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Effect of TBZ, Acetaldehyde, Citral and *Thymus Capitatus* Essential Oil on 'Minneola' Tangelo Fruit Decay

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Abstract. **Thiabendazole (TBZ), citral, acetaldehyde (AA), and *Thymus capitatus* essential oil were tested for their ability to control decay on 'Minneola' tangelo fruit sprayed with a conidial suspension of *Penicillium digitatum,* either alone, or in combination with a low dose (100 ppm) of TBZ. TBZ at 2000 ppm was used as the standard. Thyme oil, citral and AA, all at 100 ppm (v/v), were applied under reduced pressure (0.6 bar) and heated to complete vaporisation. Control treatments were performed at normal and reduced pressure. Viability of fungal spores was checked by plating the rinsing water of three fruit per experimental plot on potato-dextrose agar dishes. A colony count was performed daily. All treatments significantly reduced the viability of the pathogen, TBZ at 2000 ppm being the best. The volatile compounds provided better control of the pathogen than TBZ at 100 ppm. Thus, *T. capitatus oil,* citral and AA could be of great interest in controlling postharvest disease of citrus fruits.**

Additional index words. Acetaldehyde, citral, orange, postharvest, *Thymus capitatus* essential oil

The control of microgial infection of fruit is still carried out almost exclusively with synthetic fungicides, despite the fact that the formation of resistant strains has reduced the efficacy of chemical agents (Dave *et al.*, 1990) and has frequently led to the use of higher doses and consequent risk of residues in the fruits. It is particularly important to limit the use of fungicides in postharvest control since the interval between treatment and consumption is usually short.

This serious problem has encouraged research to find substances which can integrate with, or be used as alternatives to chemical plant protection products. Increasing interest is being shown in volatile compounds of natural origin (Davis and Smoott, 1972; Tonini and Caccioni, 1990; Shaw *et al.*, 1991). Numerous studies conducted on the antimycotic and antibacterial activity of essential oils and their fractions (Pandey *et al.*, 1983; Caccioni and Guizzardi, 1994), particularly those distilled from the genera *Thymus* and *Origanum* (Carta and Arras, 1987) provide an important basis for the practical application of these substances.

Some studies of the essential oil of *Thymus capitatus* (L.) Hoffmgg. et Link have shown that it has a toxic action against several postharvest pathogens. The minimum inhibitory concentration (MIC) was between 0.2 and 0.3g/ℓ against fungi and 0.4-0.6g/ℓ against bacteria (Arras, 1988). Since the essential oil of thyme was found to have particularly good fungitoxic properties, further studies were done on the composition of the oil in relation to the various phenological stages of the plant, to discover the compounds causing the inhibitory activity. The fungitoxic effect was mainly due to carvacrol, although not always in proportion to its content in the oil (Arras and Grella, 1992). The highly effective fungicidal activity found *in vitro* was not, however, confirmed *in vivo*, on account of the volatile nature of the constituents of the oil.

Recent research has overcome this problem by combining physical and chemical measures in treatment (Arras *et al.*, 1993). In particular, when thyme essential oil was volatized at subatmospheric pressure, the fungitoxic effect was 10 times higher than when the treatment was carried out at normal atmospheric pressure (Arras *et al.*, 1995).

Many studies have been conducted on natural compounds either present in fruit or synthesized during storage which have proved to be effective in controlling some pathogenic microorganisms. Ashtana *et al.*, (1988) and Singh *et al.*, (1983) were among the first to demonstrate that citral (composed of neral and geranial, constituent fractions of the essential oil of citrus fruits) besides having a wide spectrum of antifungal activity, had no toxic effects on the fruits at the doses used. Acetaldehyde, a highly volatile compound authorized for use as an additive by the United States Environmental Protection Agency (EPA) and which is either present in nature (Fidler, 1968) or synthesized after certain methods of conservation (Pesis and Ben-Arie, 1984; Pesis and Avissar, 1990) has a high bactericidal and fungicidal activity against many postharvest pathogens (Prasad and Stadelbacher, 1974; Stadelbacher and Prasad, 1974).

The present study investigates further the fungitoxic effect under vacuum of acetaldehyde and citral vapours and the essential oil of *T. capitatus* against *Penicillium digitatum* inoculated to fruits of the tangelo 'Minneola'. The volatile compounds were used both alone and combined with a low concentration of thiabendazole (TBZ). As control, we used TBZ at a concentration known to be effective against the pathogen (2000 ppm).

Material and Methods

Fruits of 'Minneola' tangelo ['Dancy' mandarin (*Citrus reticulata* Blanco) x 'Duncan' grapefruit (*Citrus paradisi* Macf.)] were harvested and transported within a few hours to the Institute's laboratory in Sassari, where they were selected on the basis of minimal blemishes and homogenous size, washed with ethanol and allowed to dry in air. A spore suspension of a young *P. digitatum* culture was prepared, adjusted to 10^5 conidia/mℓ and then uniformly sprayed on the fruit surface, after a low concentration (100 ppm) thiabendazole (TBZ) suspension had been applied to half the fruit sample by dipping. The fruits were divided into four groups, then placed in 15-litre dessicators and after a vacuum of 0.1 bar had been created, exposed for 6, 12 and 24 hours to one of the following vapours: acetaldehyde, citral (a mixture of two geometric isomers, geranial and neral, from the flavedo extract of lemon fruit) and *Thymus capitatus* essential oil, 0 and 100 ppm (v/v) of liquid. A separate group dipped in a 2000 ppm TBZ suspension was used as a control. Vacuum was applied only to allow a more uniform vaporization. The essential oil was obtained in our laboratory by hydrodistillation of young leaves and flowers of thyme as previously reported (Arras and Grella, 1992). After each exposure period, three fruits from each dessicator were placed in beakers filled with 100 ml of sterile water. Each beaker was put in a water bath at 25°C and shaken at 100 rpm for 1 hour, before three 100 ml water samples were taken and plated on potato-dextrose agar (PDA) Petri dishes, then incubated at 25°C. Daily counts of colony forming units (CFU) were made.

Data were subjected to analysis of variance with a 4x3x2 completely randomized factorial design, where treatments, exposure time and TBZ were the factors. Comparison of mean values was performed by Duncan's multiple range test at P=0.01.

Results and Discusssion

The results show a significant reduction in the number of *P. digitatum* conidia in tangelo fruits in all treatments as compared with the control (Table 1). When the control was tested under vacuum for a time equivalent to that of the vaporization of the volatile compounds, no significant reduction in the growth of the pathogen was found compared to control results at atmospheric pressure (Table 1). Under vacuum conditions the essential oil of *T. capitatus*, acetaldehyde and citral inhibited almost all the conidia on the fruits, with values of 0.25, 0.42 and 0.17 colony forming units (CFU) respectively (Fig. 1).

The combination of TBZ (100 ppm) and volatile substances produced results which were not significantly better than those of the single treatments in the control of the pathogen (Fig. 2). Only TBZ at 2000ppm totally inhibited the growth of conidia in the fruits but the results were not statistically significant compared with the other treatments (Table 1).

The duration of treatment with the three volatile compounds (6 - 12 - 24h) did not significantly affect the results. It is likely that the maximum efficacy of treatment was reached within 6 hours.

The application of the volatile compounds did not harm the epicarp of the fruits or change in any way the quality of the fresh fruit. When the fruits were tasted there was no sign of 'off-flavours' or 'off-odours'.

As was found in previous studies on the vaporization of thyme, the vacuum helped the dispersion of the volatile compounds and adhesion to the fruits and condia (Arras *et al.*, 1994). In this regard, the high concentrations used in acetaldehyde treatments (1-10%) by Prasad and Stadelbacher (1974) against *Botrytis cinerea* in strawberries should be noted. No data are available on treatment with citral *in vivo*.

The results are of a certain interest as they show that

Table 1. Effect of vapours of thyme oil, citral and acetaldehyde on conidial viability of *Penicillium digitatum* after 6, 12 and 24 hours of exposure. Data represent colony forming units (CFU) per plate and are the average of six replications.

Treatments	Exposure (hours)		
	6	12	24
Test + TBZ (2000 ppm)	0b[x]	0b	0b
Test	22.20a	22.00a	23.50a
Test + Vacuum	21.50a	21.90a	22.80a
Test + TBZ[a]	0.67b	0.83b	0.84b
Thymus	0.67b	0.33b	0.17b
Thymus + TBZ	0.33b	0.00b	0.00b
Citral	0.83b	0.67b	0.33b
Citral + TBZ	0.33b	0.33b	0.00b
Acetaldehyde	0.50b	0.00b	0.17b
Acetaldehyde + TBZ	0.33b	0.00b	0.00b

[a] The TBZ concentration is 100 ppm.
[x] Mean separation by Duncan's Multiple Range Test, 1% level.

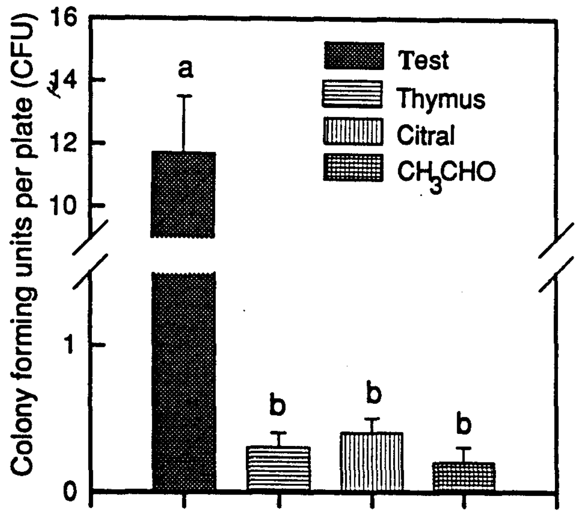

Figure 1. Effect of acetaldehyde, *Thymus capitatus* essential oil and citral on viability of *P. digitatum* conidia. Results, the mean of six replications + SE (vertical bars), include data from both fruits dipped or not dipped in a 100 ppm TBZ suspension. Mean values followed by the same letters are not significantly different at 1% level.

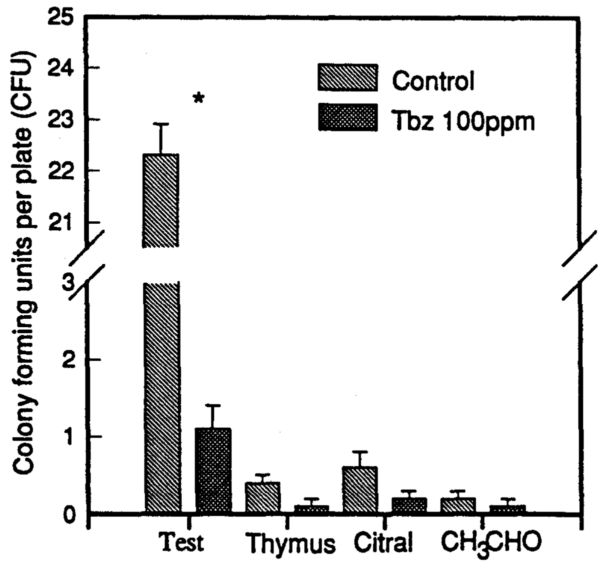

Figure 2. Influence of TBZ application on fungicide effect against *P. digitatum* of acetaldehyde, *Thymus capitatus* essential oil and citral. Average of six replications +SE (vertical bars).
* Significantly different at 1% level.

treatments using low doses of volatile compounds (100 ppm) gave similar results to treatment with TBZ at 2000 ppm, reducing the conidia population on tangelos to around zero values. This is important, considering that fruits at the time of harvesting are often contaminated by a high number of conidia and easily become infected, particularly in storage.

Conclusions

The three volatile compounds significantly inhibited the growth of *P. digitatum* as compared with the control, with no risk to the consumer or damage to the fruits. The fungicidal activity could be due to the subatmospheric environment in which vapours can more readily diffuse into the cells and adhere to the fruits and pathogens, thus having a significantly higher toxic effect than at normal atmospheric pressure.

The biggest problem is cost, as thyme essential oil is more expensive than citral and acetaldehyde, but one solution could be the use of one or more fractions of the essential oil. Moreover, treatment should be carried out under vacuum conditions, which in practical terms is not easy. However, in future it may be possible to use vacuum pre-refrigeration equipment for treatments using volatile compounds.

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