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ORIGINAL ARTICLE

A cinnamaldehyde-based formulation as an alternative to sodium hypochlorite for post-harvest decontamination of citrus fruit

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Abstract

According to the most recent regulation, published in 2018, areas or states of Brazil where citrus canker is endemic are no longer obliged to eradicate citrus trees affected by the disease as in the past 60 years. Instead, growers have to adopt a set of control measures, such as copper sprays, windbreaks, and control of the citrus leaf miner to minimize the impact of the disease on fruit quality and yield. Another important change was that all fresh Fruit commercialized out of the state of origin and to other countries have to be sanitized against the canker-causing bacterium *Xanthomonas citri* subsp. *citri* (*X. citri*). Initially, sodium hypochlorite (NaOCl) was the only product allowed in Brazil by the referred legislation. Recently, this bactericide was prohibited to be used on fresh fruit shipped to the European Union and replaced by eugenol at 2%. Although effective, NaOCl may damage fruit skin, cause corrosion of packing house equipment and react with organic matter, which generates noxious by products. Here, we evaluated an alternative to NaOCl known as PosFruit. GC/MS and ^{1}H NMR chemical analyses showed that Posfruit contains both cinnamaldehyde isomers, with the *trans* being present in larger quantities. We showed that PosFruit was as effective as NaOCl to eliminate *X. citri* from citrus fruit artificially contaminated with the bacterium. In a pilot sanitization line, treatment with 2% PosFruit reduced the *X. citri* population on contaminated fruit by 4 $\log_{10}$ CFU/mL. Furthermore, we detect neither the natural resistance of *X. citri* to PosFruit nor the persistence of the bacterium following progressive exposure to the product, which indicates that the product has multi-target action. PosFruit is a plant fortifier, residue-free, and efficient alternative to NaOCl for post-harvest decontamination of citrus fruit against *X. citri*.

Keywords Citrus canker · Fresh fruit · Sanitization

Introduction

The most profitable market within the citrus business is that of the fresh fruit, which has higher aggregated value compared with fruit used for the production of juice. However, there are important limitations to be addressed concerning the quality of the fruit, which is constantly threatened by diseases and insects. Among them, a great focus has been given to Asiatic citrus canker (ACC), a disease caused by the Gram-negative bacterium *Xanthomonas citri* subsp. *citri* (*X. citri*). ACC affects all the commercial citrus cultivars at different levels, and the only viable option to control the disease in areas where it is endemic is to apply concomitantly a set of agricultural management measures (Gottwald et al. 2002; Ference et al. 2018). This disease has a great dispersion potential through the combined action of wind and rain (Gottwald et al. 2002). It can also be introduced into new areas by contaminated vegetal parts and fruit. Therefore, in areas where the disease is not

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present or occurs at low incidence, the main strategy to prevent its establishment is to monitor the transit of plant material and inspect the citrus orchards. In case the disease is detected, eradication efforts are undertaken to eliminate the pathogen (Behlau et al. 2016).

The post-harvest sanitization of citrus fruit before their distribution and commercialization is extremely important to avoid the dissemination of the pathogen. Based on the most recent Brazilian regulation, known as Normative Instruction 21 (IN21), published by the Ministry of Agriculture Livestock and Supply (MAPA) on April 25, 2018 (Brazil 2018), areas or states where ACC is endemic are no longer obliged to eradicate canker-affected or suspect trees as before (Behlau et al. 2016). Instead, growers have to adopt a set of control measures, such as copper sprays, windbreaks, and control of the citrus leaf miner to minimize the impact of the disease on fruit quality and yield. Another important change was that all fresh fruit commercialized out of the state of origin and to other countries have to be sanitized with NaOCl. Upon arrival in the packing house, fruit harvested from orchards where ACC is present should be immersed in a 200 ppm (0.2% *v*/v) NaOCl solution at pH 7.0 for 2 min (Brazil 2018).

Until recently, NaOCl was the only product allowed in Brazil by the referred legislation. That is likely due to a high number of studies that demonstrate its efficacy and low cost. Although it is considered generally safe and has been used for nearly a century as a disinfectant for water supplies (Mishra et al. 2018), NaOCl has some important disadvantages. The chlorine dissociated from NaOCl is a volatile element that loses its effectiveness upon binding to the organic matter, which makes necessary to constantly adjust its concentration in solution (topping off) during the sanitization process. Moreover, chlorine is corrosive to industrial equipment and may also damage the fruit skin. Chlorine-based products are not friendly to the environment and to packing-house workers as it can cause injuries to eyes and skin (Gil et al. 2009; Mishra et al. 2018; Moretti 2007). Finally, some concerns are associated with the use of hypochlorite solutions as they may result in the formation of trihalomethanes that are considered carcinogens, as well as other byproducts due to its reaction with the organic matter (Richardson et al. 2000). Because of these disadvantages, since September 2019, NaOCl is no longer allowed to be used to decontaminate fresh citrus fruit to be shipped to the European Union (European-Commission 2019). Instead, fruit should be sprayed with 2% eugenol (an essential oil extracted from clove, *Syzygium aromaticum*) and kept wet for at least 2 min (Brasil 2019).

Although the Brazilian legislation restrict the options of bactericides allowed for post-harvest sanitization of citrus fruit depending on the destination of the production, it also allows the recognition of alternative fruit disinfectants, provided that their efficacy and safety are scientifically demonstrated. A number of sanitizing products are known and allowed to be safely used in other countries. For instance, in the USA, the Food and Drug Administration (FDA) approved the use of chlorine dioxide, hydrogen peroxide, peracetic acid, and ozone, beyond NaOCl, for the sanitization of fruits (no sentido de vários tipos de fruta) and vegetables in post-harvest. In addition, several studies have suggested the use of formulations containing essential oils, such as eugenol, as sanitizers due to their antimicrobial potential (Lis et al. 2017; Sakkas and Papadopoulou 2017; Uma et al. 2017; Zhao et al. 2017).

Thus, in this work, we evaluated the use of a cinnamaldehyde-based formulation known as PosFruit as an alternative post-harvest sanitizer of citrus fruit as an alternative to NaOCl. We showed that PosFruit has bactericidal action against *X. citri*, and it is as effective as NaOCl to sanitize citrus fruit artificially contaminated with *X. citri*. Using a pilot processing line, we also demonstrated that PosFruit is able to eliminate the *X. citri* population on contaminated fruit.

Materials and methods

Characterization of the sanitizing agent candidate

The commercial formulation named PosFruit (Arvensis; Zaragoza, Spain) was evaluated as a sanitizer of citrus fruit against *X. citri*. The product was obtained from Comnagro Agroespecialidades LTDA (Campinas, São Paulo, Brazil, Lot number 3690006). Pluron 444A (Catanduva, São Paulo, Brazil, Lot 2138), containing NaOCl, was used as a positive control for fruit decontamination.

Bacterial strain and growth conditions

The *X. citri* strain used for the sanitization tests was the isolate 306 (IBSBF 1594) (Schaad et al. 2006). Before being exposed to the sanitizing agents, bacteria were cultivated for 16 h in NYG/NYG-agar medium (nitrogen-yeast-glycerol 5 g/L of peptone, 3 g/L of yeast extract, 2% glycerol; for solid medium bacterial agar was added to 15 g/L) at 29 °C.

Sensitivity assays

Bacterial liquid cultures had their optical densities (O.D. 600 nm) adjusted to 0.3 using fresh NYG medium. Subsequently, cell suspensions were diluted 100 × with fresh medium to make test cultures of 5 mL containing 10^6 CFU/mL. PosFruit was added to the bacterial cultures at the final concentrations of 0.0625%, 0.125%, 0.25%, 0.5%, and 1% (*v*/*v*). As positive control, cells were exposed to Kanamycin at 20 μg/mL. Each treatment was composed of three repetitions. Tubes were kept at 29 °C for 4 h and 200 rpm. After incubation, samples of 100 μL of each tube were diluted (10 to 10^6-fold) and spread onto NYG-agar plates for CFU counting. The

absence of growth after 72 h incubation indicated bactericidal activity. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the compound which completely prevented growth of *X. citri*. This experiment was performed three times.

Sanitization tests

Preparation of the bacterial suspension and fruit

Bacteria were inoculated from plate in 250 mL Erlenmeyer flasks containing 100 mL of NYG and incubated at 29 °C under shaking (200 rpm) until cultures reached the O.D. 600 nm of 0.3 (10^8 CFU/mL). After incubation, the cultures were centrifuged at 6000 ×*g* for 7 min and the supernatant discarded. Cells were resuspended in phosphate buffer (1X PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4; pH 7.4) and the O.D. 600 nm was again adjusted to 0.3. Tahiti limes (*Citrus latifolia*, Tanaka) were used for the decontamination tests. Besides being smaller and easier to manipulate than other citrus fruit, Tahiti limes are affected by citrus canker and are important for the national and international market of fresh fruit. The limes had approximately 5 cm in diameter measured in the cross-section perpendicular to the longer axis of the fruit.

Evaluation of PosFruit as a sanitizer

Before being exposed to the bactericides, fruit were washed with 5% Wash Fruit 33 soap (Aruá, Matão, Brazil), in order to remove dust and debris from the field. After washing, fruit were dried overnight at room temperature. For each treatment, 15 Tahiti limes, divided in 5 groups (replicates), were spray-contaminated until the run-off point using the *X. citri* cell suspension, and allowed to dry at room temperature (~ 21 °C) for approximately 6 h. PosFruit was assessed at 1%, 2%, and 5% by spraying the fruit with a handgun sprayer until the run-off point. The fruit was exposed to the product for 2 min, followed by the removal of the excess using an air drier (without heat) for 30 s. NaOCl at 0.2% and 1X PBS were used as positive and negative controls, respectively, in place of PosFruit. The evaluation of the product was assessed in three independent experiments.

Effiency of PosFruit in a pilot packing line

Fruit used in these tests was not pre-washed before passing through the automated sanitization line. During the process, fruit was sprayed with PosFruit at 2% in water (*v/v*), and exposed to the product for 2 min. Fruit washed with tap water only was used as negative control. For each treatment, 15 limes were divided into 5 groups of 3 limes each (replicates), labeled using a permanent marker for identification, contaminated with *X. citri* suspension prepared as previously described, and then mixed with other limes added to give flow of fruit throughout the sanitizing line. Each of the passed through the sanitizing line separately. Marked fruit was collected at the end of the line and allowed to dry at room temperature. The evaluation of the product was assessed in three independent experiments.

Assessment of the sanitization efficiency

After the treatments, fruit was placed into plastic bags and washed manually using 100 mL of 1X PBS during 5 min. The washes were collected in 50 mL polypropylene tubes and centrifuged at 6000 ×*g* for 7 min and the supernatant discarded. Cells were resuspended in 3 mL of 1X PBS, and 100 μL were spread onto semi-selective MGY-KCC medium (16 μg/mL kasugamycin, 16 μg/mL cephalexin, and 50 μg/mL cycloheximide) (Behlau et al. 2012). Colony growth was evaluated after 72 h of incubation at 29 °C. To confirm the identity of *X. citri* colonies, we performed diagnostic PCR following the procedure described by Coletta-Filho et al. (2006).

Induction of *X. citri* resistance

The probability of resistance development in *X. citri* against PosFruit was evaluated as previously described (Oz et al. 2014), with modifications. Starting cultures of 5 mL at 10^6 CFU/mL were exposed to PosFruit at 1 ×/8 of the MIC (MIC = 0.125%; as described in Results) and incubated for 24 h at 29 °C and 200 rpm. Following, 100 μL of the bacterial suspension were plated on NYG-agar and incubated for 72 h at 29 °C for CFU counting. This process was repeated until the maximum concentration of PosFruit reached 4 × the MIC. In each step, 500 μL of the previous culture were used as inocula and transferred to fresh 5 mL cultures (identical to the starting cultures). Experiments were performed in triplicates, and repeated three times.

Assessment of *X. citri* resistance

X. citri was inoculated into 5 mL of NYG to a final concentration of 10^6 CFU/mL, followed by the addition of PosFruit at 0.9 × the MIC (MIC = 0.125%; section 3.1). The bacterial suspension was exposed to the product for 4 h at 29 °C under shaking (200 rpm). After incubation, 100 μL of the liquid culture were spread onto NYG-agar for the recovery of *X. citri*. Colonies that were able to grow were inoculated into fresh liquid NYG medium, now containing PosFruit at 1 × the MIC, and incubated as before. Aliquots of 100 μL were spread onto solid media following exposure to the product for 4 h, 4.5 h (half an hour beyond the incubation period used to define the MIC), and 24 h. Plates were incubated for up to

72 h before assessing the recovery of persistent colonies of *X. citri*. Experiments were performed in triplicates and repeated three times.

Results

Chemical characterization of PosFruit

The total ion chromatogram (TIC) of PosFruit exhibited two major peaks at 37 and 38 min (Figs. S1 and S2, supplementary material). Both peaks displayed similar mass spectra and were identified as cinnamaldehyde isomers. The ^{1}H NMR spectrum showed the presence of the *trans*-cinnamaldehyde isomer, identified in the spectrum by the presence of the *trans* coupling constant (J = 15.9 Hz) between the two hydrogens of the double bond conjugated with the carbonyl group (H_2–H_3) (Figs. S3 and S4). The *cis* isomer was not detected by the ^{1}H NMR analysis. This difference in the proportion of the isomers was confirmed with the GC/MS chromatogram. The signal at 37 min had a low intensity (*cis* isomer) and the signal at 38 min had a high intensity (*trans* isomer).

PosFruit has bactericidal activity against *X. citri*

The efficacy of PosFruit to inhibit growth of *X. citri* was evaluated by exposing bacterial cultures to various dilutions of the product prepared directly into the culture medium. PosFruit was able to inhibit *X. citri* growth in liquid medium at 0.125% after 4 h of exposure. In addition, no colonies from this treatment were recovered after plating samples of the cultures on NYG-agar (Fig. 1c). These results were similar to the positive control, kanamycin, which has bactericidal action against *X. citri* at 20 μg/mL (Fig. 1b). Even after decreasing the concentration of PosFruit to 0.06%, there was still a significant inhibition of bacterial growth reflected as a reduced cell turbidity in the culture. However, upon plating samples of this culture on solid medium, a significant amount of *X. citri* colonies were able to grow (Fig. 1, compare d with the negative and positive controls, a and b, respectively). Based on that, 0.125% was defined as the minimal inhibitory concentration (MIC) of PosFruit against *X. citri*.

Resistance and persistence of *X. citri* against PosFruit

Xanthomonas citri cells were exposed to PosFruit at the MIC (0.125%) for 4 h, 4.5 h, and 24 h, before culture samples were spread onto NYG-agar in order to allow the growth of persistent colonies. After several attempts, no colonies could be recovered, which indicated that *X. citri* is not able to persist the treatment with PosFruit. Additionally, to check if and how likely natural resistant cells could be isolated, we prepared serial dilutions of *X. citri* cultures and plated them on NYG-agar containing PosFruit at the MIC. Plates were incubated for up to a week under standard cultivation conditions, and again, no bacterial colonies could be observed.

A final approach to assess the likelihood of resistance development was to expose *X. citri* to increasing concentrations of PosFruit, starting from a sub-lethal concentration and increasing up to fourfold the MIC (Table 1). The

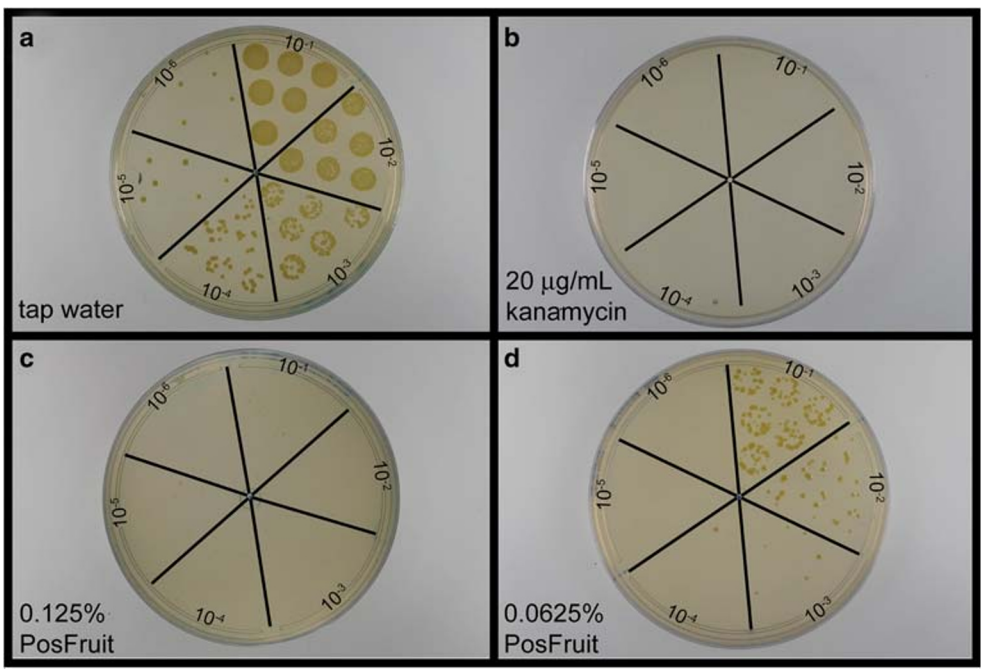

Fig. 1 Sensitivity of *Xanthomonas citri subsp. citri* to PosFruit. Bacterial substitute for suspension was exposed to different concentrations of PosFruit for a period of 4 h and plated on NYG-agar after serial dilutions (from 10^{-1} to 10^{-6}) for CFU counting. **a** Negative control (sterilized tap water). **b** 20 μg/mL kanamycin. **c** 0,125% and **d** 0.0625% PosFruit

Table 1 PosFruit resistance induction in *Xanthomonas citri subsp. citri*

Day	1	2	3	4	5	6	7	8	9	10	11	12	13
Concentration[a]	1 ×/8	1 ×/4	1 ×/2	1 ×	1 ×	2 ×	2 ×	2 ×	3 ×	3 ×	3 ×	3 ×	4 ×
Bacterial growth[b]	3	2	0	0	0	0	0	0	0	0	0	0	0

[a] 1 × minimal inhibitory concentration of PosFruit in water (MIC = 0.125%)

[b] number of experiments in which at least one of the three replicate plates showed any growth of *X. citri*. Experiments were repeated three times

rationale was to test if the product can induce genetic alteration in the course of increasing concentrations, and that could lead to PosFruit resistance in *X. citri*. The experiment was initiated by exposing *X. citri* to 1 ×/8 of the MIC (day 1). After 24 h of incubation, culture samples were spread onto NYG-agar, and *X. citri* colonies were detected in 3 out of 3 trials after 48 h of plate incubation (Table 1, day 1). Fractions of the starting cultures (day 1, after 24 h incubation) were used as inocula to prepare the subsequent cultures in which the concentration of PosFruit was increased twofold (to 1 ×/4 of the MIC). Upon plating culture aliquots from the second day, we observed growth of *X. citri* in 2 out of 3 plates. However, when the concentration of the PosFruit reached half the MIC (1×/2), bacterial growth was halted (Table 1, day 3). This process continued until the concentration of PosFruit reached four times the MIC (day 13). Neither the increase of cell turbidity in liquid cultures nor the growth of colonies on plate was detected from the third day on. Altogether, these results show that PosFruit did not induce resistance in *X. citri*.

PosFruit was efficient to sanitize *X. citri* under laboratory conditions

The efficiency of PosFruit to sanitize Tahiti limes was evaluated by artificially contaminating fruit with *X. citri* followed by their exposure for 2 min to three concentrations of the product (Fig. 2). Treatments with PosFruit at 1% showed a reduction of approximately 2 $\log_{10}$ in the number of *X. citri* CFU/mL recovered on agar plates (Fig. 2; compare panels a-1 and a-3; b). By further increasing the concentration of the product to 2%, we detected a reduction of 3 $\log_{10}$ in the number of recovered CFU/mL. At this concentration the efficacy of PosFruit was comparable with NaOCl at 0.2% used as the positive control (Fig. 2; compare panels a-1, a-2, and a-4; b). Besides some typical *X. citri* colonies, other yellowish epiphytic bacteria were also recovered after fruit sanitization with PosFruit at 2% (Fig. 2 (a-4)). These contaminants were disregarded for being false positive colonies based on PCR analysis. Finally, exposure of Tahiti limes to PosFruit at 5% completely eliminated all the bacteria, as no colonies could be recovered on NYG-agar plates following this treatment (Fig. 2 (a-5)).

PosFruit was efficient to sanitize *X. citri* in a packing line

The efficiency of 2% PosFruit to sanitize Tahiti limes against *X. citri* was subsequently evaluated using a pilot processing line (Fig. 3a). Untreated control fruit, washed with water only, led to the recovery of 4 $\log_{10}$ CFU/mL (Fig. 3b, (c-1), red circles). The identity of *X. citri* isolates was checked by PCR. A substantial amount of non-*X. citri* colonies was also recovered, and this was probably due to the fact that limes were washed only with tap water (not soap) before PosFruit application. However, when fruit was exposed to PosFruit at 2% using the pilot processing line, we observed a complete elimination of *X. citri* (Fig. 3b, (c-2)). Therefore, the reduction of the *X. citri* population on fruit exposed to this treatment was equivalent to 4 $\log_{10}$ CFU/mL when compared with the untreated fruit.

Discussion

The current Brazilian legislation that regulates the control of ACC in the country (Brazil 2019) determined for the first time in 60 years that growers in states where the incidence of the disease is high are no longer required to eradicate the affected trees. Instead, growers have to adopt a set of control measures in order to reduce the impact of the disease on fruit quality and yield. This regulation also mandates that all fresh fruit commercialized out of the producing state or country are sanitized with bactericide in the packing-house. Although the legislation allows the use of two bactericides (NaOCl and eugenol) for that purpose depending on the market, it also consents that other products are authorized upon demonstration of their efficacy. In this study, we showed that a cinnamaldehyde-based formulation known as PosFruit is an efficient bactericide to be used for post-harvest sanitization of citrus fruit against *X. citri*.

Reactive chlorine released from NaOCl is probably one of the most widespread sanitization agents used in the world to

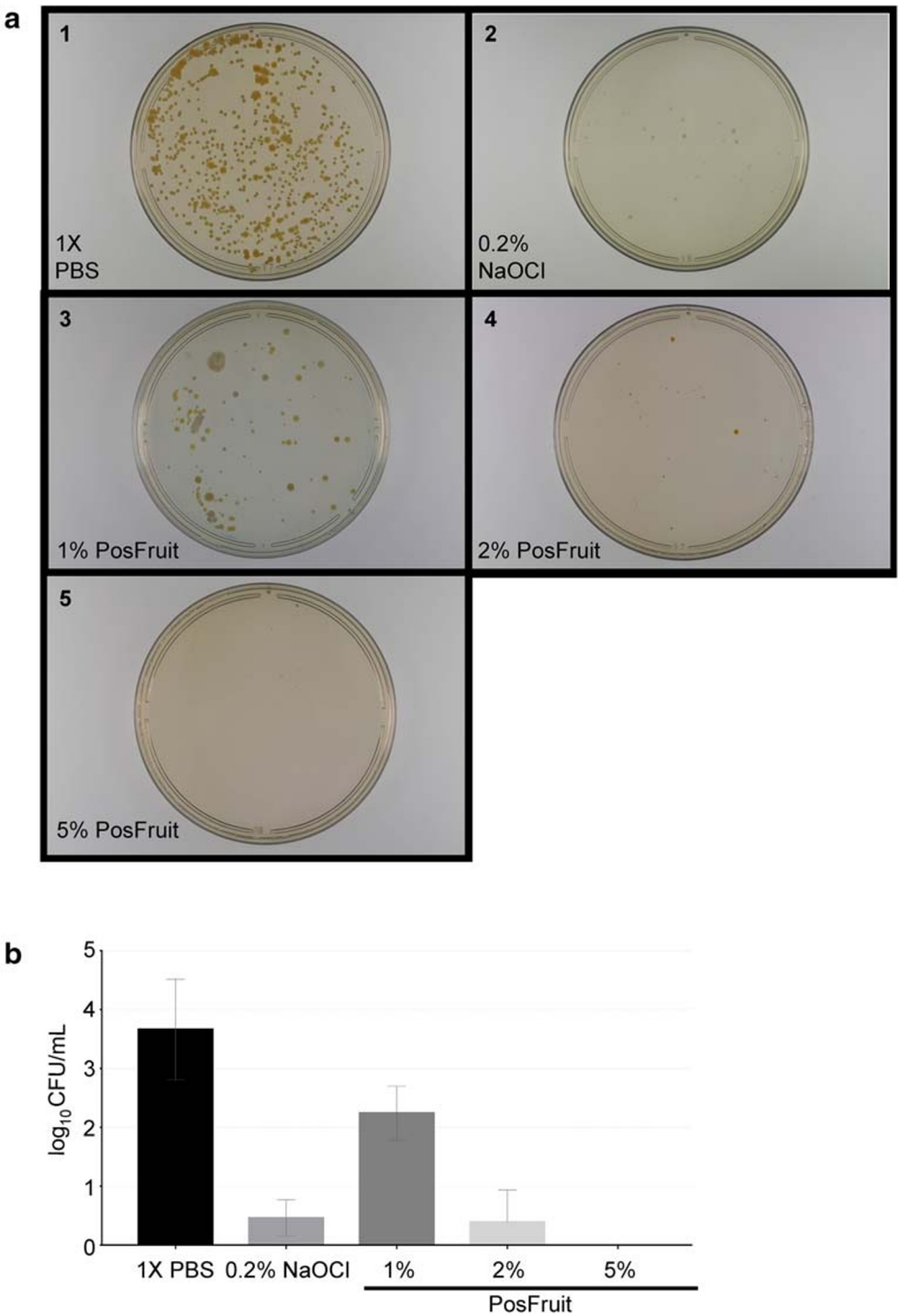

Fig. 2 Efficiency of PosFruit as a sanitizer of Tahiti limes against *X. citri* under laboratory conditions. Tahiti limes were spray-contaminated with *X. citri* and then manually exposed to 1, 2, and 5% PosFruit for 2 min. Following treatment, limes were washed and the recovery of *X. citri* was assessed on MGY-KCC agar plates after 72 h. **a** Recovery of *X. citri* on agar plates: (1) 1X PBS (negative control); (2) 0.2% NaOCl (positive control); (3) 1% PosFruit; (4), 2% PosFruit; (5), 5% PosFruit. **b** *X. citri* recovered after treatment as $\log_{10}$ colony forming units (CFU)/mL. Whiskers indicate the standard error of the mean of three independent experiments

guarantee the quality of water and freshly harvested salads, vegetables, and fruits. Chlorine has long been recognized as an active agent against many bacteria, fungi, and viruses (Goda et al. 2018; Kohler et al. 2018; Lineback et al. 2018; Da Silva et al. 2016; Rich and Slots 2015). However, concerns have been raised about the relative efficacy of chlorine-based formulations compared with other products and its safety to health and the environment (Gomes et al. 2018; Teixeira et al. 2018; Slaughter et al. 2019). In solution, hypochlorous acid (hypochlorite anion) promptly reacts with the organic matter forming byproducts potentially harmful to health such as trihalomethanes, haloacetic acids, haloketones, and chloropicrin (reviewed by Gil et al. (2009)). Although the safe level of these byproducts in food has never been demonstrated, in September 2019, the European Union banned the use

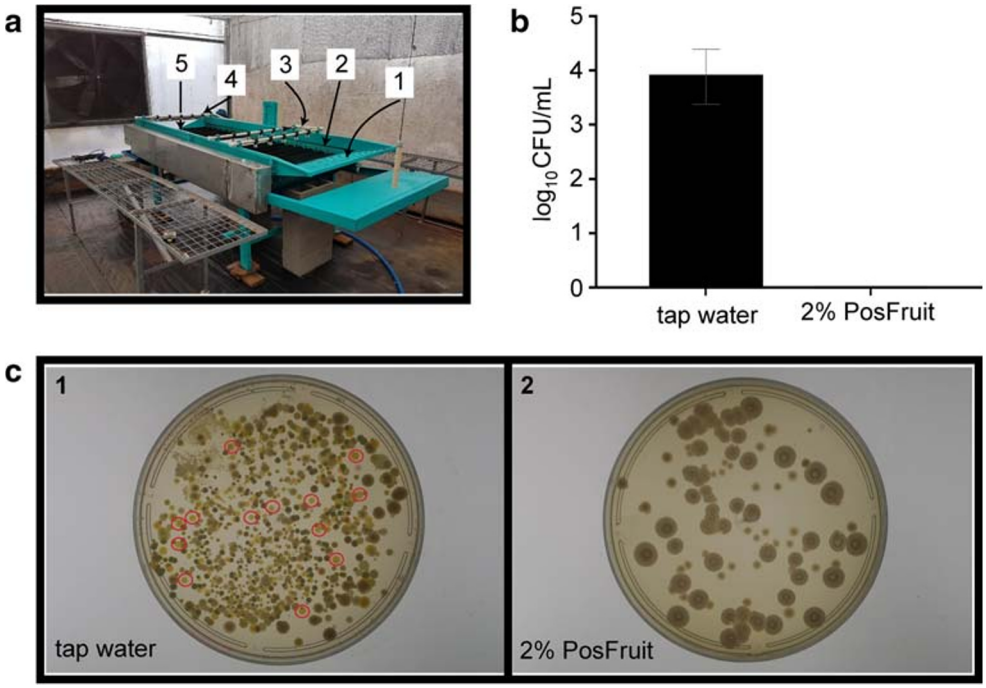

Fig. 3 Efficiency of PosFruit as a sanitizer of Tahiti limes against *X. citri* in a pilot processing line. Marked fruit were contaminated with *X. citri*, and loaded on the pilot line (**a**): 1, fruit drop point; 2, rollers; 3, water spray lines; 4, product (PosFruit) spray line, and **5**, fruit collection point. Following treatment with PosFrui at 2% for 2 min, limes were washed and the recovery of *X. citri* was assessed on MGY-KCC agar plates after 72 h. **b** *X. citri* recovered after treatment as $\log_{10}$ colony forming units (CFU)/mL. Tap water was used as negative control. Whiskers indicate the standard error of the mean of three independent experiments. **c** Plates from a representative experiment: (1) negative control, limes washed with water only (*X. citri* colonies are labeled using red circles) and (2) plate inoculated with washes from limes exposed to PosFruit at 2%

of chlorine for treating fresh citrus fruit (European-Commission 2019). Nevertheless, there are other important disadvantages of NaOCl: loss of efficiency due to its interaction with the organic matter requiring constant solution adjustments in washing tanks, risk fruit skin damage, cXorrosion of equipment, and the release of gases that are harmful to workers direct- or indirectly exposed to solutions containing chlorine (Slaughter et al. 2019).

Conversely, PosFruit has been used mainly as a plant fortifier or inducer of plant resistance. This formulation is mainly based on *trans*-cinnamaldehyde, which is the major constituent of essential oils of cinnamon (*Cinnamomum* spp.) and has been demonstrated to exhibit antimicrobial properties against several Gram-positive and Gram-negative bacteria (Vasconcelos et al. 2018; Friedman 2017). In line with this, PosFruit has also been utilized as post-harvest sanitizer for the protection of fruit against several kinds of microorganisms without leaving any residues, i.e., according to the manufacturer, fruit treated with PosFruit can be commercialized immediately after treatment as no safety period has to be observed. PosFruit was demonstrated to be an efficient bactericide against *X. citri* based on the sensitivity tests *in vitro*, and its efficacy was comparable to NaOCl. In addition, there is no risk for the development of PosFruit resistance by *X. citri*. These results are congruent with the performance of multitarget bactericides, such as chlorine, for which the emergence of microbial resistance is disregarded (assessment report on active chlorine, EU 528/2012 from January 2017).

The best cost-benefit was achieved with PosFruit at 2%. Although some *X. citri* were recovered after exposure to PosFruit at 2% for 2 min under laboratory conditions, we did not recover any *X. citri* when fruit were treated with the product at this concentration and exposure time in a pilot packing line. Nevertheless, it is important to mention that the sanitizing processes or products are not necessarily intended to thoroughly eliminate a microorganism from a fruit or vegetable but mainly to reduce its population to harmless levels or to levels that do not represent risks for dissemination as demonstrated previously (Gottwald et al. 2009; Narciso 2005).

Alternative bactericides for post-harvest disinfection of citrus fruit, especially those with satisfactory effectiveness and with less or no influence in the environment, are most welcome. Citrus farming and agriculture are generally lacking in this type of tool to deal with diseases and pests. At the same time, there is a very strong and unquestionable trend of valuing sustainability and adopting less invasive and polluting measures of control. As a plant fortifier and inducer of plant resistance, PosFruit fulfills such premises. Moreover, it can be used for the disinfection of not only Tahiti limes but also other types of citrus fruit.

Considering the epidemiology of ACC and the morphological similarity between the citrus fruits of different species, the results of this study may also be extended to oranges, lemons, tangerines, tangors, among other important citrus fruit for the fresh market worldwide.

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Authors' contributions CFCZ, GD, LBC, and TGS executed the experiments; MB designed and supervised the execution of the sensitivity and resistance tests; LLS helped with the GC/MS analyses; DCS designed, performed, and analyzed the GC/MS and NMR data; FB and HF designed and coordinated all the experiments and wrote the manuscript

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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