



RESEARCH ARTICLE - ANTS

Toxicity of plant extracts from Bahia, Brazil, to *Atta sexdens sexdens* (Hymenoptera: Formicidae) workers

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Abstract

Ants of the *Atta* and *Acromyrmex* genera (Formicidae: Myrmicinae: Attini) are the true leaf cutting-ants with species of economic importance in America Neotropical and mainly controlled by toxic baits. There are few active ingredients for use in baits, being necessary studies to indicate molecules with insecticide potential. The aim of this study was to evaluate the toxicity of *Aspidosperma spruceanum* Benth ex. Mull Arg. (leaf and bark), *Casearia arborea* (Rich.) Urb. (leaf and branch), *Casearia sylvestris* Sw. (leaf and bark), *Erythroxylum affine* A.St.-Hil. (leaf and branch), *Esenbeckia grandiflora* Mart. (leaf and bark), *Ocotea brasiliensis* Coe-Teix (bark and branch), *Simarouba amara* Aubl. (bark), *Tabernaemontana bracteolaris* Mart. ex Müll.Arg. (leaf, bark and branch) and *Zanthoxylum rhoifolium* Lam. (leaf and branch) extracts to workers of *Atta sexdens sexdens* L. (Hymenoptera Formicidae). The contact and ingestion toxicity of all extracts to this ant by was evaluated by topical application and addition in their diet, respectively. Data of contact application were submitted to analysis of variance and Tukey test while those from ingestion were compared by survival curves using the statistical test "log rank". Through contact, the leaf and branch extracts of *Z. rhoifolium* and of that of bark of *S. amara* were the most toxic ones. Through ingestion, four extracts were toxic and showed delayed action. The extract of *Z. rhoifolium* branches presented the slowest action ($S_{50} = 10$ days). This characteristic is crucial for toxic baits. The *Z. rhoifolium* leaf and branch extracts were the only ones with contact and ingestion toxicity to *A. sexdens sexdens* workers.

Introduction

Ants of the *Atta* and *Acromyrmex* genera (Formicidae: Myrmicinae: Attini) are the true leaf cutting-ants with their species utilizing fresh plant parts, mainly leaves, to cultivating fungus of the *Leucoagaricus* and *Leucocoprinus* genera (Agaricaceae: Leucocoprinae) (Della Lucia et al., 2014). The leaf cutting-ants are one of the most significant pests in Neotropical America damaging almost all cultivated plants and causing losses to agriculture, forest and pasture plants (Zanetti et al., 2000a; Zanuncio et al., 2002). Leaf cutting-ants use green leaves what justifies their importance by reducing survival, growth and reproduction of plant of economic value (Zanetti et al., 2000b; Zanetti et al., 2014).

Leaf cutting-ants are the main pests in cultivated forests of the *Pinus* and *Eucalyptus* genera (Zanetti et al., 2014). Damages by leaf cutting-ants is common in forest commercial cultivation in 2005 in 3.4 million of hectares planted with eucalyptus, 1.8 million with *Pinus* and 326 thousand with other plant species (Pereira & Santos, 2008).

The leaf cutting-ants can be controlled with different methods, but toxic baits are the most practical and efficient one (Laranjeiro et al., 1995; Zanuncio et al., 2000). On the other hand, thermal fogging with plant extracts could be an alternative to these baits, but the costs of equipments with this method is much higher compared to toxic bait application (Zanetti et al., 2014).

The active ingredient must be attractive to ants even distant from the nest to assure the efficiency of toxic baits.



Initial rejection of these baits cannot occur and its toxic compound should have delayed action at the right time to completely contaminated the ant colony besides having low toxicity to non-target organisms (Nagamoto et al., 2004; Verza et al., 2006).

The sulfuramids (*N*-ethyl perfluorooctane sulfonamide) and fipronil are the most used active ingredients in ant baits (Zanuncio et al., 2003; Della Lucia et al., 2014). The sulfuramid, categorized in annex B of the Stockholm Convention on Persistent Organic Pollutants (POPs) as an organic pollutant (United Nations Treaty Collection, 2009), is the most used active ingredient in baits to control leaf cutting-ants, what makes urgent searching for active ingredients to replace it.

The leaf-cutting ant [*Atta sexdens* Forel] (Hymenoptera: Formicidae) has attracted the attention of researchers (Zanetti et al., 2003) and studies have been developed to searching for plants with toxic substances to this pest (Peñaflor et al., 2009; Gouvêa et al., 2010). The toxicity is due to secondary metabolites in plant that can be toxic to ants, to its fungus (Morais et al., 2015) or to both.

Oil of *Ricinus communis* L. and *Jatropha curcas* L. seeds with different concentrations were toxic, through ingestion and topical application to *A. sexdens*, in laboratory tests, thus, suggesting the toxicity of these oils to this ant (Alonso & Santos, 2013).

The search for alternatives that are effective to control leaf cutting-ants is important. The objective of this study was to evaluate the toxicity of extracts of several plant species collected in the Bahia State, Brazil, to *A. sexdens sexdens* workers, through topical application and ingestion, in laboratory conditions.

Material and Methods

Plant species were collected in June 28th of 2012, in a forest fragment in Brejo Novo Farm (13°56'41"S and 40°06'33.9"W) between 617 m and 755 m of altitude at 9 km from Jequié, Bahia State, Brazil. The plant material was dried in Tecnal drying oven (TE 394/2 Model) at 40 °C for 48 h and submitted to cold maceration with methanol. Extracts from different plant parts of the following species represented by their respective abbreviations were prepared: leaf (EFAS) and branch (EGAS) of *Aspidosperma spruceanum* Benth. ex Müll. Arg.; leaf (EFCA) and branch (EGCA) of *Casearia arborea* (Rich.) Urb.; leaf (EFCS) and bark (ECCS) of *Casearia sylvestris* Sw.; leaf (EFEA) and branch (EGEA) of *Erythroxylum affine* A. St.-Hil.; leaf (EFEG) and bark (ECEG) of *Esenbeckia grandiflora* Mart.; bark (ECOB) and branch (EGOB) of *Ocotea brasiliensis* Coe-Teix; bark (ECSA) of *Simarouba amara* Aubl.; leaf (EFTB), bark (ECTB) and branch (EGTB) of *Tabernaemontana bracteolaris* Mart. Ex Müll. Arg.; and leaf (EFZR), branch (EGZR) and root (ERZR) of *Zanthoxylum rhoifolium* Lam. The extract solution was filtered and concentrated under vacuum, in rotary evaporator (Fisatom, 801 Model), at 50 °C. The masses of dry plant materials and their respective extracts and their yields were obtained (Table 1).

The biological tests were conducted using *A. sexdens sexdens* workers collected from several nests in the Myrmecology Laboratory of the Southwestern Bahia State University. Foraging workers of the similar size and width of head capsule between 1.7 and 3.0 mm were selected.

Table 1. Family, species, plant parts (Plant), abbreviation (Abbr.), mass of dry material (Mat.), mass of extract (Ext.) and yield of plant species and extracts used in bioassays with *Atta sexdens sexdens* (Hymenoptera: Formicidae) workers.

Family	Species	Plant	Abbr.	Mat. (g)	Ext. (g)	Yield (%)
Salicaceae	<i>Casearia arborea</i>	Leaf	EFCA	91.45	29.91	33
		Branch	EGCA	135.79	11.58	9
	<i>Casearia sylvestris</i>	Leaf	EFCS	91.08	34.77	38
		Bark	ECCS	147.4	19.37	13
Erythroxylaceae	<i>Erythroxylum affine</i>	Leaf	EFEA	56.50	13.23	23
		Branch	EGOB	704.75	30.41	4
Lauraceae	<i>Ocotea brasiliensis</i>	Bark	ECOB	388.48	55.14	14
		Leaf	EFTB	81.38	26.54	33
		Bark	ECTB	281.07	32.99	12
		Leaf	EFAS	54.76	19.16	35
Apocynaceae	<i>Aspidosperma spruceanum</i>	Branch	EGAS	152.57	8.35	5
		Bark	ECSA	362.27	13.07	4
Simaroubaceae	<i>Simarouba amara</i>	Leaf	EFEG	79.57	23.45	29
		Bark	ECEG	138.78	18.98	14
	<i>Esenbeckia grandiflora</i>	Leaf	EFZR	117.04	22.16	19
		Branch	EGZR	179.78	19.75	11
Rutaceae	<i>Zanthoxylum rhoifolium</i>	Root	ERZR	122.23	14.58	12

The contact toxicity test (Araújo et al., 2008) was conducted in a completely randomized design with 21 treatments (19 extracts and two controls) with three replications and each parcel having 10 ants. The extracts (Table 1) were diluted in ethanol at the concentration of 1.0 mg.mL⁻¹. Each leaf cutting-ant worker was treated topically with 1.0 µL of this solution on its pronotum using a dosing micro syringe of 10 µL (Hamilton, 701N Model). Two controls were prepared: one without application and another with the application of 1.0 µL of solvent (ethanol). The ants were conditioned in Petri dishes per treatment with a cotton ball soaked in distilled water. The ant mortality was evaluated after 24, 48 and 72 of treatment application.

In the ingestion toxicity test the ants were fed on a solid diet composed of (g.L⁻¹): glucose (50), bacteriological peptone (10), yeast extract (1.0) and agar (10) dissolved in distilled water and autoclaved at 120 °C for 15 minutes (Bueno et al., 1997). The experimental diets, except the controls, were obtained by adding the extracts EFEG, ECEG, EFEA, EFZR, EGZR, EFCA, ECCS, ECSA, EFAS (concentration of 0.2 mg.mL⁻¹) to the diet still hot, immediately after its removal from autoclave. The liquid was poured in Petri dishes previously identified and kept under refrigeration. The ant mortality was evaluated in a daily basis during 25 days. The Petri dishes were covered with paper filter, previously damped

with distilled water. The diet was replaced daily when 0.4 g of the artificial diet was put per Petri dish. The experimental design was completely randomized with 10 treatments, nine with the extracts and one control (diet without extract) with five replications using 10 ant workers each one. In both tests, the Petri dishes (90 mm diameter) were kept at a temperature of 25 ± 1 °C and relative humidity from 70 to 80%.

The data from the topical application test were submitted to analysis of variance and the averages compared by the Tukey test (p < 0.05) using the SAS Institute program (2002). The data from ingestion tests were graphically analyzed comparing the survival curves of each treatment with that of the control by the statistical test “log-rank” using the PRISMA 6.0 program (GraphPad Software). For each treatment, the average survival period of ants (S₅₀) was determined considering the day that 50% of them were still alive.

Results and Discussion

The topical application of the extracts EFZR, EGZR, ECSA, EGAS and EFCA caused higher mortality of ants than the controls. Ants treated with ECSA, EFCA, EGZR, EFZR and EGAS presented cumulative mortality between 33% and 37% (Table 2). This may be due to secondary metabolites toxic to

Table 2. Mortality (%) of *Atta sexdens sexdens* (Hymenoptera: Formicidae) workers (mean and standard deviation) treated with plant extracts at concentration of 1 mg.mL⁻¹ through topical application.

Treatment	Mortality (%)				
	1° day	2° day	2° day (cumulative mortality)	3° day	3° day (cumulative mortality)
Control with solvent	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00a
Control without solvent	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00a
EFTB	0.00±0.00	6.67±9.43	6.67±9.43	15.00±10.80	21.67±16.41a
EFEG	3.33±4.71	0.00±0.00	3.33±4.71	3.33±4.71	6.67±4.71 a
EGTB	0.00±0.00	3.33±4.71	3.33±4.71	3.33±4.71	6.67±4.71a
EGZR	16.67±9.73	24.34±9.55	41.01±15.38	0.00±0.00	41.01±13.21 b
ERZR	0.00±0.00	6.67±4.71	6.67±4.71	0.00±0.00	6.67±4.71 a
ECEG	6.67±9.43	4.17±5.89	10.83±15.32	3.33±4.71	14.17±12.47 a
ECCS	0.00±0.00	0.00±0.00	0.00±0.00	6.67±4.71	6.67±4.71 a
EFCA	10.00±8.16	8.33±11.79	18.33±19.29	19.26±7.93	37.59±13.18 b
ECSA	6.67±4.71	14.44±5.52	21.11±9.07	16.93±12.25	38.04±13.33 b
EFZR	6.67±9.43	21.67±8.50	28.33±14.34	11.11±15.71	39.44±5.47 b
EGCA	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00 a
EFCS	0.00±0.00	0.00±0.00	0.00±0.00	3.33±4.71	3.00±4.71 a
ECTB	0.00±0.00	0.00±0.00	0.00±0.00	3.33±4.71	3.00±4.71 a
ECOB	0.00±0.00	3.33±4.71	3.33±4.71	7.04±5.00	10.37±8.71 a
EGOB	0.00±0.00	0.00±0.00	0.00±0.00	6.67±4.71	6.67±4.71 a
EGEA	0.00±0.00	0.00±0.00	0.00±0.00	6.67±4.71	6.67±4.71 a
EFEA	6.67±4.71	3.70±5.24	10.37±8.62	0.00±0.00	10.74±8.17 a
EFAS	3.33±4.71	7.41±10.48	10.74±15.19	0.00±0.00	10.74±14.16 a
EGAS	3.33±4.71	14.44±13.97	17.78±18.53	21.48±18.17	39.26±12.74 b

Different letters after the mortality value on the 3rd day, show significant difference in relation to the control (Tukey at 5%).

A. sexdens sexdens and that these plants need further studies in field with thermal fogging. Besides, secondary metabolites are usually found at low concentrations in extracts and their isolation is necessary to prove their respective activities. The quassinoids, the most active substances used in traditional medicine, is the main chemical components of *S. amara* and present inhibitory effect (Fiaschetti et al., 2011). *Simarouba versicolor* was toxic to cutting-ants at concentrations of 2.0, 1.6 and 0.3 mg.mL⁻¹ (Peñaflor et al., 2009). Species of *Casearia* genus present therapeutic properties and active substances as diterpenes, called casearins (Bento et al., 2013). *Aspidosperma* has species, including *A. spruceanum*, important sources of indole alkaloids with therapeutic properties (Oliveira et al., 2009). Insecticidal activity for *C. arborea*, *S. amara* and *A. spruceanum* has not been found, but plant extracts may be active against leaf cutting-ants. For instance, *Ruta graveolens*

L. and *Ageratum conyzoides* L. extracts employed in traditional medicine, induced mortality of *A. sexdens* workers through topical application, at concentration of 1 mg.mL⁻¹ (Araújo et al., 2008).

In tests through ingestion, the extracts of leaves and branches of *Z. rhoifolium* (EFZR and EGZR), leaves of *E. grandiflora* (EFEG) and of bark of *C. sylvestris* (ECCS) caused higher mortality of ants than the control (Figure 1). Results for the EFCA, ECEG, EFAS, ECSA and EFEA extracts were similar to those of the controls. The average survival period (S_{50}) of *A. sexdens sexdens* workers per treatment varied from 8 to 15 days (Table 3).

The average survival period (S_{50}) of ants in the EFEG, ECCS, EFZR and EGZR treatments was seven, eight, eight and 10 days, respectively, evidencing their mortality after ingesting them. The values of S_{50} showed that these extracts had delayed action and therefore, they may be considered for future use in the management of cutting-ants. The delayed toxic action occurs when the mortality of ant workers is lower or equal to 15% up to the first day of evaluation, and higher or equal to 90% after the twentieth first day of evaluation (Nagamoto et al., 2004). The accumulated mortality showed that extracts with difference from control had this characteristic.

Further tests need to be develop to implement these extracts in field. An extract, to be used in field in toxic bait, must present action preferably through ingestion, delayed toxic action, lethality at low concentrations, environmental safe odorless and non repellent (Boaretto & Forti, 1997).

In ingestion tests, the toxicity of plants studied to leaf cutting-ants are not found in literature, but other plants such as *Ricinus communis* L. (Bigi et al., 2004), *Sesamum indicum* L. (Morini et al., 2005), *Cedrela fissilis* Vell (Bueno et al., 2005), *Helietta puberalla* RE Fr. (Almeida et al., 2007), *Simarouba versicolor* St. Hil. (Peñaflor et al., 2009), *Jatropha curcas* L. and *Ricinus communis* L. (Alonso & Santos, 2013)

Table 3. Mortality (%) and average survival period (S_{50}) in days of *Atta sexdens sexdens* (Hymenoptera: Formicidae) workers fed on diet containing several plant extracts at the concentration of 0.2 mg.mL⁻¹

Treatment	Accumulated Mortality (%)/day										S_{50}
	1°	2°	3°	6°	8°	10°	14°	17°	21°	25°	
Control	2	4	8	14	16	23	42	56	75	87	15 a
EFZR	2	2	4	30	56	72	86	92	94	94	8 b
EFEG	4	6	22	51	57	71	87	89	94	96	7 b
EFCA	2	4	12	24	30	38	68	78	85	85	12 a
ECCS	4	10	28	48	60	86	92	92	94	98	8 b
ECEG	2	2	8	23	31	42	66	83	85	87	11 a
EFAS	6	6	8	14	22	51	69	78	82	90	11 a
ECSA	0	2	4	14	22	32	50	62	84	94	15 a
EFEA	10	12	12	16	22	52	80	90	92	92	11 a
EGZR	6	16	26	32	34	58	80	86	98	98	10 b

S_{50} = average survival. Letters after the value of S_{50} showed significant differences according to the “log-rank test” (b = 0.01 < p < 0.05).

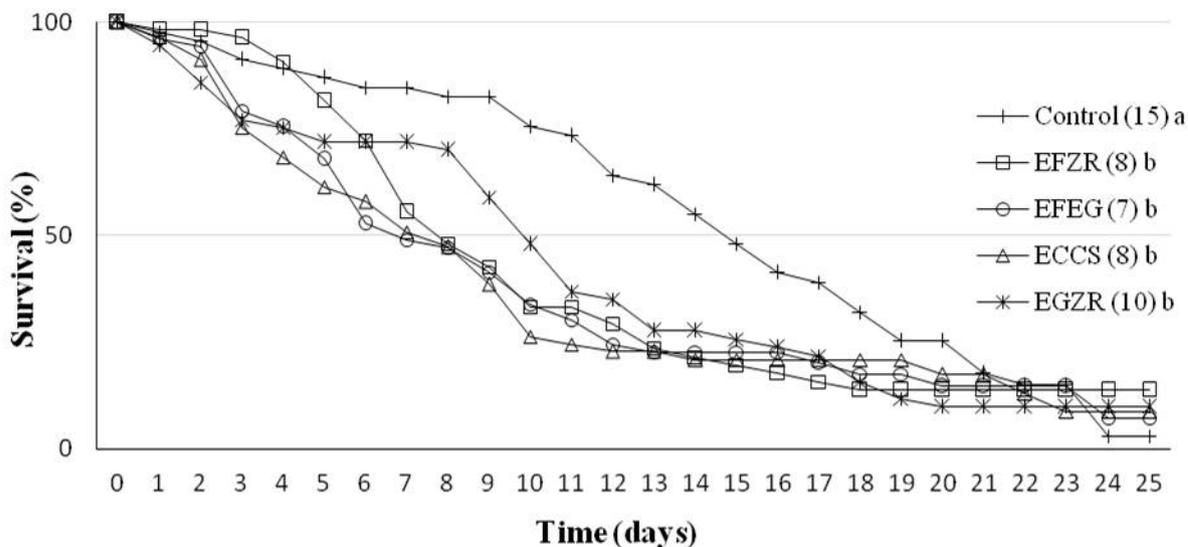


Fig 1. Survival curves for *Atta sexdens sexdens* (Hymenoptera: Formicidae) workers in ingestion test with plant extracts. Average survival period (S_{50}) is displayed besides the names of extracts, in brackets. Different lower case letters indicate difference in relation to control (data obtained with the application of “log-rank” test).

also presented toxicity to leaf cutting-ants. The toxicity of *C. sylvestris* extracts to leaf cutting ants may be due to its diterpenes, class of substances compounds with biological activities, including antifungal effect (Bento et al., 2013). The alkaloids (Januário et al., 2009) and coumarins from *E. grandiflora* were toxic to *Aedes aegypti* larvae (Oliveira et al., 2005).

Leaf and branch extracts from *Z. rhoifolium* presented contact and ingestion toxicity to leaf cutting ants. The biological activities of this plant were described (Negi et al., 2011; Medhi et al., 2013), but insecticidal activity was observed only for its fruit essential oil (Prieto et al., 2011). Alkaloids are the main active pharmacological constituents of this plant (Jullian et al., 2006), with promising molecules for the management of leaf cutting-ants (Bigi et al., 2004; Almeida et al., 2007).

The activity of the EGZR extract through topical application and delayed toxic action through ingestion to leaf cutting-ant deserve studies to define possibilities of its use in toxic baits, due to its mode of action preferably through ingestion (Boaretto & Forti, 1997). However, as this extracts showed both contact toxicity and delayed toxic action by ingestion, future studies are needed to elucidate this issue.

Zanthoxylum rhoifolium and *E. grandiflora*, are from Rutaceae family, which has other species toxic to leaf cutting-ants as *Citrus* sp. (Fernandes et al., 2002), *Raulinoa echinata* R.S. Cowen (Biavatti et al., 2005) and *Helietta puberula* R. E. Fr. (Almeida et al., 2007).

Conclusions

1- The leaves and branches of *Zanthoxylum rhoifolium*, bark of *Simarouba amara*, branches of *Aspidosperma spruceanum* and leaves of *Casearia arborea* extracts are toxic by contact to *A. sexdens sexdens*, justifying studies to elucidate its active ingredient.

2- The branches and leaves of *Z. rhoifolium*, leaves of *Esebenkia grandiflora* and bark of *Casearia sylvestris* extracts were toxic through ingestion to *A. sexdens sexdens*.

3 - The leaves and branches extracts of *Z. rhoifolium* are the only ones with contact and ingestion toxicity to *A. sexdens sexdens*.

4- The extracts of Rutaceae plants are highlighted with toxicity to *A. sexdens sexdens* and, therefore, toxicity of other species of this family to leaf-cutting should be studied.

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