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Residue levels and performance of potassium sorbate and thiabendazole and their co-application against blue mold of apples when applied as water dip treatments at 20 or 53 °C

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ABSTRACT

The efficacy of potassium sorbate (K-sorb) as an antifungal, alone or combined with thiabendazole (TBZ), and hot water at 53 °C, was studied against blue mold on apples. Results demonstrated the fungistatic effect of K-sorb: 2000 mg L^{-1} significantly reduced *Penicillium expansum* growth, and a 24 h delay in fungi development was observed in the amended liquid culture. K-sorb at 1% significantly diminished decay incidence, especially when applied at 53 °C. The performance of TBZ was not improved by the co-application of K-sorb. Treatments efficacy was related to K-sorb distribution on fruit surface. ESEM and X-ray qualitative spectra analyses revealed an irregular distribution of K-sorb over the fruit surface and areas with salt accumulation. K-sorb residues remained quite unchanged until 3 d of storage then they declined progressively. Their degradation was influenced by water temperature: it was faster on fruit treated at 53 °C. An inverse relation between *P. expansum* growth and K-sorb concentration was observed. After 72 h of incubation, *P. expansum* depleted 99% of K-sorb from liquid culture media.

The low efficacy of K-sorb treatments against blue mold decay on apple fruit may be ascribed to the low persistence of K-sorb residues, to the irregular spatial distribution of the salt on the fruit surface, and the ability of *P. expansum* to degrade the salt.

1. Introduction

Apples are subjected to several postharvest diseases that determine severe economic losses during postharvest handling, transport and commercialization. Among molds, *Penicillium* expansum is one of the most aggressive food-born pathogen and one of the leading producers of patulin, a mycotoxin responsible for severe and chronic effects on human health (Reddy et al., 2010).

At present, *P. expansum*, is effectively controlled by synthetic fungicides. On a commercial scale, blue mold disease management is based on thiabendazole (TBZ) treatment employed as drench or spray before cold storage. In recent years, fludioxonil and cyprodinil, two reduced risk fungicides, have been proposed as chemical alternatives to TBZ (Errampalli and Crnko, 2004). However, the increase of fungicide resistant strains (Baraldi et al., 2003; Li and Xiao, 2008a,b) caused by the intensive and exclusive use of only a few active ingredients, along with the new European Union directives on the sustainable use of pesticides (Directive 2009/128/CE), has led to research to identify new strategies for postharvest pest management of apples.

Integrated control programs based on food preservatives in combination with low doses of fungicides have been studied and suggested for several commodities (Gregori et al., 2008; Schirra et al., 2008, 2011; Dore et al., 2010; Mehyar et al., 2011; D'Aquino et al., 2012, 2013; Parra et al., 2014).

Among food preservatives, sorbic acid and its potassium salt are extensively used as antimicrobial agents in food. Their action, based on the ability to inhibit or delay the growth of spoilage microorganisms, is strongly influenced by microbial species and strain, substrate composition and environmental factors (Stopforth et al., 2005). Some *Penicillium* species are able to grow in the presence of high concentrations of K-sorb by degrading the salt into 1,3-pentadiene, a volatile compound not toxic for molds (Marth et al., 1966).

On naturally infected peaches K-sorb applied at 15 g L^{-1} reduced brown rot caused by *Monilinia laxa* by over 80% (Gregori et al., 2008),

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while on citrus fruit, it was able to control only recently established infections without providing any persistent protection. However, when applied in combination with fungicides, K-sorb improved their activity against *Penicillium digitatum* and *Penicillium italicum* and partially controlled *P. digitatum* TBZ resistant strains and *Geotricum citri-aurantii,* which were not controlled by fungicides alone (Palou et al., 2002; Smilanick et al., 2008).

On apples, the use of K-sorb to control postharvest decays is essentially based on its inclusion into edible coatings which are claimed to improve the efficacy of K-sorb by providing a controlled release of the active ingredient (Olivas et al., 2007; Mehyar et al., 2014).

Little information is available in the literature on the effectiveness of dip treatments with K-sorb solutions or on the combined effects of K-sorb with TBZ treatments. The present study was designed to evaluate the influence of these treatments as a function of TBZ and K-sorb concentration and dipping temperature; moreover residue levels and persistence of TBZ and K-sorb were investigated and correlated to mold growth. Experiments were performed by in *vitro* tests and on artificially inoculated apples immersed for 60 s in water or water mixtures containing K-sorb or TBZ alone or in combination at 20 or 53 °C. The active ingredients (a.i.) distribution on fruit surface was evaluated by environmental scanning electron microscopy (ESEM) and energy dispersive X-ray microanalysis (EDX).

2. Materials and methods

2.1. Chemicals

A commercial formulation of TBZ (Tecto SC, at 42,9% a.i., Syngenta Crop Protection S.p.a. Milan, Italy) and K-sorb (99% a.i., Panreac Quimica Sau Castellar de Valles, Barcelona, Spain) were used in this study. Acetone and hexane were of GC grade (Merk, Milan, Italy). NaCl was of analytical grade (Carlo Erba, Milan, Italy). Analytical standard of TBZ was purchased from Dr. Ehrenstorfer (Augsburg, Germany). A stock standard solution of the active ingredient (500 mg L^{-1}) was prepared in acetone.

2.2. Plant material

Commercially mature apples (*Malus domestica*, Borkh), *cv* 'Miali' and 'Caddina' from Sardinian germoplasm, were harvested from the experimental orchard of the CNR-Institute of Sciences of Food Production located in central-western Sardinia (Italy). Immediately after harvest, fruit were delivered to the laboratory. Fruit free from visible defects were selected and left overnight at 20 °C.

2.3. Fungal strains and growth conditions

Monosporic isolate of *P. expansum* was obtained from decayed apples *cv* 'Miali' harvested in an orchard located in central-western Sardinia (Italy). The pathogen was cultured on potato dextrose agar (PDA; Merck & Co., Whitehouse Station, NY) amended with streptomycin sulfate and oxytetracycline hydrochloride (0.1 + 0.1 g L^{-1}) to prevent growth of bacterial contaminants.

A conidial suspension of *P. expansum* isolate was prepared by scraping the surface of a 7 d old colony, grown on Petri plate (90 mm diameter, 15 mL of PDA) incubated at 25 °C. Spores were resuspended in sterile Ringer's solution (NaCl, 8.6 g L^{-1}, KCl, 0.3 g L^{-1}; and $CaCl_2$, 0.48 g L^{-1}), filtered through two layers of sterile cheesecloth, and counted with a hemocytometer. The conidial concentration was adjusted at 10^8 conidia L^{-1} by adding sterile water.

2.4. K-sorb and TBZ activity on artificially inoculated fruit

Before inoculation and treatments, fruit were disinfected by dipping for 30 s in household bleach solution (0.1 mg L^{-1}, sodium hypochlorite), rinsed with tap water and left to dry overnight.

The fruit, arranged in plastic boxes (20 fruit per box), were wounded (1 wound per fruit; diameter: 3 mm, depth: 3 mm) and allowed to dry for 3 h. Twenty microliters of a conidial suspension 10^8 conidia L^{-1} were pipetted into each wound. Before treatment, fruit were incubated at 20 °C and 90–95 % RH for 24 h.

Fungicide treatments (three replicates of 20 fruit each) were performed in: (a) water or water mixtures at 20 °C containing K-sorb at 1%, TBZ at 600 mg L^{-1}, or K-sorb at 1% plus TBZ at 600 mg L^{-1}; (b) water or water mixtures at 53 °C containing K-sorb at 1%, TBZ at 200 mg L^{-1}, or K-sorb 1% plus TBZ at 200 mg L^{-1}. Hot water treatments were carried out by dipping the fruit for 60 s in a bath fitted with 3.96 kW heating elements, an electronic recirculation pump (0.022 L s^{-1} water flow) and an electronic thermostat (OEM/HT, Carel, France).

Following treatments, fruit were left to dry at room temperature, replaced into the packing trays with the wounded side upward and stored at 20 °C and 90–95% RH. After 6 and 9 d, fruit were collected and evaluated for decay caused by blue mold. Decay incidence, for each treatment, was expressed as percentage of fruit with lesions of any size on the total. The severity of decay was determined by measuring lesion surface on 20 fruit per replicate. For lesion surface calculation the mean diameter of the lesion was measured and the following formula was applied: surface lesion area: $(D/2)\pi$, where D is the diameter of the lesion. Results were expressed in mm^2.

2.5. In vitro assays

2.5.1. Effect of K-sorb, TBZ and their co-application on mycelia growth inhibition

A monosporic isolate of *P. expansum* prepared as described above was used to test the effect of TBZ and K-sorb on mycelia growth. The fungicides were added alone or in combination to molted PDA at 45 °C. The following concentrations of a.i. were tested: TBZ 0.05, 0.1, 0.25, 0.5, 1 mg L^{-1}, K-sorb 100, 200, 400, 800, 1000, 2000 mg L^{-1}. Five microliters of a conidial suspension of *P. expansum* at the concentration of 10^9 conidia L^{-1} was dropped onto the plates containing the amended media. Five replicate plates were used for each concentration and for the control without fungicide. The radial growth (mm) of the fungal colony was measured after 7 d of incubation at 25 °C. The results were expressed as percentage of growth inhibition calculated as follows: $100\times$ $(G–g)/G$, where G is the growth of the control without fungicides and g is the growth of the fungal colony in the amended media. The experiment was repeated twice.

2.5.2. Effect of K-sorb, TBZ and their co-application on colony formation

A monosporic isolate of *P. expansum* was tested to evaluate the effectiveness of TBZ, K-sorb and their combination on colony formation. 100 μL of a spore suspension (10^6 spores L^{-1}) were spread onto the surface of PDA medium amended with TBZ and K-sorb alone or in combination with increasing TBZ and K-sorb concentrations (TBZ: 0.05, 0.1, 0.25, 0.5, 1 mg L^{-1}; K-sorb: 100, 200, 400, 800, 1000, 2000 mg L^{-1}). Plates were incubated at 23 °C for 48 h, then the number of colonies was counted. Results were expressed as percentage of colony forming units (CFU mL^{-1}) compared to control. Three replicate plates were used for each fungicide concentration. The test was performed twice.