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Morinda citrifolia compounds such elicitor substances of biochemical responses in melon plants

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Abstract

Plants have complex structural and biochemical defense mechanisms that can be activated before or after the attack of phytopathogenic microorganisms. Among these mechanisms are the proteins related to the pathogenesis (PRP) and the hypersensitive response through the production of reactive oxygen species (ROS), which exist in enzymes capable of reducing its toxicity to the plant. In addition to activation induced by biotic or abiotic factors, such as compounds or substances that stimulate biochemical activity, called elicitor substances, such as essential oils and their constituents. Studies in the area are necessary to deepen knowledge and reach the technical and economic feasibility of its use for the benefit of sustainable agriculture. The aim was to evaluate the enzymatic activity of melon plants submitted to the noni essential oil application (EO) and octanoic acid (OA). In different treatment concentrations, as well as fungicide Cerconil® (0.2%) and Yantra® plant stimulant. The enzymes that had their activity evaluated were superoxide dismutase (SOD), chitinase (QUIT), ascorbate peroxidase (APX), and catalase (CAT). Octanoic acid (0.1%) and essential oil (0.5%) were the primary activators of the enzyme superoxide dismutase among the treatments tested. The enzyme ascorbate peroxidase had its production increased by the octanoic acid at 0.1%. EO was the primary activator of the catalase enzyme at 2% concentration. The secondary metabolites activity of the *Morinda citrifolia* against microorganisms and as an activator of the defense system were confirmed, considered a potential compound for developing a bioproduct as a substitute for pesticides.

Keywords: bioproducts, Cucumis melo L., essential oil, natural compounds, reactive oxygen species

Introduction

Plants are often attacked by pathogens such as fungi, bacteria, viruses, nematodes, and insects. However, to survive, they have defense strategies that hinder or even prevent the penetration and infection of phytopathogenic agents (Fernandes et al., 2009; Shittu et al., 2019). They also react under abiotic stress (Deuner et al., 2015; He et al., 2018) or with direct action, inhibiting the pathogen or spore germination growth. Represents a simplified conception of pre-infection plant defense against the phytopathogens input (Stangarlin et al., 2011; Hashemi et al., 2021).

Between the plants' defense mechanisms induced inside of infection or abiotic stimulus stands out pathogenesis-related proteins (PRP) (Jain et al., 2018). Among PRP are chitinases, which can digest chitin which constitutes a primary compound of the insect exoskeleton and cell wall in many phytopathogens (Zarei et al., 2011; Wang et al., 2021).

Another form that plants have to defend themselves is through reactive oxygen species (ROS) production (Choi et al., 2017; Tomar et al., 2021). It consists of a hypersensitive response of the plant reacting to the microorganisms' attack or under contact with compounds that stimulate ROS activation. They can be in the singlet oxygen form ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), and superoxide anion ($O_2^{\cdot-}$) (Mittler, 2017). ROS are produced as by-products of regular cell metabolism but in small quantities. What causes the increase is the electron transport system destruction during stressful conditions.

ROS lead to the biomolecules' oxidation with a consequent loss of their biological functions and homeostatic imbalance, causing damage to plant cells and tissues like fungi (Halliwell and Whiteman 2004;

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Hasanuzzaman et al. 2021). The ROS cytotoxicity is attenuated in plants utilizing antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) (Gill et al. 2015; Sofo et al. 2015; Del Río et al. 2018). These remain inactive until they receive stimuli through the resistance-inducing signals.

Research on the natural substances used to induce defense has increased in different areas and has become essential in agriculture. Among the diversity of medicinal compounds contained in tropical biomes, noni (Morinda citrifolia) can be highlighted, one Rubiaceae of Polynesie origin. The essential oil and the primary compound (octanoic acid) are necessary due to their high fungitoxic capacity, efficiency, and yield (Dalcin et al., 2021; Osorio et al., 2018; Silva et al., 2017).

The search for compounds capable of reducing pesticide use and that allows achieving more excellent knowledge of the technical and economic viability of its use for the benefit of sustainable agriculture led to this work realization which aimed to evaluate the melon plants' enzymatic responses as a function of noni (Morinda citrifolia) essential oil application and octanoic acid.

Material And Methods

Melon plants (Cucumis melo L.) cultivation for carrying out the test

Melon plants (Cucumis melo L.), cultivar Eldorado 300, were grown in plastic vases with a five liters capacity filled with commercial substrate + sieved soil in a greenhouse environment. The design used was completely randomized with three replications in a factorial scheme where each repetition was represented by three pots containing a plant.

Material collection and essential oil extraction

Morinda citrifolia L. (noni) plants were identified in the Gurupi-Tocantins region for fruit collection. The essential oil was extracted from the ripe fruits by hydrodistillation in a modified Clevenger apparatus and stored at 4 ° C until analysis.

Treatments

Tests were carried out using the essential oil major compound, octanoic acid, commercially acquired (Sigma-Aldrich®). For the potential elicitor tests, essential oil concentrations and octanoic acid were prepared (0.05, 0.10, 0.50, 1.00, 2.00%), added Tween 80 (1%), and sterile distilled water. As a relative control was used, the fungicide Cerconil® (20% methyl thiophanate + 50% chlorothalonil) was at the concentration recommended by the manufacturer (0.2%). Another relative control is the vegetable stimulant Yantra® (potassium phosphite - K_2O: 26% e P_2O_4: 33,6%). As an absolute control, plants spread with water. The amount (10 mL) of the solution was previously established by spraying a sample until all the leaves homogeneously received the product until the dripping point. The treatments were sprayed on melon plants (Cucumis melo L.) twenty-five days after sowing with a hand spray.

Enzymatic activities

For the enzymatic activity determination, melon plants (Cucumis melo L.) were used 25 days after being sown. Samples were prepared 24 hours after treatment application, weighing 267 mg of fresh melon leaves, macerated in liquid nitrogen with 20% polyvinylpolypyrrolidone (PVPP). Then, were added of 1.5 mL extraction buffer (100 mM potassium phosphate buffer (pH 7.0), 1 mM EDTA, and 1 mM ascorbate at the buffer volume used). Finally, the samples were centrifuged at 10.000 rpm for 25 minutes at 4ºC ± 1ºC. The supernatant was collected (protein extract) and stored at -20ºC for analysis.

Superoxide Dismutase (SOD)

SOD activity was measured by adding 0.1 mL of protein extract, 0.1 mL of 50 Mm potassium phosphate buffer (pH 7.8), 0.02 mL of 0.1 mM EDTA, 0.4 mL of 70 mM L-methionine, and 0.2 mL of 1 mM NBT. The reaction was initiated by adding 2 mM riboflavin and fast tube transfer, without light protection, for a chamber illuminated by a 30-Watt lamp (30 µmol de fótons $m^{-2}s^{-1}$) for 7 minutes (Del Longo et al., 1993). Absorbance was measured at 540 nm (BioSpectro model SP-220) (Giannopolotis and Ries, 1977). According to (Beauchamp and Fridovich, 1971), the activity unit was defined as the enzyme amount required to inhibit 50% NBT reduction and the activity expressed in units per gram per mass of extract per minute (U g^{-1} E^{-1} min^{-1}).

Chitinase (QUIT)

To determine the QUIT activity, 0.2 mL of the protein extract was added, 0.6 mL of 0.1 M sodium acetate buffer (pH 5.0), and 0.2 mL of "CM-chitin-RBV®" at 2.0 mg mL^{-1}. Then, it was incubated at 40 °C for 20 minutes. The reaction stopped by adding 0.2 ml of 1 M HCL, cooling on ice, and centrifuging at 10,000 rpm for 5 minutes (Wirth & Wolf, 1990). The reaction supernatant had its absorbance measured at 550 nm (BioSpectro model SP-220). The enzyme activity was expressed in absorbance per minute units (U min^{-1}).

Catalase (CAT)

To determine CAT activity, 0.05 mL of protein extract was added; 2.95 mL of 50 mM potassium phosphate buffer (pH 7.8) was added to 20 mM hydrogen peroxide. The readings were noted according to the absorbance decline. The activity was measured at 240 nm (BioSpectro model SP-220) for 300 seconds, with readings taken every 15 seconds and calculated based on the molar extinction coefficient of 35 M-1 cm-1 (240 nm) and expressed in hydrogen peroxide micromole per gram per extract per minute (μmol H_2O_2 g^{-1} E^{-1} min^{-1}) (Havir and Mchale, 1987).

Ascorbate Peroxidase (APX)

The APX enzyme activity was performed by mixing 0.1 mL of the protein extract, 2.7 mL of ascorbate buffer (ASA) 0.5 mM, and 0.2 mL of 30 mM hydrogen peroxide. The absorbance was measured at 290 nm (BioSpectro model SP-220), 25 °C, due to the hydrogen peroxide degradation. APX activity is expressed in ascorbate micromol per gram per extract per minute (μmol ASA g^{-1} E^{-1} min^{-1}) (Asada and Takahashi, 1987).

Statistical analysis

Variance analysis was performed for all characteristics and mathematical models adjusted to quantitative treatments and comparison tests of means to qualitative ones. Quantitative data (Figures A) were preliminary for carrying out the qualitative comparison (Figures B) only with the concentrations that most stood out for each enzyme for octanoic acid and essential oil. All analyzes were performed using the Sisvar software (Ferreira, 2014).

Results and Discussion

According to the results obtained for the QUIT enzyme activity, it can be observed (**Figure 1**A) that in plants with noni essential oil treatment (EO), the values started with 442 U min^{-1}, attaining a maximum of 445.2 U min^{-1}, at a concentration of 0.5%. From this concentration, the enzymatic activity decreased to 442.1 U min^{-1} in treatment at 2%.

The octanoic acid activity (OA) has a similar trend reaching the highest value at 0.1% (449.6 U min^{-1}), decreasing up to 440.1 U min^{-1} by 2%.

Comparing the treatments in figure 1B, it can be seen that there was no statistically significant difference. Therefore, as they did not differ from the control, it can be said that the QUIT activity was not significantly enhanced with EO application, OA, fungicide, and potassium phosphite (vegetable stimulant).

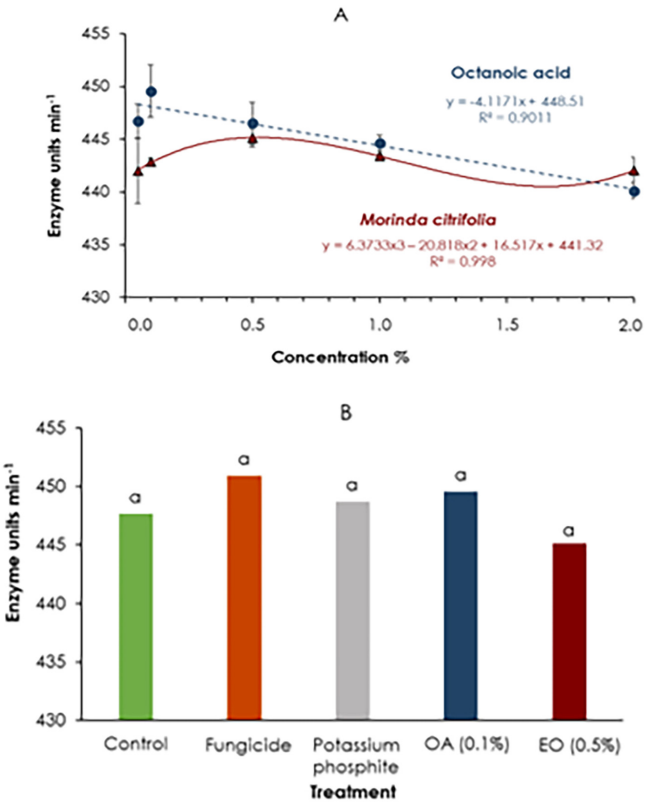

Figure 1. A: Chitinase enzymatic activity in melon leaves as a function of noni essential oil concentrations (EO) and octanoic acid (OA). B: Chitinase enzymatic activity comparison between control, fungicide, potassium phosphite, and the EO and OA highest values.
Means with the same letter do not differ by Tukey's test ($p < 0.05$), (U min^{-1})- Enzyme units per minute.

SOD enzyme activity results and melon plants submitted to alternative treatments are shown in (**Figure 2**).

In Figure 2A, it is possible to observe a concentrations polynomial trend. For example, in the EO 0.05% concentration, SOD started with 40.1 U g^{-1} E^{-1} min^{-1}, reaching the maximum value in the concentration of 0.5% (75.4 U g^{-1} E^{-1} min^{-1}). From that concentration, the enzyme activity decreased to 40.4 U g^{-1} E^{-1} min^{-1}, in the highest concentration (2%). For OA, the highest SOD value was obtained at a concentration of 0.1% (88.8 U g^{-1} E^{-1} min^{-1}), reducing approximately seven units by 0.5%. There was a significant decrease in SOD activity from this concentration until staying at 16.1 U g^{-1} E^{-1} min^{-1}, at a concentration of 2%.

In Figure 2B, the EO and OA concentrations obtained the highest SOD activity compared to the control (without application), fungicide, and treatment with potassium phosphite. In the comparison performed through the means of the Tukey test ($p < 0.05$), EO and OA were significantly more efficient for SOD enzymatic activation, followed by potassium phosphite (53.4 U g^{-1}

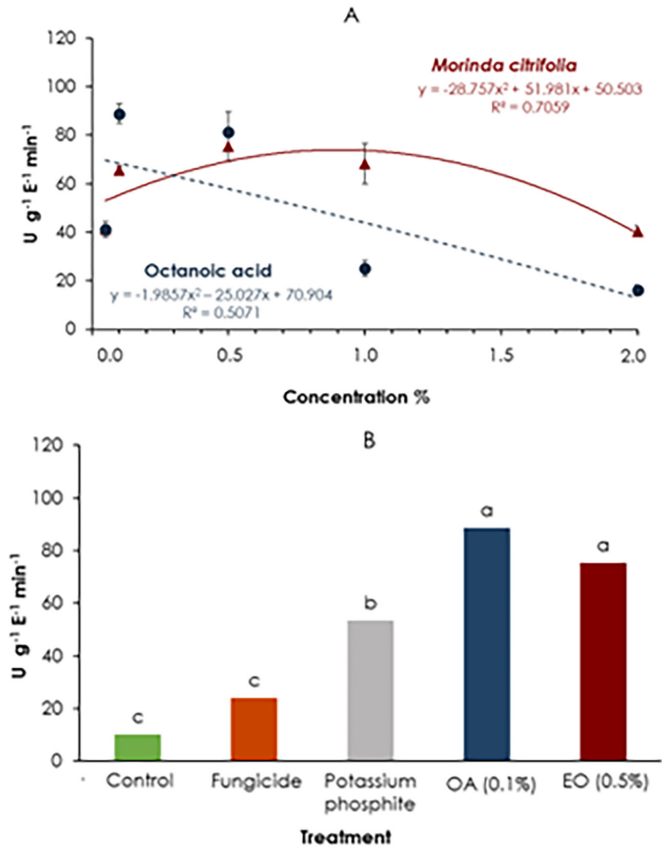

Figure 2. A: Superoxide Dismutase (SOD) enzymatic activity in melon leaves as a function of noni essential oil concentrations (EO) and octanoic acid (OA). B: Superoxide Dismutase enzyme activity comparison between control, fungicide, potassium phosphite, and the EO and OA highest values.
Means with the same letter do not differ by Tukey's test ($p < 0.05$), (U g^{-1} E^{-1} min^{-1}) - Enzyme unit per gram of extract per minute.

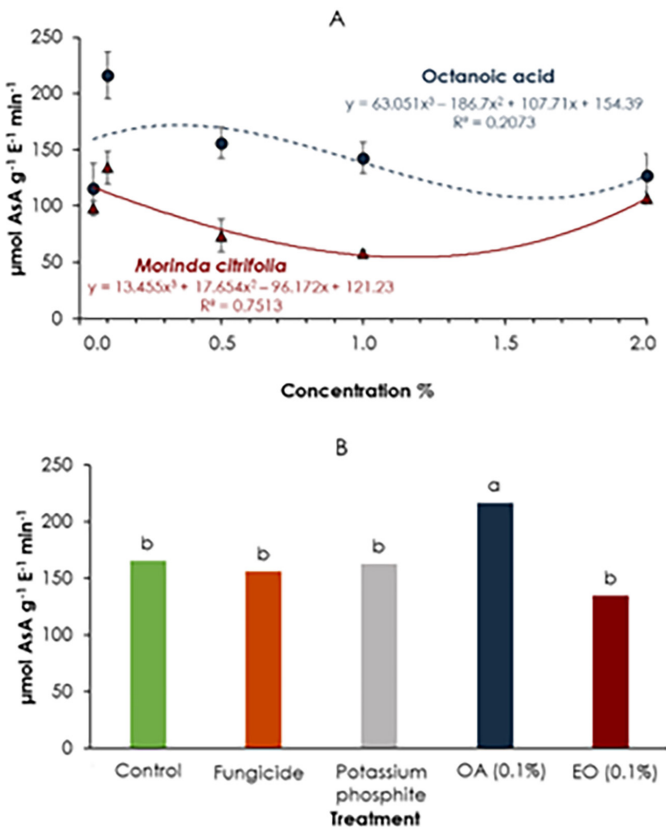

Figure 3. A: Ascorbate Peroxidase (APX) enzymatic activity in melon leaves as a function of noni essential oil concentrations (EO) and octanoic acid (OA). B: Ascorbate Peroxidase (APX) enzymatic activity comparison between control, fungicide, potassium phosphite, and the EO and OA highest values.
Means with the same letter do not differ by Tukey's test ($p < 0.05$), (µmol ASA g^{-1} E^{-1} min^{-1}) – Ascorbate micromol per gram of protein extract per minute.

E^{-1} min^{-1}). Fungicide treatment (24 U g^{-1} E^{-1} min^{-1}) and the control (10.4 U g^{-1} E^{-1} min^{-1}) obtained the lowest values for SOD activity.

Figure 3A shows the APX activity from a polynomial for both treatments. The enzymatic variation between concentrations was short in plants sprayed with EO.

For EO at the 0.05% concentration, the APX enzyme activity started with 98.1 µmol AsA g^{-1} E^{-1} min^{-1}. It achieved 134.2 µmol AsA g^{-1} E^{-1} min^{-1} in the 0.1% concentration and reduced to 58.0 µmol AsA g^{-1} E^{-1} min^{-1} to 1% of the EO. It was observed that the APX activity increased again at 2%, with 107.0 µmol AsA g^{-1} E^{-1} min^{-1}. OA treatments had only one peak, a concentration at 0.1% (216.3 µmol AsA g^{-1} E^{-1} min^{-1}), decreasing its values from that point up to 127.1 µmol AsA g^{-1} E^{-1} min^{-1}, in concentration at 2%.

The most effective EO and OA concentrations were 0.1% for both treatments. Compared with the other treatments in Figure 3B, these values demonstrate that OA was more efficient in activating the APX enzyme, differing statistically from the other treatments.

EO concentrations were shown to be more effective than OA concentrations in activating CAT (**Figure 4**).

For EO, CAT enzyme activity values started at 34.3 µmol H_2O_2 g^{-1} E^{-1} min^{-1}, in the 0.05% concentration, and achieved 46.1 µmol H_2O_2 g^{-1} E^{-1} min^{-1}, in 2%. On the other hand, activation by OA was 31.9 µmol H_2O_2 g^{-1} E^{-1} min^{-1}, in the lowest concentration, up until 36.1 µmol H_2O_2 g^{-1} E^{-1} min^{-1} to the highest.

The most excellent enzyme activity caused by EO was observed in Figure 3B. Again, the treatment differed significantly from the control, demonstrating a value of 27.7 µmol H_2O_2 g^{-1} E^{-1} min^{-1}.

Plants have defense systems responsible for recognizing specific structures so that, when faced with different types of stress, they induce the first barriers that prevent any damage. For example, the first hydrolase recruitment responsible for the recognition of chitin present in the fungal cell wall constitutes the plant's first line of defense (Martínez-Cruz et al., 2021). With the chitin oligomers identified in contact with the host plant,

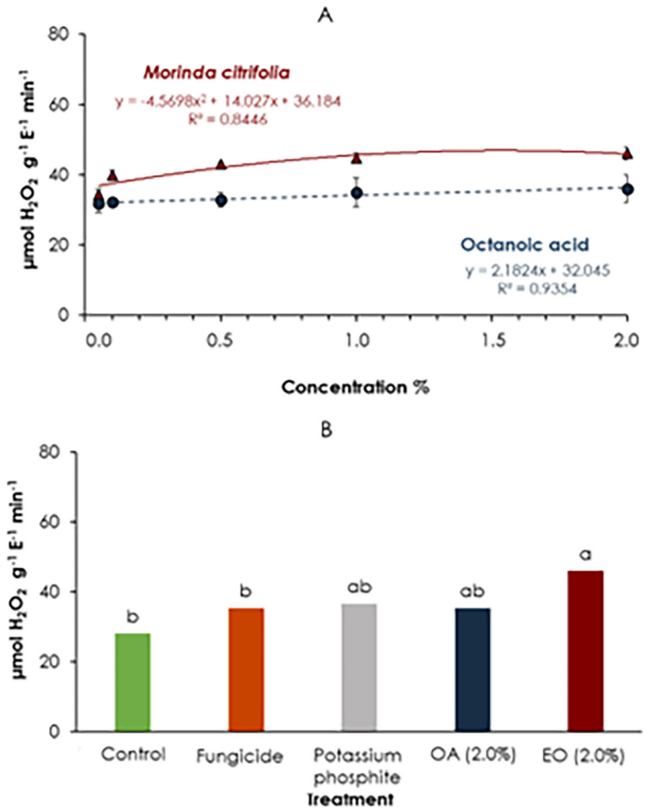

Figure 4. A: Catalase (CAT) enzymatic activity in melon leaves as a function of noni essential oil concentrations (EO) and octanoic acid (OA). B: Catalase (CAT) enzymatic activity comparison between control, fungicide, potassium phosphite, and the EO and OA highest values.
Means with the same letter do not differ by Tukey's test ($p < 0.05$), (µmol H_2O_2 g^{-1} E^{-1} min^{-1}) - Hydrogen peroxide micromol per gram of protein extract per minute.

the hydrolases stimulate the reactive oxygen species accumulation and reinforce the plant cell wall to prevent phytopathogen action (Gao et al., 2019).

The treatments applied to the melon plants did not demonstrate significant QUIT activity since all the evaluated plants had a high number of units of the enzyme. In addition, potassium phosphite, a plant stimulant used as a foliar fertilizer, is known to be a PRP protein activator (Agrichem, 2019). Yet, this stimulating effect was not observed for chitinase activity in the present work. Furthermore, this stimulating effect was not observed compared to the control.

The study by (Cardoso et al., 2017) demonstrated that potassium phosphite reduced the nematode incidence in soybeans by stimulating some other PRP protein production. This indicates that even without a specific increase in this enzyme can induce an immune response by activating other mechanisms. And in the face of abiotic stress, plants similarly increase the number of available enzymes.

Noni essential oil and octanoic acid could not significantly increase chitinase production compared to other treatments. Nevertheless, further studies with different EO have been described in the literature as being efficient antifungals. For example, (Sellamuthu et al., 2013) described that thyme EO effectively increased the QUIT enzymatic activity in avocado fruits, followed by citronella and mint essential oil. Another report demonstrates an increase in chitinase in corn plants observed with the Jacaranda mimosifolia plant extract application against Fusarium verticillioides (Naz et al., 2021).

On the other hand, the treatments were significantly effective in the SOD enzyme activity increase, mainly the EO and OA. This enzyme has the initial function of detoxification the plant. It transforms the superoxide anion into hydrogen peroxide, consumed by catalase and ascorbate peroxidase, producing water and oxygen (Buchanan et al., 2000; Soares and Machado, 2007). (Höferl et al., 2014) evidenced SOD and CAT eliciting activity using EO from Juniperus communis L. The potassium phosphite activity was also observed, however, in fewer quantities. Therefore, the ROS detoxifying action is one of the presupposed that the product is intended for.

The enzyme Ascorbate Peroxidase (APX) activity was stimulated by octanoic acid (OA). In other studies, some other compounds present in the several EO constitution were tested as antioxidant activity stimulants in raspberries. (Jin et al., 2012) developed the work using carvacrol, anethole, cinnamic acid, perillaldehyde, cinnamaldehyde, and linalool. All efficiently increased SOD activity in treatment with plants grown in an organic system. Carvacrol, anethole perillaldehyde, and cinnamaldehyde were efficient in stimulus APX. There are no OA reports as a defense mechanisms stimulant in plants. However, (Aranega-Bou et al., 2014) stated that hexanoic acid is a potent inducer of resistance in plants. This product belongs to the same chemical group as OA and is the second largest component in the noni EO constitution.

EO was the only treatment increasing the plant's antioxidant capacity through CAT enzyme production. The increase in activity of this same enzyme was also detached by (Ferreira et al., 2018). When using Lippia sidoides essential oil, compared with endophytic fungi found in the same plant, they obtained more than twice the CAT enzymatic activity.

With the results obtained from the present work, it can be said that alternative products from natural compounds were effective in stimulating the melon plants' antioxidant activity. Thus, the results demonstrated activating the plants' defense mechanism against

phytopathogens with alternative products is possible. These represent essential tools in the plant's protection against phytopathogenic microorganisms. The results also allow us to infer that its use may help reduce the use of pesticides in crops.

Conclusion

Noni EO and octanoic acid treatments efficiently induce physiological and biochemical responses that confer increased immunity with preventive treatments. Furthermore, the results were promising for controlling pathogens in melon plants with alternative products in concentrations below 2%. This is done by inducing significant amounts of enzymes in the plant detoxification process.

These results suggest that the compounds of Morinda citrifolia secondary metabolism are promising for the development of products, acting as an activator of the plant defense system against harmful microorganisms and consequent decrease in the use of pesticides.

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