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Entomotoxic activity of *Eragrostis plana* Nees extract on the central and peripheral nervous systems of cockroaches *Nauphoeta cinerea* Olivier

Atividade entomotóxica do extrato de *Eragrostis plana* Nees sobre o sistema nervoso central e periférico de baratas *Nauphoeta cinerea* Olivier

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Eragrostis plana Nees is a C4 perennial plant that exhibits toxic inhibitory action on other plants and has demonstrated an entomotoxic potential, although poorly elucidated, in cockroaches of the species *Nauphoeta cinerea*. The objective of the study was to evaluate the mechanisms involved in the entomotoxic activity of the hydroalcoholic extract of *E. plana* on the nervous system of *N. cinerea*. Aerial parts of *E. plana* were crushed to obtain the hydroalcoholic extract (EHEP). The concentrations (0.5; 2.5; 5.0; 12.5; 25.0; 50.0 100.0 μg g^{-1} per animal) were prepared and saline solution was used for the control treatment. The effect of EHEP on the behavioral (self-hygiene), electrophysiological and biochemical patterns of *N. cinera* was tested. Data were expressed as mean ± standard deviation and were analyzed by two-way ANOVA followed by Tukey's test when considered significant at $p < 0.05$. An increase in leg grooming activity was observed when the animals were treated with EHEP. The quercetin compound observed in the phytochemical profile of *E. plana* was shown to modulate the leg and antennae grooming activity of *N. Cinera*. EHEP also significantly altered locomotion and inhibited the activity of the enzyme acetylcholinesterase (AChE). Thus, this study corroborates our previous studies on the entomotoxic potential of *E. plana* and suggests a possible biotechnological potential for the species as a bioinsecticide, due to its modulations on the main signaling pathways of invertebrates.
Keywords: secondary metabolism, octopaminergic pathway, bioinsecticides.

Eragrostis plana Nees é uma planta perene C4 que apresenta ação inibitória tóxica sobre outras plantas e já demonstrou um potencial entomotóxico, embora pouco elucidado, em baratas da espécie *Nauphoeta cinerea*. O objetivo do estudo foi avaliar os mecanismos envolvidos na atividade entomotóxica do extrato hidroalcóolico de *E. plana* sobre o sistema nervoso de *N. cinerea*. Partes aéreas de *E. plana* foram trituradas para obtenção do extrato hidroalcóolico (EHEP). Foram preparadas as concentrações (0,5; 2,5; 5,0; 12,5; 25,0; 50,0 100,0 μg g^{-1} por animal) e solução salina foi utilizada para o tratamento controle. Testou-se o efeito do EHEP sobre os padrões comportamentais (auto-higiene), eletrofisiológicos e bioquímicos de *N. cinera*. Todos os dados foram expressos como média ± desvio padrão, sendo os mesmos analisados pelo teste ANOVA bidirecional, seguido do teste Tukey, quando considerado significativos com $p<0,05$. Foi observado um aumento da atividade de auto-higiene das pernas quando os animais foram tratados com EHEP. O composto quercetina observado no perfil fitoquímico de *E. plana* demonstrou modular a atividade de limpeza de pernas e antenas de *N. Cinera*. EHEP também alterou significativamente a locomoção e inibiu a atividade da enzima acetilcolinesterase (AChE). Assim, este estudo corrobora com nossos estudos anteriores sobre o potencial entomotóxico de *E. plana* e sugere um possível potencial biotecnológico para a espécie como bioinseticida, devido à mesma apresentar modulações sobre as principais vias de sinalização dos invertebrados.
Palavras-chave: metabolismo secundário, via octopaminérgica, bioinseticidas.

1. INTRODUCTION

Eragrostis plana Nees is a C4 perennial plant belonging to the Poaceae family. It is of South African origin and was accidentally introduced to Brazil in the 1950s as a contaminant of *Chloris*

gayana Kunth seeds [1-3]. It is one of the most important invaders of the southern fields, which has long caused damage in the biodiversity of the Pampa biome, altering the biological balances [4-6], as well as in the livestock and agricultural activities of Rio Grande do Sul state, and in neighboring countries of Uruguay and Argentina [7-9]. As an invasive plant, *E. plana* has characteristics that favor its proliferation [10-12] and, in addition, this species presents limitations in grazing due to its reduced palatability and low nutritional value [13, 14]. These characteristics make *E. plana* one of the most impactful invasive alien species today [15], with the species currently infesting approximately 20% of the grassland vegetation of the Pampa biome in southern Brazil, representing 3.1 million hectares [15, 16]. Controlling the expansion of this plant is largely dependent on the use of chemical herbicides, which have become the main management strategy for its containment [17]. Moreover, cutting *E. plana* at developmental stages prior to seed production as an adjuvant alternative for the gradual reduction of the soil seed bank, followed by post-cutting conservation in the form of hay [18].

Eragrostis plana has been a model for studies in work to identify bioclimatic niche change during its invasion in South America [8]. *Eragrostis plana* produces allelochemicals with allelopathic potential that inhibit and delay the germination and growth of numerous native and exotic species, supporting its expansion into the native fields of Rio Grande do Sul state [6, 14, 19]. Ferreira et al. (2008) [4] and Favaretto et al. (2015) [6] mention that species of the genus *Eragrostis* contain amino acids, phenolic compounds, and glutamic acid, all of which have recognized allelopathic potential. In addition, Favaretto et al. (2015) [6], Fiorenza et al. (2016) [19], and Rosa et al. (2024) [20] found that the presence of phenolic compounds (gallic acid, ellagic acid, caffeic acid, and chlorogenic acid), flavonoids (quercetin and rutin), and tannins (epicatechin and catechin) may be responsible for the allelopathic effect of this species. The presence of this species reduces the productivity of fields and livestock production; therefore it is necessary to study and understand the allelopathic effects of *E. plana* on species of the Pampa biome. Several studies emphasize the seed germination ecology of *E. plana* [4, 6, 8, 11, 14, 19], but little knowledge its entomotoxic potential has been explored, especially for the cockroach species *Nauphoeta cinera* Olivier.

Cockroaches exhibit considerable diversity, with more than 4,000 species classified under the suborder Dictyoptera. *Nauphoeta cinerea* Olivier, an ovoviviparous species from the Blaberidae family, is commonly employed in biomedical and biotechnology researches. This is largely due to its ease of breeding and upkeep, as well as its relative simplicity and appropriateness for specific experimental applications. There are many similarities between the nervous systems of cockroaches and other insect species [21]. Furthermore, the literature shows that the nervous system of invertebrates, such as insects, has several similarities with that of vertebrates, especially in the neurotransmission mechanism. Both begin with the depolarization of the neuronal membrane (action potential) and end with the release of neurotransmitters into the synaptic cleft. These similarities also help to understand how substances can modulate the nervous system, contributing to investigative studies [22, 23]. In addition to their responsiveness to experiments, their manipulability, and biophysical parallelism to vertebrates, make their use very convenient for a number of neurophysiological approaches [24].

Using this insect model, the effects of several neuromodulator compounds have already been described [25-27]. Each of these discoveries has contributed to the understanding of the functioning of different neurotransmission pathways. Due to some peculiarities of its nervous system, such as the importance of the octopaminergic pathway for its physiology and consequently its survival, many bioinsecticides have already been proposed in studies using this model. Also, much research in plants has focused on establishing pest control methods using natural and selective pesticides. In this regard, the octopamine pathway has emerged as an important target to improve the selectivity of new molecules insecticide and prevent toxicity to non-target organisms, due to its relevance in invertebrates and low toxicity in vertebrates [28].

The survival of insects depends on the optimal functioning of several factors, such as their locomotion, hygiene, and reproductive activities, as well as other aspects that are unique to insect. If a compound modulates one of these factors, it is possible considered a bioinsecticide in potential because it will affect the insect's ability to escape, feed, or reproduce.

The current study seeks to deepen the understanding of the mechanisms of entomotoxicity previously observed with the hydroalcoholic extract of *E. plana*, expanding the focus to new alterations in the nervous system of *Nauphoeta cinerea* [29]. Flavonoids, tannins, and phenolics present in the aqueous extract of *E. plana*, with possible allelopathic and entomotoxic activity, were analyzed in the central and peripheral nervous systems of cockroaches. Our aim is to clarify the additional modifications observed, corroborating previous results and providing new evidence on the bioinsecticidal potential of the extract.

2. MATERIAL AND METHODS

2.1 Study location

The preparation of the hydroalcoholic extract and the behavioral, electrophysiological, and biochemical experiments were conducted in the Laboratory of Neurobiology and Toxinology (LANETOX) of the Universidade Federal do Pampa (UNIPAMPA) – campus São Gabriel, São Gabriel, Rio Grande do Sul state, Brazil.

2.2 Phytochemical quantification of *Eragrostis plana* Nees

The phytochemical characterization of *E. plana* was performed by high-performance liquid chromatography (HPLC) using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) and Shimadzu auto injector (SIL-20A) equipped with reciprocating pumps (Shimadzu LC-20AT) connected to a degasser (20A5 DGU) with integrator (CBM 20A), diode array detector (SPD-M20A), and software (LC solution SP1 1.22). Chromatographic analyses were performed in reverse phase under gradient conditions using a C18 column (4.6 mm x 150 mm) packed with 5 μm diameter particles; the mobile phase used was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was 5% (B) for 2 min, 25% (B) until 10 min, 40, 50, 60, 70, and 80% (B) every 10 min [30, 31] with some modifications.

The infusion extract of *E. plana* was analyzed at a concentration of 20 mg mL^{-1}. The flow rate used was 0.7 mL min^{-1}, the injection volume was 50 μL, and the wavelength was 271 nm for gallic acid, 280 nm for catechin and epicatechin, 327 nm for caffeic acid, chlorogenic acid, and ellagic acid, and 365 nm for quercetin, apigenin, and rutin. Samples and mobile phase were filtered through a 0.45 μm membrane filter (Millipore) and then degassed in an ultrasonic bath prior to use.

Reference solutions were prepared in the mobile phase for HPLC at concentrations of 0.050-250 mg mL^{-1} for catechin, epicatechin, quercetin, apigenin, and rutin; 0.20-200 mg mL^{-1} for gallic acid, chlorogenic acid, ellagic acid, and caffeic acid. The chromatographic peaks were confirmed by comparison of their retention times with those of the reference standards and by DAD spectra (200 to 600 nm). The calibration curve for gallic acid was $Y = 13569x + 1344.9$ ($r = 0.9995$); catechin: $Y = 10932x + 1258.0$ ($r = 0.9987$), chlorogenic acid: $Y = 12573x + 1206.5$ ($r = 0.9997$); caffeic acid: $Y = 11872x + 1570.3$ ($r = 0.9996$); ellagic acid: $Y = 12653x + 1367.5$ ($r = 0.9991$); quercetin: $Y = 13620x + 1337.6$ ($r = 0.9996$), rutin: $Y = 15983x + 1321.5$ ($r = 0.9998$), apigenin: $Y = 12784x + 1372.7$ ($r = 0.9996$), and epicatechin: $Y = 16423x + 1853.2$ ($r = 0.9998$). All chromatographic operations were performed at room temperature and in triplicate.

2.3 Hydroalcoholic extract of *Eragrostis plana* Nees (EHEP)

Aerial parts (stem and leaf) of *E. plana* were collected at Universidade Federal do Pampa (UNIPAMPA) – campus São Gabriel (-30º20'11" S; -54º19'11" W, 114 m altitude). To obtain the hydroalcoholic extract (EHEP), previously dried leaves were ground in a Willey-type knife mill with a 1.70 mm sieve mesh until a powder was obtained. The material was subjected to an

extraction process by percolation in a 90% hydroalcoholic solution using 99.99% absolute alcohol (P.A.).

After filtering the solution obtained, the same was subjected to the rotary evaporator with temperature control up to 60 ºC, then lyophilized at -80 ºC until obtaining a dry residue [32]. The hydroalcoholic extract (EHEP) was obtained by diluting the dry residue in Milli-Q water, obtaining the following concentrations: 0.5; 2.5; 5.0; 12.5; 25; 50; 100 μg g^{-1} animal of the EHEP. The control treatment was obtained with the administration of saline.

2.4 Saline solution for insects

A saline solution was used, composed of 150 mM NaCl, 2 mM $CaCl_2$, 10 mM KCl and 10 mM Tris in addition to ultrapure water up to the volume of 200 mL, with pH 6.8 corrected with NaOH. The pH was adjusted with a glass electrode pH meter, previously calibrated [32].

2.5 Animal model

According to Federal Law N°. 11,794/08, the competence by Ethics Committee on Animal Use (CEUA) is linked to the evaluation of activities involving vertebrate animals, therefore, as we use invertebrates, CEUA registration is not necessary. To standardize the experiments, male cockroaches of the species *N. cinerea* Olivier were used (3 to 4 months after molting). These animals were raised in the Anipampa Animal Facility of UNIPAMPA – campus São Gabriel, in polystyrene boxes (34.4 cm wide x 41.4 cm long x 16.8 cm high), kept at a temperature of 24 ºC, with a 12-hour photoperiod, with water and food (dog food) *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}.

In all protocols, saline, EHEP, or quercetin were injected (10 μL $animal^{-1}$) into the third abdominal portion of the specimens directly into the hemolymph using a Hamilton micro syringe.

2.6 Behavioral experiments

2.6.1 Grooming: psychomotor behavior of self-cleaning

For the grooming assay, we chose to use only concentrations of 5, 50, and 100 μg g^{-1} animal, in addition to the saline solution (control treatment) [33]. These concentrations coincide with those established for Quercetin, a flavonoid already identified and quantified in *E. plana* [6, 19], which will be tested together (5, 50, and 100 μg g^{-1} per animal) [25]. These EHEP concentrations have shown to be significantly effective in previous studies [29], justifying their choice and thus maintaining a balance of concentrations between the extracts tested (EHEP and Quercetin).

2.6.2 Locomotion

Activity locomotor of cockroaches was assessed [25]. For this protocol, 30 cockroaches were used per treatment. After injection of EHEP (0.5, 2.5, 5, 12.5, 25, 50, and 100 μg g^{-1} per animal); and SSI (control treatment), the cockroaches were individually placed in white polystyrene boxes (15 cm wide x 25 cm long x 7 cm high). A Logitech for 10 minutes recorded the locomotion of the specimens® HD WEBCAM camera (Philips, Brazil) attached to the experimental apparatus and connected to a desktop computer (Dell, São Paulo, Brazil). From the recordings, the parameters were calculated by idTracker® software (Stoelting, CO, USA). In these trials, the

following parameters were evaluated: number of immobility episodes, stopping time, and total distance traveled. Data were analyzed using Matlab® software.

2.6.3 Metathoracic coxal-abductor nerve-muscle preparation from cockroaches in vivo

The electromyographic tests performed to verify the susceptibility of the peripheral nervous system (PNS) of cockroaches to EHEP were carried [34]. For this protocol, 6 cockroaches were used per treatment, which were: SSI (control treatment) and EHEP (5, 25, and 50 μg g^{-1} per animal). The insects were fixed individually on a stand, in dorsal decubitus, with the help of entomological needles. One of the legs of the third pair was suspended on a 1 g force transducer (AVS Projects, São Carlos, SP, Brazil). Nerve 5 (thoracic region) was stimulated at a rate of 0.5 Hz, 5 ms^{-1}, for 120 minutes. The recordings were obtained with an AECAD 05 amplifier (AVS Projetos, São Carlos, SP, Brazil), digitized with AQCAD software (AVS Projetos, São Carlos, SP, Brazil) and analyzed with ANCAD software (AVS Projetos, São Carlos, SP, Brazil).

2.6.4 In vitro study of acetylcholinesterase (AChE) enzyme activity

The evaluation of AChE enzyme activity was performed [33] with some modifications. This protocol used 6 cockroaches per treatment, performed in triplicate, and the SSI (control treatment) and EHEP (5, 25, 50 and 100 μg g^{-1} per animal) were administered. After 4 h of treatment, the animals were anesthetized by cooling to 5 °C.

The antennae of each animal were removed, and the heads were collected and stored in 1500 μL phosphate buffer (Kpi: pH 7.0). Samples were then homogenized in a Powerlyzer (Powerlyzer 24 Homogenizer, MO BIO) for 3 min and centrifuged (1000 rpm/15 min/4 °C). 1000 μL of the supernatant was collected and protein quantification of these samples was measured by spectrophotometry at A280 nm using a NanoVue spectrophotometer (NanoVue Plus, GE Healthcare). The volume of supernatant to be used for the measurement of the enzymatic reaction was determined by considering the values obtained in the protein quantification of the samples. The respective supernatant volumes were collected in a microtiter plate, and 50 μL DTNB (10 mM), 50 μL phosphate buffer (Kpi: pH 8.0), and 2.5 μL ACh were added, and the reaction was measured in a UV-visible spectrophotometer (λ = 412 nm, for 1 min) (evolution model 60S, Thermoscientific, New Hampshire, USA). The results were analyzed using VISION Lite software (Thermoscientific).

2.7 Experimental design and statistical analysis

The experiments were performed using a randomized block design (RBD) and animals were randomly selected. Results were expressed as mean ± standard deviation or mean ± standard error of the mean. Statistical analysis was performed with Graphpad Prism 7.0 software (Software Inc., San Diego, CA) using Tukey test or analysis of variance (one-way/two-way) ANOVA, followed by Bonferroni or Dunnett post hoc test when necessary. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Identified compounds

In this study, the following compounds were identified and determined in mg g^{-1} MS^{-1}: chlorogenic acid (46.05 ± 0.01), quercetin (40.17 ± 0.01), caffeic acid (38. 61 ± 0.02), gallic acid (29.82 ± 0.03), apigenin (23.52 ± 0.01), epicatechin (20.15 ± 0.03), ellagic acid (19.38 ± 0.01), catechin (17.93 ± 0.01) and rutin (15.84 ± 0.02). Among these compounds, it was verified as tannins: catechin, epicatechin, and apigenin; flavonoids: quercetin (possible entomotoxic role),

and rutin; phenols: chlorogenic acid, caffeic acid, gallic acid, and ellagic acid. As major compounds chlorogenic acid, quercetin (possible entomotoxic role), and caffeic acid were found.

3.2 Octopaminergic modulation of grooming behaviour

Administration of EHEP and quercetin (5, 50, and 100 µg g^{-1} per animal) significantly altered the self-cleaning movements of *N. cinerea,* especially of the legs, compared to the control treatment (Figure 1A). The control treatment (saline) showed a mean time of 120 s ± 62.752 for leg cleaning and 18 s ± 58.51 for antenna cleaning. EHEP showed a concentration-dependent effect, with the greatest increase in leg grooming observed when the lowest concentration of extract was administered (5 µg g^{-1} per animal = 575 s ± 216.902; 50 µg g^{-1} per animal = 208 s ± 52.853 and 100 µg g^{-1} per animal = 40 s ± 62.752). EHEP did not significantly alter antenna cleaning behavior, which remained constant across treatments with no statistical differences (5 µg g^{-1} per animal = 37 s ± 13.255; 50 µg g^{-1} per animal = 34 s ± 11.134 and 100 µg g^{-1} per animal = 19 s ± 19.619) (Figure 1A).

When quercetin, identified as one of the major compounds in *E. plana*, was administered to the cockroach *N. cinerea*, a significant modulation was observed of both grooming behaviors (legs and antennae) (Figure 1B). All quercetin concentrations induced an increase in leg grooming time, with the intermediate concentration (50 µg g^{-1} g per animal) being the most evident (5 µg g^{-1} per animal = 191 s ± 62.752; 50 µg g^{-1} g per animal = 384 s ± 152.555; 100 µg g^{-1} per animal = 172 s ± 63.459) (Figure 1A). The flavonoid quercetin also induced increased antenna grooming, with the lowest concentration being most effective (5 µg g^{-1} per animal = 65 s ± 62.752), while concentrations of 50 µg g^{-1} per animal and 100 µg g^{-1} per animal were not significantly different from the control (41 s ± 11.134 and 18 s ± 15.376) (Figure 1B).

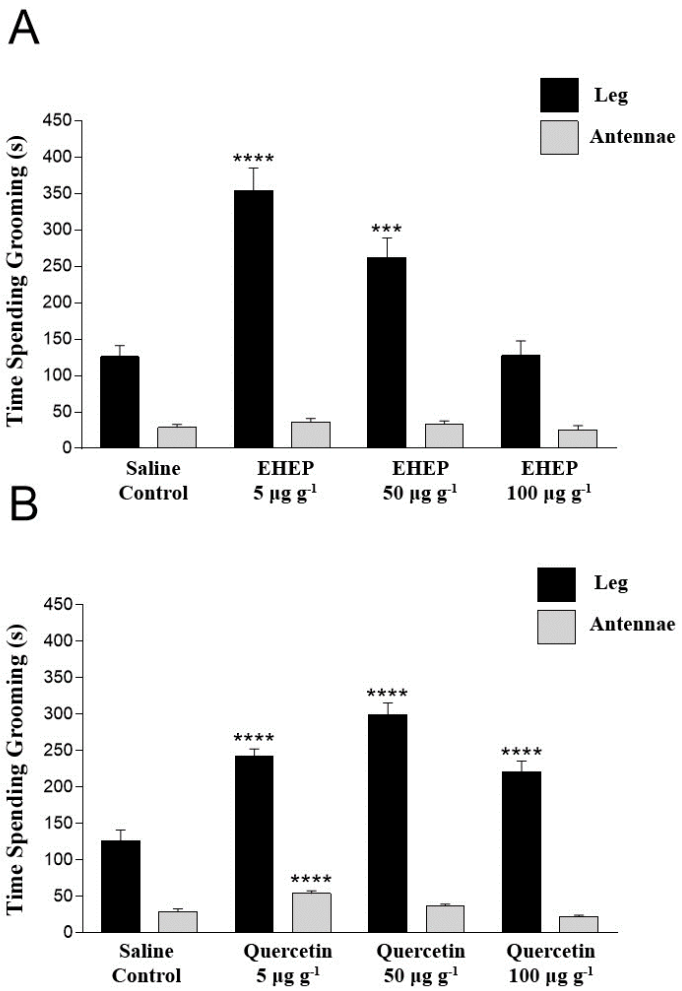

*Figure 1 - Behavioral modulation of grooming induced by Eragrostis plana Nees hydroalcoholic extract (EHEP) and quercetin in Nauphoeta cinerea Olivier. Concentrations used: saline solution (control), EHEP and quercetin (5.0, 50, and 100 µg g-1 per animal). Results were expressed as mean ± standard deviation of total grooming time (in seconds) for 30 min. Data were analyzed by One-way ANOVA and Dunnett tests, **** p<0.0001, *** p<0.0003; n=30.*

3.3 Effect of EHEP on cholinergic pathways during locomotion

The administration of EHEP (0.5, 2.5, 5, 12.5, 25, 50 and 100 µg g^{-1} per animal) significantly affected the locomotor behavior of *N. cinerea* during the in vivo assays (Figure 2). The locomotor behavioral assays showed that only the concentration of 5 µg g-*1* per animal of EHEP induced modulations on the locomotor behavior of the insects. Figure 2 demonstrates that this concentration significantly increased the distance traveled by the animals (Figure 2C) and consequently decreased the period of immobility (Figure 2A) and the stopping time (Figure 2B).

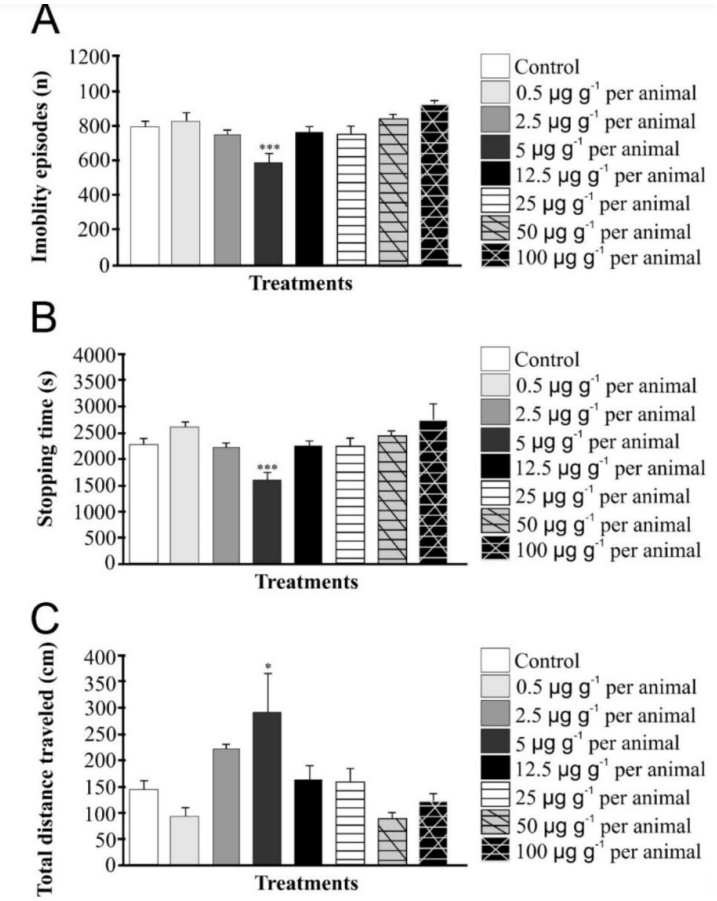

*Figure 2 - Locomotion of Nauphoeta cinerea Olivier modulated by administration of Eragrostis plana Nees hydroalcoholic extract (EHEP). (A) episodes of immobility, (B) stopping time, (C) distance traveled. Results were expressed as mean ± standard deviation by One-way ANOVA analysis and Dunnett tests, *** p< 0.0005; *** p< 0.003; * p< 0.0184; n=28, respectively.*

3.4 Decrease in contractile response induced by EHEP

EHEP treatments (5, 25, and 50 µg g-1 per animal) showed a concentration-dependent effect, with a significant decrease in the contractile response of cockroaches compared to the control treatment (100% ± 1.1%) (Figure 3). The highest concentration (50 µg g-1 per animal) of EHEP decreased the contractile response by 49 ± 13.9% compared to the control treatment after 70 min of recording. The intermediate concentration (25 µg g^{-1} per animal) decreased 73.4% ± 11.5% of the contraction at 80 min and the lowest concentration (5 µg g^{-1} per animal) showed a marked decrease in the contractile response of *N. cinerea* at 100 min of recording (85.2% ± 6%).

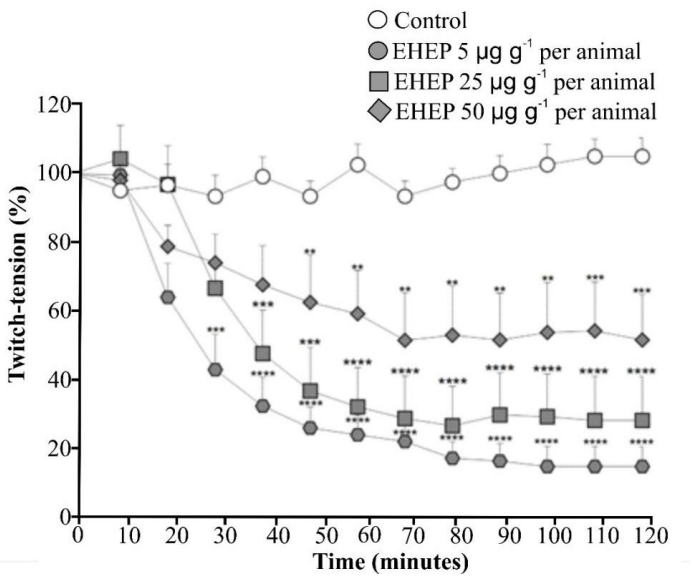

Figure 3 - Effects of Eragrostis plana Nees hydroalcoholic extract (EHEP) on the coxal-abductor nerve-muscle preparation of Nauphoeta cinerea Olivier. Points represent the mean ± standard deviation of the percent contractile response relative to the control time. Analyses were performed by Two-way ANOVA and Bonferroni tests, n=6.

3.5 Inhibition of acetylcholinesterase (AChE) enzyme activity

Administration of EHEP (5, 25, 50, and 100 µg g^{-1} per animal) demonstrated marked inhibition of AChE enzyme activity (Figure 4). For the control treatment, the enzymatic activity was 100% ± 0.35% and the incubation with EHEP demonstrated a concentration-dependent effect on the enzymatic activity of this enzyme (5 µg g^{-1} per animal = 0.98% ± 0.12%; 25 µg g^{-1} per animal = 0.78% ± 0.13%; 50 µg g^{-1} per animal = 0.76% ± 0.08%, and 100 µg g^{-1} per animal = 0.26% ± 0.21%), where the concentration of 100 µg g^{-1} per animal decreased about 99.74% the activity of AChE enzyme.

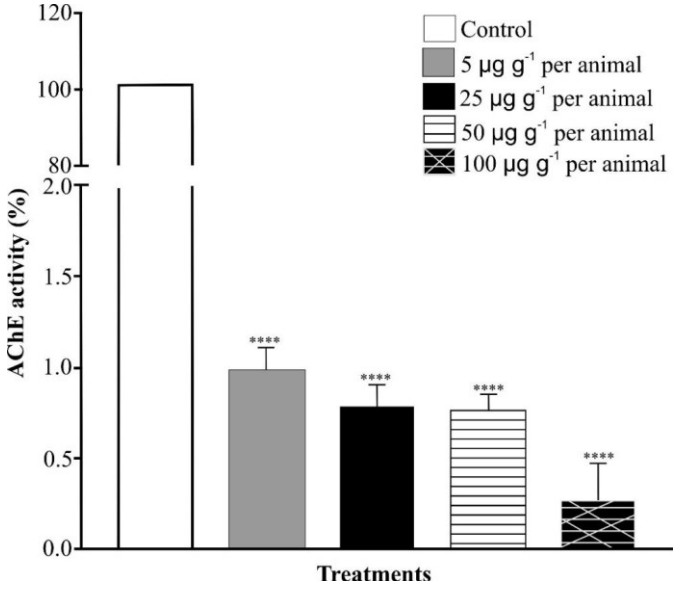

*Figure 4 - Effects of Eragrostis plana Nees (EHEP) hydroalcoholic extract on AChE enzyme activity of Nauphoeta cinerea Olivier. Results were expressed as mean ± standard deviation of percentages. Analyses were performed by One-way ANOVA and Dunnett tests, **** p < 0.0001, n=6.*

4. DISCUSSION

Phenolic compounds (gallic acid, ellagic acid, caffeic acid, and chlorogenic acid), flavonoids (quercetin and rutin), and tannins (epigenin, epicatechin, and catechin) identified in this study are often associated with the allelopathic potential of *E. plana* [19]. However, these compounds confer other effects, including reduced palatability in invertebrates, but quantified in other plant species [35, 36]. In general, flavonoids confer protection against ultraviolet radiation, environmental stress protection strategies and pigmentation [35].

Among these compounds present in *E. plana,* quercetin can be highlighted, a compound already described for its insecticidal activity and characterized by the inhibition of the enzyme acetylcholinesterase (AChE) [31, 32, 37]. *E. plana* naturally produces high concentrations of quercetin, and preparations from parts of this plant may be an excellent source to isolate this compound for use in various bioassays. However, there are few studies that describe and quantify the substances present in this species, which may be related to its biological role in relation to other species, with possibilities of acting as bioinsecticides. In this question, it is emphasized that with the detection of active principles, substances of secondary metabolism can bring ecological alternatives for the reduction of agrochemicals and can be used as natural herbicides and/or bioinsecticides [38, 39]. Quercetin, as previously highlighted, with its anticholinesterase action, highlights its importance as one of the most important compounds for *E. plana,* since this flavonoid has a strong modulation on the cholinergic pathway. This may be a crucial factor involved in the modulation observed in the tests with the AChE enzyme, where the *E. plana* extract inhibited the action of this enzyme to extreme levels (Figure 4). For future verification, it is important that the compounds of *E. plana* can be isolated and prepared in pharmacological tests, where the effects of its crude extract, containing a mixture of biomolecules, and its isolated fractions can be compared. The importance of this pathway is evident in all aspects observed during the assays, as it correlates with all other neurotransmission pathways in insects.

As shown in the literature, antenna and leg grooming behavior are modulated by two major neurotransmitters, the amines dopamine and octopamine, respectively [40, 41]. The increase in leg grooming by EHEP suggests an alteration in octopamine neurotransmission pathways (Figure 1). It is suggested that the observed concentration-dependent effect may be related to the fact that we do not know in detail the proportion of EHEP compounds in each of its concentrations. This also makes it difficult to fully understand the mechanism of action on the octopaminergic pathway. However, it is likely that quercetin is one of the compounds responsible for this effect.

Results in grooming assays highlight the strong modulation of EHEP and quercetin on leg grooming, with the concentration of 50 μg g^{-1} per animal shown to be fully equivalent for both extract and pure compound (Figures 1 A-B). In addition, the complexity of the phytochemical profile also justifies the effect of quercetin on the dopaminergic pathways that control antenna grooming, as this effect is only observed when quercetin is administered at a very low concentration. Thus, if the hydroalcoholic extract of *E. plana* acts on the dopaminergic pathways (antenna grooming), there may be a combination of compounds that together are not visibly active on this pathway at the concentrations administered (Figures 1 A-B).

Using hydroalcoholic and/or methanolic plant extracts to test the entomototoxic effect on leg and antenna grooming activity of *N. cinerea*, while also verifying stimulatory and/or inhibitory effects on the leg and/or antenna grooming activity of this cockroach species, suggests a strong involvement of dopaminergic and octaminergic modulation of the extracts in insects [24, 42-44]. Additionally, grooming in insects is induced by monoamines, and leg movements are mainly coordinated by the neurotransmitter octopamine [42].

The cholinergic system, along with the dopaminergic system, is one of the neurotransmission pathways that regulates locomotor activity in in invertebrates [42]. The decrease in the number of immobility episodes and the stopping time of insects after EHEP administration indicates a probable modulation of the cholinergic pathways, since the extract did not show to modulate the dopaminergic pathway in any of the administered concentrations and in contrast, it caused the inhibition of AChE activity in all proposed concentrations (Figure 4). The increase in the distance traveled by the insects due to EHEP treatment indicates that these animals may become disoriented. Therefore, susceptible to events that lead them to exciting and the concentration of

5 μg g^{-1} per animal, which once showed the greatest modulation on the increase in leg grooming in relation to locomotion, proves to be the concentration that most significantly alters the locomotor pattern of *N. cinerea.*

Octopamine plays an important role as a neurotransmitter and neuromodulator in both peripheral and central nervous systems of insects. The action of octopamine is mediated by the activation of protein-coupled receptors [43]. Different classes of octopamine receptors are responsible for mediating specific effects in insects, which are responsible for slowing myogenic rhythm and influences slow neuromuscular transmission mediated by motoneurons [45].

In the study with *Manilkara rufula* extracts (control, 25, 50, and 100 μg g^{-1} per animal) also was demonstrated a significant effect on leg grooming activity, especially at higher concentrations, while antenna grooming was not affected by the presence of extracts of this species; showed a modulatory effect of *M. rufula* extracts that occurred in octopaminergic pathways [44].

These data support the hypothesis that the different patterns observed in the assays of the present study are due to the involvement of the octopaminergic system. In the insect central nervous system, octopamine plays an important neuromodulator role in the regulation of insect behavior, including rhythmic behavior in grasshoppers, locomotion and hygiene in fruit flies, and feeding behavior in blowflies and cockroaches [46]. Nevertheless, the biogenic amines octopamine and dopamine modulate grooming activity in insects. This activity is also related to courtship, social signaling, and arousal [40]. Octopamine is also related to neurohormones, neuromodulators, and neurotransmitters [47]. In addition, 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 [40]. Under the experimental conditions of the present study, the increased arrest times and immobility episodes induced by EHEP are likely to be a result of octopaminergic receptor antagonism.

Although *E. plana* is considered an invasive species, ours results show high biotechnological potential. As a potent inhibitor of AChE activity, it is a promising organism for the study of new bioinsecticides with higher degradability, especially bioinsecticides. Furthermore, the effects observed with quercetin administration contribute to the elucidation of the sites of action of this metabolite in the insect nervous system.

5. CONCLUSION

Hydroalcoholic extract of *E. plana* altered the behavioral, electrophysiological, and biochemical patterns of *N. cinerea*, suggesting entomotoxicity. Phytochemical profile of this grass is quite diverse, justifying the neurotoxic effects, both on the central and peripheral nervous systems, observed on preparations of *N. cinerea.* Sublethal concentrations of this extract modulated the cholinergic pathways, inhibiting the activity of the AChE enzyme, besides inducing alterations in the octopaminergic, GABAergic, and glutamatergic pathways. These neurotransmitter systems are directly related to the vital processes of insects.

The prospection of the primary site of action of the other products of secondary metabolism of *E. plana*, especially those found in the majority is necessary. It is also fundamental to correlate the time-dependent condition to the composition of EHEP at different concentrations, for a better understanding of the way in which these biomolecules are able to modulate neurotransmission pathways in invertebrates.

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