Tropical Plant Pathology (2022) 47:608–617
https://doi.org/10.1007/s40858-022-00518-9

ORIGINAL ARTICLE

Alternative products in the management of powdery mildew (*Podosphaera xanthii*) in melon

Afonso Luiz Almeida Freires[1] · Francisco Romário Andrade Figueiredo[1] · Tatianne Raianne Costa Alves[1] · Karol Alves Barroso[1] · Igor Vinicius Pereira da Silva[1] · Jarlan Lucas Santos Silva[1] · Geovane de Almeida Nogueira[1] · Naama Jessica Assis Melo[1] · Rui Sales Júnior[1] · Andreia Mitsa Paiva Negreiros[1] · Márcia Michelle de Queiroz Ambrósio[1]

Received: 13 March 2022 / Accepted: 6 June 2022 / Published online: 11 July 2022

Abstract

Melon (*Cucumis melo* L.) is one of the main vegetable crops produced in the Brazilian Northeast region. However, despite the favorable climate for its development, melon is still affected by diseases, including foliar diseases such as powdery mildew (*Podosphaera xanthii* (Castag.) U. Braun & N. Shish.). This study aimed to determine the efficacy of alternative products (less aggressive to man and nature) for managing powdery mildew in melon. A randomized block design (RBD) was used in the experiment, with eight treatments and eight replications. Four fertilizers (Agro Mos®, Copper Crop®, Soil Set®, and Fertisilício®), the biocontrol product Suppress-L™, and raw milk (20%) were tested compared to a nontreated control and the fungicide Amistar Top®. The following variables were analyzed: incubation period (IP), disease incidence (INC), disease severity (SEV), the area under the disease progress curve (AUDPC), and melon growth variables (plant height, stem diameter, number of leaves, leaf area, physicochemical quality of fruits, and biochemical analysis of fruit). The data were subjected to analysis of variance (ANOVA), followed by mean comparison by the Scott-Knott test at 5% probability. Raw milk was the best alternative control method for powdery mildew and was as effective as chemical control (Amistar Top®), reducing the SEV by 49% and increasing the °Brix of melon by 19%. Copper Crop® was also effective in controlling powdery mildew and reduced SEV by 30%. The present study highlighted that the activities of polyphenol oxidase, peroxidase, chitinase, and β-1,3-glucanase are involved with the melon defense mechanism since the treatments with higher enzymatic activities promoted lower powdery mildew severity. Among the studied products, raw milk promoted the highest activities of the studied enzymes.

Keywords *Cucumis melo* L. · Foliar diseases · Enzymes · Fungus

Introduction

Melon (*Cucumis melo* L.) is a cucurbit crop of worldwide economic importance whose production is favored by high temperature and low humidity conditions, which results in high-quality fruits (Pandey et al. 2016; Li et al. 2017). Global melon production in 2019 totaled approximately 27.3 million tons, 46.5% of which was produced in China, the main melon-producing country (FAO 2021). In Brazil, the Northeast region stands out with 95.8% (563,378 tons) of the national melon production (IBGE 2021). However, the expansion of farming areas along with intense and successive cultivation increases the incidence and severity of plant diseases, causing yield losses worldwide (Porto et al. 2019; Sales Júnior et al. 2019). In this scenario, powdery mildew is highlighted as the main melon shoot disease on a global scale (Li et al. 2017) and is usually caused by the fungi *Podosphaera xanthii* (Castag.) U. Braun & N. Shish. (previously *Sphaerotheca fuliginea* (Schlecht.Ex.Ft.) Pollacci) and *Golovinomyces cichoracearum* (S. Blumer) (Rur et al. 2018).

The white colonies produced by these fungi on the leaf surface reduce the photosynthetic activity of plants (Dallagnol et al. 2015). Subsequently, as the disease

✉ Francisco Romário Andrade Figueiredo
romarioagroecologia@yahoo.com.br

[1] Departamento de Ciências Agronômicas e Florestais, Universidade Federal Rural Do Semi-Árido, Mossoró 59625-000, Brazil

progresses, the infected tissues become chlorotic and show premature senescence, thus reducing fruit yield and quality (Wang et al. 2016; He et al. 2017). The colonies contain very light, wind-dispersed spores (Wang et al. 2016).

P. xanthii is the predominant melon pathogen (Wang et al. 2021). This species is adapted to high temperatures and dry climates because it does not require moisture on the leaf surface to infect (Ramos et al. 2013). However, although fungicides are known to reduce disease severity (Wang et al. 2016), their use might result in the emergence of pesticide-resistant *P. xanthii* and there are restrictions imposed by melon importers pertaining to the maximum residue limit (MRL) of agrochemicals and government agencies.

Consequently, there is interest in sustainable, effective alternative products to fungicides for managing *P. xanthii* on melon, including biological control, fertilizers, and raw milk. Biological control entails using microorganisms such as the fungus *Trichoderma*, notably effective in pathogen suppression by employing mechanisms such as antibiosis, competition, parasitism, and predation through one or more antagonistic mechanisms (Martínez et al. 2016; Sarhan et al. 2020).

Another interesting approach is to supply silicon (potassium silicate) via foliar application. Silicon acts directly on the fungal pathogen through the induction of enzymes involved in plant defense, producing phenolic compounds and phytoalexins at the site of infection (Dallagnol et al. 2012). This element also acts indirectly by forming a physical barrier resulting from the increased pH and osmotic potential after water evaporation (Dallagnol et al. 2015).

Raw milk is another option that provides calcium and phosphate salts, iron, magnesium, proteins, vitamins, amino acids, and microorganisms. These components increase after milk application and remain stable on the leaves, providing protection against powdery mildew (Hamann and Krömker 1997). Raw milk has a satisfactory cost–benefit ratio, accessibility by producers, and significant environmental acceptance (Medeiros et al. 2012). Applying raw milk has been shown effective against powdery mildew in several cucurbit crops: cucumber, pumpkin, and melon (Faria et al. 2011).

Plant defense can also be induced by some commercial fertilizers, including Agro Mos®, Copper Crop®, Soil Set®, and Fertisilício®, whose composition includes nutrients and enzyme-activating amino acids. Reduction in severity of powdery mildew is associated with increase in the activity of chitinase (QUI), β-1,3-glucanase (GLU), peroxidase (PO), and polyphenol oxidase (PPO), among other enzymes related to pathogenicity (Soliman and El-Mohamedy 2017). These compounds act by inhibiting conidial germination and mycelial growth, resulting in a smaller leaf area affected by the pathogen (Sarhan et al. 2020).

This study aimed to determine the efficacy of alternative products to conventional fungicides for managing powdery mildew caused by *P. xanthii* in melon.

Material and methods

Location of the study

The study was conducted in a plant nursery at the Universidade Federal Rural do Semi-Árido (UFERSA) in Mossoró, RN, Brazil. According to the Köppen climate classification, the region has a BSwh' climate, i.e., hot, dry, and typically semi-arid. The mean annual temperature is 27 °C, with irregular annual rainfall (annual mean of 673.9 mm) and a mean air relative humidity of 68.9% (Alvares et al. 2013).

Collection, storage, and inoculation of P. xanthii

The *P. xanthii* isolate was collected from leaves of the melon hybrid *Goldex* at the Agrícola Salutaris Farm in the municipality of Afonso Bezerra, RN. Since the species is an obligate parasite, the material was sent to the plant nursery in paper bags to preserve the isolate under in vivo conditions.

Seedlings of the melon hybrid *Goldex* were provided by the TopPlant® company and transplanted 10 days after sowing (DAS) to pots containing 14.3 dm^3 of soil from a producing area in Baraúna, RN. Inoculation was performed 18 DAS when the plants had three true leaves. The plants were inoculated artificially by depositing a small amount of conidia two spots (at each side of and equidistant from, the main leaf vein) on the second true leaf of each plant (Yuste-Lisbona et al. 2010).

A drip irrigation system was used to provide high water-use efficiency (Batista et al. 2009), and macro- and micronutrients were supplied according to the nutrient requirements of the crop (Cury et al. 2014).

Experimental design

A randomized block design (RBD) was used with eight treatments and eight replications, with one plant per replicate. Treatments were as follows: 1 = control, 2 = Amistar Top®, 3 = Suppress-L™, 4 = Agro Mos®, 5 = Copper Crop®, 6 = Soil Set®, 7 = Fertisilício®, and 8 = raw milk.

Preparation and application of treatments

Suppress-L™ was used as a biocontrol. This product was multiplied in rice grains at the concentration of 10^9 conidia mL^{-1} and applied at 5 kg/ha, as recommended by the manufacturer. Amistar Top® (300 mL/ha), Agro Mos® (900 mL/ha), Copper Crop® (300 mL/ha), Soil Set® (300 mL/

ha), and Fertisilício® (600 mL/ha) were diluted in water and administered as recommended by the manufacturers. Amistar Top® (azoxystrobin and difenoconazole) was chosen because it is a fungicide commonly used by farmers in the region for this disease, and is registered in the Ministry of Agriculture, Livestock and Food Supply for this crop and pathogen. Raw milk was diluted in water and applied at a concentration of 20% (Zatarim et al. 2005). Whereas Suppress-L™ and Fertisilício® were applied every 5 days, the remaining treatments were applied weekly, as recommended by the manufacturers. The applications began 1 day after inoculation with powdery mildew. All applications were performed using a sprayer commercial hand held, and the volume applied was increased according to the crop development stage to achieve full shoot coverage.

Epidemiological components and plant evaluation

Plant evaluation was performed weekly, beginning after the first signs of *P. xanthii* (6 days after inoculation). The following epidemiological components were evaluated: incubation period (IP), disease incidence (INC), disease severity (SEV), and area under the disease progress curve (AUDPC).

The IP was defined as the number of days between inoculation and the appearance of the first disease symptoms. The INC was estimated as the percentage of symptomatic plants in each treatment. The SEV was determined using the scale proposed by He et al. (2017) with adaptations, where 0 = no visible symptom; 1 = up to 5% of the leaves covered with powdery mildew; 3 = 6–10% of the leaves covered with powdery mildew; 5 = 11–20% of the leaves covered with powdery mildew; 7 = 21–40% of the leaves covered with powdery mildew; 9 = 41–60% of the leaves covered with powdery mildew; 11 = 61–80% of the leaves covered with powdery mildew; and 13 = 81–100% of the leaves covered with powdery mildew. The SEV data was used to calculate AUDPC using the equation $AUDPC = \Sigma\ [((y_1 + y_2)/2)*(t_2 - t_1))]$, where y_1 and y_2 are two consecutive evaluations performed at times t_1 and t_2, respectively.

Melon growth analysis

The variables of plant height (PH), stem diameter (SD), and number of leaves (NL) were determined weekly. At the end of the experiment, leaf area (LA) was obtained by the leaf disk method (Lins et al. 2018).

Post-harvest analyses

The post-harvest variables examined were pulp firmness (PF), fruit weight (FW), longitudinal diameter (LD), transverse diameter (TD), and °Brix. Fruit color was used as the defining parameter for harvest (Câmara et al. 2007).

Biochemical analyses

Fully expanded melon leaves were collected from all plants of each treatment 12 h after the last application of products. These samples were stored in coolers containing ice and transported to the Laboratory of Plant Biotechnology at UFERSA, where they were weighed, processed, and stored in a freezer (−43 °C) for later analysis.

The leaf samples (0.5 g) were mechanically homogenized in 4 mL of 100 mM sodium acetate buffer (pH 5.0) using a mortar and pestle. The homogenate was kept for 25 min at 4 °C in a centrifuge at 20,000 g, and the resulting supernatant was considered as an enzyme extract to determine the activity of chitinase (QUI) and β-1,3-glucanase (GLU) (Bezerra Neto and Barreto 2011).

Chitinase activity was evaluated by the release of soluble "CM-chitin-RBV" fragments from carboxymethyl chitin marked with Remazol Brilliant Violet (CM-chitin-RBV 4 mg mL^{-1}, Loewe Biochemica GmbH). For that purpose, 200µL of the protein extract was mixed with 600 µL of the same extraction buffer and 200 µL of "CM-chitin-RBV" (2.0 mg mL^{-1}). After incubation for 20 min at 40 °C, the reaction was stopped by adding 200 µL of 1 M HCl, followed by ice cooling and centrifugation at 10,000 g for 5 min. The absorbance of the supernatant was determined at 550 nm with extraction buffer in the reference cuvette. The results were expressed as enzyme unit mg^{-1} of protein after discounting the absorbance values of the control (800 µL of extraction buffer + 200 µL of "CM-chitin-RBV") (Stangarlin et al. 2000).

The spectrophotometric determination of the activity of β-1,3-glucanase in the extracts was performed by transferring 25 µL of the enzyme extract to each test tube. Subsequently, 200 µL of the acetate buffer solution (0.1 M, pH 5.0) and 200 µL of laminarin were added to the tubes (15 mg mL^{-1}). The mixture was incubated for 30 min at 37 °C, after which the glucose content produced by the hydrolysis of laminarin was determined (Bezerra Neto and Barreto 2011). Then, the reducing sugars formed after incubation were quantified and the absorbances were read at 410 nm in a spectrophotometer. Finally, the protein concentration was analyzed by the Bradford method (Bradford 1976), and the results were expressed as enzyme unit min^{-1} mL^{-1}.

The polyphenol oxidase extract was prepared by homogenizing the frozen leaves at the maximum temperature of 4 °C in 10 mL of 0.05 M phosphate buffer (pH 7.0) containing 1 mg of polyvinylpyrrolidone-10. The homogenized material was then centrifuged at 4000 g for 20 min, and the precipitate was discarded. The supernatant was preserved in ice and used in the analyses of this enzyme (Campos et al. 2004). Polyphenol oxidase activity was determined according to the technique described by Hyodo and Yang (1971) with

the following modifications: a cold tube received 3.6 mL of 0.05 M phosphate buffer at pH 6.0, 1 mL of enzyme extract, and 0.1 mL of 0.1 M catechol. Next, the tube with the mixture was agitated in a vortex for 15 s, then incubated at 30 °C for 30 min, and transferred to an ice bath. Then, the mixture received 0.2 mL of 1.4% perchloric acid and, after agitation in a vortex, the tube rested for 10 min. Absorbance was read in a spectrophotometer at 395 nm. In the control, the enzyme extract was replaced by water. Enzyme activity was expressed as enzyme unit (UE) min^{-1} of sample. One enzyme unit was defined as the amount of enzyme that caused an increase of 0.001 absorbance units per minute (Campos et al. 2004).

In the procedure to prepare the peroxidase extract, the leaves were weighed (1 g of sample) and ground in a mortar with 50 mg of PVP and approximately 10 mL of liquid nitrogen. During this process, 3 mL of the sodium acetate buffer solution (50 mM, pH 5.0) and 1 mM of EDTA were added. Subsequently, the homogenized material was centrifuged at 10,000 mg for 10 min at 4 °C (Bezerra Neto and Barreto 2011). Then, the supernatant was transferred to Eppendorf tubes for analysis.

Peroxidase activity was determined according to the techniques described by Bezerra Neto and Barreto (2011). First, a cold tube received 1 mL of phosphate buffer (0.2 M) at pH 6.0 and 100 µL of the enzyme extract. Then, the tubes were placed in a thermostatic bath at 25 °C until the temperature stabilized. Subsequently, 100 µL of guaiacol (0.5%) and 100 µL of hydrogen peroxide (0.08%) were added to the tubes and the mixture was agitated in a vortex, after which seven spectrophotometric readings at 470 nm were made every 30 s. Enzyme activity was estimated based on the difference of absorbance per minute and weight of fresh sample. In the control, the enzyme extract was replaced by distilled water. All analyses were performed in duplicate.

Statistical analyses

The experiment was conducted twice. A preliminary ANOVA was used to determine whether there were significant differences between the two experiments and whether the data could be combined. Upon meeting the assumptions, the analysis of variance was performed, and the Scott-Knott test was applied at 5% probability. The statistical software R was used in all statistical analyses (R Core Team 2017) (Wei et al. 2017).

Results

According to the *F* test, the evaluated treatments significantly affected the IP, SEV, and AUDPC variables ($p > 0.001$) (Table 1).

Table 1 *F* test for the incubation period (PI), severity (SEV), and area under the disease progress curve (AUDPC) of melon plants after 57-day inoculation with powdery mildew

p value				
Sources of variation	DF	IP	SEV	AUDPC
Treatment	7	0.0001	0.0000	0.0000
Block	7	0.4250	0.0530	0.0045
Error	49			
CV (%)		29.99	22.96	19.58

DF, degrees of freedom; *CV*, coefficient of variation

The incubation period was longer in the plants that received Amistar Top® (mean of 16 days), delaying the onset of symptoms by 9.25 days relative to the control treatment (6.75 days) (Fig. 1A). Since all plants showed symptoms, there were no differences among treatments for the INC according to the Scott-Knott test at 5% probability (100%) (Fig. 1B).

The lowest SEV was observed in the plants treated with Amistar Top® (4.5), although this fungicide did not differ significantly from raw milk (6.0). These two treatments resulted in reductions in SEV of 62 and 49%, respectively, relative to the control (11.75). Copper Crop® (8.25) and Agro Mos® (9.25) reduced the SEV by 30 and 21%, respectively, relative to the control. These treatments also resulted in lower SEV values than Soil Set® (12.00), Fertisilício® (12.50), and Suppress-L™ (13.00) which all were not significantly different from the control (Fig. 1C).

Most treatments reduced the AUDPC, except Suppress-L™ (Fig. 1D). Amistar Top® was the most effective treatment and resulted in an AUDPC of 756. This fungicide promoted a reduction of 81.60% compared to the control (3,115). Raw milk, Copper Crop®, and Agro Mos® showed AUDPC values of 1561, 2009, and 2072 and reductions of 59, 36, and 33%, respectively (Fig. 1D).

With regard to the plant growth variables (PH, SD, and LA), only LA was significantly affected by the treatments ($P < 0.05$) (Table 2). Among fruit quality parameters, only the °Brix showed a significant difference between treatments ($P < 0.05$).

According to the Scott-Knott test, raw milk and Amistar Top® differed from the other treatments at 5% probability, providing the best LA results, with mean values of 1865.50 and 1606.01 cm^2, corresponding to increases of 48.43 and 27.78% relative to the control (Fig. 2A). The mean soluble solids in the fruits were higher than 9°Brix in all treatments, and raw milk stood out with a mean value of 11.8°Brix (Fig. 2B).

Regarding the activity of defense enzymes (PPO, POX, QUI, and GLU), there was a significant difference between treatments ($P < 0.05$) (Table 3).

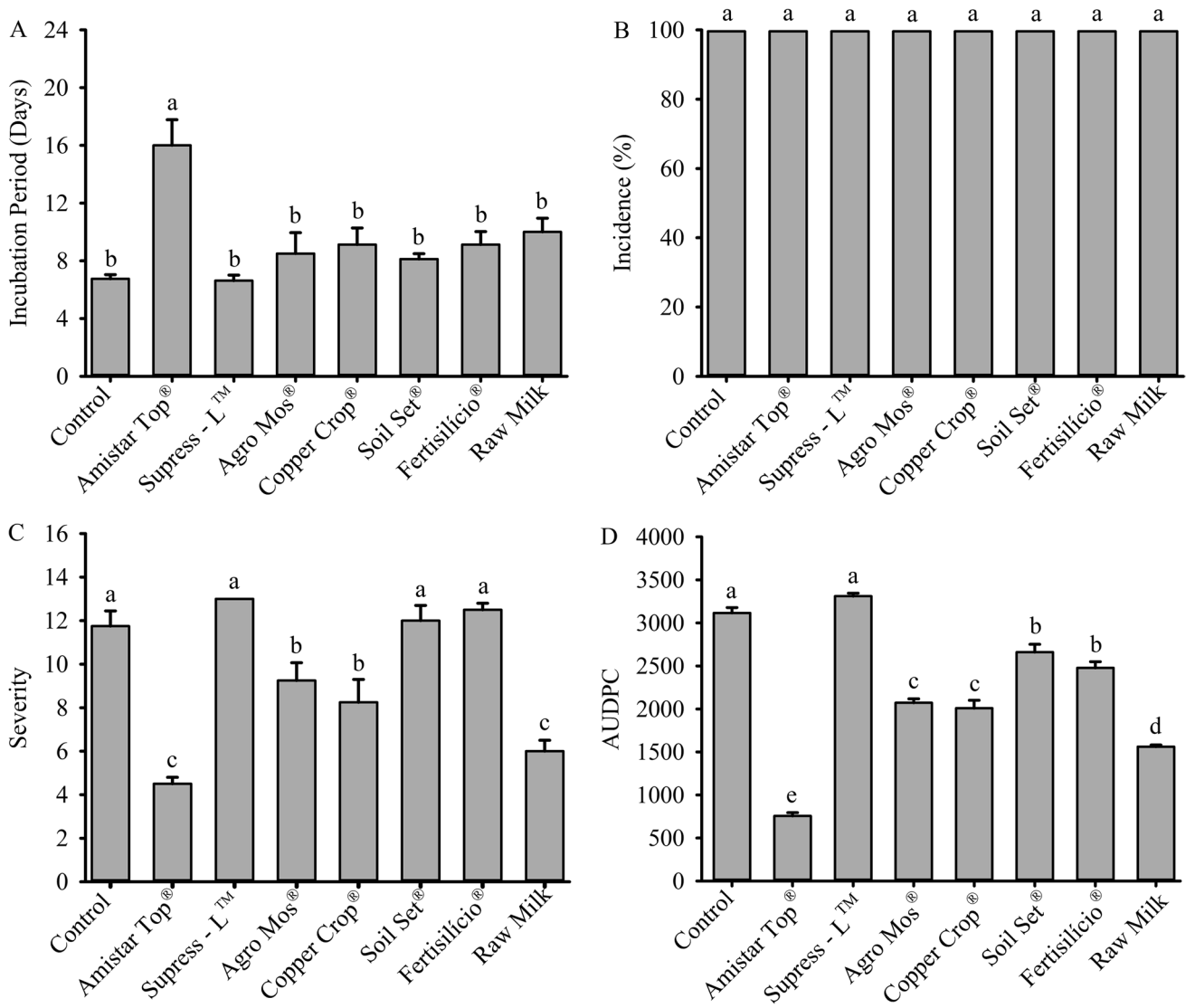

Fig. 1 Incubation period (**A**), incidence (**B**), severity (**C**), and area under the disease progress curve (AUDPC) (**D**) in melon plants after 57-day inoculation with powdery mildew. The error bars represent the standard deviation of the means. Means with different letters differ by the Scott-Knott test at 5% probability

Table 2 *F* test for plant height (PH), stem diameter (SD), leaf area (LA), and °Brix in melon plants after 57-day inoculation with powdery mildew

Sources of variation	DF	PH	SD	LA	°Brix
Treatment	3	0.3117	0.1023	0.0422	0.0220
Block	4	0.1646	0.5266	0.6902	0.3298
Error	12				
CV (%)		16.38	8.31	47.96	9.17

DF, degrees of freedom; *CV*, coefficient of variation

All treatments increased polyphenol oxidase (PPO) activity relative to the control (Fig. 3), differing by the Scott-Knott test at 1% probability. Moreover, raw milk (8.61 E.U.$^{-1}$ min^{-1} mg of sample) resulted in higher enzyme activity compared to the other treatments, showing a 46% increase compared to the control (Fig. 3A).

With regard to peroxidase (POX) activity, most treatments resulted in higher enzyme activity compared to the control, except Suppress-L™ (Fig. 3B). The highest POX activity occurred with raw milk (8.31 E.U.$^{-1}$ min^{-1} mg of sample), followed by Copper Crop® (7.25 E.U.$^{-1}$ min^{-1} mg of sample), Agro Mos® (4.92 E.U.$^{-1}$ min^{-1} mg of sample), and Fertisilício® (4.54 E.U.$^{-1}$ min^{-1} mg of sample), increasing by 4055, 3525, 2360, and 2170%, respectively (Fig. 3B).

The treatments with Fertisilício® (1.27 E.U.$^{-1}$ mg of protein), Copper Crop® (1.39 E.U.$^{-1}$ mg of protein), and raw milk (1.03 E.U.$^{-1}$ mg of protein) increased chitinase activity (QUI) by 160, 107, and 54%, respectively, relative to the control (0.67 E.U.$^{-1}$ mg of protein) (Fig. 3C).

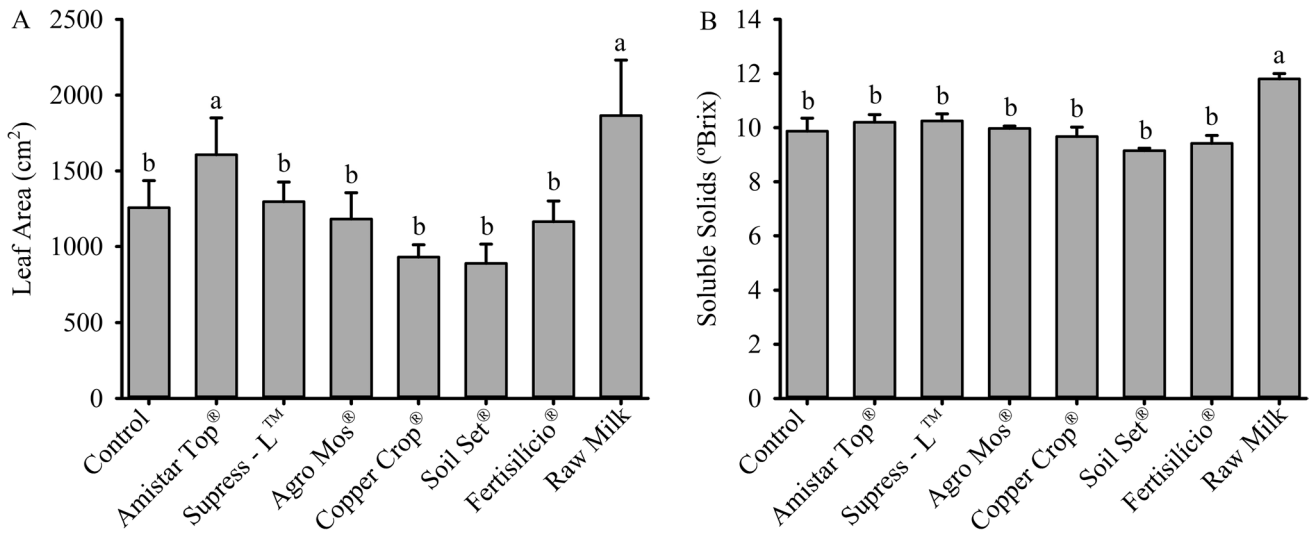

Fig. 2 Melon leaf area (**A**) and fruit °Brix (**B**) after inoculation with powdery mildew. The error bars represent the standard deviation of the means. Means with different letters differ by the Scott-Knott test at 5% probability

Table 3 *F* test for the activity of polyphenol oxidase (PPO), peroxidase (POX), chitinase (QUI), and β-1,3-glucanase in melon plants 57 days after inoculation with *P. xanthii*

Sources of variation	DF	PPO	POX	QUI	GLU
Treatment	7	0.0000	0.0000	0.0000	0.0000
Block	7	0.6056	0.1706	0.7491	0.2211
Error	49				
CV (%)		4.7	12.78	12.95	6.41

DF, degrees of freedom; *CV*, coefficient of variation

The maximum activity of β-1,3-glucanase (GLU) was obtained with raw milk (5.14 E.U.$^{-1}$ min^{-1} mL). However, Suppress-L™ (4.21 E.U.$^{-1}$ min^{-1} mL), Copper Crop® (4.00 E.U.$^{-1}$ min^{-1} mL), and Fertisilício® (3.95 E.U.$^{-1}$ min^{-1} mL) were also effective in promoting the activity of β-1,3-glucanase in relation to the control. These treatments increased enzyme activity by 46, 19, 13, and 12%, respectively (Fig. 3D).

Discussion

The present study highlights the potential of raw milk as an alternative to fungicides to control powdery mildew, the main melon leaf disease. Raw milk induced plant defense mechanisms by promoting higher activities in three of the four enzymes studied (PPO, POX, and GLU). In addition to raw milk, the results documented that the fertilizer Copper Crop® was another alternative product that provided high protection.

Raw milk reduced disease severity and AUDPC through various action mechanisms, with its constituents acting directly against the fungus, especially phosphate and calcium, which degrade the cell wall of hyphae. Additionally, its constituents provide a slightly basic pH and cause disturbances in fungal development at the onset of the disease, in addition to stimulating the growth of antagonistic microorganisms on the leaf surface (Kamel et al. 2017). These results agree with Kamel and Afifi (2020), who observed a reduction in the AUDPC (499.3) after milk application in cucumber. On the other hand, indirect action occurs through the expression of enzymes that strengthen the plant defense system due to the biosynthesis of phytoalexins and phenols to form lignin, which acts as a barrier against pathogen penetration (Bettiol 1999; Zatarim et al. 2005; Sudisha et al. 2011; Li et al. 2015). Moreover, Bettiol and Astiarraga (1998) and Medeiros et al. (2012) found evidence of suppression of fungal mycelia, bacteria, and yeast strains on the leaf surface after milk application, which also explains the efficacy of this material.

Control of powdery mildew by applying raw milk was also documented by Kamel and Afifi (2020) in an experiment conducted with cucumber plants grown under greenhouse conditions. In a study with pumpkin cultivars, Barickman et al. (2017) observed lower powdery mildew severity after copper application. However, the same study stressed that plants treated with azoxystrobin showed a significant reduction in severity (13%) compared to copper, corroborating the results of the present study.

Due to the increase in plant resistance and the limited potential for environmental contamination, milk is widely used (Sudisha et al. 2011). Moreover, in agricultural crops such as common bean (*Vigna unguiculata* L. Walp), resistance usually depends on plant age and reaches its maximum

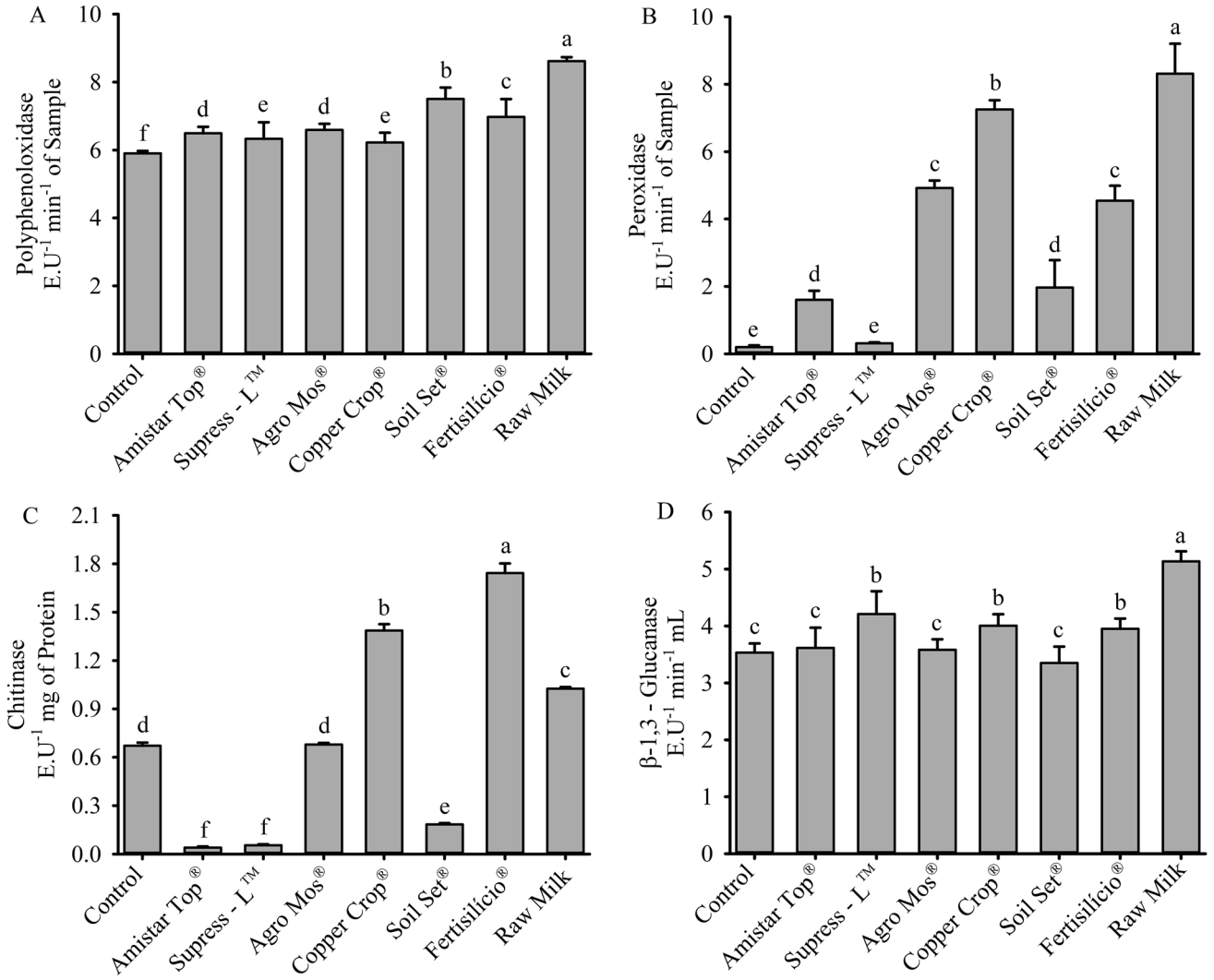

Fig. 3 Activity of polyphenol oxidase (PPO) (**A**), peroxidase (POX) (**B**), chitinase (QUI) (**C**), and β-1,3-glucanase (GLU) (**D**) after the application of treatments and powdery mildew inoculation in melon plants. The error bars represent the standard deviation of the means. Means with different letters differ by the Scott-Knott test at 5% probability

at the beginning of the crop cycle (Ebrahim et al. 2011). However, under the conditions of the present study, protective values were also observed at the end of the melon crop cycle. Substances like raw milk cannot be legally used by farmers for disease control in some countries, including the USA, until reviewed and registered as a fungicide. However, milk is widely used for disease control in several countries, e.g., China, Brazil, and India (Sudisha et al. 2011). In Brazil, it is possible to use this tool, mainly in organic production.

Copper Crop® was effective due to the action of the cupric ion in the cell membrane, resulting in the denaturation of structural proteins and promoting enzyme reactions that block the respiratory activity, consequently inhibiting spore germination (Torre et al. 2018). Moreover, heavy metals such as copper can induce, by stress, enzymes related to plant defense (Chmielowska et al. 2010). Copper Crop® is a suitable alternative to a copper-based fungicide because it has complexed amino acids that facilitate absorption by the plant, and is an important alternative, as it increases the fresh and dry mass of the shoot, contributing to nutrition and, consequently, increasing plant vigor and disease resistance. In Brazil, this copper-based product is already being used by farmers in the agricultural market.

Amistar Top®, a conventional fungicide, was the only treatment that delayed the onset of symptoms. However, disease incidence was not affected by this fungicide or any other treatment due to the rapid spread of this fungus. The fungicide delays the onset of the disease due to its protective and curative properties, inhibiting the action of enzymes involved in fungal mitochondrial respiration and paralyzing sterol biosynthesis in the membranes through the activity of C-14 demethylase (Krämar and Schirmer 2007; Souders

et al. 2019). Efficacy of azoxystrobin, one of the two active ingredients in Amistar Top®, was also observed by Barickman et al. (2017) in pumpkin cultivars affected by powdery mildew.

Therefore, the present study highlighted several beneficial effects, with raw milk resulting in increased PPO and GLU activity due to the high concentration of amino acids, which promote GLU activity, the presence of tyrosine (a PPO constituent), and glycine activity (Martins Filho 1987; Sudisha et al. 2011; Teixeira et al. 2017). In addition to raw milk, Fertisilício® also favored the phenolic compounds, increasing the content of protective enzymes such as PPO (Hasan et al. 2020).

The higher POX activity obtained with raw milk is attributed to the composition of this product since lactoperoxidase, an enzyme of the peroxidase family, is naturally found in the mammary glands of bovines. Moreover, the phosphate ion found in milk and applied to the leaves also significantly increases POX activity (Kussendrager and Van Hooijdonk 2000; Walters et al. 2005). High values of this enzyme are also important to ensure the balance of reactive oxygen species (Berger et al. 2016). From this perspective, another study reported a similar finding with millet after infection with downy mildew (*Sclerospora graminicola*) (Sudisha et al. 2011), with amino acids and raw milk promoting a fivefold increase in POX activity. The eliciting capacity of amino acids and raw milk also effectively reduced the severity of powdery mildew in *Vitis vinifera* L. and *Cucurbita pepo* L. (Bettiol 1999; Crisp et al. 2006).

Although the literature relates the application of silicon (Si) to the increase in enzyme activity only when applied to the roots (Dallagnol et al. 2015), the present study proved that foliar application also effectively increased QUI activity. The levels of this enzyme are usually low in plants, as observed in the control treatment. However, the application of inducers such as Fertisilício® favors enzyme activity through signal transduction, a biochemical process that induces plant defense and increases the activity of PR proteins such as QUI. QUI activity was also increased after the foliar application of Si under stress caused by *Sporisorium scitamineum* (Deng et al. 2020).

Larger leaf area on plants treated with raw milk likely is due mainly to phosphate and other salts (Ca, Fe, and Mg), components that induce cell division and growth (Epstein and Bloom 2006), and control of powdery mildew may have also contributed. Amistar Top® also increased leaf area, probably due to control of powdery mildew and perhaps also growth enhancement which has been documented for this and other strobilurin fungicides in some plants. Furthermore, raw milk positively affected fruit °Brix. As a potassium-rich material, raw milk plays a key role in solute transport through the phloem, favoring sugar accumulation in fruits (Rangel et al. 2018).

Raw milk was the best alternative method to control powdery mildew in melon, showing equivalent efficacy to chemical control (Amistar Top®), reducing disease severity by 49% compared to the control, and increasing the °Brix of melon by 19.5%. Copper Crop® also controlled powdery mildew in melon, reducing severity by 30%. Suppress-L™ and Fertisilício® were not effective for managing powdery mildew under the environmental conditions of this study. The present study highlighted that the activities of polyphenol oxidase, peroxidase, chitinase, and β-1,3-glucanase are involved with the plant defense mechanism of melon plants since the treatments with the highest enzyme activity values had the lowest powdery mildew severity. Among the treatments tested, raw milk promoted the highest activities of the studied enzymes.

Author contribution Performed the experiment: (A. L. A. F), (F. R. A. F), (T. R. C. A), (K. A. B), (I. V. P. S), (G. A. N.), (J. L. S. S), and (N. J. A. M). Coordinated the research project: (M. M. Q. A) and (A. L. A. F). Wrote the paper: (A. L. A. F), (F. R. A. F), (A. M. P. N), (R. S. J), and (M. M. Q. A). Performed the statistical analysis: (A. L. A. F), (F. R. A. F), and (A. M. P. N). Approved the final version and revision of the manuscript: (M. M. Q. A) and (A. L. A. F). Both authors read and approved the manuscript.

Funding The authors thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for granting the scholarships.

Data availability Data are available upon request.

Declarations

Conflict of interest The authors declare no competing interests.

References

Abdel-monaim MF, Ismail ME, Morsy KM (2012) Induction of systemic resistance in soybean plants against *Fusarium* wilts disease by seed treatment with benzothiadiazole and humic acid. African Journal of Biotechnology 11:2454–2465

Ahammed GJ, Yang Y (2021) Mechanisms of silicon-induced fungal disease resistance in plants. Plant Physiology Biochemistry 165:200–206

Alvares CA, Stape JL, Sentelhas PC, Gonçalves JDM, Sparovek G (2013) Ko¨ppen's climate classification map for Brazil. Meteorologische Zeitschrift 22:711–728

Barickman TC, Horgan TE, Wilson JC (2017) Efficacy of fungicide applications and powdery mildew resistance in three pumpkin cultivars. Crop Protection 101:90–94

Batista PF, Pires MMML, Santos JS, Queiroz SOP, Aragão CA, Dantas BF (2009) Produção e qualidade de frutos de melão submetidos a dois sistemas de irrigação. Horticultura Brasileira 27:246–250

Berger LRR, Stamford NP, Willadino LG, Laranjeira D, Lima MAB, Malheiros SMM, Oliveira WJ, Stamford TCM (2016) Cowpea resistance induced against *Fusarium oxysporum* f. sp. *tracheiphilum* by crustaceous chitosan and by biomass and chitosan obtained from *Cunninghamella elegans*. Biology Control 92:45–54

Bettiol W (1999) Effectiveness of cow's milk against zucchini squash powdery mildew (*Sphaerotheca fuliginea*) in greenhouse conditions. Crop Protection 18:489–492

Bettiol W, Astiarraga BD (1998) Control of *Sphaerotheca fuliginea* in zucchini squash with residue of glutamic fermentation of molasses and product of milk fermentation. Fitopatologia Brasileira 23:431–435

Bezerra Neto E, Barreto LP (2011) Análises químicas e bioquímicas em plantas. UFRPE, Recife

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248–254

Câmara MJT, Negreiros MZ, Medeiros JF, Bezerra Neto F, Barros Júnior AP (2007) Produção e qualidade de melão amarelo influenciado por coberturas do solo e lâminas de irrigação no período chuvoso. Ciência Rural 37:58–63

Campos ÂD, Ferreira AG, Hampe MMV, Antunes IF, Brancão N, Silveira EPD, Osório VA (2004) Augustin E (2004) Atividade de peroxidase e polifenoloxidase na resistência do feijão à antracnose. Pesquisa Agropecuária Brasileira 39:637–643

Chmielowska J, Veloso J, Gutiérrez J, Silvar C, Díaz J (2010) Cross-protection of pepper plants stressed by copper against a vascular pathogen is accompanied by the induction of a defence response. Plant Science 178:176–182

Crisp P, Wicks TJ, Troup G, Scott ES (2006) Mode of action of milk and whey in the control of grapevine powdery mildew. Australasian Plant Pathology 35:487–493

Cury TN, Maria IC, Bolonhezi D (2014) Biomassa radicular da cultura de cana-de-açúcar em sistema convencional e plantio direto com e sem calcário. Revista Brasileira de Ciência do Solo 38:1929–1938

Dallagnol LJ, Rodrigues FA, Tanaka FAO, Amorim L, Camargo LEA (2012) Effect of potassium silicate on epidemic components of powdery mildew on melon. Plant Pathology 61:323–330

Dallagnol LJ, Rodrigues FA, Pascholati SF, Fortunato AA, Camargo LEA (2015) Comparison of root and foliar applications of potassium silicate in potentiating post-infection defences of melon against powdery mildew. Plant Pathology 64:1085–1093

Deng Q, Wu J, Chen J, Shen W (2020) Physiological mechanisms of improved smut resistance in sugarcane through application of silicon. Frontiers in Plant Science 11:1–13

Ebrahim S, Usha K, Singh B (2011) Pathogenesis related (PR) proteins in plant defense mechanism. Science Against Microbial Pathogens 2:1043–1054

Epstein E, Bloom AJ (2006) Nutrição Mineral de Plantas: Princípios e perspectivas. Editora Planta, Londrina

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS - FAO (2021) The agricultural production domain covers. http://www.fao.org/faostat/en/#data/QCL. Acessado em 16 de Agosto de 2021

Faria GS, Verzignassi JS, Tessmann DJ, Lorenzetti ER, Gasparatto F (2011) Controle de oídio em pepino parternocárpico com produtos alternativos em cultivo protegido. Summa Phytopathologica 37:205–207

Hamann J, Krömker V (1997) Potencial of specific milk composition variables for cow health management. Livestock Production Science 48:201–208

Hasan KA, Soliman H, Baka Z, Shabana YM (2020) Efficacy of nano-silicon in the control of chocolate spot disease of *Vicia faba* L. caused by *Botrytis fabae*. Egyptian Journal of Basic and Applied Sciences 7:53–66

He LM, Cui K, Ma D, Shen R, Huang X, Jiang J, Um W, Liu F (2017) Activity, translocation, and persistence of isopyrazam for controlling cucumber powdery mildew. Plant Disease 101:1139–1144

Hyodo H, Yang SF (1971) Ethylene-enhanced synthesis of phenylalanine ammonia lyase in pea seedlings. Plant Physiology 47:765–770

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA - IBGE (2021) https://sidra.ibge.gov.br/tabela/5457#resultado. Acessado em 16 de Agosto de 2021

Kamel SM, Afifi MMI (2020) Controlling cucumber powdery mildew using cow milk and whey under greenhouse conditions. Egyptian Journal of Phytopathology 48:58–70

Kamel SM, Ketta HA (2017) Emeran AA (2017) Efficacy of raw cow milk and whey against cucumber powdery mildew disease caused by *Sphaerotheca fuliginea* (Schlecht.) Pollacci under plastic house conditions. Egyptian Journal of Biological Pest Control 27:135–142

Krämar W, Schirmer U (2007) Modern crop protection compounds. Wiley, Weinheim

Kussendrager KD, Van Hooijdonk ACM (2000) Lactoperoxidase: physicochemical properties, occurrence, mechanism of action and application. British Journal of Nutrition 84:19–25

Li H, Wang Y, Liu F, Yang Y, Wu Z, Cai H, Zhang Q, Wang Y, Li P (2015) Effects of chitosan on control of postharvest blue mold decay of apple fruit and the possible mechanisms involved. Scientia Horticulturae 186:77–83

Li B, Zhao Y, Zhu Q, Zhang Z, Fan C, Amanullah S, Gao P, Luan F (2017) Mapping of powdery mildew resistance genes in melon (*Cucumis melo* L.) by bulked segregant analysis. Scientia Horticulturae 220:160–167

Lins HA, Silva TS, Ribeiro RMP, Souza MF, Freitas MAM, Albuquerque JRT, Silva DV (2018) Crescimento inicial do melão após aplicação de herbicidas em pós-emergência. Revista Brasileira de Herbicidas 17:611

Martínez FD, Santos M, Carretero F, Marín F (2016) *Trichoderma saturnisporum*, a new biological control agent. Journal of the Science of Food and Agriculture 96:1934–1944

Martins Filho J (1987) Como e porque amamentar seu filho. Editora Sarvier, São Paulo

Medeiros FHV, Bettiol W, Souza RM, Alves E, Pinto ZV, Lost R (2012) Microorganisms, application timing and fractions as players of the milk-mediated powdery mildew management. Crop Protection 40:8–15

Pandey S, Ansari WA, Atri N, Singh B (2016) Standardization of screening technique and evaluation of muskmelon genotypes for drought tolerance. Plant Genetic Resources 16:1–8

Porto MAF, Ambrósio MMQ, Nascimento SRC, Cruz BLS, Torres TM (2019) Interaction of *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* as root rot pathogens of *Cucumis melo*. Summa Phytopathologica 45:355–360

Ramos ARP, Santos RL, Amaro ACE, Fumes LAA, Boaro CSF, Cardoso AII (2013) Eficiência do silicato de potássio no controle do oídio e no desenvolvimento de abobrinha de moita. Horticultura Brasileira 31:432–438

Rangel PP, Pérez LS, Robles MAG, Espinoza FHR, Garay AVA, Hernández MF, Amador BM (2018) Increasing doses of potassium increases yield and quality of muskmelon fruits under greenhouse. Horticultura Brasileira 36:184–188

Rur M, Rämert B, Hökeberg M, Vetukuri RR, Grenville-briggs L, Liljeroth E (2018) Screening of alternative products for integrated pest management of cucurbit powdery mildew in Sweden. European Journal of Plant Pathology 150:127–138

Sales Júnior R, Senhor RF, Michereff SJ, Negreiros AMP (2019) reaction of melon genotypes to the root´s rot caused by *Monosporascus*. Revista Caatinga 32:288–294

Sarhan EAD, Abd-elsyed MHF, Ebrahiem AMY (2020) Biological control of cucumber powdery mildew (*Podosphaera xanthii*) (Castagne) under greenhouse conditions. Egyptian Journal of Biological Pest Control 30:1–7

Soliman MH, El-mohamedy RSR (2017) Induction of defense-related physiological and antioxidant enzyme response against powdery mildew disease in okra (*Abelmoschus esculentus* L.) plant by using chitosan and potassium salts. Mycobiology 45:409–420

Souders CL II, Xavier P, Rodriguez VP, Ector N, Zhang J, Martyniuk CJ (2019) Sub-lethal effects of the triazole fungicide propiconazole on zebrafish (Danio rerio) development, oxidative respiration, and larval locomotor activity. Neurotoxicology and Teratology 74:106809

Stangarlin JR, Pascholati SF, Labate CA (2000) Efeito de *Phaeoisariopsis griseola* na atividade de ribulose-1,5-bifosfato carboxilase-oxigenase, clorofilase, μ-1,3-glucanase e quitinase em cultivares de *Phaseolus vulgaris*. Fitopatologia Brasileira 25:59–66

Sudisha JKA, Amruthesh KN, Niranjana SR, Shetty HS (2011) Elicitation of resistance and defense related enzymes by raw cow milk and amino acids in pearl millet against downy mildew disease caused by *Sclerospora graminicola*. Crop Protection 30:794–801

Teixeira WF, Fagan EB, Soares LH, Umburanas RC, Reichardt K, Dourado Neto D (2017) Foliar and seed application of amino acids affects the antioxidant metabolism of the soybean crop. Frontiers in Plant Science 8:1–14

Torre AL, Iovino V, Caradonia F (2018) Copper in plant protection: current situation and prospects. Phytopathologia Mediterranea 57:201–236

Walters D, Walsh D, Newton A, Lyon G (2005) Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. Phytopathology 95:1368–1373

Wang Y, Wu D, Huang J, Tsao S, Hwu K, Lo H (2016) Mapping quantitative trait loci for fruit traits and powdery mildew resistance in melon (*Cucumis melo*). Botanical Studies 57:19

Wang S, Yan W, Yang X, Zhang J, Shi Q (2021) Comparative methylome reveals regulatory roles of DNA methylation in melon resistance to *Podosphaera xanthii*. Plant Science 309:110954

Wei T, Simko V, Levy M, Xie Y, Jin Y, Zemla J (2017) R Package, "corrplot." Statistician 56:24

Yuste-lisbona FJ, López-sesé AI, Gómez-guillamón ML (2010) Inheritance of resistance to races 1, 2 and 5 of powdery mildew in the melon TGR-1551. Plant Breeding 19:72–75

Zatarim M, Cardoso AII, Furtado EL (2005) Efeito de tipos de leite sobre oídio em abóbora plantadas a campo. Horticultura Brasileira 23:198–201