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Efeitos do óleo essencial de *Zanthoxylum caribaeum* contra o percevejo do algodão *Dysdercus peruvianus*

Effects of *Zanthoxylum caribaeum essential oil against cotton bug Dysdercus peruvianus*

Efectos del *Zanthoxylum caribaeum* sobre el insecto del algodón *Dysdercus peruvianus*

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Resumo

Cultivos agrícolas necessitam de proteção contra uma variedade de diferentes insetos conhecidos popularmente como pragas. Algumas dessas pragas estão se tornando cada vez mais resistentes aos pesticidas convencionais, portanto, novas alternativas de controle são necessárias. Neste trabalho, foram analisados os efeitos do óleo essencial da planta *Zanthoxylum caribaeum* no desenvolvimento do percevejo do algodão *Dysdercus peruvianus*. Após análise por cromatografia e espectrometria de massa, o óleo essencial de *Z. caribaeum* apresentou 54 substâncias, sendo os principais constituintes Sylvestrene, Muurola-4 (14), 5-trans-dieno, Isodauceno e α-Pineno. Esses compostos aumentaram significativamente a mortalidade dos insetos e interromperam a metamorfose e a muda, frequentemente de forma dose-dependente. Além disso, ninfas com deformações de pernas, asas e antenas foram observadas. De acordo com os dados, os componentes presentes no óleo essencial de *Z caribeum* parecem ser candidatos promissores ao desenvolvimento de inseticidas verdes para uso em futuros programas de manejo integrado de pragas (MIP).

Palavras-chave: Percevejo manchador; Rutaceae; Atividade inseticida das plantas; *Gossypium* sp; Biopesticidas.

Abstract

Agricultural crops need protection from a variety of different insects popularly known as pests. Some of these pests are becoming increasingly resistant to conventional pesticides, so new control alternatives are needed. In this work, the effects of the essential oil of the plant *Zanthoxylum caribaeum* on the development of cotton stink bug *Dysdercus peruvianus* were analyzed. After analysis by chromatography and mass spectrometry, the essential oil of *Z. caribaeum* presented 54 substances, the main constituents being Sylvestrene, Muurola-4 (14), 5-trans-diene, Isodaucene and α-Pinene. These compounds significantly increased insect mortality and interrupted metamorphosis and molting, often in a dose-dependent manner. In addition, nymphs with deformed legs, wings and antennae were observed. According to the data, the components present in the essential oil of *Z. caribeum* appear to be promising candidates for the development of green insecticides for use in future integrated pest management (MIP) programs.

Keywords: Cotton stainer; Rutaceae; Insecticidal activity of plants; *Gossypium sp;* Biopesticides.

Resumen

Los cultivos agrícolas necesitan protección contra una variedad de insectos diferentes conocidos popularmente como plagas. Algunas de estas plagas son cada vez más resistentes a los pesticidas convencionales, por lo que se necesitan nuevas alternativas de control. En este trabajo se analizaron los efectos del aceite esencial de la planta *Zanthoxylum caribaeum* sobre el desarrollo de la chinche apestosa del algodón *Dysdercus peruvianus*. Después de análisis por cromatografía y espectrometría de masas, el aceite esencial de Z. caribaeum presentó 54 sustancias, siendo los principales constituyentes Silvestreno, Muurola-4 (14), 5-trans-dieno, Isodauceno y α-Pineno. Estos compuestos aumentaron significativamente la mortalidad de los insectos e interrumpieron la metamorfosis y la muda, a menudo de forma dependiente de la dosis. Además, se observaron ninfas con patas, alas y antenas deformadas. Según los datos, los componentes presentes en el aceite esencial de Z. caribeum parecen ser candidatos prometedores para el desarrollo de insecticidas verdes para su uso en futuros programas de manejo integrado de plagas (MIP).

Palabras clave: Chinche manchador del algodón; Rutaceae; Actividad insecticida de las plantas; *Gossypium* sp; Biopesticidas.

1. Introduction

Conventional synthetic insecticides, such as carbamates, pyrethroids, organochlorines, and organophosphates, are used widely in controlling plant diseases and pests of grains of economic importance in agriculture (Viegas, 2003; Dambolena et al., 2016). Despite their rapid and effective action against crop pests, they have high economic costs by the indiscriminate use that leads to the development of resistance by insects and toxicity to human populations and beneficial insects (Mello et al., 2007; Chaubey et al., 2019). One possible way to avoid the side effects of insecticides in the field is to use natural products, plants with insecticidal activity that selectively target plant pests, avoiding non-target animals, and are environmentally safe (Isman 2005; Chaubey et al., 2019; Bignon et al., 2020). Essential oils (EOs) derived from aromatic plants by steam distillation have been used for ages by humans for flavoring foods, drinks and perfumes (Isman, 2005, 2011). Essential oils are volatile compounds of plant secondary metabolism with aromatic components giving smell, or scent to a plant. They are also responsible for several biological activities and effect against mites, insects, and fungi (Nogueira et al., 2014 a, b; Ranasinhe et al., 2002). Also, many essential oils have been known to disrupt the development, reproduction and behavior of insects of health and economic importance (Koul 2008, Nogueira et al, 2014 a, b). In recent years, the use of essential oils (EOs) derived from aromatic plants as low-risk insecticides has increased considerably due to its popularity with organic producers and environmentally conscious consumers and some commercial products are already available in USA and EU (Isman, 2019).

Rutaceae is a large plant family, containing *ca.* 160 genera, 1900 species (Groppo et al, 2008), and distributed mainly in the tropics and subtropics (Judd et al, 2009). Some species have significance in medicine, ecology and in the economy (Januário et al, 2009), and are used traditionally for treating venereal diseases, malaria, dysentery, skin and urinary infections (Boehme et al, 2008). In Brazil, this family has about 33 genera and 193 species (Pirani and Groppo 2013), including Z*anthoxylum caribaeum* Lamarck that is largely present in the neotropics, occurring in every Brazilian state (Pirani, 2002, 2013).

Dysdercus peruvianus Guérin-Méneville (Heteroptera: Pyrrhocoridae), commonly known as cotton stainer, is an economically important species of the Order Hemiptera, and a pest of cotton (*Gossypium hirsutum* L. – Malvaceae). Feeding cotton by *D. peruvianus* reduces seed weight, oil content and inoculates microorganisms (bacteria and fungi entomopathogenics) into the spots produced in the fiber which result in economic loss (Milano et al., 1999). *D. peruvianus* are also a common model to study pest phytophagous physiology since its easily rearing in laboratory colonies (Brisolla, 1992). In the present work, the effects of the essential oil from leaves of *Z. caribaeum* on the development of *D. peruvianus* was studied.

2. Methodology

Plant Material

Z. caribaeum leaves were collected in Restinga de Jurubatiba National Park, Rio de Janeiro state, Brazil. Plant material was identified by the botanist Dr. Marcelo Guerra Santos and a voucher specimen number 15.497 was recorded at the herbarium in the Faculdade de Formação de Professores (Universidade do Estado do Rio de Janeiro, Brazil).

Extraction and chemical analysis of the EO from leaves of Z. caribaeum

Essential oil extraction was performed immediately after collection. Leaves were collected and turbolized with distilled water. The material was hydrodistillated for 4h in 5.0 L round-bottom flask in a Clevenger-type apparatus. Residual water was removed by filtration with sodium sulfate (Na2SO4) and the EO obtained was stored at 4 °C until further analysis (Amaral et al., 2013). The essential oil composition was then analyzed by gas chromatography/mass spectrometry analysis and flame ionization gas chromatography (Gonzalez et al., 2014, Adams, 2007).

Insect colony

The D. peruvianus colony was rearing at 26 ± 1°C and 60% relative humidity at the Laboratory of Insect Biology (UFF) as described by Fernandes et al. (2013) and Gonzalez et al. (2014). Genetic biodiversity property was authorized under number (A0E95C4) at National Management System of Genetic Heritage and Associated Traditional Knowledge (SISGEN) of Brazilian Ministry of the Environment.

Insect bioassays

From 1 g of the pure and fresh essential oil from the leaves of Z. caribaeum, a serial dilution in acetone produced final concentrations of 500 mg, 250 mg, 125 mg, 62.5 mg and 31.25 mg of oil/mL. For experimental groups, 1 μL of undiluted fresh oil or 1 μL of each dilution were topically applied to the ventral abdominal cuticle of randomly chosen fourth-instar nymphs of D. peruvianus (Tietbohl et al., 2014). Therefore, the dose administered were 500 µg, 250 µg, 125 µg, 62.5 µg or 31.25 µg/insect in each experimental group. Insects in negative control group were untreated while insects in positive control group were topically applied solely with acetone. The biological evaluation of the different treatments was undertaken during the entire time (30 days) required for development from the fourth-instar nymphs to the adult stage. Parameters recorded were, weight, toxicity (mortality for 24 h immediately after treatment), lethality (mortality during 30 days of treatment), intermolt period (range), molt, metamorphosis and malformations. All experiments were repeated three times with the average of three groups of 10 insects (n = 10).

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Statistical analysis

The results were analyzed for significance with the one-way ANOVA and Tukey's test according to Stats Direct Statistical Software, version 2.2.7 for Windows 2000 (Armitage et al., 2002). The differences between the treated groups from the control insects were not significant statistically when p>0.05. (Armitage et al., 2002). Lethal dose (LD) was estimeted using Statgraphics Centurion XV version 15.1.02 program with upper and lower confidence limit 95%. Probability levels are indicated in the text and tables.

3. Results

The essential oil obtained by hydrodistillation of *Z. caribaeum* fresh leaves furnished clear light yellowish essential oil in 0.1% yield (w/w) containing fifty-four substances identified by GC/MS and CG/FID analysis. The major constituents were monoterpenes (41.2 %) and sesquiterpenes (47.3%). The main substances found were Sylvestrene (11.30%), Muurola-4(14), 5-trans-diene (8.40%), Isodaucene (8.30%) and α-Pinene (7.60%). (Table 1).

Table 1: Chemical composition of essential oil of *Zanthoxylum caribaeum* fresh leaves extracted by hydrodistillation and analyzed by chromatography/mass spectrometry coupled to flame ionization gas chromatography.

RI	SUBSTANCES	% AREA
802	Hexanal	0.2
851	Hexenol	0.4
863	n-Hexanol	0.3
928	α-Tujeno	0.3
938	α-Pinene	7.6
950	Camphene	0.4
974	Sabinene	0.8
979	β-Pinene	0.7
994	Myrcene	4.0
1009	α-Carene	3.7

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1019	α-Terpinene	1.0
1027	θ-Cymene	0.5
1035	Sylvestrene	11.3
1038	B-Z-ocimene	0.4
1049	B-E-ocimene	2.0
1060	γ-Terpinene	1.8
1091	Terpinolene	0.4
1103	Linalool	2.0
1141	p-trans-Menth-2-en-ol	0.3
1154	Citronellal	0.3
1180	Terpine-4-ol	0.6
1194	α-Terpineol	1.0
1203	trans-Carveol	0.3
1211	B-cyclocitral	0.9
1230	Citronellol	0.5
1234	E-ocimene	0.4
1340	δ-Elemene	0.7
1353	α-Cubenene	0.4
1398	β-Elemene	4.0
1444	α-Guaiene	1.4
1449	Myltayl-4(12)-ene	0.4
1455	cis-Muurola-3,5-diene	0.6
1459	A-humulene	1.1
1466	Γ-gurjunene	0.6
1475	4,5-di-epi-Aristolochene	0.8
1481	Γ-muurolene	0.9
1491	Muurola-4(14),5-trans-diene	8.4
1496	Viridiflorene	3.0

1506	Isodaucene	8.3
1514	Premnaspirodiene	0.8
1525	Selinene-7-epi-α	2.8
1531	Zonarene	0.4
1563	Germacrene B	0.7
1583	Spathulenol	0.6
1635	Cubenol-1-epi-	0.9
1427	β-caryophyllene	4.3
1522	Cubebol	1.2
1554	Elemol	0.8
1590	Ledol	0.8
1649	Cubenol	0.6
1653	α-Muurolol	0.4
1662	α-cadinol	1.7
1693	Germacra-4(15),5, 10(14)-trien-1-α-ol	0.7
2115	β-Phytol	1.0
	Total identified	90.40
	Hydrocarbon monoterpenes	34.9
	Oxygenated monoterpenes	6.3
	Hydrocarbon sesquiterpenes	39.6
	Oxygenated sesquiterpenes	7.7
	Others	1.9

**RI* retention indices on DB-5MS column in reference to n-alkanes. Source: Authors

The weight of the insects did not vary significantly after treatment with the *Z caribaeum* EO when compared to the controls. None of the tested treatments deterred insect feeding. Also, no significant differences in the morphology or development occurred comparing untreated insects with those treated only with the acetone solvent (results not shown).

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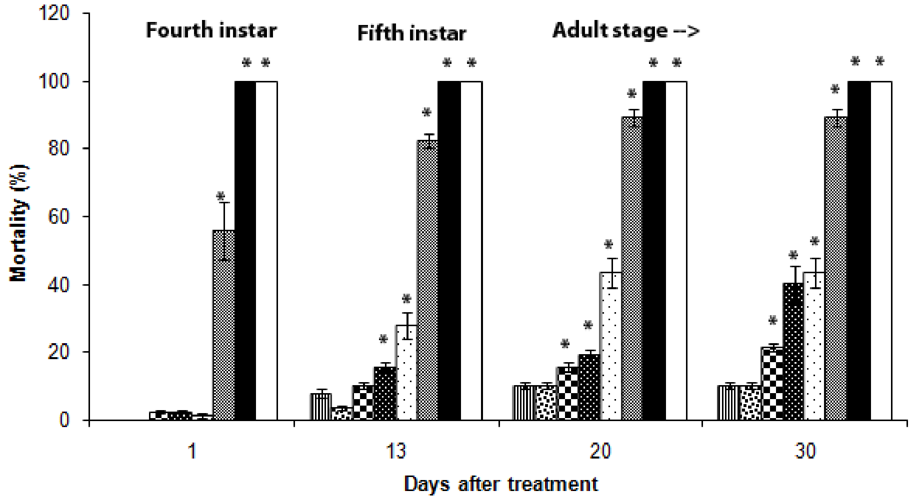
In contrast, topical treatment of *D. peruvianus* fourth instar nymphs with the *Z. caribaeum* EO and its dilutions induced significant increases in mortality (Fig. 1). Insects treated with pure EO and the 500 µg /insect dilution showed 100% mortality rates (p<0.0001) already by 24 h after treatment while no insects died in both positive e negative control groups at the same time. In all other experimental groups, and both controls (negative and positive), the mortality rates gradually increased during insect development stages from fourth instars to adults (Fig.1). Dose of 250 µg /insect produced 55.57±8.39% (p<0.001) mortality after 24 h and 88.90±2.52% (p<0.001) by the end of the experiments (30 days) when both controls groups reached 10.00±1.00% mortality (Fig.1). Doses of 125 µg /insect, 62.5 µg /insect and 31.25 µg /insect induced low, none significant levels of mortality of 1.10±0.58%, 2.23±0.58% and 2.23±0.58% (p>0.05), respectively, by 24 h after treatment. However, the same doses induced significant levels of mortality of 43.33±4.36% (p<0.001), 40.00±5.57% (p<0.001) and 21.23±1.00% (p<0.01), respectively, after 30 days (Fig.1). Toxicities of EO from *Z. caribaeum* leaves was also expressed as the estimated doses at which 50% to 100% of test insects were killed at both 2nd and 30th days after treatment and is referred as lethal doses (LD).

Figure 1: Mortality of *Dysdercus peruvianus* after topical treatment of fourth-instar nymphs with pure *Zanthoxylum caribaeum* essential oil and its serial dilutions.

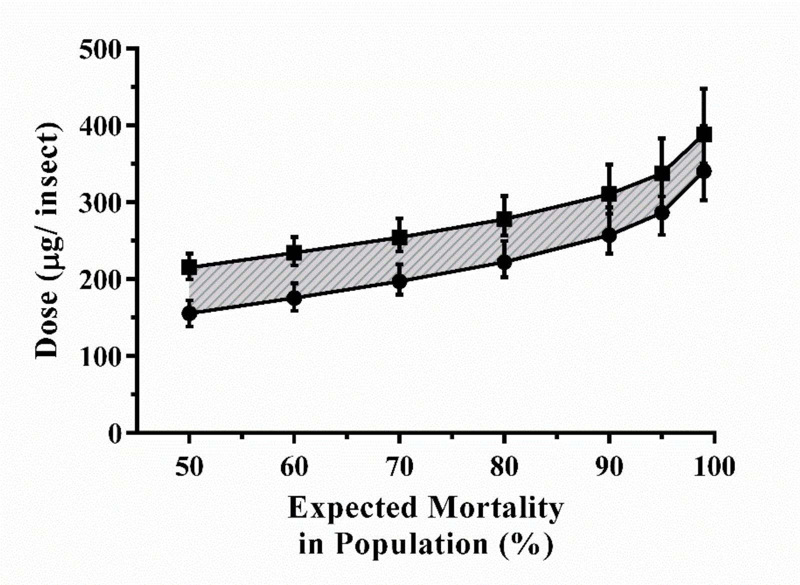


Each group represents means ±SD percentage of at least three experiments. Asterisks represents groups with statistical difference compared with negative control (non-treated) and positive control (solvent only) groups. A= Negative control; B= Positive control; C= 31.25 µg /insect; D= 62.5 µg mg/insect; E= 125 µg /insect; F= 250 µg /insect; G= 500 µg /insect and H= pure oil.

Source: Authors.

Toxicities of essential oil of leaves from *Z. caribaeum* were also expressed as the concentrations at which LD_{50}, LD_{90} and LD_{100} (Fig 2) and its lower and upper limits at 2nd day of treatment were 215.343 (199.779 to 233.231), 310.756 (285.025 to 348.902) and 388.551 (350.149 to 447.596), respectively. At 30 days of treatment these values were 155.540 (138.595 to 172.602), 257.495 (232.998 to 293.512) and 340.623 (302.713 to 399.351), respectively. Thus, by our results the effects of *Z caribeum* essential oil and its dilutions on *D.peruvianus* mortality were displayed in a dose response manner (Fig. 2).

Figure 2: Determination of median lethal dose (LD) of *Zanthoxylum caribaeum* essential oil on *Dysdercus peruvianus* at 2nd (■) and 30th (●) days of treatment.



Source: Authors.

Regarding the effects of the EO on insect development, Table 2 shows that in the negative and positive control groups, 82.2±4.16% and 96.7±1.15% ($p>0.05$) insects moulted to fifth instar, respectively, during an intermolt period from the 4th to 12th day after treatment. In the same groups, 82.20±4.16% and 90.00±1.00% ($p>0.05$) of fifth instar nymphs metamorphosed to adults in 12th-21th days after treatment. Dose of 250 µg /insect of Z. caribaeum EO reduced moulting from fourth to fifth instar nymph to only 13.3±1.53% (p<0.001) and metamorphosis to adults to 11.1±2.52 (p<0.001). Also, 2.2±0.53% (p<0.01) of overaged fourth instar nymphs were observed. In this group, the intermoult period was advanced from 1st-9th days after treatment but the range of metamorphosis was the same as the control groups (Table 2). In the group treated with 125 µg/insect, 72.20±4.04% (p<0.001) of insects reached the fifth instar after a longer intermolt period from 1st-12th days after treatment (p<0.001). In this group, all of the 56.7±4.36% (p<0.001) surviving insects reached the adult stage in the same period (12th-21th days) as the control groups (Table 2). In the group treated with 62.5 µg/insect, 60.00±1.53% (p<0.001) insects reached both fifth instar and metamorphosis to adults after longer intermoulting period of 1st-12th days (p<0.001) and 13th-24thdays (p<0.001), respectively. However, in this group 24.4±2.52%(p<0.001) of insects remained as overaged fourth instar nymphs (Table 2). Dose of 31.25 µg/insect did not affect the percentage moulting to fifth instars (87.8±2.52) (P >0.05). In this experimental group, the intermoult period to fifth instar nymphs was 13 days (1-14) and in control 1 group 8 days (4-12 days) (Table 2). This dilution also significantly reduced the metamorphosis process to adults to 70.0±7%(p<0.001) during a longer intermoult period from 12th-24thdays (p<0.001). In this group, 2.20±0.53%(p<0.01) and 6.6±1.15 (p<0.01) of surviving insects remained as overaged fourth and fifth instar nymphs, respectively. Additionally, doses of 250 µg /insect, 125 µg/insect and 62.5 µg/insect induced the appearance, respectively, of 1.00±0.58%, 3.00±1.73% or 2.00±1.15% insects with body deformities such as absence or malformation of legs, wings or antennae (Figs 3, 4). In summary, the EO and its dilutions partially inhibited both molting and metamorphosis and induced longer intermolt period together with overaged fourth or fifth-instar nymphs, often in a dose-response manner.

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Table 2: Development of *Dysdercus peruvianus* after topical treatment (1µL) of fourth-instar nymphs with pure *Zanthoxylum caribaeum* essential oil and its serial dilutions. Negative Control: non-treated insects; Positive Control: Insects treated with only acetone. No significance: *P* > 0.05. Significance: **a** – P < 0.01; **b** – P < 0.001; **c** – P < 0.0001. The data without letters, did not show statistical significance. * All results are expressed ± SD.

***Groups**	% Moulting/range days		% Metamorphosis /range		% Over-aged nymphs		%Deformed Insects	
	from 4^{th} to 5^{th} instar	range days	from 5^{th} to adult	range days	4^{th} instar	5^{th} instar	5^{th} instar	adults
<u>Negative Control</u>	82.2±4.16	4^{th}-12^{th}	82.2±4.16	13^{th}-20^{th}	0	0	0	0
<u>Positive Control</u>	96.7±1.15	4^{th}-$9^{th\ \mathbf{b}}$	90.0±1.00	12^{th}-21^{th}	0	0	0	0
<u>Essential oil/insect</u>:	-	-	-	-	0	0	0	0
0.500 mg	0	-	-	-	0	0	0	0
0.250 mg	13.3±1.53^{c}	1^{st}-9^{th}	11,1±2,52^{c}	12^{th}-21^{th}	2.2±0.53^{a}	0	0	1±0.58
0.125 mg	72.2±4.04^{b}	1^{st}-$12^{th\ b}$	56.7±4.36^{b}	12^{th}-21^{th}		0	0	3±1.73
0.0625 mg	60.0±1.53^{b}	1^{st}-$12^{th\ b}$	60.0±1.53^{b}	13^{th}-24^{thb}	24.4±2.52^{c}	0	0	2±1.15
0.03125 mg	87.8±2,52	1^{st}-14^{th}	70.0±7.00^{b}	12^{th}-24^{thb}	2.2±0.53^{a}	6.6±1.15^{a}	0	0

Source: Authors.

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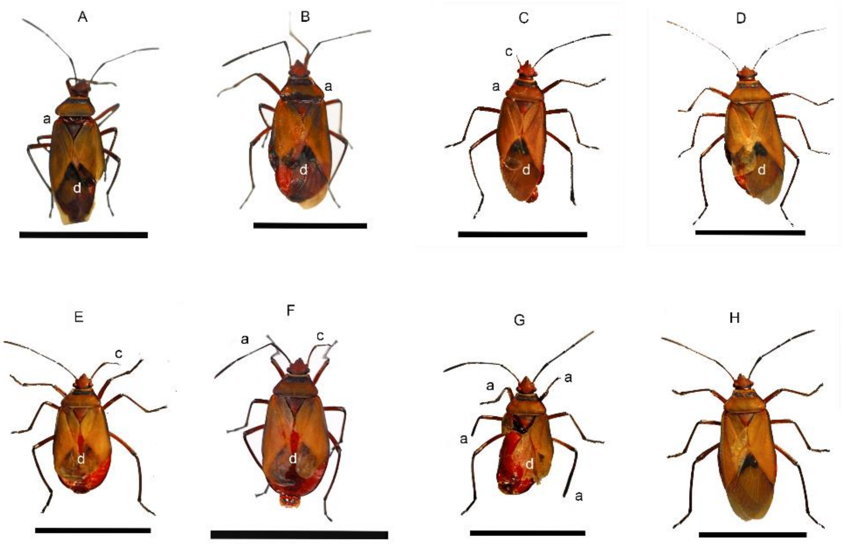
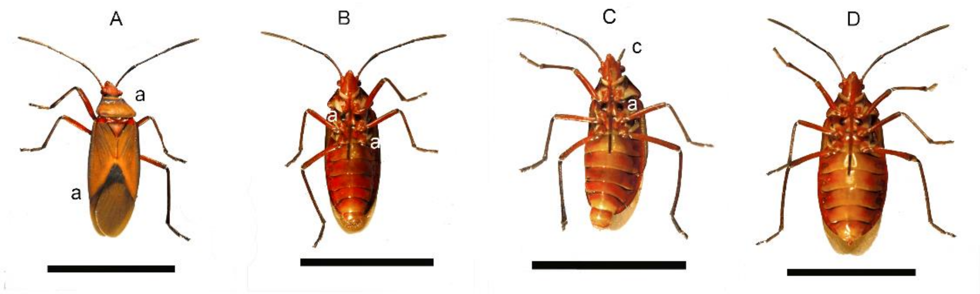
Figure 3: Malformation of adults *Dysdercus peruvianus* body after topical treatment with *Zanthoxylum caribaeum* essential oil. (**A**) Dorsal view: absence of left leg (a) and wing malformation (d); (**B**) absence of right leg (a) and wing malformation (d); (**C**) dorsal view: absence of the left leg (a) absence of left antennal flagellum (c), wing malformation (d); **(D)** wing malformation (d); **(E)** wing malformation (d) and absence of right antennal flagellum (c); **(F)** absence of both antennal flagella (c), left leg malformation (a), wing malformation (d); **(G)** left and right leg malformation (a), second leg absence of tarsus (a) and wing malformation (d) (**H**) dorsal view of control insect (untreated).



Source: Authors.

Figure 4: Malformation of adult *Dysdercus peruvianus* body after topical treatment with *Zanthoxylum caribaeum* essential oil. (**A**) Dorsal view: absence of the right prothoracic leg and the left metathoracic leg (a); (**B**) Ventral view: absence of the right prothoracic leg and the left metathoracic leg (a); (**C**) ventral view: absence of the left prothoracic leg (a) and pedicel and flagellum of the right antenna (c); **(D)** ventral view of control insect (untreated).



Source: Authors.

4. Discussion

The biological effect of different essential oils on several organisms is directly related to their compositions and often biological activities of an essential oil is determined by one or two of its major components, however synergistic effect may occur in other circumstances Chaubey (2019). The major compounds found in this work are the same found by Nogueira et al. (2014a) for leaves of *Z. caribeum*, with slightest differences concerning their percentage composition with sylvestrene, muurola-4(14),5-trans-diene, isodaucene and α-pinene as major compounds. Those differences may be due to environmental factors that may involve geographical origin, or as a consequence of stress produced by variation of abiotic factors such as wind, air humidity and salinity along different seasons in the sandbank area (Gershenzon and Dudareva, 2007, Van Vuuren et al, 2007, Zoghbi et al, 2014). Besides, the comparison of effects of a particular essential oil in different organisms or biological systems can help to enlight some aspects of its mode of action and feasibility for use to control of insect populations (Lopez et al, 2011).

The yield 0.1% (w/w), chemical profile and insecticidal effects of the EO of fresh leaves of *Z. caribaeum* (Rutaceae) are also quite like another species of the genus. EO of *Z. rhoifolium* fresh leaves also contains β-Elemene (31.70%), β-Caryophyllene (12.1%), Phytol (1.26%) and exhibited insecticidal effects against *Bemisia tabaci* (Gennadius 1889) (Hemiptera: Aleyrodidae) (Christofoli et al. 2015). Besides, α-Pinene (2.30%), β-Pinene (2.60 %), α-Terpinene (1.40%), Z-β-Ocimene (1.80%), E-β-Ocimene (1.90%), α-Cadinol (12.30%), Elemol (2.40%) and α-Cubenene (1.70%) were also present in EO of *Z. monophyllum* (Rutaceae) which has larvicide activity against *Aedes albopictus* (SKUSE, 1894) (Díptera: Cullicidae), *Anopheles subpictus* (Grassi, 1899) (Diptera: Culicidae) and *Culex tritaeniorhynchus* (Giles, 1901) (Diptera: Culicidae) (Pavela & Govindarajan 2016). Also, α-Pinene (1.31%), Linalool (12.94%), β-Elemene (3.32%), Spathulenol (4.59%) were identified in OE of *Z. schinifolium* leaves which increased mortality in maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) (Wang et al 2011). The major terpene in *Z. caribeum* EO, sylvestrene, was previoulsly described in EO of *Schinus terebinthifolia* (Anacardiaceae) fruits, an EO able to kill mosquitos' vectors of Malaria (Kweka et al. 2011). Muurola-4(14) and 5-trans-diene are a bicyclic sesquiterpenes present in EO of *Haplopappus foliosus* (Asteraceae) which shown insecticidal activity against *Musca domestica* (Linnaeus, 1758), (Diptera: Muscidae) (Urzua et al 2010) and Isodaucene obtained from EO of *Etlingera elatior* (Zingiberaceae)is able to trigger antennal depolarization in *A. aegypti* females followed by inhibition of oviposition (Bezerra-Silva et al. 2016). The enantiomers α-Pinene and β-Pinene have been associated with several bioactivities as antiviral, antifungal, antimicrobial and used as a natural insecticide, although studies show low insecticide effect against *S. zeamais* (Wang et al. 2011, Yildirim et al. 2013). Moreover, the major component of *Z. caribeum* EO, sylvesterene, were identified in EOs with antimicrobioal activity (Hsouna et al., 2011).

Our results showed that a single topical treatment of pure or diluted *Z. caribeum* EO with much lower doses significant increased mortality of *D. peruvianus* in a dose-response and time-dependent manner. Also, the EO partially inhibited moulting and metamorphosis and induced longer intermoult periods together with the arising of overaged fourth- and fifth-instar nymphs which cannot metamorphose or reproduce (Mordue et al., 1985). Consequently, few adult insects emerged after treatment often with deformities in wings, legs, and antennae. These changes are suitable to the proposal to control insect populations and are usually induced by compounds- mainly terpenes- that interfere with the molt process and act either by a neuroendocrine route, inhibiting the synthesis of the molting hormone, ecdysone or by directly disrupting chitin synthesis (Hummelbrunner & Isman, 2001). In most cases biological activities of a specific essential oil is determined by one or two of its major components, however synergistic or additive effects frequently occurs. Most studies have been used whole EOs or only their major compounds for the investigation (Senthil et al., 2006) and few studies have worked on the interactive effects of the several oil components **(**Park et al., 2017). Thus, the information about the effects of interaction among individual compounds on the functionality of EOs has been limited and are needed to clarify possible applications in selecting EOs or in seeking the best combination of EOs or individual compounds to achieve efficient insecticidal effects since essential oils may be more efficacious than the pure compounds derived from them due to synergism or joint action of active compounds with different modes of action (Liu et al., 2020).

For example, Yildirim (2013) tested separately the insecticidal capacity of 28 hydrocarbon and oxygenated terpenes in *S.zeamais* from 24h to 96h after treatment. The mortality rate was highly significant by dosage rate and exposure time. α-Pinene, β-Pinene, Myrcene, α-Carene, Linalool, Citronellal, Terpine-4-ol and α-terpineol - which are also present in the essential oil from *Z. caribaeum* - were *a*mong the terpenes tested and shown potential to increase toxic effect acting synergistically with the major metabolites. It has been suggested that the evolution of insect resistance to EO mixtures is slow in contrast to resistance to pure compounds - which develops rapidly - due to the difficulty of developing several adaptations simultaneously (Siskos et al. 2009). In addition, synergism may help in reducing the pesticide load due to the use of smaller absolute amounts in the mixture to achieve satisfactory levels of efficacy (Hummelbrunner & Isman 2001). In the same way, the treatment of *D. peruvianus* with *Myrcyaria floribunda* EO resulted in high mortalities and disrupted metamorphosis as well as overaged nymphs, juvenoid insects and insect deformations (Tietbohl, et al 2014, Tietbohl et al 2019).

Thus, insect growth regulators (IGRs) as EOs that are environmentally friendly has been integrated into modern vector and crop control programs since they are ecologically safer, degradable, and target specific (Isman 2005; Mello et al. 2007; Fernandes et al. 2013; Gonzalez et al. 2014; Fernandes et al. 2014, Duprat et al. 2017, Tietbohl et al. 2019, Feder et al. 2019, Rosado et al. 2019). In the case of EOs, some, or their chemical components, are already in use as flavoring components in foods and beverages and are even exempt from pesticide registration. This regulatory status and the vast availability of EOs from the flavor and scent industries, has made it possible to fast track commercialization of EO-based green pesticides (Koul et al 2008) and studies with EO, like this, can help in the development of new formulations. The commercial development of bioinsecticides based on the essential oil of plants can be produced in different ways as a mixture of essential oils, as a single essential oil, a single terpenoid constituent, a blend of terpenoids, synthetically produced, that emulate those in a plant essential oil or even as a novel (non-natural) blend of terpenoids obtained from different plant sources (Isman 2019). It is worth to note that by our results, the action of the *Z. caribaeum* EO is not restricted only to the hematophagous pests, *Rhodnius prolixus* (Stål, 1859) (Hemiptera: Reduviidae) and *Rhipicephalus microplus* (Aragão 1936) (Acarina:Ixodidae) (Nogueira et al, 2014 a e b) but has potential for application to a wide spectrum of both vector and agricultural pests.

5. Final Considerations

Thus, according to our observations, the essential oil of the leaves of *Z. caribaeum* and its components are promising candidates for integrated pest management programs that are environmentally friendly or as an alternative tool in the protection of stored grains. Our results also point to the viability of its components being used as growth regulators for insects against agricultural pests. Currently, more detailed research on the mechanism of action of its components and the interaction between them is underway in our laboratory. Field assessment and investigation of the effects of essential oil on non-target organisms are also necessary

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