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Original Article

The repellent activity evaluation of the ethanolic extract of the tipi herb against the *Bothrops moojeni* serpents

Avaliação da atividade repelente do extrato etanólico da erva tipi frente a serpentes *Bothrops moojeni*

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Abstract

The serpent study involves physiological, behavioral and interaction factors with an extremely dynamic and complex environment. The relation of these animals with biodiversity is still quite preliminary, little studied and sustained by popular practices related to traditional medicine, esoteric and mystical currents. Therefore, this study proposed to know about the effect of 80% crude alcoholic extract of *Petiveria alliacea* L., popularly known as Tipi Herb, and its effects associated with the repellency of the *Bothrops moojeni* serpent. A complex of boxes was built for the observation of the snake's displacement, carried out for a total period of six days, the first three for defining the exploratory pattern and the other two for testing the alcoholic extract of Tipi, and finally on the sixth day, one cycle with the presence of a live attractant as bait. It was observed that the animal had no inhibition behavior and moved through the complex in a way like what occurs in nature and showed behavior to avoid remaining in the presence of the extract both with the presence of a live attractant or without it, preferring to move in the other compartments. The results show that this proposed tool is valid for the study of displacement of *Bothrops moojeni* snakes, in the same way that it added values on the importance of cerrado biome biodiversity and on the popular medicine practices of Tipi Herb, since the repellent effect on the snake was significant in the two tests performed.

Keywords: repellent activity, *Petiveria alliacea* L., *Bothrops*, tipi.

Resumo

O estudo das serpentes envolve fatores fisiológicos, comportamentais e de interação com um ambiente extremamente dinâmico e complexo. A relação destes animais com a biodiversidade ainda é bastante preliminar, pouco estudada e muito alimentada por práticas populares ligadas a medicina tradicional, correntes esotéricas e místicas. Nessa linha que este estudo propôs conhecer sobre o efeito do extrato bruto alcoólico a 80% da *Petiveria alliacea* L, popularmente conhecida como Erva Tipi, e seus efeitos associados à repelência da serpente *Bothrops moojeni*. Foi construído um complexo de caixas para a observação do deslocamento da serpente, realizado por um período total de seis dias, sendo os três primeiros para definição do padrão exploratório e os outros dois para o teste ao extrato alcóolico de Tipi e finalmente no sexto dia um ciclo com presença de um atrativo vivo. Foi observado que o animal não teve comportamento de inibição e se deslocou pelo complexo de forma análoga ao que ocorre na natureza e demonstrou comportamento de evitar se manter na presença do extrato tanto com a presença de um atrativo vivo como sem ele, preferindo se deslocar nos outros compartimentos. Os resultados mostram que essa ferramenta proposta é valida para estudo de deslocamento das serpentes *Bothrops moojeni*, da mesma maneira que agregou valores na importância da biodiversidade do Cerrado e sobre as práticas populares da Erva Tipi, uma vez que o efeito repelente sobre a serpente foi significativo nos dois testes realizados.

Palavras-chave: atividade repelente, *Petiveria alliacea* L., *Bothrops*, tipi.

1. Introduction

Evidence of the presence of snakes from desert and tropical regions to the planet's temperate forest zones (Underwood, 1979; Kochva, 1987) is associated with the high number of species and the complexity of ecological relationships existing among its representatives (Greene, 1997).

In this context, considering that Brazilian biomes comprise the third greatest richness of the Reptilia class in the world, with the suborder *Ophidia* 10 of the 18 families of snakes cataloged, being distributed in 75 genera and 457 species (Costa et al., 2022), it is important to expand the understanding of the factors, behaviors and characteristics

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that involve and influence the environmental relationships of animals in these habitats (Pough and Groves, 1983).

Elements such as diets, hunting techniques, defense, predation, forms and habits of displacement, specialization in the use of resources available in micro-habitats, in addition to evolutionary characteristics such as Jacobson's organ, loreal pits, inoculating devices, which together they present close associations with the behavioral patterns, routines and habitat preferences of these animals (Kochva, 1987; Sazima, 1988; Young, 2003; Costa and Bérnils, 2018).

As it is a fact that eventual encounters between snakes and human beings can be dangerous and are surrounded by fears about the clinical outcomes and sequelae of snakebites, the development of several popular practices is observed, using medicinal plants seeking to produce therapeutic effects or for chase these animals away from places where their presence is unwanted, in a manner analogous to methods used for insect repellency (Dey and De, 2012; Giovannini and Howes, 2017; Silva et al., 2012; Fernandes et al., 2016).

Thus, the importance of understanding the factors, behaviors and characteristics involved in the environmental relationships of serpents in these habitats is emphasized (Martins et al., 2002). These relationships involve habits and adaptations on diets, hunting and predation techniques, forms and habits of displacement and specializations in the use of available resources of micro-habitats (Sazima, 1988).

It is noticed that simultaneously with the diversity of serpents, there are a number of relationships and behaviors, whose understanding still needs scientific elucidation. Studies present that there are groups of evolutionary characteristics with consequent behavioral manifestations between species that allow us to observe that the presence of specialized organs as Jacobson's Organ, loreal pits, inoculating devices, may also be contributing to the establishment of forms of relationships, preferences for habitats and other behaviors in these animals (Kochva, 1987).

The comprehension of these mechanisms and their relationships contribute to the behavioral understanding of species and increase arguments to prove the importance of maintaining habitats (Young, 2003; Costa and Bérnils, 2018; Gouveia et al., 2015).

Considering possible contacts between animals and human beings in different environments, noteworthy the clinical situation triggered by accidents with these animals and the associations with popular practices, using medicinal plants, in which it aims to produce therapeutic effects and sometimes repellent, that is, to scare these animals away from places where the presence is unwanted.

In general, they are practices with a great deal of traditional cultural concepts, including esoteric values. Although they are uncommon actions, when compared to the formulas and practices used for insect control and repellency (Fernandes et al., 2016), there is a belief in the effectiveness of these repellent effects in these animals, when these products or practices are used (Dey and De, 2012; Giovannini and Howes, 2017).

Considering the biodiversity of the Brazilian cerrado and the potential of bioprospecting studies for the development of natural products (Lima et al., 2018), it is strategic to consider traditional knowledge as a prior source for pharmaceutical recognition on the use of plant species and direct research in favor of establish the pharmacological profile and possible therapeutic applications of the existing plant genetic heritage (Tinoco et al., 2015).

Among the natural potentialities existing in the cerrado flora, the plant *Petiveria alliacea* L. stands out in this work, belonging to the *Phytolaccaceae* family, which is common in several regions of the world, also found in South America and in the cerrado Tocantinense, popularly named as Erva Guinea, Tipi Herb, Amansa-senhor or Garlic Herb (Trevisan et al., 2021). It is a sub-shrub herbaceous plant, associated with forest environments, with traditional uses associated with mystical, esoteric, spiritual, religious rituals and for its medicinal effects (Luz et al., 2016).

Phytochemical studies of these extracts present substances with robust pharmacological potential such as alkaloids, sterols, triterpenes, saponins, tannins, lipids and coumarins, glutamic dipeptides and cysteine derivatives, in addition to the presence of essential oils such as petiverine and polysulfides (Duarte and Lopes, 2005; Castellar et al., 2013). Traditionally, this vegetable is used as an analgesic, anti-inflammatory, antipyretic, hypoglycemic, antibiotic, antifungal, antitumor, and sedative in several ethnobotanical studies (Lopes-Martins et al., 2002; Oluwa et al., 2017; Oliveira, 2012; Pacheco et al., 2013; Giovannini and Howes, 2017). Adding the records on the antivenom activity (Vásquez et al., 2015) and the repellent activity against snakes (Houghton and Osibogun, 1993; Trevisan et al., 2021).

In this line, this work proposes a tool that seeks collaborate with observational studies of displacement of *Bothrops moojeni* snakes, in an artificial environment, while evaluating the repellent effect raised by traditional knowledge for *Petiveria alliacea* L.

2. Materials and Methods

2.1. Alcoholic extract of Petiveria alliacea L.

Samples of *Petiveria alliacea* L. were collected in the region of Palmas - Tocantins, from September 2018 to April 2019, coinciding with the rainless season (September) and the rainy season (april).

For a better understanding of the collection region, it can be adopted that the collections happened in a radius of 18 km, with the geographical coordinates 10.02'.38" south and 048°.17'.32" west longitude (obtained using global positioning equipment) and an average altitude of 217 m, which determines the city center of Palmas-Tocantins.

The exsiccate of the species was deposited for botanical identification and registration at the Botanical Identification Center of the Federal University of Tocantins under the identification HTO 12150. The study was approved by the Animal Ethics Committee of the Federal University of Tocantins under number 23101-005186/2018-98, the authorization from SISBIO for the collection of animals for scientific purposes has number 52416-4 and for the collection of botanical material the number 66129-1. The project was registered with the National System for the Management of Genetic Heritage and Traditional Knowledge number AE97D0F.

The aerial parts of *Petiveria alliacea* L. were collected, basically containing leaves, flowers, and stems, which were gently cleaned with water initially and later, with a 10% alcoholic solution, to remove possible fungal contaminants and other microorganisms. After 12 hours of drying at room temperature (± 26°C) and protected from light, they were analyzed macroscopically to remove solid contaminations, deteriorated parts and other plants that were accidentally collected, ensuring that there were no visual contaminants. They were then returned to the drying environment at a temperature of 23°C ± 5°C in a ventilated environment and protected from direct light for 5 days. The milling was fulfilled in an industrial electric knife mill, following as a reference the method described by Simões et al. (2001).

The milled vegetable was mixed in an amber glass volumetric flask in the proportion of 5 g of powdered raw material for 100 mL of solvent, 80% ethanol (v/v). The solution was lightly stirred every hour for the first 6 hours and after every 6 hours for 48 hours.

Then the solution was filtered and concentrated to 80% in a rotary evaporator adjusted to 120 rpm, with a bath at 55°C ± 2°C and a negative pressure of 0.5 atm. To ensure total removal of the solvent, the concentrate was kept in an open flask in the water bath at 30°C ± 2°C for sixty minutes. Only then the concentrate was frozen at -70°C and dried by sublimation in Terroni® LS 3000 Lyophilizer. The total lyophilized extract was kept frozen at -20°C ± 2°C until experimental use.

2.2. Characterization of the conditions of the study environment and the capture of animals

The conditions of the test environment of this study were reproduced according to the prevailing environmental conditions in the region of animal capture, city of Palmas, geopolitical capital of the State of Tocantins, located in the region of the geographical coordinates 10°25'S and 048°10W, central Brazil.

The region has an average altitude of 276 m, with a humid tropical climate, has an average annual temperature of 28°C, average annual rainfall of 1.700 mm, two defined seasons, one of which is dry between April and September and one of which is wet from October to March (Lima et al., 2018).

This region presents relief, vegetation, and environmental conditions characteristic of the Cerrado, including an important part of this biodiversity hotspot, considered a priority for preservation due to the high and important endemic concentration of specimens and the constant attacks of anthropic degradation (Guarim Neto and Morais, 2003; Santos et al., 2020).

2.3. Method of searching, capturing, and caring for serpents in captivity

The fourteen serpents used in the study were captured in the Tocantins Cerrado region near the city of Palmas, through the active search with walks and exploration of vegetation, in the swamps, hiding places in rocks, depressions, burrows and cavernous shelters formed by the relief.

After being captured, the animals were transported to the UFT-TO's Phytochemistry Laboratory in the city of Porto Nacional where they received sanitary management, identification of the species, sex and the presence of bodily injuries. The animals were kept for 5 days in observation, in individual polypropylene cages 45 cm long x 32 cm wide x 28 cm high, with perforated lid and closing lock, regardless of the size of the serpent. The serpents were kept in boxes lined with brown paper and a permanent source of water. Captured animals that were not able to be part of the study were returned to the wild. (Three animals were returned due to the presence of ticks on the body, or healing wounds).

The boxes used for packaging were considered safe and easy to maintain because they are transparent, resist impacts, have a locking lock on the lid and allow a quick inspection and good ventilation. The captivity of serpents was maintained under normal conditions of the season, between 33 and 35°C, relative humidity of 55% to 60%, with natural light/dark cycle (day/light and night/dark), conditions that was reproduced during the test.

The serpents were captured at different times of the year and kept separately in the boxes, noting that none were kept in captivity for more than 60 days. These cares were aimed at minimizing the impacts of capture and interfering with the ability of these animals to survive in their natural environment in the future, as well as minimizing the damage caused by the absence of serpents in the ecosystems of the capture region.

The captured serpents were confirmed as *Bothrops moojeni* and cataloged according to sex and morphometric characteristics of snout-vent length (SVL), tail length (TAL), total length (TOL), in addition to checking the condition of the tip of the tail being whitish or not (having a caudal luring mechanism) (Neill, 1960; Martins et al., 2002; Andrade et al., 2006). To collect this information, the animals were lightly sedated using isoflurane, which is considered safe and quickly recovered, and when necessary, as a precaution, Lutz Loop fixation was used after sedation (Valente, 2013; Simões et al., 2001).

In this study, 14 snakes (8 males and 6 females), 10 adults, and 4 young were used (caudal trap present). The young population classified considering the caudal luring, the caudal snout-vent length (SVL), the total length (TOL), and tail length (TAL) (Matias et al., 2011). The collection period, sex and morphometric data are shown in Chart 1, should be noted that the population of serpents used, were all the same species (*Bothrops moojeni*) and with several morphometric characteristics.

2.4. Characteristics of the equipment for assessing the displacement of serpents

The proposed structure and methodology for analyzing the pattern of the serpent's displacement consisted of keeping the animal in a complex that is both safe for the animal and for the observers. And still, that allows the animal to develop the exploration initiative without interference or inhibition of the external environment or

Chart 1. Morphometric variables of the *Bothrops moojeni* serpents captured to perform the experiment*.

Animal	Capture	Sex	Total Length (TOL)	Tail Length (TAL)	Length (SVL)	Caudal Luring
1	October 2018	Male	62.00 ± 1cm	6.78 ± 1cm	55.22 ± 1cm	Present
2	December 2018	Female	68.08 ± 1cm	7.04± 1cm	61.04 ± 1cm	Absent
3	February 2019	Male	88.20 ± 1cm	7.56± 1cm	80.64 ± 1cm	Absent
4	March 2019	Male	63.40 ± 1cm	6.92 ± 1cm	56.48 ± 1cm	Present
5	April 2019	Female	42.40 ± 1cm	4.30 ± 1cm	38.10 ± 1cm	Present
6	April 2019	Male	54.60 ± 1cm	4.90 ± 1cm	49.70 ± 1cm	Present
7	May 2019	Female	87.00 ± 1cm	8.64 ± 1cm	78.36 ± 1cm	Absent
8	May 2019	Male	68.30 ± 1cm	6.42 ± 1cm	61.88 ± 1cm	Absent
9	June 2019	Female	78.00 ± 1cm	7.78 ± 1cm	70.22 ± 1cm	Absent
10	June 2019	Female	82.10 ± 1cm	8.09 ± 1cm	73.91 ± 1cm	Absent
11	July 2019	Male	89.35 ± 1cm	8.89 ± 1cm	80.46 ± 1cm	Absent
12	August de 2019	Male	92.80 ± 1cm	8.56 ± 1cm	84.24 ± 1cm	Absent
13	September 2019	Female	108.00 ± 1cm	9.56 ± 1cm	98.44± 1cm	Absent
14	December 2019	Male	112.00 ± 1cm	10.30± 1cm	101.70± 1cm	Absent

*Data collect between Oct/2018 and Dez/2019.

the structure of the captivity, while the realization and characteristics of this displacement can be computed in some way.

After several measurements and material tests, an appropriate final structure was reached for the observation and performance of the tests using two types of environments (boxes), with a central rectangular room of greater volume (48L) with rectangular dimensions of 60 cm long, 45 cm wide and 20 cm high, and four sides of smaller volume (12L) with 30 cm long, 20 cm wide and 20 cm high. The material that showed the best cost-benefit was the transparent polypropylene polymer, replacing glass due to the simplicity of cleaning, transparency, and safety for handling. On the floor of the five lateral rooms, four perpendicular lines were discreetly drawn and so each room had four equal horizontal quadrants that were marked in I, II, III and IV. In the central room the lines crossed at angles that ensured that the accesses to the side compartments were in the center of the side wall.

The lateral environments were interconnected to the center by cylindrical tubes of polyvinyl chloride (PVC), 50 mm in diameter and 10 cm in length, which were installed in the center of the side walls of the central environment, interconnecting all environments and allowing free movement of snakes between the compartments, at most 1 cm above the floor, so that snakes were not required to climb to transit inside. It is important to note that the cylinders were not considered for displacement counting, so they did not receive any marking, they were only the passage from one environment to another. Figure 1 presents a schematic drawing of the displacement evaluation complex described and used in the experiment.

All compartments were kept with transparent lids and drilled on the entire surface, with 0.5 cm holes in diameter, to ensure air renewal, without unleash the animals.

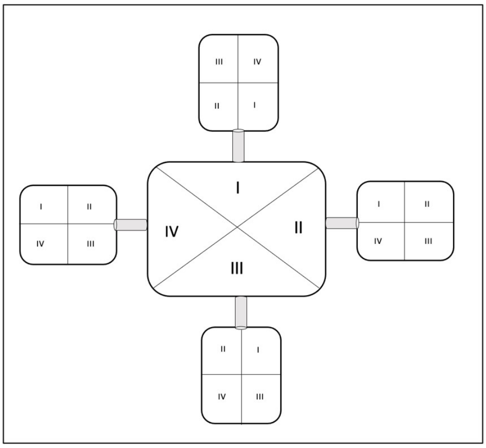


Figure 1. Schematic model of the Displacement Assessment Complex. Layout showing how the complex for analyzing and observing the movement of snakes during the experiment was built and quadrants that were marked on the floor (I, II, III and IV).

2.5. Serpents' ambience in the Displacement Assessment Complex

For the ambience of the animals in the proposed complex, each serpent was always inserted in the central compartment at eight in the morning (morning period), being kept for 24 hours, before starting to record its exploratory behavior. It is important to note that the entire structure was cleaned with soap and water and, subsequently, 70% ethyl alcoholic solution, 12 hours before the beginning of the experiment.

2.6. Serpents' displacement pattern analysis

The tests were always started at 8 am (morning period) and observation followed continuously in 24-hour cycles.

To arrive at the total result of the displacement of animals per cycle, a point was attributed for each new quadrant explored. And it was considered a new quadrant explored, every time during the movement of the animal, more than two thirds of its body entered the new quadrant, thus avoiding in the summation, the inclusion of small movements of position of the animal.

The experiment was carried out indoors in an exclusive and private room in the laboratory, where the temperature was maintained between 33°C and 35°C, the relative humidity between 55% and 60%, throughout the test period and the lighting regime natural (day/bright and night/dark). At night, the only source of indirect lighting in the environment was a 5-watt red lamp that was kept on, producing a penumbra to allow the observer to see.

Each animal used in the experiment served as its own control, as it was observed for three uninterrupted 24-hour cycles (three days) to determine its pattern of displacement in the complex. And immediately after the end of the third cycle, there were three more uninterrupted cycles (three cycles of 24 hours), but now with one of the compartments containing a permanent sample of the extract under test.

During the training period, the extract deposition containers were kept in all four side compartments. At the beginning of the third cycle, the sample of 100 mL of the extract under test was placed in the container on the floor at the back of box of one of the compartments, and in the others, the same volume was added, but only the extract diluent vehicle. The extracts were placed at 8:00 am and replenished at 6:00 pm.

The obtained results were computed hourly, counting the explored quadrants based on the displacements of each snake in the standardization period and later, the same counting was carried out in the test period.

At the end of the second cycle (test), which ended at 8:00 am, the snakes were kept in the complex and a third observation cycle was started at 18:00 pm, lasting 12 hours at night, involving the last five snakes captured. It has been placed on the back of two lateral compartment (box side 2 and 4), a cylindrical cage with a live mouse was placed as a live attractant for the snake.

This cylinder was made of PVC material, 10 cm long, 5 cm in diameter, and completely perforated by 8 mm holes, perfectly safe for the animal, at the same time ventilated and impenetrable for the snake.

This 12-hour nocturnal observation cycle was carried out by depositing at the bottom of two of the four lateral compartments, a live mouse, one facing the other and serving, and the other two compartments without live attractants, with only the cage. In one of these lateral compartments, together with the attractant, the repellent was added, between the cage and the opening of the box. In the others, the same procedure was performed, but only with a vehicle – without the extract.

The results were computed based on the exploratory displacements of the snakes and grouped as the sum of the explored fields in each quadrant of the box, per hour of the nightly monitoring period, which lasted a total of 12 hours.

2.7. Extra care for serpents during the experiment

To avoid interference in the results due to the occurrence of the moult, the serpents were monitored for the color of their skin to suspend the tests if there were changes in these aspects. It is expected that a few days before the moulting, the serpents start to show albescent skin, justified by the interposition of liquid between the old and the new epidermal layer, during this period they reduce their activities, withdrawing to quiet corners. They become active again after moulting the skin, with a renewed appearance and more vivid colors (Andrade et al., 2006).

To minimize the impacts of feeding and reproductive status, no animal was kept together in captivity or fed during the experiment, only water was freely available, and the test was only performed after ten days of the animal being approved and kept in captivity, which guaranteed at least 15 days without food for all animals. The serpents were manipulated exclusively by hook and were not kept in captivity for more than 45 days, to minimize interferences in their behaviors or in the skills of hunting and defending themselves in the natural habitat.

Seven days after conducting the behavioral tests, the serpents were returned to the natural environment, always in the middle of the afternoon towards the end of the day, in the same region where they were collected.

2.8. Analysis of results

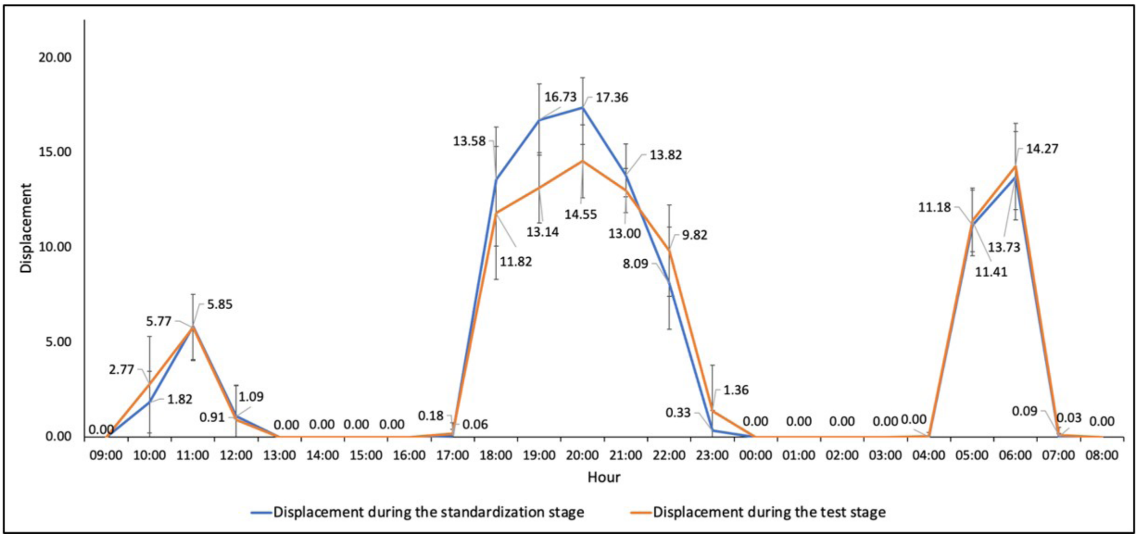
The results were analyzed considering the means and standard deviations, and application of the one-way ANOVA test, followed by *Tukey's Post-Hoc* test to determine the statistical difference between the means of the control group and the extract under test, considering the significance level α=0.05 for statistical significance, 95% confidence interval.

3. Results and Discussion

The displacement results of *Bothrops moojeni* serpents showed in Graph 1 demonstrate that the animals were not intimidated to the point of inhibiting nocturnal displacement habits, maintaining a behavior like that presented in the natural environment. The observation and recording of displacements were performed every hour, for three 24-hour cycles, to understand the displacement pattern (orange line) of the animal in the complex. Afterwards, the displacement was computed in three more cycles of 24 hours, in the presence of the extract of *P. alliacea* (Tipi Herb) in the blue line.

These values represent both the maintenance of the animal's circadian cycle, being active essentially at night, and in agreement with other studies, as well as the feasibility of the complex to observe and measure the snake's displacement in the periods proposed for the experiment.

By observing the pattern of displacement presented in the boxes of the complex, it is possible to see that the snakes moved at different distances according to the period of the day, interspersing periods of rest with periods of exploration, with the highest intensities of displacement



Graph 1. Circadian displacement of serpents in the Displacement Assessment Complex, in the absence and presence of the test extract of tipi herb.

occurring at night between 6 pm and 10 pm, in smaller proportions between 5 and 6 am, moving to perform discrete or no displacement during the rest of the period, as shown in Graph 1.

The results obtained in this work were like those reported for snakes of the genus *Bothrops* in the natural environment, that is, the snakes reproduced their displacement activity in the artificial environment. These facts collaborate in favor of the potential use of the tool to assist in studies on the movement of these animals in controlled environments (Oliveira and Martins, 2001; Hartmann et al., 2003).

There were no statistical differences between the results obtained from the displacement of serpents when evaluated by sex and ontogeny (adults and young people) and between the standardization period and the test period, that is, the snakes showed similar exploratory displacement behavior as showed at Graph 1. The study by Stuginski (2009), which analyzed the movement of snakes in captivity, based on the understanding of the temperature factor, also reported similar behaviors, not noticing differences in the pattern of movement between males and females.

The importance of this study is highlighted by the success in evaluating the displacement of these animals in an artificial environment, considering that the understanding of the factors involved in the displacement of serpents is complex, while adding a tool for the study of their behavior. It can also be noted that the results obtained do not differ from other scientific studies that addressed the issue of habitat with the behavior of serpents. Allowing, then, to suggest that there are ways to register the circadian cycle of these animals, as well as the behavioral habits of displacement and exploration (Pinho and Pereira, 2001; Cruz, 2006; Turci et al., 2009; Bernarde and Abe, 2010).

The presentation of the alcoholic extract of P. *alliacea* to the animals, which in the test period was placed in box side 2, showed significantly reduced mean displacement rates in this box when compared to the displacement rates obtained in the same box in the test period without the extract (mean displacement rate of 11.20 ± 4.41sd in the test, versus mean displacement rate of 20.51± 1.65sd in the standardization), as shown in Graph 2.

The serpents did not stop circulating through the Displacement Assessment Complex, they only modified their exploration behavior, not accessing compartment number two (where the extract of *Petiveria alliacea* L., embedded in cotton, was present). The statistical test revealed that the mean scores obtained in the central box, and boxes sides 1, 3 and 4, when compared to each other, were not different. However, when compared with the values of lateral boxes when compared with box side 2 (which have the extract under test), the differences were statistically significant as show in Table 1.

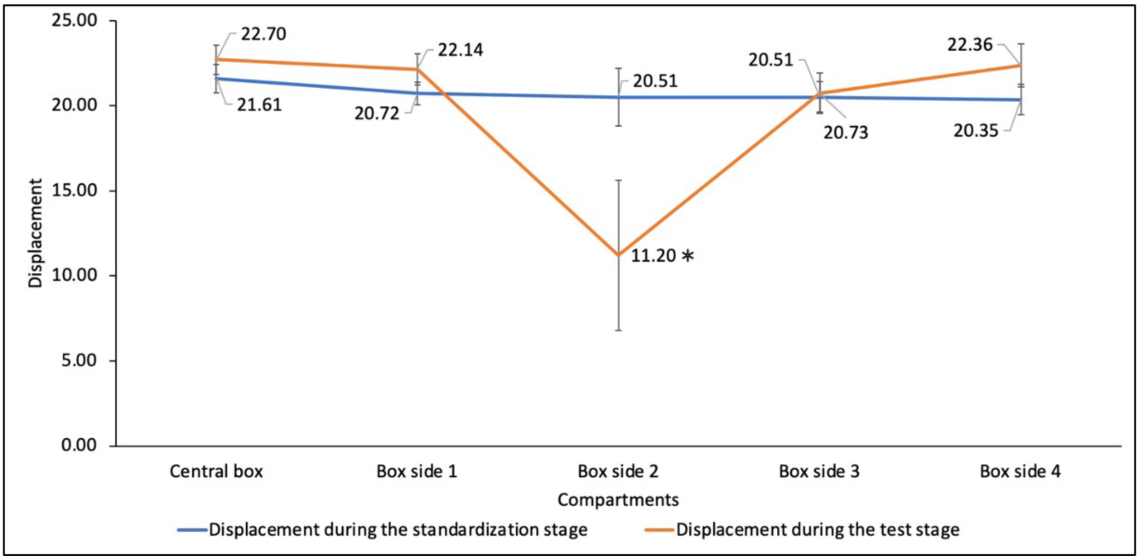
The results suggest the existence of repellent bioactivity in caused by the extract of the *P. alliacea* (*Tipi Herb)* under test, which was successful in away chasing away or discouraged the animal from exploring that compartment (box side 2). Thus, it is inferred that there is component(s) exhaled by the extract that stimulated the animals to move away and look for other environments, that is, it produced a form of repellency to the animal.

To increase confidence in the results of the repellent activity of the extract under test, the third test cycle was carried out including a live attractant in two of the complex boxes (box side 2 with attractant plus repellent and box side 3 with attractant without repellent, with the extract diluent only) and the other two boxes (3 and 4)))) remaining empty. It can be seen from the results in Graph 3, that the extract was efficient in discouraging the snake to move through the box side 3 in search of the attraction.

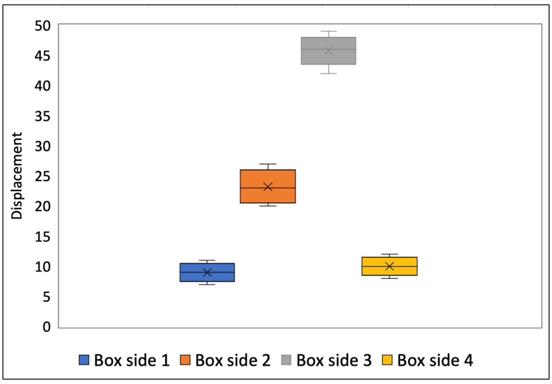
The analysis of displacement means presented in Graph 3 was performed using the ANOVA hypothesis test, considering the significance level $\alpha = 0.01$, with a result of

Table 1. Values of student's T test by analyzing the average of the exploratory behavior of serpents when compared to compartment 2, with *P. alliacea* extract.

T-Test	Central Box	Lateral Box side 1	Lateral Box side 3	Lateral Box side 4
Lateral Box side 2 Extract in test	1.6809 E^{-14}	4.6463 E^{-13}	2.0112 E^{-11}	2.1628 E^{-13}



Graph 2. Exploration scores of *Bothrops moojeni* serpents in the Exploratory Evaluation Complex, in the standardization stage (first three days) and in the test stage (last two days). Data presented as average ± standard deviation (sd).



Graph 3. Average displacement rates of the serpent *B. moojeni* in the Displacement Assessment Complex in the presence of live attractants and repellent extract of *P. alliacea*. Source: Trevisan et al., 2021. Central box: compartment used in the center of the complex and connecting all compartments; box side 1: empty; box side 2: with live attractant plus repellent based on *P. alliacea*; box side 3: with live attractant plus the diluent vehicle of the extract under test; box side 1 and 4: empty.

$p < 0.0001$, accepting the alternative hypothesis that not all means are equal. To identify which means are significantly different, the Tukey test was applied, confirming that, with $p < 0.001$, the means of boxes side 2 and 3 are different, and only the means between boxes side 1 and 4 (empty boxes) and between boxes side 2 and central there are no significant differences, with $p = 0.9$ for the two cases.

Results such as those obtained in this study are relevant because they collaborate to highlight the importance of the olfactory function of snakes and even suggest that they are used to avoid situations with potential danger for the safety of the animal, in addition to ensuring its survival as a predator, together with a tool to study these behaviors and their relationships (Andrade et al., 2006).

Phytochemical studies of *P. alliacea* have already detected the presence of sterols, triterpenes, saponins, alkaloids, tannins, coumarins, lipids, flavonoids and derivatives, which highlight the importance of bioprospecting and the pharmacological potential of this plant. Furthermore, the presence of metabolites such as polysulfides, thiosulfinates, glutamic dipeptides, cysteine sulfoxide derivatives, dibenzyl trisulfide and n-dipropyl disulfide, including essential oils that are present in practically all parts of the plant and associated with the strong odor of garlic characteristic of the vegetable (Ayedoun et al., 1998; Castellar et al., 2013; Luz et al., 2016).

These facts support the indication that this repellent property observed in this study may be related to the presence of these compounds that are exhaled by the extract and that influence the snake's behavior from the olfactory sense.

These findings also contribute positively demonstrating the importance of recognizing the traditional customs associated with the repellent activity of this plant against serpents. The various popular accounts attribute the use of *P. alliacea* for therapeutic, esoteric, and anti-ophidic purposes and it is amplified through the present study by bringing to light an important evidence of repellency effect.

It is a fact that the expansion and understanding of which substances are associated with these effects, as well as how the animal is stimulated to move away from the presence of the extract, or how it could be applied to minimize the impact of the snakebite accident, still lack studies. The use of the tool and the demonstration of the repellent activity of the alcoholic extract of *P. alliacea* (Tipi Herb), contributes to raising the importance of the biological value added to the plant and consequently to the cerrado biome.

4. Conclusion

We conclude that the use of the crude extract of *Petiveria alliacea L.* is efficient in promoting repellent effects on the *Bothrops moojeni* serpent, through the evident change in the displacement rates of these animals in the complex built for the test. Thus, we can infer that the exhaled content of the test extract drove the animal away, even when stimulated by the presence of a live attractant in the compartment.

The outcomes of this study are important because show the importance of bioprospecting studies, in especial of this plant, where the importance of the biodiversity of the Cerrado hot spot is recognized, the possibilities of environmental services of plants such as *P. alliacea* that can be cultivated in rural properties and scare away snakes, reducing the opportunity of snake accidents.

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