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Birational control of corn leafhopper and activation of resistance with compounds from *Morinda citrifolia.*

Controle biorracional de cigarrinha do milho e ativação de resistência com compostos de *Morinda citrifolia*.

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ABSTRACT

The potential of plant compounds in pests and diseases alternative control has been widely researched. This study is the first to investigate the use of the plant *Morinda citrifolia* L.(noni) for managing maize leafhoppers, a major issue in plantations. The effects of essential oil and octanoic acid on resistance induction were evaluated with quantification of phytoalexins, potential phytotoxicity, and toxicity to corn leafhopper, *Dalbulus maidis*. The chromatographic analysis identified octanoic acid as the primary compound, constituting 58.43% of the essential oil. The elicitor potential was more pronounced in plants treated with *M. citrifolia* compounds than in those treated with commercial resistance-activating products. The essential oil effectively controlled *D. maidis*, resulting in 50% mortality after 48 hours without damaging the leaf area. These findings highlight the potential of *M. citrifolia* in the biorational control of the stunting complex, with effective vector control and resistance induction.

Keywords: Alternative control, *Dalbulus maidis*, Noni, Octanoic acid, Phytoalexins.

RESUMO

O potencial dos compostos vegetais no controle alternativo de pragas e doenças tem sido amplamente pesquisado. Este estudo é o primeiro à investigar o uso da planta *Morinda citrifolia* L. (noni) no manejo de cigarrinhas do milho, um grande problema nos cultivos. Os efeitos do óleo essencial e do ácido octanóico na indução de resistência foram avaliados com a quantificação de fitoalexinas, potencial de causar fitotoxicidade e toxicidade para cigarrinha do milho, *Dalbulus maidis*. A análise cromatográfica identificou o ácido octanóico como o principal composto, constituindo 58,43% do óleo essencial. O potencial elicitor foi mais pronunciado em plantas tratadas com compostos de *M. citrifolia* do que naquelas tratadas com produtos comerciais de ativação de resistência. O óleo essencial controlou efetivamente *D. maidis*, resultando em 50% de mortalidade após 48 horas sem danificar a área foliar. Essas descobertas destacam o potencial de *M. citrifolia* no controle biorracional do complexo de nanismo, com controle efetivo do vetor e indução de resistência.

Palavras-chave: Ácido octanóico, Controle alternativo, *Dalbulus maidis*, Fitoalexinas, Noni.

INTRODUCTION

With the increasing challenge of emerging diseases in corn cultivation, the search for sustainable and effective alternatives becomes increasingly crucial. The increase in population density and the necessary global supply of the second most important commodity for Brazil (OLIVEIRA et al., 2022) have driven the cultivation of corn (*Zea mays* L). It presents itself as a multifunctional product in agri-food systems and this expands its demand. However, the increase in cultivation area and reduction of fallow time have stimulated the emergence of emerging diseases (SABATO et al., 2020).

The occurrence of diseases and pests, such as the stunting transmitted by *Dalbulus maidis*, have shown a gradual increase over the years, causing losses of up to 90% in final yield (HAMZAT et al., 2022). The stunting complex is caused by pathogens of the mollicutes class, specifically *Spiroplasma kunkelii* and *Maize bushy stunt phytoplasma* (COSTA et al., 2023). With cultivation at different times, migratory capacity and constant exposure to non-specific molecules tend to intensify the problem of insect resistance emergence (OLIVEIRA et al., 2022).

The pesticides in use present a series of harmful effects, which include ecological risks, toxicity, insect resistance (ROSIC et al., 2020) and food contamination (GERAGE et al., 2017). Given this, the use of natural compounds with multiple functions emerges as a promising strategy (VELOSO et al., 2020). Among the species with potential use in alternative controls, Noni (*Morinda citrifolia* L.) stands out due to its fungistatic and insecticidal properties (NOSÉ et al., 2021). In the context of the crops to be treated, the mobilization of structural barriers and the induction of resistance are actions combined by several factors. These protection mechanisms can be signaled by the synthesis of phytoalexins, which are important indicators of plant immunity and act in the protection and increase of plant resistance (MATIELLO & BONALDO, 2013).

Therefore, the research aimed to seek alternative ways in the management of insect pests, contribute with technical knowledge, ensure food safety and low environmental impact. Evaluate the induction of resistance with the spraying of noni essential oil and octanoic acid, analyze possible toxic effects in corn plants and the mortality of *D. maidis*. To establish acceptable standards of the use of potential biorationals, such as active principles of products, more studies are needed focused on the viability and efficacy of these. The use of *M. citrifolia* compounds in corn leafhopper has not been reported, making this study even more relevant in the prospecting of new potentially active molecules.

MATERIALS AND METHODS

Hydrodistillation and sample preparation

Morinda citrifolia L. plants were collected from the Gurupi region, Tocantins, Brazil. The essential oil was extracted from the ripe fruits by steam hydrodistillation in a modified Clevenger-type apparatus and stored at 4 °C until analysis. The octanoic acid, was purchased from an industry specialized in the production of this isolated compound (Sigma–Aldrich®, São Paulo/SP, Brazil). For the elicitor tests, essential oil and octanoic acid concentrations were prepared adding Tween 80.

Gas chromatography-mass spectrometry (GC-MS) analysis

Qualitative analyses were performed through gas chromatography coupled with mass spectrometry (GC-MS) using the Shimadzu QP2020 model equipped with a mass detector model QP2010 Plus operated under the programmed temperature 50–280 °C (3 °C/min), silica capillary column (30 m × 0.25 mm × 0.25 μm), helium carrier gas, splitless injection of solution in hexane, mass spectrometer with an impact energy of 70 eV. The spectra were compared with those from the National Institute of Standards and Technology (NIST) and Wiley 229 library databases. The retention index of each constituent was compared with the standards by Adams (2007) and values were expressed as percentages.

Phytotoxicity of essential oil of *M. citrifolia*

Corn seeds (cultivar 30A37-PM) were cultivated in 8-L pots with soil and commercial substrate in equal parts. The experiment was carried out under greenhouse. Thirty days after sowing, manual spray triggers were used to apply 10 mL of 0.25–3% essential oil solutions in each pot. After 24 h of application, the scale of phytotoxicity was determined according to Sarmento-Brum et al. (2014): 0% = absence of phytotoxicity, 1–25% = mild leaf necrosis or mild chlorosis of the plant, 26–50% = moderate leaf necrosis or moderate chlorosis of the plant, 51–75% = high leaf necrosis or high chlorosis of the plant, and 76–100% = wilt and dryness of the plant.

Effect of *M. citrifolia* essential oil and octanoic acid on the phytoalexins induction

Phytoalexins were quantified following Bonaldo et al. (2004). Seeds of *Sorghum bicolor* (cultivar Buster (Atlântica Sementes®)) were wrapped in moistened germination paper and incubated in the dark (96h). They were then exposed to light (4h) for spraying essential oil and octanoic acid concentrations 0.625-7.5 mg mL^{-1}, Biozyme® (Arysta Lifescience, Salto de Pirapora/SP, Brazil) was used as a positive control. The seedlings were maintained under fluorescent light (60h), the mesocotyls were then excised, weighed, added to 80% acidified methanol (0.1% HCl), and stored (96 h, 4°C) for absorbance reading at 480nm.

To quantify glyceolin, soybean seeds (Glycine max, cultivar Monsoy 8644-IPRO, Intacta®) were sown in trays containing a sand and kept in a greenhouse for 10 days. Cotyledon fragments were placed on moistened filter and sprinkled with essential oil and octanoic acid concentrations and Acorda® (JUMA AGRO®, Mogi Guaçu/SP, Brazil). Kept in the dark (20 h), were weighed and placed in water under orbital agitation (150 rpm, 1h). The supernatant absorbance was read in a spectrophotometer at 285 nm (LORENZETTI et al., 2018). All treatments were performed in triplicates.

Essential oil toxicity of *M. citrifolia* on *D. maidis*

The *Dalbulus maidis* collected from crops in the region were kept in cages with anti-aphid screens in a greenhouse at room temperature. After identification, healthy corn plants with four expanded leaves were placed for oviposition. Later adult insects were collected for laying for 4 to 6 days, with a small number of insects per greenhouse. After the hatching of the nymphs, approximately 12 days, new plants were added to maintain the creation.

Five adult insects were collected and placed in Gerbox boxes covered with organza fabric, contained corn leaves for feeding, these leaves had moistened cotton swabs positioned transversely to avoid dehydration 750 µL of each treatment concentrations of 0.25-3.0 µg mL^{-1} were sprayed. Survival was assessed in triplicate for 72 h at a 24-h interval.

Statistical analysis

The experiment was entirely randomized, the results for phytotoxicity, and phytoalexins were subjected to regression analysis using the curve-fitting procedures in SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA). The models were selected using the parsimony criterion, and the assumptions of normality and homogeneity of variance were verified.

RESULTS

Essential oils were extracted from ripe fruits using hydrodistillation. The chromatographic analysis showed that the highest composition was of carboxylic acids, including octanoic acid and derivatives (65.69%) and hexanoic acid and derivatives (23.57%). Table 1 shows compounds with peak area above 1%.

Table 1 - *Morinda citrifolia* essential oil components obtained using chromatography coupled to mass spectrometry (GC-MS).

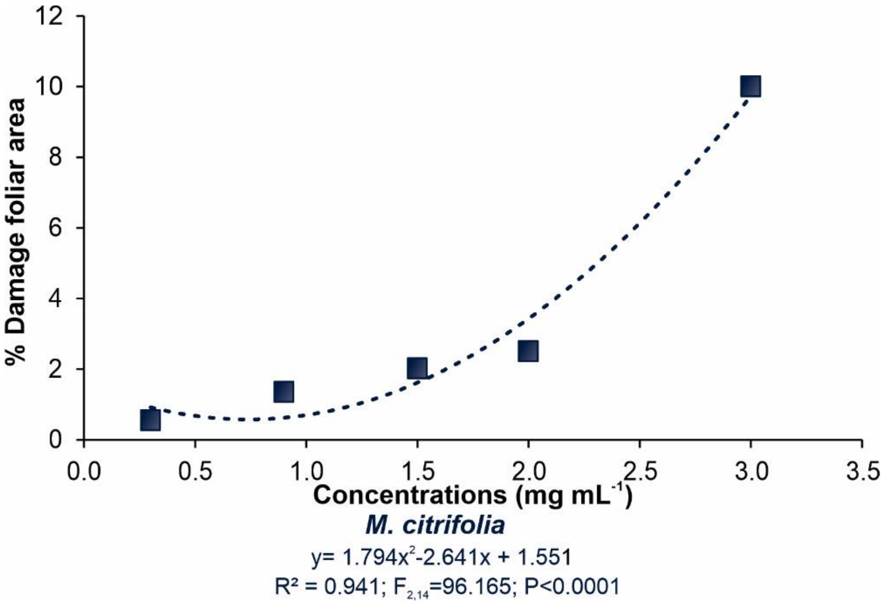
CHEMICAL CLASS	COMPOUND NAME (IUPAC)	%	R.T. (MIN)	I.T.	CAS NUMBER
Fatty Acid	Octanoic acid	58.43	14.239	12.78	124-07-2
Fatty Acid	Hexanoic acid	9.46	8.435	7.81	53896-26-7
Fatty Acid Esters	Pent-4-enyl hexanoate	8.17	14.449	14.35	30563-33-8
Fatty Acid Methyl Ester	Methyl octanoate	7.26	10.606	10.52	111-11-5
Fatty Acid Methyl Ester	Methyl hexanoate	4.63	5.305	5.265	106-70-7
Carbonate	3-methylbut-3-enyl 2-methylpropyl carbonate	4.6	19.646	19.50	
Fatty Acid	Pent-4-enyl butanoate	1.57	7.779	7.39	

Fatty Acid	Pent-4-enyl 4-chlorobutanoate	1.33	8.931	8.8	
Fatty Acid	3-methylbut-2-enyl hexanoate	1.31	15.361	15.15	76649-22-4
-	Others	3.24			

% = Peak Area, RT = Retention time, I.T. = Retention index

The percentage of damaged leaf area in plants treated with different concentrations of *M. citrifolia* essential oil is shown in Figure 1.

Figure 1 - Toxicity of corn plants as a function of increasing concentrations of *Morinda citrifolia* essential oil.

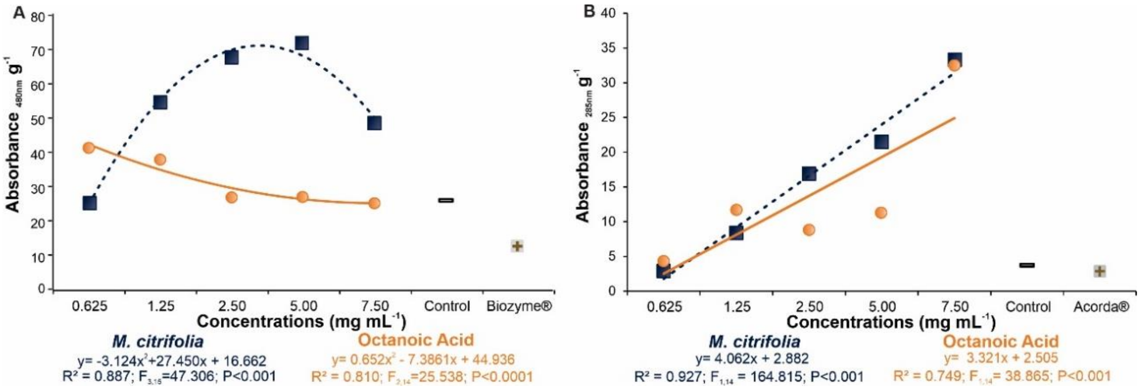


Fonte: Dias (2023)

The essential oil concentrations herein used did not result in intense high levels of chlorosis and necrosis in the corn leaves. The highest percentage of damaged leaf area (10%) occurred under 3.0 mg mL^{-1}. The best concentration for further studies was 1.5–2.5 mg mL^{-1}, which caused no significant injuries to corn (< 5% of leaf area) (Figure 1).

Figure 2 demonstrates the induction assays for phytoalexin production in soybean and sorghum.

Figure 2 - Production of 3-deoxyanthocyanin phytoalexins in sorghum mesocotyls (A) and glyceolin in soybean cotyledons (B)



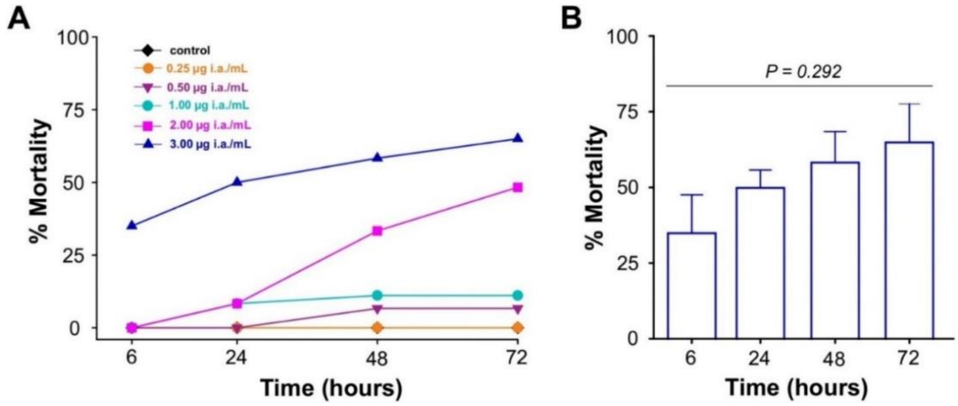
Fonte: Dias (2023)

The resistance activator (Biozyme®) decreased the production of 3-deoxyanthocyanin in sorghum mesocotyls (12.8 abs_{480nm} g^{-1}) compared to the control (26.1 abs_{480nm} g^{-1}). Noni treatment induced the production of a significant amount of phytoalexins. For *M. citrifolia*, there was an increasing trend up to 5.0 mg mL^{-1}, and 2.5 mg mL^{-1} (67.3 abs_{480nm} g^{-1}) was the second highest concentration in phytoalexin production.

The vegetable stimulant Acorda® had the lowest stimulation of glyceolin production in soybean cotyledons (2.8 $abs_{285\ nm}$ g^{-1}). The healthy plant under normal conditions showed a value of 3.7 $abs_{285\ nm}$ g^{-1}. The two treatments at 7.5 mg mL^{-1} linearly and significantly increased the amount of phytoalexin, with octanoic acid (32.4 $abs2_{85\ nm}$ g^{-1}) and *M. citrifolia* (33,4 $abs_{285\ nm}$ g^{-1}). For noni essential oil, the concentration of 2.5 mg mL^{-1} resulted in a value approximately eight times higher (16.8 abs_{285nm} g^{-1}) than that of the plant with the pathogen.

The influence of different concentrations of *M. citrifolia* essential oil on the mortality of *D. maidis* over exposure time is shown in Figure 3.

Figure 3 - Mortality of the leafhopper *Dalbulus maidis* submitted to different concentrations of *Morinda citrifolia* essential oil over time (Figure 3A). Mortality over time at the highest concentration (3mg i.a./mL) (Figure 3B).



Fonte: Dias (2023)

The toxicity of essential oil was directly related to its concentration and time of contact with the compound. Concentrations below 1.0 mg/mL affected less than 10% of the population within 72 h (Fig. 3A). The 2.0 mg/mL concentration had a mortality rate of 33% and 48% after 48 h and 72 h of exposure. The 3.0 mg/mL concentration had a lethality of 35% in the first 6 h after essential oil application (Fig. 3B), which increased up to 72 hours, and resulted in a mortality rate of 65% of the leafhopper population.

DISCUSSION

The composition of essential oils is commonly discussed. It varies with the season, climate, collection time, and biotic or abiotic stresses, which interfere with the levels of the components derived from the secondary metabolism of plants. Analyses of composition of oils extracted from noni fruits collected from the same region of Brazil (VELOSO et al., 2020) and others collected in Malaysia (PIARU et al., 2011) showed content variations of up to 20%, but maintaining fatty acids as major compounds. Evidence of compound stability and antimicrobial activity support their use as alternative controls for plant diseases.

Foliar diseases threaten large crops and negatively affect yield by reducing the photosynthetic area (ARELLANO et al., 2021). This scenario includes important emerging diseases such as stunting, transmitted by *D. maidis*. The mechanisms of action depend on the biochemical responses of the pathogens in plant biosynthetic pathways. They interfere with protein synthesis and respiration, degrade membrane integrity and cytoplasmic contents, and inactivate mitochondrial activity (LAGROUH et al., 2017).

In addition to antimicrobial action, the phytotoxic effects of alternative controls for foliar application were also tested. The phytotoxicity described by Werrie et al. (2020), such as the negative effects caused by the biostimulation of compounds, may be related to cellular dysfunction and requires careful evaluation (RAVEAU et al., 2020). The *M. citrifolia* essential oil at concentrations below 3.0 mg/mL did not result in levels of chlorosis or necrosis that affected the photosynthetic mechanisms. The dose-dependent phytotoxic effects of terpene-rich essential oils were compared by Abd-ElGawad (2021), and the same pattern was maintained in different cultures, confirming that analyses to determine the optimal concentrations are necessary.

The phytoalexins in sorghum and soybean cultures were quantified using spectrophotometric analysis and compared to investigate the induction of defense mechanisms. In addition to investigating the potential elicitors of phytoalexins from sorghum and soybeans, the protection of *Cucumis sativus* was investigated by Bonaldo et al. (2004). These low molecular weight chemical compounds are produced in cytoplasmic inclusions and are considered disease controllers by disrupting the plasma membrane and inactivating the pathogen's enzymes (LORENZETTI et al., 2018). Essential oil elicitor activity of *Lippia sidoides* Cham. potentiated the accumulation of deoxyanthocyanin and glyceolin, as well as noni essential oil induced a higher quantity than Biozyme® and Acorda® fertilizers (FERREIRA et al., 2018), both classified as efficient biostimulants, containing seaweed extract and neem oil (RAFIEE et al., 2016).

Noni essential oil and octanoic acid are revealed as activators of latent defense mechanisms in sorghum and soybeans. The diversity of study subjects, including the main research crop, corn, allows us to hypothesize that these compounds also induce corn phytoexins (kauralins and zealexins) consisting of terpenoids (SCHMELZ et al., 2011). The strategy of correlating studies across different crops was proposed due to the lack of methods for identifying and quantifying corn phytoalexins through color variations captured in a spectrophotometer. The available identification techniques suggest that they involve the activation of latent defense mechanisms, and due to similarities in biosynthetic pathways, these compounds remain as elicitor molecules. The biosynthetic genes for these phytoalexins have already been characterized. However, their regulatory mechanisms are unknown, and there is evidence of a correlation between biosynthetic genes and stress response regulators (FU et al., 2017).

The association among resistance induction, and action against insect pests demonstrates the capacity and complexity of the mechanisms involved in the use of natural compounds as broad-spectrum bioproducts. The corn leafhopper is considered a primary pest in the Neotropics and is responsible for direct damage (feeding and reproduction) and indirect damage by the transmission of diseases caused by mollicutes and viruses (POZEBON et al., 2022). The toxicity of *M. citrifolia* essential oil was verified within 72 h. Thus, lethality depends on dose and time and systemic action occurs after contact with the compound. The potential pest control of plant compounds has proven the susceptibility of insect populations of the same order and family to essential oil from rosemary, and sesquiochemicals by repellency and lethality (NIU et al., 2022, HARIZIA et al., 2020). Despite the poorly described mechanisms of action, the insecticidal activities of plant compounds have led to their potential for incorporation into products.

Systemic stunt infections are caused by mollicut-class bacteria that colonize the phloem. The main consequences are the impairment of chlorophyll levels and limitation of nutrient production and absorption pathways (COSTA et al., 2023). With the need for instant action owing to the high population numbers and displacement that spread the disease in the early stages of maize development, the movement of the essential oil, which started in the first six hours of contact, for alternative controls is remarkable and of rapid action, which allows for the suggestion of consecutive applications to optimize efficiency.

The present study showed that *M. citrifolia* is effectivene as an eco-friendly option for managing corn leafhoppers and activating plant defense mechanisms. Although the insecticidal action has indirectly contributed to reducing mollicute transmission by *D. maidis*, the direct effectiveness against these pathogens has not yet been proven. Therefore, new research is essential to optimize the use of these compounds in the management of stunting complexes and to develop efficient bioproducts that advance science and sustainable agriculture.

CONCLUSION

This study is pioneering in the research on the use of *Morinda citrifolia* L. (noni) in the management of corn leafhoppers. The results demonstrated that the essential oil effectively controls the pest, resulting in 50% mortality in *Dalbulus maidis* in 48 hours without causing toxic effects on corn plants. This insecticidal activity also ensures the reduction of the transmission of mollicutes, causing the stunting complex. Octanoic acid and the essential oil activated plant defense mechanisms, increasing the production of phytoalexins. The discovery of new resistance elicitors has significant implications for pest and disease management. These results contribute to increased productivity, implementation of phytosanitary measures, and global food security. The compounds of *M. citrifolia* offer a promising and sustainable alternative to conventional methods of pest control and resistance induction. It is hoped that these findings will inspire future research for the formulation of effective bio-products, contributing to the advancement of science and sustainable agriculture.

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