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Entomotoxic potential of hydroalcoholic extract of *Eragrostis plana* Nees in experimental model of *Nauphoeta cinerea* Olivier

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ABSTRACT. The objective of this study was to evaluate the entomotoxic activity of the hydroalcoholic extract of *Eragrostis plana* Nees in an experimental model of cockroaches (*Nauphoeta cinerea* Olivier), for a possible bioinsecticide. For this purpose, aerial parts of *E. plana* were grounded to obtain the hydroalcoholic extract (EACA), after preparing the concentrations of (0.5; 2.5; 5.0; 12.5; 25.0; 50.0 100.0 μg g^{-1} per animal) a saline solution was used to control the treatment. To determine the receptors and pathways involved in responses to EACA doses of *E. plana*, a treatment with the drug octopamine and its inhibitor phentolamine were prepared. The effect of EACA on behavioral activities (*grooming*) and on cardiac activity were tested. All data were expressed as mean ± standard deviation, being analyzed by the two-way ANOVA test, followed by the Tukey test, considered significant when $p<0.05$. After carrying out the experiment, it was found that EACA induced a significant effect on leg grooming at all concentrations, except for the concentration of 100 μg g^{-1} per animal, but the same did not modulate antenna grooming. Regarding the receptors and pathways involved, it was observed that octopamine alone and together with the extract, had a significant effect on leg grooming, while phentolamine, together with the extract, influenced antennae grooming. Regarding the effect of the extract on the semi-isolated heart, it was noticed that the same doses that affected grooming, affected heart rate by decreasing it. At no concentration, at the end of the treatment, there was no recovery of the heart rate. The results point to a direct modulation of octopaminergic and cholinergic pathways in the insect nervous system.

Keywords: annoni-2 grass; grooming; semi-isolated heart; insecticidal activity.

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Introduction

Annoni-2 grass (*Eragrostis plana* Nees) is a Poaceae of South African origin that was accidentally introduced in Brazil in the 1950s as a seed contaminant of *Chloris gayana* Kunth, also known as Rhodes grass (Zenni & Ziller, 2011), being one of the greatest invasive of the southern fields, which for the longest time has been causing damage to both the biodiversity of the 'pampa biome' and the livestock activity of Rio Grande do Sul (RS) and also for its southern neighbour countries like Uruguay and Argentina (Nachtigal, Neumann, Costa, & Garlich, 2009). Like any other invasive plant, its characteristics are rapid growth, long reproductive phase, presence of allelopathy and seed bank in the soil (Medeiros & Focht, 2007).

Eragrostis plana is featured amongst the most invasive exotic species with the greatest impact due to its high rusticity, adaptation to poor soil and great capacity for seed multiplication (Goulart, Nunes, Kupas, & Merotto Junior, 2012). These attributes make the species easy to disperse and difficult to control, reaching about 20% of the vegetation in the pampa biome's fields of southern Brazil, which is equivalent to 3.1 million acres (Goulart et al., 2012; Cicconet, Alba, Spironello, & Cunha, 2015). Control the dispersion of this species is restricted to the control of chemical herbicides (Nachtigal et al., 2009).

Eragrostis plana has been a model of studies on identifying the changes in bioclimatic niche during the invasion of South America (Barbosa, Pillar, Palmer, & Melo, 2013). Several studies emphasize the allelopathic potential in germination and initial growth of other plant species (Ferreira, Medeiros, & Soares, 2008; Fiorenza et al., 2016), however studies on its entomotoxic potential have been little explored. In a study carried out by Fiorenza et al. (2016) with methanolic extract of dried leaves of *E. plana*, phenols (gallic acid, ellagic acid, caffeic acid, chlorogenic acid), flavonoids (quercetin and rutin) and tannins (epicatechin and catechin) were found to be potential in allelopathic effect.

Over the past century, most natural insecticides have been replaced by their synthetic counterparts. Among the main reasons of synthetic insecticides popularization were their low cost, wide spectrum of use in covering different insects' orders, and longevity in the field. However, even though these characteristics seemed desirable at first, they revealed catastrophic consequences for the environment, given that the chemical insecticides do not choose between insect species, also affecting within the insect species itself. In addition, the simplicity of its chemical structures favors resistance to the insects (Libersat & Pflueger, 2004), which, usually, requires an even greater increase in dosage and application frequency to overcome its last dosage, putting the environment at risk. For these reasons, the research for natural molecules with insecticidal potential has become more of a necessity than an alternative.

Bioinsecticidal compounds generally modulate some aspect of the insect's neurotransmission pathways, such as the cholinergic and octopaminergic ones, for example. Other neurotransmission pathways, such as glutamatergic and GABAergic, are also evaluated as possible foci of bioinsecticide action. Neuromodulation affects several physiological aspects of the animal, because the same neurotransmitter has different functions in different physiological processes (Osborne, 1996; Raymond-Delpech, Matsuda, Sattelle, Raun, & Sattelle 2005; Leal et al., 2018; Santos et al., 2019; Borges et al., 2020; Leal et al., 2020). Most insecticides exert their effect on insects and other arthropods by inducing: (1) neurostimulation that leads to hyperactivity; (2) uncoordinated spontaneous movements that lead to paralysis due to energy consumption; and (3) paralysis in neuromuscular processes (Rattan, 2010).

Cockroaches are quite diverse representing more then 4000 species that make up the suborder Dictyoptera. *Nauphoeta cinerea* Olivier is an ovoviviparous cockroach in the Bladeridea family. This species is frequently used in biomedical research not only for its easiness of reproduction and maintenance, but also because of its relative simplicity and suitability for certain experimental procedures. There are many similarities between the cockroach`s nervous system and that of other insect species (Huber, Masler, & Rao, 1990). In addition, its susceptibility to manipulation experiments and biophysical parallelism with vertebrates make its use very convenient on a number of neurophysiological approaches (Stankiewicz, Dabrowski, & Lima, 2012). Through this insect model, the effects of several neuromodulatory compounds have already been described. Each of these findings contributed to the understanding of the functioning of several neurotransmission pathways. Due to some peculiarities of its nervous system, such as the importance of the octopaminergic pathway for its physiology and consequently survival, many bioinsecticides have already been proposed in studies with this model (Adedara et al., 2015; Leal et al., 2018; Santos et al., 2019; Borges et al., 2020; Leal et al., 2020).

We know that the survival of the insect depends on the ideal functioning of some factors such as its locomotion, grooming and reproduction activities, in addition to other aspects that are particular to the insect. When a compound modulates some of these factors, we can already consider it as a possible bioinsecticide, as they will be influencing the insect's ability to escape, feed or perpetuate itself (Nicolaus & Lee, 1999; Delpuech, Bardon, & Boulétreau, 2005; Bonilla-Ramirez, Jimenez-Del-Rio, & Velez-Pardo, 2011; Hampel, Franconville, Simpson, & Seeds, 2015).

Studies have been conducted on identification of *E. plana* regarding the changing bioclimatic niche during its invasion in South America. The allelopathic potential in the germination and growth of other plants has been demonstrated in several studies, but little has been done to explore its entomotoxic potential, which emphasizes the importance of improving the selectivity of new insecticide molecules by targeting the octopamine pathway.

In this study, the entomotoxic activity of the hydroalcoholic extract of the *E. plana* Nees at different concentrations on the nervous and cardiac system of *N. cinerea* Olivier was investigated.

Material and methods

Behavioral and heart rate modulation experiments were conducted at the Laboratory of Neurobiology and Toxinology (Lanetox) at the Federal University of Pampa – São Gabriel campus site, São Gabriel/RS.

Plant extract

Aerial parts (stem and leaf) of *E. plana* were collected at the *Universidade Federal do Pampa* (UNIPAMPA) - São Gabriel campus site. To obtain the hydroalcoholic extract (EACA), the leaves were dried out and grounded in a willey mill until it all turned into powder. The material was subjected to the extractive process by percolation in a 90% hydroalcoholic solution using 99.99% absolute alcohol (P.A.).

After obtaining a filtered solution, it was submitted to a rotary evaporator with a controlled temperature of up to 60°C, after that it was lyophilized at -80°C until a dry residue was obtained (Carrazoni et al., 2016). The hydroalcoholic extract (EACA) was obtained by diluting the dried-out residue in milli-Q water, obtaining the following doses: 0.5; 2.5; 5.0; 12.5; 25; 50; 100 μg g^{-1} of EACA per animal, for grooming and semi-isolated heart tests. The control treatment was obtained with the administration of saline solutions.

Animal model

To make sure the experiment was standard, male cockroaches of the species *N. cinerea* were used (3 to 4 months after moulting), using a 'n' sample of 30 cockroaches; these animals were raised in the vivarium of Unipampa - São Gabriel campus site, in special insect boxes kept at a temperature of 20-24°C, in cycles of 12 hours of light and 12 hours of darkness, fed with dog food and drinking water *ad libittum*. All experimental animals were fed with dog Chow with the following nutritional composition: Crude Protein (min.) 180 g kg^{-1} (18%); Etheric Extract (min.) 50 g kg^{-1} (5%); Linolenic acid (min.) 2000 mg kg^{-1}; Linoleic acid (min.) 10 g kg^{-1} (1%); Calcium (max.) 23 g kg^{-1} (2.3%); Calcium (min.) 10 g kg^{-1} (1%); Phosphorus (minimum) 8000 mg kg^{-1}; Fibrous Matter (max.) 60 g kg^{-1} (6.0%); Mineral Matter (max) 100 g kg^{-1} (10%); Saponin 7 mg kg^{-1}; Humidity 120 g kg^{-1} (12%); Sodium (min.) 2500 mg kg^{-1}.

Saline solution for insects

A saline solution was used, composed of NaCl 150 mM, $CaCl_2$ 2 mM, KCl 10 mM and Tris 10 mM Tris in addition to ultra pure water up to a volume of 200 mL, with pH 6.8 corrected with NaOH. The pH was adjusted with a previously calibrated glass electrode pHmeter (Carrazoni et al., 2016).

Grooming activity

The grooming activity (legs and antennae cleansing) followed the methodology described by Carrazoni et al. (2017). The extract and the tested drugs were injected into the third abdominal portion with the aid of a Hamilton syringe, with 10 μL of the extract and/or drug being tested. The animal was placed in a circular arena (Open Field, Insight), used in behavioral tests (circular container with a 30 cm height and radius of 20 cm). Cockroaches had never been to open field before. For this activity, an 'n' sample of 30 cockroaches were used for each dosage of EACA and each treatment was monitored for 30 minutes. Also, to determine the receptors and pathways involved in responses to EACA, solutions of two drugs were prepared: octopamine and phentolamine at concentrations of 1.0 μg g^{-1} of animal and 0.01 μg g^{-1} of animal, respectively. The tests were carried out during the daytime.

Semi-isolated heart

The preparation of the semi-isolated heart in cockroaches followed the methodology recommended by Rodrigues et al. (2012), where cockroaches were put under anesthesia for 5 to 8 minutes in cooling condition and for each test dosage, 10 adult male cockroaches were used. The cockroaches were fixed on Styrofoam plates with the help of entomological pins. The ventral part of the animal was directed upwards where the abdominal cuticle was cut up and removed with the aid of sterilized surgical scissors and forceps. The viscera were pushed aside so that the heart could be exposed.

The heart was bathed with 200 μL g^{-1} per animal saline solution at 37°C for 5 minutes to stabilize the heart rate. After this, different treatments were applied to the heart (being 190 μL g^{-1} of saline solution per animal and 10 μL $g^{-1}$of EACA) and the heart rate was checked and monitored for 30 minutes. The heart was bathed again with 200 μL g^{-1} of saline solution per animal, where heart rate monitoring and counting took place for 5 minutes, to observe if heart rate recovery occurred. The entire procedure takes around 40 minutes, with the help of a camera and the VLouge program.

Results and discussion

The application of different doses of EACA in *N. cinerea* cockroaches affected leg grooming activity, when compared to the control treatment, except at the highest concentration, i.e., 100 μg g^{-1} of animal (Figure 1).

In cockroaches that were submitted to the saline solution (control) the average time of continuous cleansing was 110±5 s for 30 minutes for legs and 20±1 s for 30 minutes of antenna (n=30), not differing statistically from the treatment group of 100 μg g^{-1} of EACA animal, with an approximate time of 133±5 s for 30 minutes for legs and 18±1 s for 30 minutes for antenna (n=30). Additionally, EACA induced leg grooming activity at concentrations from 0.5 to 50 µg g^{-1} per animal when compared to the control treatment, with an increased growth up to the concentration of 2.5 to 25 µg g^{-1} of animal (Figure 1). As for the antenna grooming, no statistical differences were found in the different doses of EACA when compared to the control treatment (Figure 1).

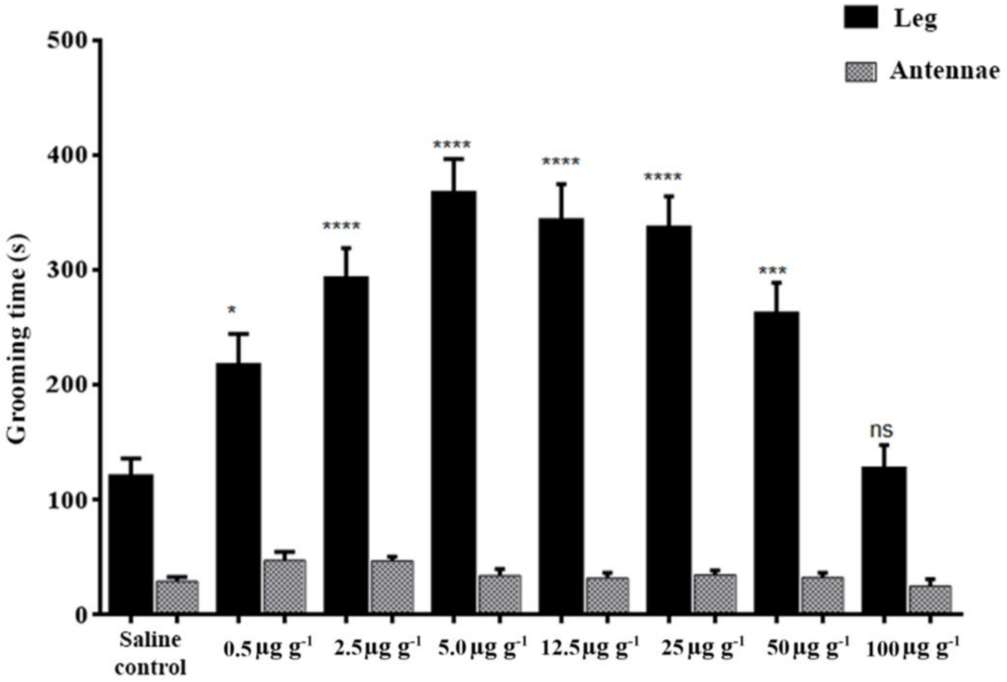

Figure 1. Behavioral modulation of grooming induced by the hydroalcoholic extract of *Eragrostis plana* Nees in *Nauphoeta cinerea* Olivier. Concentrations used: saline solution (control) and EACA (0.5; 2.5; 5.0; 12.5; 25; 50 e 100 µg g^{-1} per animal). Results are expressed as mean ± standard deviation of total grooming time (in seconds) with the duration of 30 minutes. Data were analyzed by One-way Anova followed by Dunnett`s post-test where * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Tukey port-test was applied with the aim of comparing significance between treatments where $p < 0.0001$ (n = 30).

Studies by Carrazoni et al. (2017), Lorensi et al. (2019), Santos et al. (2019) and Borges et al. (2020) using hydroalcoholic and/or methanolic plant extract testing the entomotoxic effect on grooming activity of the leg and antennae of *N. cinerea* also found stimulatory and/or inhibitory effects on the grooming activity of the legs and/or antennae of this cockroaches` species, and for all of these studies it suggests a strong involvement of dopaminergic and octaminergic modulation of extracts in insects, emphasizing what Libersat and Pflueger (2004) stated that grooming in insects is induced by monoamines and the cockroaches` legs movements are primarily coordinated by the neurotransmitter octopamine.

Octopamine plays an important role as a neurotransmitter and neuromodulator in both the peripheral and central nervous system of insects. The action of octopamine is mediated by the activation of protein-coupled receptors (Farooqui, 2012). According to Evans and Maqueira (2005), different classes of octopamine receptors are responsible for mediating specific effects in insects, which are responsible for the deceleration of the myogenic rhythm and influence the slow neuromuscular transmission mediated by motoneurons.

These data support the hypothesis that the different patterns observed during the trials of the present study originated from the involvement of the octopaminergic system. In relation to the central nervous system of insects, octopamine plays an important neuromodulatory role in the regulation of insect behaviour, such as rhythmic behaviors in locusts, locomotion, and hygiene in fruit flies, feeding behaviors of blowflies, cockroaches, among other species (Farooqui, 2012). Furthermore, the biogenic amines, octopamine and dopamine, modulate the cleaning activity in insects, this activity is also related to courtship behavior, social signaling and arousal (Osborne, 1996; Libersat & Pflueger, 2004). Octopamine is also related to neurohormones, neuromodulators and neurotransmitters (Papaefthimiou & Theophilidis, 2011). Additionally, octopamine plays a role in the fight-or-flight response; thus, it is related to arousal in insects with an increase in its level during stress (Libersat & Pflueger, 2004). Consequently, under the experimental conditions of the present study, the increase in stopping times and episodes of immobility induced by EACA may probably be the result of antagonism of octopaminergic receptors.

Octopamine and phentolamine were used in the present study, the latter is a selective blocker for octopaminergic receptors (Borges et al., 2020). When octopamine (1 μg g^{-1} per animal) was tested on cockroaches (n=30), an increase in leg grooming activity was observed, with a mean value of 210±10 s for 30 minutes, when compared to the control treatment; in the same way, octopamine (1 μg g^{-1} per animal) was administered 15 minutes before the application of EACA (5 μg g^{-1} per animal). It was verified that EACA reinforced the effect obtained by octopamine in leg grooming, resulting in an average time of 300±10 s for 30 minutes; although this result still showed lower grooming activity when compared to treatment with EACA 5 μg g^{-1} per animal (Figure 2).

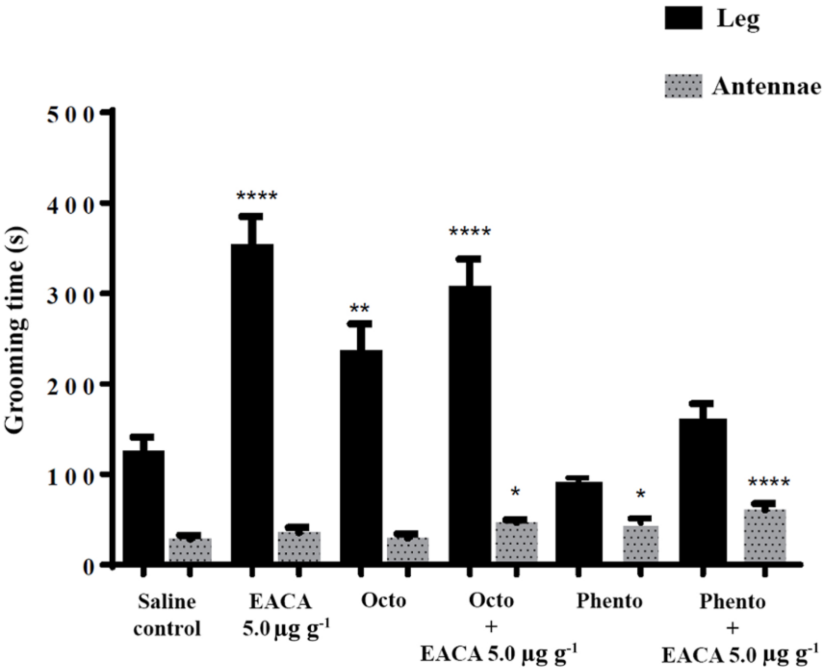

Figure 2. Behavioral modulation of grooming induced in *Nauphoeta cinerea* Olivier with the action of octopamine (octo) (1.0 μg g^{-1} per animal) and phentolamine (phento) (0.01 μg g^{-1} per animal) together with the extract of EACA. Results are expressed as mean ± standard deviation of total grooming time (in seconds) for 30 minutes. Data were analyzed by One-way Anova followed by Dunnett's post-test where * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Tukey post-test was applied with the aim of comparing significance between treatments where $p < 0.0001$ (n = 30).

The presence of octopamine in the antennae grooming was not significantly different from the control group; however, when this drug was applied associated with EACA, a statistical difference was observed (Figure 2). A similar test was performed with octopamine's receptor inhibitor, phentolamine. The application of this drug at a concentration of 0.01 μg g^{-1} per animal in cockroaches (n=30) induced a decrease in leg grooming activity, with mean value of 80 ± 1 s for 30 minutes. The administration of phentolamine, at the same concentration above, 15 minutes after the application of EACA resulted in a leg grooming activity of 120 ± 5 s for 30 minutes. Possibly, the presence of EACA reversed the inhibitory potential of phentolamine, showing an octopaminergic agonist-like effect (Figure 2). Additionally, for antennae cleaning activity, treatment using phentolamine alone or associated with EACA showed an increase in antennae grooming activity when compared to control (Figure 2).

In the study developed by Borges et al. (2020), extracts of *Manilkara rufula* (control, 25, 50 and 100 μg g^{-1} per animal) also showed a significant effect on leg grooming activity, especially at higher doses, while antenna grooming was not affected by the presence of the extracts of this species; evidencing a modulatory effect of *M. rufula* extracts that occurred in octopaminergic pathways.

Decreased leg clearance observed in our trials suggests octopamine-like activity, generating a selective boost of octopaminergic receptors. Therefore, under the experimental conditions of the present study, EACA contributed to increase the effect of the octopamine neurotransmitter, even managing to overcome the effects of the phentolamine inhibitor. This activity demonstrates that one of the main sites for the effect of EACA is indeed the octopaminergic receptors (Figure 2).

Weisel-Eichler, Haspel, and Libersat (1999) emphasizes that in some studies, the neurotransmitter dopamine is related to antennae grooming and octopamine to leg grooming. Through our study, we again had proof that leg grooming is derived from an octopaminergic activity. Likewise, we validate other studies that emphasize the action of phentolamine as an octopaminergic receptor blocker (Weisel-Eichler et al., 1999; Libersat and Pflueger, 2004; Carrazoni et al., 2016; 2017; Lorensi et al., 2019; Santos et al., 2019; Borges et al., 2020).

In trials of the semi-isolated heart of *N. cinerea*, a time-dependent response induced by different doses of EACA was obtained with the heart rate of the cockroaches being significantly modulated after treatment with EACA (Figure 3).

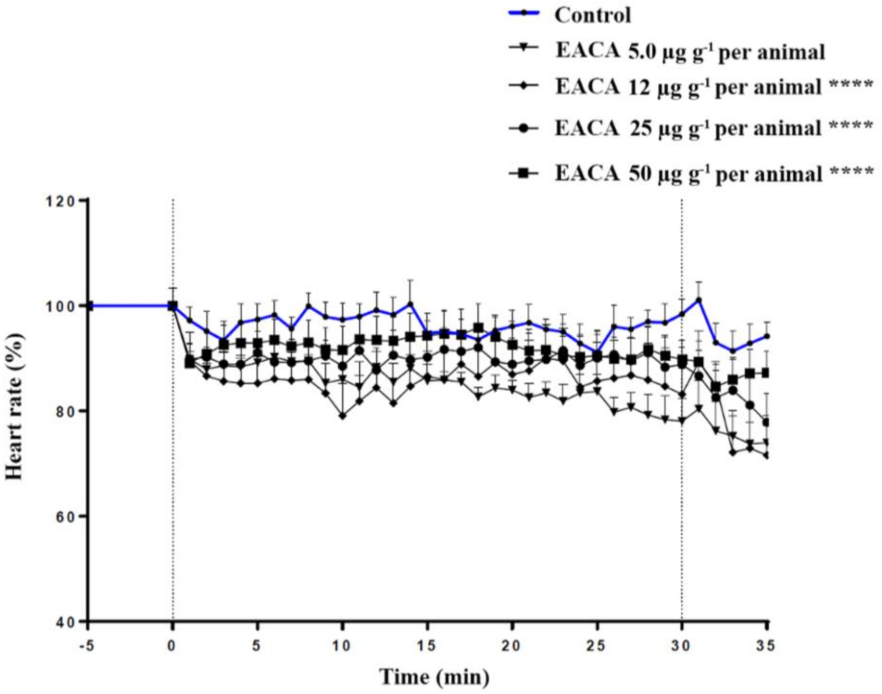

Figure 3. Chronotropic response of different treatments of the aqueous extract of *Eragrostis plana* Nees on the heart rate of *Nauphoeta cinerea* Olivier cockroaches. Concentrations used: saline solution (control) and EACA (5.0; 12.5; 25; 50 µg g^{-1} per animal). Results are expressed as mean ± standard deviation of the 35-minute total time. Data were analyzed by One-way Anova followed by Dunnett`s post-test where * $p < 0.05$, ** $p < 0.01$, **** $p < 0,0001$. Bonferroni's post-test where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (n = 10).

In control preparations, the chronotropic response was 100±5 beats min.$^{-1}$. Concentrations of 5.0; 12.5; 25; 50 µg g^{-1} of animal caused a decrease in the cockroaches' heart rate (n=10) around 90±10 beats min.$^{-1}$, significantly different from the control, but not different from one another. Additionally, after washing the heart with saline, there was no recovery of heart rate at different concentrations of EACA; therefore, EACA concentration with 5.0; 12.5; 25 and 50 µg g^{-1} per animal did not cause a significant effect on the heart rate of cockroaches of this species. However as observed for the grooming activity (Figure 2), at the lowest concentrations of EACA, especially at the concentration of 50 µg g^{-1} per animal, greater effects on heart rate were observed. Borges et al. (2020) also observed a negative chronotropic effect in relation to the control (98±2 beats min.$^{-1}$) at all concentrations of *M. rufula* extracts used (25, 50 and 100 µg g^{-1} per animal) (n=6). However, after washing the saline solution, heart rate was reversed.

The same study using octopamine (1.0 µg g^{-1} per animal) and phentolamine (0.01 µg g^{-1} per animal) showed a reduction in heart rate in relation to the control, both remaining at 95±5 beats min.$^{-1}$ with no recovery of heart rate after washing it (Figure 4). Borges et al. (2020) suggested in their work, and it can be seen in Figure 4, the involvement of the octopaminergic pathway.

In insects, the heart represents one of the main sites of octopaminergic action. Papaefthimiou and Theophilidis (2011) showed in their studies that the concentration of octopamine influences the heart rate in insects, being able to accelerate the heartbeat when in high concentrations or decrease it in low concentrations. In the present study, octopamine and phentolamine (selective blocker of octopaminergic receptors) were tested, to analyse which receptor is involved in the heart rate of *N. cinerea* and it was verified that the effect on heart rate induced by EACA is modulated by octopaminergic pathways, due to the similarity of EACA with the agonist (Figure 4). In addition, it has been shown that octopamine levels increase during stress, increasing cardiac contraction in insects (Papaefthimiou & Theophilidis, 2011). Such behaviour was attested in the treatment with EACA, but not being observed in relation to the control treatment (Figure 4).

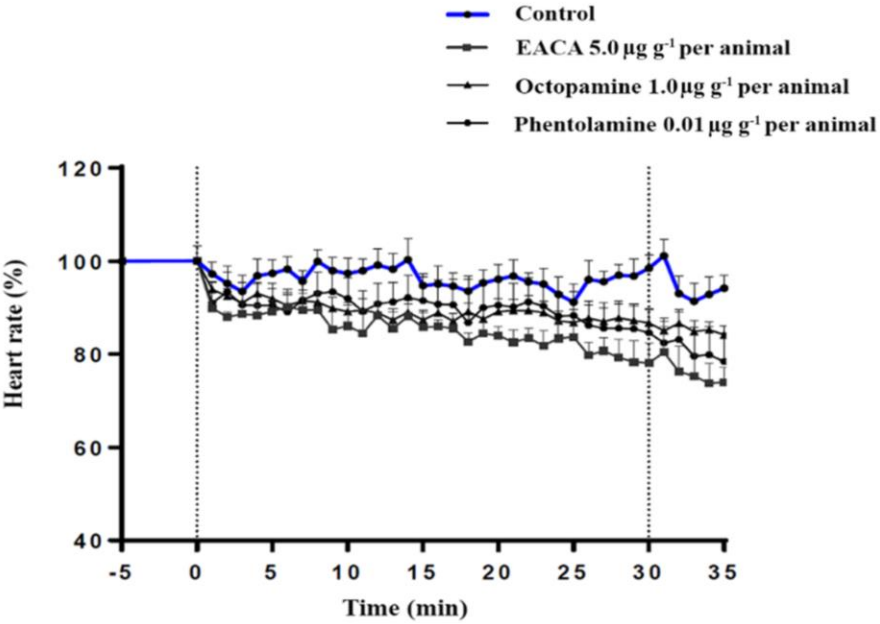

Figure 4. Chronotropic response of different treatments of the aqueous extract of *Eragrostis plana* Nees, on the heart rate of *Nauphoeta cinerea* Olivier cockroaches. Concentrations: saline solution (control), EACA (5.0 µg g^{-1} per animal), octopamine (1.0 µg g^{-1} per animal) and phentolamine (0.01 µg g^{-1} per animal). Results are expressed as mean ± standard deviation of the 35-minute total time. Data were analyzed by One-way Anova followed by Dunnett`s post-test where * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$. Bonferroni's post-test where * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (n = 10).

Lorensi et al. (2019) with the objective of investigating the toxic effects of the ethanolic extract of *Prasiola crispa*, an algae from Antarctica, by determining the effects on the heart rate of *N. cinerea* cockroaches and identifying possible action mechanisms of the plant extract, observed a significant decrease in the animals heart rate after administration of the ethanolic extract of *P. crispa* (200 µg g^{-1} per animal) when compared to the control treatment (saline solution) with indication of bioinsecticide potential of the extract of *P. crispa*. Also, Borges et al. (2020) tested the influence of different compounds on the heart rate modulation of *N. cinerea* using the semi-isolated heart *in vivo* preparation for 30 minutes and found that octopamine (5 µM) did not affect the heart rate of this species.

Our intention in the present study was to evaluate the concomitant action of all the metabolites present in the *E. plana* extract. Based on our data, we can suggest that one or more EACA compounds have a strong influence on the octopaminergic pathway and, with that, we generate possibilities for future tests with the compounds in isolation. *E. plana* extract, as observed in a study by Fiorenza et al. (2016), has quercetin in its composition, the same flavonoid observed in the methanol extract of *Arauacaria angustifolia* and which demonstrated insecticidal effects in studies by Carrazoni et al. (2017), using cockroaches of the species *Phoetalia pallida* as an invertebrate model. Based on this data, we suggest a possible action of quercetin present in EACA on the effects observed in our trials. To confirm this hypothesis, we opened the opportunity to evaluate this compound in isolation on the same preparations. In the same way, knowing that quercetin influences the cardiac mechanism of cockroaches (Carrazoni et al., 2017), it is necessary to verify, in the future, the action of EACA in other possible neurotransmission pathways, such as the cholinergic pathway.

In conclusion, the present study contributes to highlight the entomotoxicity activity of the *E. plana* extract, due to behavioral disturbances and electrophysiological aspects of the octopaminergic pathways on the *N. cinerea* cockroach. Furthermore, this study establishes that *N. cinerea* can be considered an eminent biological model for testing insecticidal compounds e, according to several researchers, such as Jung and Park (2007), Rosa et al. (2007), Rigon, Salomani, Cutti, & César (2013), and Fiorenza et al. (2016), flavonoids have already been discovered, including quercetin, which may have bioinsecticidal properties.

Conclusion

The present study demonstrated the potential insecticidal activity induced by the hydroalcoholic extract of *E. plana* on grooming activity and hear rate using cockroaches of the species *N. cinerea* as an experimental model, and the change in the behavior of cockroaches of this species by octopaminergic pathways in the nervous system of the insects.

Future studies are projected to use the secondary metabolites (in the form of drugs) found in *E. plana* to further investigate the possible entomotoxic activity in the *N. cinerea* cockroaches' species.

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