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Potential of aromatic and medicinal plant extracts from Cerrado biome to control the velvetbean caterpillar *Anticarsia gemmatalis*

[Potencial de extractos de plantas aromáticas y medicinales del bioma Cerrado para controlar la oruga *Anticarsia gemmatalis*]

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Abstract
Anticarsia gemmatalis was treated with aromatic and medicinal plant extracts from Cerrado biome: *Acisanthera* sp., *Adenocalymma nodosum*, *Bidens sulphurea*, *Lepidoploa aurea*, *Dimorphandra mollis*, and *Salvertia convallariaeodora*. Extracts of astilbin from *D. mollis* or *L. aurea* were the most toxic to eggs and have reduced the sex ratio of *A. gemmatalis* pupae. Extracts of *B. sulphurea*, astilbin from *D. mollis* or *S. convallariaeodora* reduced the weight of male pupae and those of astilbin from *D. mollis*, the weight of female pupae of *A. gemmatalis*. The viability of the stages from egg to caterpillar was lower with extracts of astilbin from *D. mollis* or *L. aurea*; from caterpillar to pupa with *Acisanthera* sp. or astilbin from *D. mollis* and from pupa to adult with *Acisanthera* sp., *A. nodosum*, *B. sulphurea* or astilbin from *D. mollis*. Extracts of astilbin from *D. mollis* and *L. aurea* presented greater potential for future studies on toxicity of *A. gemmatalis*.

Keywords: aromatic and medicinal plants, botanical extracts, Cerrado, ethnopharmacological use, insecticidal plants, lepidopteran defoliator

Resumen
Anticarsia gemmatalis fue tratada con extractos de plantas aromáticas y medicinales del bioma Cerrado: *Acisanthera* sp., *Adenocalymma nodosum*, *Bidens sulphurea*, *Lepidoploa aurea*, *Dimorphandra mollis*, y *Salvertia convallariaeodora*. Extractos de astilbin de *D. mollis* o *L. aurea* fueron los más tóxicos para los huevos y han reducido la proporción sexual de pupas de *A. gemmatalis*. Extractos de *B. sulphurea*, astilbin de *D. mollis* o *S. convallariaeodora* redujeron el peso de las pupas macho y el de astilbin de *D. mollis*, el peso de las pupas hembras de *A. gemmatalis*. La viabilidad de los estadíos de huevo a oruga fue menor con extractos de astilbin de *D. mollis* o *L. aurea*; de oruga a crisálida con *Acisanthera* sp. o astilbin de *D. mollis* y de pupa a adulto con *Acisanthera* sp., *A. nodosum*, *B. sulphurea* o astilbin de *D. mollis*. Extractos de astilbin de *D. mollis* y *L. aurea* presentan un mayor potencial para futuros estudios sobre la toxicidad sobre *A. gemmatalis*.

Palabras Clave: Cerrado, extractos botánicos, lepidópteros defoliadores, plantas aromáticas y medicinales, plantas insecticidas, uso etnofarmacológico

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DEDICATION

†To Prof. Fernando Petacci (Federal University of Goiás, *Campus* Catalão, Brazil) who died in June 15, 2012. Our sincere feelings.

INTRODUCTION

Adverse effects of synthetic insecticides, such as residues in foods, environmental contamination, toxicity to the applicator, phytotoxicity, development of resistant insect populations and biological unbalance have motivated the search for alternatives to be incorporated to the integrated pest management (IPM) (Choung *et al*., 2010; Di Marzio *et al*., 2010; Fouad *et al*., 2012). The use of aromatic and medicinal plants with insecticidal properties is an ancient and sustainable practice in pest control (Morse and McNamara, 2004; Asogwa *et al*., 2010; Rattan, 2010), since their products may be more biodegradable and selective than synthetic insecticides (Isman, 2006; Jansen *et al*., 2010; Kamaraj *et al*., 2010).

The activity of aromatic and medicinal plant extracts can be tested by the immerging foods or placing them in extract solutions, or applying them in artificial diets; or in pests, preys or hosts (Pavela, 2010; Tavares *et al*., 2010a; Tavares *et al*., 2010b). Extracts from insecticidal aromatic and medicinal plants can inhibit feeding, cause deterrence, reduce food intake, delay the development and cause malformation, sterility and mortality of insects (Sahayaraj, 1998; Pipithsangchan and Morallo-Rejesus, 2005; Chermenskaya *et al*., 2010). Aromatic and medicinal plant extracts do not usually cause high mortality of insects even at high concentrations, which explains their wide use to reduce the growth of such populations (Shaalan *et al*., 2005; Charleston *et al*., 2006; Birah *et al*., 2010).

The velvetbean caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Noctuidae) reduces the productivity of soy culture *Glycine max* L. (Fabaceae) in North and South America (Walker *et al*., 2000; McPherson *et al*., 2008; Lourenção *et al*., 2010). This insect can also damage other vegetables, such as alfalfa *Medicago sativa* L. (Fabaceae), cotton *Gossypium hirsutum* L. (Malvaceae), pastures, rice *Oryza sativa* L. and wheat *Triticum aestivum* L. (Poaceae) (Panizzi *et al*., 2004; Tavares *et al*., 2011a). New varieties of soybean can synthesize toxic substances, such as protease inhibitors that act on defoliating caterpillars and flavonoids, but present difficulties to integrate productivity characteristics and resistance to insects and avoid resistant insect populations by selection process (Kraemer, 2001; Shukle and Wu, 2003). The transgenic lineage of soybean expressing the gene *cry Ac* of the *Bacillus thuringiensis* Berliner, 1915 (Bacillales: Bacillaceae) can confer resistance to defoliating caterpillars (Macrae *et al*., 2005; Miklos *et al*., 2007; McPherson and MacRae, 2009). However, Brazil has cultivated the transgenic soybean resistant to glyphosate, in no-tillage, but susceptible to defoliating caterpillars (Morjan and Pedigo, 2002; Cox *et al*., 2009). Besides, this cultivation system may, in some cases, increase the number of pests due to the high toxicity of this herbicide to natural enemies (Jackson and Pitre, 2004; Berman *et al*., 2010).

The Brazilian Cerrado (Savannah-type) biome is important for its flora and diversity. Therefore, it must be an area of preservation and the search for active biological substances must be a priority (Myers *et al*., 2000; Basso *et al*., 2005). Natural compounds, which are sources of insecticidal substances of agricultural interest, can be lost due to the extinction of Cerrado species (Tavares *et al*., 2009; Tavares *et al*., 2011b). Thus, monitored biological studies should be conducted on extracts, fractions and substances from aromatic and medicinal plants of this region (Zvinavashe *et al*., 2009; Petacci *et al*., 2012).

The present work aimed to evaluate mortality and viability at the stages from egg to caterpillar, caterpillar to pupa and from pupa to adult; the weight of male and female pupae and sexual ratio of *A. gemmatalis* after treatment with botanical extracts from Cerrado aromatic and medicinal plants.

MATERIAL AND METHODS

Experimental site

The experiment was carried out at the Laboratory of Biological Control of Insects (LCBI) of the Institute of Biotechnology Applied to Agriculture (BIOAGRO) of the Department of Animal Biology (DBA), at the Federal University of Viçosa (UFV), in the municipality of Viçosa, Minas Gerais State, Brazil; at 25.0 ± 1.0 ºC, 70 ± 10% R.H. and 12-h photoperiod.

Aromatic and medicinal plants collection

Aromatic and medicinal plants of *Acisanthera* sp. (Melastomataceae), *Bidens sulphurea* (Cav.) Sch.Bip., *Lepidoploa aurea* Mart. ex DC. (Asteraceae), *Dimorphandra mollis* Benth (Fabaceae), *Adenocalymma nodosum* (Silva Manso) L.G.Lohmann

[= *Memora nodosa* (Silva Manso) Miers] (Bignoniaceae) and *Salvertia convallariaeodora* A.St.-Hill. (Vochysiaceae) were collected in February 2009 at the Cerrado region (18°9' South latitute, 47°56' West longitude, at an altitude of 835 m above sea level), in the municipality of Catalão, Goiás State, Brazil. Samples of these plants were deposited at the herbarium of the Institute of Biological Sciences (ICB), at the Federal University of Goiás (UFG), *Campus* Samambaia, in Catalão (Table Nº 1).

Obtaining the crude extracts

Six hundred grams of fresh material (leaves or flowers) of each species of aromatic and medicinal plants were extracted at room temperature, with 1.0 L of ethanol for seven days, and measurement of the yield of extracts after vacuum concentration (Table Nº 1). Unprocessed aromatic and medicinal plant extracts were transferred to 50.0 mL plastic tubes.

Table Nº 1
Plants, yield and record number of six species of aromatic and medicinal plants deposited in the herbarium of the Institute of Biological Sciences of the Federal University of Goiás, *Campus* Samambaia in Catalão, Goiás State, Brazil

Plants	Part	Yield (%)	Register
***Acisanthera* sp.**	**Leaves**	**7.09**	**43249**
Bidens sulphurea	**Flowers**	**10.73**	**43248**
Astilbin from *Dimorphandra mollis*	**Flowers**	**7.60**	**43247**
Lepidoploa aurea	**Leaves**	**3.91**	**29981**
Adenocalymma nodosum	**Flowers**	**6.97**	**43253**
Adenocalymma nodosum	**Leaves**	**8.78**	**43253**
Salvertia convallariaeodora	**Leaves**	**5.48**	**87659**

Extraction of astilbin from flowers of Dimorphandra mollis

The flavonoid astilbin [(2*R*,3*R*)5,7,3',4'-tetrahydroxy-2,3-dihydroflavonol-3-*β*-*O*-rhamnoside] was isolated from *D. mollis* flowers. Six hundred grams of the plant material were extracted three times with dichloromethane, and twice with methanol. The extract was dried under low pressure (2.67 kPa) in a rotating evaporator.

Astilbin was crystallized from the methanolic extract. The purity of this compound was determined by recycle HPLC (polymeric package column, Schimadzu, Asahipak GS-310 P; 21.5 cm ID × 50.0 cm of length, eluded in ethanol, flow rate 7.0 mL.min^{-1}, detector UV in 290 nm) and by its melting point. White astilbin crystals were obtained from the addition of methanol and water, at the ratio of 8:2 in volume, mp. 190 - 191 ºC; APCI +/MS *m/z* = 451 $[M + H]^+$; Spectrums ^1H and ^{13}C NMR Spectra were obtained, $[\alpha]\ ^{25}D = -12.8$ º (*c* 1.0, methanol) (Han *et al.*, 1998; Pereira *et al*., 2002).

Preparation of solutions from crude extracts

The solutions were prepared after the dilution of the extracts with absolute ethanol Merck KGaA until reaching concentrations of 0.1% and 0.01% (w.w^{-1}). These solutions were shaken in a Branson sonicator 2510 for 220 min in SET DEGAS and uniformed with a Fisher Vortex Genie 2™ at speed eight for 10 min (Tavares *et al*., 2009).

Obtaining the eggs of Anticarsia gemmatalis and extracts application

White sheets of paper (size A4) containing one-day-old eggs of *A. gemmatalis* were taken from the breeding cage (30.0 cm length × 30.0 cm width × 30.0 cm height), where adults of this insect are fed with a nutritive solution (10.5 g of honey; 1.05 L of distilled water; 350.0 mL of beer; 60.0 g of sucrose; 1.05 g of ascorbic acid and 1.05 g of nipagin), damped in cotton (Ferreira *et al*., 2008). These sheets were cut and separated into groups with 10 eggs for each piece of paper. Each of these pieces of paper was placed into a 50.0 mL plastic cup. A total of 0.1 mL of each solution, either absolute ethanol or distilled water, was applied over the eggs after one day of collection using

a micropipette. The treated eggs of *A. gemmatalis* were deprived of direct sun light for 2 h to allow alcohol evaporation or drying of water (Tavares *et al*., 2009).

Two grams of solid artificial diet (125.0 g of bean *Phaseolus vulgaris* L. (Fabaceae) grains; 62.4 g of yeast; 100.0 g of wheat germs; 100.0 g of soy protein; 50.0 g of casein; 35.0 g of Agar; 5.0 g of nipagin; 6.0 g of ascorbic acid; 3.0 g sorbic acid; 6.0 mL of formaldehyde at 40.0% and 10.0 mL of vitamin solution) were added to each plastic cup, when necessary, to feed the caterpillars of *A. gemmatalis* (Ferreira *et al*., 2008). Later, these plastic cups were sealed with a PVC film and an elastic band with 30 thin holes for air entrance and placed in polystyrene cup supports with capacity for 36 cups.

Experimental design and data analysis

The experimental design was carried out with 36 treatments and five replications. Each replication was a piece of paper with 10 eggs of *A. gemmatalis*. Treatments were: extracts of *Acisanthera* sp. leaves at 0.1% on one-day-old eggs (T1), *Acisanthera* sp. leaves at 0.01% on one-day-old eggs (T2), *Acisanthera* sp. leaves at 0.1% on two-day-old eggs (T3), *Acisanthera* sp. leaves at 0.01% on two-day-old eggs (T4), *B. sulphurea* flowers at 0.1% on one-day-old eggs (T5), *B. sulphurea* flowers at 0.01% on one-day-old eggs (T6), *B. sulphurea* flowers at 0.1% on two-day-old eggs (T7), *B. sulphurea* flowers at 0.01% on two-day-old eggs (T8), astilbin from *D. mollis* flowers at 0.1% on one-day-old eggs (T9), astilbin from *D. mollis* flowers at 0.01% on one-day-old eggs (T10), astilbin from *D. mollis* flowers at 0.1% on two-day-old eggs (T11), astilbin from *D. mollis* flowers at 0.01% on two-day-old eggs (T12), *L. aurea* leaves at 0.1% on one-day-old eggs (T13), *L. aurea* leaves at 0.01% on one-day-old eggs (T14), *L. aurea* leaves at 0.1% on two-day-old eggs (T15), *L. aurea* leaves at 0.01% on two-day-old eggs (T16), *A. nodosum* flowers at 0.1% on one-day-old eggs (T17), *A. nodosum* flowers at 0.01% on one-day-old eggs (T18), *A. nodosum* flowers at 0.1% on two-day-old eggs (T19), *A. nodosum* flowers at 0.01% on two-day-old eggs (T20), *A. nodosum* leaves at 0.1% on one-day-old eggs (T21), *A. nodosum* leaves at 0.01% on one-day-old eggs (T22), *A. nodosum* leaves at 0.1% on two-day-old eggs (T23), *A. nodosum* leaves at 0.01% on two-day-old eggs (T24), *L. aurea* leaves at 0.1% on one-day-old eggs (T25), *L. aurea* leaves at 0.01% on one-day-old eggs (T26), *L. aurea* leaves at 0.1% on two-day-old eggs (T27), *L. aurea* leaves at 0.01% on two-day-old eggs (T28), *S. convallariaeodora* leaves at 0.1% on one-day-old eggs (T29), *S. convallariaeodora* leaves at 0.01% on one-day-old eggs (T30), *S. convallariaeodora* leaves at 0.1% on two-day-old eggs (T31), *S. convallariaeodora* leaves at 0.01% on two-day-old eggs (T32), distilled water on one-day-old eggs (T33), distilled water on two-day-old eggs (T34), absolute ethanol on one-day-old eggs (T35) and absolute ethanol on two-day-old eggs (T36).

The mortality per group of eggs of *A. gemmatalis* was corrected and the control efficacy of the extracts was evaluated with the Abbott correction (1925) using distilled water and absolute ethanol treatments as control. Abbott (1925):

$$100 \times (Tr - T) \div Tr$$

where:
Tr = mortality in the treatment;
T = mortality in the control.

Later, the data were submitted to ANOVA and the averages were compared by the Tukey test ($P < 0.05$).

Caterpillars at the pre-pupa stage from treated eggs of *A. gemmatalis* were transferred to 500.0 mL plastic cups sealed with a plastic lid full of holes and covered by thin organza. These caterpillars were separated in the plastic cups per treatment and replication. Thin sand, sterilized for eight h in an oven at intensity eight, was placed at the bottom of the cup with the substrate for pupation characteristic of *A. gemmatalis* (Peng *et al*., 1997). Caterpillars submitted to the treatments with astilbin extract from flowers of *D. mollis* or the control with ethanol were also evaluated (Figure Nº 1).

Pupae of *A. gemmatalis* were sexed by the external morphology technique (Conti and Waddil, 1982), with the aid of a table magnifying glass, weighed in a TECKNAL scale, 1.0 g precision, and individualized in 50.0 mL plastic cups with PVC films and elastic bands with holes for air entrance. This material was placed in polystyrene supports with capacity for 36 cups. Data from sex ratio and weight of two-day-old male and female pupae were submitted to ANOVA and the averages were compared by the Tukey test ($P < 0.05$).

Adults of *A. gemmatalis* were placed of PVC pipe cages with bottom sealed by PVC film and elastic bands; the top part was sealed with a plastic plate fixed with three strips of tape and the sides in the inner part of these cages were coated with sheets of A4 white paper. These adults were separated in the cages per treatment and replication. A Petri dish with a lump of

cotton dampened with nutritive solution was place at the bottom of the cage to feed the adults of *A. gemmatalis* (Ferreira *et al*., 2008). Viability data (%) of the stages from egg to caterpillar, caterpillar to pupa and from pupa to adult were submitted to the ANOVA, and the averages were compared by the Tukey test ($P < 0.05$).

Figure Nº 1
Caterpillar of the same age from the eggs treated with astilbin extract from flowers of *D. mollis* (left) or absolute ethanol (control) (right) with topical application to the groups of eggs of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae)

Observational data were not normally distributed and were transformed using a logarithmic transformation before analysis with ANOVA.

RESULTS

The yield of astilbin extract from *D. mollis* flowers (7.60%) was higher than that from leaves of *Acisanthera* sp. (2.20%), flowers of *B. sulphurea* (3.70%), leaves of *L. aurea* (3.91%), flowers (6.97%) or leaves of *A. nodosum* (4.80%), or leaves of *S. convallariaeodora* (3.99%) (Table Nº 1).

The mortality of *A. gemmatalis* eggs was higher with botanical extracts at 0.1% than at 0.01% and higher effects were observed on one-day-old eggs, compared to two-day-old eggs. Astilbin extracts from *D. mollis* flowers or *L. aurea* leaves were the most effective on *A. gemmatalis* eggs. Extracts from leaves of *Acisanthera* sp., flowers of *B. sulphurea*, flowers or leaves of *M. nodosa*, or leaves of *S. convallariaeodora* were not effective at 0.01% on two-day-old eggs of this pest. Flower or leaf extract from *A. nodosum* caused similar toxicity to *A. gemmatalis* eggs ($F = 4.54$, $P < 0.05$, df = 35, 5) (Table Nº 2).

Table Nº 2
Mortality[1] (%) of groups of eggs of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) after being treated by topical application of extracts from Cerrado aromatic and medicinal plants

Treatments	Concentration (%) / age of eggs (days)			
	0.01 / One	0.01 / Two	0.1 / One	0.1 / Two
***Acisanthera* sp.**	50.0 ± 6.0 aA	0.0 ± 0.0 bB	75.0 ± 9.0 aA	66.7 ± 8.0 aA
Bidens sulphurea	50.0 ± 6.0 aA	0.0 ± 0.0 bB	75.0 ± 9.0 aA	50.0 ± 6.0 aA
Astilbin from *Dimorphandra mollis*	80.0 ± 9.6 aA	80.0 ± 9.6 aA	80.0 ± 9.6 aA	80.0 ± 9.6 aA
Lepidoploa aurea	80.0 ± 9.6 aA	75.0 ± 9.0 aA	80.0 ± 9.6 aA	80.0 ± 9.6 aA
***Adenocalymma nodosum* (flowers)**	50.0 ± 6.0 aA	0.0 ± 0.0 bB	75.0 ± 9.0 aA	66.7 ± 8.0 aA
***Adenocalymma nodosum* (leaves)**	50.0 ± 6.0 aA	0.0 ± 0.0 bB	66.7 ± 8.0 aA	66.7 ± 8.0 aA
Salvertia convallariaeodora	50.0 ± 6.0 aA	0.0 ± 0.0 bB	50.0 ± 6.0 aA	50.0 ± 6.0 aA
Distilled water	0.0 ± 0.0 bB	0.0 ± 0.0 bB	0.0 ± 0.0 bB	0.0 ± 0.0 bB
Absolute ethanol	0.0 ± 0.0 bB	0.0 ± 0.0 bB	0.0 ± 0.0 bB	0.0 ± 0.0 bB
CV (%)	32.02			
ANOVA	***F*= 4.54**			

[1]Abbott (1925). Averages followed by the same lower case letter per column or upper case letter per line, do not differ by the Tukey test ($P < 0.05$), CV = Variation Coefficient.

The sex ratio in pupae of *A. gemmatalis* was higher for the botanical extracts of leaves of *Acisanthera* sp., flowers of *B. sulphurea*, astilbin from *D. mollis* flowers or leaves of *S. convallariaeodora*, compared to the extracts made from leaves of *L. aurea*, or flowers or leaves of *A. nodosum* ($F = 4.05$, $P < 0.05$, df = 33, 5) (Table Nº 3).

Table Nº 3
Sex ratio[1] (%) of pupae from the groups of eggs of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) treated with extracts from Cerrado aromatic and medicinal plants

Treatments	Concentration (%) / age of eggs (days)			
	0.01 / One	0.01 / Two	0.1 / One	0.1 / Two
***Acisanthera* sp.**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**
Bidens sulphurea	**0.5 ± 0.0 aA**	**0.5 ± 0.0 aA**	**0.5 ± 0.0 aA**	**0.5 ± 0.0 aA**
Astilbin from *Dimorphandra mollis*	**0.5 ± 0.0 aA**	**-**	**0.5 ± 0.0 aA**	**0.5 ± 0.0 aA**
Lepidoploa aurea	**-**	**0.3 ± 0.0 bB**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**
***Adenocalymma nodosum* (flowers)**	**0.2 ± 0.0 bB**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**
***Adenocalymma nodosum* (leaves)**	**0.9 ± 0.1 aA**	**0.9 ± 0.1 aA**	**0.1 ± 0.0 bB**	**0.7 ± 0.0 aA**
Salvertia convallariaeodora	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**
Distilled water	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**
Absolute ethanol	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**	**0.7 ± 0.0 aA**
CV (%)	**25.67**			
ANOVA	***F*= 4.05**			

[1]Total of females ÷ total of males + total of females. Averages followed by the same lower case letter per column or upper case letter per line, do not differ by the Tukey test ($P < 0.05$). CV = Variation Coefficient.

The weight of male pupae of *A. gemmatalis* was lower for extracts made from flowers of *B. sulphurea*, astilbin from *D. mollis* flowers or leaves of *S. convallariaeodora*, compared to those made from leaves of *Acisanthera* sp., leaves of *L. aurea*, or flowers or leaves of *A. nodosum* ($F = 4.91$, $P < 0.05$, df = 31, 5) (Table Nº 4). On the other hand, the weight in female pupae was lower than the weight in male pupae of *A. gemmatalis*. The weight of female pupae of *A. gemmatalis* was lower for the astilbin extract from *D. mollis* flowers, compared to those from leaves of *Acisanthera* sp., flowers of *B. sulphurea*, leaves of *L. aurea*, flowers or leaves of *A. nodosum*, or leaves of *S. convallariaeodora* ($F = 3.84$, $P < 0.05$, df = 33,6) (Table Nº 4).

The viability of the stage from egg to caterpillar of *A. gemmatalis* was lower for the astilbin extracts from *D. mollis* flowers or leaves of *L. aurea*, compared to those from flowers of *B. sulphurea*, flowers or leaves of *A. nodosum*, or leaves of *S. convallariaeodora*. However, the viability of this stage was lower for extracts from leaves of *Acisanthera* sp. at 0.1% or 0.01% on one-day-old eggs or at 0.1% on two-day-old eggs or extracts of this plant at 0.01% on two-day-old eggs of *A. gemmatalis* ($F = 4.69$, $P < 0.05$, df = 35, 6) (Table Nº 5).

The viability from caterpillar to pupa of *A. gemmatalis* was lower for the extract from leaves of *Acisanthera* sp. or astilbin from *D. mollis* flowers, compared to those from flowers of *B. sulphurea*, leaves of *L. aurea*, flowers or leaves of *A. nodosum*, or leaves of *S. convallariaeodora*. The viability from caterpillar to pupa was only affected by extracts from flowers of *B. sulphurea* on one-day-old eggs of this pest ($F = 5.02$, $P < 0.05$, df = 33, 5) (Table Nº 5).

The viability from pupa to adult on two-day-old eggs of *A. gemmatalis* was affected by extracts from flowers or leaves of *A. nodosum*. However, this stage presented lower viability with extracts at 0.1% from leaves of *Acisanthera* sp. or flowers of *B.*

sulphurea, compared to those at 0.01% of these plants ($F = 3.01$, $P < 0.05$, df = 33, 8) (Table Nº 5).

Table Nº 4
Weight (mg) of two-day-old male or female pupae from the groups of eggs of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) treated with extracts from Cerrado aromatic and medicinal plants

Treatment	Concentration (%) / age of eggs (days)			
	0.01 / One	0.01 / Two	0.1 / One	0.1 / Two
	MALE PUPAE			
Acisanthera sp.	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA
Bidens sulphurea	227 ± 10 bA	227 ± 10 bA	227 ± 10 bA	227 ± 10 bA
Astilbin from *Dimorphandra mollis*	227 ± 10 bA	-	227 ± 10 bA	227 ± 10 bA
Lepidoploa aurea	-	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA
Adenocalymma nodosum (flowers)	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA
Adenocalymma nodosum (leaves)	-	-	253 ± 11 aA	253 ± 11 aA
Salvertia convallariaeodora	227 ± 10 bA	227 ± 10 bA	227 ± 10 bA	227 ± 10 bA
Distilled water	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA
Absolute ethanol	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA	253 ± 11 aA
CV (%)	24.51			
ANOVA	$F = 4.91$			
	FEMALE PUPAE			
Acisanthera sp.	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA	-
Bidens sulphurea	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA
Astilbin from *Dimorphandra mollis*	219 ± 9 bA	-	219 ± 9 bA	219 ± 9 bA
Lepidoploa aurea	-	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA
Adenocalymma nodosum (flowers)	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA
Adenocalymma nodosum (leaves)	239 ± 10 aA	239 ± 10 aA	239 ± 9 aA	239 ± 10 aA
Salvertia convallariaeodora	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA
Distilled water	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA
Absolute ethanol	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA	239 ± 10 aA
CV (%)	24.40			
ANOVA	$F = 3.84$			

Averages followed by the same lower case letter per column or upper case letter per line, do not differ by the Tukey test ($P < 0.05$), CV = Variation Coefficient.

Table Nº 5
Viability (%) from eggs to caterpillar, from caterpillar to pupa and from pupa to adult after topical application of extracts from Cerrado aromatic and medicinal plants to the groups of eggs of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae)

Treatment	Concentration (%) / age of eggs (days)			
	0.01 / One	0.01 / Two	0.1 / One	0.1 / Two
	EGG TO CATERPILLAR			
Acisanthera sp.	40 ± 3 bB	100 ± 7 aA	40 ± 3 bB	40 ± 3 bB
Bidens sulphurea	100 ± 7 aA	100 ± 7 aA	100 ± 7 aA	100 ± 7 aA
Astilbin from *D. mollis*	40 ± 3 bA	40 ± 3 bA	40 ± 3 bA	40 ± 3 bA
Lepidoploa aurea	40 ± 3 bA	40 ± 3 bA	40 ± 3 bA	40 ± 3 bA
A. nodosum (flowers)	65 ± 4 abA	65 ± 4 abA	65 ± 4 abA	65 ± 4 abA
Adenocalymma nodosum (leaves)	65 ± 4 abA	65 ± 4 abA	65 ± 4 abA	65 ± 4 abA
Salvertia convallariaeodora	65 ± 4 abAB	100 ± 7 aA	65 ± 4 abAB	100 ± 7 aA
Distilled water	100 ± 7 aA	100 ± 7 aA	100 ± 7 aA	100 ± 7 aA
Absolute ethanol	100 ± 7 aA	100 ± 7 aA	100 ± 7 aA	100 ± 7 aA
CV (%)	26.74			
ANOVA	*F* = 4.69			
	CATERPILLAR TO PUPA			
Acisanthera sp.	56 ± 1 bA	56 ± 1 bA	56 ± 1 bA	56 ± 1 bA
Bidens sulphurea	82 ± 3 abA	100 ± 4 aA	82 ± 3 abA	100 ± 4 aA
Astilbin from *D. mollis*	82 ± 3 abA	-	82 ± 3 abA	82 ± 3 abA
Lepidoploa aurea	-	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA
A. nodosum (flowers)	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA
Adenocalymma nodosum (leaves)	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA
Salvertia convallariaeodora	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA
Distilled water	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA
Absolute ethanol	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA
CV (%)	24.22			
ANOVA	*F* = 5.02			
	PUPA TO ADULT			
Acisanthera sp.	91 ± 4 aA	91 ± 4 aA	50 ± 2 bB	50 ± 2 bB
Bidens sulphurea	74 ± 3 abAB	74 ± 3 abAB	50 ± 2 bB	50 ± 2 bB
Astilbin from *D. mollis*	74 ± 3 abA	-	74 ± 3 abA	74 ± 3 abA
Lepidoploa aurea	-	83 ± 3 aA	83 ± 3 aA	83 ± 3 aA
A. nodosum (flowers)	74 ± 3 abAB	50 ± 2 bB	74 ± 3 abAB	50 ± 2 bB
Adenocalymma nodosum (leaves)	100 ± 4 aA	74 ± 3 abAB	100 ± 4 aA	74 ± 3 abAB
Salvertia convallariaeodora	91 ± 4 aA	91 ± 4 aA	91 ± 4 aA	91 ± 4 aA
Distilled water	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA
Absolute ethanol	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA	100 ± 4 aA
CV (%)	24.72			
ANOVA	*F* = 3.91			

Averages followed by the same lower case letter per column or upper case letter per line, do not differ by the Tukey test ($P < 0.05$), CV = Variation Coefficient.

DISCUSSION

The yield from extracts of Cerrado plants were 2.08% to 5.80% ($m.m^{-1}$) of the extracts from 12 species of the Asteraceae family from the Cerrado in the state of Minas Gerais, Brazil (Tavares *et al*., 2009). *Piper tuberculatum* Jacq. (Piperaceae), from the Amazon, presented an extract yield of 4.45% ($m.m^{-1}$) (Navickiene *et al*., 2007), corroborating the efficacy of the method of botanical extract manufacture. The yield of astilbin extract from flowers of *D. mollis* collected in Goiás State, Brazil was similar to that of the extract of the same plant collected from Rio Claro and Corumbataí, São Paulo State, Brazil which demonstrates that *D. mollis* has maintained its extract yield, regardless of where it was collected.

The highest efficacy of the botanical extracts at 0.1% confirms the need for the use of higher concentrations of these extracts on Lepidoptera (Hoffmann-Campo *et al*., 2006; Navickiene *et al*., 2007; Baskar *et al*., 2010). The fact that two-day-old eggs of *A. gemmatalis* were less affected agrees with the results for the older eggs of *Diatraea saccharalis* F., 1794 (Lepidoptera: Pyralidae) and *S. frugiperda*, which have a more impermeable external coating that hindered the penetration of the botanical extracts (Tavares *et al*., 2009; Tavares *et al*., 2010a). The highest efficacy of astilbin extracts from in high mortality of caterpillars of *A. gemmatalis* and *S. frugiperda* when a diet contaminated by them was used (Pereira *et al*., 2002). Extracts with the lowest insecticidal efficacy, including those from *Acisanthera* sp., *B. sulphurea*, *A. nodosum* and *S. convallariaeodora*, should not be discarded, but evaluated for different concentrations of solvents, extraction methods, extract concentration and edaphoclimatic and genetic factors of the plant (Nascimento *et al*., 2003; Sidhu *et al*., 2004; Khalil *et al*., 2006). Similar toxicity levels in extracts from flowers or leaves of *A. nodosum* suggest a similar chemical composition in both parts of this plant. On the other hand, extracts from seeds of *P. tuberculatum* were more toxic to *A. gemmatalis* than the stem or leaves of this plant, suggesting that chemical composition may vary in different parts of this plant (Navickiene *et al*., 2007).

The highest sex ratio in pupae of *A. gemmatalis* for botanical extracts from leaves of *Acisanthera* sp., flowers of *B. sulphurea*, astilbin from flowers of *D. mollis* or leaves of *S. convallariaeodora* is in agreement with the higher number of males (1.2:1 - males: females) of *Carmenta theobromae* Busck, 1910 (Lepidoptera: Sesiidae) (Morillo *et al*., 2009) or *Heliconius charithonia* L., 1767 (Lepidoptera: Nymphalidae) (68% males) (Fleming *et al*., 2005). On the other hand, lower sex ratio in pupae of *A. gemmatalis* for the extract from flowers or leaves of *A. nodosum*, or leaves of *L. aurea* agrees with reports for *Ascia monuste monuste* L., 1764 (Lepidoptera: Pieridae) (0.76:1 - males:females) with cabbage *Brassica oleraceae* L. (Brassicaceae) as food (Liu, 2005). The lower proportion of males of *A. gemmatalis* may contribute for reducing the possibility of mating and increasing the total of infertile postures.

Weight reduction in pupae of *A. gemmatalis* by the use of astilbin extract from flowers of *D. mollis* is in agreement with the lower weight of pupae of *Plutella xylostella* L., 1758 (Lepidoptera: Plutellidae) after feeding with extracts from seeds of *Peganum harmala* L. (Zygophyllaceae) (Abbasipour *et al*., 2010) and *A. gemmatalis* with rutin from soy plants (Salvador *et al*., 2010). The weight of pupae of *Helicoverpa armigera* Hübner, 1805 (Lepidoptera: Noctuidae) was reduced from 68.2 mg to 41.5 mg with the use of extracts from seeds of *Catharanthus roseus* L. (Apocyanaceae), suggesting the urgency in smaller adults and less reproductive, which would reduce the total lineage and damage to crops (Satyan *et al*., 2009).

The lowest viability from egg to caterpillar of *A. gemmatalis* for astilbin extracts from flowers of *D. mollis* or leaves of *L. aurea* reveals their continuous effect on the larval stage of this pest. However, astilbin extracts were toxic for defoliating caterpillars (Pereira *et al*., 2002) and reduced the population of non-targeted organisms (worker bees and ants) (Cintra *et al*., 2002; Cintra *et al*., 2005a; Cintra *et al*., 2005b), suggesting that this compound should be used with caution. The lowest viability from egg to caterpillar of *A. gemmatalis* for extracts from leaves of *Acisanthera* sp. only at 0.01%, on two-day-old eggs of this pest suggests that the resilience of these older eggs can make the penetration of these extracts difficult.

The lowest viability from caterpillar to pupa of *A. gemmatalis* for astilbin extracts from flowers of *D. mollis* is similar to that reported for neonatal caterpillar of *A. gemmatalis* after feeding with methanolic or hydroalcoholic extracts of *Siphoneugena densiflora* Berg (Myrtaceae) or flowers or fruits of *Vitex polygama* Cham. (Verbenaceae) (Gallo *et al*., 2006) or with substances of *Aristolochia pubescens* Willd (Aristolochiaceae) applied to their

mesothorax (Nascimento *et al*., 2003). The lowest viability from caterpillar to pupa in subjects from one-day-old eggs treated with extracts from flowers of *B. sulphurea* suggests greater resistance from two-day-old eggs to this pest, corroborating the studies on extracts from 12 species of plants from this family, besides neem *Azadirachta indica* A. Juss. (Meliaceae) and pyroligneous extract on *D. saccharalis* and *S. frugiperda* (Tavares *et al*., 2009; Tavares *et al*., 2010a).

The lowest viability from pupa to adult after treatment of two-day-old eggs of *A. gemmatalis* with extracts from flowers or leaves of *A. nodosum* suggests the recovery of the one-day-old eggs after being treated with these extracts. It corroborates the idea that concentrated botanical products are more toxic to insects than the more diluted ones (Hoffmann-Campo *et al*., 2006; Navickiene *et al*., 2007; Baskar *et al*., 2010). The toxic effects from flowers of *B. sulphurea* on the biological parameters of *A. gemmatalis* agree with the reports of insecticidal activities of plants of the Asteraceae family, such as *Chrysanthemum* spp. (Asteraceae), probably due to the presence of pyrethrin in its composition (Tavares *et al*., 2009). These substances have originated the synthetic pyrethroids, which are not toxic to mammals (Costa *et al*., 2008; Wolansky and Harrill, 2008; Weiner *et al*., 2009).

CONCLUSION

In conclusion, this research demonstrates that astilbin extracts from flowers of *D. mollis* and leaves of *L. aurea* have caused a great impact on the biological aspects of *A. gemmatalis*. Therefore, they could be used in future studies for pest management programs after the non-targeted organisms have been evaluated.

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