Synergistic Effects of Combining Biocontrol Agents with Silicon against Postharvest Diseases of Jujube Fruit

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ABSTRACT

The synergistic effects of biocontrol yeasts Cryptococcus laurentii and Rhodotorula glutinis combined with silicon (Si) against Alternaria alternata and Penicillium expansum molds were investigated in jujube fruit (Chinese date, Zizyphus jujuba) stored at 20 and 0°C, respectively. Combinations of C. laurentii and R. glutinis at 5 × 10^7 cells/ml with 2% Si was most effective in controlling the diseases caused by A. alternata and P. expansum on jujube fruit stored at 20°C. When fruits were stored at 0°C, combining C. laurentii and R. glutinis with Si was as effective against P. expansum as was Si or the yeasts applied alone and was more effective in controlling A. alternata. Si may have a fungistatic effect by directly inhibiting pathogen growth, but it did not greatly influence the growth of the antagonists.

Biological control utilizing antagonistic microorganisms as a promising alternative to synthetic fungicides has achieved considerable success (8, 16, 34). Many biological control agents have been used to effectively reduce various postharvest diseases of fruits (1, 14). However, these antagonists of fungal pathogens, especially under semicommercial conditions, usually are not as effective as chemical fungicides (5, 8, 31) because they are affected by many factors (30). To enhance the biocontrol activity of these antagonists, various strategies have been proposed, such as adding calcium chloride (29, 35), chitosan (10), 2-deoxy-D-glucose (11, 15), and other nutrient compounds (9, 17) to biocontrol treatments and combining antagonists with salicylic acid (25), sodium bicarbonate (36), ammonium molybdate (21, 32), potassium sorbate (18), and fungicides (13, 23). Previous research has indicated that these chemical compounds play an important role in the inhibition of postharvest decay of fruits and in enhancing the efficacy of postharvest biocontrol agents.

Silicon (Si), as a quantitatively major inorganic constituent of higher plants, has a multitude of functions in plants and influences plant development and growth and crop yield (12). Although Si application can decrease the severity of plant diseases caused by fungal infections (2, 20, 26), there is no information concerning the efficacy of Si in combination with biocontrol agents to prevent postharvest diseases in fruit and vegetables. The objective of this study was to (i) evaluate the efficacy of Si, alone or in combination with biocontrol agents, for control of the molds Penicillium expansum and Alternaria alternata, which cause postharvest decay in jujube fruit (Chinese date, Zizyphus jujuba), (ii) determine the effects on disease control of timing of Si treatment and pathogen inoculation, and (iii) investigate the effects of Si treatment on the growth of the pathogens in vitro and the population dynamics of the biocontrol agents in wounds or on the surface of jujube fruit.

MATERIALS AND METHODS

Antagonists. Biocontrol yeasts Cryptococcus laurentii (Kuffer.) Skinner and Rhodotorula glutinis (Fresen.) FC. Harrison were isolated previously (19). These antagonists were identified by the CABI Bioscience Identification Services (International Mycological Institute, Egham, UK). The yeast cells were cultured in nutrient yeast dextrose broth (8 g of nutrient broth, 5 g of yeast extract, and 10 g of dextrose in 1 liter of water) for 48 h at 25°C. Washed yeast cell suspensions were obtained by centrifugation at 6,000 rpm (2,500 × g) for 10 min, resuspended in sterile distilled water, and centrifuged again. The concentration of the yeasts was determined with a hemacytometer and adjusted to 5 × 10^7 cells per ml.

Pathogen. Alternaria alternata (Fr.) Keissler and Penicillium expansum Link were isolated from infected jujube fruit and maintained on potato dextrose agar (PDA) for 14 days at 20°C. The spores of both pathogens were suspended in 5 ml of sterile distilled water containing 0.05% (vol/vol) Tween 80 and adjusted to 5 × 10^5 spores per ml.

Fruit material. Jujube fruits without physical injuries or infections were harvested at commercial maturity and sorted based on size. The firmness of jujube fruit was 52.5 N as determined with a penetrometer (FT-327, UC Fruit Firmness Tester, Effegi, Milan, Italy), and total soluble solids content was about 21% as determined with an Abbe refractometer (model 10481, Mark II, Leica, Bannockburn, Ill.). Fruits were held at 0°C and used within 15 days.

Chemical substance. The Si used in this experiment was sodium silicate (Sigma Chemical Co., St. Louis, Mo.). The pH of the Si solution was about 12.
FIGURE 1. Effects of different concentrations of silicon on controlling diseases caused by A. alternata and P. expansum in jujube fruits stored at 20°C. Within the same day, columns with the same letter are not significantly different according to the least significant difference test (P < 0.05). Bars represent the standard deviation of the treatment mean for pooled data.

Effect of Si concentrations on fruit decay. Jujube fruits were disinfected with 2% (vol/vol) sodium hypochlorite for 2 min, washed with tap water, and air dried prior to use. The fruits were wounded (3 mm deep and 3 mm wide) with a sterile nail and inoculated with 20 µl of the conidial suspensions of A. alternata or P. expansum at 10^5 spores per ml. After 24 h, 30 µl of Si at different concentrations (0, 0.5, 1, and 2%) was put into the wound. Treated fruits were placed in plastic boxes (250 by 150 by 50 mm) that were placed in plastic film bags to maintain high relative humidity (about 95%) and stored at 20°C. Disease incidence (percentage of samples showing lesions) and lesion diameter were determined after 4, 5, and 6 days for A. alternata and after 3, 4, and 5 days for P. expansum. Each treatment was replicated three times with 15 fruits per replicate, and the experiment was repeated twice.

Effect of Si treatment time on fruit decay. Jujube fruits were disinfected and wounded as described above. Thirty microliters of 2% Si was put into the wound 6 h before or 6, 12, 24, and 48 h after inoculation with 20 µl of conidial suspension at 10^5 spores per ml. The treated fruits were stored at 20°C as described above. Disease incidence and lesion diameter were determined after 4, 5, and 6 days for A. alternata and after 3, 4, and 5 days for P. expansum. Each treatment was replicated three times with 15 fruits per replicate, and the experiment was repeated twice.

Effect on fruit decay of combining Si with biocontrol agents. The experiments included two parts. For the room temperature experiment (20°C), jujube fruit were disinfected and wounded as described above. Thirty microliters of suspensions of C. laurentii and R. glutinis at 5 × 10^7 cells per ml or of sterile distilled water was put into each wound. Fruits were air dried, and the wounds were inoculated with 20 µl of conidial suspensions of A. alternata or P. expansum at 10^8 spores per ml. After incubation at 20°C for 24 h, 30 µl of 2% Si were put into the same wounds. The fruits treated with water served as controls. Each treatment was replicated three times with 15 fruits per replicate. Treated fruits were stored as described above at 20°C for 7 days. Disease incidences and lesion diameters were measured at different times.

For the low-temperature (0°C), sound fruit were dipped in the suspensions of C. laurentii and R. glutinis both at 5 × 10^7 cells per ml or in sterile distilled water for 2 min. After 2 h, the fruits were dipped in Si solution at 2% for 4 min. The fruits treated with water served as controls. There were 40 fruits and three replications for each treatment. All fruits were stored as described...
FIGURE 3. Effects of time interval between inoculation of pathogens and treatment with 2% silicon on controlling diseases caused by A. alternata and P. expansum in jujube fruits stored at 20°C. Within the same day, columns with the same letter are not significantly different according to the least significant difference test (P < 0.05). Bars represent the standard deviation of the treatment mean for pooled data.

Population dynamics of yeasts in fruit wounds. Jujube fruits were disinfected and wounded as described above. Thirty microliters of suspensions of C. laurentii and R. glutinis at 10⁷ cells per ml was put into each wound. After 24 h, 30 μl of 2% Si was added to the same wound. Treated fruits were stored at 20°C as described above. Fruit samples were taken at different times using the method reported by Janisiewicz et al. (17). The wound tissue (10 mm deep and 10 mm wide) removed from five fruits was placed in a mortar with 10 ml of sterile distilled water and ground with a pestle, and 100 μl of serial 10-fold dilutions were plated on nutrient yeast dextrose agar (NYDA). Colonies of C. laurentii and R. glutinis were counted after incubation at 20°C for 72 h and were expressed as log CFU per wound. Each treatment was replicated three times, and the experiment was repeated three times.

Population dynamics of yeasts on fruit surfaces. Jujube fruits were dipped in the suspensions of C. laurentii and R. glutinis for 2 min. After they were air dried, the fruits were dipped in a 2% Si solution for 4 min and stored at 0°C as described above. Five fruits from each treatment were removed at different times (0, 10, 20, and 30 days) after treatment according to the method of Benbow and Sugar (4). The fruits were put in beakers containing 100 ml of sterile distilled water and shaken at 200 rpm for 30 min, and 100 μl of serial 10-fold dilutions were plated on NYDA. Colonies of C. laurentii and R. glutinis were counted after incubation at 20°C for 72 h and were expressed as log CFU per fruit. Fruits treated with sterile water were used as controls and only those lesions resembling the ones induced by the yeasts used in the treatments were counted. Each treatment was replicated three times, and the experiment was repeated three times.

Effect of Si on growth of fungal pathogens in vitro. The inhibition effect of Si on the growth of P. expansum and A. alternata was assayed on PDA using the method of Droby et al. (9). Si solutions were filtered through a 0.45-μm pore size Milipore filter before being added to autoclaved PDA. Agar plugs (5 mm diameter) containing mycelia were cut from the growing edge of 7-day-old cultures of the fungi and were placed in the center of petri dishes containing PDA with different concentrations (0, 0.5, 1, and 2%) of Si. Radial growth of the pathogens was observed at 6, 9, 12 days after incubation. Each treatment was replicated three times, and the experiment was repeated twice. Results were expressed as the percent inhibition relative to growth in control PDA not containing Si.

Statistical analysis. All data were analyzed as a one-variable general linear model procedure (analysis of variance). Mean separations were performed using the least significant difference method. Differences at P = 0.05 were considered significant. Results presented were pooled across repeated experiments.

RESULTS

Effect of Si concentrations on fruit decay. The results indicated that Si concentration significantly affected decay caused of jujube fruit by A. alternata and P. expansum (Fig. 1). Although disease incidence increased with increased storage time, reduction of disease incidence and lesion diameter was significantly greater with 2% Si (wt/vol) that with other Si concentrations. No decay caused by P. expansum was found, and only 20% of the fruits were infected by A. alternata after 3 days of storage (Fig. 1).

Effect of Si concentration on growth of pathogens in vitro. The concentrations of Si used in the experiments were 0, 0.5, 1, and 2% (wt/vol) of Si in jujube fruits stored at 20°C for 30 days, and disease incidence was determined. The entire experiment was repeated twice.

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2). There was a significant positive correlation between Si concentration and antifungal effect.

**Effect of Si treatment time on fruit decay.** The timing of Si application also affected disease control. Application of Si before pathogen inoculation resulted in significantly higher A. alternata and P. expansum disease incidence than did Si treatment after pathogen inoculation (Fig. 3). Application of Si 24 h after pathogen inoculation resulted in more effective disease control than did other treatments after 3 days at 20°C.

**Effect on fruit decay of combining Si with biocontrol agents.** Although application of Si and biocontrol agents alone could inhibit A. alternata and P. expansum growth in jujube fruits after 4 days at 20°C, combining Si with biocontrol agents resulted in more effective control of disease (Fig. 4). C. laurentii at $5 \times 10^7$ cells per ml in combination with 2% Si was most effective among all treatments for decreasing decay. Si combined with C. laurentii was more effective for controlling disease than was Si combined with R. glutinis. No decay produced by P. expansum was found in jujube fruits treated by combining Si with C. laurentii after 6 days at 20°C. However, at 0°C, application of Si alone or in combination with biocontrol agents resulted in significantly lower incidence of decay compared with use of the biocontrol agents alone (Table 1). The difference in biocontrol efficiency of the yeasts against the pathogens at 20 and 0°C may be attributed to the fungistatic effect of Si, which directly inhibited pathogen growth, or to the fact that the growth of biocontrol agents is suppressed at low temperatures (29, 30).

**Effect of Si on growth of biocontrol agents in vivo.** Population dynamics of biocontrol agents in the wounds of jujube fruits at 20°C are presented in Figure 5. Si at 2% (vol/wt) significantly inhibited the growth of C. laurentii during the first 48 h of the incubation period but then stimulated the yeast growth by the end of the study (96 h). Compared with its effect on C. laurentii, Si only slightly inhibited the population of R. glutinis in jujube wounds.

**Population dynamics of biocontrol agents in vivo.** Because C. laurentii is better able to adapt to low temperatures, its population on the surface of jujube fruits at 0°C was higher than that of R. glutinis (Fig. 6). Si significantly decreased the number of yeast cells at the fruit surface, particularly in the early storage periods. Populations of C. laurentii and R. glutinis in all treatments tended to increase with prolonged storage time, but the only significant differences ($P = 0.05$) found were between C. laurentii and R. glutinis plus Si after 30 days of storage.

### TABLE 1. Efficacy of silicon in combination with biocontrol agents to control decay of natural infected jujube fruit kept at 0°C for 30 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P. expansum</th>
<th>A. alternata</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.9 A</td>
<td>52.2 A</td>
<td>66.1 A</td>
</tr>
<tr>
<td>Si</td>
<td>4.4 B</td>
<td>21.1 B</td>
<td>27.8 B</td>
</tr>
<tr>
<td>C. laurentii</td>
<td>8.9 AB</td>
<td>45.6 A</td>
<td>54.5 A</td>
</tr>
<tr>
<td>C. laurentii plus Si</td>
<td>6.7 AB</td>
<td>24.4 B</td>
<td>31.1 B</td>
</tr>
<tr>
<td>R glutinis</td>
<td>11.1 AB</td>
<td>45.6 A</td>
<td>56.7 A</td>
</tr>
<tr>
<td>R. glutinis plus Si</td>
<td>11.7 AB</td>
<td>26.7 B</td>
<td>38.4 B</td>
</tr>
</tbody>
</table>

*Values in the same column with different letters are significantly different according to a least significant difference test ($P = 0.05$).
DISCUSSION

Yeasts as biocontrol agents have been widely used to control postharvest diseases in various fruit because they do not produce antibiotics (8, 16, 33). In recent years, many researchers have focused on an integrative approach to improving the biocontrol efficacy of these yeasts against pathogenic fungi; application of these biological control agents alone has not provided commercially acceptable control of plant diseases (11, 24). The combination of biological control agents with selected chemicals produces a synergistic effect that enhances their efficacy for postharvest disease control (9, 36). Compounds such as food additives or non-chemical fungicides are preferred because they pose a minimal risk to human health and can be useful in the management of fungicide-resistant fungal strains (22). The results of our previous studies indicated that combining R. glutinis or C. laurentii at 10^7 cells per ml with sodium bicarbonate (238 mmol/liter) or ammonium molybdate (15 mmol/liter) provided a more effective control of postharvest blue mold (caused by P. expansum) in jujube fruits than did using the antagonistic yeasts alone (32). In the present study, the yeasts C. laurentii and R. glutinis at 5 × 10^7 cells per ml in combination with 2% Si was the most effective among all treatments in controlling blue mold and black mold rot (caused by A. alternata) in jujube fruits stored at 20°C (Fig. 4). Application of Si with the yeasts produced a synergistic effect on control of jujube fruit decay.

The mechanisms by which Si in combination with the biocontrol agents provided better disease control compared with Si or the yeasts alone are not fully understood. Several modes of action of Si combining with the biocontrol agents possibly involved in the synergistic effectiveness on disease control can be suggested. First, Si can directly inhibit pathogen growth. The growth of A. alternata and P. expansum on PDA was completely stopped at Si concentrations ≥1% (Fig. 2). Second, Si did not greatly influence the growth of the antagonists. These yeasts survived in the wound of jujube fruits at high and relatively stable levels, especially after 96 h at 20°C (Fig. 5). The mode of action of antagonistic yeasts has been considered to be mainly competition for nutrients and space (16), and it is beneficial for biocontrol agents to maintain high populations in wounds or on the surface of fruit. Third, Si may induce host defense responses. In a recent study (unpublished data), we found that activities of phenylalanine ammonia lyase, polyphenol oxidase, and peroxidase in sweet cherries treated with Si increased significantly compared with those in wounded or nonwounded untreated control fruit. Other researchers have suggested that Si acts by stimulating the natural defense mechanisms of the plant (3), and enhanced resistance to pathogen infections in Si-treated plants may be related to the accumulation of phenolic and phenol-like compounds (6, 12). Chérif et al. (7) demonstrated that several enzymes, including chitinase, had increased activity in cucumber

FIGURE 5. Influence of 2% silicon on population dynamics of biocontrol agents in wounds of jujube fruits stored at 20°C. Bars represent standard deviations of the means.

FIGURE 6. Population dynamics of biocontrol agents on the surface of jujube fruits stored at 0°C during storage periods of 30 days. Bars represent standard deviations of the means.
plants treated with Si and challenged by *Pythium ultimum*. Takahashi (27) stated that Si-induced disease resistance in rice can be attributed to the formation of a silicated epidermal cell layer. Recent research suggested that the defense mechanisms mobilized by Si include the accumulation of lignin and generally phenolic compounds, chitinases, and peroxidases (12).

Si combined with *C. laurentii* was more effective for controlling blue mold and black mold rot than was Si combined with *R. glutinis*, particularly for control of blue mold (Fig. 4). This effect can be attributed to the difference in biocontrol efficacy of the two yeasts. When jujube fruits were stored at 0°C, the combination of *C. laurentii* and *R. glutinis* with Si was just as effective against blue mold as was application of Si or the yeasts alone, but the combination was more effective for controlling black mold rot (Table 1). These different outcomes may be due to the difference between the two pathogens in sensitivity to low-temperature stress (28). Further study is needed to determine the precise mechanism(s) by which Si influences the physiological processes of jujube fruits.

ACKNOWLEDGMENTS

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REFERENCES


33. Wilson, C. L., and M. E. Wisniewski. 1989. Biological control of