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Termiticidal Activity of *Libidibia ferrea* var. *ferrea* and of the Association With *Isaria* spp. Against *Nasutitermes corniger*

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Abstract

Nasutitermes corniger (Motschulsky) is an urban termite pest that is controlled by chemical applications. We investigated the effect of the association of *Isaria farinosa* (Holm: Fries) Fries, *I. fumosorosea* (Wize) Brown & Smith, and *I. javanica* (Frieder & Bally) Samson & Hywell-Jones with the extracts of *Libidibia ferrea* var. *ferrea* (Mart. Ex Tul.) L. P. Queiroz in the control of *N. corniger*. The following experiments were performed: the toxicity of aqueous and methanolic extracts on the biological aspects of fungi, action of extracts on workers and soldiers, and fungus-extract combination on workers of termite. The aqueous extracts of the leaves and pods of *L. ferrea* var. *ferrrea* were more efficient than the methanol extracts, demonstrating termiticide activity at 10, 25, 50, 100, and 200 mg mL^{-1}, with 100% worker mortality after the third and fourth days and 100% soldier mortality by the third through sixth day. Lethal concentrations (LC_{50}) varied from 0.624 to 0.710 mg mL^{-1} for workers and from 0.146 to 1.410 mg mL^{-1} for soldiers. The extracts were compatible with the fungal strains at the lowest concentrations. Associations of the extracts with *I. farinosa* ESALQ1355 demonstrated efficient control of termite workers. The results demonstrate that *L. ferrea* var. *ferrrea* extracts, either alone or in association with *I. farinosa* ESALQ1355, functioned in the *in vitro* control of *N. corniger*, representing a viable alternative to be further tested in controlling those termites in urban areas.

Keywords: biological control, entomopathogenic fungi, plant extracts, termites

1. Introduction

Nasutitermes corniger (Motschulsky) (Isoptera: Termitidae) is an arboreal termite widely distributed in the Americas, from southern Mexico to northern Argentina. In Brazil, and in most of South America, it is considered one of the most important urban pests, being responsible for enormous damage to structural, decorative, and utilitarian wooden objects. Infestations by that termite have been reported in many Brazilian states with frequent occurrences in the states of Bahia, Pernambuco, Paraíba, Ceará, Pará, and Amazonas, causing serious damage to historic buildings, constructions, and tree trunks, (Milano & Fontes, 2002; Albuquerque, Matias, Couto, Oliveira, & Vasconcellos, 2012; Mello, Costa, A. C. Silva, A. M. B. Silva, & Bezerra-Gusmão, 2014).

Termites have usually been controlled by chemical insecticides, but those compounds are toxic to other living organisms and can cause serious problems in health human and the general environment (Chen, Ohmura, Doi, & Aovama, 2004). Additionally, those chemical treatments result in the selection for resistant insects that become

successively more difficult to control (Pourseyed, Tavassoli, Bermousi, & Mardani, 2010). Entomopathogenic fungi and plant extracts have been shown to be efficient alternatives for controlling insect pests, as they minimize their multiplication and help maintain insect populations in check, but without negatively impacting other organisms or the environment (Marques, Monteiro, & Pereira, 2004; Lv et al., 2011; Sabbour & Abdel-Rahman, 2013).

Plants naturally produce bioactive substances, and can serve as potential alternative sources of natural insecticides (Luna et al., 2005; Omena et al., 2007), with many reports in the scientific literature of the *in vitro* control of *N. corniger* workers and soldiers by plant extracts (Santana et al., 2010; Souza et al, 2011; Lima et al., 2013). *Libidibia ferrea* (Mart. Ex Tul.) L. P. Queiroz (Fabales: Fabaceae) (= *Caesalpinia ferrea*) is a widely distributed tree native principally found in northern and northeastern regions of Brazil (Queiroz, 2010), and insecticidal activity has been documented on nymphs and adults of the cochineal *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) (Lopes et al., 2018).

Species of *Isaria* Persoon are known to be efficient in the *in vitro* control of termites, and certain strains of *Isaria fumosorosea* (Wize) Brown & Smith (= *Paecilomyces fumosoroseus*) and *Isaria javanica* (Frieder & Bally) Samson & Hywell-Jones (= *Paecilomyces javanicus*) (Hypocreales: Cordycipitaceae) have been patented for controlling the subterranean termites *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) and *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) (Wright, Connick, & Jackson, 2003). The efficiency *in vitro* of these fungi was confirmed by *C. formosanus*, *Coptotermes gestroi* Wasmann (Isoptera: Rhinotermitidae) and *N. corniger* (Yanagawa, Yokohari, & Shimizu, 2008; Lopes, Svedese, Portela, Albuquerque, & Luna-Alves Lima, 2011; Wright & Lax, 2013; Passos et al., 2014; Lopes et al., 2017).

The use of entomopathogenic fungi associations with plant oils and extracts could increase their pest control efficacy and (Marques, Monteiro, & Pereira, 2004; Santos, R. L. S. Oliveira, Costa, Tiago, & N. T. Oliveira, 2015; Silva, Alves, Luna-Alves Lima, & Lima, 2015). As such, the present work evaluated the *in vitro* insecticide potentials of the association of *Isaria* spp. with the extracts of *L. ferrea* var. *ferrea* against *N. corniger*.

2. Materials and Methods

The bioassays were carried out at the Biological Control Laboratory of the Agronomic Institute of Pernambuco, Recife, Pernambuco, Brazil (8º03′50.2″S, 34º55′29.2″W).

2.1 Nasutitermes corniger

The *N. corniger* termites were collected from nests on the Campus of the Federal University of Pernambuco (UFPE) in Pernambuco State, Brazil (8°03′07″S, 34°56′59″W). The species was identified in the Biology Department of the Federal Rural University of Pernambuco (UFRPE) by Dr. Auristela Correia de Albuquerque. The adult workers and soldiers could be distinguished and selected based on their physical characteristics (such as body size and color) to standardize the development states of the castes used in the biological tests.

2.2 Species of Isaria Used in the Experiments

The *I. javanica* URM4993, *I. farinosa* ESALQ1355, and *I. fumosorosea* ESALQ1297, kept in the URM Culture Collection (WDCM604) of Federal University of Pernambuco (UFPE) and the ESALQ Collection of Microorganisms at "Escola Superior de Agricultura Luiz de Queiroz" (ESALQ) University of São Paulo (USP), were tested for biological activity based on pathogenicity data on *N. corniger* reported by Lopes et al. (2017). The fungal strains were cultivated in Sabouraud medium (SAD) (Peptone-Dextrose-Agar) for 12 days; afterwards, the conidia of each strain were transferred to 10 mL of Tween 80 (0.1%) solution and quantified using the Neubauer chamber, and the final concentration adjusted at 1×10^7 conidia mL^{-1}. This conidia solution was used for experiments of compatibility with extracts and to obtain LC_{50} values using *N. corniger* workers, following the procedures in Lopes et al. (2017) (Table 1).

Table 1. *Isaria* strains used in the experiments

Strains	Origin	Host	Germination (%)	LC_{50} (IC)[1](conidia mL^{-1})
Isaria farinosa ESALQ1355	ESALQ/Collection	*Brassolis sopharea*	91	6.66×10^4 (12.47-3.10)
Isaria javanica URM4993	Micoteca/URM	*Lonomia obliqua*	98	7.22×10^5 (20.25-2.43)
Isaria fumosorosea ESALQ1297	ESALQ/Collection	*Bemisia tabaci*	91	4.60×10^5 (13.10-1.38)

Note. [1] 95% confidence interval.

Source: Lopes et al. (2017).

2.3 Obtaining the Plant Extracts

The leaves and pods of *L. ferrea* var. *ferrea* were collected from specimens growing in Euclides da Cunha' Square, Recife, Pernambuco State, Brazil (8°3′30″S, 34°54′12″W); the specimens were identified at the Botany Department of the Agronomy Institute of Pernambuco/IPA and subsequently deposited in the Dárdano de Andrade Lima Herbarium. The plant material was washed in distilled water to remove any surface impurities, dried at room temperature, and shredded. Aqueous extracts of the leaves and pods of *L. ferrea* var. *ferrea* (AELLf and AEPLf) were obtained by mixing 20 g of the shredded plant material with 80 mL of a 0.15 M solution of NaCl to obtain a final concentration of 20% (w/v). The suspensions were agitated for 16 h at 4 °C, filtered, and subsequently centrifuged at 10.000 rpm for 15 min at 4 °C. Methanol extracts of the leaves and pods (MELLf and MEPLf) were obtained by mixing 20 g of the shredded material with 80 mL of methanol. The mixtures were then agitated for 24 h, filtered, and the solvent evaporated in a rotary evaporator. The extracts (200 mg mL^{-1}) were subsequently diluted with distilled water (containing 0.1% Tween 80) to final concentrations of 100 mg mL^{-1}, 50 mg mL^{-1}, 25 mg mL^{-1}, and 10 mg mL^{-1} (Lopes et al., 2018).

2.4 Effects of the Plant Extracts on the Fungal Strains

The effects of the plant extracts on the fungal strains were determined by evaluating fungal germination, mycelial growth, and sporulation. Each experiment was performed on the three strains of *Isaria*, employing four extracts (Aqueous-AELLf and AEPLf; Methanol-MELLf and MEPLf) at five different concentrations (10 mg mL^{-1}, 25 mg mL^{-1}, 50 mg mL^{-1}, 100 mg mL^{-1}, and 200 mg mL^{-1}), as well as the control (without extract), with three repetitions, totaling 63 treatments. The conidia of each strain were added to SAD (liquid, 45 °C) and with a solution Tween 80 (0.1%). To evaluate spore germination, 1 mL of a conidia suspension (1×10^8 conidia mL^{-1}) of each strain was added to 9 mL of each plant extract concentration, with Tween 80 (0.1%) as the control. After 1h, we inoculated 0.1 mL of each suspension separately into Petri dishes containing SAD to be incubated at 26±1 ºC and 80±10% RH. Germination was determined after 16 h by observing 500 conidia (both germinated and non-germinated), with percentage germination being calculated by the formula: G = n × 100/500 (where G is the germination percentage and n is the number of germinated conidia), according to Alves and Pereira (1998). Evaluations of mycelial growth involve inoculating 0.3 mm filter paper discs with 0.01 mL of the conidia suspensions (1×10^8 conidia mL^{-1}) of the different species, which were then placed into Petri dishes with SAD and five concentrations of the plant extracts to be incubated at 26±1ºC and 80±10% RH for 12 d. Mycelial growth was quantified by determining colony diameters. To evaluate fungal sporulation, fragments of those colonies were transferred to test tubes containing 10 mL of Tween 80 (0.1%) solution. The suspensions were then agitated for approximately 2 min in a Vortex mixer and the conidia were counted using a Neubauer chamber.

2.5 Efficiencies of the Extracts Against Nasutitermes corniger

The effects of the extracts on *N. corniger* termites were analyzed following the methodology described by Kang et al. (1990) (modified). Filter paper disks (4 cm diameter) were impregnated with 0.2 mL of the extracts (10 mg mL^{-1}, 25 mg mL^{-1}, 50 mg mL^{-1}, 100 mg mL^{-1}, and 200 mg mL^{-1}) and Tween 80 (0.1%) solution (control). The impregnated disks were then dried at room temperature and placed in Petri dishes (90 × 15 mm) together with a small amount of moistened cotton (to maintain humidity levels) and a fragment of the termite nest (to serve as shelter for the insects). A total of 20 insects (four soldiers and 16 workers) were carefully transferred to the dishes; that pre-established 1:4 ratio is based on the natural interdependence of *N. corniger* casts and helps guarantee the maximum survival of those insects outside their intact nests, according to Vasconcellos and Bandeira (2006). The dishes were maintained at 26±1 °C, with a relative humidity of 80±10% in the absence of light. The experiment included 21 treatments (four extracts × five concentrations, plus the control) with five repetitions, totaling 100 insects per treatment (20 soldiers and 80 workers). Mortality was evaluated daily until the death of the last insect; those results were used to determine the percentage survival and the Lethal Concentration (LC_{50}).

2.6 Effects of Plant Extracts Associated With Isaria spp. Fungi on Nasutitermes corniger Termites

The effect of the association of each extract at its LC_{50} with the fungal extract at LC_{50} (Tables 1 and 3) was analyzed according to Kang et al. (1990). The paper filter discs (4 cm in diameter) were impregnated separately with 0.2 mL of the combination of the agents, the extracts, the fungi, and the 0.1% Tween 80 (control) solution. The impregnated discs were then dried at room temperature and placed in Petri dishes (90 × 15 mm) along with a piece of moistened cotton to maintain activity and provide a nest fragment for termite shelter. A total of 16 workers were transferred to each Petri dish, being maintained at 26±1 °C and relative humidity of ±80% and in the dark. These experiments were therefore composed of 21 treatments (four extracts, three strains and 12

association of the agents, plus the control) with five replications, totaling 100 insects per treatment. The percentage of mortality was evaluated after four days. To confirm the mortality of workers was caused by the fungi, the dead insects were disinfected on the surface in 70% ethanol and 4% sodium hypochlorite and rinsed in sterilized distilled water and then kept in a humid chamber (26±1 °C and 80±10% RH) to be examined for fungal growth.

2.7 Statistical Analyses

The level of toxicity of extracts on *Isaria* spp. was determined by the Biological Index (IB), obtained by means of the formula IB = 47 [CV] +43 [ESP] +10 [GERM]/100 (where, CV = the percentage of vegetative growth; ESP = % of sporulation; and GERM = % conidia germination), all in relation to the control. The IB index may vary from: 0-41 (toxic), 42-66 (moderately toxic) and more than 66 (compatible) according to Rossi-Zalaf, Alves, Lopes, Silveira, Tanzini (2008). The survival rates (%) were determined for each treatment and the data were submitted to the Log-Rank test, using the Kaplan-Meyer method by pairs of isolates, using the SAS Proc. Lifetest (SAS Institute, 1999-2001). The mean Lethal Concentrations (LC_{50}) was determined after the fourth day of treatment, using Proc Probit software (SAS Institute, 1999-2001). Data on mortality of termites caused by the combination of fungi + extracts were submitted to analysis of variance (ANOVA) using the SAS ANOVA Proc (SAS Institute 1999-2001) and the means were compared by the Tukey test at a 5% level of probability. The control data of the termites are presented graphically using Software GraphPad Prism (Software Prism, 2016).

3. Results

Table 2 illustrates the effects of aqueous and methanol extracts of *L. ferrea* var. *ferrea* on the biological parameters of the different fungal strains. The aqueous extracts were compatible with the fungal strains at the concentrations tested, except the extract of pods (200 mg mL^{-1}) for *I. farinosa* ESALQ1355 and leaf extract (200 mg mL^{-1}) for *I. javanica* URM4993, which were classified as toxic according to the Biological index. In general, the methanolic extracts did not present a fungicidal effect on the strains, being only the extract of pod classified as moderately toxic in the concentrations 100 and 200 mg mL^{-1} for *I. fumosoresea* ESALQ1297 and the leaf extract in the concentration 200 mg mL^{-1} for *I. farinosa* ESALQ1355, causing significant decreases in the means of mycelia growth and sporulation, mainly.

Table 2. Compatibility of *Isaria* with extracts of leaves and pod of *Libidibia ferrea* var. *ferrea*

Extract	Concentration (mg mL^{-1})	Strains/[1]IB Values and Classification		
		I. farinosa ESALQ1355	*I. javanica* URM4993	*I.fumosorosea* ESALQ1297
AEPLf	10	89.94 C	92.27 C	96.25 C
	25	82.82 C	92.27 C	92.11 C
	50	81.63 C	97.73 C	92.89 C
	100	81.88 C	97.70 C	88.05 C
	200	44.66 T	67.94 C	72.44 C
AELLf	10	92.38 C	97.81 C	94.76 C
	25	93.73 C	92.50 C	94.06 C
	50	86.99 C	101.95 C	92.66 C
	100	86.99 C	96.71 C	89.77 C
	200	86.47 C	40.93 T	90.78 C
MEPLf	10	105.90 C	99.43 C	81.53 C
	25	102.70 C	98.43 C	79.95 C
	50	101.42 C	95.78 C	79.35 C
	100	83.28 C	105.42 C	60.06 MT
	200	42.67 MT	68.12 C	45.64 MT
MELLf	10	98.04 C	97.71 C	91.51 C
	25	96.83 C	93.92 C	91.17 C
	50	92.59 C	92.65 C	115.69 C
	100	82.55 C	101.10 C	85.07 C
	200	49.36 MT	76.71 C	85.07 C

Note. [1]Biological Index: C compatible (above 66), MT moderately toxic (42-66), T toxic (0-41).

The survival of *N. corniger* treated with aqueous extracts of *L. ferrea* var. *ferrea* was shown in (Figure 1). The

extracts presented significant insecticidal activity *in vitro* against *N. corniger,* reducing termite survival in relation to control treatments, which showed a life time of up to 11 days. Survival tests have reported that the aqueous extracts of *L. ferrea* var. *ferrea* were more effective causing death of termites in a lower time (3 to 5 days) than the pod extracts treatments. Differences in daily survival between the control group and the treatments reinforce the findings on insecticidal effect of aqueous extracts.

The AELLf treatment was the most effective, causing complete termite death in three days at concentrations of 50 mg mL^{-1} (LC_{50} = 0.624 mg mL^{-1}) among both workers (Figure 1a; Table 3) and soldiers (LC_{50} = 0.146 mg mL^{-1}) (Figure 1b; Table. 3); the other concentrations resulted in insects survival until the fifth day. The AEPLf treatment resulted in the death of 100% of the insects by the fourth day of exposure at a concentration of 100 mg mL^{-1} (LC_{50} of 0.710 mg mL^{-1}) (Figure 1c; Table 3), promoting the death of 100 % of the workers (Figure 1c) by the third day (200 mg mL^{-1}) and 100% of the soldiers by the fourth day with the concentration of 50 mg mL^{-1} (LC_{50} = 1.410 mg mL^{-1}) (Figure 1d; Table 3).

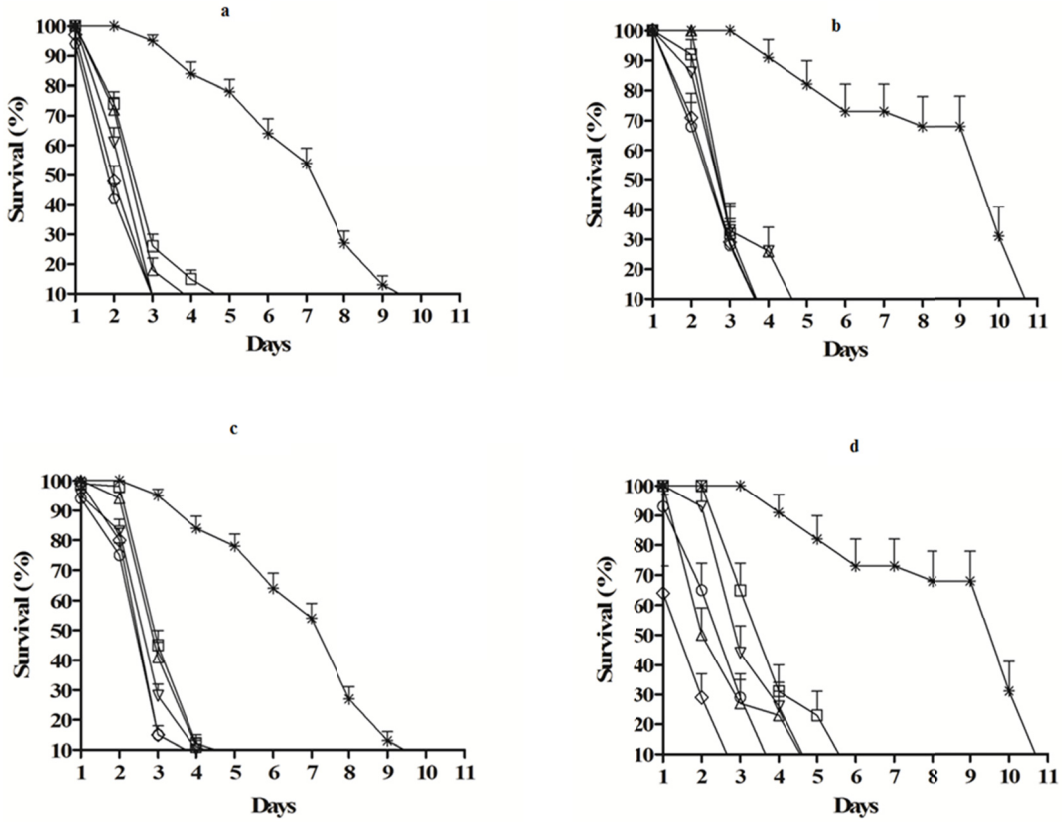


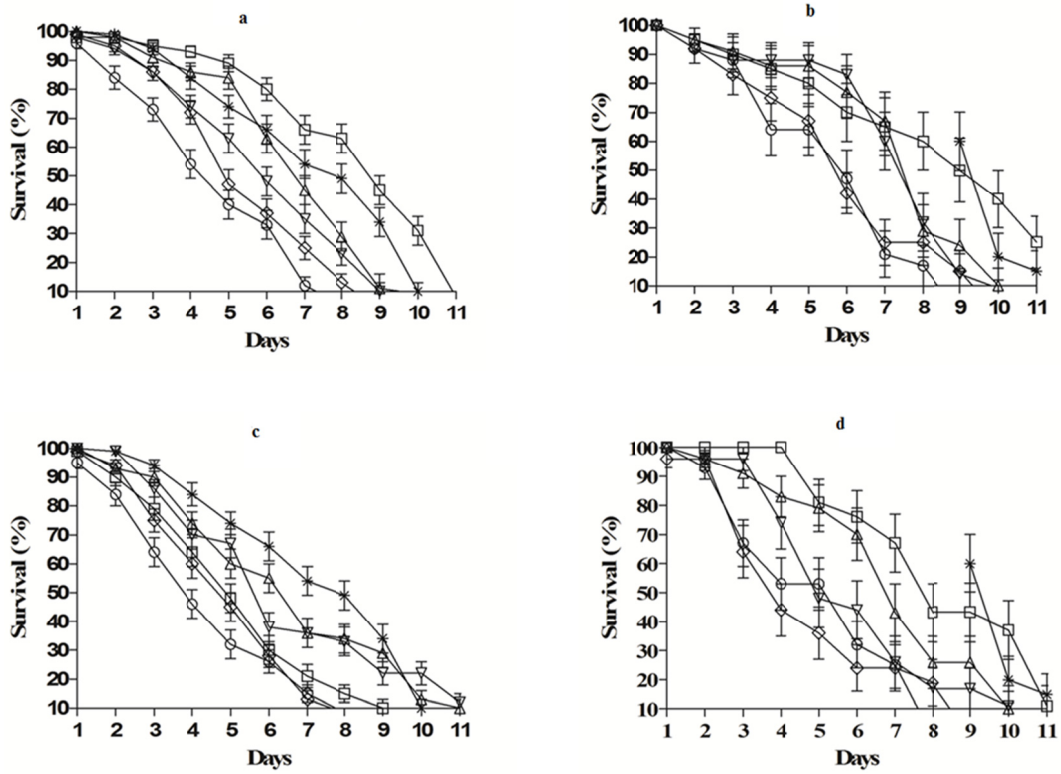
Figure 1. Daily survival (%) of workers and soldiers of *Nasutitermes corniger* treated with aqueous extracts of *Libidibia ferrea* var. *ferrea* evaluated until death of the last individual by the Log-Rank test: AELLf effects on workers (a) and soldiers (b). AEPLf effects on workers (c) and soldiers (d), at concentrations of 10 (⊟), 25(⧋), 50 (▽), 100 (◊), 200 mg/mL (⊖), and the control (✳). Each point represents the mean±SE of five repetitions

Table 3. Lethal concentrations (LC_{50}) of *Libidia. ferrea var. ferrea* on *Nasutitermes corniger*

Extracts	Workers			Soldiers		
	LC_{50} (IC)[1] (mg mL^{-1})	Regression equation	$(\chi^2)^2$	LC_{50} (IC) (mg mL^{-1})	Regression equation	(χ^2)
AEPLf	0.710 (1.163-0.297)	Y=5.14954+1.00908*logX	28.40	1.410 (2.098-0.698)	Y=4.71308+1.92107*logX	29.05
AELLf	0.624 (0.844-0.363)	Y=5.49411+2.41777*logX	57.12	0.146 (0.657-0.00)	Y=5.9102+1.09267*logX	77.36
MEPLf	0.255 (1.116-0.000)	Y=5.19769+0.033384*log	62.02	2.871 (4.092-1.815)	Y=4.15853+ 1.8367*logX	25.34
MELLf	1.279 (2.978-0.021)	Y=4.96053+0.36901*logX	75.57	8.003(23.465-4.365)	Y=4.10687+0.98879*logX	21.65

Note. [1]95% confidence interval. [2]Significant analyses (p = 0.05) by the Chi-square test.

The MELLf treatment caused the death of 100% of the workers and soldiers by the seventh day at 200 mg mL^{-1} concentration (Figure 2a). Similarly, other concentrations of that extract (50 and 100 mg mL^{-1}) caused the death of 100% of the workers after the ninth day while soldiers survived between 7 and 8 d (50, 100 and 200 mg mL^{-1}) (Figure 2b). MEPLf extracts (100 and 200 mg mL^{-1}) caused the death of 100% of the workers after eight days, while the other concentrations caused the deaths of all of the workers between the ninth and 10th day (Figure 2c), with 100% of the soldiers dying after the eight day (100 and 200 mg mL^{-1}); exposure to the other concentrations allowed soldiers to survive between nine and 11 d (10, 25, 50 mg mL^{-1}), (Figure 2d). The LC_{50} values of MELLf and MEPLf were 1.279 mg mL^{-1} and 0.255 mg mL^{-1} for workers, and 2.871 mg mL^{-1} and 8.003 mg/mL for soldiers respectively (Table 3).



Fiure 2. Daily survival (%) of workers and soldiers of *Nasutitermes corniger* treated with methanol extracts of *Libidibia ferrea* var. *ferrea* evaluated until death of the last individual by the Log-Rank test: MELLf effects on workers (a) and soldiers (b). MEPLf effects on workers (c) and soldiers (d), at concentrations of 10 (⊟), 25(△), 50 (▽), 100(◊), 200 mg/mL (⊖) and the control (⊣). Each point represents the mean±SE of five repetitions

Figures 3 and 4 illustrate the *in vitro* effects of associations of extracts with fungal strains on mortality of *N. corniger* workers. Associations of the AELLf and AEPLf extracts with *I. farinosa* ESALQ1355 (Figure 3a; Figure 4a) were effective in causing an increase in mortality, causing the death of 70% more workers as compared to the extracts and fungal strains used alone (p = 0.05). Associations of the plant extracts with *I. javanica* URM4993 (Figure 3b; Figure 4b) and *I. fumosorosea* ESALQ1297 (Figure 3c; Figure 4c) did not demonstrate synergistic effects against *N. corniger*, as no significant statistical differences were observed between the percent mortalities of the termites in the fungus+extract associations and their isolated applications (p = 0.05).

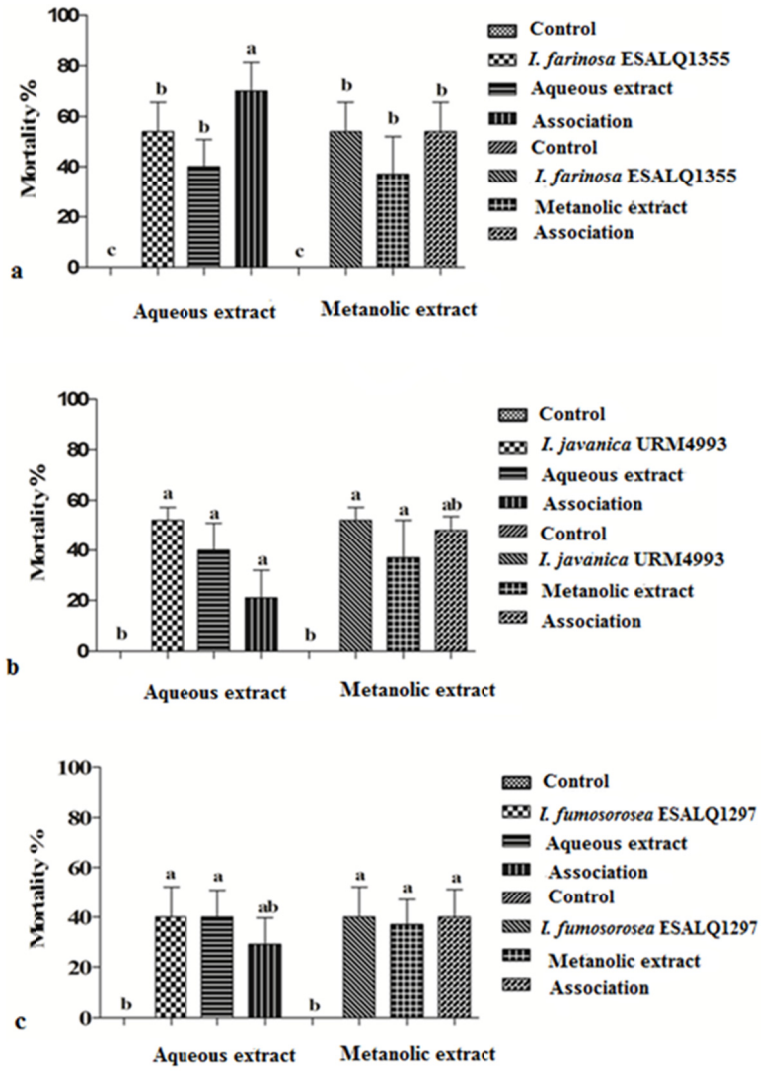


Figure 3. Effects of the associations of fungal strains with aqueous and methanol extracts of the leaves of *Libidibia ferrea* var. *ferrea* against *Nasutitermes corniger* workers: *Isaria farinosa* ESALQ1355 (a), *Isaria javanica* URM4993 (b), and *Isaria fumosorosea* ESALQ1297 (c). Different letters in the bars indicate statistically significant differences

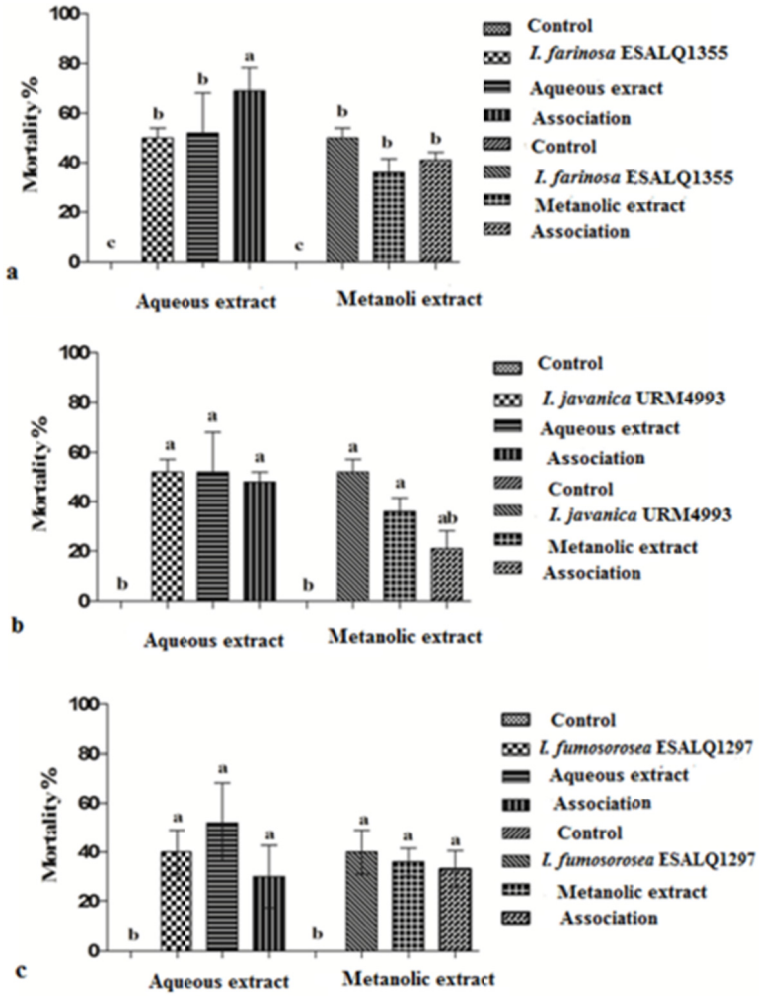


Figure 4. Effects of the associations of fungal strains with aqueous and methanol extracts of the pods of *Libidibia ferrea* var. *ferrea* against *Nasutitermes corniger* workers: *Isaria farinosa* ESALQ1355 (a), *Isaria javanica* URM4993 (b), and *Isaria fumosorosea* ESALQ1297 (c). Different letters in the bars indicate statistically significant differences between them by the Tukey test (p = 0.05)

4. Discussion

The extracts were compatible with the fungi, except in concentrations of 100 and 200 mg mL^{-1}, in general, were classified as moderately toxic to toxic, and caused decrease in growth and fungal sporulation. The effect of biological products on *Isaria* spp. was analyzed. Marques, Monteiro, and Pereira (2004) observed that neem oil reduced mycelial growth and sporulation but did not affect the germination of *I. farinosa.* Matsuura and Matsunaga (2015) reported that the fungicidal pheromones ethylene n-butyl-n-butyrate and 2-methyl-1-butanol, extracted from the termite queens of *Reticulitermes speratus* (Kolbe) (Isoptera: Rhinotermitidae) significantly reduced conidia germination, growth, and sporulation of *I. farinosa.* However, Xu, Ali, and Huang (2011) verified that the secondary metabolic compound 20-Hydroxyecdysone did not have any negative effect on germination, mycelial growth and the production of conidia of *I. fumosorosea*.

Our data demonstrated the insecticidal efficiency of the *L. ferrea* var. *ferrea* extracts, on *N. corniger* workers and soldiers, under laboratory conditions. Plants demonstrating resistance to termite attacks are being considered potential alternative sources of natural insecticides, and their bioactive compounds are less damaging to humans and the natural environment than industrial chemicals (Luna et al., 2005; Omena et al., 2007).

The insecticidal actions of the extracts AELLf and AEPLf on *N. corniger* workers and soldiers were greater than those of the MELLf and MEPLf extracts, because they caused the death of the insect in shorter times of survival and with lower values of LC_{50}, with the extract of AELLf considered the most insecticidal. Similarly, Santana et al. (2010) reported that *N. corniger* survived only to the fourth day when treated with extracts of *Bowdichia*

virgilioides Kunth (Fabales: Fabaceae) at a concentration of 100 mg mL^{-1} (LC_{50} 7.2 mg mL^{-1}), while ethyl acetate extracts of *Anadenanthera colubrina* (Vell.) Brenan (Fabales: Fabaceae) at concentrations of 25 mg mL^{-1}, 50 mg mL^{-1}, and 100 mg mL^{-1} (LC_{50} 17.3 mg mL^{-1}) occasioned the death of 100% of the insects by the seventh day of treatment. Likewise, Soares, Lemos, Cardoso, Medeiros, and Araújo (2008) tested the effects of neem (*Azadirachta indica* A. Juss.) (Sapindales: Meliaceae) and citronella (*Cymbopogon winterianus* Jowitt) (Poales: Poaceae) extracts at concentrations of 1 mg mL^{-1}, 5 mg mL^{-1}, and 10 mg mL^{-1} against *N. corniger* and reported that the neem extract was the most efficient, with higher mortality by the third day of treatment. Araújo et al. (2012) demonstrated that a lectin isolated from *Crataeva tapia* L. (Brassicales: Capparaceae) was effective against *N. corniger*, causing the death of all of those insects after six days (LC_{50} = 0.475 mg mL^{-1}).

Possibly, the insecticidal activity of the tested extracts of *L. ferrea* var. *ferrea* on *N. corniger* may be related to the toxicity of their primary and secondary chemical compounds. Previous studies of the extracts of the leaves, stems, and bark of *L. ferrea* var. *ferrea* reported the presence of flavonoids, saponins, tannins, gallic acid, coumarins, steroids, and phenolic compounds (Wyrepkowski et al., 2014). Bioactive compounds such as alkaloids, tannins, terpenoids, glycosides, phenolics, and phenylpropanoids can have attractive, deterrent, and insecticidal properties (Cheng, Chang, Wu, & Chang, 2007; Melo-Santos, Araújo, Rios, & Regis, 2009). Primary metabolites such as lectins have been tested for controlling *N. corniger* in the laboratory, with the lectins extracted from *Myracrodruon urundeuva* Fr. (Sapindales: Anacardiaceae) causing 100% mortality among workers (LC_{50} = 0.374 mg mL^{-1} and 0.974 mg mL^{-1}) and soldiers (LC_{50} = 0.432 mg mL^{-1} and 0.787 mg mL^{-1}) (Napoleão et al., 2011). Similarly, a lectin extracted from *Bauhinia monandra* Kurz (Fabales: Fabaceae) demonstrated termiticide activity against workers and soldiers of an arboreal termite after the 12th day of exposure (LC_{50} = 0.09 mg mL^{-1} and 0.395 mg mL^{-1} respectively), demonstrating its biotechnological potential for controlling termite-pests (Souza et al., 2011). The greater observed efficiencies of aqueous extracts of *L. ferrea* var. *ferrea* in comparison to methanol extracts in controlling *N. corniger* are related to the different primary and secondary compounds encountered in those extracts, as well as their quantities, although our data indicates that both types of extracts contain toxic or anti-feeding compounds that affect both workers and soldiers.

The most important steps in selecting an entomopathogenic fungus for potential use as a bioinsecticide include determining its virulence, reproductive aspects, and production when grown in artificial medium (Ambethgar, 2009; Lopes, Svedese, Portela, Albuquerque, & Luna-Alves Lima, 2011). The effects of pathogens can often be increased when associated with plant extracts—although it will always be necessary to determine what extract concentrations are compatible with the fungus itself.

The associations of the extracts AELLf and AEPLf with *I. farinosa* ESALQ1355 may have increased the fungus capacity to infect termites, making it more pathogenic, which suggests a synergistic action of the agents in the control of *N. corniger*. Termites live and work in varied environments that could make them susceptible to infections and to the rapid transmission of illnesses—thus favoring pathogen propagation. On the other hand, the initiation and epizootic spread of diseases within the termite nest could be slowed by inherent defense mechanisms (mechanical and/or chemical) such as their excretion of chemicals against entomopathogenic fungi (Chouvenc, Su, & Kenneth, 2011; Hamilton, Lay, & Bulmer, 2011). The use of plant extracts in association with *I. farinosa* ESALQ1355 may have stressed those termites and facilitated the penetration of the fungal conidia into tegument. As such, associations of entomopathogenic fungi with chemical insecticides or plant extracts can amplify their effects against insect pests while diminishing environmental damage (Amjad, Bashir, Afzal, Sabri, & Javed, 2012; Silva, Alves, Luna-Alves Lima, & Lima, 2015). The increased potential for fungal control of *N. corniger* observed when *I. farinosa* ESALQ1355 was associated with aqueous extracts of *L. ferrea* var. *ferrea* therefore corroborates previous reports in the literature of associations of agents in insect control.

Integrated insect control, combining entomopathogenic fungi and chemical control products such as insecticides or plant extracts, takes advantage of their synergetic interactions—so that low doses of those products can be effectively used against insect pests while preserving their natural enemies, decreasing environmental pollution, and decreasing the risk of selecting for resistant insects (Marques, Monteiro, & Pereira, 2004; Ambethgar, 2009). Previous studies report the synergism of the interaction between entomopathogenic fungi and bioactive products in the control of insect pests. In this sense, Xu, Ali, and Huang (2011) demonstrated that the mortality of *Plutella xylostella* L. (Lepidoptera: Plutellidae) caused by the fungus *I. fumosorosea* when associated with different concentrations of 20-hydroxyecdysone was directly related to the concentrations of each component in the solution, and that cumulative effects were observed with greater exposure times. Similarly, Santos, Oliveira, Costa, Tiago, and Oliveira (2015) examined the insecticidal action of extracts (aqueous and hydro-ethanolic) of *R. communis* and *P. pyramidalis* applied together with the fungus *F. incarnatum-equiseti* against *D. opuntiae*, and

found the greatest efficiency to be the association of the fungus with the aqueous extract of *R. communis*—with 100% mortality of *D. opuntiae* females.

5. Conclusion

The termite, *N. corniger,* is a serious pest in urban areas throughout the world, and efficient but environmentally friendly methods of controlling them are desperately needed, while diminishing the use of chemical insecticides. Our results demonstrated the *in vitro* termiticide actions of extracts of *L. ferrea* var. *ferrea* against *N. corniger* soldiers and workers, and the potential efficiency of the association of AELLf or AEPLf with the fungus, *I. farinosa* ESALQ1355, against termite workers. As such, those agents appear to be viable options for continued testing for controlling *N. corniger* in urban areas.

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