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# Botanical insecticide formulation with neem oil and D-limonene for coffee borer control

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**Abstract** – The objective of this work was to evaluate the insecticidal activity of a botanical formulation with neem (*Azadirachta indica*) oil and D-limonene for the control of *Hypothenemus hampei*, as well as to determine the fatty acid composition of neem oil. Ninety-five percent neem oil was extracted from cold-pressed *A. indica* seeds, and D-limonene, from citrus peel. Humic extract (organic carbon), potassium, calcium, magnesium, and sulfur were included as additives. The analysis of neem oil by gas chromatography coupled to mass spectrometry showed that linoleic acid (49.28%) is the main fatty acid in its composition. Field tests were carried out on 'Topázio MG 1190' coffee (*Coffea arabica*) trees, with four applications of the formulated compound every 20 days for a period of 64 days. For the evaluation of insecticidal activity, the botanical formulation was applied to filter paper or topically on the insect's dorsal side, showing a mortality of 63.34 and 100% after 48 hours, respectively. In the field, insecticidal activity caused a 62.4% reduction in the incidence of the *H. hampei* population, when compared with the control. The evaluated botanical formulation is efficient in controlling *H. hampei* in coffee plants.

**Index terms:** *Hypothenemus hampei*, alternative control, coffee crop, control programs, mass spectrometry, pest arthropods.

## Formulação de inseticida botânico com óleo de nim e D-limoneno para controle da broca-do-café

**Resumo** – O objetivo deste trabalho foi avaliar a atividade inseticida de formulação botânica com óleo de nim (*Azadirachta indica*) e D-limoneno no controle de *Hypothenemus hampei*, bem como determinar a composição de ácidos graxos do óleo de nim. O óleo de nim 95% foi extraído de sementes de *A. indica* prensadas a frio, e o D-limoneno, da casca de frutas cítricas. Extrato húmico (carbono orgânico), potássio, cálcio, magnésio e enxofre foram adicionados como aditivos. A análise do óleo de nim por cromatografia gasosa acoplada à espectrometria de massa mostrou que o ácido linoleico (49,28%) é o principal ácido graxo na sua composição. Os testes em campo foram realizados em cafeeiro (*Coffea arabica*) 'Topázio MG 1190', com quatro aplicações do composto formulado, a cada 20 dias, por um período de 64 dias. Para análise da atividade inseticida, a formulação botânica foi aplicada em papel filtro ou topicamente no dorso do inseto, tendo mostrado mortalidade de 63,34 e 100% após 48 horas, respectivamente. Em campo, a atividade inseticida causou redução de 62,4% na incidência da população de *H. hampei*, em comparação ao controle. A formulação botânica avaliada é eficiente no controle de *H. hampei* em cafeeiro.

**Termos para indexação:** *Hypothenemus hampei*, controle alternativo, cafeeiro, programas de controle, espectrometria de massa, artrópodes-praga.



## Introduction

The coffee borer, *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Curculionidae: Scolytinae), is considered one of the most important coffee (*Coffea* spp.) pests, specialized in consuming and reproducing inside fruit seeds (Vega et al., 2015). This causes losses in the weight of beans and/or in the quality of coffee, reflecting negatively on the product's commercial value (Damon, 2000).

The control of the coffee borer still depends heavily on the application of pesticides, which have limitations due to adverse effects on human health and the environment, as well as to insecticide resistance (Nascimento & Melnyk, 2016). Therefore, controlling this insect pest has been a challenge for coffee farmers, increasing the search for alternative control methods, especially of integrated ones.

Natural products, such as essential oils, plant extracts, and fixed oils (fatty acids), are a promising source of natural insecticides due to the complexity and diversity of their chemical composition. For this reason, natural products from plants have been recently explored as an alternative to synthetic insecticides against *H. hampei*. Celestino et al. (2016), for example, studied castor (*Ricinus communis* L.) oil as a botanical insecticide and compared it with a commercial insecticide containing azadirachtin – extracted from neem (*Azadirachta indica* A.Juss.) oil –, observing a lethal concentration (LC<sub>50</sub>) of 3.49 and 6.71% (v/v), respectively, against *H. hampei*. The composition of the used castor oil included: 1.10% palmitic, 4.50% linoleic, 4.02% oleic, 0.50% stearic, and 88.04% ricinoleic fatty acids.

Depieri & Martinez (2010) observed how spraying aqueous solutions of neem oil and aqueous extracts of neem seeds and leaves on coffee fruits reduced the attack by *H. hampei*. The authors concluded that the aqueous seed extract was more promising than neem oil and the leaf extract, but did not evaluate their compositions. Santos et al. (2010) verified the insecticidal potential of the acetonic leaf extract of *Piper hispidum* Kunth (25.0 mg mL<sup>-1</sup>), which caused 65% *H. hampei* mortality, but also did not assess its composition. Zorzetti et al. (2012) found that the ethanolic extract of *Tephrosia purpurea* (L.) Pers. leaves (10%, v/v) and of *Moringa oleifera* Lam. seeds (10%, v/v) caused 96 and 62% mortality of *H. hampei*, respectively.

Although Cloyd et al. (2009) did not specifically study *H. hampei*, they observed that, similarly to neem oil consisting predominantly of azadirachtin, the clarified hydrophobic extract of neem oil containing mostly fatty acids may also show cytotoxic activity against the citrus mealybug, *Planococcus citri* (Risso, 1813).

Another essential oil used as a bioinsecticide is obtained from citrus peel, specifically from ones of its compounds, D-limonene. Campolo et al. (2016), for example, evaluated the larvicidal effect of lemon peel [*Citrus lemon* (L.) Burm.f.] against *Aedes albopictus* (Skuse, 1894). According to Isman (2020), next to clarified neem oil, limonene from orange [*Citrus sinensis* (L.) Osbeck] oil was the most used as a bioinsecticide in the state of California, USA. Moreover, other essential oils with limonene have also shown insecticidal activity, including the essential oil from *Baccharis dracunculifolia* D.C. used against *Rhipicephalus microplus* (Canestrini, 1888) (Lage et al., 2015) and *Cochliomyia macellaria* (Fabricius, 1775) (Chaaban et al., 2018). Therefore, the obtained results are indicative that both neem oil and limonene have potential as botanical insecticides.

The objective of this work was to evaluate the insecticidal activity of a botanical formulation with neem oil and D-limonene for the control of *H. hampei*, as well as to determine the fatty acid composition of neem oil.

## Materials and Methods

Bored coffee fruits, at different ripening stages, were collected from plants of the Mundo Novo IAC 379-19 coffee (*Coffea arabica* L.) cultivar, planted for seven years in the municipality of Patrocínio, in the state of Minas Gerais, Brazil (18°54'41"S, 46°56'56"W).

The individual LC<sub>50</sub> of neem oil, D-limonene, total humic extract (organic carbon), potassium, calcium, magnesium, and sulfur was evaluated at six concentrations (% w/v in water) (Table 1). The 95% neem oil, extracted from cold-pressed *A. indica* seeds containing azadirachtin (A+B), was obtained from the Bioneem company (Araçuaí, MG, Brazil), and D-limonene, extracted from orange peel, from the Cutrale company (Araraquara, SP, Brazil). The other substances were used as additives and were extracted from their respective raw materials (Table 1). The total

humic extract was obtained by the method described by Wershaw et al. (1990), consisting of a sequence of acid-base extractions from a soil sample: after an initial acid extraction using 0.1 mol L<sup>-1</sup> HCl, the formed sediment was separated and redissolved with 0.1 mol L<sup>-1</sup> NaOH, then the formed supernatant was separated and acidified using 6.0 mol L<sup>-1</sup> HCl, and finally the formed sediment was separated and characterized as humic acid. Calcium and magnesium were obtained by hot water extraction (5% w/v) from a fossil lithothamnium sample (calcareous algae), following basic extraction (Na<sub>2</sub>CO<sub>3</sub>, 2% w/v) for 2 hours (Soares et al., 2012). The potassium and sulfur solutions were prepared by solubilization in water of potassium chloride (25%, w/v) and sulfur powder (15%, w/v), respectively. Then, the solutions were left under mechanical stirring at 60°C for 6 hours, being stored in a polyethylene bottle after vacuum filtration.

To assess the lethal concentration of each substance, 600 adults of *H. hampei* were distributed into six groups, receiving different concentrations (% w/v in water), with ten replicates of ten individuals each. Insects were kept in 9 cm Petri dishes, containing filter paper, without food. A volume of 0.15 mL of each substance concentration was sprayed, together, on the insects using the 1.5 L Vonder hand sprayer (Grupo OVD, Curitiba, PR, Brazil). Sterile distilled water was used as a control. The dishes were closed with Parafilm (Sigma Aldrich, St. Louis, MO, USA) and incubated under bio-oxygen demand (BOD), in the dark, at 25±2°C. The number of dead individuals (with complete immobility) was obtained for 48 hours, and the percentage of confirmed mortality caused by each substance was determined.

Based on the obtained results for mortality due to the LC<sub>50</sub>, the efficacy scales of the substances were prepared. The substances that caused the highest

mortality were selected for use in the subsequent formulation of the botanical insecticide. In the calculation of the LC<sub>50</sub> of each substance, the probit regression model was used to evaluate probability and, then, multiple regression with a confidence interval of p≤0.05 was applied in both tests by the Statistica, version 13.3, software (TIBCO Software Inc., Palo Alto, CA, USA).

The botanical insecticide was composed of neem oil, D-limonene, total humic extract (organic carbon), potassium, calcium, magnesium, and sulfur, being prepared with the LC<sub>50</sub> concentrations of each substance against *H. hampei*. After each component of the formulation was added, the mixture was stirred, left to stand, filtered, and stored in polyethylene bottles at room temperature (20 to 27°C) for a maximum period of 30 days.

The botanical insecticide was evaluated in two different assays. In the first, six different concentrations of the formulation in water (0.50, 0.62, 0.75, 0.87, 1.0, and 1.12%, w/v) were sprayed on a filter paper inside 9 cm Petri dishes, where ten adult insects were placed and kept without food. Four replicates for each treatment were used.

In the second assay, four different concentrations of the botanical insecticide in water (0.62, 0.75, 0.87, and 1%, w/v) were applied topically on the dorsal side of ten insect adults, with five replicates per treatment. Sterile distilled water was used as a control. In both assays, 0.15 mL of the formulation were applied using a 1.5 L hand sprayer.

All Petri dishes were closed with Parafilm (Sigma Aldrich, St. Louis, MO, USA) and incubated under BOD, in the dark, at 25±2°C. The number of dead individuals in the same experiment was determined in two intervals of 24 hours (24 and 48 hours) after spraying. Mortality (%) values were corrected with the

**Table 1.** Class, origin, and concentration of the substances used to determine the lethal concentration of the evaluated botanical insecticide against the coffee borer, *Hypothenemus hampei*.

Substance	Class	Origin	Concentrations (% w/v)
Neem oil	Botanical	<i>Azadirachta indica</i>	0, 10, 12.9, 15, 20, 25
D-limonene	Botanical	<i>Citrus</i>	0, 5, 6.9, 10, 15, 20
Total humic extract (organic carbon)	Biosorbent	Algae/Peat	0, 5, 8.5, 10, 15, 20
Potassium	Nutrient	Fertilizer	0, 25, 30, 37.5, 40, 45
Calcium and Magnesium	Nutrient	Algae/ <i>Lithothamnium</i>	0, 10, 14, 16.7, 18, 20
Sulfur	Nutrient	Fertilizer	0, 4, 6.25, 10, 15, 20

formula presented in Abbott (1925). The experiments were performed in a completely randomized design. The mortality data for each concentration were subjected to the analysis of variance by the F-test. Means were compared by Tukey's test ( $p < 0.05$ ) after observing the assumptions of normality, linearity by Shapiro-Wilk's test, and error independence conferred by causality and homoscedasticity by Levene's test, using the R, version 3.6.3, software (R Core Team, 2020), at a confidence interval of  $p \leq 0.05$ .

The used 95% commercial neem oil was analyzed by gas chromatography coupled to mass spectrometry, according to the methodology of Reifenhath et al. (2012) and Adams (2007). The material (0.02 g) was dissolved in methanol 37% HCl-CHCl<sub>3</sub> (1:8:40 mL) (Vetec, Sigma-Aldrich Brasil Ltda., São Paulo, SP, Brazil) for 1 hour. The solvent was removed under nitrogen flow and resolved in 150  $\mu$ L CHCl<sub>3</sub> and 40  $\mu$ L N,O-bis(trimethylsilyl)trifluoroacetamide (Sigma-Aldrich, St. Louis, MO, USA). The extracts were analyzed by the GCMS-QP2010 gas chromatography mass spectrometer (Shimadzu Corporation, Kyoto, Japan), using a J&W DB-5 capillary column, with 30 m x 0.25 mm internal diameter and 0.25  $\mu$ m (Agilent, Santa Clara, CA, USA), with helium as a carrier gas, at a flow rate of 1.0 mL min<sup>-1</sup>. Detector and injector temperatures were 220 and 240°C, respectively. The injection volume was 1.0  $\mu$ L, and the division ratio was 1:20. The oven temperature was set at 60 to 240°C, at 3°C min<sup>-1</sup>. The electron impact energy was 70V, and the fragments were collected from 40 to 650 *m/z*. Fatty acids were identified by comparing their MS spectra to those in the Wiley registry of mass spectral data (McLafferty & Stauffer, 1994) and/or in the NIST/EPA/NIH Mass Spectral Library webbook (Wallace, 2019).

The same botanical formulation used in the previous analyses was evaluated against *H. hampei* adult insects under field conditions. However, the following synthetic insecticides were used as a control and applied in a mixture: 1.0 L Voliam Targo (18 g L<sup>-1</sup> abamectin + 45 g L<sup>-1</sup> chlorantraniliprole) (Syngenta Proteção de Cultivos Ltda., Paulínia, SP, Brazil); and 1.0 L Lorsban 480 BR (480 g L<sup>-1</sup> chlorpyrifos) (Dow AgroSciences Industrial Ltda., Barueri, SP, Brazil). These products were chosen according to the standard methodology used for coffee borer control on the property where they were tested.

The tests were carried in the municipality of Monte Carmelo, in the state of Minas Gerais, Brazil (18°72'05"S, 47°53'58"W) on 13-year-old 'Topázio MG 1190' coffee (*C. arabica*) trees, in a total area of 3.0 ha. Four applications of the botanical formulation (0.75% w/v) were performed every 20 days. A strip-plot design was used, with three treatments (botanical insecticide, synthetic insecticides, and water as control) and ten replicates each, totaling 30 plots of 50 m each. The mathematical model used for the application of the analysis of variance was the same as that adopted in the completely randomized design:  $y_{ij} = m + t_i + e_{ij}$  in which  $y_{ij}$  is the observed value for the characteristic analyzed (treatment  $i$  and repetition  $j$ ),  $m$  in the overall mean,  $t_i$  is the effect of treatment  $i$ , and  $e_{ij}$  is the random experimental error common to all observations; where all tests of assumptions for normality and homoscedasticity by Levene's test were performed and accepted.

The field experiment started in the second phenological year, in the third stage, 57 days after coffee flowering, specifically at fruit expansion, with sampling when the natural occurrence of the insect was above 3% (Souza et al., 2016). The initial determination of pest status was performed in December 2017, by analyzing the dried fruits of the 2016–2017 crop, collected from 20 plants from the center of each plot, with ten plants in each direction.

Spraying started in December 2017, totaling four applications every 20 days, which ended in the fourth phenological phase, at the end of fruit granulation, in February 2018, totaling 64 days. For the applications, the Arbus 2000 TF jet sprayer (Jacto, Pompéia, SP, Brazil) was used, with a Jacto JA-2 nozzle, an empty cone, application angle of 80°, and spray flow rate of 400 L ha<sup>-1</sup>.

The incidence of insects was measured every 15 days. The first evaluation was carried out before the application of the formulation, with fruit expansion in December 2017, and the last one, at the end of fruit granulation in March 2018, totaling five evaluations throughout 92 days. Grain samples were collected from plagiotropic branches in the middle third of 20 plants from the center of each plot, with ten plants in each direction. An average of 500 random fruits were collected in each plot, and the number of bored grains per sample was determined to calculate the percentage of pest insect attack and infestation.

The obtained data were subjected to the analysis of variance by Tukey's test, observing the assumptions of chance and independence due to the arrangement of the plots, normality by Shapiro-Wilk's test, and homoscedasticity by Levene's test, in order to assess insect medium attack and infestation. Statistical analyzes were performed using the R, version 3.6.3, software (R Core Team, 2020), with a confidence interval of 1%.

## Results and discussion

Among the components of the formulation, neem oil presented the lowest  $LC_{50}$  (Table 2). However, the obtained value is very close to that found for neem oil against *H. hampei* by Martinez (2011), confirming its insecticidal activity.

Calcium and magnesium presented the second best  $LC_{50}$  result. This is the first known time that the insecticidal activity of this compound on *H. hampei* was evaluated, showing a promising potential. Therefore, calcium homeostasis and signaling are attractive targets for future evaluations of an insecticide (Lümmen, 2013).

Although D-limonene, a monoterpene and the main compound present in essential oils from citrus fruits, also has insecticidal properties (Feng et al., 2020), its use against *H. hampei* had not yet been investigated. In the present study, no expressive results were obtained, differently from other researches that described both irritant and repellent properties of essential oils on insects. Plant extracts of *Anethum graveolens* L. seeds with 30% limonene, for example, showed an irritant and toxic effect against *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) (Emilie et al., 2015).

The  $LC_{50}$  of the evaluated additives (potassium, sulfur, and total humic extract) indicated low insect lethality. However, the use of these additives as a potentiating agent favors the action of botanical substances. Adjalle et al. (2009) observed that the inclusion of additives in the applied formulations resulted in an increased photostabilization and dissipation of half-life, improving compound homogeneity. Novais et al. (2007) found that humic substances with reducing properties form stable complexes with iron, copper, calcium, and magnesium, highlighting both their use as an additive and their stabilizing action in the formulation.

A significant variation in mortality was observed for the concentrations of 0.75, 0.87, 1.0, and 1.12% of the botanical insecticide in relation to the control treatment after the first 24 hours of spraying (Table 3). The highest mortality values 48 hours after spraying and in the total evaluation time were obtained at the concentrations of 0.75 and 1.12%. Similar results were reported by Santos et al. (2013a), who observed *H. hampei* mortality by contact using essential oil extracted from *Schinus terebinthifolius* Raddi (Anacardiaceae).

There was an increase in *H. hampei* mortality at all concentrations of the botanical insecticide applied on the dorsal side of the insect after 24 hours (Table 4). Mortality values ranged from 10.53 to 28.95% after 48 hours of spraying. Total evaluation time showed that insect mortality reached its limit (100%), highlighting the effectiveness and lethality of the dorsal topical application of the botanical insecticide.

Some studies have also shown satisfactory results in the effectiveness of neem oil for coffee borer control. Celestino et al. (2016) obtained 40.8% mortality of coffee borer females by spraying an insecticide with 0.3% azadirachtin at a concentration of 3.0% (v/v).

**Table 2.** Lethal concentration ( $LC_{50}$ ) of the substances present in the evaluated botanical insecticide against the coffee borer, *Hypothenemus hampei*.

Substance	$LC_{50}$ ( $\mu\text{g mL}^{-1}$ )	-95.0% $LC_{50}$	+95.0% $LC_{50}$	$R^2$	p-value	Predicted ( $r^2$ )
Neem oil	6,632.0	2,953.9	10,310.1	0.62	0.002	0.79
D-limonene	51,210.0	8,658.1	93,761.8	0.42	0.023	0.65
Total humic extract (organic carbon)	23,400.0	5,278.8	41,521.2	0.45	0.016	0.67
Potassium	74,550.0	48,666.5	100,433.5	0.80	0.000	0.90
Calcium and magnesium	6,837.1	2,671.9	10,181.8	0.62	0.002	0.78
Sulfur	19,687.5	2,803.2	36,571.7	0.40	0.026	0.64

However, a higher mortality rate was observed in the present study at a lower concentration of 0.75% (w/v), which suggests that the used botanical formulation had an improved insecticidal activity.

A higher mortality was observed in the initial 24 hours after the botanical insecticide was applied (Tables 3 and 4). After this period, there was a decrease in insect mortality, which may be related to the high degradation of the essential compounds, whose volatility causes a reduction in botanical actives. Santos et al. (2013b) reported a 55% mortality of *H. hampei* in the first 24 hours after the topical application of the lowest dilution (0.5 mg mL<sup>-1</sup>) of a *Piper alatabaccum* Trel. & Yunck. extract.

The insecticidal action potential of the botanical formulation against *H. hampei* was fast, i.e., its action in 24 hours was better than in 48 hours. This is a characteristic considered desirable for an insecticide. The speed with which the formulation causes mortality can be a great advantage since, under field conditions, the insect's contact with the product can be relatively quick (Santos et al., 2013a).

The analysis of neem oil composition showed the following fatty acids: tetradecanoic acid or myristic acid, 9,12-octadecadienoic acid or linoleic

**Table 3.** Effect of the concentration (%) of the botanical insecticide applied to filter paper surface against the coffee borer, *Hypothenemus hampei*, in 24 hours, 48 hours, and total hours evaluated<sup>(1)</sup>.

Concentration of the formulation (% w/v)	Mortality (%)		
	24 hours	48 hours	Total
1.12	52.94aA	10.39aB	63.34aA
1.00	32.36abA	7.64abB	40.00abA
0.87	38.24abA	5.10abB	43.33abA
0.75	41.18abA	12.16aB	53.33aA
0.62	2.94cA	3.73bcA	6.67cA
0.50	17.65bcA	0.00cB	17.65bcA
Control	0.00c	0.00c	0.00c
p-value	0.00	0.001	0.00
SW	0.20	0.05**	0.06
L	0.10	0.51	0.22
CV (%)	12.78	22.40	29.43
DF	27		

<sup>(1)</sup>Means followed by equal letters, lowercase in the columns and uppercase in the rows, do not differ significantly by Tukey's test, at 5% probability. Control, water; p-value, probability of concentrations in the analysis of variance; SW, probability by Shapiro-Wilk's test; L, probability by Levene's test; CV, coefficient of variation; and DF, degree of freedom. \*\*Significant at 1% probability.

acid, 11-octadecenoic acid or vaccenic acid, and heptacosanoic acid or heptacosylic acid (Table 5). In their assays, Pinto & Lanças (2010) analyzed unrefined neem oil, purchased locally and without prior treatment, using high-resolution gas chromatography coupled to mass spectrometry, and identified the presence of linoleic acid (14.08%) in its composition. However, in the present study, the neem oil used in the botanical insecticide formulation had higher acid percentages.

To evaluate insecticidal activity in the field, the concentration of 0.75% of the botanical formulation was used, based on the data on its insecticidal activity

**Table 4.** Effect of the concentration (%) of the botanical insecticide applied on the dorsal side of adults of the coffee borer, *Hypothenemus hampei*, in 24 hours, 48 hours, and total hours evaluated<sup>(1)</sup>.

Concentration of the formulation (% w/v)	Mortality (%)		
	24 hours	48 hours	Total
1.00	76.32aB	23.68abC	100.00aA
0.87	71.05aA	28.95aB	100.00aA
0.75	89.47aA	10.53bB	100.00aA
0.62	78.95aA	21.05abB	100.00aA
Control	0.00bA	0.00cA	0.00bA
p-value	0.00	0.00	0.004
SW	0.22	0.29	0.12
L	0.70	0.36	0.41
CV (%)	17.14	38.28	5.52
DF	24		

<sup>(1)</sup>Means followed by equal letters, lowercase in the columns and uppercase in the rows, do not differ significantly by Tukey's test, at 5% probability. Control, water; p-value, probability of treatment in the analysis of variance; SW, probability by Shapiro-Wilk's test; L, probability of Levene's test; CV, coefficient of variation; and DF, degree of freedom. Significant at 5% by the analysis of variance.

**Table 5.** Fatty acid composition of neem (*Azadirachta indica*) oil.

Retention time (min) <sup>(1)</sup>	Fatty acid		Composition (%)
43.04	Tetradecanoic acid or myristic acid	14:0	10.32
48.38	9,12-octadecadienoic acid or linoleic acid	18:2	49.28
48.55	11-octadecenoic acid or vaccenic acid	18:1	35.05
49.28	Heptacosanoic acid or heptacosylic acid	27:0	5.35

<sup>(1)</sup>Time in minutes that the compounds spend in a chromatography column.

on filter paper surface and when directly applied on the dorsal side of *H. hampei*. Areas treated with the botanical insecticide presented lower insect incidence than those with the control treatment, indicating a significant difference between them (Table 6). However, when comparing the mean infestation of insects in areas treated with the botanical insecticide with that of areas treated with the chemical insecticide, no significant differences were observed. Therefore, the obtained data suggests that the botanical insecticide is able to control *H. hampei*, compared with the traditionally used pesticides, with proximity between the treatments.

Depieri & Martinez (2010) concluded that spraying neem oil on *H. hampei* insects can lead to death by ingestion associated to repellency caused by contact, resulting in a low incidence of bored fruits. The authors reported an average mortality of 58%, close to that found in the present study, which is indicative that the reduction in insect incidence observed here is probably due to the botanical formulation. However, future studies are needed to confirm insect mortality within the fruit.

Therefore, the data of the present study showed the applicability of the evaluated botanical insecticide in controlling *H. hampei*, at a concentration of 0.75%, sprayed at 3.0 L ha<sup>-1</sup> with a 400 L ha<sup>-1</sup> nozzle volume.

**Table 6.** Mean effect of treatments on the infestation ( $\pm$  standard error) of adults of the coffee borer, *Hypothenemus hampei*, in a field assay<sup>(1)</sup>.

Treatment	Insect incidence (%)	Insect incidence (%) in relation to the control
Synthetic insecticide <sup>(2)</sup>	7.4 $\pm$ 1.47a	68.35
Botanical insecticide (0.75% w/v)	8.8 $\pm$ 2.45a	62.40
Control	23.4 $\pm$ 1.75b	0.00
p-value <sup>(3)</sup>		0.00
SW		0.97
L		0.40
CV (%)		37.47
DF		29

<sup>(1)</sup>Means followed by equal letters, in the columns, do not differ significantly by Tukey's test, at 1% probability. <sup>(2)</sup>Synthetic insecticide: 18 g L<sup>-1</sup> abamectin, 45 g L<sup>-1</sup> chlorantraniliprole, and 480 g L<sup>-1</sup> chlorpyrifos. <sup>(3)</sup>p-value, probability of treatment in the analysis of variance. p-value = 3.13x10<sup>-06</sup>. Control, water; SW, probability by Shapiro-Wilk's test; L, probability by Levene's test; CV, coefficient of variation; and DF, degree of freedom. Significant at 5% by the analysis of variance.

## Conclusions

1. The botanical formulation composed of neem oil (*Azadirachta indica*), D-limonene, total humic extract (organic carbon), potassium, calcium, magnesium, and sulfur is efficient in the control of the coffee borer, *Hypothenemus hampei*.

2. In the field, the concentration of 0.75% of the botanical formulation shows the best insecticidal activity by contact and dorsal application.

3. The used neem oil has a high concentration of 49.28% linoleic acid.

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