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Citrus bliss: potassium, sodium, and calcium silicates secrets for post-harvest diseases of fruit defense

Meriem Hamrani,[1-3] Rachid Lahlali,[4] Rabea Ziri,[3] Rachid Ezzouggari,[4,5] Najiba Brhadda,[3] Félicie Lopez-Lauri,[2] Fouad Mokrini,[6] Essaid Ait Barka,[7] Mohammed El Guilli[1]

[1]Plant Pathology and Postharvest Quality Laboratory, Regional Center for Agronomical Research of Kenitra, Morocco; [2]UPRI/ERIT PSII, Avignon University, France; [3]Plant, Animal and Agro-Industrial Production Laboratory, Ibn Tofail University, Kenitra, Morocco; [4]Phytopathology Unit, Department of Plant Protection, National School of Agriculture of Meknes, Morocco; [5]Laboratory of Biotechnology, Conservation and Valorization of Natural Resources (LBCVNR), Faculty of Sciences Dhar El Mehraz, Sidi Mohamed Ben Abdallah University, Fez, Morocco; [6]Biotechnology Unit, Regional Center of Agricultural Research, INRA-Morocco, Rabat, Morocco; [7]Unit of Induced Resistance and Plant Bioprotection, University of Reims Champagne-Ardenne, Reims, France

Correspondence: Rachid Lahlali, Phytopathology Unit, Department of Plant Protection, National School of Agriculture of Meknes, Km10, Rte Haj Kaddour, BP S/40, Menkes 50001, Morocco
E-mail: rlahlali@enameknes.ac.ma

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Abstract

Biotic stress significantly challenges the global citrus industry. Major post-harvest issues include diseases caused by *Penicillium digitatum, Penicillium italicum, Geotrichum citri-aurantii, Alternaria alternata,* and *Phytophthora citrophthora*. The negative impact of chemical fungicides on the environment and health necessitates eco-friendly alternatives. This study examines the effectiveness of sodium, potassium, and calcium silicates against common citrus diseases. *In vitro* tests evaluated mycelial growth inhibition using silicate concentrations from 0 to 10,000 ppm after 7 days at 25°C. Sodium silicate showed the highest efficacy, completely inhibiting *P. digitatum* and *P. italicum* at 2000 ppm. Potassium and calcium silicates achieved 100% inhibition against *Penicillium* spp. at a concentration of 1%. *In vivo* tests on Sidi Aissa clementines assessed the preventive and curative effects of 1, 2, and 6% silicate salt solutions. Sodium silicate prevented 41% of brown rot, 72% of sour rot, and 100% of green mold at 6%. Calcium silicate at 6% significantly reduced blue mold and black rot by 32% and 74%, respectively. Sodium silicate was most effective in curative treatments, suggesting its potential as a pre- or post-harvest spray to control *P. digitatum, P. italicum,* and *G. citri-aurantii*.

Introduction

Citrus fruits consistently rank among the world's top ten most valuable crops, with cultivation spanning over 10.2 million hectares globally, yielding approximately 161.8 million tons annually (Pereira Gonzatto and Scherer Santos, 2023). Leading countries in citrus cultivation include China, Brazil, the United States, South Africa, Peru, Turkey, and Morocco (Zhong and Nicolosi., 2020). Notably, Morocco has seen a steady rise in citrus production across various varieties during the 2022/2023 period. Tangerines/mandarins witnessed a significant increase, reaching 927,000 metric tons (MT), while oranges reached 783,000 MT and lemons/limes reached 35,000 MT. This uptick in production has made a substantial economic impact, driven by the export of 425,000 MT of tangerines/mandarins, 80,000 MT of oranges, and 7,000 MT of lemons/limes (USDA, 2023).

Citrus fruits face susceptibility to various pathogens during post-harvest stages, including handling, shipping, storage, and marketing, resulting in substantial losses and economic setbacks within the citrus industry (Wang *et al*., 2020). Biotic stress, notably infections stemming from pre- and post-harvest decay fungi, presents a formidable challenge in citrus cultivation (Ezzouggari *et al*., 2024). In recent times, brown rot, alternaria rot, sour rot, and green, and blue mold have emerged as the most pervasive diseases afflicting harvested citrus fruits (Saito and Xiao, 2017; Salvador López *et al*., 2022). Green mold, attributed to *P. digitatum*, stands out as the most prevalent (36.3%), followed by blue mold caused by *P. italicum* (23.3%), and sour rot initiated by *G. citri-aurantii* (18.7%) (Saito and Xiao, 2017). Alongside these ailments, alternaria rot poses a significant threat, particularly in hot and humid climates, affecting citrus fruits through various species of the *Alternaria* genus, with *A. citri* among the most common pathogens (Soylu and Kose, 2015; Saito and Xiao, 2017). Moreover, brown rot, attributable to *Phytophthora* spp., holds considerable economic importance, affecting all citrus species and exhibiting increased prevalence during periods of ample rainfall in the later stages of fruit development (Ramallo *et al*., 2019). In Morocco, fungal diseases are the most frequent sorting gaps in packinghouses. A study conducted in the Berkane region revealed that *Geotrichum*, *Penicillium* spp., and *Phytophthora* are major contributors, with average sorting gap rates of 40.3, 31.2, and 28.6%, respectively (Ben Yazid *et al*., 2020).

Frequently, fungicides serve as a remedy to mitigate postharvest damage inflicted on citrus by diverse fungi (Radouane *et al*., 2023). However, the extensive application of these chemical agents has fostered the emergence of highly resistant pathogen strains and the buildup of chemical residues in food, posing heightened risks to human health and the environment (Cheng *et al*., 2020). Consequently, the pressing need to explore alternative methods for disease management has led to the investigation of various approaches beyond synthetic fungicides. These methods include physical treatments such as hot water treatment, hot air treatment, and light irradiation (Kahramanoğlu *et al*., 2020; Kahramanoğlu *et al*., 2020); the use of antagonistic microorganisms like yeast, bacteria, and fungi (Wang *et al*., 2022; Ezzouggari *et al*., 2024); incorporation of nanomaterials (Ruffo *et al*., 2019); application of chitosan (El Guilli *et al*., 2016); plant extracts and essential oils (Bhatta, 2022); and the utilization of salts (Palou, 2018).

Silicon, ranked as the second most abundant element on Earth after oxygen (Mvondo-She *et al*., 2021), holds the status of a Generally Recognized as Safe (GRAS) compound (Nikagolla *et al*., 2019). Currently, silicon has garnered attention for its beneficial effects on plants, enhancing their resilience against both biotic and abiotic stressors (Yavaş and Ünay, 2017). In the foreseeable future, silicon is poised to emerge as a sustainable solution for addressing challenges in horticulture. Among silicon compounds, potassium, calcium, and sodium silicates have been under scrutiny for their potential application in plant systems (Valdebenito-Sanhueza *et al*., 2018). Silicate presence instigates resistance against fungal and bacterial pathogens through mechanisms such as the establishment of physical barriers (Datnoff *et al*., 2001) and the accumulation of phenolic and phytoalexin compounds (Seebold *et al*., 2001).

The presence of silicate can induce resistance to both fungal and bacterial pathogens through the formation of a physical barrier (Datnoff *et al*., 2001) and the accumulation of phenolic and phytoalexin compounds (Seebold *et al*., 2001). Additionally, this resistance is linked to the activation of genes responsible for producing glucanase, peroxidases, and chitinases (Rodrigues *et al*., 2005). The specific objective of this study was to evaluate and compare the efficacy of various silicate forms potassium, sodium, and calcium silicates in combating postharvest citrus fruit diseases caused by assorted fungi.

Materials and Methods

Fungal pathogen and preparation of inoculum

The pathogenic fungi employed in this study, namely *Penicillium digitatum*, *Penicillium italicum*, *Geotrichum citri-aurantii*, *Alternaria alternata*, and *Phytophthora citrophthora* were sourced from the Plant Pathology and Postharvest Quality Laboratory at the Regional Center of Agricultural Research of Kénitra, Morocco. Fungal solutions were prepared by harvesting colonies grown on potato dextrose agar (PDA) medium and transferring them into glass test tubes containing 0.05% Tween 80 solution (Soto-Muñoz et *al*., 2020). The concentrations of the suspensions were standardized to 10^5 conidia/ml for *P. digitatum* and *P. italicum,* and 10^7 conidia/mL for *G. citri-aurantii* and *A. alternata*, utilizing a hemacytometer (Moscoso-Ramírez and Palou, 2014).

Plant material

The plant material utilized in this research comprised mature and healthy Citrus fruits of the Sidi Aissa clementine variety, harvested from the Sidi Allal Tazi experimental domain in Morocco. After harvesting, the samples were transported to the Plant Pathology and Postharvest Quality Laboratory at the Regional Center of Agricultural Research in Kenitra. Before commencing each experiment, the fruits underwent immersion in a 10% sodium hypochlorite solution for 2 minutes, followed by two rinses with sterile distilled water. Subsequently, two wounds were created on opposite sides along the equatorial axis of the fruits, with each wound measuring 2-3 mm in depth and 5 mm in width, utilizing a sterile cork-borer.

Soluble silicate compounds

In this study, three types of silicate: potassium silicate, sodium silicate, and calcium silicate, were evaluated through both *in vitro* and *in vivo* trials. Table 1 shows detailed information, such as the molecular formula, food additive E-number, and molecular weight of each silicate.

In vitro evaluation of the effectiveness of soluble silicate

The aim of this experiment was to evaluate the effects of soluble sodium silicate (Na_2SiO_3), potassium silicate (K_2SiO_3), and calcium silicate ($CaSiO_3$) on the mycelial growth of the aforementioned fungi. Different volumes of the three silicates were individually mixed into a sterilized PDA medium before solidification, resulting in final concentrations of 0, 500, 2000, 4000, 6000, and 10000 ppm. These solutions were then poured into sterile 90 mm-diameter Petri dishes. Each dish was centrally inoculated with 17 µL of fungal suspension, while mycelial plug disks (5mm) were used for *P. citrophthora*. Five plates were prepared for each silicate concentration and each fungus. Following a 7-day incubation period at 25°C, the linear growth of the fungi was measured using the methodology outlined by Abd-El-Kareem *et al*. (2019).

In vivo evaluation of the effectiveness of soluble silicate

The study utilized prepared fruits of the Sidi Aissa clementine variety to investigate the efficacy of Na, K, and Ca silicates in controlling postharvest citrus diseases. Two treatments were administered: preventive and curative, with a 24-hour interval between inoculation and treatment. The fruits underwent treatment by immersion for 2 minutes in silicate solutions prepared at various concentrations (1%, 2%, and 6%). Fruits immersed solely in sterile distilled water served as the negative control, while those treated with the fungicide Imazalil at 0.2% were designated as positive controls. Each fruit wound received a 40 µL conidial suspension, and ten repetitions were carried out for each type and concentration of silicate. Subsequently, all fruits were stored at 25±1°C and maintained at a relative humidity for 7 days.

Assessment methodology and determination of the inhibitory concentration 50

The inhibition rate of fungal development was determined in both *in vitro* and *in vivo* trials according to the flowing formula:

$\% IC = ((dc - dt) / dc) \times 100$, where:

- % I represent the percentage of inhibition,
- dc is the control development rate,
- dt is the treatment development rate.

The concentration that induces 50% inhibition of fungal growth [inhibitory concentration 50 (IC50)] was determined through regression analysis of the log-probit transformed data.

Statistical analysis

The *in vitro* and *in vivo* results were analyzed using XLSTAT 2016 version. A one-way ANOVA was performed, and Duncan's test was used to compare means between treatments. The statistical significance was judged at the 95% level of confidence ($p = 0.05$). Data were presented as a mean ± standard deviation.

Results

In vitro antifungal activity of sodium, potassium, and calcium silicates against fungal diseases in citrus fruits

The *in vitro* results revealed pronounced inhibitory effects on fungal mycelium growth upon the application of sodium, potassium, and calcium silicates ($p<0.0001$) (Figures 1 and 2). Specifically, sodium silicate completely arrested colony growth of *Penicillium* spp. and *G. citri-aurantii* at concentrations of 2000 and 4000 ppm, respectively. Potassium silicate demonstrated significant inhibition, reducing mycelial growth by 72% for *P. italicum* and achieving complete inhibition (100%) for *P. digitatum* at 4000 ppm. Calcium silicate, when applied at a concentration of 10000 ppm, led to a 55% reduction in *G. citri-aurantii* mycelium growth. Regarding *A. alternata,* the most effective inhibition of radial mycelial growth was observed with sodium silicate (100%), followed by potassium silicate (30%), both at the highest concentration of 10000 ppm. Notably, complete inhibition (100%) of *P. citrophthora* was exclusively achieved with sodium silicate at 6000 ppm.
Various concentrations of the three silicates were employed to establish the IC50 for each fungus, as outlined in Table 2. The results showed that sodium silicate displayed a slightly lower IC50 value compared to potassium and calcium silicate. Additionally, the IC50 values varied depending on the specific fungus.

Preventive activity bioassay

The effectiveness of silicate salts in preventing post-harvest citrus diseases was assessed following a 7-day treatment period, with the findings presented in Figures 3 and 4. Notably, sodium silicate exhibited superior protective effects compared to other silicates, particularly at a 1% concentration. Sodium silicate demonstrated efficacy rates of 53.12% and 81.61% against *A. alternata* and *P. citrophthora,* respectively, achieving complete inhibition (100%) against *P. digitatum* and *G. citri-aurantii*. Potassium silicate, applied at a 6% concentration, significantly suppressed the growth of blue and green molds by 32.86% and 68.91%, respectively. Additionally, at the same concentration, potassium silicate mitigated disease symptoms caused by *A. alternata* and *P. citrophthora* by up to 43.35%. In the case of calcium silicate, its efficacy against green and blue molds surpassed 37.26%, notably reaching 42.44% for sour rot at a concentration of 6%. These results underscore the potential of silicate salts as effective preventive measures against various post-harvest citrus diseases.

Curative activity bioassay

Regarding curative activity, a significant and noteworthy difference emerged when citrus fruits underwent treatment 24 hours after inoculation, distinguishing between treated and untreated fruits (Figures 5 and 6). The results revealed that *P. digitatum* was effectively suppressed by sodium silicate at a concentration of 2% ($p<0.0001$), a level of inhibition comparable to that achieved by the fungicide imazalil at 0.2% (2000 ppm). Across all other pathogens, a substantial inhibition rate exceeding 52.11% was observed at a concentration of 6%. Potassium silicate, at a 6% concentration, demonstrated a 40% inhibition rate against *P. citrophthora*. Conversely, calcium silicate, at a concentration of 6%, exhibited approximately 20% and 26% reductions in brown rot and green mold, respectively. However, no inhibitory effects were observed for potassium and calcium silicate in controlling black rot and sour rot at any of the tested concentrations.

Discussion

The primary post-harvest diseases affecting citrus fruits include green mold, blue mold, and sour rot, caused by *P. digitatum, P. italicum,* and *Geotrichum citri-aurantii,* respectively. Less prevalent but still significant are *A. alternata* and *P. citrophthora.* These pathogens pose a considerable threat to citrus exports due to the substantial production losses they induce and their detrimental impact on citrus quality. Addressing citrus rot presents a significant challenge, leading to the exploration of various solutions such as beneficial yeasts, bacteria, fungi, biofungicides, plant extracts, essential oils, chitosan, and GRAS substances, along with synthetic elicitors. Physical approaches like heat exposure or irradiation are also under consideration (Palou *et al*., 2016; Papoutsis *et al*., 2019). Despite the promising inhibitory capabilities of certain methods, their use is limited due to their high cost and strict environmental requirements, including specific pH, particular temperature, and precise water activity coverage (Li *et al*., 2019). The use of organic and inorganic salts classified as GRAS compounds or as food additives by national or international legislation constitutes a promising and non-polluting alternative to conventional fungicides for controlling post-harvest diseases of fresh horticultural produce, including fresh citrus fruits (D'Aquino and Palma, 2019). These salts are highly soluble, easy to handle and apply, and widely available at relatively low costs, making them suitable for commercial implementation in existing fresh produce packing warehouses (Palou, 2018). This makes them well-suited for addressing issues related to fruit residues, improving water quality, and meeting the requirements of organic agriculture.

Hence, our aim was to evaluate the efficacy and consistency of various post-harvest treatments utilizing GRAS substances such as potassium silicate, sodium silicate, and calcium silicate as a practical approach to combat citrus rot. This study employs a sequential methodology to optimize the application parameters of these silicate salts (potassium, sodium, and calcium silicates). Initially, their impact on linear growth was assessed, followed by two *in vivo* tests focusing on both preventive and curative actions against citrus diseases. Preliminary *in vitro* trials were conducted to demonstrate the antifungal effectiveness of the treatment compounds. All types of silicates tested significantly reduced mycelial growth during the *in vitro* experiments. Notably, sodium silicate exhibited complete suppression of *Penicillium* spp., *G. citri-aurantii, P. citrophthora,* and *A. alternata* at concentrations of 2000, 4000, 6000, and 10000 ppm, respectively. Moreover, potassium silicate demonstrated complete inhibition of mycelial growth in *P. digitatum* and *P. italicum* at concentrations of 4000 and 10000 ppm, respectively. Furthermore, only calcium silicate-enriched Petri dishes at 10000 ppm exhibited the ability to completely suppress *P. digitatum* and *P. italicum.* However, potassium silicate and sodium silicate were identified as the least effective soluble silicate types in inhibiting the growth of *G. citri-aurantii, A. alternata*, and *P. citrophthora.* These findings contrast with those of Biggs *et al*. (1997), who reported the effectiveness of calcium silicate incorporated into PDA in inhibiting the growth of *Monilinia fructicola* (G. Wint.) Honey, the causal agent of brown rot in peach fruit when compared to the control group. The *in vivo* experiments revealed variable outcomes, indicating disparities depending on the types of silicates, application methods (preventive or curative), and specific pathogens. The three silicate salts have the capacity to prevent the development of green and blue molds, sour rot, alternaria rot, and brown rot.

However, potassium and calcium silicate treatments are not effective in controlling the development of sour rot and alternaria rot in the curative activity; the results underscore the crucial importance of timing in the application. Indeed, applying the salts before harvest allows for extended interaction with pathogens present on the fruit, potentially altering their inoculum density and the environmental conditions within wounds, thus potentially inducing tissue resistance (Youssef *et al*., 2012). Abd-El-Kareem *et al*. (2019), evaluating the same silicate salts in controlling *Fusarium solani* and *Rhizoctonia solani*, the causal agents of black root disease of strawberry plants, found that the complete inhibition of mycelium growth of *R. solani* and *F. solan*i was achieved with concentrations of 4 g/L and 6 g/L, respectively. Additionally, a similar effect of sodium silicate showed in this study at the concentration of 10000 ppm has been reported by Zhao *et al*. (2023), which resulted in complete suppression of 100% on *P. italicum, G. citri-aurantii, C. gloeosporioides* and *P. digitatum.* Furthermore, in a previous study, 90 mM of potassium silicate reduced the severity of green and blue molds by 74% and 67%, respectively. Additionally, its curative effects on diminishing gray mold and blue mold on 'Valencia' oranges by 23% and 40%, respectively, were also documented (Moscoso-Ramírez and Palou, 2014). Effective inhibition of fungal growth was observed with the incorporation of 100 and 200 mM of sodium silicate into PDA, achieving rates of 52% and 90%, respectively, after a seven-day incubation at 25°C, as demonstrated by Li *et al*. (2009). According to Mbili (2012), potassium silicate decreased significantly grey mold incidence on "Golden Delicious" apples, showing a reduction of 75% at a concentration of 10 mg/l to 72.5% at a concentration of 100 mg/l. Other studies report that potassium silicate exhibits the lowest efficacy among GRAS salts, resulting in a 50% inhibition of growth in *C. gloeosporioides* at the highest concentration of 2% (Martínez-Blay *et al*., 2020). However, calcium silicate did not show a significant effect on inhibiting *Rigidoporus microporus* (Hadi *et al*., 2021).

Further investigation is warranted into the potential of GRAS salts for controlling citrus rots, particularly in terms of their capacity to halt or slow down fruit-to-fruit transmission. Additionally, there is a need for research to evaluate the effectiveness of aqueous solutions containing these salts in eradicating or deactivating foodborne microorganisms on citrus surfaces, especially concerning regulatory frameworks like the Food Safety Modernization Act in the USA (Soto-Muñoz *et al*., 2020). It is also crucial to conduct compatibility studies with common fruit sanitizers such as chlorine derivatives and peracetic acid. Beyond serving as alternatives to banned fungicides like propiconazole in the EU, these salts exhibit promise in reducing other significant postharvest citrus ailments such as green and blue molds (Radulović et *al*., 2023). Importantly, their commercial application in citrus packinghouses as aqueous solutions would necessitate minimal facility modifications, leveraging their designation as GRAS compounds or food additives. This classification facilitates their registration as postharvest antifungal treatments, potentially expediting industry adoption and compliance with fruit safety standards.

Conclusions

This study highlights the effectiveness of sodium, potassium, and calcium silicates as viable, eco-friendly alternatives to traditional chemical fungicides in combating the primary post-harvest ailments of citrus fruits. Sodium silicate emerged as the most effective treatment, significantly inhibiting key pathogens such as *Penicillium digitatum*, *Penicillium italicum*, and *Geotrichum citri-aurantii*. It demonstrated strong preventive and curative effects, particularly against brown rot, sour rot, and green mold. Calcium silicate also showed promising results in reducing blue mold. This proposition carries significant weight in tackling citrus rots, particularly *P. digitatum*, solely responsible for approximately 90% of total post-harvest citrus fruit losses, and sour rot induced by *G. citri-aurantii.* Additionally, their straightforward application and widespread availability render them accessible choices for citrus growers seeking sustainable disease control strategies. Nonetheless, further research is imperative to fine-tune application protocols and comprehend the long-term ramifications before widespread implementation. The potential antifungal prowess of this compound could be augmented through synergistic combinations with other treatments such as heat, minimal doses of fungicides, and biological antagonists. Nevertheless, the findings presented underscore the promising role of GRAS compounds in enhancing the sustainability and resilience of citrus fruit production systems.

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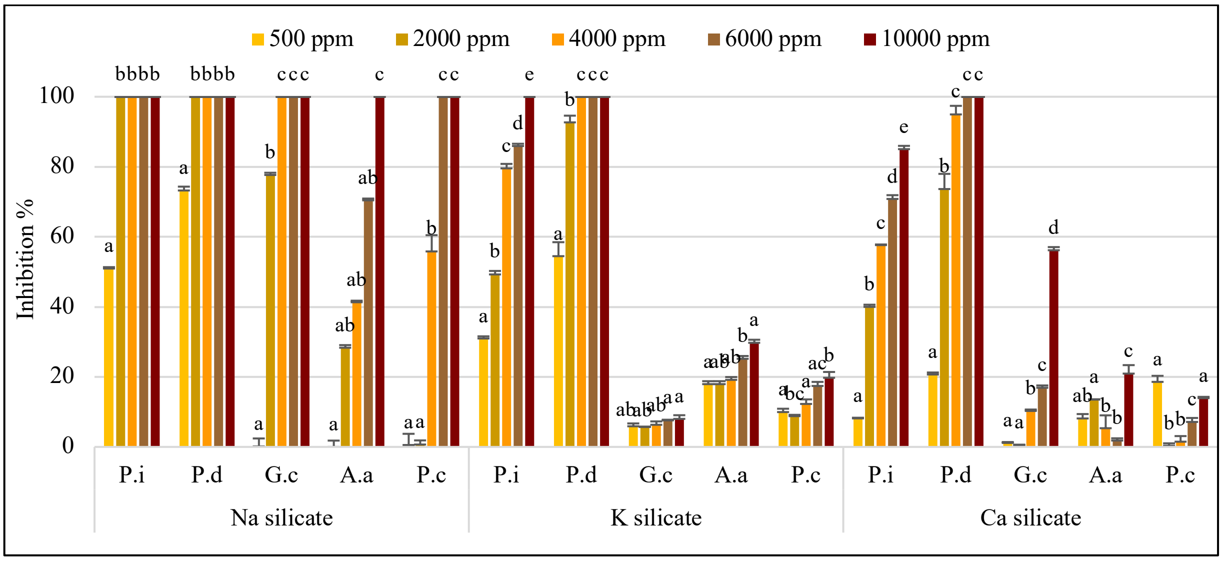

Figure 1. Percentage of growth inhibition of *P. italicum, P. digitatum, G. citri-aurantii, A. alternata* and *P. citrophthora* induced by Sodium, Potassium, and Calcium Silicates. Data represent average inhibition rate (%) ± standard deviation. Values having the same letter, above histogram bars, are not significantly different according to the Duncan test ($p<0.05$).

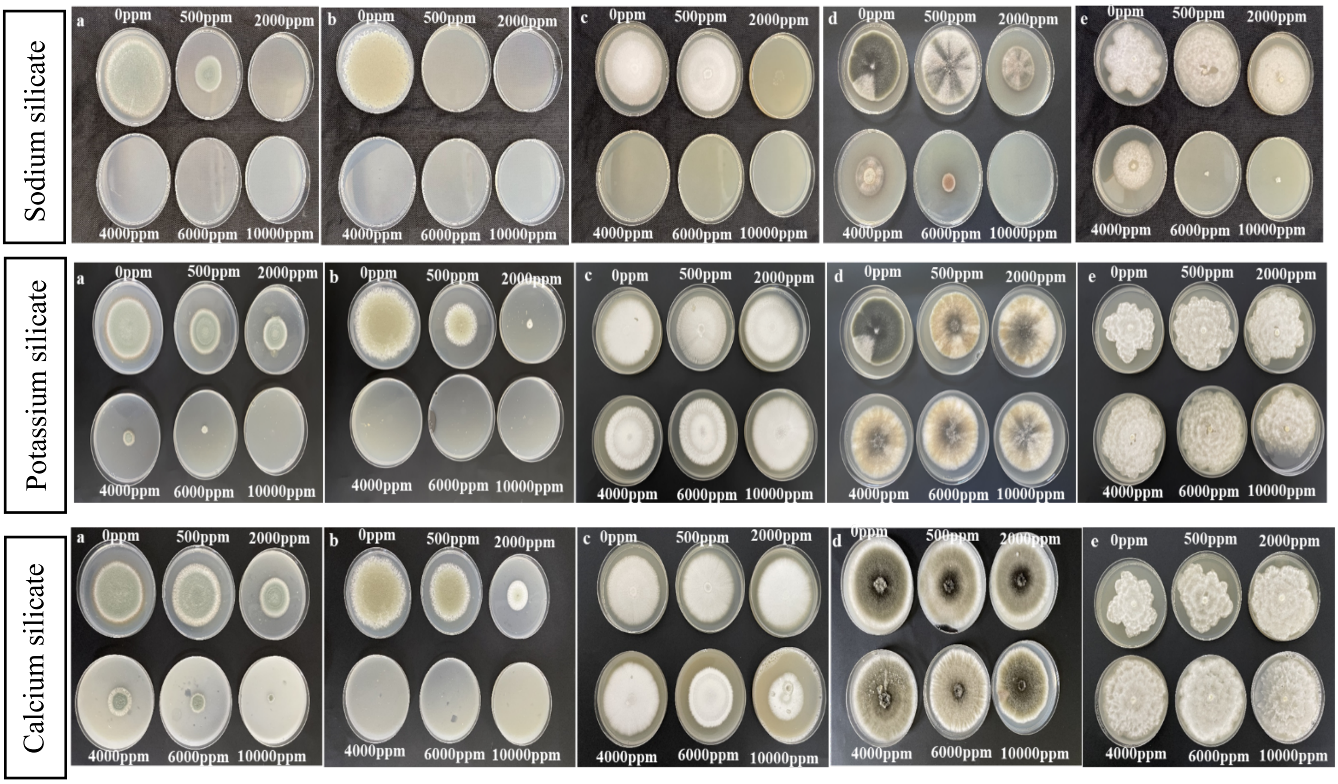

Figure 2. Effectiveness of silicate salts at various concentrations on the radial mycelial growth of *P. italicum* (a), *P. digitatum* (b), *G. citri-aurantii* (c), *A. alternata* (d) and *P. citrophthora* (e) on PDA plates after 7 days at 25°C.

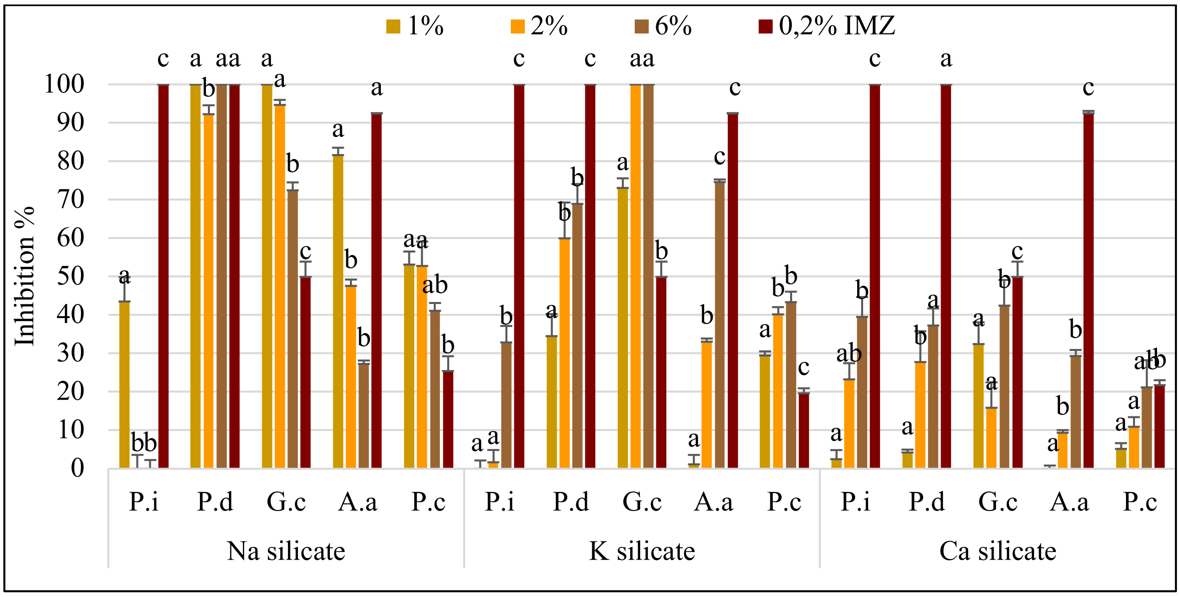

Figure 3. Preventive activity of sodium, potassium, and calcium silicates at different concentrations for controlling blue and green molds, sour rot, black rot and brown rot on artificially inoculated clementine 'Sidi Aissa' fruits. Data represent average inhibition rate (%) ± standard deviation. Values having the same letter, above histogram bars, are not significantly different according to the Duncan test (p<0.05).

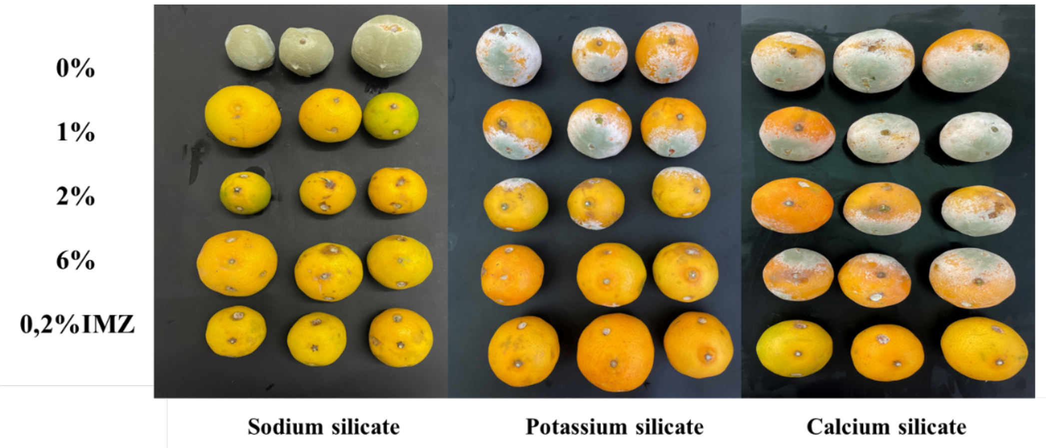

Figure 4. *In vivo* silicates effect of sodium, potassium and calcium silicate on the severity of green mold on artificially inoculated clementine 'Sidi Aissa' fruits (preventive treatment).

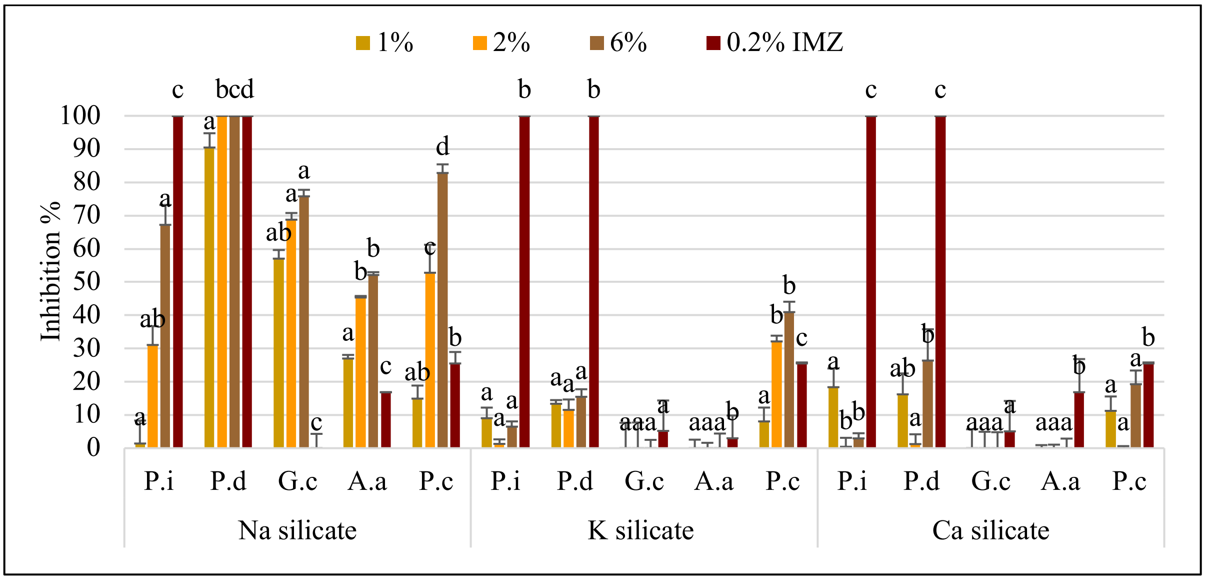

Figure 5. The curative activity of sodium, potassium, and calcium silicates at different concentrations for controlling blue and green molds, sour rot, black rot, and brown rot on artificially inoculated clementine 'Sidi Aissa' fruits. Data represent average inhibition rate (%) ± standard deviation. Values having the same letter, above histogram bars, are not significantly different according to the Duncan test ($p<0.05$).

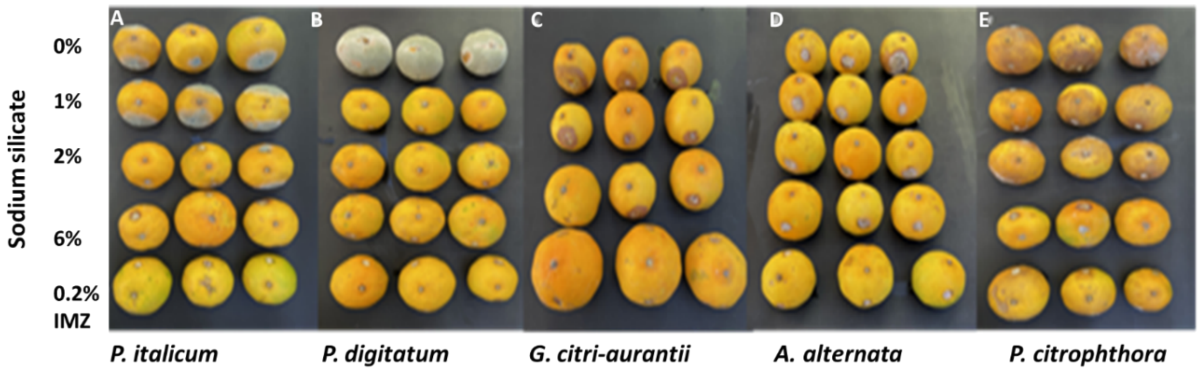

Figure 6. *In vivo* effect of sodium silicate on the severity of different pathogens evaluated on artificially inoculated clementine 'Sidi Aissa' fruits (curative treatment).

Table 1.The properties of the soluble silicate employed in this study.

Compounds	Chemical formula	Molecular weight (g/mol)	E-number[1]	Purity
Potassium silicate	K_2SiO_3	154,280	E-560	99%
Sodium meta silicate	Na_2SiO_3	122,063	E-211	99%
Calcium silicate	Ca_2SiO_4	172,239	E-552	99%

[1]E-number refers to a code assigned by the European Union.

Table 2. Inhibitory concentration 50 values of sodium, potassium, and calcium silicates of each fungus.

IC 50 (ppm)			
Pathogen	**Na silicate**	**K silicate**	**Ca silicate**
P. italicum	826.65	2897.58	4488.82
P. digitatum	651.17	1082.47	2178.19
G. citri-aurantii	1809.08	148284	10378.01
A. alternata	4694.02	24405.71	36321.92
P. citrophthora	2207.05	34857.91	75515.66
Mean	10187.97	206527.67	128882.60

IC, inhibitory concentration; Na, sodium; K, potassium; Ca, calcium.