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Chemical composition and bioactivity of essential oil from *Morinda citrifolia* L. fruit

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Morinda citrifolia **has aroused the interest of several research institutions due to its pharmacological properties. The compounds biosynthesized can be explored as an alternative for control measures of plant pathogens causing leaf lesions in maize. The study aim at evaluating the potential effects of essential oil obtained from ripe fruits of *M. citrifolia* on *Bipolaris maydis* and *Exserohilum turcicum* isolated from maize plants. Ripe fruits were subjected to extraction of essential oil by hydrodistillation method and chemical composition was determined by gas chromatography/mass spectrometry. The yield of essential oil was 0.17% (v/w), and the main constituents identified were octanoic acid constitutes 82.2%, hexanoic acid 8.3%, 3-methylbutyl octanoate 4.2%, and ethyl etanoate 2.5%. Mycelial growth control *in vitro* and *in vivo* of *B. maydis and E. turcicum* spots diseases in maize plants was evaluated and the fruit of *M. citrifolia* was found to have potential essential oil with fungicidal activity at concentration 0.25%. With area under the disease progress curve (AUDPC) values lower than those observed in treatments with fungicide, the preventive control of leaf spot in *B. maydis* plants using essential oil of *M. citrifolia* showed biological activities and therefore a source of molecules to be exploited that can minimize the severity of diseases.**

Key words: *Bipolaris maydis, Exserohilum turcicum*, fungitoxicity, phytopathogenic, *Zea mays.*

INTRODUCTION

Morinda citrifolia, also known as "Noni", is a tropical, exotic and medicinal plant belonging to Rubiaceae family

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(Arunachalam, 2018). It is a native species from Southeast Asia and Australia that is being used for centuries to cure or prevent illnesses (Lopes et al*.,* 2018; Wu et al*.,* 2019). Since it comprise phytochemicals that ensure effectiveness against pathologies (García-Vilas et al*.,* 2015), it has attracted the industry to employ it as part of various products, a natural source of medicines and chemical reagents, as well as a green insecticide, based on advances in the elucidation of certain benefits of the plant (Ali et al*.,* 2016; Assi et al*.,* 2017).

Just like other medicinal herbs, *M. citrifolia* produce several bioactive compounds (Almeida et al., 2019), its chemical composition is not yet fully reported, insofar as research is directed to the characterization of molecules new ones are discovered (Wen et al*.,* 2018; Rodrigues et al*.,* 2017). Among the main products of its own metabolism is the synthesis of essential oil, which is a complex mixture, formed of organic structures (Zhang et al*.,* 2018). It has awakened the interest of the scientific community through the plant diseases combat in order to reduce the adverse impacts of pesticides (Gimenez et al*.,* 2018). Several works with essential oils of medicinal plants have indicated their potential in the control of phytopathogenic bacteria and fungi (Gormez et al*.,* 2015; El Ouadi et al*.,* 2017).

The leaf diseases that occur in crop corn (*Zea mays* L.) involve one of the essential oils that can cause a fungitoxic effect (Karimi et al., 2016). The corn is a commodity of high socioeconomic value in all Brazil's regions with large production in Tocantins State of north Brazil, and around 4 tons of productivity per hectare (Companhia Nacional de Abastecimento - CONAB, 2018). However, one of the limiting factors at plant development has been the presence of phytopathogens responsible for leaf injuries (Chagas et al., 2015). So, it has promoted the greater use of pesticides in the control of diseases, and as a result, environmental damage and agrochemicals resistance organisms are recurrent (Hawkins et al., 2018).

Most of the reports about chemical composition and biological activity of *M. citrifolia* is derived from juice and fruits extracts used in the control of pathogenic micro-organisms in mammals (Torres et al., 2017; Thongchai et al., 2019). In agreement with the literature, few studies demonstrated the potential to use essential oil of this species against phytopathogens (Dalcin et al., 2017; Silva et al., 2017). Therefore, the aim of this study was to evaluate the essential oil potential extracted from ripe fruits of *M. citrifolia* in controls *in vitro* and *in vivo* of *Bipolaris maydis* and *Exserohilum turcicum* isolated from maize plants.

MATERIALS AND METHODS

This study was carried out in the Laboratory of Phytopathology of the Federal University of Tocantins, Gurupi Campus, Tocantins, Brazil.

Morphological characterization of fungi

The fungi *B. maydis* and *E. turcicum* were initially isolated from maize plants with symptoms of the disease as follows: plant parts were cut and washed in water, thereafter subjected to asepsis with alcohol (70%) for 30 s, sodium hypochlorite (1%) for 40 s, and distilled/sterilized water. The leaves fragments were grown in potato, dextrose, and agar (PDA) culture medium. The plates were sealed, identified and taken to incubation chamber at 25°C with a photoperiod of 12 h for 7 days. The morphological characterization of fungi was verified through observations of the macroscopic and microscopic characteristics with supporting specialized literature.

Plant material and hydrodistillation

M. citrifolia originating in Asia was collected in municipality of Gurupi (11°43'30" latitude S, 49°04'34" longitude W), Tocantins, Brazil. *M. citrifolia* essential oil was extracted from the ripe fruits by hydrodistillation method and 0.2 kg of material was deposited for extraction in one liter of distilled water for two hours of boiling in a Clevenger apparatus and stored at 4°C until further analysis, before antifungal experiments were conducted.

Effect of *M. citrifolia* essential oil on mycelia *B. maydis* and *E. turcicum*

To verify the effect of essential oil on the mycelial growth of the phytopathogen, 100 µL of each of the eight solutions (0.5, 2.0, 3.5, 5.0, 6.5, 9.5 and 11%) was spread on the surface of the culture medium. Thereafter, a disk of mycelium-agar around 5 mm in diameter was placed in the center of the plates and incubated in BOD at 25°C for 10 days according to the methodology of Ferreira et al. (2018). The evaluations were carried out by measuring the diameter of the mycelial outlining of two orthogonal axes with each other over the center of the plates, resulting in arithmetic mean, and measured every 2 days (2, 4, 6, 8, and 10 days). The Petri plate control contained only PDA. All experiments were conducted in triplicate.

Phytotoxicity of essential oil of *M. citrifolia*

The experiment for testing the phytotoxicity was carried out under greenhouse conditions (relative humidity of 70 to 80% and temperature at 27 to 33°C) according to the methodology used by Santos et al. (2013). For planting of maize, pots were used and then sowed. After sowing, the pots were irrigated daily until the growth of the seedlings reached four definite leaves or till 30 days of planting. Only the manual spray trigger was used for the application of treatments. Each pot was sprayed with 20 mL of 0.5 to 11% solutions. That was done to both controls, one containing Tween 80% (1%) in sterile distilled water and the other containing just sterile distilled water. After 24 h of application, the evaluation was performed according to the scale of phytotoxicity adapted by 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.

Preventive effect of essential oil

To assess the preventive effect of essential oil, a completely randomized experimental design in a factorial design was used with three replicates, where the factors were a type of oil and the

Table 1. Relative percentage (area %), obtained by gas chromatography attached to a mass spectrometry detector, of the components of ripe noni (*Morinda citrifolia*) fruit essential oil.

CN	RI	(%)
3-methyl-3-butenyl-1-acetate	888	-
2-heptanone	897	-
Methyl hexanoate	922	-
Hexanoic acid	987	8.26
Ethyl etanoate	999	2.48
Methyl octanoate	1123	-
Octanoic acid	1177	82.24
Ethyl octanoate	1196	-
Isopenthyl hexanoate	1259	1.6
3-methil-2-butenyl hexanoate	1292	-
3-methylbutyl octanoate	1457	4.25
3-methylbutyl-2-enyl octanoate	1489	-
Essential oil content (%)		0.2

CN = Compost number; RI = Retention index; *-Not quantified (values < 0.05).

following five concentrations of the solutions of oil: 0.002; 0.01; 0.05 and 0.25% v/v. As a negative control, plants sprayed with just sterile distilled water were used, whereas plants sprayed with 0.1% *Azoxistrobin-Ciproconazole* (fungicide) served as the positive control. For each treatment, 20 mL was sprayed on each pot, and after 24 h, it was inoculated with 20 mL of 10^4 mL^{-1} *B. maydis* and *E. turcicum.* The severity of the disease was assessed every 2 days after the inoculation (five assessments in total) (Ferreira et al., 2018). The average rates of incidence and severity observed were transformed into an area under the severity progress curve (AUDPC), determined using the equation proposed by Shaner and Finney (1977).

Gas chromatography-mass spectrometry (GC-MS) analysis

Qualitative analyses were performed through gas chromatography coupled with mass spectrometry (GC-MS) using the Shimadzu GC2010 model equipped with selective detector for the mass Model QP2010Plus, with the equipment operated under the following conditions: fused silica capillary column RTX-5MS (30 m × 0.25 mm × 0.25 µm film thickness), with the following schedule of temperature in the column: 60 to 240°C (3°C/min), temperature of the injector 220°C, helium gas carrier, injection with rate of split (1:100) with injected volume of 1 µL of a solution 1:1000 in hexane. For the mass spectrometer (MS), the following conditions were used: impact energy of 70 V and temperature of the source of ions and the interface at 200°C. A homologous series of n-alkanes (C_9H_{20}…$C_{26}H_{54}$) was injected under the same conditions as for samples. The constituents were identified by comparing their spectra of masses with those from the databases from the Nist and Wiley 229 libraries and also by comparing between their rates of retention calculated using those reported in the literature (Adams, 2007). The quantification of the levels of the compounds, expressed as a percentage based on the standardization of areas, was obtained by using a gaseous chromatograph equipped with a detector flame (DIC), using a diagnostic Shimadzu GC-2010, in the following experimental conditions: a capillary column RTX-5MS (30 m × 0.25 mm × 0.25 µm film thickness); temperature of the injector at 220°C; temperature of the DIC 300°C; programming the column: initial temperature of 60°C with a heating rate of 3°C/min up to 240°C, then increasing to a heating rate of 10°C/min up to 300°C and remaining at this temperature for 10 min; nitrogen drag gas (1.18 mL min^{-1}); rate of split 1:50; pressure in the column of 115 kPa, and injected volume of 1 µL, diluted in hexane (1:100 v/v).

Statistical analysis

The *in vitro* bioassay data were submitted to regression by calculating the determination coefficient (R^2) using SigmaPlot software (version 10). And the results obtained in the *in vivo* bioassay of the preventive control were analyzed by analysis of variance based on the coefficients at 5 or 1% of probability, using ASSISTAT software (Silva and Azevedo, 2002).

RESULTS

As for the chemical composition of the essential oil of *M. citrifolia*, 12 chemical constituents were identified, the main constituent being octanoic acid with 82.24% (Table 1). In the essential oil there was a higher number of compounds with ester function, however octanoic and hexanoic acids were the main constituents. According to Wall et al. (2017), the presence of esters in fruits of *M. citrifolia* confer the characteristic flavour of ripe fruits.

In vitro test

The essential oil of *M. citrifolia* presented fungitoxic potential, inhibiting the mycelial growth of the phyto-pathogens evaluated (Figure 1). It was observed that the increase in the concentration of essential oil promoted the reduction in mycelial growth, leading to 100% inhibition in the mycelial growth of *B. maydis* and *E. turcicum* from the concentrations of 8.0 and 5.0% respectively.

From the regression equations generated, it is possible

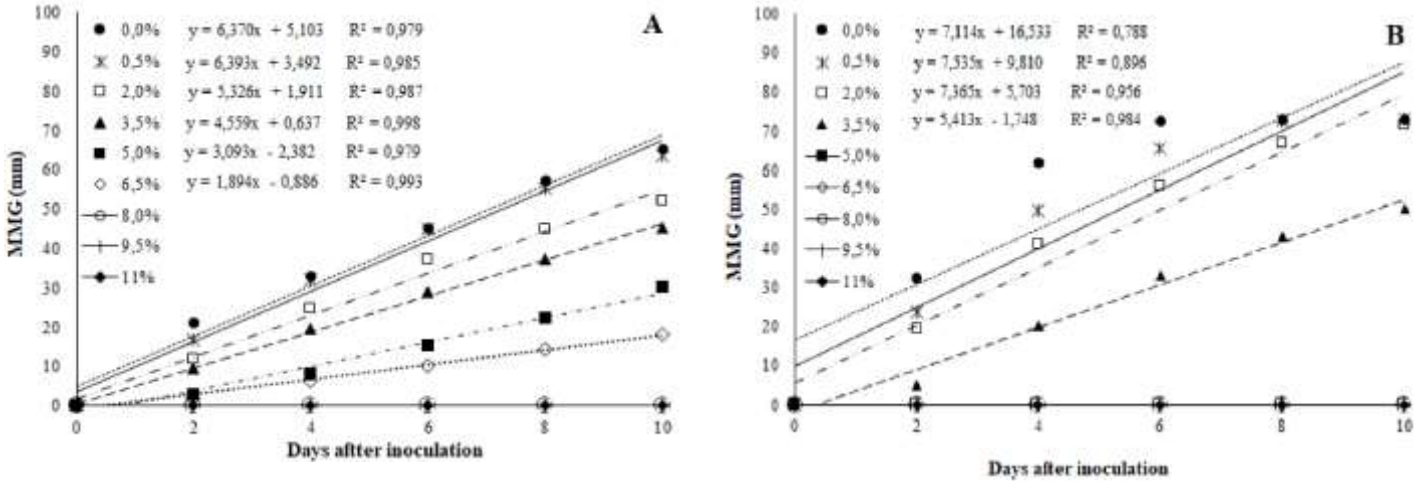

Figure 1. Mean mycelial growth (MMG) of (A) *Bipolaris maydis* and (B) *Exserohilum turcicum*, submitted to different concentrations of essential oil of *Morinda citrifolia*.

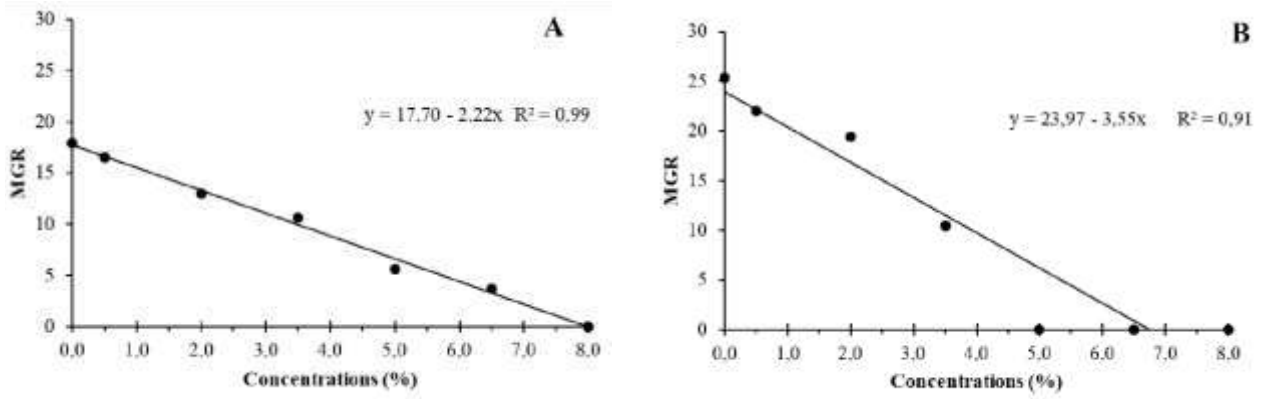

Figure 2. Mycelial growth rate (MGR) of (A) *Bipolaris maydis* and (B) *Exserohilum turcicum*, submitted to different concentrations of essential oil of *Morinda citrifolia*.

to note that although *B. maydis* showed whole inhibition of mycelial growth from the concentration of 8.0% of the essential oil, the development of the fungus was smaller, with a variation of around 6.35 mm.day^{-1} in the control treatment, and 1.90 mm.day^{-1} at the concentration of 6.5% of essential oil. Meanwhile, the medium mycelial growth of *E. turcicum* phytopathogen ranged from around 7.11 mm.day^{-1} in the control treatment to 5.41 mm.day^{-1} at the concentration of 3.5% essential oil.

The rate of mycelial growth (MGR) showed that the essential oil of *M. citrifolia* interfered with the development of the evaluated phytopathogens as the concentrations increased (Figure 2). There was a high adjustment to the linear model, represented by the determination coefficients for the two equations generated. The MGR were 99 and 90% for the phytopathogens *B. maydis* and *E. turcicum*, respectively.

According to the adjusted equation, with each addition of 1% in the essential oil concentration, a decrease was estimated in the mycelial growth rate of at least 2.22 and 3.55 mm.day^{-1} for fungi *B. maydis* and *E. turcicum*, respectively. Although *B. maydis* showed higher MGR, the essential oil of *M. citrifolia* was more efficient in inhibiting the mycelial growth of *E. turcicum*, because this phytopathogen was altogether suppressed at the concentration of essential oil lower than that observed for total inhibition of growth mycelial of *B. maydis*.

In vivo test

Phytotoxicity

A phytotoxic effect was observed from the concentration

Table 2. Phytotoxicity in maize plants in order to the application of different concentrations of the *Morinda citrifolia* essential oil.

Treatment	Characteristics
H_2O	Phytotoxicity absence
H_2O + Tween	Phytotoxicity absence
0.25%	Phytotoxicity absence
0.50%	51 – 75% High chlorosis and necrosis on the leaves
0.75%	51 – 75% High chlorosis and necrosis on the leaves
1.0%	76 – 100% Wilt and dryness of the plant
1.5%	76 – 100% Wilt and dryness of the plant

Table 3. Area under disease progress curve (AUDPC) for the preventive control of *Bipolaris* and *Exserohilum* spots in order of different concentrations of (*Morinda citrifolia*) essential oil.

Treatment	AUDPC[1,2]	
	***Bipolaris* spot**	***Exserohilum* spot**
Fungicide	168.00[a]	162.33[a]
0.0%	218.67[a]	242.00[a]
0.002%	207.83[a]	214.67[a]
0.01%	189.67[a]	172.17[a]
0.05%	116.50[ab]	158.83[a]
0.25%	41.33[b]	35.67[b]
CV (%)	17.01	16.64

[1]Means followed by the same letter in the column do not differ from each other by the Tukey test at 5% probability. [2]Original data were transformed to $\sqrt{x}$.

of 0.5% of essential oil of *M. citrifolia* in corn plants, presenting symptoms of necrosis and chlorosis in the leaves, at levels varying from 51 to 75% of the affected leaf blade (Table 2).

The high degree of phytotoxicity caused by the essential oil in the tested concentrations allowed to stipulate that the concentration of 0.25% of essential oil of *M. citrifolia* would be the maximum concentration adopted in the preventive control of foliar diseases in maize plants.

Preventive control

In the preventive control *in vivo*, a significant difference was observed using different concentrations of essential oil (Table 3). The essential oil of *M. citrifolia* was effective in preventing the symptoms of *Bipolaris* and *Exserohilum* stains in corn plants, with AUDPC values at concentrations of 0.05 and 0.25% lower than the values observed in the treatment with fungicide.

The lower medial values of AUDPC, demonstrating a lower disease severity, were observed with the concentration of 0.25% of essential oil, for both diseases. The treatment with the fungicide did not a present statistical difference of the control treatment. This may be indicative that phytopathogens may have acquired resistance to the product. The lowest AUDPC values were observed for *Bipolaris* stain than for the *Exserohilum* stain, which demonstrates greater efficacy of the essential oil of *M. citrifolia* in the preventive control of the first disease.

DISCUSSION

In the essential oil of ripe fruits of *M. citrifolia*, octanoic and hexanoic acid were identified 82.24%, 8.26% as major constituents. Nevertheless, Osorio et al. (2018) obtained 64.03%, 8.64% octanoic and hexanoic acid in Noni, respectively. As for the essential oil content in fruits of *M. citrifolia*, no reports were found in the literature; however, Palioto et al. (2015) found 2.19% of lipids in works with pulp of mature fruits of *M. citrifolia* in Paraná. While the free fatty acid content found by Rosyida et al. (2019) was 0.65%. One of those responsible for this discrepancy in the chemical composition of essential oils is the genetic factor (Shing et al., 2018). But also, abiotic factors like luminosity, temperature, precipitation, nutrition; season, and harvest techniques, or even biotic factors resulting from microorganism-plant, plant-insect,

and plant-plant interactions, as well as the age and stage of development. They may interfere with the quality and quantity of secondary metabolites. These factors may present correlations among themselves and do not act in isolation (Huang et al*.,* 2018).

The essential oil of *M. citrifolia* presented a potential for inhibition of mycelial growth and reduction of mycelial growth rate index of *B. maydis* and *E. turcicum* phytopathogens. This is due to the high number of constituents; essential oils seem to have no specific cellular targets (Yang et al*.,* 2016). As typical lipophiles this feature allows them to interact with the lipid bilayers more quickly so that they alter permeability and cause disturbances in these structures (Lima et al*.,* 2019). The integrity of the cytoplasmic membrane is a critical factor for any organism growth. The essential oil constituents can interfere with electron flow in the cytochrome pathway and reduce ATP production, besides affecting enzyme systems, coagulate the cytoplasm, extravasation of macromolecules intracellular vital to the homeostasis cellular (Hazrati et al*.,* 2018). It should be kept in mind that essential oils are complex compounds, so their biological effects might be the result of synergism of all molecules or only those of the main molecules present at the highest levels according to gas chromatographical analysis. In this way, to affirm that the action of one or two main components of the oil seems questionable, since it is possible that other in minor quantity molecules modulate the activity of the main components (Chouhan et al*.,* 2017).

However, essential oil concentrations tested *in vitro* promoted a phytotoxic effect in maize plants when tested in vivo, and it is necessary to evaluate concentrations of essential oil of *M. citrifolia* below 0.5%. Evaluating the potential of this oil to cause phytotoxicity in corn plants, Silva et al. (2017) observed that the concentration above 1% was not phytotoxic. This indicates variability in the species at higher concentrations. The phytotoxic effect due to this arises from the formation of free radicals responsible for lipid peroxidation in plant cells. When the plant fails to maintain the rate of damage repair, oxidative stress causes a collapse in metabolism, leading to cell death, with the onset of chlorosis and necrosis in leaf tissue (Synowiec et al*.,* 2017).

From the essential oil phytotoxicity test, it was possible to determine which concentrations of essential oil of *M. citrifolia* could be used in the preventive control of the *Bipolaris* spot and the *Exserohilum* spot in maize plants. It was observed that the application of the essential oil presented values of AUDPC at concentrations of 0.05 and 0.25%, for both types of leaf spots, lower than the values observed for applications with the fungicide (*Azoxystrobin:Cyproconazole*). This demonstrates the fungitoxic potential of the essential oil in minimizing the severity of the disease. But also, a possible induction of resistance of the pathogen to the fungicide molecules, since, *Azoxystrobin:Cyproconazole* is the main used suspension in the control of foliar diseases in corn.

Conclusion

Under such conditions, it was possible to conclude that ripe fruits of *M. citrifolia* are promising sources of essential oils with fungitoxic activity inhibiting mycelial growth and the severity of foliar diseases in crop maize. The major component of noni essential oil was octanoic acid. Concentrations above 0.5% of the noni essential oil were phytotoxic to the maize. The preventive application at 0.25% concentration of Noni essential oil was efficient in the controls of both fungi spots.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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