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EFFECTS OF ASTILBIN FROM *DIMORPHANDRA MOLLIS* (FABACEAE) FLOWERS AND BRAZILIAN PLANT EXTRACTS ON *SITOPHILUS ZEAMAIS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Botanical extracts can be an alternative to synthetic insecticides for pest management, owing to their efficiency and environmental safety. The lethal times (LT_{50} and LT_{90}), loss of grain weight, and repellence of *Sitophilus zeamais* (Coleoptera: Curculionidae) were evaluated when extracts from 5 plant species from the Brazilian Cerrado biome [*Adenocalymma nodosum* (Bignoniaceae) leaves, astilbin from *Dimorphandra mollis* (Fabaceae) flowers, *Psychotria prunifolia* (Rubiaceae) leaves, *Senna obtusifolia* (Fabaceae) leaves, and *Tithonia diversifolia* (Asteraceae) flowers] were applied to a surface and to food. The weevils exposed to a surface treated with extracts from *T. diversifolia* flowers (106.43 h) and *P. prunifolia* leaves (119.68 h) at 2% ($m.v^{-1}$) presented the lowest LT_{50} and LT_{90} values, respectively. Corn grains treated with the extract from *P. prunifolia* leaves at 2% ($w.w^{-1}$) exhibited fastest mortality of *S. zeamais* individuals with lower values of LT_{50} and LT_{90} than the other treatments. All the botanical extracts repelled *S. zeamais* after 30 min of application; however, the extracts from *S. obtusifolia* leaves, *P. prunifolia* leaves, and *T. diversifolia* flowers at 2% ($m.v^{-1}$) repelled this insect after 6 min. Moreover, at 90 and 120 min after the beginning of the experiment, the extracts from *P. prunifolia* leaves and *T. diversifolia* flowers repelled the adult weevils. The extracts from *A. nodosum* leaves, *P. prunifolia* leaves, and *T. diversifolia* flowers applied to corn grains showed class III repellency according to the Preference Index for *S. zeamais* and less grain weight loss, when compared with astilbin from *D. mollis* flowers and *S. obtusifolia* leaves. Therefore, it was concluded that the extracts from *T. diversifolia* flowers and *P. prunifolia* leaves were the most promising in controlling *S. zeamais*, with the potential for application in programs for the integrated management of this pest.

Key Words: alternative control, Asteraceae, botanical insecticides, maize weevil, Rubiaceae, toxicity

RESUMO

Extratos botânicos pode ser uma alternativa aos inseticidas sintéticos para o manejo de pragas, devido à sua eficiência e segurança ambiental. O tempo letal (TL_{50} e TL_{90}), perda de peso de grãos e repelência de *Sitophilus zeamais* (Coleoptera: Curculionidae) foram avaliados quando extratos de cinco plantas do bioma Cerrado brasileiro [*Adenocalymma nodosum* (Bignoniaceae), astilbina de flores de *Dimorphandra mollis* (Fabaceae), folhas de *Psychotria prunifolia* (Rubiaceae), folhas de *Senna obtusifolia* (Fabaceae) e flores de *Tithonia diversifolia* (Asteraceae)] foram aplicados sobre uma superfície e alimento. Os gorgulhos expostos a uma superfície tratada com extratos de flores de *T. diversifolia* (106,43 h) e folhas de *P. prunifolia* (119,68 h) a 2 % ($m.v^{-1}$) apresentaram o menor TL_{50} e TL_{90}, respectivamente. Grãos de milho tratados com extrato de *P. prunifolia* a 2% ($p.p^{-1}$) apresentaram mortalidade mais rápida de indivíduos de *S. zeamais* com menores valores de TL_{50} e TL_{90} que os outros tratamentos. Todos os extratos botânicos repeliram *S. zeamais* após 30 min de aplicação; no entanto, os extratos de folhas de *S. obtusifolia*, folhas de *P. prunifolia* e flores de *T. diversifolia* a 2% ($m.v^{-1}$) repeliram esse inseto após 6 min. Além disso, aos 90 e 120 min após o início do experimento, os extratos de folhas de *P. prunifolia* e flores de *T. diversifolia* repeliram os gorgulhos adultos. O extrato de folhas de *A. nodosum*,

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folhas de *P. prunifolia* e flores de *T. diversifolia* aplicadas aos grãos de milho mostraram classe de repelência III de acordo com o Índice de Preferência para *S. zeamais* e uma perda de peso de grãos menor, quando comparado com astilbina de flores de *D. mollis* e folhas de *S. obtusifolia*. Portanto, concluiu-se que os extratos de flores de *T. diversifolia* e folhas de *P. prunifolia* foram os mais promissores no controle de *S. zeamais*, com potencial para aplicação em programas de manejo integrado dessa praga.

Palavras Chave: Asteraceae, controle alternativo, inseticidas botânicos, gorgulho do milho, Rubiaceae, toxicidade

Synthetic insecticides are used to protect agricultural and forest crops as well as stored grains and byproducts (Tavares et al. 2010a, 2010b). However, the repetitive use of the same active component may lead to resistant insect populations and toxic residues adhering to food (Pimentel et al. 2010; Tavares et al. 2012). The use of botanical extracts and essential oils to control insects is increasing because these natural products present smaller effects on native fauna and flora, greater availability, and faster degradation, when compared with synthetic compounds (Tavares et al. 2011; Fouad et al. 2014).

Maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) is an important insect pest, especially of corn grain, *Zea mays* L. (Poaceae), both in the field and in storage (Tavares et al. 2013a). The extracts from several plant parts (bark, flowers, leaves, roots, seeds, and stems) of the families Euphorbiaceae, Fabaceae, Lamiaceae, Monimiaceae, Verbenaceae, and Zingiberaceae have shown efficacy against the adults and larvae of *S. zeamais* (Fouad et al. 2012; Tavares et al. 2013a). However, only a few bioprospection studies of compounds useful as natural insecticides have been carried out (Tavares et al. 2009, 2013b).

Cerrado, a type of savannah and an important Brazilian biome, is rich in endemic flora. Plants from Cerrado are used in agriculture, folk medicine, and pharmaceutical industry, thus motivating the search for insecticides from these plants. *Adenocalymma nodosum* (Silva Manso) L.G. Lohmann [= *Memora nodosa* (Silva Manso) Miers] (Bignoniaceae) and *Psychotria prunifolia* (H.B.K) Steyerm. (Rubiaceae) are plants used in folk medicine to treat several human diseases (Soares et al. 2010; Tavares et al. 2013b). *Dimorphandra mollis* Benth. (Fabaceae) contains the flavonoid astilbin and its seeds are toxic to cattle, *Bos primigenius* Bojanus (Artiodactyla: Bovidae) (Cintra et al. 2002, 2005). *Senna obtusifolia* (L.) Irwin & Barneby (Fabaceae) has anti-fungal and anti-bacterial properties (Doughari et al. 2008), and *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) has antidiabetic, anti-inflammatory, and anti-malarial properties (Ambrósio et al. 2008).

Botanical extracts of the families Asteraceae, Bignoniaceae, Fabaceae, and Rubiaceae affect the biology, behavior, and reproduction of coleopteran and lepidopteran pests (Asawalam et al. 2008; Tavares et al. 2012), and reduce the growth of their populations (Fazolin et al. 2010). The aim of the present study was to identify the compound astilbin, extracted and purified from *D. mollis* flowers, and determine its lethal time to kill 50% (LT_{50}) and 90% (LT_{90}) of the maize weevil, *S. zeamais*, and its repellency potential, besides other ethanolic extracts from 4 Cerrado plant species at 2% ($m.v^{-1}$ or $w.w^{-1}$).

Material and Methods

Experimental Site

The experiment was carried out in the Laboratory of Integrated Pest Management-Grains (LIPM-Grains), Department of Agricultural Engineering (DEA), the Federal University of Viçosa (UFV), Viçosa, Minas Gerais, Brazil, in an environmentally controlled room at 25 ± 1 °C, 70 ± 10% RH and a 12:12 h L:D photoperiod.

Sitophilus zeamais and Corn Grains

The weevils *S. zeamais* were obtained from stock cultures of the Laboratory of Integrated Pest Management-Grains (LIPM-Grains), DEA-UFV, where this species was reared with corn grains (13% humidity). Individual weevils were isolated for 24 h without food before the experiment.

The corn was grown and harvested at the UFV Experiment Farm where no insecticide was used, and stored in an environmentally controlled chamber at 5 °C until the beginning of the experiment.

Plants Collection and Deposit

Adenocalymma nodosum leaves, *D. mollis* flowers, *P. prunifolia* leaves, *S. obtusifolia* leaves, and *T. diversifolia* flowers were removed with a pocketknife from the flowering plants in February 2013 in a part of Cerrado (a protected area) at S 18° 9'-W 47° 56', and an altitude of 835 m in Catalão, Goiás, Brazil. These materials were placed in twenty plastic bags inside polystyrene $(C_8H_8)_n$ boxes lined with ice cubes and taken to the laboratory, where they were stored at 2 °C in a refrigerator. The plants were identified after examination of their reproductive organs, according to keys and descriptions (Watson & Dallwitz 1992 onwards).

Samples of these plants (a voucher specimen and 2 duplicates) were deposited in the herbarium of the Institute of Biological Sciences (ICB) at the Federal

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University of Goiás (UFG), Samambaia campus in Goiânia, Goiás, Brazil (Table 1).

Preparation of Crude Extracts

A total of 600 g of fresh material of each plant species were left under direct sunlight in 1-L ethanol (C_2H_6O) for 7 days to extract its compounds, and the yield of the plant extracts was verified on a dry weight basis after concentration in vacuum (Table 1). The crude extract obtained after the extraction was stored in 50-mL plastic tubes covered with black paper and kept at 2 °C in a refrigerator.

Extraction of Astilbin from *D. mollis* Flowers

Astilbin (Fig. 1) was isolated from *D. mollis* flowers. This compound was extracted thrice with dichloromethane (DCM, CH_2Cl_2) and twice with ethanol, before being concentrated in vacuum. The ethanolic extract of astilbin was separated in a recycling High Performance Liquid Chromatography (HPLC) [Asahipak GS-310 P polymetric column, mobile phase of methanol (CH_3OH) with a flow of 7 $mL.min^{-1}$, and UV detection at 290 nm] and recrystallized with methanol to yield yellow crystals. The structural characterization of astilbin was based on comparisons with the physical and spectral data of its crystals (De Brito et al. 1995; Han et al. 1998).

Analysis of Nuclear Magnetic Resonance (NMR) ^{1}H (200 MHz) and of NMR ^{13}C (50 MHz) (Figs. 2A and 2B) was carried out in a Brüker spectrometer (ARX-200) with tetramethylsilane (TMS, $C_4H_{12}Si$) as an internal standard. The mass spectra were obtained in positive mode Atmospheric Pressure Chemical Ionization (APCI-MS) in a mass spectrometer (Micromass Quattro LC). The melting point of the astilbin crystals was determined as 190–191 °C, $[\alpha]^{25}_{D}$= –12.8° (*c* 1.0, methanol), m/z= 451 $[M + H]^+$ (APCI-MS, positive mode), and the NMR of ^{1}H and ^{13}C NMR were compatible with the published data (De Brito et al. 1995; Han et al. 1998). The yield of the plant extracts was calculated by dividing the mass obtained from that of the initial material after concentration in a vacuum (Tavares et al. 2013c).

Preparation of Solutions from Crude Extracts

Solutions were prepared after dilution of the dry botanical extracts with absolute ethanol (Merck KGaA) until 2% ($m.v^{-1}$ or $w.w^{-1}$) concentration. These solutions were shaken with a Branson 2510 sonifier for 220 min in SET DEGAS and homogenized with a Fisher Vortex Genie 2™ shaker at "speed eight" for 10 min (Tavares et al. 2009).

Toxicity on a Treated Surface

A total of 50 µL of the botanical extracts (*A. nodosum* leaves, astilbin from *D. mollis* flowers, *P. prunifolia* leaves, *S. obtusifolia* leaves, and *T. diversifolia* flowers) at 2% ($m.v^{-1}$) (Table 1) or absolute ethanol (control) were applied to disks of filter paper (5 cm diam) with a precision micro-syringe (Hamilton 1 mL), and left for 10 min at 25 ± 1 °C to evaporate the solvent. Subsequently, the treated papers were placed in Petri dishes (5 cm diam) with twenty 90-day-old unsexed *S. zeamais* adults. These dishes were placed in an environmentally controlled chamber (25 ± 1 °C, 70 ± 10% RH, and 12:12 h L:D photoperiod). The mortalities of the weevils were evaluated after 1, 3, 6, 9, 12, 24, 48, 72, 96, and 120 h (Tapondjou et al. 2005). A total of 10 replications was carried out.

Toxicity on Treated Corn Grains and Weight Loss of the Corn

The toxicities of botanical extracts (*A. nodosum* leaves, astilbin from *D. mollis* flowers, *P. prunifolia* leaves, *S. obtusifolia* leaves, and *T. diversifolia* flowers) mixed with food (corn grains) were evaluated by applying 50 µL of the extract at 2% ($w.w^{-1}$) (Table 1) or absolute ethanol (control) in 10 g of corn grains. Plastic jars (100 mL) with the respective treatments were shaken for 15 s to mix the extracts or ethanol with the corn grains. The treated grains were left for 10 min at 25 ± 1 °C to evaporate the solvent. Subsequently, twenty 90-day-old unsexed *S. zeamais* adults were left to feed on the corn grains, treated or untreated, in an environmentally controlled chamber (25 ± 1 °C, 70 ± 10% RH, and 12:12 h L:D photoperiod). The mortalities of the weevils

TABLE 1. SPECIES, BOTANIC FAMILY, PLANT PART, YIELD OF THE EXTRACT RESIDUE (%, $W.W^{-1}$), AND VOUCHER CODE FOR EACH SPECIES COLLECTED FROM THE BRAZILIAN CERRADO BIOME AND DEPOSITED IN THE HERBARIUM OF THE INSTITUTE OF BIOLOGICAL SCIENCES AT THE FEDERAL UNIVERSITY OF GOIÁS, SAMAMBAIA CAMPUS, GOIÂNIA, GOIÁS, BRAZIL.

Species	Family	Part	Yield (%, $w.w^{-1}$)	Voucher
Adenocalymma nodosum	Bignoniaceae	Leaves	3.91	43253
Astilbin (isolated from *D. mollis*)	Fabaceae	Flowers	7.60	43247
Psychotria prunifolia	Rubiaceae	Leaves	1.64	43236
Senna obtusifolia	Fabaceae	Leaves	4.90	43752
Tithonia diversifolia	Asteraceae	Flowers	7.56	43419

D. mollis = *Dimorphandra mollis*.

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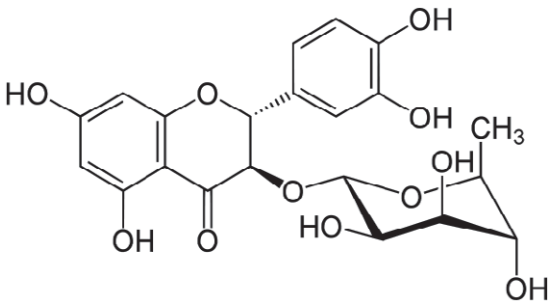


Fig. 1. Chemical structure of astilbin (2R,3R)5,7,3¢,4¢-tetrahydroxy-2,3-dihydroflavonol-3-β-O-rhamnoside.

were recorded after 1, 6, 9, 12, 24, 48, 72, 96, and 120 h (Betancur et al. 2010). The weight loss of the corn grains offered to the insects was recorded after 30 days (Tavares et al. 2013b). A total of 10 replications was carried out.

Insect Repellency

Repellency tests were conducted in Petri dishes (14 cm diam) containing filter paper (Whatman no 1) of the same size as the dishes. To half of the papers, 1 mL of each of the botanical extracts (*A. nodosum* leaves, astilbin from *D. mollis* flowers, *P. prunifolia* leaves, *S. obtusifolia* leaves, and *T. diversifolia* flowers) at 2% ($m.v^{-1}$) was uniformly applied (Table 1) and absolute ethanol (control) was applied to the rest of the filter papers. The dishes were left for 10 min at 25 ± 1 °C to evaporate the solvent. Subsequently, twenty 90-day-old unsexed *S. zeamais* adults were placed in the center of each dish. The numbers of weevils in the control (NC) and treatment (NT) dishes were recorded after 30, 60, 90, and 120 min (Islam et al. 2009). A repellency assay was carried out in an environmentally controlled chamber (25 ± 1 °C, 70 ± 10% RH, and 12:12 h L:D photoperiod). A total of 10 replications was carried out.

Statistical Analyses

The data on the toxicity of the botanical extracts on contaminated surfaces and grains, determined with six treatments (five plant extracts and one control) and 10 replications, were analyzed by using regression equations, and the 95% confidence limits were calculated by PROBIT analysis (Finney 1971). The LT_{50} and LT_{90} of the insects were obtained by PROC PROBIT (Supplier: UFV). The total insects in the NC and NT plates were evaluated 30, 60, 90, and 120 min after the beginning of the repellency experiment, and the results were submitted to Analysis of Variance (one-way ANOVA) and the means were compared with the "*t*" test at 5% probability (SAS Institute 1997).

The percentage of repellency (PR) was obtained by using the equation: PR = [(NC – NT) ÷ (NC + NT)] × 100, and classified into class 0 (PR ≤ 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), class III (PR = 40.1–60%), class IV (PR = 60.1–80%), and class V (PR = 80.1–100%) (Benzi et al. 2009).

Results

Extracts

The yield of astilbin from *D. mollis* flowers (7.60%) was higher than that from *P. prunifolia* leaves (1.64%), *A. nodosum* leaves (3.91%), *S. obtusifolia* leaves (4.90%), and *T. diversifolia* flowers (7.56%) (Table 1). The signals in the spectra with their multiplicities and coupling constant (number that determines the strength of the force exerted in an interaction) found for astilbin (Fig. 1) were characteristic of flavonoids (Figs. 2A and 2B).

Toxicity of the Treated Surface

The extracts from all 5 plant species investigated caused mortality of *S. zemais* adults. The LT_{50} for *S. zeamais* on the surface treated with the extracts from *T. diversifolia* flowers was approximately 1.12-, 1.29-, 1.34-, and 1.52-fold lower (106.43 h) than that for *S. zeamais* on the surface treated with the extracts from *P. prunifolia* leaves, *A. nodosum* leaves, astilbin from *D. mollis* flowers, and *S. obtusifolia* leaves, respectively (Table 2). On the other hand, the LT_{90} for *S. zeamais* adults on grains treated with extracts from *P. prunifolia* leaves (167.56 h) was approximately 1.45-, 1.52-, 1.72-, and 2.16-fold lower than that for *S. zeamais* adults on grains treated with the extracts from *A. nodosum* leaves, *T. diversifolia* flowers, astilbin from *D. mollis* flowers, and *S. obtusifolia* leaves, respectively (Table 2).

Toxicity of the treated grains

The extract from *P. prunifolia* leaves mixed with corn grains was more toxic (LT_{50}= 89.73 h and LT_{90}= 171.19 h) to the adult maize weevils than those from *T. diversifolia* flowers, *S. obtusifolia* leaves, *A. nodosum* leaves, and astilbin from *D. mollis* flowers (the toxicity was 1.25-, 1.25-, 1.36-, and 1.54-fold higher with respect to LT_{50} and 1.04-, 1.14-, 1.31-, and 1.47-fold higher with regard to LT_{90}, respectively) (Table 2).

Repellency

The botanical extracts from Cerrado repelled *S. zeamais* adults after 30 min of exposure (Fig. 3A) and those of *S. obtusifolia* leaves (P = 0.03), *P. prunifolia* leaves (P = 0.0008), and *T. diversifolia* flowers (P = 0.005) repelled this weevil after

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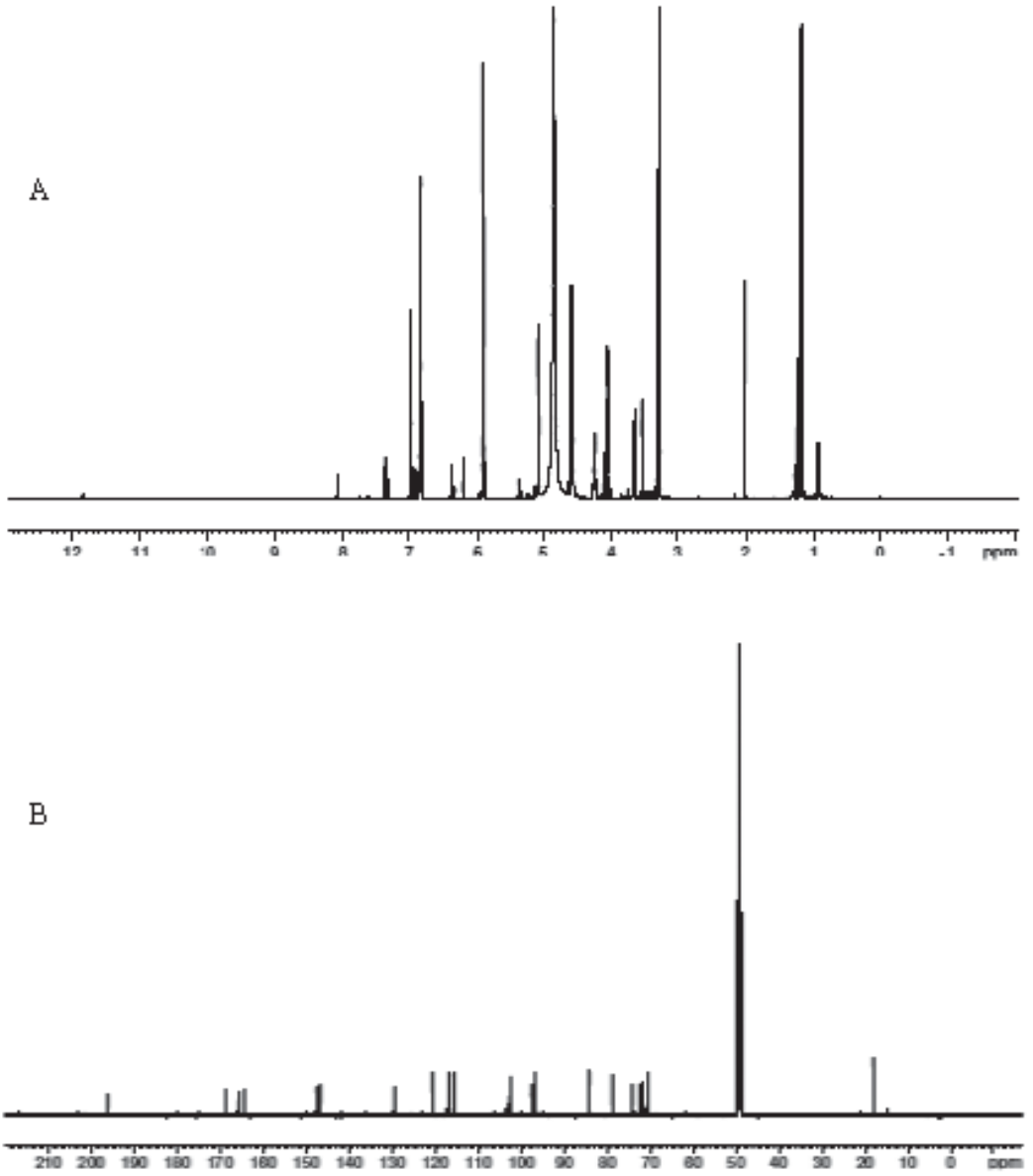


Fig. 2. A. Nuclear Magnetic Resonance (NMR) ^{1}H (200 MHz) spectrum of astilbin, and of NMR ^{13}C (50 MHz) spectrum of astilbin.

60 min of exposure (Fig. 3B). Furthermore, the extracts from *P. prunifolia* leaves [(P = 0.004) and (P = 0.02)] and *T. diversifolia* flowers [(P = 0.02) and (P = 0.04)] repelled the maize weevil adults after 90 and 120 min, respectively (Figs. 3C and 3D).

The extracts from *A. nodosum* leaves exhibited class III repellency after 30 min, while those from *P. prunifolia* leaves and *T. diversifolia* flowers exhibited similar class of repellency after 60 and 90 min of treatment against *S. zeamais* adults, respectively (Table 3).

Grain Weight Loss

The extracts from *P. prunifolia* leaves (3.14 ± 0.48%), *T. diversifolia* flowers (3.28 ± 0.45%), and *A. nodosum* leaves (4.78 ± 0.23%) exhibited higher grain weight loss caused by maize weevil feeding after 30 days (P = 0.0002) (Table 4).

Discussion

The yield of astilbin extract from *D. mollis* flowers collected from Catalão was found to be similar

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TABLE 2. PLANT EXTRACTS, LT_{50} (H) AND LT_{90} (H) (VARIATION INTERVAL), MEAN ± STANDARD ERROR (SE) OF MORTALITY, X^2, AND *P* OF THE EFFECTS OF THE EXTRACTS FROM PLANTS COLLECTED FROM THE BRAZILIAN CERRADO BIOME ON A TREATED SURFACE OR CORN GRAINS ON *SITOPHILUS ZEAMAIS* ADULTS.

Extracts	LT_{50} (h)	LT_{90} (h)	Mean ± SE	χ^2	*P*
	Treated surface of filter paper				
A. nodosum leaves	137.70 (121.33-175.79)	243.10 (186.51-430.47)	5.19 ± 1.00	8.23	0.83
Astilbin (isolated from *D. mollis* flowers)	143.07 (122.85-191.76)	288.20 (208.77-572.48)	4.21 ± 0.80	11.81	0.54
P. prunifolia leaves	119.68 (112.20-132.70)	167.56 (146.67-218.03)	8.77 ± 1.55	8.47	0.81
S. obtusifolia leaves	161.67 (132.17-252.90)	362.23 (237.48-997.80)	3.66 ± 0.78	7.67	0.86
T. diversifolia flowers	106.43 (94.06-126.27)	255.09 (194.74-402.50)	3.38 ± 0.47	13.67	0.40
	Treated corn grains				
A. nodosum leaves	122.09 (111.85-141.11)	225.05 (178.30-338.90)	6.30 ± 1.09	4.19	0.99
Astilbin (isolated from *D. mollis* flowers)	138.02 (120.99-178.04)	252.18 (190.79-460.70)	4.89 ± 0.94	7.88	0.85
P. prunifolia leaves	89.73 (81.95-98.68)	171.19 (144.88-222.73)	4.45 ± 0.57	6.99	0.90
S. obtusifolia leaves	112.24 (101.01-131.15)	194.94 (161.84-290.01)	4.24 ± 0.62	9.71	0.78
T. diversifolia flowers	111.94 (104.48-123.31)	178.05 (147.60-219.17)	7.06 ± 1.09	4.97	0.97

A. nodosum = *Adenocalymma nodosum*, *D. mollis* = *Dimorphandra mollis*, *P. prunifolia* = *Psychotria prunifolia*, *S. obtusifolia* = *Senna obtusifolia*, and *T. diversifolia* = *Tithonia diversifolia*.

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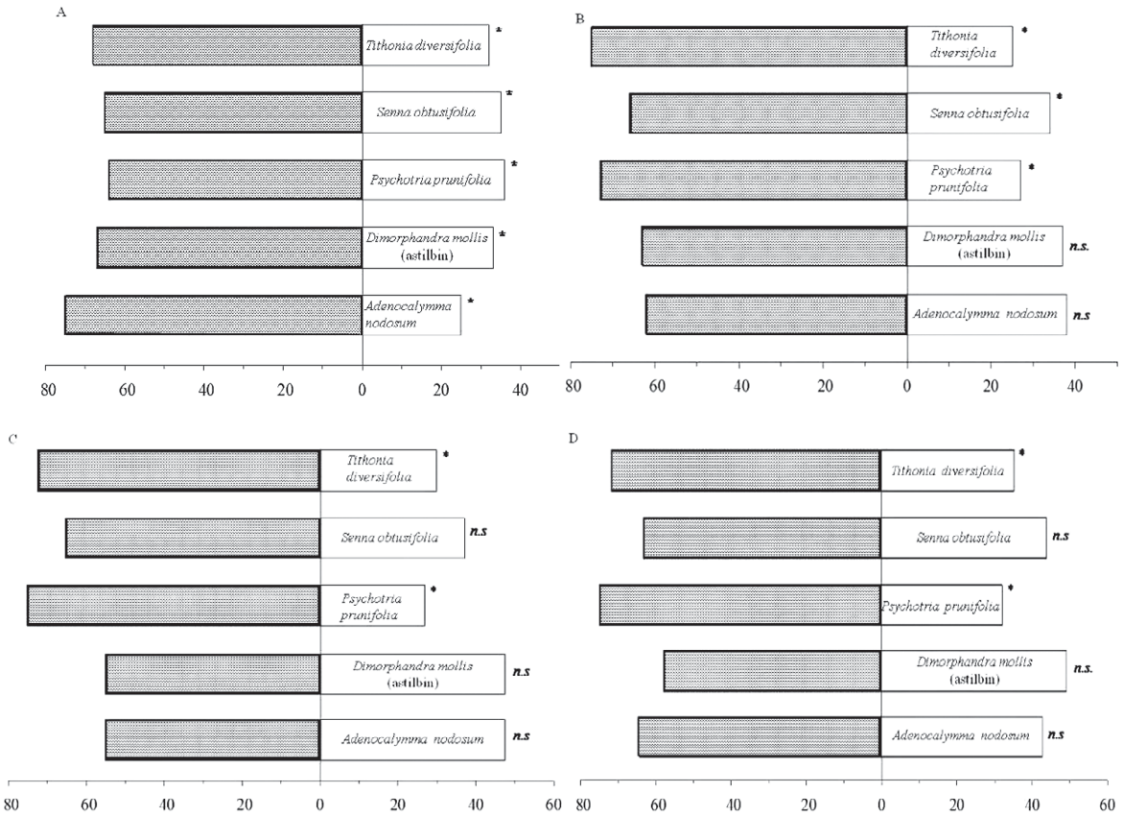


Fig. 3. Selection by *Sitophilus zeamais* adults of treated and control corn grains after 30 (A), 60 (B), 90 (C), and 120 (D) min of exposure. *Significant values; *n.s.* non-significant values at 5% probability by the "*t*" test. Treatments consisted of ethanol extracts of *T. diversifolia* flowers, *S. obtusifolia* leaves, *P. prunifolia* leaves, astilbin isolated from the extract of *D. mollis* flowers, and *A. nodosum* leaves, all from the Brazilian Cerrado biome. In the control, the corn grains had been treated with absolute ethanol.

to that from the same plant collected from Rio Claro and Corumbataí, São Paulo, Brazil (Cintra et al. 2002, 2005), demonstrating that *D. mollis* contains the same amount of astilbin, irrespective of its geographical location. The yield of the extract from *A. nodosum* leaves collected on Feb 2012 from Catalão was similar to that extracted from this plant collected on Feb 2011 in the same municipality (Menezes et al. 2014), which indicates that content of the extract in this species is similar over time and over a wide geographic ranges. These results show that plants collected from Cerrado may produce high yields of extracts.

The higher susceptibility of *S. zeamais* adults to the extract of *T. diversifolia* flowers on a treated surface with a lower LT_{50} value (106.43 h) agrees with the previous finding indicating 100% mortality of *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) adults with extracts of this plant at 3, 4, and 5% ($m.v^{-1}$) (Adedire & Akinneye 2004). Furthermore, the LT_{50} values for *S. zeamais* adults exposed to a surface treated with essential oils of *Lantana camara* L. (Verbenaceae) and *Tephrosia vogelii* Hook (Fabaceae) were noted in 6 days (with rates of 7.5–10% $w.w^{-1}$) and between 7 and 8 days (with rates of 2.5–5% $w.w^{-1}$), respectively (Ogendo et al. 2005). Sesquiterpene lactones [containing sesquiterpenoids (made up of 3 isoprene units) and a lactone ring], such as eudesmanolides and germacranolides, were isolated from *Tithonia rotundifolia* (Mill.) S. F. Blake (Asteraceae), which might be responsible for the insecticidal activity of the extracts of this plant (Bohlmann et al. 1981). In addition, chromone (a derivative of benzopyran with a substituted keto group on the pyran ring), dinorxanthane sesquiterpene, and other substances isolated from *T. diversifolia* roots have been noted to exhibit insecticidal activities (Kou & Lin 1999). It has been reported that the extracts from *T. diversifolia* have low toxicity to mammals when compared with synthetic insecticides (Bohlmann et al. 1981).

In the present study, the extract from *P. prunifolia* leaves mixed with corn grains presented toxicity to *S. zeamais* adults in a shorter time (LT_{50} and LT_{90}),

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TABLE 3. PLANT EXTRACTS, NUMBER OF *SITOPHILUS ZEAMAIS* (COLEOPTERA: CURCULIONIDAE) ADULTS REPELLED BY TREATED FILTER PAPER SURFACE AFTER 30, 60, 90, AND 120 MIN, AND CLASS OF REPELLENCY OF THE EXTRACTS FROM PLANTS COLLECTED FROM THE BRAZILIAN CERRADO BIOME.

	Time (min)			
Extracts	30	60	90	120
Adenocalymma nodosum leaves	50 III	24 II	8 I	20 I
Astilbin (isolated from *Dimorphandra mollis* flowers)	34 II	26 II	8 I	8 I
Psychotria prunifolia leaves	28 II	46 III	48 III	40 II
Senna obtusifolia leaves	30 II	35 II	28 II	18 I
Tithonia diversifolia flowers	36 II	50 III	42 III	34 II

Repellency Class: class I (PR = 0.1%–20.0%), class II (PR = 20.1%–40.0%), and class III (PR = 40.1%–60.0%) (Benzi et al. 2009).

similar to the hexanic $CH_3(CH_2)_4CH_3$ extract from *T. vogelii* mixed with food, which reduced the population of *S. zeamais* by 50% after 5 days of application (Koona et al. 2007). Medicinal herbs have shown anti-feeding and insecticidal activity against stored grain pests (Liu et al. 2007). In a previous study, the extract from *Peumus boldus* Molina (Monimiaceae) leaves at 15 and 20% ($m.v^{-1}$) caused 100% mortality of the maize weevil after 15 days of contact with treated food (Bustos-Figueroa et al. 2009). It has been reported that monoterpene indole alkaloids and strictosamide (alkaloid glycoside), besides being important as chemotaxonomic markers for the taxonomy of the species of this genus (Simões-Pires et al. 2006; Petacci et al. 2012), are the main compounds of the extracts from the leaves of the genus *Psychotria* (Kerber et al. 2008). These compounds also present biological activities against microorganisms that cause diseases in humans and act as a stimulant of the central nervous system (Figueiredo et al. 2010). Furthermore, the extract from *Cinchona ledgeriana* Moens. (Rubiaceae) leaves was noted to contain abundant monoterpene indole alkaloids that reduced the growth as well as development and increased the mortality of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae that were fed on a treated artificial diet (Aerts et al. 1992).

In the present study, the greatest effect of the extract from *P. prunifolia* leaves on corn grains suggested a higher efficiency of ingestion owing to the protective cuticle that prevents contact of the insecticide with its action site (Lopez et al. 2009). Products with a greater residual effect or sequential application, such as extracts from *T. diversifolia*, are recommended because they have better efficiency when applied against weevils (Thornham et al. 2007), and the use of insecticides acting on ingestion can increase the mortality and repellency of Coleoptera pests in stored grains (Bozsik 2006).

The greater repellency effect of the extract from *P. prunifolia* leaves and *T. diversifolia* flowers on *S. zeamais* adults, especially after 1 h of treatment, suggested a wide spectrum of action from the extracts of these plants. In a previous study, the crude extract of *T. diversifolia* also repelled immature *Chlosyne lacinia* Geyer (Lepidoptera: Nymphalidae) on a treated surface (Ambrósio et al. 2008).

The highest class of repellency (class III) achieved with extracts from *P. prunifolia* leaves and *T. diversifolia* flowers against the maize weevil was found to be similar to the repellency effect exhibited by 40.1–60% concentration of the extracts from *Ocimum kenyense* Ayob. ex A. J. Paton, *Ocimum grattisimum* L. (Lamiaceae), and *Ricinodendron heudelotii* (Baill) Pierre ex Pax (Euphorbiaceae) leaves on this insect (Bekele et al. 1997; Asawalam et al. 2008).

The least weight loss in corn grains treated with the extracts from *P. prunifolia* leaves and *T. diversifolia* flowers after 30 days of feeding confirmed

TABLE 4. MEAN WEIGHT LOSS ± STANDARD ERROR (%) AND VARIATION INTERVAL OF CORN GRAINS TREATED WITH EXTRACTS FROM PLANTS COLLECTED FROM THE BRAZILIAN CERRADO BIOME AFTER 30 DAYS OF FEEDING BY *SITOPHILUS ZEAMAIS* ADULTS AND CONTROL.

Extracts	Weight loss	Variation interval
Adenocalymma nodosum leaves	4.78 ± 0.23 AB	4.55-5.01
Astilbin (isolated from *Dimorphandra mollis* flowers)	5.48 ± 0.56 A	4.92-6.04
Psychotria prunifolia leaves	3.14 ± 0.48 B	2.66-3.62
Senna obtusifolia leaves	5.30 ± 0.34 A	4.96-5.64
Tithonia diversifolia flowers	3.28 ± 0.45 B	2.83-2.83
Absolute ethanol (control)	6.00 ± 0.48 A	5.52-6.48

Means followed by the same capital letter per column do not differ by the Tukey test at 5% probability.

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that secondary compounds in the extracts of these plants produced repellent or anti-feeding activity in *S. zeamais* adults. The presence of rotenones (substance classified by the World Health Organization as moderately hazardous) in the *R. heudelotii* extracts repelled *S. zeamais* and *C. maculatus*, and decreased the weight loss in corn and common bean *Phaseolus vulgaris* L. (Fabaceae) grains treated with the extracts of this plant (Udo & Epidi 2009), suggesting that the anti-feeding activity of the botanical extracts can be efficient in protecting stored grains (Nerio et al. 2010). In conclusion, the extracts from *T. diversifolia* flowers and *P. prunifolia* leaves caused higher mortality and repellency on *S. zeamais* adults in corn grains, and thus have the potential for use in the integrated management of this pest in storage.

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