desiccators were kept sealed for 24 h and then the fruit rinse water was plated in Petri dishes to test spore viability. The results showed that the untreated control rinse water exhbited a high rate of fungus growth whereas the water from the treated fruit registered a significant reduction of fungal growth. SEM observations indicated that the thyme oil injured fungus hyphae.

Prevention of postharvest microbiological alterations in crops is relegated almost exclusively to synthetic chemical agents like thiabendazol, imazalil and benomil (Eckert and Ogawa, 1985). Yet, because of the short interval between produce treatment and marketing and the diminished effectiveness of many compounds due to the appearance of resistant strains (Eckert, 1988; Gullino et al., 1985; Romano et al., 1983), the use of low-toxicity fungicides in postharvest pathogen control to safeguard human health is extremely important, and it is necessary to develop viable alternatives to integrate or replace standard chemical agents. The present study tested the effectiveness of vacuum conditions and the essential oil of *Thymus capitatus* Hoffman et Link to control *Botrytis cinerea* Pers. in postharvest mandarin fruits.

Materials and Methods

Healthy fruits of cv. Carvalhal (Citrus Deliciosa Tan.) were washed in alcohol, inoculated with a 10^s conidia/ml suspension of B. cinerea and placed in 15-liter glass desiccators under vacuum. The T. capitatus essential oil was extracted in late July by vapour distillation of stemless leaves and flowers in the apparatus of Clevanger as reported in Farmacopea europea and analysed by gas chromatography (GC) with Varian 3300 chromatograph by split (1/50) injection in a fused-silica column (50 m long, 0.2 mm i.d., Supelco NS-54 bonded stationary phase) with helium as the carrier gas. Oven temperature range was 60° (10 min) to 200°C (10 min) with a 3_C/min step; the detector (FID) and injector were both at 250°C. The main constituents detected were: alpha-pinene, \(\beta\)-pinene, myrcene, linalol, borneol, p-cimene, transcaryophillene, v-terpinene and carvacrol, the latter making up about 70% of the whole oil, as already reported in a two year research on a wild thyme in Sardinia (Arras and Grella, 1992). The thyme oil was heated at 50°C and applied as vapour spray both at atmospheric pressure and under vacuum (0.6 bar) at 50, 100 and 200 ppm concentrations to test its fungicide effectiveness. The mandarin fruits were tested in two lots, 25 fruits per lot, at each concentration one lot was inoculated and the other used as the untreated control. The glass dessicators were opened after 24 h and four sample fruits were removed and washed individually for 1 h in a beaker containing 100 ml sterile water under 100 rpm shaking at 25°C. Four 100-ml samples of fruit rinse water were taken, individually plated in Petri dishes containing potato dextrose agar (PDA) and incubated at 24°C. The pathogen-colony count was taken daily; the data were analysed by the split-plot method and the Duncan's multiple range test used for mean separation with P = 0.01.

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Results

Thymus capitatus essential oil most effectively controlled the pathogen when applied under vacuum, its efficacy in comparison to control increasing 3.8 times at 50 ppm and 15.8 times at 100 ppm; no pathogen growth was detected at 200 ppm (Fig. 1).

These data indicate the greater diffusion of oil volatiles and the greater efficacy of oil against *B. cinerea* under vacuum. In addition, the oil markedly reduced microbe flora growth on the fruit epicarp.

Thyme oil neither injured fruit epicarp nor induced off-flavours in comparison to the typical taste characteristics exhibited by freshly harvested fruits. Scanning electron microscope (SEM) observations showed damage to pathogen hyphae (Figs. 2 and 3).

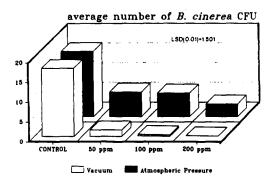


Fig. 1. Average number of *Botrytis cinerea* colony forming units (CFU) after *Thymus capitatus* essential oil treatment both in vacuum and at atmospheric pressure.



Fig. 2. Healthy *Botrytis* cinerea culture (control).



Fig. 3. Botrytis cinerea culture after Thymus capitatus essential oil fumigation. Note severe injury in the hyphae cell wall.

Discussion and Conclusions

The reported findings indicate that thyme oil was notably effective against *B. cinerea*, especially under vacuum conditions where the volatiles are more uniformly distributed, as reported elsewhere by Arras *et al.* (1993), and probably have greater adhesion to fruit epicarp. Yet practical application is still far from immediate, given the high cost of this essential oil. Future studies should focus on the determination of those oil fractions that are particularly active as fungicides, apart from those already known like carvacrol and others (Arras and Picci, 1986), and on the antagonistic and synergistic effects of the oil's individual compounds.

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