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Efficacy of Low-Toxicity Alternatives for Citrus Disease Management: Potassium Sorbate and Sodium Benzoate against *Alternaria alternata*

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Abstract

The integration of generally recognized as safe (GRAS) salts as low-toxicity solutions represents a crucial eco-friendly alternative to fungicides, harnessing their potent antimicrobial effects against various crop pathogens. In this study, the effectiveness of potassium sorbate, sodium benzoate, sodium bicarbonate, and sodium tetraborate was investigated against *Alternaria alternata*, a significant pathogen causing diseases worldwide in citrus. The inhibitory effects of various salt concentrations on this pathogen were evaluated *in vitro* using modified potato dextrose agar (PDA) and *in vivo* through artificial inoculation of 'Maroc Late' orange fruits, under both curative and preventive treatments. Initial screening of different active ingredients against three *A. alternata* isolates established imazalil as a commercial reference for comparative analysis. Results showed that potassium sorbate and sodium benzoate were the most potent inhibitors, suppressing the fungus *in vitro* by 71% and 67% at 2,000 ppm, respectively, revealing a very low value of IC_{50} (3 ppm). These two salts yielded comparable outcomes to imazalil (100% suppression) in the curative treatment, achieving significant reductions in severity of 80% and 100% at a low concentration of 2% (w/v). Additionally, fruits treated preventively with 4% (w/v) potassium sorbate and sodium benzoate reduced disease symptoms by up to 100%. The current study highlights GRAS salts that are similarly effective to imazalil and could serve as alternatives to conventional fungicides registered for managing Alternaria diseases of citrus.

Keywords: Alternaria diseases; alternatives; citrus fruit; fungicides; GRAS salts

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INTRODUCTION

Concurrently with the swift rise in the global population, the substantial reduction of post-harvest losses of fruits and vegetables was suggested as a primary low-cost strategy to address the worldwide food crisis and combat hunger, especially in developing countries. Citrus fruits, prized for their nutritional properties, are in high demand in more than 140 countries (Chen, 2019). Morocco stands out as one of the world's leading producers of citrus fruits, with a particular emphasis on the Souss-Massa region and the Gharb, identified as key areas for Moroccan citrus cultivation (Maroc Citrus, 2023). In the 2022/2023 season, the recorded production quantities for tangerines/mandarins and oranges were 927,000 and 783,000 metric

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tons, respectively (USDA, 2023). However, fruit losses in packinghouses reached up to 65%, primarily due to physical damage, insect pests, fungal infections, and physiological disorders. These losses, which include both quality downgrades and outright rejection of fruit during sorting, significantly impact export levels (Belabess et al., 2020).

In addition to the notable losses that occur during transportation from the fields to packinghouses and subsequent distribution to consumers, fungal pathogens have emerged as the predominant contributors to these losses, impacting the quality and shelf life of harvested citrus fruits. According to Palou and Smilanick (2019), a group of fungi infiltrates the fruit during its growth phase, lying dormant until post-harvest stages when optimal conditions trigger their development. One such pathogenic fungus is *Alternaria alternata*, a quiescent pathogen that threatens the citrus industry (Zelmat et al., 2021; Bastianel et al., 2023; Taycir et al., 2023). In a study conducted in California by Saito and Xiao (2017), the prevalence of post-harvest pathogens in mandarin fruit was assessed at both the presorting stage and after cold storage. Alternaria rot, caused by *Alternaria* species, was identified as the dominant decay in presorted fruit, accounting for 53.5% and 83.1% of decayed fruit in 2015 and 2016, respectively.

During the post-harvest stage, *A. alternata* manifests in two prominent forms of disease: Alternaria black rot (ABR) and Alternaria brown spot of tangerines (ABS) (Güney et al., 2023). The ABR holds greater economic significance due to the ability of its causal agent to induce internal symptoms stealthily in fruits, without visible signs. This insidious nature inflicts heavy post-harvest decay and poses a critical risk to traders. In contrast, ABS is primarily reported as a preharvest disease and is considered one of the most damaging citrus diseases (Garganese et al., 2016). It induces necrotic lesions on leaves, fruits, and young shoots, often resulting in premature defoliation and fruit drop, significantly compromising yield and fruit quality (Timmer et al., 2003; Aiello et al., 2020). According to several studies, *A. alternata* synthesizes host-specific toxins (HSTs) in citrus, which play a crucial role in pathogenicity (Masunaka et al., 2005). These mycotoxins have been identified as potential hazards to both human and animal health (Escrivá et al., 2017).

At present, fungicide application is the main strategy used to prevent the occurrence of citrus-associated species of *Alternaria* (Camiletti et al., 2022, 2024). Indeed, numerous active ingredients have been reported as effective, such as difenoconazole and azoxystrobin (Shehata et al., 2018). Fludioxonil, pyrimethanil, imazalil, and propiconazole also exhibit promising potential in combating Alternaria rot on mandarin fruit during storage (Wang et al., 2023). Likewise, there has been a growing focus on exploring alternative methods in response to increasing legislative constraints on fungicide usage, apprehensions regarding chemical residues, and the emergence of resistant strains of pathogens (Palou et al., 2016; Wisniewski et al., 2016; Vitale et al., 2021). Biological control agents (BCAs) (Ferreira et al., 2020), plant extract (Maghsoodi and Taheri, 2021), essential oils (EOs) (Devite et al., 2023), and cultivation of the resistant varieties (Zelmat et al., 2022) are the current major methods discussed to manage Alternaria diseases of citrus around the world.

Post-harvest treatment with low-toxicity products such as GRAS salts has generated great research interest due to their potential commercial availability, ease of application, relatively low cost, high solubility in water, and excellent management of fungal pathogens (Palou, 2018; Soto-Muñoz et al., 2020). Furthermore, GRAS salts demonstrate no negative effects on the quality properties of citrus fruit (Martínez-Blay et al., 2020). They are increasingly utilized alternatives in the citrus industry, proving particularly valuable in complementing the efficacy of conventional fungicide treatments.

Positive effects of various organic and inorganic salts in controlling post-harvest diseases of citrus have been described in previous research. *Geotrichum citri-aurantii* and *Colletotrichum gloeosporioides* are prominent pathogens affecting citrus, which can be effectively mitigated through the application of salts (Soto-Muñoz et al., 2020). Sodium bicarbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), and potassium sorbate ($C_6H_7KO_2$) have shown effectiveness against the main post-harvest fungal pathogens of citrus. Sodium bicarbonate can effectively reduce the severity of *Penicillium italicum* Wehmer and *Penicillium digitatum* (Pers.:Fr.) Sacc., responsible for blue and green molds on citrus fruits (Montesinos-Herrero et al., 2016). It effectively communicates that salts can destroy fungus by modifying spore microstructure and hyphal cell membranes, leading to enhanced membrane permeability, nucleic acid leakage, and a synergistic inhibition of pathogen hyphal growth and spore germination (Zhao et al., 2023).

Considering the paramount importance of minimizing losses in the citrus supply chain and recognizing the vast export potential, a recent study brought attention to the extensive diversity, occurrence, and prevalence of *A. alternata* in Morocco (Zelmat et al., 2021). However, imazalil stands as the sole registered fungicide officially sanctioned for treating Alternaria rot on citrus fruit during the post-harvest phase (ONSSA, 2024). In addition, there is currently a broad discussion regarding the removal of imazalil from the list of approved chemicals in Morocco. This underscores the necessity for supplementary strategies to manage Alternaria diseases in citrus post-harvest practices, aiming to preserve fruit quality and mitigate their impact on market supply. The present work was conducted to elucidate the efficacy of GRAS salts, including potassium sorbate, sodium tetraborate, sodium bicarbonate, and sodium benzoate, as potential post-harvest alternatives with low toxicity for managing Alternaria rot on citrus fruit.

MATERIALS AND METHOD

Fungal isolates and suspensions preparation

The isolates employed in this study were sourced from infected citrus fruits and previously identified as *A. alternata*, the causal agent of citrus post-harvest diseases in Morocco (Zelmat et al., 2021). The selection of these isolates was based on both their host specificity and geographical origin, as detailed in Table 1.

Fungal suspensions were prepared from seven-day-old *A. alternata* cultures grown on potato dextrose agar (PDA) at a controlled temperature of 25 °C, under a photoperiod of 16 hours of darkness and 8 hours of light. Initially, conidia were harvested by adding sterilized distilled water containing Tween 20 (0.02%, v/v) to the plate surface and gently scraping with a sterile scalpel. To remove mycelium fragments, the suspension underwent filtration through 2 layers of sterile paper and was then adjusted to a concentration of 10^6 conidia ml^{-1} using the hemocytometer technique.

Pathogenicity test

The pathogenicity test was conducted using detached leaves from the rootstock ‘Rough lemon’. The leaves were surface-sterilized in 1% (v/v) sodium hypochlorite for 1 minute, rinsed twice with sterile distilled water, dried, and placed on moist sterile filter paper in petri dishes. The upper surface of each leaf was wound with a sterile scalpel, and 5 µl of *Alternaria* inoculum (10^6 conidia ml^{-1}) was applied to the wound. Control leaves received 5 µl of sterile distilled water. Each isolate was tested in triplicate, and the leaves were incubated at 25 °C for 7 days.

Screening and selecting an effective active ingredient against *A. alternata*

The research was initiated with a fundamental *in vitro* evaluation of 6 fungicides against 3 distinct strains of *A. alternata* from citrus. The primary objective was to select the most efficacious active ingredient to act as a positive control for comparison with GRAS salts. Additionally, the *A. alternata* strain exhibiting greater resistance to fungicides was employed for subsequent investigations. Table 2 presents the active ingredients assessed against *A. alternata* isolates.

In vitro efficacy of active ingredients against A. alternata

The methodology employed to assess the *in vitro* efficacy of fungicides began with the

Table 1. *A. alternata* isolates used in this study

Isolates	Hosts	Geographical origin	Accession number
Sds9	Oranges fruit	Sidi Slimane (Market)	-
Elm1	Oranges fruit	Kénitra (Field)	MW616575
G4	Lemon fruit	Kénitra (Market)	-

Table 2. Characteristics of the active ingredients tested *in vitro* and *in vivo* against *A. alternata*

Active ingredients	Acronym	Chemical family	Active ingredient content (g l^{-1})
Carbendazim	CRB	Benzimidazoles	500
Chlorothalonil	CLT	Chloronitriles	720
Thiophanate methyl	TPM	Benzimidazoles	450
Difenoconazole	DFN	Triazoles	250
Azoxystrobin	AZX	Strobilurins	250
Imazalil	IMZ	Imidazoles	500

Table 3. Characteristics of the salts tested *in vitro* and *in vivo* against *A. alternata*

Products	Acronym	Formula	E-number
Sodium benzoate	SB	$C_7H_5NaO_2$	E 211
Sodium bicarbonate	SBC	$NaHCO_3$	E 500
Potassium sorbate	PS	$C_6H_7KO_2$	E 202
Sodium tetraborate	ST	$Na_2B4O_7.H_2O$	E 285

Note: E-number is the code number assigned to food additives approved by the European Union (EU, 2011)

formulation of individual stock solutions for each product. Subsequently, precise volumes were withdrawn and incorporated into sterilized PDA culture media to achieve concentrations of 0, 0.001, 0.01, 0.1, 1, 10, and 100 ppm. The resultant solutions underwent thorough mixing and were uniformly dispersed within petri dishes.

In vitro assays were carried out using the poisoned food technique. Briefly, in each experiment, 5 mm mycelial discs of the pathogen were extracted from the periphery of colonies and subsequently positioned at the center of plates amended with the solidified mixture (Balai et al., 2020). Cultures grown on PDA medium without fungicides were maintained as controls. The plates were then incubated at a temperature of 25 °C for 7 days. Notably, each trial underwent three replications.

Curative effect of active ingredients against A. alternata

Curative treatments were applied using fungicides that demonstrated *in vitro* inhibitory effects against *A. alternata*. In a concise summary, mature fruits of the 'Maroc Late' oranges variety, uniform and free from defects, underwent disinfection with a 10% (v/v) sodium hypochlorite solution for 2 minutes, followed by two rinses with sterile distilled water. After drying, the fruits were punctured at two equidistant points (3 mm deep) using a sterile round scalpel handle. Subsequently, each wound was inoculated with 20 µl of an aqueous suspension of *A. alternata*, previously adjusted to a concentration of 10^6 spores ml^{-1}. Following a 24-hour incubation period at 25 °C, the fruits were immersed for 1 minute in each fungicide solution prepared at varying concentrations of 500, 1,000, and 1,500 ppm. Afterward, they were placed in plastic containers moistened with sterile distilled water to maintain optimal humidity for pathogen development. Control fruits were treated only with sterile distilled water under identical conditions and then incubated at 25±2 °C for 7 days. The study employed a completely randomized design, with each treatment independently replicated 5 times and randomly assigned to experimental units. This approach enabled accurate comparison of treatment effects.

Assessment of the efficacy of low-toxicity GRAS salts against *A. alternata*

Table 3 presents the assortment of salts investigated for their impact on the *A. alternata* strain, previously selected for its heightened chemical resistance.

In vitro efficacy of low-toxicity GRAS salts against A. alternata

To evaluate the impact of natural salts on the *in vitro* mycelial growth of *A. alternata*, each product was dissolved in sterile distilled water and added to autoclaved PDA culture medium to attain final concentrations of 0, 500, 1,000, 1,500, and 2,000 ppm. The medium was then meticulously blended and poured into 90 mm diameter petri dishes. *In vitro* assays were carried out using the poisoned food technique, as described in previous fungicide experiments conducted *in vitro*.

Curative and preventive effect of low-toxicity GRAS salts against A. alternata

In examining the impact of various salts on the development of *A. alternata* on 'Maroc Late' orange fruits, a curative treatment was implemented. Initially, sound fruits underwent a 2-minute disinfection with a 10% (v/v) sodium hypochlorite solution, followed by the infliction of wounds at two equidistant points and inoculation with 20 µl of the pathogenic agent *A. alternata* suspension, adjusted to 10^6 conidia ml^{-1}. After a 24-hour incubation at 25 °C, the inoculated fruits were immersed for 2 minutes in salt solutions prepared at concentrations of 2% and 4% (w/v). The control groups included the first set of fruits treated with sterile distilled water as the negative control, and the second set treated with the selected fungicide imazalil (2,000 ppm) as the positive control. Subsequently, all samples were incubated at a temperature of 25 °C for 7 days. This study comprised 10 repetitions for each treatment, arranged in a completely randomized design to ensure unbiased assignment and reliable comparison of results. The salts exhibiting the most promising results were subsequently evaluated preventively against *A. alternata* using the same conditions applied in the curative treatment. In this phase, fruits were treated 24 hours before inoculation with the pathogenic agent.

Evaluation method

The quantification of *A. alternata* mycelial growth inhibition on PDA and fruits was determined through the application of a standardized formula by Arora and Upadhyay (1978) (Equation 1).

$$\% \text{ Inhibition} = \frac{\text{T0-T}}{\text{T0}} \text{ x } 100\% \qquad (1)$$

Where T0 represents the average mycelial growth of *A. alternata* on the PDA medium and fruits devoid of fungicides or salts; T represents the average mycelial growth of *A. alternata* on the PDA medium and fruits in the presence of fungicides or salts.

The linear regression analysis method was used to compute the effective concentrations needed to inhibit 50% of *A. alternata* growth. The values were then presented as IC_{50}.

Data analysis

Statistical analyses were performed using the XLSTAT software. The results obtained from the studies assessing the effectiveness of chemical control underwent a three-factor analysis of variance (ANOVA). In the case of the salt-related findings, a two-way analysis of variance was employed. For both experiments, mean value comparisons were carried out using the Tukey test, with a 95% confidence interval.

RESULTS AND DISCUSSION

Pathogenicity test

A pathogenicity test was performed to evaluate the pathogenic potential of *A. alternata* isolates on citrus leaves. Artificial inoculation demonstrated that the isolates were capable of causing disease. As depicted in Figure 1, typical symptoms appeared as dark brown lesions developing around the sites of inoculation.

An effective active ingredient controlling *A. alternata* on citrus fruit

In vitro efficacy of various fungicides on A. alternata growth rate

The *in vitro* assessments of fungicides showed their capacity to minimize the *A. alternata* growth rate compared to the control (Figure 2). Difenoconazole, azoxystrobin, and imazalil demonstrated significant inhibitory effects against all three strains of *A. alternata* ($p < 0.0001$). Imazalil, at a concentration of 10 ppm, achieved complete inhibition of mycelial growth for the strains (100%), with a mean IC_{50} of 23.38 ppm (Table 4). Difenoconazole exhibited inhibition percentages exceeding 70% for all three strains when applied at a low concentration of 1 ppm (IC_{50} = 26.95). Azoxystrobin also impeded the *in vitro* mycelial growth of *A. alternata*, with inhibition rates ranging from 58 to 78% at 10 ppm (IC_{50} = 63.23). On the other hand, the findings revealed that the G4 strain displayed a relatively low inhibition rate in response to the major fungicides, recording higher values of IC_{50} (Table 4). This strain demonstrated significantly greater resistance to the tested active substances compared to the Elm1 and Sds9 strains ($p < 0.0001$). Consequently, further studies were performed using G4 *A. alternata* strain sourced from lemon fruit.

In alignment with the results of this study, difenoconazole was tested against the ABR of citrus, unveiling a minimal IC_{50} value of 20.44 ppm (Shehata et al., 2018). Likewise, in a recent study by Wang et al. (2023), the fungicide resistance profiles of 100 isolates of *A. alternata* and *A. arborescens* from mandarin fruit were examined. The study found that all isolates were sensitive to imazalil, with effective concentration (EC_{50}) values of 0.492±0.133 µg ml^{-1} for *A. alternata* and 0.327±0.180 µg ml^{-1} for *A. arborescens*.

In vivo efficacy of various fungicides on A. alternata development

Based on the *in vitro* findings, difenoconazole, azoxystrobin, and imazalil were selected for the assessment of their curative impact on *A. alternata* development. As illustrated in Figure 3, all 3 tested active substances exhibited

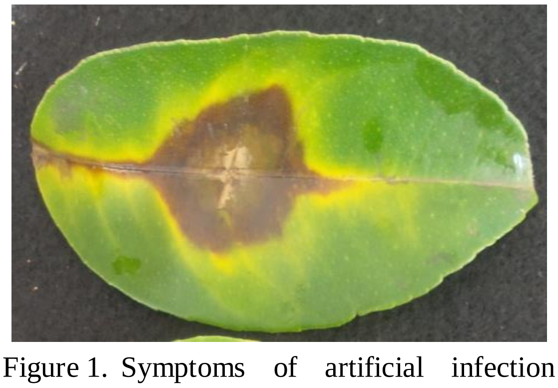

Figure 1. Symptoms of artificial infection caused by *Alternaria* spp. on 'Rough lemon' leaves

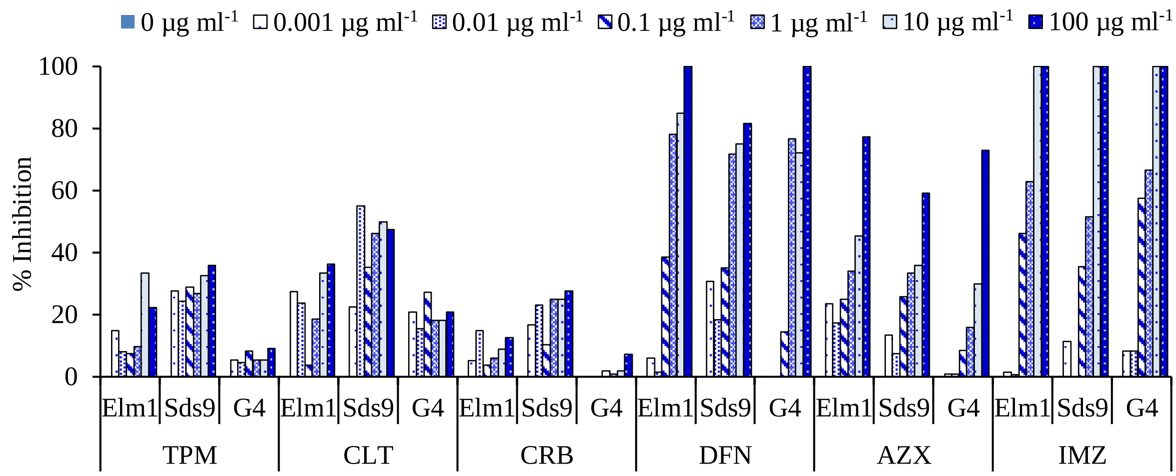

Figure 2. Inhibitory effect of fungicides on the *in vitro* mycelial growth of *A. alternata*
Note: TPM = Thiophanate methyl; CLT = Chlorothalonil; CRB = Carbendazim; DFN = Difenoconazole; AZX = Azoxystrobin; IMZ = Imazalil

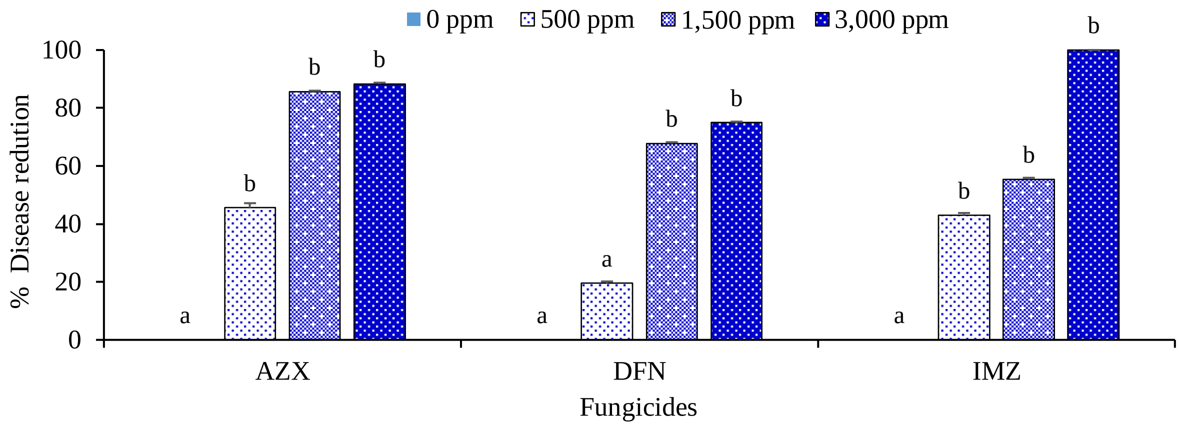

Figure 3. Inhibitory effect of fungicides on the *A. alternata* development in curative treatment
Note: AZX = Azoxystrobin; DFN = Difenoconazole; IMZ = Imazalil. Standard deviations of the means are represented by bars. Means sharing the same letter are considered statistically non-significantly different based on the Tukey test at a significance level of 5%

a notable reduction in the expression rate of *A. alternata* lesions on citrus fruits. Compared to the control, inhibition percentages of 20%, 43%, and 46% were observed at a lower concentration (500 ppm) for difenoconazole, imazalil, and azoxystrobin, respectively. Significantly, at a higher concentration (3,000 ppm), a substantial decrease in *A. alternata* development was achieved with imazalil (100%), followed by azoxystrobin (88%) and then difenoconazole (75%). No statistically significant difference was noted among the three tested fungicides (p = 0.121). The lowest IC_{50} values were recorded for azoxystrobin (1,068.55 ppm) and imazalil (1,282.19 ppm) (Table 4).

Similarly, Wang et al. (2023) identified imazalil as one of the most effective fungicides for controlling Alternaria rot in mandarin fruit. Control tests on inoculated fruit confirmed its efficacy, as imazalil significantly reduced both disease incidence and severity, highlighting its potential for managing post-harvest Alternaria rot. Azoxystrobin and fludioxonil were found to be less effective than imazalil. Indeed, multiple studies have shown that imazalil remains the most effective fungicide against citrus diseases and continues to be widely used in the global citrus industry (Erasmus et al., 2011; Besil et al., 2016; Savage et al., 2025).

Effective low-toxicity GRAS salts for controlling *A. alternata* on citrus fruit

In vitro efficacy of GRAS salts on A. alternata growth rate

The examination of the impact of natural salts on the mycelial growth of *A. alternata* was conducted *in vitro*, employing ascending concentrations ranging from 0 to 2,000 ppm.

Table 4. IC_{50} values of the evaluated fungicides *in vitro* for *A. alternata* strains in ppm

	Isolates	CRB	CLT	TPM	DFN	AZX	IMZ
In vitro	Elm1	304.58	159.87	670.75	23.96	48.24	23.38
	Sds9	195.96	106.94	278.70	26.95	74.86	25.60
	G4	1,037.59	744.76	736.95	31.46	63.23	17.80
	Mean IC_{50}	304.58	159.87	670.75	26.95	63.23	23.38
In curative treatment					1,622.25	1,068.55	1,282.19

Note: IC_{50} = Effective concentration for 50% inhibition of *A. alternata* growth. CRB = Carbendazim; CLT = Chlorothalonil; TPM = Thiophanate methyl; DFN = Difenoconazole; AZX = Azoxystrobin; IMZ = Imazalil

Table 5. IC_{50} values of the evaluated salts *in vitro* and *in vivo* for *A. alternata*

	PS	SB	SBC	ST
In vitro (ppm)	3.16	3	5.66	35.28
In curative treatment (%)	1.78	1.72	4	2.72
In preventive treatment (%)	2.65	2.04	3.96	-

Note: PS = Potassium sorbate; SB = Sodium benzoate; SBC = Sodium bicarbonate; ST = Sodium tetraborate

As depicted in Figures 4 and 5, potassium sorbate, sodium benzoate, Sodium bicarbonate, and sodium tetraborate exhibited inhibition percentages of 71%, 67%, 41%, and 5%, respectively, at 2,000 ppm. Compared to the control group, the first three salts significantly curtailed the mycelial growth of *A. alternata* ($p < 0.0001$), exhibiting estimated IC_{50} values of 3.16, 3.34, and 5.66 ppm in order (Table 5).

The results demonstrated statistically the ability of potassium sorbate and sodium benzoate to inhibit the mycelial growth of *A. alternata*, with an IC_{50} below 3.4 ppm. This aligns with previous research indicating the antifungal efficacy of these compounds. For instance, a study reported that potassium sorbate and sodium benzoate exhibit high inhibitory activity that is effective in inhibiting both *A. alternata* and *Botrytis cinerea in vitro*. Specifically, potassium sorbate exhibited a reduction in *A. alternata* mycelial growth with increasing concentrations, achieving percentages of 69% and 88% inhibition at concentrations of 1% and 2%, respectively (Fagundes et al., 2013). Further research has highlighted the superior efficacy of potassium sorbate over sodium benzoate in inhibiting fungal growth. For example, potassium sorbate was found to be more effective than sodium benzoate in inhibiting the mycelial growth of *Coniella granati*, a pathogen affecting pomegranate fruit (Kara et al., 2023). Moreover, in a study on the antifungal potential of eco-friendly chitosan-sodium benzoate in inhibiting the development of *Rhizopus stolonifer* isolated from jackfruit, the application of sodium benzoate at 2.0% individually resulted in 98% mycelial growth inhibition of *R. stolonifer* (Coronado-Partida et al., 2023).

In vivo efficacy of GRAS salts on *A. alternata* development

Curative treatment

The curative test consisted of treating *A. alternata*-inoculated citrus fruits with 0%, 2%, and 4% concentrations of each salt. Salt application brought about a remarkable reduction in disease severity, effectively alleviating symptoms caused by *A. alternata*, as vividly depicted in Figure 6. Both potassium sorbate and sodium benzoate showed notable inhibition of disease lesions on fruit wounds ($p < 0.0001$). Specifically, at a low concentration of 2% (w/v), potassium sorbate showed a substantial inhibition percentage of approximately 86%, while sodium benzoate exhibited an even higher inhibition percentage of around 92%. These two compounds also recorded the lowest values of IC_{50} (1.78 and 1.72%, respectively) (Table 5). Similar positive outcomes were observed in fruits treated with imazalil at a concentration of 2,000 ppm, resulting in a 91.68% inhibition of the pathogen. At the 4% (w/v) concentration, sodium benzoate achieved the highest inhibition rate at 100%, surpassing the imazalil inhibition rate (91.68%). At the higher concentration of 4% (w/v), sodium benzoate achieved the highest inhibition rate at 100%, surpassing the imazalil (91.68% of pathogen inhibition). Additionally, treatment with 4% (w/v) potassium sorbate also led to a greater reduction in disease (94.91%) compared to imazalil (91.68%). Sodium tetraborate and sodium benzoate exhibited the least effectiveness in disease control, with respective rates of 51.6% and 32%, highlighting their comparatively lower performance in managing the disease.

The potassium sorbate and sodium benzoate were statistically similar or superior to the chemical control for Alternaria diseases. These findings followed Soto-Muñoz et al. (2020), who found that the effectiveness of sodium benzoate in suppressing *G. citri-aurantii* surpassed that of the fungicide PCZ used as a positive control. The curative treatment, involving the immersion dipping of fruit from various orange varieties in sodium benzoate (3% w/v) for 60 seconds, led to a reduction in sour rot incidence and severity by up to 90%. Furthermore, sodium benzoate emerged as one of the four most effective coating ingredients in minimizing black rot severity caused by *A. alternata* in cherry tomato fruit, achieving a reduction of 70% (Fagundes et al., 2013). Similarly, the severity of the anthracnose disease was curatively suppressed using coatings containing 2% potassium sorbate and 2% sodium benzoate individually, achieving inhibition rates exceeding 60% on 'Nadorcott' mandarins artificially inoculated with *C. gloeosporioides* (Martínez-Blay et al., 2020). In an evaluation of the curative activity of sodium benzoate and other food additives for controlling citrus post-harvest green and blue mold disease, sodium benzoate was identified as the most effective compound (Montesinos-Herrero et al., 2016).

GRAS salts demonstrate a variety of antimicrobial mechanisms that contribute to their effectiveness against post-harvest pathogens. These compounds act not only by directly suppressing fungal development but also by enhancing the host's natural defense responses (Zhao et al., 2025). For instance, they can disrupt fungal nutrient absorption and energy pathways, leading to structural collapse of hyphae and inactivation of spores (Martínez-Blay

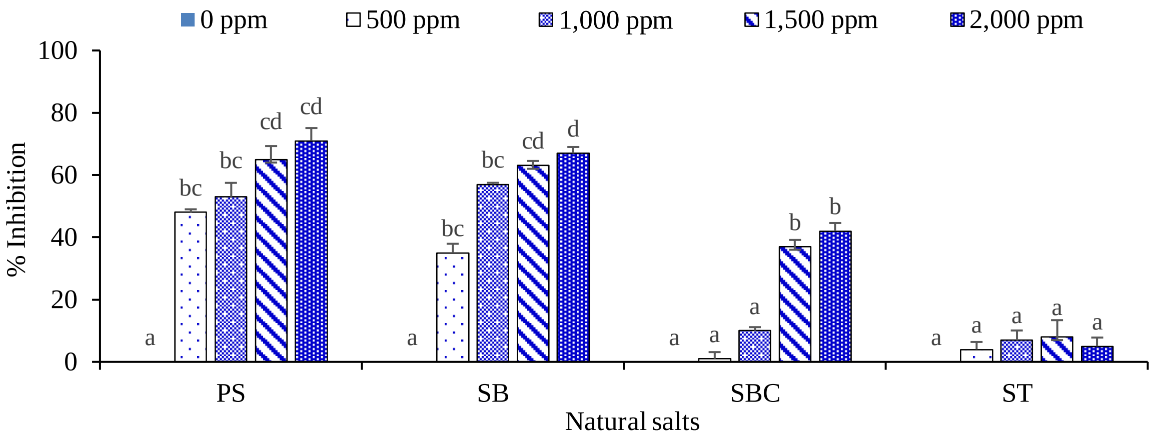

Figure 4. Inhibitory effect of natural salts on the *in vitro* mycelial growth of *A. alternata*

Note: PS = Potassium sorbate; SB = Sodium benzoate; SBC = Sodium bicarbonate; ST = Sodium tetraborate. Standard deviations of the means are represented by bars. Means sharing the same letter are considered statistically non-significantly different based on the Tukey test at a significance level of 5%

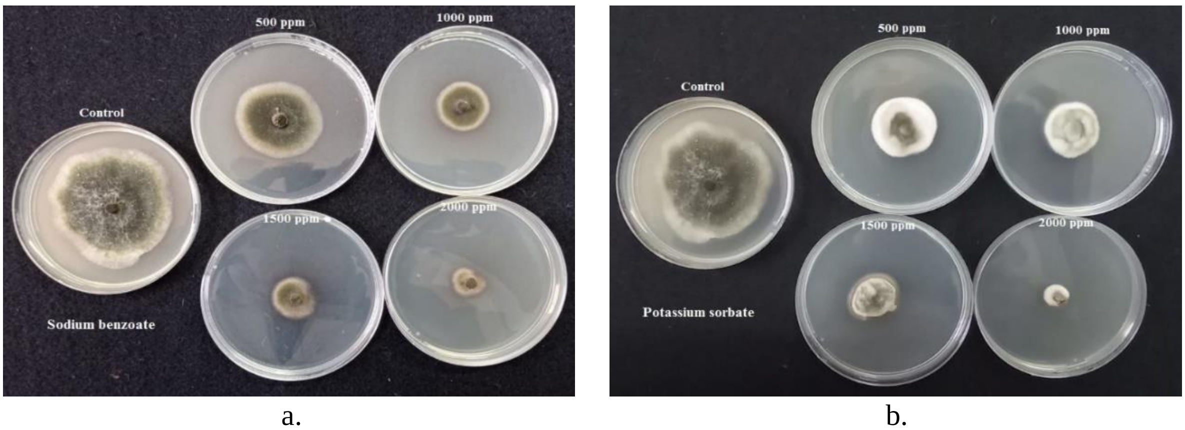

Figure 5. Inhibitory effect of sodium benzoate (a) and potassium sorbate (b) on the *in vitro* mycelial growth of *A. alternata*

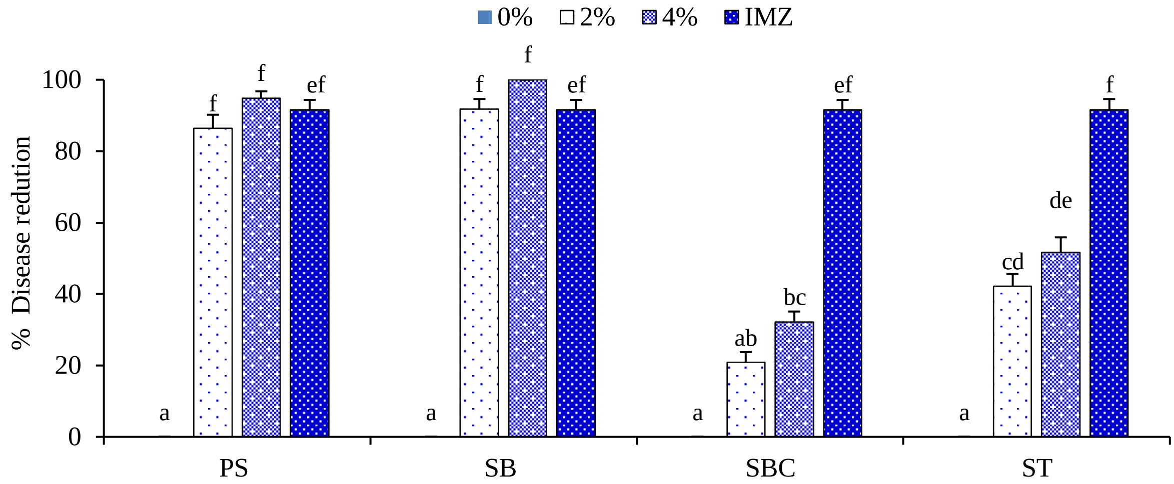

Figure 6. Inhibitory effect of GRAS salts on the *A. alternata* development in curative treatment
Note: PS = Potassium sorbate; SB = Sodium benzoate; SBC = Sodium bicarbonate; ST = Sodium tetraborate; IMZ = Imazalil. Standard deviations of the means are represented by bars. Means sharing the same letter are considered statistically non-significantly different based on the Tukey test at a significance level of 5%

et al., 2020). Some GRAS salts also trigger resistance responses in the host fruit by activating oxidative metabolism and the phenylpropanoid biosynthetic pathway, thereby fortifying the plant's defense system (Zhao et al., 2023).

Sodium benzoate exhibits its antifungal activity through several mechanisms. Romli et al. (2023) reported that sodium benzoate disrupts fungal metabolism, alters membrane permeability, and denatures cellular proteins, ultimately inhibiting mycelial growth and spore germination. The efficacy of sodium benzoate has been shown to increase under acidic conditions and at higher concentrations (Gürgen and Yıldız, 2024). Furthermore, sodium benzoate induces oxidative stress within fungal cells, promoting lipid peroxidation and diminishing the activity of antioxidant enzymes, which further compromises cell viability (Walczak-Nowicka and Herbet, 2022).

Potassium sorbate similarly acts by targeting the fungal cell membrane. According to Gürgen and Yıldız (2024), potassium sorbate increases membrane permeability, leading to the leakage of essential intracellular contents and disruption of metabolic functions. Romli et al. (2023) corroborated these findings, noting that potassium sorbate also inhibits fungal development by impairing metabolism and denaturing cellular proteins. In addition, Awaad et al. (2023) demonstrated that potassium sorbate, when combined with chitosan, exhibited a synergistic antifungal effect against *Rhodotorula mucilaginosa* and *Candida albicans*, achieving complete inhibition of yeast growth by the ninth day of storage.

Preventive treatment

Findings reported in Figures 7 and 8 denote an impressive suppression of the pathogen by the salts administered as preventive treatments. Relative to untreated fruits, sodium benzoate emerged as a star performer, completely thwarting disease development on fruits (100% inhibition) at a concentration of 4% (w/v), mirroring the efficacy of imazalil with an IC_{50} of 2.65%. At this concentration, potassium sorbate showcased a significant 60% reduction in severity induced by the pathogenic agent *A. alternata*, boasting a minimal IC_{50} value of 2.04 (Table 5).

Potassium sorbate and sodium benzoate were effective in reducing the severity of the disease at a concentration of 4% (w/v). These two salts exhibited a high level of protection from *A. alternata* pathogen. Accordingly, the post-harvest potential of these salts was recently reviewed by Palou (2018), who outlined the effectiveness in controlling fresh fruit decay. Guimarães et al. (2019) and Lyousfi et al. (2023) describe that certain GRAS salts have demonstrated remarkable efficacy in preventing various pathogens during the storage period. The utilization of GRAS salts helps to prevent post-harvest fungal losses in different fruit crops, including citrus (Soto-Muñoz et al., 2020; Zhao et al., 2023). Their application is particularly advantageous due to their safety profile and environmental friendliness, making them suitable alternatives to conventional synthetic fungicides.

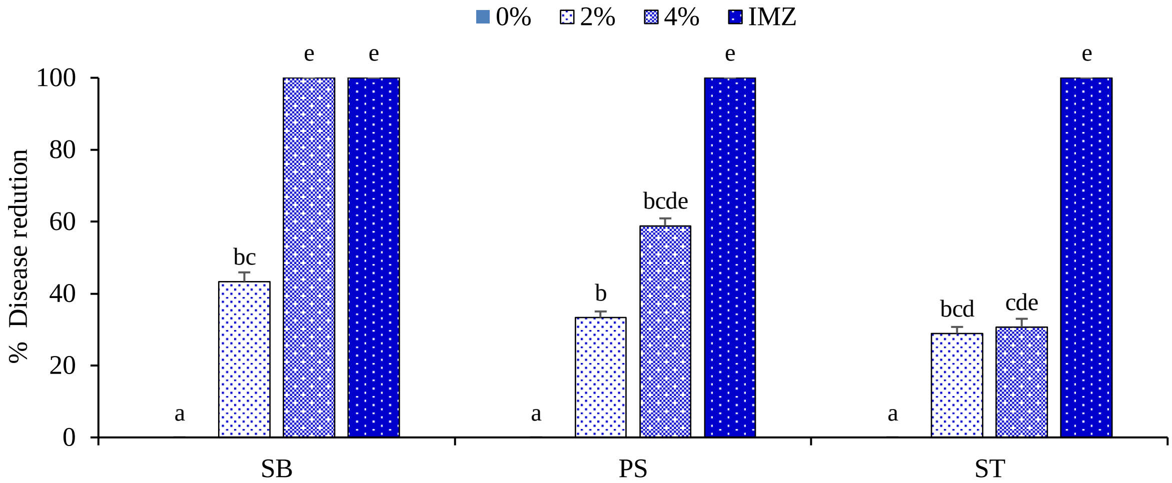

Figure 7. Inhibitory effect of natural salts on the development of *A. alternata* in preventive treatment

Note: SB = Sodium benzoate; PS = Potassium sorbate; ST = Sodium tetraborate; IMZ = Imazalil. Standard deviations of the means are represented by bars. Means sharing the same letter are considered statistically non-significantly different based on the Tukey test at a significance level of 5%

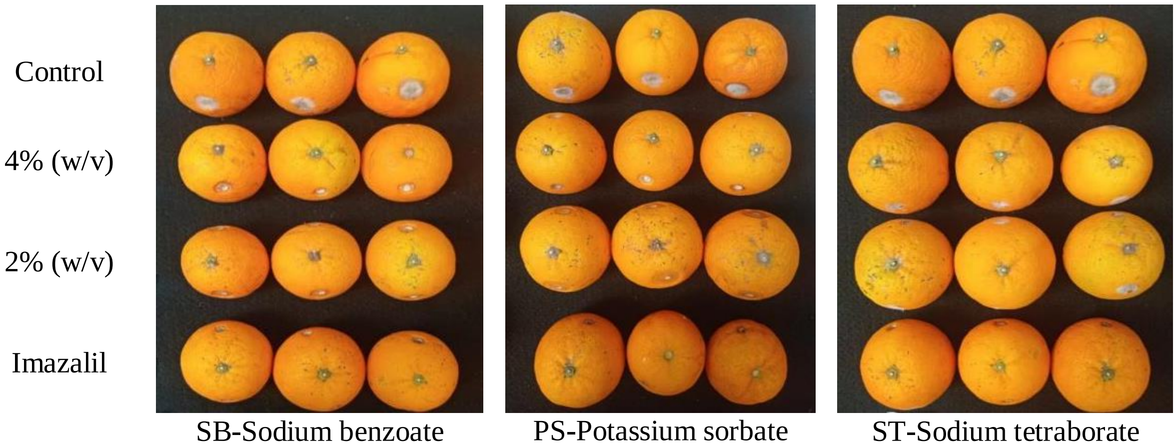

Figure 8. Inhibitory effect of sodium benzoate, potassium sorbate, and sodium tetraborate on the *in vivo* development of *A. alternata*

CONCLUSIONS

The management of post-harvest Alternaria diseases involves finding an efficacious alternative product to advance the development of eco-friendly environmental strategies. The findings derived from the present study represent a foundational database, delineating effective and promising salts as potential low-toxicity interventions for combating Alternaria disease of citrus in Morocco. Research findings underscore the efficacy of GRAS salts in impeding the mycelial growth of *A. alternata*. These compounds demonstrated the capacity to inhibit *in vitro* colony development at low concentrations. Furthermore, potassium sorbate and sodium benzoate showed significant effectiveness, offering high levels of protection against *A. alternata*, both in curative and preventive treatments. Moreover, it is shown that these two salts exhibit a similar efficacy to imazalil, which is commonly employed for managing Alternaria rot in citrus fruits after harvesting.

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