

Extratos de *Dicksonia sellowiana* Hook. e *Nepholepis cordifolia* (L.) Lellinger como potenciais pesticidas verdes: atividade em insetos

***Dicksonia sellowiana* Hook. and *Nepholepis cordifolia* (L.) Lellinger extracts as potential green pesticides: insecticidal activity**

Extractos de *Dicksonia sellowiana* y *Nepholepis cordifolia* (L.) Lellinger como posibles plaguicidas ecológicos: actividad en insectos

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Resumo

Conhecidos como inseticidas verdes, os produtos naturais de origem vegetal têm se revelado uma promissora alternativa aos inseticidas convencionais. O presente trabalho teve como objetivo avaliar a atividade inseticida dos extratos etanólicos brutos das samambaias,

Dicksonia sellowiana e *Nephrolepis cordifolia* sobre *Oncopeltus fasciatus* (Hemiptera). As folhas foram coletadas no Parque Nacional de Itatiaia, secas, maceradas com etanol 96% e o extrato concentrado em evaporador rotatório. A partir do extrato bruto foi preparada uma solução na concentração de 50 mg/mL, usando acetona como solvente. A análise qualitativa do perfil de terpenoides e substâncias fenólicas foi realizada por meio de cromatografia em camada delgada. Foram utilizados insetos no 4º ínstar em grupos de 10 animais em triplicatas por tratamento. Em cada grupo foi topicamente aplicado à cutícula abdominal dos insetos 1 µL das seguintes soluções: extrato de *D. sellowiana*, de *N. cordifolia*, controle água e controle acetona. Após 21 dias todos os insetos do grupo controle negativo (água) haviam atingido a idade adulta. O extrato de *N. cordifolia* foi responsável por 63% ($p < 0,0001$) de mortalidade nos insetos, em torno do 16º dia após o tratamento, enquanto que o extrato de *D. sellowiana* apresentou 50% ($p < 0,0001$) no 21º dia após o tratamento. Os extratos também promoveram atrasos no período de muda e metamorfose dos insetos. Os extratos de *D. sellowiana* e *N. cordifolia* apresentaram similaridade de 18% no perfil de terpenoides e 0% para substâncias fenólicas. *N. cordifolia* e *D. sellowiana* apresentam um potencial promissor para a pesquisa de substâncias para uso como inseticidas verdes.

Palavras-chave: Samambaias; Insetos-praga; Bio inseticidas; Inseticidas vegetais; Atividade inseticida.

Abstract

Known as green insecticides, natural plant-based products have become a promising alternative to conventional insecticides. The primary objective of the present study was to analyze, under laboratory conditions, the insecticidal activity of crude ethanol extracts of the fern species *Dicksonia sellowiana* and *Nephrolepis cordifolia* against *Oncopeltus fasciatus* (Hemiptera). Fern leaves were collected from Itatiaia National Park (Brazil), dried and ground using 96% ethanol, with the extract concentrated in a rotary evaporator. The crude extract was used to prepare a 50 mg/mL solution, with acetone as solvent. Qualitative analysis of the terpenoid and phenolic substance profile in the extracts was performed by thin-layer chromatography (TLC). Fourth-instar insects were used, in groups of 10 animals per treatment, with three repetitions. Four treatments were used: *D. sellowiana* and *N. cordifolia* extracts, acetone control and water control. Then, 1 µL either of the respective solution was topically applied to the insect abdominal cuticle. After 21 days, all the insects in the water control group had reached adult. The *N. cordifolia* extract was responsible for 63% ($p < 0.0001$) of insect mortality around 16 days after treatment, whereas the *D. sellowiana* exhibited 50% ($p < 0.0001$) on the 21st day post-treatment. The extracts also caused delays in insect molting and metamorphosis. The *D. sellowiana* and *N. cordifolia* extracts exhibited 18% similarity in the terpenoid profile and 0% for

phenolic substances. *N. cordifolia* and *D. sellowiana* show potential for research on selective biodegradable substances for use as green insecticides.

Keywords: Ferns; Insect pests; Bioinsecticides; Plant insecticides; Insecticidal activity.

Resumen

Conocidos como insecticidas verdes, los productos naturales de origen vegetal han demostrado ser una alternativa prometedora a los insecticidas convencionales. El presente documento tiene por objeto evaluar la actividad insecticida de los extractos etanólicos brutos de helechos, *Dicksonia sellowiana* y *Nephrolepis cordifolia* en *Oncopeltus fasciatus* (Hemiptera). Las hojas se colectaron en el Parque Nacional Itatiaia (Brasil), se secaron, se maceraron con 96% de etanol y el extracto se concentró en un evaporador rotativo. A partir del extracto crudo se preparó una solución a una concentración de 50 mg/mL, utilizando acetona como disolvente. El análisis cualitativo del perfil de las sustancias terpenoides y fenólicas se realizó mediante cromatografía en capa fina. Se usaron insectos en su 4° estadio en grupos de 10 animales por triplicado por tratamiento. En cada grupo se aplicaron tópicamente las siguientes soluciones a la cutícula abdominal de los insectos 1 µL: extracto de *D. sellowiana*, de *N. cordifolia*, control de agua y control de acetona. Después de 21 días todos los insectos del grupo control de agua habían alcanzado la edad adulta. El extracto de *N. cordifolia* fue responsable del 63% ($p < 0,0001$) de la mortalidad de los insectos alrededor del 16° día después del tratamiento, mientras que el extracto de *D. sellowiana* presentó un 50% ($p < 0,0001$) al 21° día después del tratamiento. Los extractos también promovieron retrasos en el período de muda y metamorfosis de los insectos. Los extractos de *D. sellowiana* y *N. cordifolia* mostraron una similitud del 18% en el perfil terpenoide y del 0% para las sustancias fenólicas. *N. cordifolia* y *D. sellowiana* presentan un potencial prometedor para la investigación de sustancias para su uso como insecticidas verdes.

Palabras clave: Samambaias; Insectos-plaga; Bio insecticidas; Insecticidas vegetales; Actividad Insecticida.

1. Introduction

There are an estimated 5 to 10 million different species of insect (Lewinsohn et al., 2012). Despite their economic and ecological importance, approximately 10% of insect species are considered pests worldwide, causing significant damage to major crops and heavy production losses (Zucchi et al., 1992), in addition to being harmful to domestic animals, humans and other plants (Gallo et al., 2002).

Most chemical insecticides available are harmful to non-target organisms, such as humans and wild animals (Rizzati et al., 2016). Given the need for new substances that act efficiently and specifically, with non-neurotoxic mechanisms of action and low persistence, green insecticides have become a viable alternative (Koul et al., 2008). One of the most prominent is insect growth regulators (IGRs), which can be hormonal or chitin synthesis inhibitors. Hormonal IGRs typically function by inhibiting or mimicking the juvenile hormone (JH), the latter causing abnormal reactions such as extraneous stages in the life cycle, for example, the juvenoids. The aim is to prolong the larval and nymph stages, disrupting development and resulting in sterile adults (Ferreira, 1999). Anti-juvenile hormone agents inhibit JH production, blocking the insect molting (Quistad et al., 1982). Chitin synthesis inhibitors interfere in the formation of the exoskeleton (ecdysis) and chitin production, inhibiting insect development.

Plants produce a variety of substances that can act as IGRs, including essential oils, terpenoids and phenolic substances (Feder et al., 2019). As such, due to their efficacy/dose, environmental safety and lack of human toxicity, plants have become a promising source of green insecticides and an alternative to synthetic pesticides (Tietbohl et al., 2014).

Ferns have an estimated 11,916 different species (PPG I, 2016), making them the second largest group of vascular plants. Ferns are promising candidates as a source of green insecticides, since several species produce substances such as steroids (Oliveira, 2012; Xavier et al., 2016), which have insecticidal potential (Lovatto, Schiedeck & Mauch, 2013), including *Dicksonia sellowiana* Hook (Dicksoniaceae) and *Nephrolepis cordifolia* (L.) Lellinger (Lomariopsidaceae). However, studies that assess the biological activity of ferns remain scarce (Santos et al., 2010).

Over the years, *Oncopeltus fasciatus* (Dallas, 1852) (Hemiptera: Lygaeoidea) has become a popular laboratory species because it is easy to grow and tolerates a wide range of conditions. It has also become one of the most important species worldwide in terms of entomology and physiology-related issues (Chipman, 2017).

The primary objective of the present study was to analyze, under laboratory conditions, the insecticidal activity of crude ethanol extracts of the fern species *Dicksonia sellowiana* and *Nephrolepis cordifolia* against *Oncopeltus fasciatus*.

2. Material and methods

Botanical material

Dicksonia sellowiana (22°22'35.9" S 44°45'38.1" W) and *Nephrolepis cordifolia* (22°28'29.0"S 44°34'48.0"W) were collected from Itatiaia National Park. The first species in October 2016 (end of the dry season) and the second in April 2017 (end of the rainy season). The collections were authorized for scientific purposes, under license number 53534-2, issued on 11/28/2016 by the Chico Mendes Institute for Biodiversity Conservation (ICMBio). Part of the botanical material was collected in line with the techniques described by Fidalgo & Bononi (1989) and all the exsiccates were deposited in the Faculdade de Formação de Professores Herbarium (RFFP) at Universidade do Estado do Rio de Janeiro (UERJ), Brazil.

Extract preparation

The leaves of the fern species were dried in an oven at 50°C, then ground and weighed. The dry plant material was macerated in 96% (v/v) ethanol at a proportion of 1L of solvent to 100g of macerated plant for approximately 45 days to obtain the concentrated extract. The crude extract was used to prepare a 50 mg/mL solution, with acetone as solvent. Each extract was submitted to ultrasound for three minutes to ensure total solubilization in the solvent.

Insect bioassays

The *O. fasciatus* colonies were established in the Laboratory of Insect Biology of the Universidade Federal Fluminense, which followed the methodology already in place in the lab (Duprat et al., 2017).

To evaluate the toxicity of the extracts, randomly chosen fourth instars of *O. fasciatus* were used, in groups of 10 insects per treatment, with three repetitions. Four treatments were used: *D. sellowiana* extract (DS), *N. cordifolia* extract (NP), acetone control (AC) and water control (WC). Then, 1 µL either of the respective solution was topically applied to the insect abdominal cuticle. Detailed evaluations of the various treatments were made from the day after treatments (1st day) and throughout the time for development from the fourth instars to the adults. Evaluations recorded were the toxicity (mortality over the 24 h following

treatment), lethality, range of intermolt period, molting and metamorphosis (Fernandes et al., 2013).

Qualitative analysis of the plant extracts

Qualitative analysis of the extracts was performed by thin-layer chromatography, using chemical markers for terpenoids (beta-sitosterol and fridelinol) and phenolic compounds (rutin and kaempferol). Samples of extract (4 µl) diluted in methanol (50 mg/ml) were applied to silica gel (F254) chromatography plates (SILICYCLE inc.). For the terpenoid plate, a hexane:acetate (7:3) mobile phase and vanillin-sulfuric reagent were used, with reading performed in a darkroom at a UV wavelength of 365nm. An acetate:formic acid:acetic acid:H₂O (100:11:11:26) mobile phase and NP/PEG reagent (methanol solution with 1% 2-aminoethyl diphenylborinate (p/v) + ethanol solution with 5% polyethylene glycol (p/v)) were used for the phenolic compound plate, with reading carried out in a darkroom at a 254nm UV wavelength.

Statistical analysis

Data normality was analyzed using the Shapiro-Wilk test. The Kruskal-Wallis and Dunn's post hoc tests were applied in accordance with the PAST program (Paleontological Statistics software package for education and data analysis), version 3.10. Analyses were performed in triplicate, with 3 tubes for each treatment, containing 10 insects each. Differences between the control groups and treated insects were considered statistically significant when $p < 0.05$. The p -values were specified throughout the results.

3. Results

Molting time

After 21 days, all the insects in the water control group (NC) had reached adulthood, ending the experimental model (Table1). No significant differences were observed between the control and treatment groups in the intermolt period ($X^2 = 3.993$; $p = 0.2584$). However, molting in controls began two days after treatment, while the experimental groups took an average of 6 days to start molting. Six days after treatment, all the controls had reached 5th

instar nymph, whereas the *Dicksonia sellowiana* (DS) and *Nephrolepis cordifolia* (NP) groups took 14 and 9 days, respectively. Metamorphosis onset was at 9 days after treatment in controls and approximately 12 days in the experimental group, but not all the insects reached this stage. These findings indicate a delay in molting.

Table 1: Analysis of mortality, molting and metamorphosis after topical treatment of *Oncopeltus fasciatus* 4th instar nymphs with 50 mg/mL of crude fern leaf extract.

Groups	%Mortality	%Molting		Molting and metamorphosis range days	
		from 4 th to 5 th instar	from 5 th instar to adult	from 4 th to 5 th instar	from 5 th instar to metamorphosis
WC	10 ^a	90 ^a	90 ^a	2 – 7	9 – 21
AC	20 ^b	80 ^a	80 ^{ab}	2 – 7	8 – 21
DS	50 ^b	73 ^a	43 ^{bc}	6 – 18	10 – >21
NP	63 ^c	53 ^a	23 ^c	5 – 10	11 – >21

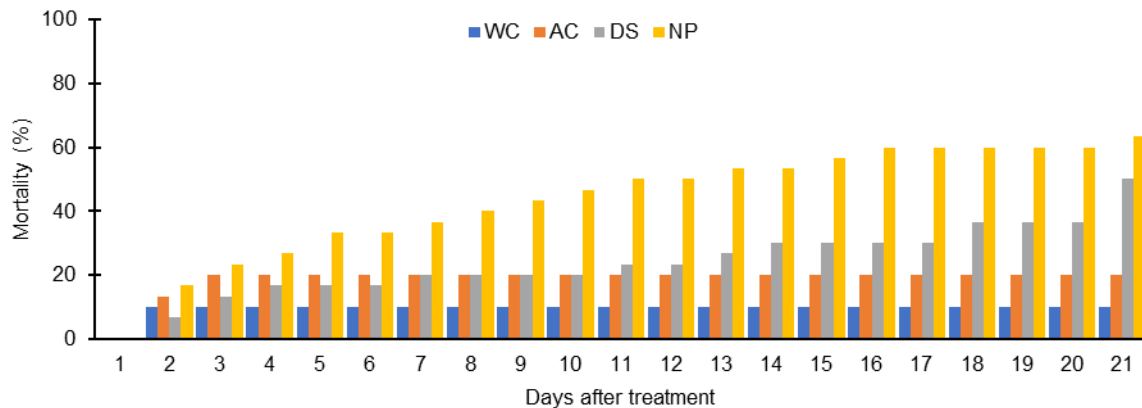
Treatments=4, repetitions=3, number of insects per treatment=10. WC= water control, AC= acetone control, DS= *Dicksonia sellowiana* extract, NP= *Nephrolepis cordifolia* extract. Values with the same letter in the columns do not differ ($p < 0.05$) according to the Kruskal-Wallis and Dunn's post hoc tests. Source: Authors.

Mortality

According to mortality analysis, the following significant intergroup differences ($X^2 = 49.38$; $p = 0.0001$) were observed: WC and AC ($p = 0.0005927$), WC and DS ($p = 1.39E^{-05}$), WC and NP ($p = 8.29E^{-13}$), AC and NP ($p = 0.0001981$) and DS and NP ($p = 0.004948$) (Table 1).

Topical application of the *N. cordifolia* and *D. sellowiana* extracts in *O. fasciatus* treatment showed considerable toxicity. At the end of the observation period, the *N. cordifolia* extract was responsible for 63% ($p < 0.0001$) of insect mortality around 16 days after treatment. The *D. sellowiana* extract was equally toxic to *O. fasciatus*, whose mortality reached 50% ($p < 0.0001$), albeit only on the 21st day post-treatment. Although the results obtained were significant for both treatments, *O. fasciatus* exhibited greater sensitivity to the *N. cordifolia* extract in terms of mortality when compared to the *D. sellowiana* extract (Figure 1).

Figure 1: Mortality after topical treatment with crude fern leaf extracts on *Oncopeltus fasciatus* 4th instar nymphs.



Dicksonia sellowiana (DS) and *Nephrolepis cordifolia* (NP), Water Control (WC), Acetone Control (AC). Source: Authors.

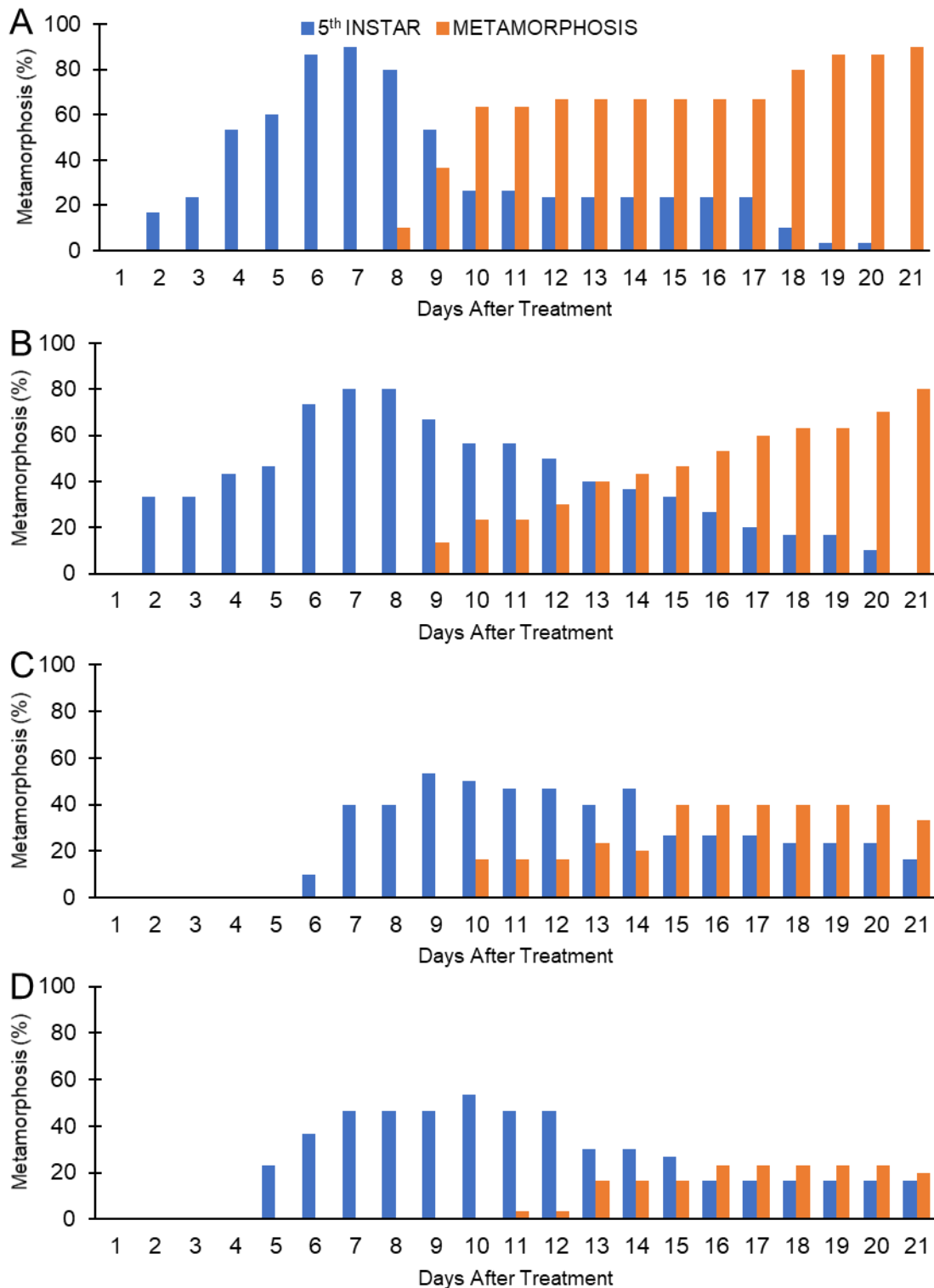
Development

Analysis of development from 4th and 5th instar nymphs showed no significant intergroup differences ($X^2= 3.79$; $p= 0.2704$). However, as shown in Table 1, significant differences ($X^2= 11.27$; $p= 0.006279$) were observed from 5th instar nymphs to metamorphosis, as follows: WC and NP ($p= 0.0006803$), WC and DS ($p= 0.02743$) and AC and NP ($p= 0.03697$).

At the end of the observation period (21 days after the test), 90% of insects in the WC (figure 2A) and 80% in the AC had reached metamorphosis (figure 2B).

At the end of the test, 33% of insects in the group treated with *D. sellowiana* extract had achieved metamorphosis and 17% remained in 5th instar nymph (Figure 2C), whereas only 20% of those in the *N. cordifolia* extract group reached metamorphosis and 17% remained in 5th instar nymphs (Figure 2D). No morphological changes were observed in the adult insects. Delayed metamorphosis was observed in insects treated with *D. sellowiana* and *N. cordifolia* extracts, with nymphs identified in both groups on the last day of the test (21st day of monitoring).

Figure 2: Metamorphosis of *Oncopeltus fasciatus* 5th nymphs after topical application of crude fern leaf extracts.



A: water control (WC); B: acetone control (AC); C: *Dicksonia sellowiana* (DS) leaf extract D: *Nephrolepis cordifolia* (NP) leaf extract.). Source: Authors.

Qualitative analysis of the plant extracts

The retention factors observed in TLC suggest the presence of 9 terpenoids in *Dicksonia sellowiana* and *Nephrolepis cordifolia*; however, similarity of the terpenoid profile between the species was only 18% (Table 2). With respect to phenolic substances (Table 2), 8 are suggested for *Dicksonia sellowiana* and 6 for *Nephrolepis cordifolia*, with no similarity between the species.

Table 2: Retention factors (Rfs) in thin-layer chromatography for terpenoids and phenolic substances.

Rf	Terpenoids		Rf	Phenolic substances	
	DS	NF		DS	NF
0.03	1	1	0.13	1	0
0.15	1	0	0.22	1	0
0.22	1	1	0.38	1	0
0.36	0	1	0.39	0	1
0.48	0	1	0.43	0	1
0.49	1	0	0.44	1	0
0.51	0	1	0.53	1	0
0.62	1	0	0.57	0	1
0.64	0	1	0.65	1	0
0.66	1	0	0.72	1	0
0.8	1	0	0.76	0	1
0.82	0	1	0.85	0	1
0.86	1	0	0.86	1	0
0.88	0	1	0.91	0	1
0.95	0	1			
0.96	1	0			

Dicksonia sellowiana (DS) and *Nephrolepis cordifolia* (NP). Substance present (1) absent (0). Source: Authors.

4. Discussion

This is the first study to assess the insecticidal action of fern extracts against *Oncopeltus fasciatus*, with the *N. cordifolia* and *D. sellowiana* extracts responsible for 63% and 50% of insect mortality, respectively. These results are higher or similar to the findings already reported for angiosperms. *Clusia fluminensis* ethanol extracts achieved 33.3% mortality and delayed nymph and adult development in *O. fasciatus* (Duprat et al., 2017), while *M. subsericea* extracts caused 66% mortality in addition to delaying development and inhibiting molting. Other extracts induced permanent (overaged) or extranumerary nymphs, both incapable of metamorphosis (Fernandes et al., 2013).

Tietbohl et al. (2014) reported 100% mortality in *Dysdercus peruvianus* (Guerin-Meneville, 1831) (Hemiptera: Pyrrhocoridae) and *Oncopeltus fasciatus*, both in the 4th instar, using 1 μ L of pure *Myrciaria floribunda* (Myrtaceae) leaf essential oil per insect. These data suggest that essential oils are more potent and efficient than plant extracts because they are concentrates of the target substances of bioinsecticide research.

Several studies have assessed the insecticidal activity of the main insect pest orders (Diptera, Lepidoptera, Hemiptera and Coleoptera) using fern and lycophyte species (Lima, 2019). There are no studies for the genus *Dicksonia*, only a species from the family Dicksoniaceae, *Cibotium barometz* (L.) J.Sm analyzed by Huang et al. (2010), who found no significant insecticidal activity for the leaf methanol extracts of the species against *Musca domestica* L. and *Aedes albopictus* Skuse (Diptera: Culicidae).

Extracts of two species from the genus *Nephrolepis* have been assessed for insecticidal activity, *N. cordifolia* (Huang et al., 2010) and *Nephrolepis pectinata* (Willd.) Schott (Potenza et al., 2004). Xavier et al. (2016) analyzed the insecticidal potential of methanol *N. cordifolia* extracts on *Spodoptera litura* 3th instar larvae (Order Lepidoptera) and observed 50% mortality, with no emergence of adults. Huang et al. (2010) studied the potential of *N. cordifolia* methanol extract against *M. domestica* and obtained positive results, with 83.3% insect mortality. Toxicity of a chemical substance to insects does not qualify it as an insecticide; its efficacy even at low concentrations and lack of toxicity to mammals and humans must also be taken into account (Viegas Júnior, 2003).

According to the retention factors (R_fs) obtained in thin-layer chromatography (TLC), the *Nephrolepis cordifolia* and *Dicksonia sellowiana* extracts exhibited different terpenoid and phenolic substance profiles. These substances are described in the literature as having insecticidal action (Corrêa & Salgado 2011). Terpenoids act in plant defense against

herbivores and as repellents, toxins or modifiers of insect development (Fürstenberg-Hägg et al., 2013), while phenolic substances repel herbivores by affecting their digestive system with toxic or ovicidal substances, in addition to influencing the photosensitivity of insects (Zagrobelny et al., 2004).

Oliveira (2012) reported the presence of phenolic compounds, flavonoids, tannins, steroids, terpenoids and coumarins in aqueous, hydroalcoholic and crude extracts of *Dicksonia sellowiana* hexane fractions. However, phytochemical tests conducted by Xavier et al. (2016) indicated the presence of alkaloids, steroids, tannins, flavonoids, cardiac glycosides and phenolic compounds in *N. cordifolia*.

5. Conclusion

N. cordifolia and *D. sellowiana* show potential for research on selective biodegradable substances for use as green insecticides. New tests using extracts of these species are needed in order to better understand the physiological mechanisms of insect interaction with the extract components and acquire data that can contribute to research on obtaining more selective substances.

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