SHORT COMMUNICATION

EFFECT OF A NOVEL POTASSIUM BICARBONATE-BASED FORMULATION AGAINST PENICILLIUM DECAY OF ORANGES

K. Youssef1, S.M. Sanzani2, A. Myrta3 and A. Ippolito2

1Agricultural Research Center, Plant Pathology Research Institute, 9 Gamaa St., 12619 Giza, Egypt
2Dipartimento di Scienze del Suolo, della Pianta e degli Alimenti, Università degli Studi Aldo Moro, Via Amendola 165/A, 70126 Bari, Italy
3Certis Europe, Boulevard de la Woluwe 60, 1200 Brussels, Belgium

SUMMARY

The efficacy of the novel potassium bicarbonate formulation Karma (Certis Europe) for controlling Penicillium decay of orange fruit was tested. In vitro trials were carried out by amending potato dextrose agar (PDA) medium with different Karma concentrations, thereby revealing a complete inhibition of Penicillium digitatum, P. italicum, and P. ulaiense growth at 0.3, 0.3, and 0.2% (w/v), respectively. In vivo trials using dipping and spraying application strategies were conducted on Valencia late and Tarocco, two sweet orange cultivars with different degrees of susceptibility to Penicillium rot. Fruit treated with unfertilulated potassium bicarbonate (PB) or water served as controls. When applied by dipping, Karma and PB at 3% significantly reduced the incidence of Penicillium decay of cv. Valencia late oranges, i.e. by 79 and 31%, respectively. On the other hand, when applied by spraying, 6% Karma and PB were needed to completely inhibit decay incidence. On cv. Tarocco oranges, Karma and PB applied at 3% by dipping reduced the percentage of Penicillium decay, by a significant 87 and 68%, respectively. However, when applied by spraying at 6%, no difference was observed between the two treatments. Overall, Karma performed better than PB in controlling Penicillium rots and dipping proved to be the best application strategy.

Key words: Citrus sinensis, postharvest decay, potassium bicarbonate, spray, dipping, penicillium rot.

Green, blue, and whisker molds, caused by Penicillium digitatum (Pers.:Fr.) Sacc., P. italicum Wehmer, and P. ulaiense H.M. Hsieh, H.J. Su et Tzean are the most common postharvest diseases affecting citrus production worldwide (Eckert and Eaks, 1989; Youssef et al., 2010a). The severity of losses due to these fungi varies depending on production area, cultivar, climatic conditions, and postharvest handling practices (Youssef et al., 2011; Iqbal et al., 2012). Postharvest diseases are commonly controlled by synthetic fungicides; however, the demand for alternative control means is increasing, since they have a higher affordability, a lower risk of fungal resistance development and a lesser impact on the environment and human health (Elmer and Reglinski, 2006; Mari et al., 2010). For instance, inhibitory effects of organic and inorganic salts on postharvest diseases have been reported for several fruit commodities (Sanzani et al., 2009; Mari et al., 2010; Ippolito and Sanzani, 2011; Romanazzi et al., 2012; Youssef and Roberto, 2014). Among these salts, carbonate, bicarbonate, chelate, sorbate, silicate, etc. have comprehensively been tested in small and large scale experiments against postharvest rots of citrus fruit (Palou et al., 2008, 2009; Smilanick, 2011; Youssef et al., 2012a, 2012b). These compounds are gaining particular interest since they belong to the “Generally Recognized As Safe” (GRAS) category and, as such, are allowed in the agro-food industry. In some instances, salts are marketed as formulations. For example, Karma (Certis Europe, Italy), a soluble powder containing 85% (w/w) potassium bicarbonate and ≤ 15% surfactants (sodium lauryl sulfate and docusate sodium), recommended against diseases of a wide range of fruit and vegetable crops, has commercially been available in Switzerland since 2008 (Milling et al., 2012). Although several studies have been conducted in which these salts were applied in their purest form, few reports regarding testing of commercial products exist. Thus, the present study was undertaken to (i) evaluate the in vitro ability of Karma to inhibit the growth of P. digitatum, P. italicum, and P. ulaiense, and (ii) assess its effectiveness against postharvest sweet orange decay during cold storage and shelf-life.

To test in vitro the effect of Karma on mycelial growth, an aqueous solution of the formulation was 0.45 μm filtered (Millipore, USA) and added to molten PDA (45°C, pH 5.6) before pouring into 90 mm Petri dishes, to achieve final concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5% (w/v) and a pH 8.3 (0.1%) - 8.9 (0.5%). Non-amended PDA served as a control. Dishes were seeded in the center with...
a 5 mm mycelial plug taken from the edge of actively growing colonies of *P. digitatum* (CBS319.48), *P. italicum* (CBS112437), and *P. ulaiense* (CBS261.94), and incubated for 6 days at 24±1°C. For each concentration, five Petri dishes were prepared as replicates and the entire experiment was repeated twice. Growth was calculated as the average of the two orthogonal diameters; the results were expressed as minimum inhibitory concentrations (MIC) and the salt concentration that caused a 50% reduction (ED50) was determined using SAS probit analysis (Sas Institute, USA) according to Arslan et al. (2009). The percentage of reduction in the colony diameter was calculated as follows:

\[
\text{Reduction (\% of colony diameter)} = \left( \frac{dc - dt}{dc} \right) \times 100
\]

where: \( dc \) = average diameter in the control, and \( dt \) = average diameter in the salt-amended plates.

In vivo experiments were conducted on fruits from 25-year-old sweet orange [*Citrus sinensis* (L.) Osbeck] plants of cvs Valencia late and Tarocco, harvested in a commercial orchard (ALSIA, Azienda Agricola Sperimentale Dimostrativa Pantanello, Metaponto, Italy) and selected for uniform size, color and absence of any disease or defect. Commercial potassium bicarbonate (PB) (A&Z Food additives Food Co., China) and imazalil (Deccozil 50, Decco Italia, Italy) were included for comparison. All fruits were wounded singly (3 mm deep×3 mm wide) before treatment. Then they were dipped in a 3% solution of Karma or PB for about 10 min, or sprayed with 6% solutions until dripping. These concentrations were chosen following previous trials (Youssef et al., 2012a). Fruits treated by water and imazalil (0.05%, v/v) by both spray and dipping served as controls. After treatment, the fruits were left to dry for 2 h at room temperature. For
effects on each cultivar and treatment, the fruits were divided into 5 boxes (replicates) weighing around 8 kg each. Finally, the fruits were stored at 8±1°C (cv. Tarocco) or 6±1°C (cv. Valencia late) for two weeks followed by two (cv. Tarocco) or six (cv. Valencia late) days of shelf-life at 20±2°C and 90-95% relative humidity. For each box, incidence (infected wounds, %) and severity (lesion diameter, mm) of naturally occurring infections by fungal pathogens were recorded. Pathogens were identified visually but, in case of doubt, isolations and morphological identifications were carried out. All the experimental data were subjected to one way variance analysis (ANOVA) using Statistica 6.0 software. Mean values of treatments were compared using Fisher’s protected least significant difference (LSD) test and assessed at P ≤ 0.05 level. Disease incidence data were arcsine-transformed before statistical analysis.

A complete growth inhibition for *P. digitatum*, *P. italicum*, and *P. ulaiense* was achieved at 0.3, 0.3, 0.2% concentrations, respectively, which corresponded to their MICs (Table 1); on the other hand, the ED50s, inferred by Probit analysis, were 0.12, 0.12, and 0.08% (w/v), respectively. Discs re-seeded onto fresh PDA medium revived their growth (data not shown).

In *in vivo* trials, naturally occurring rots were mainly caused by *Penicillium* spp. Other postharvest pathogens (*Alternaria* spp., *Botrytis* spp., *Phytophthora* spp., etc.) were of minor importance. On cv. Valencia late fruits treated by dipping, no infections were observed during cold storage (data not shown). After two days of shelf-life, all treatments completely prevented decay as compared to the water control (Fig. 1A). After four days of shelf-life, Karma and PB significantly reduced the incidence of Penicillium decay as compared to the water control, i.e. by 79 and 31%, respectively (Fig. 1A). The results obtained after six days of shelf-life confirmed Karma’s ability to reduce the incidence of Penicillium decay more efficiently than PB (Fig. 1A).

Regarding disease severity, after two days of shelf-life, Karma and PB did not behave differently from imazalil (Fig. 1B); after four and six days of shelf-life, the two substances still reduced disease development up to 48%, with no significant differences between them. As to spray applications of Karma and PB at 6%, during cold storage no infections were observed (data not shown). After two, four, and six days of shelf-life, both substances completely inhibited rot development, the same as imazalil (Fig. 1C). Similar results were obtained considering disease severity (Fig. 1D). Concerning cv. Tarocco oranges, after one week of cold storage, Karma applied by dipping at 3% almost completely inhibited the incidence of decay, as imazalil did (Fig. 2A). At the end of cold storage, Karma still significantly reduced the percentage of Penicillium decay by 87%, whereas PB exerted a lower (68%) reduction. After two days of shelf-life, Karma reduced the incidence of Penicillium decay by around 50%, as compared to the water control, although no significant differences were recorded as compared to PB (Fig. 2A).

### Table 1. Effect of different Karma concentrations on colony diameter (mm) of *Penicillium* spp. after 6 days incubation at 24±1°C on amended PDA. For each pathogen, concentrations followed by the same letters are not statistically different according to Fisher’s protected least significant difference test (P ≤ 0.05).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Karma concentration (%)</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. digitatum</em></td>
<td>56.5 a</td>
<td>39.8 b</td>
<td>15.4 c</td>
<td>0.0 d</td>
<td>0.0 d</td>
<td>0.0 d</td>
<td></td>
</tr>
<tr>
<td><em>P. italicum</em></td>
<td>54.7 a</td>
<td>31.6 b</td>
<td>20.7 c</td>
<td>0.0 d</td>
<td>0.0 d</td>
<td>0.0 d</td>
<td></td>
</tr>
<tr>
<td><em>P. ulaiense</em></td>
<td>54.2 a</td>
<td>32.5 b</td>
<td>0.0 c</td>
<td>0.0 c</td>
<td>0.0 c</td>
<td>0.0 c</td>
<td></td>
</tr>
</tbody>
</table>

Regarding disease severity, after one week of cold storage, Karma, like imazalil, completely inhibited Penicillium growth (Fig. 2B), whereas PB did not cause a significant reduction as compared to the water control. After 14 days of refrigeration, the two compounds behaved similarly and not differently from imazalil; however, after 2 days of shelf-life, they still significantly reduced Penicillium decay as compared to the water control, although imazalil proved to be the most effective treatment. Concerning spray applications, after one week of cold storage, all treatments completely reduced disease incidence (Fig. 2C). After two weeks of cold storage, Karma and PB behaved similarly, reducing rot incidence by 77%, whereas in imazalil-treated fruits, Penicillium rot was reduced by 95%. Results were further confirmed even after two days of shelf-life, when Karma and PB sprayed at 6% significantly reduced Penicillium decay by 67 and 59%, respectively, although to a lesser extent than imazalil (Fig. 2C). Similar results were obtained when disease severity was taken into account (Fig. 2D).

Results of the present investigation show that the most common *Penicillium* spp. attacking citrus fruit could be significantly controlled *in vitro* with very low doses of Karma. In fact, a complete growth inhibition of *P. digitatum*, *P. italicum*, and *P. ulaiense*, was achieved with 0.3, 0.3, 0.2% Karma, respectively. For PB and imazalil a MIC of 0.25 and 0.025%, respectively, has been reported (Youssef et al., 2012a). Since discs re-seeded onto fresh PDA revived their growth, Karma’s inhibitory effect on *Penicillium* spp. appeared to be fungistatic. These results are in agreement with those obtained by Smilanick et al. (1999), who found that PB significantly reduced germination of *P. digitatum* spores, but once the spores were removed from the solution, they had a 99% germination rate. A certain difference in *Penicillium* spp. susceptibility was observed, *P. ulaiense* being the most susceptible. A different susceptibility of *Penicillium* spp. attacking citrus was also reported by Caccioni et al. (1998), who tested essential oils. The higher sensitivity of *P. ulaiense* to alternative control means could be of some interest, considering that, although it is a slow-growing species appearing late on the fruits, it has been reported to be resistant to imazalil, thiabendazole, and o-phenylphenols (Holmes et al., 1994).
Interesting results also came from *in vivo* experiments. The trials were conducted under naturally occurring infections, since in this way it is possible to simulate a commercial application (Smilanick *et al.*, 1997; Sanzani *et al.*, 2012) and to test the efficacy of the control methods on latent, quiescent, and incipient infections (Youssef *et al.*, 2012b). However, before treatment application, the fruits were wounded to enhance infection rate. A different degree of susceptibility to *Penicillium* spp. was observed between the two cvs. Indeed, blood (pigmented) oranges such as cv. Tarocco are known to be much more susceptible to decay than the non-pigmented ones (Schirra *et al.*, 1997), while cv. Valencia late fruits, which are tough, have a thick peel and ripen in a dry period, are not prone to postharvest decay (Chalutz *et al.*, 1981). The above consideration might explain the fact that cv. Tarocco is subject to earlier infection, i.e. during the first week of cold storage, as compared with cv. Valencia late oranges, for which an initial infection was observed only after two days of shelf-life. For both orange cvs, Karma’s good performance against *Penicillium* decay by dipping or spraying was recorded during storage. Its efficacy proved to be better than that afforded by PB at the same concentration, particularly as far as disease incidence is concerned.

No phytotoxicity symptoms were observed on orange peels in any of the trials. This novel formulation was recently tested at different concentrations (4 to 43 g/l) against sweet cherry rots, reducing brown rot, gray mold, and total rots by 75, 92 and 76%, respectively at 26 g/l (Feliziani *et al.*, 2013). In addition, Armicarb (Agronaturalis, UK), another formulated potassium bicarbonate, was successfully tested against *Botrytis cinerea* on grapevine, *Venturia inaequalis* on apple, and *Leveillula taurica* on tomatoes and peppers (Milling *et al.*, 2012). The best application strategy proved to be dipping, since spray applications required at least a double concentration to obtain a comparable level of control. The better control efficacy of dipping could be ascribed to the more prolonged contact between the fruit and the salt solution. Moreover, dipping is more suitable application strategy for...
orange fruits, since it is already included in their postharvest handling procedures. However, since a contact time of 10 min might be too long for commercial application, further trials are in progress to reduce it without compromising treatment efficacy.

Our results are in agreement with those by Mlikota Gabler and Smilanick (2001), who found that dipping grape berries into bicarbonate solutions provided a higher level of control than spraying with the same solutions. Furthermore, considering the direct effect of Karma on Penicillium spp. growth, this salt might even exert a sanitizing effect on citrus wash water, which is an important source of pathogen inoculum (Fallanaj et al., 2013). Indeed, postharvest decay can effectively be controlled by an integrated approach based on adequate harvest and handling practices, effective sanitation of fruits and packinghouse facilities, efficient postharvest antifungal treatments, and maintenance of a proper storage and transportation environment (Sanzani et al., 2009; Romanazzi et al., 2012).

Although the mode of action of bicarbonate salts is still under investigation, current studies have linked their antifungal activity to the perturbation of pH, osmotic pressure, and ion balance of sensitive fungi (Mecteau et al., 2002; Nigro et al., 2006). In fact, the reduction of fungal cell turgor pressure results in collapse and shrinkage of hyphae and spores, and consequently in the inability of fungi to sporulate (Fallik et al., 1997). However, it must be mentioned that salt solutions with a similar pH behaved very differently against rots (Youssef et al., 2012b). Moreover, bicarbonates are known to inactivate extracellular enzymes from Penicillium spp. (Fallik et al., 1996) and a certain resistance-inducing effect cannot be excluded. In fact, when bicarbonates were applied at 3% to a wound close to, but separate from the one inoculated with the pathogen, a significant reduction in disease incidence and severity was observed (Youssef et al., 2014). This hypothesis is further supported by the better results obtained in our trials on cv. Valencia late, the most resistant orange cultivar under investigation. This multi-site mode of action is of particular interest, since the risk of developing resistance in pathogen populations tends to be lower.

In conclusion, this study confirms that the use of synthetic fungicides can efficiently be reduced/replaced by low-toxicity substances such as potassium bicarbonate for controlling postharvest citrus decay during cold storage and shelf-life. Moreover, the use of formulations such as Karma significantly improves control efficiency and can easily be included in an IPM program throughout the packing line. Further studies regarding the impact of Karma on the flavor and quality characteristics of oranges are currently under way.

REFERENCES


Received February 6, 2014
Accepted May 6, 2014