Scientia Horticulturae 198 (2016) 86–90

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Evaluation of alternative means to control postharvest *Rhizopus* rot of peaches

Ehab A. Salem [a], Khamis Youssef [b,*], Simona Marianna Sanzani [c]

[a] Food Irradiation Department National Centre for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt
[b] Agricultural Research Center, Plant Pathology Research Institute, 9 Gamaa St., 12619 Giza, Egypt
[c] Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form 5 November 2015
Accepted 6 November 2015
Available online 14 December 2015

Keywords:
Calcium chloride
Lemongrass oil
Peach
Rhizopus stolonifer

ABSTRACT

The objective of the present research was to test the activity of calcium chloride and lemongrass oil, alone or in combination, against *Rhizopus stolonifer* on peaches. The inhibitory effect was evaluated both *in vitro* and *in vivo*. Results showed that *in vitro* pathogen growth decreased as treatment's concentration increased, reaching a complete inhibition at 1.5 ml/l and 20 g/l for lemongrass and calcium chloride, respectively. However, taking into account phytotoxicity phenomena, lower concentrations were tested *in vivo*. A 70% reduction of both rot incidence and severity was achieved using lemongrass oil at 1.5 ml/l; whereas, in presence of $CaCl_2$, the disease reduction was much lower, reaching a maximum at 1.5 g/l of 30 and 59% for incidence and severity, respectively. The combination of the two treatments gave the best performance against rot, and the control effect proved to be synergic as far as disease severity concerns. The observation using scanning electron microscopy (SEM) confirmed the ultra-structure modification in *R. stolonifer* after treatment. New strategies are needed to reach the critical goal of controlling *Rhizopus* rot of peaches with no fungicide residues on fruit. In this context, the integration of calcium chloride with lemongrass essential oil might be promising, although further trials are needed.

1. Introduction

Rhizopus species are considered among the most devastating fungi during storage of various horticultural commodities (Bautista-Banos et al., 2014). On peach fruit, *Rhizopus* rot is second only to brown rot by *Monilia* spp. in causing serious economic losses. Indeed, in 2012 peaches and nectarines had a worldwide production of 21,083,151 tons, and Italy and Egypt ranked among the 20 top producers with around 1,331,621 and 2,85,194 tons, respectively (FAOSTAT, 2015).

Rhizopus stolonifer (Ehrenb.: Fr.) Vuill is one of the most common and fastest-growing species, particularly in moist conditions, and therefore, considered one of the most devastating (Bautista-Baños et al., 2014). *Rhizopus* rot appears particularly on mature fruit, when temperatures are higher than 5 °C (*i.e.*, during processing at room temperature, shelf-life, or at consumer's home) and spreads quickly from infected to healthy fruit (Ogawa et al., 1995). Due to the wide array of hosts and its fast penetration and colonization, *R. stolonifer* has become an important target of control by synthetic fungicides. However, the appearance of resistant strains and the concern for residues on fruit and in the environment forced producers to evaluate safer alternatives as hydrocooling, hot water dips, waxes, and biocontrol agents (Yang and Jiang, 2015). However, no satisfying solution has still been found.

In recent years, there has been an increasing interest toward the use of plant extracts, as essential oils that might play an important role in crop protection, being antibacterial, antiviral, antifungal, insecticides, and effective against herbivores (Bakkali et al., 2008). Essential oils of anise, ammi, ziziphora and cinnamon proved to control grey mould and maintain quality of peach fruit (Mohammadi and Aminifard, 2012). Similarly, the essential oil of lemongrass (*Cymbopogon citratus* L.) showed antimicrobial activities on various commodities, including strawberry, comparable or even superior to synthetic fungicides (Tzortzakis and Economakis, 2007; Asghari et al., 2009).

Among alternative control means of postharvest diseases, even organic and inorganic salts proved to be particularly promising (Ippolito and Sanzani, 2011). Calcium dips have been used to improve firmness and extend the shelf-life of a wide range of fruit and vegetables (Babak and Forney, 2015). Indeed, the increased calcium content of the cell wall of fruit tissue can delay softening and

* Corresponding author. Fax: +20 235723146.
E-mail addresses: youssefeladawy@agr.uniba.it, youssefeladawy@arc.sci.eg (K. Youssef).

http://dx.doi.org/10.1016/j.scienta.2015.11.013
0304-4238/

mould growth, and decrease the incidence of physiological disorders (Poovaiah, 1986). Postharvest applications of calcium chloride and sodium bicarbonate, particularly in combination with the antagonist *Aureobasidium pullulans,* were effective in controlling *Botrytis cinerea* on sweet cherries (Ippolito et al., 2005). Moreover, calcium chloride proved to control blue mould and bitter rot of apple, and to preserve the quality of fig fruit (*Ficus caica* L.) during storage and shelf-life (Conway et al., 1992; Biggs, 1999; Irfan et al., 2013). Finally, calcium chloride was successfully tested against gray mould of 'Italia' table grapes and postharvest decay of 'Comune' clementine and 'Valencia late' orange fruit (Youssef et al., 2012a,b; Youssef and Roberto, 2014a).

Therefore, the objective of this research was to test the activity of calcium chloride and lemongrass oil, alone or in combination, against rot by *R. stolonifer* on peach fruit.

2. Materials and methods

2.1. Pathogen

The present study was performed during the seasons 2013–2014. The *R. stolonifer* strain R1 used in all trials came from a peach fruit collected in a market in Giza (Egypt). It was identified based on morphological and biochemical characteristics as reported by Lima et al. (2014), and deposited in the fungal collection of the Mycology and Plant Diseases Survey Department of the Plant Pathology Research Institute, ARC, Egypt. The isolate was maintained in its monoconidial form on potato dextrose agar (PDA) slants at 4 °C. To fulfill Koch's postulate, the strain was used to inoculate peach fruit (*Prunus persica* L.) Batsch cv. Early Grand. The fruit were selected for uniform size, color and absence of any disease or defect. They were surface disinfested in 70% ethyl alcohol for 1 min, washed with running tap water, dried, and wounded (4 mm depth × 6 mm wide) along the equatorial axis. Inoculation was performed by inserting a 5 mm agar plug, taken from the margin of 10-day-old PDA culture of the fungus, into fruit wounds. Control fruit were inoculated using agar plugs without fungus. The fruit were placed into carton boxes, incubated at 22 ± 2 °C, and surveyed at 7 days post-inoculation (dpi). Three replicates were used and each replicate contained 10 fruit. The rot incidence (%) was recorded according to the formula: Number of rotted fruits/Total number of fruits × 100. Rot severity was expressed as average lesion diameter (mm), and each value was calculated as the average of the two orthogonal diameters of the rotted area.

2.2. In vitro *effect of lemongrass oil and calcium chloride against* R. stolonifer

Lemongrass oil (LO) was obtained from Sekem Group (Cairo, Egypt). Its chemical constituents were determined by Gas Chromatography Mass Spectrometry (GC–MS) as reported by Onyambu et al. (2015), and included in Table 1. Calcium chloride ($CaCl_2$) was obtained from El Nasr Pharmaceutical Chemicals Co. (Abu Zaabal, center Khanka, Qaliubiya, Egypt). LO (with 1% Tween 80 as emulsifying agent) and $CaCl_2$ (dissolved in sterile distilled water) were 0.45 μm filtered (Millipore, Bedford, MA, USA), and added to molten PDA at 45 °C before pouring into 90 mm Petri dishes, to achieve final concentrations of 0.5, 1, 1.5, 2 ml/l, and 1, 2, 10, 20 g/l for LO and $CaCl_2$, respectively. Non-amended PDA served as a control. The plates were inoculated in the center with a 5 mm plug taken from the edge of actively growing colonies of *R. stolonifer*. For each concentration, 5 Petri dishes were prepared as replicates and the entire experiment was repeated twice. The growth of the fungus was measured as the average of the two orthogonal diameters when the control plates reached full growth at 24 ± 2 °C. The percentage of reduction in colony diameter was also calculated.

Table 1
Chemical constituents of lemongrass oil determined by gas chromatography–mass spectrometry (GC–MS).

Concentration (%)	Constituents
Myrecene	9.99
α-Terpenene	0.60
Linalool	1.01
Nerol	1.49
Borneol	1.62
Neral (Citral A)	40.3
Geranail (Citral B)	41.67
Methyl geranate	0.31
Geranyl acetate	0.95
Methyl-2,4-decadienoate	1.28
n-Hexadecane	0.57
α-Cadinol	0.22
Hexadecanoate	0.33

2.3. In vivo *effect of lemongrass oil and calcium chloride against* Rhizopus *rot*

Healthy peach fruit were sterilized, wounded twice at the opposite side on the equatorial surface, and inoculated with *R. stolonifer* as reported above. Then fruit were dipped for 3 min in 0.5, 1, 1.5, 2 ml/l of LO (using 1% Tween 80 as emulsifying agent), or 0.5, 1, 1.5 g/l of $CaCl_2$. Combined effect of the two treatments on incidence and severity of *Rhizopus* rot was also evaluated. Fruit were dipped in 1.5 g/l $CaCl_2$ for 3 min, air dried for 2 h in a laminar flow hood, and dipped in 2 ml/l of LO for 3 min. Fruit treated with sterilized water were used as a control. Finally, all fruit were air dried in laminar flow hood for 2 h, packed in plastic nets, put in perforated sterilized carton boxes and stored for a week at 24 ± 2 °C. For each treatment, 5 replicates were used and each replicate contained 10 fruits. The rot incidence (%) was recorded according to the formula: Number of rotted wounds/Total number of wounds × 100. Rot severity was expressed as average lesion diameter (mm), and each value was calculated as the average of the two orthogonal diameters of the rotted area.

2.4. Scanning electron microscopy

R. stolonifer was grown in Potato Dextrose Broth (PDB) amended with: (i) $CaCl_2$ at 1.5 g/l, (ii) 2 ml/l LO, or (iii) with the two combined treatments. PDB without any addition served as a control. Collected fungal mats were fixed in 2.5% glutaraldehyde at 4 °C for 24 h and post-fixed in 1% osmium tetraoxide for 1 h at room temperature (Harley and Fergusen, 1990). Then they were dehydrated with ascending concentrations of acetone, critical point dried, and finally sputter coated with gold. Examination and photographing was done through scanning electron microscope (JSM-1200 EX, Joel, Peabody, MA, USA) in National Centre for Radiation Research and Technology (Cairo, Egypt).

2.5. Synergic interaction calculation

Limpel's formula, as described by Richer (1987), was used to determine the presence of synergic interactions between treatments:

$$E_e\,(\%) = (X + Y) - \left(\frac{X \times Y}{100}\right)$$

where E_e is the expected effect from additive responses of two controlling treatments used in combination, and X and Y are the found percentages of reduction of disease incidence and severity relative