

Alternative products in the management of green mold in postharvest oranges

Produtos alternativos no manejo do bolor verde em pós-colheita de laranja

Productos alternativos en el manejo del moho verde en poscosecha de naranja

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Abstract

Green mold, caused by the fungus *Penicillium digitatum*, is the primary issue in the post-harvest phase of oranges that causes significant losses. Consequently, the objective of this work was to evaluate the effects of alternative products in the management of green mold in postharvest oranges. Four *in vitro* experiments were conducted. Three assessed the effects of different concentrations of each alternative product (potassium phosphite, *Ascophyllum nodosum* extract, and organomineral fertilizer) on the production of fresh mycelial weight of *P. digitatum*; and a fourth evaluated the effects of alternative products compared to that of the conventional product (benzimidazole fungicide). Subsequently, ‘Valencia’ oranges were subjected to the treatments, which consisted of distilled water (inoculated and non-inoculated control), potassium phosphite, *A. nodosum* extract, organomineral fertilizer, and

benzimidazole fungicide. The fruits were inoculated with *P. digitatum* and evaluated daily for the incidence and severity of green mold over seven days. Furthermore, the effects of the treatments on the physicochemical quality of fruits were evaluated for the following attributes: skin and pulp color, firmness of the pulp, pH, titratable acidity (TA), soluble solids (SS), and the SS/TA ratio. Potassium phosphite and *A. nodosum* extract inhibited the *in vitro* development of *P. digitatum*. The alternative products reduced the incidence and severity of green mold on oranges without compromising the physicochemical quality of the fruit. Therefore, the evaluated products can be used in the postharvest treatment of oranges and are considered promising alternatives for the management of green mold.

Keywords: *Penicillium digitatum*; Citrus; Alternative control; Physicochemical quality.

Resumo

O bolor verde, causado pelo fungo *Penicillium digitatum*, é o principal problema na fase de pós-colheita em laranjas, causando perdas expressivas. Neste contexto, o objetivo deste trabalho foi avaliar o efeito de produtos alternativos no manejo do bolor verde em pós-colheita de laranja. Foram realizados quatro experimentos *in vitro*: três para avaliar o efeito de diferentes concentrações de cada produto alternativo (fosfito de potássio, extrato de alga *Ascophyllum nodosum* e fertilizante organomineral) sobre a produção de massa fresca micelial do *P. digitatum*; e o quarto para avaliar o efeito dos produtos alternativos em comparação ao produto convencional (fungicida benzimidazol). Posteriormente, laranjas ‘Valência’ foram submetidas aos tratamentos: água destilada (controle inoculado e não-inoculado), fosfito de potássio, extrato de alga *A. nodosum*, fertilizante organomineral e fungicida benzimidazol. Os frutos foram inoculados com o *P. digitatum* e avaliados diariamente quanto à incidência e severidade do bolor verde, ao longo de sete dias. Além disso, avaliou-se o efeito dos tratamentos sobre a qualidade físico-química dos frutos, nos seguintes atributos: cor da casca e polpa, firmeza da polpa, pH, acidez titulável (AT), sólidos solúveis (SS) e ratio (relação SS/AT). O fosfito de potássio e o extrato de *A. nodosum* inibiram o desenvolvimento *in vitro* do *P. digitatum*. Os produtos alternativos reduziram a incidência e severidade do bolor verde da laranja, sem comprometer a qualidade físico-química dos frutos. Portanto, os produtos avaliados podem ser utilizados no tratamento pós-colheita de laranjas e são considerados alternativas promissoras para o manejo do bolor verde.

Palavras-chave: *Penicillium digitatum*; Citros; Controle alternativo; Qualidade físico-química.

Resumen

El moho verde, causado por el hongo *Penicillium digitatum*, es el principal problema en la etapa de poscosecha en naranjas, ocasionando pérdidas considerables. En este contexto, el objetivo de este estudio fue evaluar el efecto de productos alternativos en el manejo del moho verde en la poscosecha de naranja. Fueron realizados cuatro experimentos *in vitro*: tres para evaluar el efecto de diferentes concentraciones de cada producto alternativo (fosfito de potasio, extracto de alga *Ascophyllum nodosum* y fertilizante organomineral) sobre la producción de masa fresca micelial del *P. digitatum*; y el cuarto para evaluar el efecto de los productos alternativos en comparación con el producto convencional (fungicida bencimidazol). Más adelante, naranjas ‘Valência’ fueron sometidas a los siguientes tratamientos: agua destilada (control sin inocular e inoculado), fosfito de potasio, extracto de alga *A. nodosum*, fertilizante organomineral y fungicida bencimidazol. Los frutos fueron inoculados con *P. digitatum* y evaluados a diario a lo largo de siete días en cuanto a la incidencia y severidad del moho verde. Además, se evaluó el efecto de los tratamientos sobre la calidad fisicoquímica de los frutos en los siguientes atributos: color de la cáscara y pulpa, firmeza de la pulpa, pH, acidez titulable (AT), sólidos solubles (SS) y ratio (relación SS/AT). El fosfito de potasio y el extracto de *A. nodosum* inhibieron el desarrollo *in vitro* de *P. digitatum*. Los productos alternativos redujeron la incidencia y severidad del moho verde de la naranja, sin comprometer la calidad fisicoquímica de los frutos. Por lo tanto, los productos evaluados pueden utilizarse en el tratamiento poscosecha de naranjas y son considerados alternativas prometedoras para el manejo del moho verde.

Palabras clave: *Penicillium digitatum*; Cítricos; Control alternativo; Calidad fisicoquímica.

1. Introduction

Brazil is one of the largest orange producers and exporters of concentrated fruit juice. National production reached expressive values in the 2019-2020 harvest, reaching more than 18.3 million tons. The southeastern region is the leading producer, contributing 15.5 million tons (IBGE, 2020). Despite the expressive production numbers, one of the main obstacles in the commercialization of this product is postharvest losses.

In general, attaining postharvest durability of fruits and vegetables is challenging. Plant material characteristics associated with inadequate handling conditions in the various stages of the production chain can cause a high incidence of disease, which affects the quality and shelf life of the products (Roma-Almeida, Rezende & Pascholati, 2019). According to the

FAO (Food and Agriculture Organization of the United Nations), one-third of the world's food production is lost or wasted annually, of which fruits and vegetables represent 45% (FAO, 2019).

In citriculture, green mold caused by the fungus *Penicillium digitatum* is considered the biggest issue during postharvest, resulting in significant losses. This disease is related to the handling conditions of fruits because the pathogen penetrates from wounds on the surface of the plant. The first sign is a soft, watery, and slightly discolored spot that is later covered by numerous green colored spores, which are easily dispersed into the air, facilitating the spread of the disease to other fruits (Fischer, Lourenço & Amorim, 2008).

The control of green mold must be conducted in the field and during postharvest stages through practices aimed at reducing the pathogen and the careful handling of the fruits to avoid lesions. Fungicides from the benzimidazole and imidazole groups are recommended for chemical treatment (Agrofit, 2019). However, these substances can adversely affect human health and the environment because of the presence of residue on the fruits, in addition to possibly selecting resistant pathogens because of continuous use.

Because of the current demand for more sustainable production, studies on alternative products in disease control are essential to expanding the tools available for integrated management.

Phosphites are compounds initially marketed as foliar fertilizers. Many formulations can be found on the market for different applications, such as resistance inducers, plant activators, biostimulants and fungicides. The concentration of the phosphite varies among commercially available sources. In general, products indicated for disease control have a higher content of PO_4^{3-} or phosphite than do fertilizers (Roma-Almeida, Rezende & Pascholati, 2019). The fungicidal and resistance-inducing action has been attributed to phosphites because of their ability to, directly or indirectly, negatively affect the development of phytopathogens. Directly, these compounds inhibit the germination of the fungal spores, mycelial growth, and penetration into plant tissue. Indirectly, they stimulate resistance mechanisms in the plant, such as the production of lignins, phytoalexin, and hydrolytic enzymes (Brackmann *et al.*, 2008).

Seaweed extracts are natural substances obtained from species that live under extreme conditions, and consequently, have developed defense mechanisms, such as the production of biologically active substances (Peres *et al.*, 2012). Products derived from seaweed extracts have been used in agriculture because of their direct and indirect effects in controlling plant diseases. The direct effects include a total or partial reduction in the development of

pathogenic microorganisms and the promotion of beneficial microorganisms, which contribute to plant development or are antagonistic to pathogens. Indirect effects are related to the stimulation of plant response to stress, such as the synthesis and increase of enzymatic activity, hormones, and defense compounds (Carvalho & Castro, 2014).

Organomineral fertilizers are derived from organic residues from plant and animal production systems. In addition to providing plant nutrition, they have demonstrated benefits associated with the induction of resistance to diseases by stimulating the synthesis and accumulation of phenolic compounds with fungistatic properties. Certain nutrients, when present in these products, act as co-factors of enzymes that participate in many plant defense metabolic routes. Mineral nutrition also stimulates other structural changes in plants, such as increases in the thickness of the cell wall, silification and lignification of the tissues, and prevention or delay of the entry of the pathogens in the tissues (Amaral, 2008).

Because of the potential of these alternative products for plant disease control, the goal of this study was to evaluate the effects of potassium phosphite, *Ascophyllum nodosum* extract, and organomineral fertilizer in the management of green mold in postharvest oranges.

2. Methodology

The experiments were conducted at the Phytopathology Laboratory (*in vitro* and *in vivo* assays) and the Bromatology Laboratory (physical-chemical analyses) of the Federal Institute of Education, Science, and Technology of Southern Minas Gerais (IFSULDEMINAS), *Campus Machado*, Brazil.

The *P. digitatum* was isolated from symptomatic fruits collected in the region, after which it was maintained on PDA (Potato Dextrose Agar) at 25 °C, in a biochemical oxygen demand (B.O.D.) incubator to provide appropriate conditions for its development.

Three commercially available alternative products were used: potassium phosphite, *A. nodosum* extract, and organomineral fertilizer. Potassium phosphite is a commercial product that has 20% K₂O, with a density of 1.42 g mL⁻¹. The product based on *A. nodosum* extract, was an LS (liquid suspension) formulation with a 100% concentration of seaweed extract, equivalent to 48% dry matter. The fertilizer is classified as a foliar class "A" organomineral fertilizer, presenting 8.5% total organic carbon, 6% nitrogen, 2.5% molybdenum, and 0.15% cobalt. This fertilizer is produced from the following raw materials: urea, sodium molybdate, cobalt sulfate, monosodium glutamate fermentation residue, and water.

The alternative products were compared with a conventional product. A systemic fungicide from the benzimidazole chemical group was used, which is registered for use in orange postharvest and contains thiabendazole (2- (thiazol-4-yl) benzimidazole) at a concentration of 485 g L⁻¹ (48.5 % m/v).

2.1 *In vitro* effects of the alternative products on the production of fresh mycelial weight

Three *in vitro* assays were performed separately to evaluate the production of a fresh mycelial weight of *P. digitatum* submitted to different dosages of alternative products: potassium phosphite (0, 5, 10, 20, and 30 mL L⁻¹); *A. nodosum* extract (0, 25, 50, 75, and 100 mL L⁻¹); and organomineral fertilizer (0, 3, 10, 15, and 20 mL L⁻¹). The ideal dose of each alternative product was calculated based on the results obtained. The fourth *in vitro* assay was conducted to compare alternative products to the conventional product (benzimidazole fungicide), and the products were applied at the following levels: control (0 mL L⁻¹); potassium phosphite (19.5 mL L⁻¹); *A. nodosum* extract (91.5 mL L⁻¹); organomineral fertilizer (3 mL L⁻¹), and benzimidazole fungicide in the dosage indicated by the manufacturer for the postharvest treatment of green mold in citrus crops (10.3 mL L⁻¹).

The methodology used consisted of volumes pipetted into erlenmeyer flasks containing 100 mL of an autoclaved potato-dextrose liquid medium to obtain the alternative product concentrations. Subsequently, three mycelium discs (5 mm) from *P. digitatum* colonies seven days of age were inserted. The material was kept under constant agitation for five days at 80 rpm and a temperature of 25 °C (± 2 °C). At the end of the experiments, the pathogen mycelium was separated from the liquid medium using a vacuum pump and filter paper, after which the weight of mycelium (g) was measured on an analytical balance. The same methodology was used in all *in vitro* tests.

The experimental design was in randomized blocks, and each treatment consisted of four replicates containing one bottle per replicate. The data were analyzed using the SISVAR 5.7 statistical program (Ferreira, 2019). The data from the assays in which doses of alternative products were tested were subjected to an analysis of variance (ANOVA), and based on the significance of the treatments ($p \leq 0.05$), a regression analysis was performed to estimate the ideal dose of each product. For data adjusted to the 2nd ($y = ax^2 + bx + c$) and 3rd degree ($y = ax^3 + bx^2 + cx + d$) polynomial equations, the ideal dose was estimated using the equations:

$$\text{Ideal dose (2nd degree)} = \frac{-b}{2a} \quad \text{Ideal dose (3rd degree)} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

The data from the comparison assay between alternative and conventional products were subjected to an analysis of variance (ANOVA) and the means were compared using the Scott-Knott test at 5% probability ($p \leq 0.05$).

2.2 Evaluation of the alternative products to control green mold in oranges

Physiologically mature fruits of ‘Valencia’ orange trees from the orchards of the Horticulture Sector of IFSULDEMINAS *Campus* Machado were harvested and immediately sanitized with 0.5% sodium hypochlorite solution for three minutes, washed in autoclaved distilled water, and allowed to dry at room temperature for 24 hours.

The fruits were subsequently immersed for five minutes in solutions containing autoclaved distilled water (inoculated control and non-inoculated control), potassium phosphite (19.5 mL L⁻¹), *A. nodosum* extract (91.5 mL L⁻¹), organomineral fertilizer (3 mL L⁻¹), and benzimidazole fungicide (10.3 mL L⁻¹). After drying at room temperature for 24 hours, the fruits were superficially wounded with a histological needle (0.6 mm diameter and 2 mm depth) in four equidistant regions and later inoculated with *P. digitatum* spores. The fruits were sprayed with a suspension of 10⁶ conidia mL⁻¹ of the pathogen (Eckert & Brown, 1986). Wounded fruits without inoculation (non-inoculated control) were used as controls.

The oranges were kept in a humid chamber (plastic boxes closed within a plastic bag with cotton wool moistened with autoclaved distilled water) for 36 hours and stored in a B.O.D. at 25 °C. After removal from the humid chamber, the fruits were kept in the B.O.D. at 25 °C and evaluated daily for seven days for the incidence and severity of green mold. The incidence was obtained by the percentage of sick fruits. Severity was evaluated considering the number of wounds with signs and/or symptoms of the disease in each fruit. The area under the disease progress curve (AUDPC) was calculated using the equation proposed by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i)$$

Where n is the total number of assessments, and y_i and y_{i+1} are two consecutive evaluations of severity performed at times t and t_{i+1} , respectively.

The experiment was conducted in randomized blocks and each treatment consisted of eight replicates containing three fruits per replicate. The data were submitted to an analysis of variance (ANOVA) and the means were compared by the Scott-Knott test at 5% probability ($p \leq 0.05$), using the SISVAR statistical program 5.7 (Ferreira, 2019).

2.3 Effects of the alternative products on the physical-chemical quality of oranges

The oranges were harvested and cleaned, as described in item 2.2, and subjected to the following treatments for five minutes: autoclaved distilled water (control); potassium phosphite (19.5 mL L^{-1}); *A. nodosum* extract (91.5 mL L^{-1}); organomineral fertilizer (3 mL L^{-1}), and benzimidazole fungicide (10.3 mL L^{-1}). They were then placed on plastic trays and stored at $21 \text{ }^\circ\text{C}$ ($\pm 2 \text{ }^\circ\text{C}$) for 16 days. After 1, 8, and 16 days of storage, the fruits were evaluated for physical-chemical attributes: skin and pulp color, firmness of the pulp, pH, titratable acidity, soluble solids, and the SS/TA ratio.

The skin and pulp color were evaluated for three fruits per plot using a Minolta colorimeter, model Chroma Meter CR-400 (D_{65} illuminant, and the CIEL $*a^*b^*$ color system), at two points in the equatorial region of the fruits, before and after removing the skin. The mean of the a^* and b^* values were obtained to calculate the hue angle according to the methodology proposed by Minolta (1994).

The firmness of the pulp was evaluated for three fruits per plot after removing the skin using a SoilControl penetrometer, model PDF-200, equipped with an 8 mm diameter tip, in the Peak-hold function (maximum peak) with the fruits in vertical and horizontal positions. The mean values were calculated and expressed in Newtons.

The pH, titratable acidity, and soluble solids analyses were performed in triplicate on the fruit juice for three fruits per plot. The pH was evaluated using the Lucadema pH meter, model LUCA-210. Titratable acidity was determined according to the methodology proposed by Carvalho *et al.* (1990), and the results expressed in g of citric acid per 100 mL of sample. The content of SS was determined using an Atago digital refractometer, model SMART-1, and the data obtained in $^\circ\text{Brix}$. The ratio value was obtained by the relationship between SS and TA (SS/TA).

The experimental design was randomized blocks and each treatment consisted of eight replicates containing three fruits per replicate, for each storage period, totaling 72 fruits per treatment. The data were submitted to an analysis of variance (ANOVA) and the means

were compared by the Scott-Knott test at 5% probability ($p \leq 0.05$) using the SISVAR statistical program 5.7 (Ferreira, 2019).

3. Results and Discussion

3.1 *In vitro* effects of the alternative products on the production of fresh mycelial weight

Significant differences were observed in the production of fresh mycelial weight of *P. digitatum* in the different tests with the alternative products (Table 1).

Table 1. Summary of analysis of variance for fresh mycelial weight of *Penicillium digitatum* (g) in the different tests with alternative products: potassium phosphite (0, 5, 10, 20, and 30 mL L⁻¹); *A. nodosum* extract (0, 25, 50, 75, and 100 mL L⁻¹); and organomineral fertilizer (0, 3, 10, 15, and 20 mL L⁻¹).

		Pr>Fc		
VF	DF	Potassium phosphite	<i>A. nodosum</i> extract	Organomineral fertilizer
Treatment	4	0,0000*	0,0001*	0,0220*
Block	3	0,7340 ^{NS}	0,1588 ^{NS}	0,3216 ^{NS}
Residue	12			
Total	19			
CV (%)		37,19	26,37	18,41

* Significant at 5% probability

^{NS} Not significant

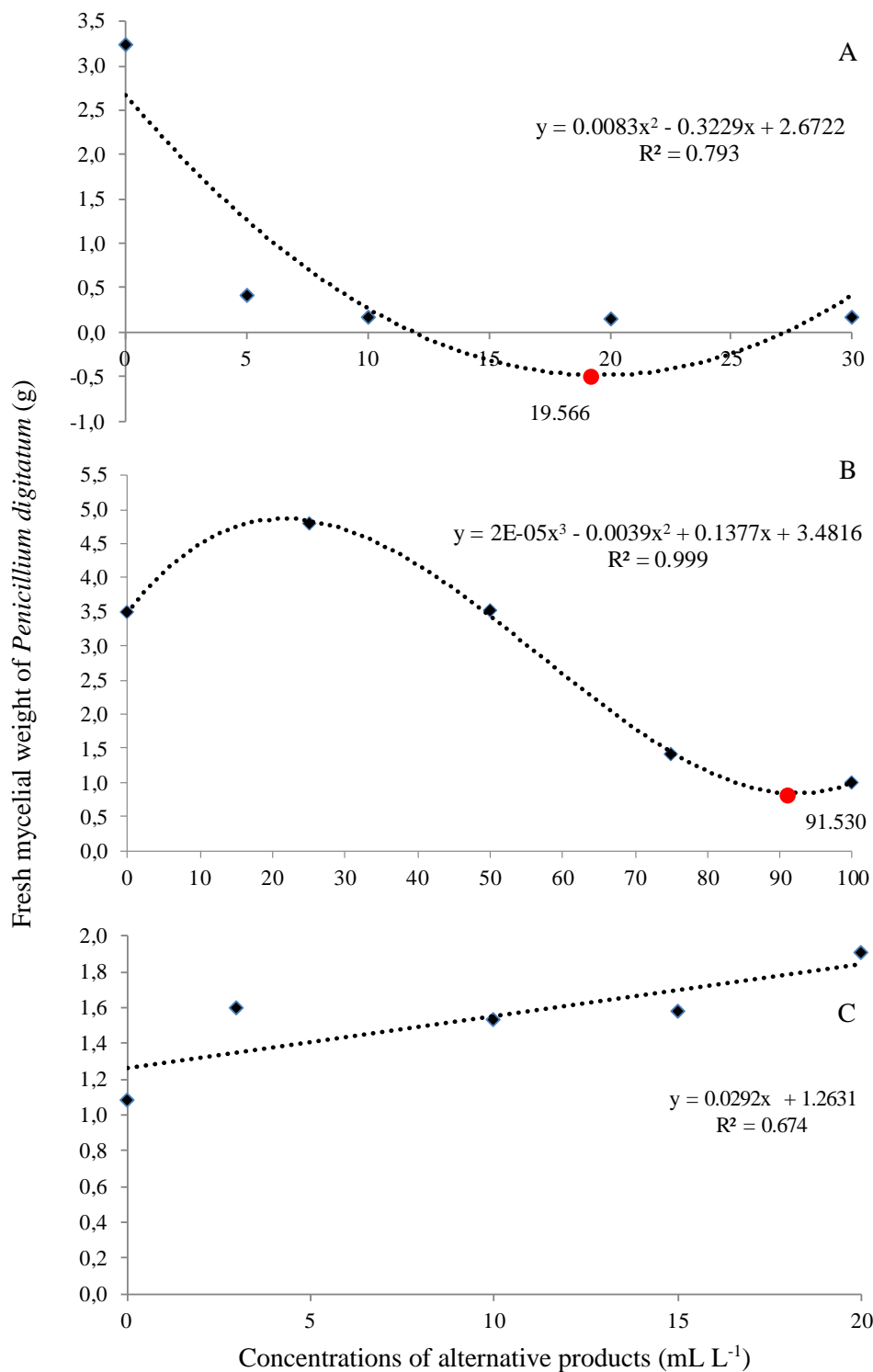
Source: Authors.

Figure 1A shows that potassium phosphite significantly interfered in the *in vitro* development of *P. digitatum* because there was less production of fresh mycelial weight for all the evaluated concentrations of the product (5, 10, 20, and 30 mL L⁻¹) compared to that of the control treatment. The polynomial regression analysis indicated that as the product dose increased, the production of the fresh mycelial weight of the pathogen declined ($R^2 = 0.793$). The polynomial equation allowed for the determination of the ideal concentration of potassium phosphite to obtain efficiency in inhibiting the pathogen with the lowest product expenditure. The ideal dosage was 19.566 mL L⁻¹.

A significant increase in fresh mycelial weight was observed when the fungus was subjected to a concentration of 25 mL L⁻¹ of *A. nodosum* seaweed extract in relation to that of the treatment that did not receive the product. The concentration of 50 mL L⁻¹ did not statistically differ from that of the control. On the other hand, the concentrations of 75 and 100 mL L⁻¹ interfered with the development of the pathogen, reducing the production of the fresh mycelial weight (Figure 1B). The R² value of 0.999 indicated the adequate adjustment of the data to the polynomial equation obtained, and the estimated ideal product dose was 91.530 mL L⁻¹.

Unlike the results presented above, the organomineral fertilizer induced the production of the *P. digitatum* fresh mycelial weight at all evaluated concentrations. Figure 1C shows that increasing concentrations of the product resulted in progressive increases in the production of the *P. digitatum* fresh mycelial weight (R² = 0.674).

Figure 1. Fresh mycelial weight of the fungus *Penicillium digitatum* after five days of cultivation in a potato-dextrose medium at a temperature of 25 °C, plus different concentrations of alternative products: 0, 5, 10, 20, and 30 mL L⁻¹ of potassium phosphite (A), 0, 25, 50, 75, and 100 mL L⁻¹ of *Ascophyllum nodosum* seaweed extract (B) and 0, 3, 10, 15, and 20 mL L⁻¹ of organomineral fertilizer (C). The equations represent the polynomial (A and B) and linear (C) regression analyses.

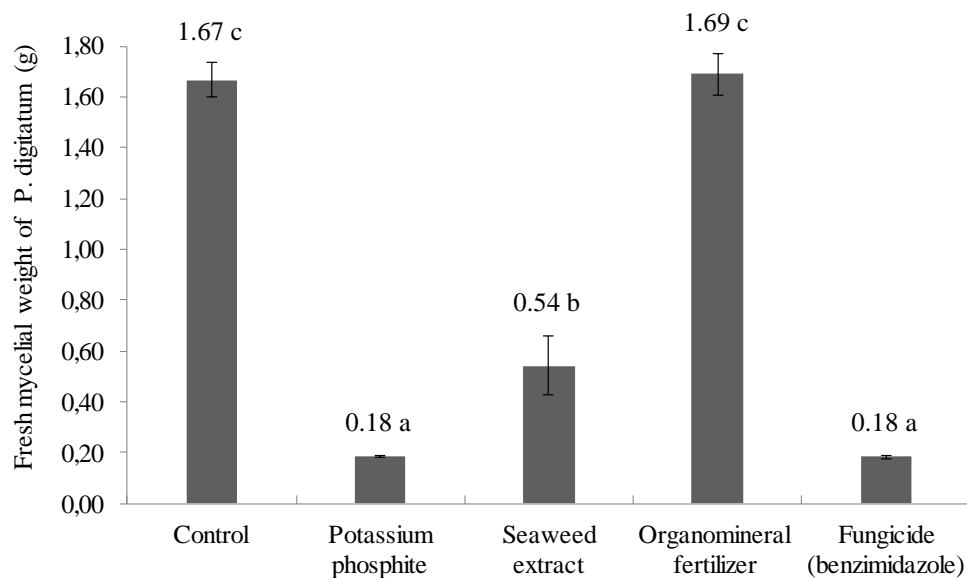


Source: Authors.

● Estimation of the ideal dose

Figure 2 represents the effects of the alternative products on the *in vitro* development of *P. digitatum* compared to that of the conventional product (benzimidazole fungicide). Potassium phosphite at a concentration of 19.5 mL L⁻¹ significantly interfered with the production of the *P. digitatum* fresh mycelial weight, with an inhibitory effect similar to that of the benzimidazole fungicide. *A. nodosum* seaweed extract at a concentration of 91.5 mL L⁻¹ partially inhibited the development of the pathogen, with the production of the fresh mycelial weight that was superior to that of the benzimidazole fungicide but inferior to that of the treatment that received no product. On the other hand, the organomineral fertilizer (3 mL L⁻¹) was unable to inhibit the *in vitro* development of the pathogen because the production of the fresh mycelial weight was superior to that of the fungicide and did not differ from that of the control treatment.

Figure 2. Fresh mycelial weight of the fungus *Penicillium digitatum* after five days of cultivation in a potato-dextrose medium at a temperature of 25 °C, plus different treatments: control (0 mL L⁻¹), potassium phosphite (19.5 mL L⁻¹), *Ascophyllum nodosum* seaweed extract (91.5 mL L⁻¹), organomineral fertilizer (3 mL L⁻¹) and benzimidazole fungicide (10.3 mL L⁻¹). Means followed by the same letter do not differ according to the Scott-Knott test ($p \leq 0.05$). The bars indicate the standard error of the mean.



Source: Authors.

The inhibitory effect of potassium phosphite on the *in vitro* growth of phytopathogens has also been observed in other studies. Melo (2017) identified that 0.1% potassium phosphite

inhibits the *in vitro* development of *Colletotrichum gloeosporioides* isolated from ‘Tommy Atkins’ mangoes. The author also found that potassium phosphite significantly interfered in the permeability of the pathogen membrane at concentrations of 0.3% and 0.4%.

Potassium phosphite also promoted *in vitro* inhibition of phytopathogens that caused postharvest diseases in table grapes (Roma, 2013). Mycelial growth inhibition reached approximately 95% for *Rhizopus stolonifer* (soft rot) and *C. gloeosporioides* (ripe grape rot), and 30% for *Botrytis cinerea* (gray mold), by using potassium phosphite ($P_2O_5 = 28\%$; $K_2O = 26\%$) at a concentration of 10 mL L^{-1} . Furthermore, sporulation was inhibited at approximately 100% for the fungi *R. stolonifer* and *C. gloeosporioides*, and at concentrations of 25 and 50 mL L^{-1} the inhibition of germination tended to 100% for all phytopathogens evaluated.

Alexandre *et al.* (2014) evaluated the *in vitro* effect of different types of phosphites (potassium, calcium, magnesium, zinc, and copper) on mycelial growth, germination, and conidia production of the fungus *Colletotrichum tamarilloi*, which is responsible for anthracnose in postharvest jilo. Potassium phosphite stood out among the other phosphites because of the direct effect on the development of the fungus for all parameters analyzed.

Melo (2017) also evaluated the effect of *A. nodosum* seaweed extract (0.1%, 0.3%, 0.5%, and 1.0%) on the fresh mycelial weight of *C. gloeosporioides* isolated from ‘Tommy Atkins’ mangoes. The seaweed extract at 0.1% inhibited the development of the pathogen but at 0.5% and 1.0% there was a significant increase in the fresh mycelial weight in relation to the treatment that did not receive the product. The concentration of 0.3% did not differ statistically from that of the control. Physiologically, *A. nodosum* extract significantly interfered in the selective permeability of the plasma membrane of the fungus hyphae in all tested concentrations, in addition to decreasing the cellulolytic capacity of *C. gloeosporioides*.

Ribeiro, Serra, and Araújo (2016) reported a reduction of approximately 50% in the mycelial growth of *C. gloeosporioides*, an etiological agent of anthracnose in papaya, using *A. nodosum* seaweed extract at a dosage of 20 and 40 mL L^{-1} . Paiva *et al.* (2020) obtained 100% inhibition of *Rhizopus stolonifer* mycelial growth, the causal agent of soft rot in strawberries, using the seaweed extract in the concentration of 40 mL L^{-1} .

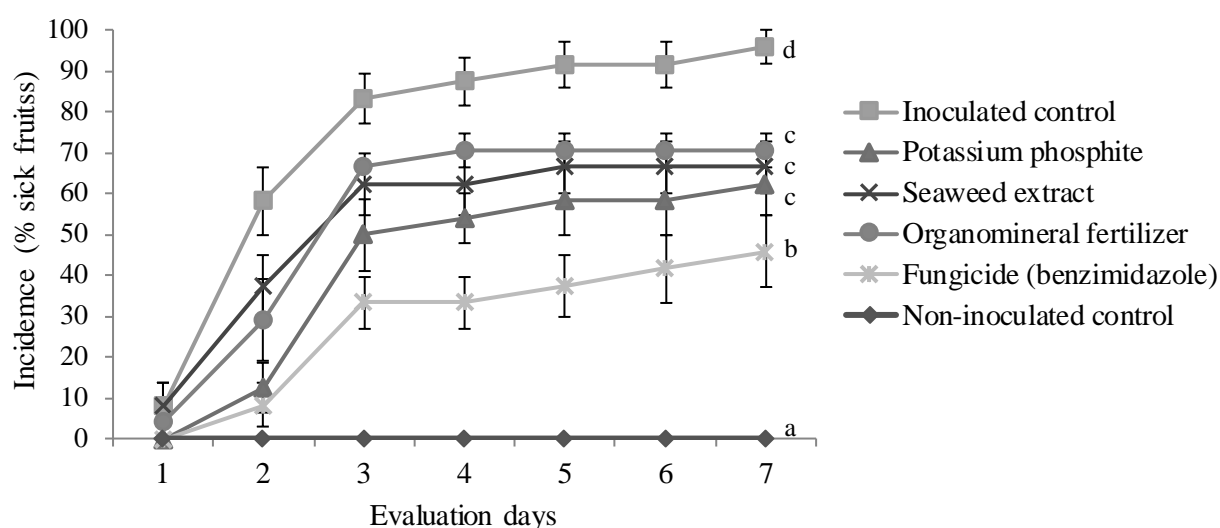
Other studies have obtained results in contrast with that of this research. Peres *et al.* (2012) observed that the alcoholic extract of *A. nodosum* seaweed had no significant effect on the inhibition of the growth of the fungus *Aspergillus flavus*. The *A. nodosum* extract induced the mycelial growth of *Monilinia fructicola* (causal agent of brown rot in stone rosacea), which was proportional to the increase of the doses applied (Oliari *et al.*, 2014).

3.2 Evaluation of the alternative products to control green mold in oranges

The *in vivo* experiment showed, as expected, that there was no development of the disease in ‘Valencia’ oranges, which were not inoculated with *P. digitatum*. Regarding the alternative products, there was a significant reduction in the incidence of green mold in the fruits after the third day of evaluation. The application of potassium phosphite (19.5 mL L^{-1}), *A. nodosum* seaweed extract (91.5 mL L^{-1}), and organomineral fertilizer (3 mL L^{-1}) promoted a reduction 35, 30, and 26%, respectively, of the incidence of the disease in oranges compared to that of the control treatment that was inoculated with the pathogen (Figure 3).

Compared to the conventional treatment, the incidence of green mold in oranges treated with the benzimidazole fungicide was lower in relation to alternative products. However, the conventional product was unable to fully inhibit the development of the disease in the fruits because the incidence of green mold in this treatment was approximately 45% at the end of the evaluation period.

Figure 3. Incidence of green mold on ‘Valencia’ oranges submitted to treatments with distilled water (inoculated control and non-inoculated control), potassium phosphite (19.5 mL L^{-1}), *Ascophyllum nodosum* seaweed extract (91.5 mL L^{-1}), organomineral fertilizer (3 mL L^{-1}), and benzimidazole fungicide (10.3 mL L^{-1}), inoculated with *Penicillium digitatum*, maintained under $25 \text{ }^{\circ}\text{C}$, and evaluated over seven days. Means followed by the same letter do not differ according to the Scott-Knott test ($p \leq 0.05$) after the third day of evaluation. The bars indicate the standard error of the mean.



Source: Authors.

Regarding the severity of the green mold, there was a reduction in the number of lesions with the development of the disease in oranges treated with potassium phosphite (19.5 mL L⁻¹) and *A. nodosum* seaweed extract (91.5 mL L⁻¹) compared to that of the control group inoculated with the pathogen. These alternative products had a similar effect as the benzimidazole fungicide on the 6th and 7th evaluation days. In contrast, the organomineral fertilizer (3 mL L⁻¹) was effective in controlling the severity of the disease only from the 2nd to the 5th day of evaluation. After the 6th day, this treatment did not differ from that of the control group inoculated with the pathogen (Table 2).

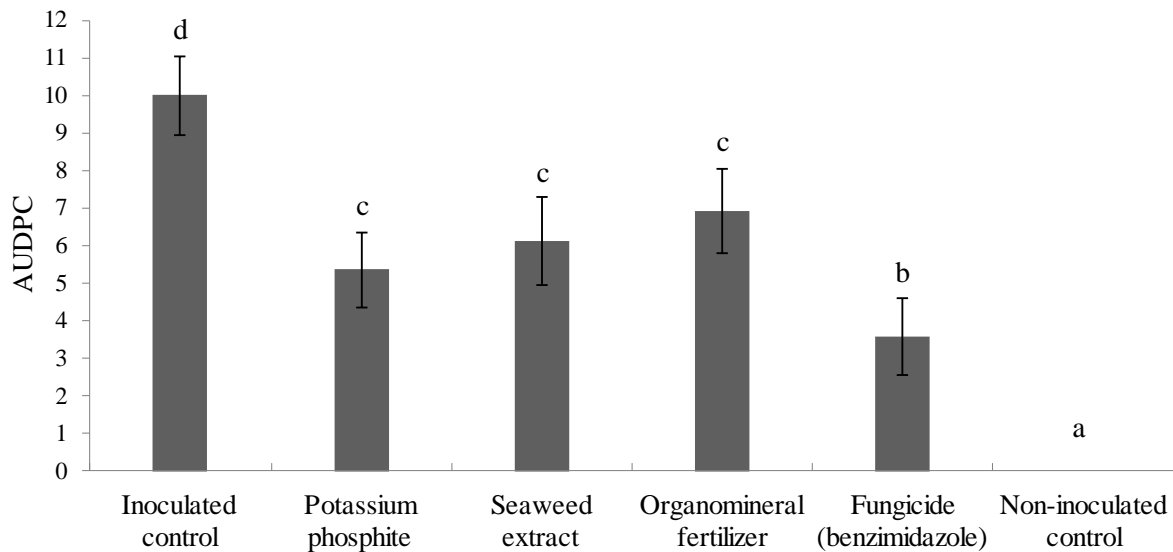
Table 2. Average number of green mold lesions on ‘Valencia’ oranges submitted to treatments with distilled water (inoculated control and non-inoculated control), potassium phosphite (19.5 mL L⁻¹), *Ascophyllum nodosum* seaweed extract (91.5 mL L⁻¹), organomineral fertilizer (3 mL L⁻¹), and benzimidazole fungicide (10.3 mL L⁻¹), inoculated with *Penicillium digitatum*, maintained under 25 °C, and evaluated over seven days. Means followed by the same letter in column do not differ according to the Scott-Knott test ($p \leq 0.05$) after the third day of evaluation. The bars indicate the standard error of the mean.

Treatments	Average number of green mold lesions / fruit						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Inoculated control	0.1 a	0.8 b	1.5 c	1.7 c	2.1 d	2.4 c	3.2 c
Potassium phosphite	0.0 a	0.1 a	0.8 b	0.9 b	1.2 c	1.3 b	2.0 b
Seaweed extract	0.1 a	0.6 b	1.0 b	1.0 b	1.1 c	1.4 b	1.8 b
Organomineral fertilizer	0.0 a	0.3 a	0.9 b	1.0 b	1.5 c	1.9 c	2.4 c
Fungicide (benzimidazole)	0.0 a	0.1 a	0.5 a	0.5 a	0.8 b	1.0 b	1.4 b
Non-inoculated control	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a

Source: Authors.

The area under the disease progression curve (AUDPC) was also reduced in treatments using alternative products compared to that of the inoculated control, but it was superior to the treatment with the benzimidazole fungicide (Figure 4)

Figure 4. Area under the disease progression curve (AUDPC) of green mold in ‘Valencia’ oranges submitted to treatments with distilled water (inoculated control and non-inoculated control), potassium phosphite (19.5 mL L^{-1}), *Ascophyllum nodosum* seaweed extract (91.5 mL L^{-1}), organomineral fertilizer (3 mL L^{-1}), and benzimidazole fungicide (10.3 mL L^{-1}), inoculated with *Penicillium digitatum*, maintained under $25 \text{ }^{\circ}\text{C}$, and evaluated over seven days. Columns followed by the same letter do not differ according to the Scott-Knott test ($p \leq 0.05$). The bars indicate the standard error of the mean.



Source: Authors.

The reduction in the efficiency of the treatment of diseases using fungicides has been attributed to the resistance phenomenon. Fischer *et al.* (2009) conducted studies in the citrus packinghouses of São Paulo and found that 39% of *P. digitatum* isolates were resistant to fungicides in the benzimidazole group. In organic and conventional orchards in São Paulo, 47.3% of *P. digitatum* isolates were resistant to benzimidazole (Fischer *et al.*, 2011). The results obtained by applying the conventional product (benzimidazole fungicide) in this research reinforced the need for studies on alternative methods as tools for the integrated management of diseases, enabling the expansion of available resources and the reduction of losses to the producer.

Studies have shown the potential of potassium phosphite to control postharvest diseases. Cerioni *et al.* (2013) studied the curative action of potassium phosphite (20 g L^{-1}) on lemons, which exhibited 80% and 90% control of the green (*P. digitatum*) and blue (*Penicillium expansum*) molds, respectively, in fruits previously inoculated with the pathogens. Approximately 100% control of these diseases was obtained when the potassium

phosphite was applied after previously treating the fruit with hydrogen peroxide solution (20 g L⁻¹) and copper sulfate (6 mmol L⁻¹).

Potassium phosphite (1.27 g L⁻¹ of P₂O₅ and 1.18 g L⁻¹ of K₂O) promoted a reduction in the incidence of rot in ‘Gala’ apples inoculated with *Penicillium* sp. after product application (Sautter *et al.*, 2008). Blum *et al.* (2007) reported a similar efficiency in controlling blue mold (*P. expansum*) by treating apples with potassium phosphite (1.5 mL L⁻¹) and benomyl fungicide.

Roma (2013) verified the protective and curative effects of potassium phosphite (2.5, 5, and 10 mL L⁻¹) in ‘Italia’ grape berries. There was a reduction in the incidence of soft rot (*Rhizopus stolonifer*), gray mold (*Botrytis cinerea*), and ripe grape rot (*Colletotrichum gloeosporioides*) at all doses tested compared to that of the control. The concentration of 10 mL L⁻¹ showed the most significant disease control.

Melo (2017) observed a reduction in the severity of anthracnose in mangos treated with potassium phosphite and inoculated with *C. gloeosporioides* compared to that of the control. In this study, all phosphite doses tested (0.1%, 0.2%, 0.3%, and 0.4%) were efficient in reducing the diameter and growth rate of anthracnose lesions in fruits, as well as the area under the disease progression curve. The application of potassium phosphite in papaya also promoted the reduction of anthracnose lesions in fruits inoculated with *C. gloeosporioides* when using the dosages of 1.5 mL L⁻¹ (Lopes, 2008) and 150 mL ha⁻¹ (Demartelaere *et al.*, 2017). Ferraz *et al.* (2016) observed a reduction in the diameter of anthracnose lesions in guavas obtained from conventional and organic cultivation systems, treated with potassium phosphite, and inoculated with *C. gloeosporioides* at all tested doses (0.5, 1.0, 1.5, and 2.0 mL L⁻¹).

Amaral *et al.* (2017) evaluated other phosphites (calcium, calcium and boron, and ammonium) in addition to potassium phosphite associated with a modified atmosphere, which was effective in reducing the severity of papaya pod rot caused by the fungus *Lasiodiplodia theobromae*. Alexandre *et al.* (2014) also evaluated different dosages of phosphites (potassium, calcium, magnesium, zinc, and copper) in the control of anthracnose of jilo, obtained from an area with a history of the disease. Potassium phosphite provided the best results, with 36.3% disease control and a lower incidence than that of the copper oxychloride fungicide during 20 days of storage.

The control of postharvest diseases using seaweed extract has also been reported in the literature. Melo (2017) evaluated the severity of anthracnose in mangoes treated with *A. nodosum* seaweed extract and inoculated with *C. gloeosporioides*. The extract at a

concentration of 1.0% promoted a reduction in the diameter of anthracnose lesions and growth rate on the fruits, presenting a higher efficiency than that of the strobilurin fungicide. The area under the disease progression curve was also reduced by treating the mangoes with 1.0% seaweed extract, presenting results similar to those of the fungicide.

In a study conducted by Gomes and Serra (2013), the application of a commercial product based on *A. nodosum* seaweed extract (100 mL L⁻¹) in peppers 48 hours before the inoculation of *C. gloeosporioides* promoted the reduction of the severity of anthracnose lesions in the fruits. Ribeiro, Serra and Araújo (2016) also observed a reduction in anthracnose lesions in papaya fruits treated with *A. nodosum* seaweed extract (40 mL L⁻¹) 72 hours before the inoculation of the fungus *C. gloeosporioides*. In contrast, Dias (2019) evaluated different doses of *A. nodosum* seaweed extract (0.1%, 0.3%, 0.5%, and 1.0%), and found no significant differences between treatments or the control regarding the inhibition of anthracnose lesions on papaya fruits. The reduction in the incidence and severity of green mold in oranges by potassium phosphite and *A. nodosum* seaweed extract could be attributed to the action of these products in inhibiting the development of *P. digitatum*, as evidenced in the *in vitro* assays.

According to King *et al.* (2010), pathogen cells exposed to phosphite exhibit changes in the expression of genes that encode proteins involved in protein and energy metabolism and oxidative stress, compromising the morphology and physiology of the microorganism. *A. nodosum* seaweed extract, in turn, has biologically active substances (amino acids, oligosaccharides, and compounds similar to plant hormones), responsible for activating the defense mechanisms of the plants and inhibiting the development of phytopathogens (Carvalho & Castro, 2014; Khan *et al.*, 2009). Melo (2017) reported that both *A. nodosum* extract and potassium phosphite acted as resistance inducers in mangos during postharvest by providing an increase in the total content of proteins, phenols, and the activity of the enzymes β -1,3-glucanase, chitinase, peroxidase, superoxide dismutase, catalase, polyphenol oxidase, and phenylalanine ammonia-lyase.

Unlike potassium phosphite and seaweed extract, studies on the effect of organomineral fertilizer on postharvest disease control are scarce in the literature. Organomineral fertilizer was applied to papaya fruits with the same composition as the product used in this study (8.5% total organic carbon, 6% nitrogen, 2.5% molybdenum, and 0.15% cobalt) to verify the potential to control diseases caused by infections from the field. The application of organomineral fertilizer (3 mL L⁻¹) enabled a 52% reduction in disease

incidence compared to that of the control over four days of fruit storage and a 50% reduction in disease severity after eight days of storage (Mafra *et al.*, 2020).

Because of the diversity of the composition of organomineral fertilizers on the market and the different pathogens that affect fruits, further studies that involve the application of these products for disease management are needed, especially during postharvest.

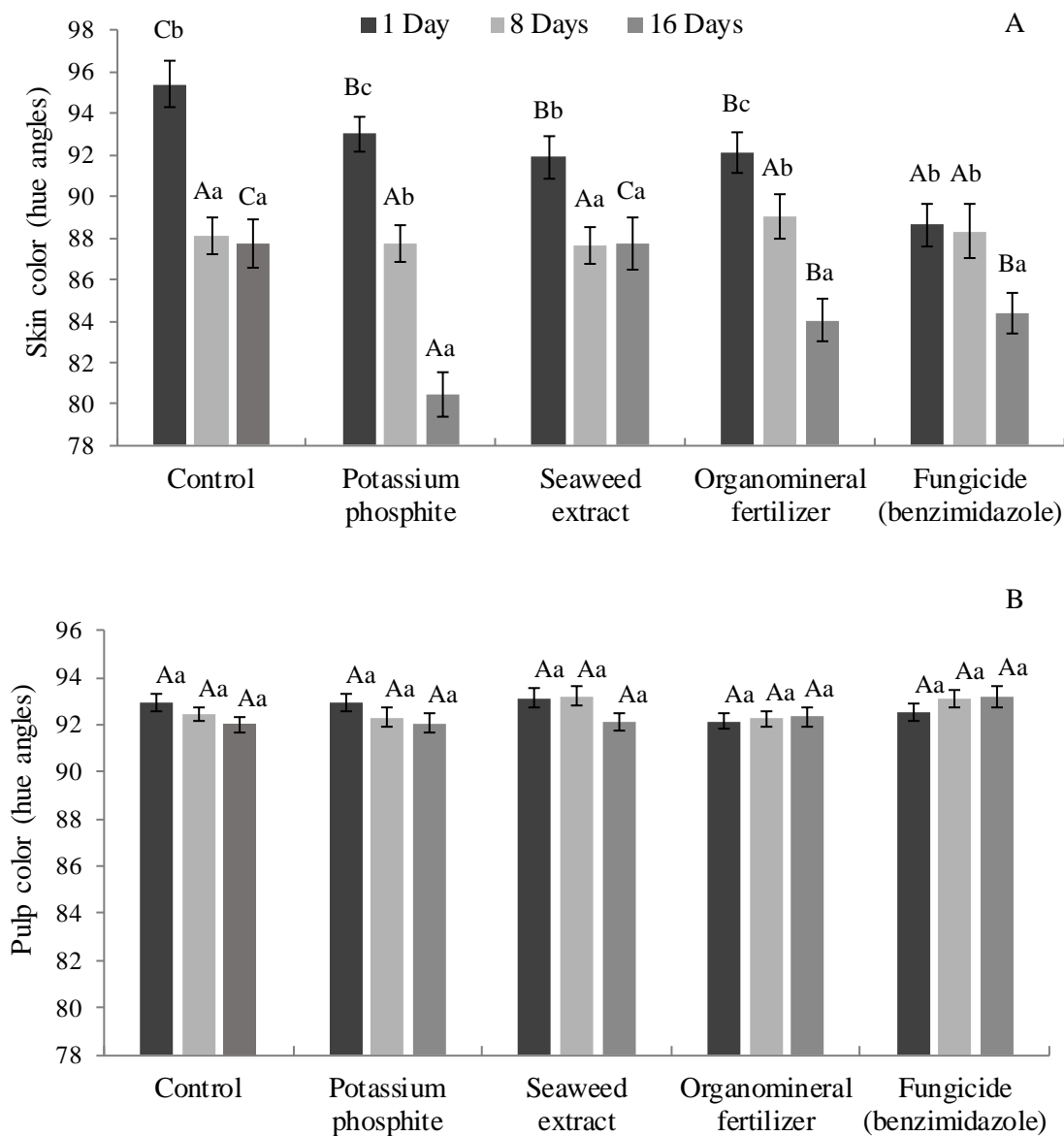
The results obtained in this research suggest that potassium phosphite, the *Ascophyllum nodosum* seaweed extract, and the organomineral fertilizer are considered alternatives to manage green mold in postharvest oranges and can be used in isolation or included in integrated strategies for disease control. However, this study was limited to investigating the effect of alternative products to control green mold in orange in only one exposition time. Therefore, other tests should be conducted to investigate different times to exposure of alternative products in oranges in the management of this disease. Moreover, the mechanisms of action of these compounds on the pathogen, such as possible changes in the cell membrane, in the morphology of the hyphae and in the enzymatic activity should be studied.

3.3 Effects of the alternative products on the physical-chemical quality of oranges

The colorimetry analysis showed that, on the first day of storage, the hue angle of the skin of the fruits treated with the alternative products was lower compared to that of the control group (Figure 5A). However, according to the color diagram, the hue angle of 0° corresponds to red, 90° to yellow, 180° to green, and the values between these ranges correspond to the mixture of these primary colors (Minolta, 1994). Therefore, on the first day of storage, the skin color of the control fruits was closer to the green range. In contrast, the fruits treated with alternative products were closer to the yellow range. There was a reduction in the hue angle value in all treatments throughout the storage period, indicating that the color of the skin of the fruits migrated to the yellow and orange ranges.

Regarding the color of the pulp, Figure 5B shows that the application of alternative products did not affect this parameter. The color angle was similar to that of the control treatment and the benzimidazole fungicide, which, on average, exhibited values close to the yellow range, in addition to remaining stable throughout the storage period.

Figure 5. Average values of skin (A) and pulp (B) color of ‘Valencia’ oranges subjected to the control (distilled water), potassium phosphite (19.5 mL L⁻¹), *Ascophyllum nodosum* seaweed extract (91.5 mL L⁻¹), organomineral fertilizer (3 mL L⁻¹), and benzimidazole fungicide (10.3 mL L⁻¹) treatments, and stored for 16 days at 21 °C. Columns followed by the same uppercase letter do not differ in terms of treatment and those followed by the same lowercase do not differ in terms of the storage period based on the Scott-Knott test ($p \leq 0.05$). The bars indicate the standard error of the mean.

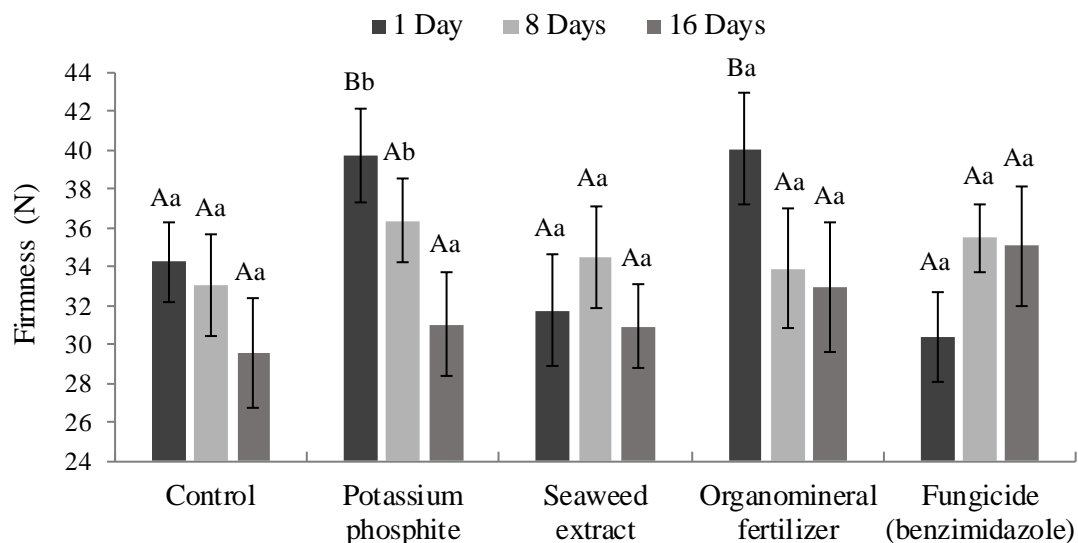


Source: Authors.

The firmness of the orange pulps treated with *A. nodosum* seaweed extract was similar to that of the control and benzimidazole fungicide throughout the storage period. In contrast,

the application of potassium phosphite and organomineral fertilizer increased the firmness of the fruits on the first day of storage (Figure 6). This increase in firmness contributed to the conservation of the fruits because it provided greater resistance to transport, storage, and handling. During the storage period, the firmness of the pulp was constant in all treatments, apart from potassium phosphite. Despite the reduction of this parameter in fruits treated with phosphite on the last day of storage, the firmness was similar to that of the control group and benzimidazole fungicide (Figure 6).

Figure 6. Average values of firmness of the pulp ‘Valencia’ oranges subjected to the control (distilled water), potassium phosphite (19.5 mL L⁻¹), *Ascophyllum nodosum* seaweed extract (91.5 mL L⁻¹), organomineral fertilizer (3 mL L⁻¹), and benzimidazole fungicide (10.3 mL L⁻¹) treatments, and stored for 16 days at 21 °C. Columns followed by the same uppercase letter do not differ in terms of treatment and those followed by the same lowercase do not differ in terms of the storage period based on the Scott-Knott test ($p \leq 0.05$). The bars indicate the standard error of the mean.

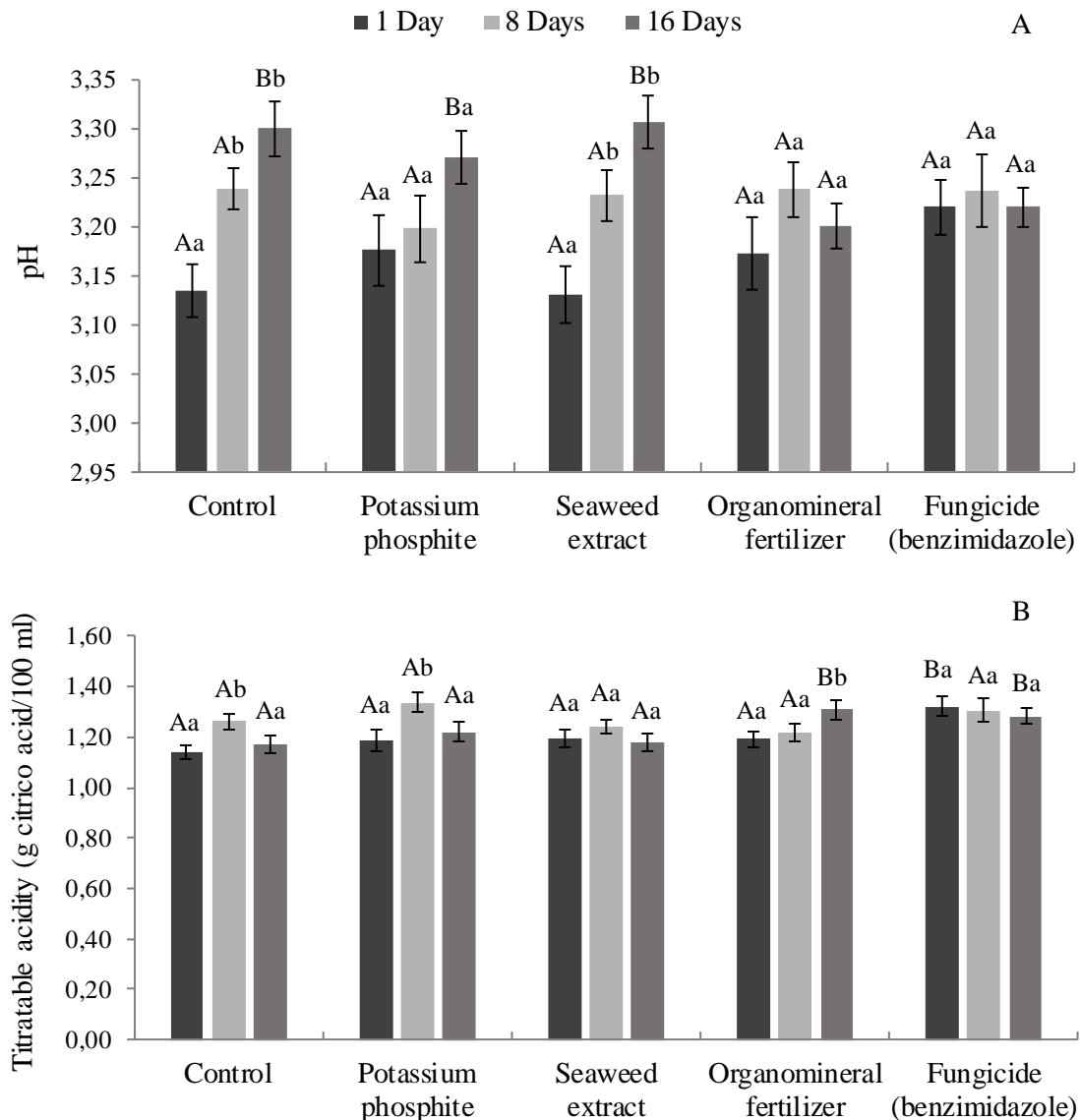


Source: Authors.

The pH of oranges treated with potassium phosphite and *A. nodosum* seaweed extract did not differ from that of the control throughout the storage period. Furthermore, there was a progressive increase in this parameter in the control and seaweed extract groups. In contrast, oranges treated with organomineral fertilizer and benzimidazole fungicide maintained a constant pH with values lower than that of the control group on the 16th day of storage (Figure 7A). Likewise, the TA of the fruits treated with potassium phosphite and *A. nodosum* seaweed

extract did not differ from that of the control group during storage. Regarding oranges treated with organomineral fertilizer, at the end of the storage period, the acidity was higher than that of the control treatment but similar to that of the benzimidazole fungicide treatment (Figure 7B).

Figure 7. Average values of pH (A) and titratable acidity (B) of ‘Valencia’ oranges subjected to the control (distilled water), potassium phosphite (19.5 mL L⁻¹), *Ascophyllum nodosum* seaweed extract (91.5 mL L⁻¹), organomineral fertilizer (3 mL L⁻¹), and benzimidazole fungicide (10.3 mL L⁻¹) treatments, and stored for 16 days at 21 °C. Columns followed by the same uppercase letter do not differ in terms of treatment and those followed by the same lowercase do not differ in terms of the storage period based on the Scott-Knott test ($p \leq 0.05$). The bars indicate the standard error of the mean.

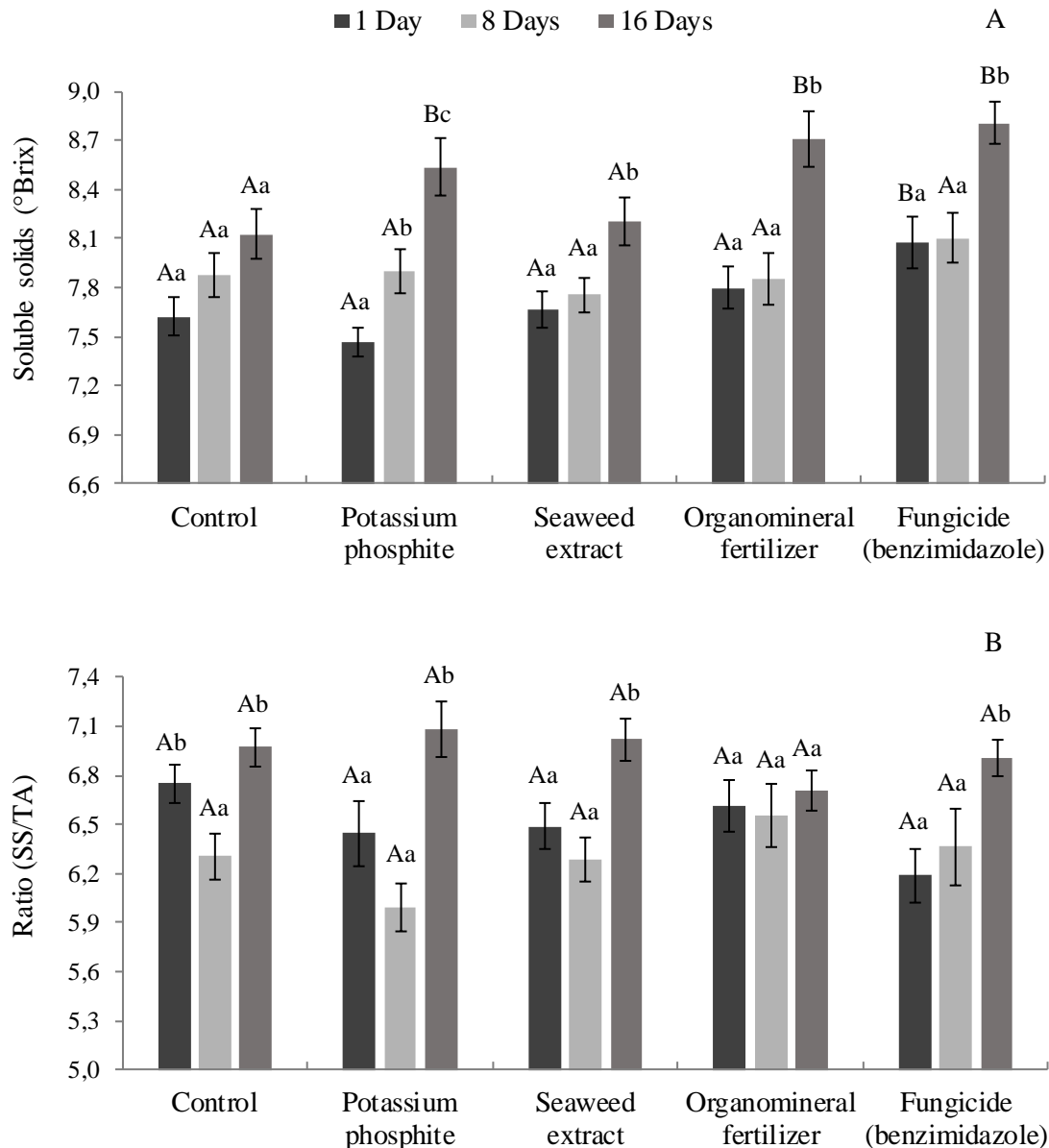


Source: Authors.

Regarding the SS content and the SS/TA ratio, Embrapa established that these parameters must be between 9 to 10 °Brix and 8.5 to 10, respectively, in ripe oranges. Figure 8 shows that these parameters were below the recommended values in all treatments. Despite this, the evaluated alternative products maintained the SS content similar to that of the control treatment until the eighth day of storage. At the end of the storage period, this parameter increased in oranges treated with potassium phosphite and organomineral fertilizer, with values similar to that of the benzimidazole fungicide treatment. Moreover, the treatment of oranges with alternative products kept the SS/TA ratio similar to that of the control through 16 days of storage.

The SS/TA ratio is one of the forms most often used to evaluate the taste of fruits, given that it is more representative than the isolated measurement of SS or acidity (Chitarra & Chitarra, 2005). Therefore, the ratio is commonly used as the main quality parameter in citrus postharvest. The results of this research indicated that the postharvest application of the evaluated alternative products provided oranges with physicochemical characteristics similar to that of the control or benzimidazole fungicide, with the latter being one of the chemicals registered for use in the postharvest treatment of green mold in citrus.

Figure 8. Average values of soluble solids (A) and ratio (B) of ‘Valencia’ oranges subjected to the control (distilled water), potassium phosphite (19.5 mL L⁻¹), *Ascophyllum nodosum* seaweed extract (91.5 mL L⁻¹), organomineral fertilizer (3 mL L⁻¹), and benzimidazole fungicide (10.3 mL L⁻¹) treatments, and stored for 16 days at 21 °C. Columns followed by the same uppercase letter do not differ in terms of treatment and those followed by the same lowercase do not differ in terms of the storage period based on the Scott-Knott test ($p \leq 0.05$). The bars indicate the standard error of the mean.



Source: Authors.

Other studies indicated the effects of postharvest treatment with alternative products on the physical-chemical quality of different plant species. Treatment with potassium

phosphite (concentration of 1.27 g L⁻¹ P₂O₅ and 1.18 g L⁻¹ K₂O) on ‘Gala’ apples did not affect the levels of total SS or TA and provided greater firmness of fruits after a storage period of eight months in a controlled atmosphere. The treatment of the ‘Fuji’ cultivar maintained the levels of total SS and firmness and promoted a reduction in fruit acidity (Sautter *et al.*, 2008).

Demartelaere *et al.* (2017) reported that the application of potassium phosphite (150 mL ha⁻¹) did not affect the physicochemical characteristics of papaya fruits regarding pH, SS, TA, and the SS/TA ratio after 12 days of storage. Amaral *et al.* (2017) evaluated the physicochemical characteristics of Sunrise Solo papayas treated with different phosphites (potassium, calcium, calcium and boron, ammonium) and doses (0.3, 0.6, 0.9, 1.25, and 1.5 g L⁻¹) and inoculated with *Lasiodiplodia theobromae*. There were no significant differences in the acidity content of the fruits after ten days of storage at ambient and under a modified atmosphere. Fruits treated with phosphites differed from that of the control group regarding SS and pH, but treatments did not compromise quality because the values followed the standards established in legislation.

Roma (2013) observed little influence of the application of potassium phosphite (10 mL L⁻¹) on the physical-chemical quality of ‘Italia’ grapes during eight days of storage. Discreet changes in skin color and pH of the fruits were observed on the 4th day after phosphite application. However, the SS content, TA, and the SS/TA ratio did not differ from that of the control treatment during the entire storage period.

Melo (2017) evaluated the physical-chemical parameters of ‘Tommy Atkins’ mangoes treated with different concentrations of *A. nodosum* seaweed extract (0.1%, 0.3%, 0.5%, and 1.0%) and phosphite potassium (0.1%, 0.2%, 0.3%, and 0.4%) over 12 days of storage. The fruits treated with the seaweed extract showed greater hue angles of pulp color compared to that of the control, indicating that there was a delay in the ripening process. However, the treatments with potassium phosphite (0.1%, 0.3%, and 0.4%) presented smaller hue angles (h°), indicating that the fruits ripened depending on the application of the product. Both the seaweed extract and potassium phosphite provided increased fruit firmness and maintenance of SS. Furthermore, the pH and acidity values indicated that the products delayed the senescence process in the fruits.

Dias (2019) considered that the postharvest treatment of papaya with *A. nodosum* seaweed extract should be further investigated. In the study conducted by the author, seaweed extract (0.1%, 0.3%, 0.5%, and 1.0%) did not interfere with the quality standard required for

consumption, considering the pH values, TA, and SS. However, there was a significant loss of fresh weight in the fruits over ten days of storage.

Mafra *et al.* (2020) found no significant difference in the analysis of pH, TA, SS, and firmness of 'Formosa' papaya pulp submitted to treatment with *A. nodosum* seaweed extract (80 mL L⁻¹), potassium phosphite (50 mL L⁻¹), and organomineral fertilizer (3 mL L⁻¹). The organomineral fertilizer evaluated in the study had the same composition as the product used in this research.

4. Conclusion

Potassium phosphite and *Ascophyllum nodosum* seaweed extract, at the concentrations of 19.5 and 91.5 mL L⁻¹, respectively, inhibited the production of a *Penicillium digitatum* fresh mycelial weight. Potassium phosphite (19.5 mL L⁻¹), *Ascophyllum nodosum* seaweed extract (91.5 mL L⁻¹), and organomineral fertilizer (3 mL L⁻¹) reduced the incidence and severity of mold green in oranges. In general, the application of the products postharvest did not compromise the physical-chemical quality of the fruit.

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