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BIOACTIVITY OF *Cymbopogon citratus* (DC.) STAPF ESSENTIAL OIL ON *Dalbulus maidis* (DELONG & WOLCOTT) (HEMIPTERA: CICADELLIDAE)

BIOATIVIDADE DO ÓLEO ESSENCIAL DE Cymbopogon citratus (DC.) STAPF SOBRE Dalbulus maidis (DELONG & WOLCOTT) (HEMIPTERA: CICADELLIDAE)

BIOACTIVIDAD DEL ACEITE ESENCIAL DE Cymbopogon citratus (DC.) STAPF SOBRE Dalbulus maidis (DELONG & WOLCOTT) (HEMIPTERA: CICADELLIDAE)

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ABSTRACT:

This work aimed to evaluate the insecticidal potential of *Cymbopogon citratus* essential oil as an alternative to conventional products in controlling adult individuals of *D. maidis*. The treatments were composed of 0.1 %, 0.5 %, 1.0 %, 1.5 %, and 2.0 % v/v of *C. citratus* essential oil, and the use of two controls: one positive, composed of the chemical insecticide Perito 070 SG (acephate) at 1.0 $kg \cdot ha^{-1}$, and two negative controls, Tween 80® 0.5% v/v and distilled water. The bioassays were performed in triplicate, with three replicates of ten individuals per replicate, totaling 30 individuals per treatment. Maize plants were grown in bottles with a capacity of 2 L, and ten adult individuals were transferred. The treatments were sprayed, and the plants were covered with voile fabric. At the concentrations of 0.1 %, 0.5 %, and 1.0 % v/v, there was no significant difference relative to the negative control. In contrast, at the concentrations of 1.5 % and 2.0 % v/v, there was a significant difference from the negative controls, with mortality similar to the positive control, being a potential biopesticide in the control of *D. maidis* and a potential candidate as a bioherbicide in the control of volunteer corn plants because it caused toxicity on corn plants from concentrations of 1.0 % v/v.

KEYWORDS: lemongrass; corn leafhopper; grain crops; pest insect.

RESUMO:

Este trabalho teve como objetivo avaliar o potencial inseticida do óleo essencial de Cymbopogon citratus *como alternativa aos produtos convencionais no controle de indivíduos adultos de D. maidis Os tratamentos foram compostos por ólo essencial de* C. citratus *nas concentrações de 0,1 %; 0,5 %; 1,0 %; 1,5 %; e 2,0 % v/v, bem como a utilização de duas testemunhas: uma positiva, composta pelo inseticida químico Perito 070 SG (acefato) 1,0* $kg \cdot ha^{-1}$*, e dois controles negativos, Tween 80® 0,5 % v/v e água destilada. Os bioensaios foram realizados em triplicata, com três repetições de dez indivíduos por replicata, totalizando 30 indivíduos por tratamento. As plantas de milho foram transplantadas em frascos com capacidade para 2 L e foram transferidos 10 indivíduos adultos. Os tratamentos foram pulverizados e cobertos com tecido voil. Foi observado que para as concentrações de 0,1 %; 0,5 % e 1,0 % não houve diferença significativa em relação ao controle negativo, enquanto nas concentrações de 1,5 % e 2,0 % v/v houve diferença significativa em relação aos controles negativos, mostrando mortalidade semelhante ao controle positivo, sendo um potencial pesticida no controle de* D. maidis *e potencial candidato como bioherbicida no controle de plantas voluntárias de milho por ter causado toxicidade a partir de concentrações de 1,0 % v/v.*

PALAVRAS-CHAVE: *capim-limão; cigarrinha-do-milho; cultura de grãos; insetos-praga.*

REVISTA DESAFIOS ISSN: 2359-3652

RESUMEN

Este trabajo tuvo como objetivo evaluar el potencial insecticida del aceite esencial de Cymbopogon citratus *como alternativa a los productos convencionales en el control de individuos adultos de* D. maidis*. Los tratamientos estuvieron compuestos por aceite esencial de C. citratus en concentraciones de 0.1 %; 0,5 %; 1,0 %; 1,5 %; y 2,0 % v/v, así como el uso de dos controles: uno positivo, compuesto por el insecticida químico Perito 070 SG (acefato) 1,0 kg·ha^{-1}, y dos controles negativos, Tween 80® 0,5 % v/v y agua destilada. Los bioensayos se realizaron por triplicado, con tres repeticiones de diez individuos por réplica, totalizando 30 individuos por tratamiento. Las plantas de maíz se trasplantaron a frascos de 2 L de capacidad y se trasladaron 10 individuos adultos. Los tratamientos fueron rociados y cubiertos con tela voile. Se observó que para concentraciones de 0,1 %; 0,5 % y 1,0 % no hubo diferencia significativa con relación al control negativo, mientras que a las concentraciones de 1,5 % y 2,0 % v/v hubo diferencia significativa con relación a los controles negativos, mostrando una mortalidad similar al control positivo. siendo un pesticida potencial en el control de* D. maidis *y un candidato potencial como bioherbicida en el control de plantas voluntarias de maíz ya que causó toxicidad a partir de concentraciones de 1. 0% v/v.*

Palabras clave: *hierba de limón; chicharrita de maíz; cultivos de cereals; insecto plaga.*

REVISTA DESAFIOS ISSN: 2359-3652

INTRODUCTION

Corn (*Zea mays* L.) is a versatile cereal with several applications, such as human and animal food and as raw material press in the chemical, pharmaceutical, beverages, and fuel industries (Castellucci *et al.*, 2015; Sologuren, 2015). This grain is the second most important commodity in the Brazilian economy (Oliveira and Frizzas, 2022). According to the second estimate of vintage in grains from the Company National in Supply, there is an expectation of production of 126.4 million tons in the vintage 2022/2023, being what at first vintage there was one reduction of 3.1 % of the planted area due to the increase in the production cost and the high pressure of the occurrence of corn leafhopper (CONAB, 2022).

The corn leafhopper (*Dalbulus maidis*. L. (DeLong & Wolcott) (Hemiptera: Cicadellidae)) causes severe losses in corn culture. This species is an important insect pest because it sucks plant sap and transmits the mollicutes spiroplasma (*Spiroplasma kunkelii*) and phytoplasma (maize bushy stunt -MBS), and maize streaked thin virus (MRFV), the causal agent of "stripes virosis". These three pathogens reduce plant development and productivity by up to 70 % (Waquil, 2007; Sabato, 2017; 2018). The corn grasshopper acquires the pathogens when it feeds on infected plants and transmits them to healthy plants (Oliveira and Sabato, 2018).

The species *D. maidis* has biological, ecological, and behavioral characteristics confer adaptive benefits to new environments. A typical strategist with a fast growth time between the egg and the adult and high fecundity, *D. maidis* produces at least two generations during the corn cycle at average temperatures of 29 °C (Waquil *et al.*, 1999). In addition, it preferentially uses the whorl of the maize, seeking meristematic tissues that are nutritionally richer for food and oviposition. It is noteworthy that, despite being found on other grasses, this insect only reproduces in corn plants (Marín, 1987).

The kind of control primarily used by the farmers in the control of this pest is the use of synthetic molecules, being at seed treatment with neonicotinoids and carbamates or in the plant with neonicotinoids, organophosphates, and pyrethroids (AGROFIT, 2012). Synthetic chemical pesticides are fundamental to public health and agriculture, controlling disease vectors and agricultural pests. Nonetheless, as disadvantages, these molecules can affect several non-target organisms. Furthermore, its indiscriminate use and lack of rotation of the active ingredients make the target insects resistant, making it more challenging to control this and other pests (Georgieva *et al.*, 2021). The toxicity and environmental impact are also problems caused by the

V.11, n.5, julho/2024. ISSN n° 2359-3652

indiscriminate use of these chemicals, which makes necessary the search for products of origin natural origin, and less aggressive to the environment and human and animal health (Rezende-Teixeira *et al.*, 2022).

Through this problem and the search for alternatives to chemical control, using natural products such as essential oils to control pests becomes one alternative to be used in Integrated Pest Management (IPM) (Campos *et al.*, 2018). These molecules have low environmental and animal toxicity and are mentioned as efficient, low-risk, broad-spectrum pesticides. Several modes of action for these molecules were proposed, such as contact and ingestion. In addition, its rapid volatilization and limited permanence in the field, with minimization or elimination of residues, are an advantage relative to contamination issues commonly found in the application of synthetic pesticides (Corrêa and Salgado, 2011). Plant secondary metabolites such as essential oils act as biopesticides due to their complex chemical composition, so the insects are hit by many different compounds, affecting them at the cell level, and sublethal doses may cause reduced fecundity and viability and deformities in the parental and filial generations. It also makes it difficult for the target species to develop resistance to all the compounds present in the essential oil (Chowanski *et al.*, 2016).

The species *Cymbopogon citratus* (DC.) Stapf, from the Poaceae family, is native to tropical regions of Asia, and its distribution occurs in the Americas and Africa. In Brazil, it is popularly known as lemongrass, citron grass, fragrant grass, and lemongrass. It is a perennial and aromatic plant, forming tussocks due to the intense tillering, with a height of 1 – 2 m, good climate adaptation, ease in cultivation, and good oil essential yield (Haber and Clemente, 2013). Li *et al.* (2020) reported the use of *C. citratus* oil essential in 14.6 % of patents regarding the production of insect repellents based on essential oils.

Lemongrass essential oil has pharmacological properties, including anti-inflammatory, antimicrobial, antioxidant, and antiparasitic activities. Citral, identified as the major compound in *C. citratus* essential oil, is being reported in other studies, with content ranges of 35.8 wt.% to 48.1 wt.% for the cis and trans isomers (Fogné *et al.*, 2017) and 32.02 wt.% citral, followed of myrcene, with 14.24 wt.% (Kimutai *et al.*, 2017). However, regardless of the major compounds, the literature describes it as a bioinsecticide with biological effects such as repellent, anti-food, and contact toxicity.

In this sense, the present work aimed to evaluate the insecticidal potential of *Cymbopogon citratus* essential oil on adult individuals of *Dalbulus maidis*.

REVISTA DESAFIOS ISSN: 2359-3652

MATERIALS AND METHODS

The experiments were conducted between October 03 to November 28, 2022. *D. maidis* individuals were obtained from maize plants in field conditions. They were bred in acrylic cages (30 cm x 30 cm x 50 cm) with the sides and top lined with voile fabric to allow air circulation and to prevent the insects from escaping. Plants of corn in stages V3-V4 were put inside the cages as a food source and to allow for oviposition. The breeding was conducted under controlled conditions (temperature of 25±2 °C, photoperiod of 14 of light and 10 h of dark, and relative humidity of 50±5 %).

The essential oil used in this work was extracted from the dry leaves of *C. citratus* and stored under refrigeration (4±2 °C). Its major compound was the cis (neral) and trans (geranial) isomers of citral, with contents of 20.54 wt.% and 31.42 wt.%, respectively, followed by myrcene, with 40.33 wt.%. The detailed chemical composition of the oil used in this study was provided in previous work (Vicenço *et al.*, 2020).

The bioassay was based on the methodology proposed by Albarracin *et al.* (2021), with modifications in using cages coated with voile and in a photoperiod of 14:10 h (light:dark). The maximum and minimum temperatures recorded during the bioassays varied between 20.1 °C and 36.5 °C.

Tests were carried out with adult individuals of *D. maidis*, all of the same generation. Two treatments were used as negative controls: distilled water and Tween-80® (polysorbate) 0.5 % v/v. Essential oil treatments were emulsified with Tween-80® (0.5% v/v), testing five concentrations of *C. citratus* essential oil (0.1 %, 0.5 %, 1.0%, 1.5 %, and 2.0 % v/v) according to the methodology of Vicenço *et al.* (2021). As a positive control, the chemical insecticide Expert 970 SG (active ingredient acephate) was used at the dose of 1 $kg \cdot ha^{-1}$. According to the Brazilian Ministry of Agriculture, this active ingredient is listed as one of the 91 products used to control the corn leafhopper (AGROFIT, 2012).

Corn plants (V2-V3 stage) were conditioned in plastic cups in a hydroponic system with nutritive solution based on the methodology of Gonçalves *et al.* (2016) and transferred to transparent bottles with a capacity of 2 L. After, ten *D. maidis* individuals, with ages between 30 – 33 days were transferred to the bottles with the aid of a manual aspirator. Then, the treatments were sprayed with an airbrush (Worker, model 974047), and the bottles were covered with voile tissue to prevent the insects from escaping. The

REVISTA DESAFIOS ISSN: 2359-3652

V.11, n.5, julho/2024. ISSN n° 2359-3652

mortality percentage of the individuals was evaluated in 24 h, 48 h, and 72 h after the application of treatments.

The experimental setup of all tests followed a completely randomized design. The tests were carried out in triplicate, and each replicate was composed of ten individuals, totaling 30 individuals per treatment. Mortality data were submitted for analysis of variance (ANOVA), followed by Tukey's multiple range test at a 5 % significance level using the Agrostat® software.

RESULTS AND DISCUSSION

In the treatment with *C. citratus* essential oil at the concentrations of 0.1 %, 0.5 %, and 1.0 % 1.5 % v/v, no differences in the mortality of *D. maidis* individuals were observed in the first 24 h. At the concentrations of 1.5 % and 2.0 % v/v, it was possible to observe mortality of 66.6 % in the first 24 h, not differing statistically from the positive control, which had 100 % mortality of *D. maidis* individuals (Table 1).

Table 1 - Mortality percentages in 24 h, 48 h, and 72 h of *Dalbulus maidis* individuals when exposed to increasing concentrations of *Cymbopogon citratus* essential oil.

Treatment	Mortality (%)		
	24 h	48 h	72 h
Water	6.6 b	13.3 c	13.3 c
Tween-80® (0.5 % v/v)	0.0 b	10.0 c	20.0 c
Acephate (1.0 % v/v)	100.0 a	100.0 a	100.0 a
0.1 % v/v	10.0 b	10.0 c	20.0 c
0.5 % v/v	0.0 b	0.0 c	0.0 c
1.0 % v/v	20.0 b	10.0 c	30.0 bc
1.5 % v/v	66.6 a	66.6 ab	70.0 ab
2.0 % v/v	66.6 a	70.0 a	70.0 ab
F-value	16.85 **	17.97 **	14.52 **
p-value	<0.0001	<0.0001	<0.0001
Coefficient of variation (%)	48.00	40.44	39.44

** – Significant at 1.0 % probability of error. Means followed by the same letter in the columns do not differ statistically by Tukey's test at a 5 % error probability. Source: authors (2023).

At 48 h, no changes were observed in the mortality percentages of the insects in the concentrations of 0.1 %, 0.5 %, and 1.0 % v/v. It was verified that the mortality of individuals in the two negative controls (water and Tween-80®) was equal to and below 20 %. On the other hand, 70 % insect mortality was observed when exposed to lemongrass essential oil at 2.0 % v/v (Table 1).

Regarding the positive control, 100 % mortality occurred after the first 24 h, not differing from treatments with essential oil at the concentrations of 1.5% and 2.0% v/v. Looking at the mortality rate of insects when submitted to the treatment with essential

REVISTA DESAFIOS ISSN: 2359-3652

oil at the concentration of 0.1 % v/v, it was possible to observe that 20 % of the insects died after 72 h, not differing from negative controls nor the treatment with essential oil at a 0.5% v/v (Table 1).

Silva *et al.* (2020) verified 100 % mortality of *Rhipicephalus microplus* larvae (Acari: Ixodidae) when exposed to the essential oil of *C. citratus* at a concentration of 1.0 %, not differing from the positive control (cypermethrin 5 %) after 48 h of exposition. Olivero-Verbel *et al.* (2010) described the repellent activity of *C. citratus* essential oil after 4 h of exposure to *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) at a concentration of 0.021 $mL \cdot L^{-1}$ (0.21 % v/v).

Studies pointed out that lemongrass essential oil applied topically on adults of *Sitophilus granarius* L. 1785 (Coleoptera, Curcullionidae) had an LD_{50} of 4.03 µg per insect and a mortality percentage of 42.3 %, in which the insects showed muscle contractions and changes in locomotion. The increase in toxicity was dose-dependent; when exposed to LD_{90} (10.95 µg per insect), mortality was observed in 85.8 % of the insects, with paralysis without recovery (Plata-Rueda *et al.*, 2020).

Radünz *et al.* (2022) evaluated the insecticidal activity of *C. citratus* essential oil against adult individuals of *Sitophilus zeamais* Motschulsky 1885 (Coleoptera: Curculionidae), also reporting mortality and repellency effects at all concentrations tested. The LD_{50} was reached after 6.86 h, 19.12 h, 33.27 h, 48.52 h, and 56.03 h of exposure, respectively, for the doses of 5.0, 2.5, 1.5, 1.0, and 0.5 $L \cdot t^{-1}$, applied on corn kernels.

According to Castilho-Morales *et al.* (2021), the essential oil of species from the *Cymbopogon* genus has ovicidal and larvicidal activities against *Aedes aegypti*. In addition, Soonwera and Phasomkusolsil (2016) reported morphological abnormalities in the juvenile stages of *Aedes aegypti* and *Anopheles dirus*, being observed mortality of larvae and deformed pupae, as well as the occurrence of incomplete emergence of adults attributed to the biological activity of *C. citratus* essential oil.

Li *et al.* (2020) highlighted citral as the major compound of lemongrass essential oil. In bioassays, concentrations of 5 % v/v and 10 % v/v of this substance caused 100 % mortality of *Sarcoptes scabiei* (DeGeer, 1778) var. canis (Bourguignon, 1853) in 25 min and 10 min, respectively, with a miticidal efficacy superior to the positive control, benzyl benzoate 25 % v/v, which reached a miticidal effect after 66 min. In all citral concentrations tested (0.1, 0.5, 1.0, 5.0, and 10% v/v), there was a significant reduction in the hatching rate of eggs in *S. scabiei*.

The bioactivity of lemongrass essential oil observed in this study was also observed in the works by Loko *et al.* (2021), in which the essential oils of *C. citratus* and *C. nardus* had average repellent activities greater than the positive control, with average repellency of 67.6 % at 5.0 μL·cm^{-2} of *C. citratus* and 56.3 % at 20.0 μL·cm^{-2} of *C. nardus*, while the positive control (pirimiphos-methyl) had an average repellency of 45.4 % at 0.5 μL·cm^{-2} in the control of *Dinoderus porcellus* L. (Coleoptera: Bostrichidae).

The observed results indicate that, despite not wholly controlling all individuals of *D. maidis* like the positive control (acephate), *C. citratus* essential oil at 1.5 % and 2.0 % v/v may be an interesting strategy to control this pest according to Integrated Pest Management (IPM) procedures. The IPM method aims primarily at pest population control rather than its complete elimination (Vicenço *et al.*, 2020), as commonly occurs with synthetic pesticides (as the positive control, acephate).

It is important to note that essential oils contain several compounds that can exert repellent and insecticidal activities. Studies demonstrated that these compounds could cause adverse effects on various pests at all levels of biological organization. The toxicity of essential oil in insects, nematodes, fungi, bacteria, and other organisms can be observed in both lethal and sublethal levels. However, the capacity of action of essential oils is little understood since biopesticides have multiple ways of action because of their complex composition, i.e., the presence of many compounds. In addition, due to the short action half-life, the pest insects do not easily develop resistance to the essential oils and their components (Chowanski *et al.*, 2016).

The results obtained in the present study bring novel information on possible alternatives for controlling *D. maidis*, especially regarding alternatives to synthetic chemicals, also aiming to avoid the emergence of resistance in such pest insects (Neves *et al.*, 2021). Moreover, applying synthetic pesticides to control *D. maidis* in large corn crops is economically unfeasible, especially in South America (CABI, 2021).

It was observed that the essential oil of *C. citratus* induced phytotoxicity in corn plants from the concentration of 1.0 % v/v after 24 h of spraying (Figure 1). The plants lost the green pigmentation in leaves, passing to a dark tonality. After 48 h, there was wilting of the leaves and dehydration of the plant tissues, featuring phytotoxicity at the concentration of 1.0 % v/v and greater.

Figure 1. Visual aspect of *Zea mays* plants after exposure to Tween-80® 0.5% v/v and *C. citratus* essential oil 1.5 % v/v by spraying.

Tween-80® 0.5% v/v   *C. citratus* essential oil 1.5 % v/v

Source: authors (2023).

There are records in the literature of the herbicidal activity of various essential oils (Angelini *et al.*, 2003; Agodia *et al.*, 2017; Issa *et al.*, 2020). Brum *et al.* (2014) commented that *C. citratus* essential oil caused phytotoxicity in watermelon plants at concentrations of 2.0 % and 4.0 % v/v and in bean plants at 4.0 % v/v. Dudai *et al.* (1999) reported that *C. citratus* essential oil inhibited seed germination and seedling growth of amaranth palmer (*Amaranthus palmeri*), black mustard (*Brassica nigra*), and wheat (*Triticum aestivum*).

Many allelochemical compounds that are highly phytotoxic are produced by the metabolic pathway of terpenoids (Duke *et al.*, 2000). Furthermore, the oil's ability to harm membrane permeability through hydrophobic interactions results in the loss of macromolecules and metabolic imbalances, ultimately causing cell death (Kaur *et al.*, 2010; Laosinwattana *et al.*, 2018).

It is important to point out that *D. maidis* only reproduces on species of the *Zea* genus (Ramos, 2021). Thus, it could be possible to use lemongrass essential oil as a method of control of voluntary plants (popularly known as *tigueras*) in corn, making unfeasible possible green bridges and, consequently, reducing this pest insect population (Cota *et al.*, 2021). Poonpaiboonpipat *et al.* (2013) also suggested the use of *C. citratus* essential oil for weed suppression since this oil showed vigorous phytotoxic activity against *Echinochloa crus-galli*, being a source and basis for the development of natural herbicides. The results obtained by Somala *et al.* (2022) also commented on

V.11, n.5, julho/2024. ISSN n° 2359-3652

using essential oils formulated as nanoemulsions not only as herbicides but also as natural insecticides.

The present work reinforces the idea that *Cymbopogon citratus* essential oil can be used as a biopesticide in the control of *Dalbulus maidis* following the IPM practices, with a green alternative to help mitigate the problems of plant health, environmental pollution, and issues to human and animal health along with food security in the sense of producing healthier foods.

CONCLUSION

C. citratus essential oil at 1.5 % and 2.0 % v/v had a mortality percentage like the positive control, with a similar performance. Thus, this essential oil may be considered a potential biopesticide in the control of *D. maidis*. The essential oil of *C. citratus* at concentrations below 1.0 % may be used as an alternative control of *D. maidis*. However, the induction of phytotoxicity on corn plants at the concentrations of 1.5 % and 2.0 % v/v may give the possibility of using *C. citratus* essential oil as a bioherbicide, which can be aimed at controlling volunteer plants in corn crops. Controlling volunteer plants would reduce the reproduction possibilities for this pest insect, and this strategy may be considered a potential and interesting alternative for controlling *Dalbulus maidis* in corn.

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