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Research Article

Effect of chitosan and silicon oxide treatments on postharvest Valencia Late (*Citrus × sinensis*) fruits

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Abstract

The efficacy of chitosan and silicon oxide to prevent postharvest weight loss and fungi infection in 'Valencia Late' oranges was tested. Three silicon oxide concentrations (0.1%, 0.2%, 1%) were applied as preharvest treatments. Chitosan treatments were performed at the same concentrations in postharvest fruit. Preharvest applications were carried out by tractor spraying, while fruit were submerged for 30 seconds in baths with the chitosan concentrations in the postharvest applications. In both cases, a positive control (water treatment) and negative control (fungicide) were included. Treated fruit were stored in a chamber to simulate commercial storage conditions (4 ºC, 90% RH) for 9 weeks. After this time, the weight loss and damage caused by fungi due to natural infection were evaluated. Both silicon oxide and chitosan applications were effective in controlling natural infection by *Penicillium* species but had no positive effect on weight loss.

More Information

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Introduction

Citrus fruit production has increased worldwide since the past decade [1]. One of the main problems that citrus distribution chains face is the appearance of postharvest diseases, which can sometimes imply up to 80% harvest losses [2]. *Penicillium digitatum* (Pers.:Fr.) Sacc and *Penicillium italicum* Wehmer are the most economically important postharvest citrus fruit diseases in the main production areas [3,4]. Other fungi that can affect postharvest citrus fruit are *Botrytis cinerea* Pers ex Fr [5] and *Geotrichum candidum* Link ex Pers [6]. In addition, weight loss due to respiration and evaporation through skin [7], as well as chilling injury and peel pitting characterized by the collapse of epidermal and subepidermal cells on fruit surfaces, can also cause significant losses [8].

Therefore, the use of several products in pre- and postharvest applications has been studied to improve shelf-life parameters, and the effect of chitosan on several fruit and vegetables has been reported [9,10]. Chitosan stimulates plant defenses against postharvest pathogens and interferes with fungal growth [11]. It is especially effective in improving postharvest characteristics in grape [12], guava [13], *Luffa cylindrica* (L.) M. Roem [14], strawberry [15] and tomato [16]. Several studies have pointed out its efficacy in prolonging the shelf life of citrus fruit, such as oranges (*Citrus × sinensis* (L.) Osbeck) 'Fortune' and 'Valencia' [11] and 'Navel' [17], in *Citrus tankan* Hayata [5], mandarins (*Citrus reticulata* Blanco) 'Or' and 'Mor', and in grapefruit (*Citrus paradisi* Macf.) 'Star Ruby' [18]. Chitosan effectiveness has been found in both postharvest treatments and *in vitro* experiments against *P. digitatum* [19] and *P. italicum* [20]. It is also efficient in preventing damage from other postharvest fungi diseases like *Penicillium expansum* Link and *B. cinerea* [17], *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. and *Aspergillus niger* Tiegh [5]. Therefore, the safe use of chitosan in agriculture and food production has been reported [21].

Other compounds have been tested for similar purposes. Saberi, et al. [22] analyzed several coatings based on pea starch and guar gum in Valencia oranges. These new coatings lowered weight loss, firmness loss and respiration rates compared to commercial wax or uncoated fruit. Several metallic salts have been reported as being useful for reducing decay damage and preventing postharvest disease. He, et al. [23] evaluated the activity of zinc oxide nanoparticles against *P. expansum* and *B. cinerea*. Other metal oxides like

MgO and CaO display antimicrobial activity against potential postharvest fungi like *A. niger* and *R. stolonifer* [24]. Sodium silicate is effective against *Alternaria alternata* (Fr.: Fr.) Keissl., *Fusarium semitectum* Berk. et Ravenel, *Trichotecium roseum* (Pers.: Fr.), *P. expansum* and *Sclerotinia sclerotiorum* (Lib.) De Bary in melon, pear and carrot [25-27]. A recent study has described a significant reduction in the postharvest deterioration of physico-chemical characteristics on mango after a preharvest potassium silicate treatment [28]. Likewise, several studies on citrus fruit have employed silicon salts. Liu, et al. [29] reported the antifungal effect of sodium silicate on controlling green mold in *C. reticulata*. Finally, treatments with 90 mM of potassium silicate reduce the incidence of *P. digitatum* and *P. italicum* in 'Valencia' and 'Lanelate' oranges [30].

However, information about the specific effect of silicon oxide (SiO_2) on preventing postharvest infections of *Penicillium* species in stored citrus fruit is lacking. The low toxicity of this substance allows its use in agricultural products intended for human consumption [31]. Therefore, this study aims to test the efficacy of silicon oxide preharvest applications in Valencia Late (*Citrus × sinensis*) fruit, and to compare these treatments to chitosan postharvest applications.

Materials and methods

Experimental site and plant material

This study was carried out in a commercial orchard of 'Valencia Late' oranges located in Picassent, Valencia province, Spain (39º21'51" N 0º32'24" W, 150 m altitude). This plot covers a total surface area of 21500 m^2. Soil characteristics were calcareous sandy-clay loam with a pH of 8.06 and 5.2% limestone. The general site climate was Mediterranean xeric-oceanic, with long-term average annual rainfall of 440 mm and an average annual air temperature of 17.3 ºC. The experiment was conducted on 30-year-old trees managed under standard cultural and drip irrigation conditions. The 'Valencia Late' orange trees were grafted onto the Carrizo citrange rootstock.

Treatments

The silicon treatments were carried out in the preharvest stage (1 week before harvest), while the chitosan ones were performed in the postharvest stage, on the fruit that did not undergo the silicon preharvest treatments. Both treatments consisted of three different product concentrations (low, medium, high), along with a negative control (fungicide treatment) and a positive control (treated only with water). For the silicon treatments, a commercial product based on monoxide silicon (formula) (SILIK©) was used. Concentrations were 0.1% (1 g/l; low silicon), 0.2% (2 g/l; medium silicon) and 1% (10 g/l; high silicon). The employed fungicide was mancozeb (NUFOZEBE©) at 0.25% (2.5 g/l). These five treatments were randomly distributed in a single row per treatment, with three border rows between them. Applications were applied by a sprayer tractor at constant 10 bar pressure, which delivered 2000 liters/ha. Thirty fruit per tree and three trees per treatment (90 fruit per treatment) were sampled for the silicon treatment evaluation.

The chitosan treatments were performed with a commercial product based on chitosan chlorhydrate (NANDA©). Concentrations were 0.1% (1 g/l; low chitosan), 0.2% (2 g/l; medium chitosan) and 1% (10 g/l; high chitosan). The used fungicide was imazalil (FRUITGARD©) at 0.25% (2.5 g/l). Treatments were applied to freshly harvested fruit that were randomly selected from the trees that received no previous treatment. These treatments were prepared in different containers and 30 fruit per treatment were submerged in solution for 30 seconds.

Measurements

After all the applications, the silicon- and chitosan-treated fruit were weighed. Then fruit were distributed into boxes (15 fruit per box) and labeled (2 boxes per treatment) (Figure 1). Boxes were stored under commercial storage conditions, namely inside a chamber for 9 weeks at 4 ºC and 90% relative humidity (RH). Two evaluations were made at 6 and 9 weeks after being left in the chamber. During each evaluation, all the fruit were weighed to assess weight loss, whereas the presence of postharvest fungi caused by natural infection (*Penicillium* spp. and other species) was assessed. The employed infection damage scale ranged between 0 and 3 (0: no symptoms; 1: slight presence; 2: medium presence, only in one fruit hemisphere; 3: considerable presence, sporulation affecting more than one fruit hemisphere).

Statistical analysis

All the statistical analyses were performed with R [32] and RStudio [33]. Fisher's Exact Test for Count Data was used to compare damage frequencies, while Holm's correction method was followed for the *post hoc* analyses between treatments [34]. The initial fruit weights were not homogenous for the different treatments. Therefore, in order to homogenize the initial weights for all the treatments, fruit were analyzed separately in three size groups (small, medium and large fruit) using quantiles for 1/3 and 2/3. The fruit with the exact quantile weight were included in the medium-sized fruit class. Weight loss was measured individually for each

Figure 1: Valencia Late orange fruits before entering the storage chamber.

fruit as the initial weight minus weight at 6 and 9 weeks. Both the ANOVA and Kruskal-Wallis tests, with Tukey *post hoc* test, were performed to assess differences between treatments.

Results

All the observed symptoms corresponded to the *P. digitatum* and *P. italicum* postharvest diseases by natural infection (Figure 2). As infection was not forced, the percentage of damaged fruit was low, even in the positive controls (17.8% and 18.8% for the silicon oxide and chitosan positive controls, respectively). Positive effects to prevent *Penicillium* infection were observed at the two highest silicon concentrations (Figure 3). In this case, the medium (2 g/l) and high concentration (10 g/l) silicon oxide treatments showed the lowest frequency of damaged fruit after 9 storage weeks with significant differences with the positive control. Specifically, the medium silicon oxide concentration treatment showed three damaged fruit (3.3%; Holm *p* value = 0.024) and the high concentration silicon oxide treatment only one (1.1%; Holm *p* value = 0.0014), while the positive control had 16 damaged fruit (17.8%). Moreover, the observed number of damaged fruit was bigger in the negative control (fungicide) than in the three silicon oxide treatments. This indicates that all the silicon treatments, including the lowest concentration treatment (1 g/l), were effective in reducing the number of damaged fruit. In fact, the medium and high concentration silicon oxide treatments showed significant differences with fungicide treatment.

Figure 2: Valencia Late orange fruits affected with *Penicillium digitatum* (green mold) and *Penicillium italicum* (blue mold).

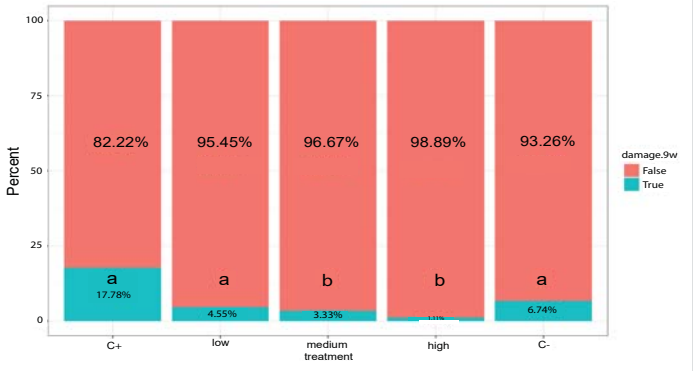

Figure 3: Damage frequencies of the silicon treatments after 9 storage weeks. 'False' refers to the fruit that did not show any symptoms. 'True' denotes the fruit that presented at least one symptom type on the considered 'damage scale'. Total Fisher test p = 2e-04.

The chitosan treatments obtained similar results to the silicon oxide treatments in damaged fruit frequency terms (Figure 4). In this case, only the high concentration chitosan treatment showed significant differences compared to the positive control. Specifically, the positive control presented 17 damaged fruit (18.8%), while only two damaged fruit were identified for the high concentration chitosan treatment, along with a significant difference (2.2%; Holm *p* value = 0.00038). The frequency of the damaged fruit observed in the medium concentration chitosan treatment was similar to the fungicide treatment (6.7% and 7.8%, respectively), and no significant differences were found. These two treatments did not show any significant differences with the positive control. The low concentration chitosan treatment showed no effect on preventing postharvest *Penicillium* infection, with a similar damaged fruit frequency as in the positive control (15.6%).

No significant effect in weight loss was found for any treatment with the small, medium or large fruit (Tables 1-4). After 9 storage weeks, the highest and lowest weight losses were recorded in the positive (20.00 g) and the negative (18.54 g) controls with the silicon oxide treatments, but without any significant differences. The three silicon oxide treatments showed intermediate weight losses between the positive and negative controls (Figure 5). Similar tendencies were observed for the small (Table 1) and large fruit (Table 2).

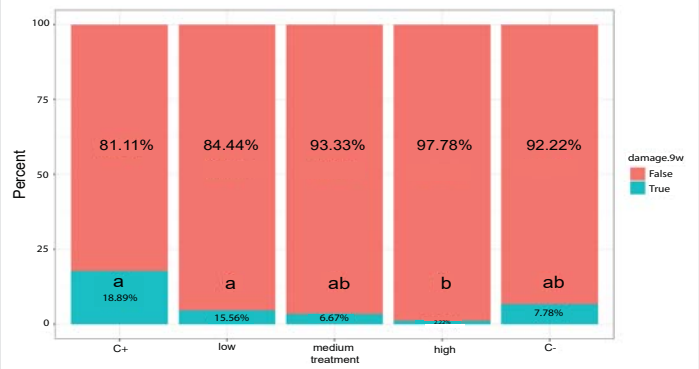

Figure 4: Damage frequencies of the chitosan treatments after 9 storage weeks. 'False' refers to the fruit that did not show any symptoms. 'True' denotes the fruit that displayed at least one symptom type on the considered 'damage scale'. Total Fisher test p = 0.00083.

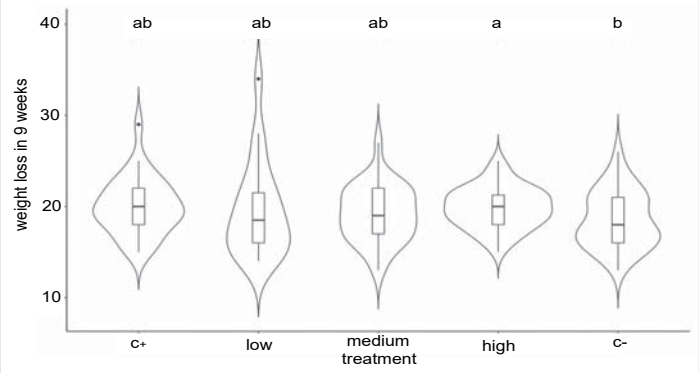

Figure 5: Violin plot of weight loss in the silicon treatments after 9 storage weeks. Different letters represent significant differences in the Kruskal-Wallis *post hoc* test (KW) for alpha = 0.05.

Table 1: Effect of silicon treatments on weight loss in the small fruit after 9 storage weeks. The Kruskal-Wallis *post hoc* test (KW) letters should be used because the residuals did not meet the normality requirement for the ANOVA. Shapiro.p = 4.9e-06. Different letters mean significant differences for alpha = 0.05. q stands for the studentized range in the Tukey test. All the treatments were run 1 week before harvest: C+, only water; low, silicon 1 g/l; medium, silicon 2 g/l; high, silicon 10 g/l; C-, mancozeb 2.5 g/l.

Treatment	N	Mean	sd	se	skew	kurtosis	Shapiro	HSD	KW
C+	40	17.5250	3.81	0.60	1.78	5.77	0.00	a	a
low	47	17.1277	2.11	0.31	0.10	-0.29	0.16	a	a
medium	24	17.8750	3.21	0.65	0.71	1.44	0.19	a	a
high	11	18.0000	1.95	0.59	0.59	0.58	0.83	a	a
C-	22	17.9091	3.29	0.70	0.44	-0.60	0.43	a	a

Table 2: Effect of silicon treatments on weight loss in the large fruit after 9 storage weeks. The Kruskal-Wallis *post hoc* test (KW) letters should be used because the residuals did not meet the normality requirement for the ANOVA. Shapiro.p = 1.86e-09. Different letters mean significant differences for alpha = 0.05. q stands for the studentized range in the Tukey test. All the treatments were run 1 week before harvest: C+, only water; low, silicon 1 g/l; medium, silicon 2 g/l; high, silicon 10 g/l; C-, mancozeb 2.5 g/l.

Treatment	N	Mean	sd	se	skew	kurtosis	Shapiro	HSD	KW
C+	21	22.33	2.99	0.65	0.36	-0.10	0.39	a	ab
low	21	23.71	4.19	0.91	0.12	-0.72	0.63	a	a
medium	37	21.97	2.94	0.48	1.01	1.50	0.01	a	ab
high	35	21.20	3.20	0.54	0.48	0.57	0.21	a	b
C-	32	22.00	5.73	1.01	2.76	11.22	0.00	a	b

Table 3: Effect of chitosan treatments on weight loss in the small fruit after 9 storage weeks. The Kruskal-Wallis *post hoc* test (KW) letters should be used because the residuals did not meet the normality requirement for the ANOVA. Shapiro.p = 1.25e-11. Different letters mean significant differences for alpha = 0.05. q stands for the studentized range in the Tukey test. All the treatments were run on the same day after harvest: C+, only water; low, chitosan 1 g/l; medium, chitosan 2 g/l; high, chitosan 10 g/l; C-, imazalil 2.5 g/l.

Treatment	N	Mean	sd	se	skew	kurtosis	Shapiro	HSD	KW
C+	26	16.85	8.17	1.60	3.36	14.18	0.00	a	ab
low	31	16.65	4.70	0.84	0.55	0.01	0.26	a	a
medium	21	17.29	4.12	0.90	0.48	0.27	0.10	a	a
high	43	14.60	4.08	0.62	0.83	0.44	0.02	a	b
C-	26	17.19	4.30	0.84	1.83	6.61	0.00	a	a

Table 4: Effect of the chitosan treatments on weight loss in the large fruit after 9 storage weeks. The Kruskal-Wallis *post hoc* test (KW) letters should be used because the residuals did not meet the normality requirement for the ANOVA. Shapiro.p = 4.99e-10. Different letters mean significant differences for alpha = 0.05. q stands for the studentized range in the Tukey test. All the treatments were run on the same day after harvest: C+, only water; low, chitosan 1 g/l; medium, chitosan 2 g/l; high, chitosan 10 g/l; C-, imazalil 2.5 g/l.

Treatment	N	Mean	sd	se	skew	kurtosis	Shapiro	HSD	KW
C+	29	21.62	7.05	1.31	2.76	10.38	0.00	a	ab
low	29	21.59	4.38	0.81	0.58	-0.46	0.11	a	a
medium	41	19.44	4.60	0.72	1.16	1.53	0.01	a	b
high	20	20.10	3.02	0.68	1.10	0.08	0.00	a	ab
C-	28	21.21	4.17	0.79	0.74	0.44	0.22	a	a

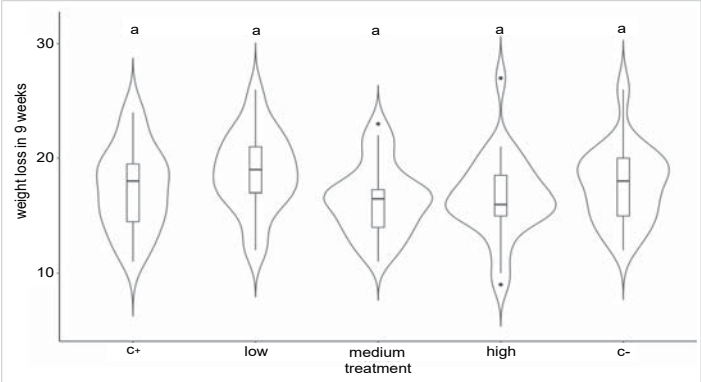

Figure 6: Violin plot of weight loss in the chitosan treatments after 9 storage weeks. Different letters represent significant differences in the Kruskal-Wallis *post hoc* test (KW) for alpha = 0.05.

The weight loss of the small fruit was minor, but with a larger percentage of weight loss than for the large fruit. However, no significant differences between treatments were observed in any fruit group.

No significant differences in weight loss in the three chitosan treatments and the two controls after 9 storage weeks (Figure 6), or between the chitosan treatments and the control for the small (Table 3) and large (Table 4) fruit, were observed.

Discussion

The results showed less damage caused by the postharvest pathogens after the silicon oxide and chitosan treatments. The two postharvest disease species by natural infection (*P. digitatum* and *P. italicum*) found in this study are considered to be the most frequent postharvest fungi in the navel fruit group [17].

We observed a lower infection level in all the treatments, even in the positive control. This was probably due to: infection being natural and not inoculated; low storage temperatures (4 ºC); a low inoculum level in the field that year. Other variables like variety ('Valencia Late') and fruit rind quality, which can vary annually, can influence the overall infection level. In any case, the highest chitosan concentration (1%)

came over as being better able to avoid natural postharvest *Penicillium* infections. This result agrees with similar studies conducted on 'Valencia' oranges stored at 4 ºC for 8 weeks, which checked the efficacy of postharvest chitosan treatment against natural *Penicillium* infection [35]. Other studies report the efficacy of these treatments at concentrations between 0.1 and 2% [9]. The incidence of postharvest *Penicillium* species is generally higher when artificial inoculation is carried out [5] and Chien, et al. [10] recorded more than 40% infected fruit after a postharvest chitosan treatment and the subsequent *Penicillium* inoculation in citrus Tankan and 'Murcott' tangor, respectively. These studies were conducted under relatively warm storage conditions (24 ºC and 15 ºC, respectively), which enhanced the growth and spread of postharvest disease. This would also explain the results obtained by Zeng, et al. [17], who reported a *P. italicum* incidence of up to 20% in navel orange fruit treated at the 2% chitosan concentration after 23 storage days at 20 ºC.

To the best of our knowledge, this is the first report of the effect of silicon oxide preharvest applications on preventing postharvest diseases in citrus fruit. Therefore, silicon oxide could be considered a new citrus postharvest disease control method. In fact, we only found data about the antimicrobial effects of silicon oxide from the *in vitro* tests. García-Saucedo, et al. [36] did not detect any effect of the silicon oxide particles tested *in vitro* against yeast *Saccharomyces cerevisiae* Meyen ex E.C. Hansen. Likewise, no effect against fungus *Candida albicans* (Robin) Berkhout was observed in a similar experiment [37]. However, silicon compounds apart from silicon oxide have already been tested to prevent *Penicillium* spp. and other fungi infections Liu, et al. [29] reported the effect of sodium silicate on inhibiting *P. digitatum* growth on *C. reticulata*. These authors suggested that any silicon compound could have effects against fungal pathogens. Youssef, et al. [38] observed minor fruit decay in orange fruit after applying a preharvest sodium silicate treatment, and potassium silicate postharvest applications showed preventive and curative antifungal activity against green mold and blue mold [30]. Finally, a recent study discussed the characteristics of chitosan/silicate nanocomposites obtained by inserting chitosan chain into silicate interlayers and their possible uses to control *P. digitatum* on orange fruit [39]. For all these reasons, silicon oxide must be added to silicon-based compounds as an effective control method against postharvest diseases in citrus fruit.

No tested chitosan concentration was effective in preventing the fruit weight loss associated with long-term decay. Previous studies carried out on harvested chitosan-treated fruit have reported less weight loss than controls. Perhaps chitosan application in a 30-second bath could be less effective than other types of postharvest treatments, such as applying a wax layer. This was the case of the study conducted by Hernández, et al. [35], who reported greater weight loss on orange fruit covered with chitosan compared to fruit covered with commercial wax. In contrast, several studies in which treatments consisted in immersing fruit for longer times and being left to dry report a positive effect even at low chitosan concentrations. Chien and Chou, [5] recorded minor weight loss in Tankan citrus fruit treated with 0.1% and 0.2% chitosan compared to the fungicide control, whereas Chien, et al. [10] observed less weight tangor fruit loss after a 0.1% chitosan treatment. In this study, not even the 1% chitosan concentration was able to significantly reduce weight loss compared to the control.

Silicon oxide treatments are not effective in controlling weight loss. To date, several available studies have focused on other silicon compounds applied to both pre- and postharvest treatments. In these studies, silicon-based treatments achieved less weight loss than their controls. Bi, et al. [25] reported lower decay incidence and less decay severity in Hami melons (*Cucumis melo* L. var. *inodorus* Jacq.) after several postharvest sodium silicate treatments. After conducting experiments on Hami melons, the authors reported high solubility for the applied sodium silicate to explain their results. Perhaps our different silicon oxide solubility could hinder a layer from being formed on fruit. Weight loss reduction is related to greater water vapor resistance because of the hydrophobic characteristics of the film formed by the applied compounds [40]. As we applied silicon oxide in our preharvest treatments, the layer that would have covered fruit could have been lost before storage owing to unexpected factors like weather conditions. The silicon oxide effect on reducing *Penicillium* infections has been related to cell wall strengthening [41], which could increase their resistance to senescence. However, our work is the first weight loss study to be carried out after a preharvest silicon oxide application, and the results showed no effect.

In a recent study, Mohamed, et al. [28] reported reduced weight loss in mango after a preharvest potassium silicate application. These authors noted that the higher concentration chitosan and potassium silicate mixture led to less weight loss. Finally, it is likely that the silicon oxide concentration herein used did not suffice to cover fruit and to, therefore, confer the appropriate physical characteristics. The above-cited study suggested that the formation of a silicon layer to totally cover the fruit stomata was the main cause of reduced fruit respiration. So, it would be interesting to perform further studies at higher silicon oxide concentrations.

Although we did not observe an effect on weight loss, the results herein obtained with natural infection in 'Valencia Late' oranges indicated that the preharvest silicon oxide and chitosan treatments were effective in preventing *Penicillium* postharvest infection, and in subsequently improving harvested fruit quality. It would be advisable to carry out further studies by applying artificial *P. digitatum* and *P. italicum* infections or higher silicon oxide concentrations to acquire more conclusive data.

Conclusion

Silicon oxide at 0.2% and 1% in a preharvest application to Valencia Late orange fruit was effective in preventing the natural infection of *Penicillium* species after 9 storage weeks. The three tested silicon oxide concentrations showed a lower damaged fruit incidence than the fungicide control. Likewise, the 1% chitosan concentration in the postharvest application tested under the same conditions was also effective in preventing *Penicillium* infection. However, the silicon oxide and chitosan treatments at the herein tested concentrations were not effective in preventing senescence-related fruit weight loss. Further studies are required to match the most appropriate concentration of silicon oxide applications to control postharvest disease and senescence-related weight loss.

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