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RELENTLESS ACTION OF THE EXTRACT OF THE FUNGUS *GANODERMA LUCIDUM* ON *NASUTITERMES* CUPINS

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Abstract: Termites are xylophagous organisms that live in organized colonies, feeding mainly on wood and cellulose materials. These little creatures play a crucial role in nature, decomposing dead organic matter. However, when they invade homes and man-made structures, they can cause significant and costly damage, causing economic impacts for the timber sector, forest species and crops such as corn, sugar cane, rice, cassava and pastures. Currently, these insects are controlled with industrial chemical insecticides, which can cause harm to humans and impact the environment. It is necessary to look for new formulas to combat these organisms, such as the use of substances extracted from fungi and plants. With this in mind, we sought to evaluate the effects caused by the extract of the fungus *Ganoderma lucidum* on Nasutitermes termites, in order to find out about its potential as a termiticide or repellent, which could serve as an input for the production of organic substances that could be used as termiticides. The extracts were obtained using the cold technique with aqueous, ethanolic and hydroalcoholic solvents (1:1) at concentrations of 0.5%, 1.5% and 5%. Repellency and mortality tests were carried out. The results showed that all the extracts tested had a satisfactory effect on termites, although the ethanolic extract had the best results in all the tests. In terms of repellency, the ethanolic extract was the most efficient in all the concentrations tested, with the 5% concentration showing the highest mortality rate (96.6%). The aqueous extract also had a significant effect, although less pronounced. These results suggest that the *Ganoderma lucidum fungus* could be a promising tool for integrated pest management, helping to reduce the use of chemical insecticides and their negative impacts. Future studies are recommended to deepen the understanding of the mechanisms and action of the extracts and their applicability to different pests.

Keywords: Termites; Extracts; Fungi

INTRODUCTION

The constant increase in the population of insects in agricultural crops has worried farmers, as the damage always affects production and compromises the harvest. Due to factors such as the laws and inspections that prohibit the use of pesticides, the high cost of insecticides, especially for small and medium-sized producers, and the rapid proliferation of pests, there is a need for alternatives to combat crop pests.

Silva et al (2001) states that the use of pesticides in the Brazilian countryside has had a number of consequences, both for the environment and for the health of rural producers. In general, these consequences are conditioned by intrinsically related factors, such as the inappropriate use of these substances, the high toxicity of certain products, the lack of use of protective equipment and the precariousness of surveillance mechanisms, aggravated by the low socioeconomic level and culture of the vast majority of these workers.

Nowadays, people have shown an interest in consuming increasingly organic and pesticide-free products, and with this, the interest in managing crop pests with less environmental impact meets the need to look for alternative methods with less impact or risk to human health and the environment.

Constantino, 2002, points out that termites, usually known as termites, are among the most abundant insect species in tropical and subtropical regions of the planet. These eusocial insects, of the order Isoptera, with around 2,900 cataloged species, are known for the economic damage they cause in urban areas, due to their attacks on buildings and wood in general, and are considered agricultural and forestry pests. But from an ecological point of view, they are very beneficial to nature, as they play a fundamental role in the chemical and physical constitution of the soil.

In Brazil, the main families found are: Kalotermitidae, Termopsidae, Rhinotermitidae, Termitidae and Serritermitidae, of which only Kalotermitidae, Rhinotermitidae and Termitidae are economically important. Termites can be considered beneficial insects, as they help decompose organic matter and contribute to nutrient cycling and soil aeration. However, they can also stand out as pests of forest species and some crops, such as corn, sugar cane, rice, cassava and pastures, among others (Gallo et al, 2002).

The use of fungi as biological control against agricultural pests has been intensifying, since in many situations they can replace chemical control with advantages. Due to the ban on the use of chlorinated insecticides from 1985 onwards, other products began to be used, as well as an increase in research into biological control agents, seeking a more rational control that is less harmful to man and the environment (Zanetti et al., 2002).

Wood-degrading fungi use enzymes to degrade the components of the cell wall. Each chemical component can be metabolized by different enzymes, so we can classify wood rot according to the type of chemical component that is attacked (Castro e Silva, 1996; 2001; 2002).

According to Carraro, 1997, chemical insecticides belong to the class of substances that are considered non-biodegradable. When accumulated in the human body, even in relatively small doses, insecticides cause serious damage to health, such as cancer, neurological disorders, cirrhosis, genetic mutations and congenital malformations.

Almeida, 2012, states that plant and fungal extracts have a great diversity of chemical constituents that can act forcefully on microorganisms, as well as being biodegradable. In this way, these extracts become a method of control that has less impact on humans and other living beings that are directly or indirectly affected by the recalcitrant compounds used in synthetic insecticides. When compared to synthetic insecticides, organic extracts show advantages in terms of lower toxicity, rapid biodegradation, as well as having compounds that pests cannot inactivate (Quarles, 1992).

The use of organic extracts as insecticides not only offers a cheaper method of control, but is also an option currently being sought by companies due to the cost of chemical products and the environmental impact they have on the agricultural sector. The advantages of using fungal extracts are the optimization of biomass yields and quality production, as they allow the control of nutrients, pH, temperature and other environmental factors. Furthermore, the microorganisms can be cultivated on a large scale in fermenters, without harming the ecosystem.

Among the agents with the potential to produce bioactive substances and biotic resistance inducers is the fungus *Ganoderma lucidum*, a medicinal mushroom traditionally used in oriental medicine for thousands of years. Known as "Lingzhi" or "Reishi" in East Asia and as the "King Mushroom" in Brazil (Cao et al., 2012), this mushroom synthesizes antibiotics, immune system activating substances, antivirals, antiallergics and many other compounds of medicinal interest (Lindequist et al., 2005; Boh et al., 2007).

Research with *G. lucidum* on termites has not yet been published, with only minimal work applied to plants. However, there are numerous. There have been many reports on the great potential and action of compounds from this fungus in controlling diseases in animals and humans. Their characteristics reveal a potential for investigating elicitor molecules with possible use for activating plant defense mechanisms. Furthermore, the lack of studies in the literature on resistance inducers obtained from *G. lucidum* fungal biomass justifies the importance of this study.

The aim of this study is to evaluate the effects caused by the extract of the fungus *Ganoderma lucidum* on Nasutitermes termites, in order to understand its potential as a termiticide or repellent, which could serve as an input for the production of organic substances that could be used as termiticides.

METHODOLOGY

The work was carried out at the Fungal Studies Laboratory/LABEF in partnership with the Biotechnology Teaching and Research Laboratory/LNEBIOTEC at the Parintins Higher Studies Center/CESP/UEA. The extracts were obtained from fungi of the species *Ganoderma lucidum*, which are stored in the form of dehydrated carpophores in the CESP Mycotheque.

- Termites: the termites were captured from tree nests around CESP/UEA. For the tests, we chose to capture worker termites, since they are the ones that bring the food to the colony. Identification took place using Constantino's (1999) dichotomous key. For each bioassay/treatment, 30 termites were used per plate. To avoid stress, the termites were always collected moments before the bioassay period and taken to the laboratory in screw-top test tubes.

Figure 01: Arboreal termite nest

Source: authors

- **Preparation of the extracts:** for the bioassays, three extracts of the fungus were tested (alcoholic, aqueous and hydroalcoholic) obtained from an extraction process using solvents with different polarity gradients. This stage follows the guidelines proposed by Castro and Silva, 2002. The extracts were obtained using the cold extraction technique with a 96% ethanolic solvent to obtain the alcoholic extract, and a hydroalcoholic solution in a ratio of 1:1 (water: ethanol) to obtain the hydroalcoholic extract; distilled water previously boiled in the microwave was used to obtain the aqueous extract. The extracts were left to stand for 72 hours in an amber container to avoid possible chemical reactions with light. The solvents were evaporated in a FANEM 515 electric oven at a temperature of 35±2 ºC and the dried crude extract duly stored (Figure 02).

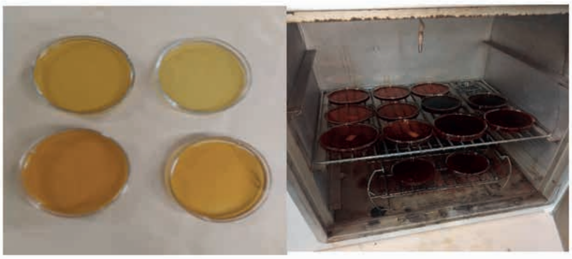

Figure 02: *G. lucidum* crude extract and solvents prepared for drying and dried in the oven.

Source: authors

The fungi were dehydrated in an oven at 40 °C and then ground in a knife mill and passed through a 60 mesh sieve. For every 100 grams of crushed material, 250 mL of the following solvents were added: ethanol, water (aqueous extract) and hydroalcoholic and left to stand for a period of 72 hours in an amber container to avoid possible chemical reactions with light and stored in a refrigerated chamber at a temperature of 4 ºC. Concentrations of 0.5%, 1.5% and 5% were tested for each type of extract.

Bioassays: the bioassays used termites of the genus Nasutitermes, collected in the vicinity of CESP/UEA, and the experimental design used a factorial model to study the interactions between the variables tested. Initially, the concentration of the fungal extract (stock solution) was obtained by mixing 50 mg of the dry extract in 2 mL of distilled water. All the tests were carried out in triplicates, with a control (plate without extract).

Repellency and Mortality: The tests were conducted in observation arenas, with the extracts applied in different concentrations, in aqueous, alcoholic and hydroalcoholic forms. The arena was divided into a treated zone and a control zone, with the termites being introduced into the center of the arena, always using a control plate sprayed in the same proportion with distilled water. The test was carried out in triplicate for each concentration and always including the control. 30 Nasutitermes termites were placed in each Petri dish, between the treated and untreated zone and observed at intervals of 10, 20 and 30 minutes.

Figure 03: Bioassay arenas.

Source: authors

The mortality test was carried out in disposable 90x15mm Petri dishes. After conditioning the 30 termites per dish, 5ml of the extracts were sprayed at a distance of 15cm in triplicates to observe the activity of the extracts, and for all the concentrations used, a control dish was always used, spraying in the same proportion of distilled water. The plates were then taken to an experimental termitary, where they were kept at 28°C and relative humidity of around 85-92% (with a cotton pad soaked in water). To assess the toxicity of the sprayed extracts, counts were taken over a 12-hour period.

Statistical analysis: a non-parametric statistical model (Tukey test) was used to evaluate the results of the treatments. In inferential statistics, the hypotheses tested were to see if there was a difference in termite mortality in the different concentrations of the different solvents used. The descriptive statistics presented the quantitative parameters that describe the samples tested.

RESULTS AND DISCUSSION

The results obtained from the application of the extracts of the fungus *Ganoderma lucidum* on *Nasutitermes termites* showed promising effects, both in terms of behavioral changes and in the late mortality of the exposed individuals. The data was analyzed based on systematic observations made over five consecutive days, after the application of different concentrations (0.5%, 1.5% and 5%) of the hydroalcoholic, aqueous and alcoholic extracts.

The results for the mortality rates are listed in Table 1, where you can see the data obtained after the termites had been exposed to the tested extracts for 3 days (30 termites were used for each concentration).

With regard to mortality, in general, the 5% concentration of the alcoholic extract is where the highest percentage of termite mortality occurred, killing 100% of the termites in the first 24 hours (Table 1). At this concentration there was no statistical difference in mortality between the three extracts tested.

At a concentration of 1.5%, for both the aqueous and alcoholic extracts, mortality was also quite significant, between 86% and 94%, 72 hours after spraying the extract. It should be noted that 48 hours after applying the extract at a concentration of 5% of the aqueous extract, a 99% mortality rate was achieved. In general, for all the concentrations tested, for all the extracts, there was a positive relationship between the increase in concentration and the increase in the mortality rate.

Extract	Concentration (%)	Time (days)	Control Ind. dead	% Dead individuals
Alcoholic	0,5	3	2	83%
	1,5 5	3 1	1 2	94% 100%
Hydroalcoholic	0,5	3	2	70%
	1,5 5	3 2	1 2	86% 100%
Aqueous	0,5	3	2	66
	1,5 5	3 2	2 2	70% 99%

TABLE 1- Final comparison of termite mortality at different concentrations, time and exposure to *Ganoderma lucidum* **extracts**

Concentration (%)	**Aqueous**		
	Aqueous	**Ethanolic**	**Hydroalcoholic**
0,5	Attractant(c)	**Repellent(a)**	Attractant(c)
1,5 **5**	Attractive(c) **Repellent(a)**	**Repellent(a)** **Repellent(a)**	Neutral **Repellent(a)**

In the aqueous extract, there was no statistical difference in the repellency index, with the highest percentage of repellency index occurring for the 1.5% concentration (54%) compared to 33% and 19% for the 5% and 0.5% concentrations respectively.

TABLE 2: Repellency index of the extracts at different concentrations. Equal letters in the column mean that there is no statistical difference at the 95% significance level (p>0.05) using the Tukey test.

The repellency test was carried out within 30 minutes for all treatments. During the first few moments after applying the extracts of *G. lucidum* extracts, a particularly important behavior of the termites was observed, the initial avoidance of contact with the treated discs, for all the extracts, which is a typical behavioral repellency characteristic.

At all concentrations, the ethanolic extract obtained from the carpophore of the fungus *G. lucidum* proved to be repellent to termites. In a way, this result corroborates the killing potential obtained in the toxic effect tests of the bioactive compounds that make up the carpophore extracts.

The aqueous extract showed a higher percentage of insects in the "untreated areas" at a concentration of 5%, proving to be repellent. At concentrations of 0.5% and 1.5% there was an increase in preference for the treated areas, indicating that termites prefer these compounds, with 40.8% and 23% of individuals in the untreated areas respectively.

The percentage of insects that chose the area treated and untreated with the hydroalcoholic extract showed no significant differences at the 5% probability level, and was neutral at the 1.5% concentration, with 50% of individuals in the treated and untreated areas. It should be noted that at a concentration of 0.5% the result was attractive with a percentage of 64.1% in the treatment and repellent at a concentration of 5%.

The repellency test was carried out in arenas, interconnected by transparent tubes. The observation of the insects circulating inside the communication tubes between the plates and inside the plates with extract and without extract indicates that the termites might avoid those containing the extracts, although this is a group choice rather than an individual one. The termites' preference for a product that causes them mortality is somewhat unexpected, even if it depends on the type of product and is not always immediate, as Rosales (2001) observes.

CONCLUSION

The aim of this study was to evaluate the potential of the crude extract of the fungus *Ganoderma lucidum* on *Nasutitermes* worker termites, with a view to understanding its potential as a termiticide or repellent, which could be used as an input in the production of organic substances that could be used as termiticides. The results showed that all the extracts tested, alcoholic, hydroalcoholic and aqueous, at concentrations of 0.5%, 1.5 and 5% showed significant toxic effects on the exposed individuals, especially the ethanolic extracts.

According to the results obtained, the ethanolic, hydroalcoholic and aqueous extracts of ***Ganoderma lucidum*** have repellent potential against *Nasutitermes sp termites*, with the ethanolic concentrations being more efficient than the aqueous and hydroalcoholic extracts, which only had a repellent effect at 5%. However, the 5% and 1.5% ethanolic treatments were more effective at repelling termites.

In terms of termite mortality, all the extracts proved to be efficient, but the ethanolic extract was the one that showed the greatest termite mortality in the first 24 hours of the test, killing 100% of the individuals.

Thus, based on the data obtained, it can be concluded that the fungus *Ganoderma lucidum* has bioactive compounds with high insecticidal potential against termites, and may represent a variable and less aggressive alternative to conventional chemical control methods. The gradual action of the extracts can be advantageous in integrated management programs, especially considering the social behavior of termites, which favors the spread of toxic agents.

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