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Effect of extracts of *Trichilia silvatica* C. DC., on development and reproduction parameters of *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)

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The objective of this study was to evaluate the effects of the methanolic extracts from the leaves, bark and flowers of *Trichilia silvatica* on *Spodoptera frugiperda*. Also, it was use in evaluating the total phenolic and flavonoid content of methanolic extracts. We also reported chemical study on the most active extract. Corn leaves were immersed in a 1% methanolic extract solution and fed to second instars of *S. frugiperda*. The extract of the *T. silvatica* (LTS) leaves decreased the viability of the larva, prolonged larval duration, affected the pupal biomass, decreased the period of oviposition and the number of eggs as well as affected the egg viability. The methanolic extract of the *T. silvatica* (BTS) bark decreased the larval viability, oviposition period, number of eggs and egg viability. The flower extract of *T. silvatica* (FTS) decreased the larval viability and period of oviposition. In relation to the constituent contents, the methanolic extract of the leaves showed highest total phenol (233.37 mg gallic acid/g of extract) and flavonoid (53.17 mg quercetin/g of extract) content. The chemical study of the FTS resulted in α-tocopherol, sitosterol 3-O-glucopyranoside, mustakone and N-metilproline. Our results indicate that the extracts affected the biology of *S. frugiperda*, with LTS being the most promising.

Key words: Meliaceae, methanolic extracts, insecticides, plant-derived compounds.

INTRODUCTION

The *Trichilia* genus consists of about 70 species, mainly distributed in tropical America and Africa, of which 43 species occur in Brazil. Chemical investigations have revealed the presence of limonoids as the main bioactive

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Abbreviations: LTS, Leaves of *Trichilia silvatica*; **BTS,** bark of *Trichilia silvatica*; **FTS,** flower of *Trichilia silvatica*.

agents (Champagne et al., 1992). The limonoids of Meliaceae are very complex with a high degree of oxidetion and rearrangement exhibited in the parent limonoid structure. Compounds belonging to this group express a wide range of biological activities like insecticidal deterrence, insect antifeedant and growth regulating capacity on insects (Champagne et al., 1989; Kubo and Klocke, 1982; Mikolajczak and Reed, 1987; Mikolajczak et al., 1989; Nakatani et al., 1981; Simmonds et al., 2001; Xie et al., 1994) as well as antiviral and analgesic, and several other pharmacological effects on humans (Romin et al., 1992; Vaz et al., 1997).

Biological studies conducted on plant extracts of *Trichilia connaroides*, *Trichilia prieureana*, *Trichilia roka*, *Trichilia triphyllaria*, *Trichilia casaretti, Trichilia catigua*, *Trichilia claussenii*, *Trichilia elegans and Trichilia pallid* demonstrated insecticidal activity on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Bogorni and Vendramim, 2003; 2005; Mikolajczak and Reed, 1987; Roel and Vendramim, 1999; Roel et al., 2000a; 2000b). Some previous studies have focused on nortriterpenoids (limonoids) from the family Meliaceae because of their potent effects on insect pests linked to low toxicity (Carpinella et al., 2002; 2003; Champagne et al., 1989; Klocke et al., 1989; Kubo and Klocke, 1982).

Trichilia silvatica C. DC., is a small evergreen tree occurring in Brazil, commonly known as "*cachuá*", "*cachuá-blank*" in Santa catarina and "*rosa-blank*" in Bahia, Brazil. Chemical studies performed on the extracts of the *T. silvatica* leaves described the isolation of the sesquiterpenes spathulenol, veridiflorol, humulene oxide, (2*S*,3*S*,6*R*,7*R*)-humulene-2,3;6,7-diepoxide, (2*R*,3*R*,6*R*,7*R*)-humulene 2,3;6,7-diepoxide, mustakone, the steroid, and the triterpenes*α*-amyrin, *β*-amyrin, pseudotaraxasterol and lupeol (Soares et al., 2013). The extract *n*-butanol of leaves demonstrated growth inhibition of *Streptococus salivarius* and *Streptococus mutans* (Figueiredo, 2010). Recently, we reported the chemical composition, relating to the anti-edematogenic and antioxidant activity of the essential oil from the *T. silvatica* leaves in particular, being characterized by the GC-MS high number of sesquiterpenes, which exhibited antioxidant and *in vivo* anti-inflammatory effects (Formagio et al., 2012).

Although the literatures show several studies about the limonoids isolation and insecticidal effect of different species of *Trichilia* species, there is no investigation of the effect of *T. silvatica* on *S. frugiperda*. Thus, a search for new plant-derived extracts to develop alternatives to conventional insecticides and reduced negative impacts on health and the environment, we continue our investigation of this plant.

The main goal of the present study was to evaluate the total phenolic and flavonoid content and the effect of the methanolic extract of the leaves, bark, and flowers of *T. silvatica* on the development and survival of *S. frugiperda.* The active extract was also investigated and the chemical composition was reported with the use of chromatographic methods. The compounds thus isolated were characterized by NMR spectral data and compared with those reported in the literature.

MATERIALS AND METHODS

Plant materials

The leaves, bark and flowers of *T. silvatica* were collected in May 2012 from Dourados (22°14′16″S E 54°48′02″W, average elevation of 452 m), in the southern part of Mato Grosso do Sul State, Brazil. The species were identified by Dra. ZefaValdevina Pereira, and the voucher of *T. silvatica* (DDMS 4662) was deposited at the Herbarium of the Federal University of Grande Dourados, Dourados, MS.

Preparation of crude extracts, fraction and isolation of the constituents

The dried plant materials (leaves, bark and flowers) were successively extracted via maceration with methanol. The extract was then filtered, concentrated under pressure in a rotoevaporatorat and lyophilised to obtain methanol crude dry extract from the leaves (LTS), bark (BTS) and flowers (FTS). The most active extract in the development and survival of *S. frugiperda*, was dissolved in MeOH-H_2O 1:1 and separated with *n*-hexane, chloroform and ethyl acetate, which was then tested for insecticidal activity. The solvent was evaporated to give the hexane (5.5 g), chloroform (5.4 g), ethyl acetate (6.2 g) and hydromethanol (7.5 g) fractions. The hexane fraction (2.58 g) was purified on chromatographic column of silica gel, eluting with a mixture of *n*-hexane: ethyl acetate in increasing polarity, to afforded α-tocopherol (6.2 mg). The chloroform fraction (3.8 g) was placed in a chromatographic column on silica gel and eluted with a mixture of *n*-hexane, ethyl acetate and methanol in increasing polarity, to give sitosterol 3-*O*-glucopyranoside (145 mg) and mustakone (14.5 mg). The hydromethanol fraction (3.4 g) was purified on Sephadex LH-20 (25 g) using H_2O, H_2O-MeOH (1:3, 1:1) and MeOH, affording N-prolinebetaine (17 mg).

Determination of total phenol content

The total phenolic content in the samples, methanol extract leaves crude (LTS), bark (BTS) and flowers (FTS), was determined using the Folin-Ciocalteu method (Meda et al., 2005). Specifically, 100 µL of samples in methanol (1 g/L) was mixed with 1.0 mL of distilled water and 0.5 mL of Folin-Ciocaleu's (1:10 v/v) reagent. After 3 min, 1.5 mL of a saturated solution of Na_2CO_3 (2%) was added. After 30 min, the absorbance was measured at 765 nm using a spectrophotometer. Quantification was carried out using a standard curve of gallic acid prepared in 80% methanol, and the results were expressed in milligrams of gallic acid equivalent per gram of extract. The equation for the gallic acid curve was y= 0.1073x + 6.2733 with a correlation coefficient of R =0.9912. The methanol solution was used as a blank. All of the assays were carried out in triplicate.

Determination of total flavonoids

To determine the level of flavonoids, 500 µL of samples (methanol extract leaves crude (LTS), bark (BTS) and flowers (FTS) was mixed with 1.50 mL of 95% ethanol, 0.10 mL of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.10 mL of acetate sodium ($NaC_2H_3O_2.3H_2O$)

(1 M) and 2.80 mL of distilled water. The tubes were maintained at room temperature for 40 min. The optical density was measured at 415 nm using a spectrophotometer. The same procedure was used for the analysis of the blank (Lin and Tang, 2007). To calculate the concentration of flavonoids, we prepared a calibration curve (2.5, 5.0, 10.0, 20.0, 25.0, 50.0, 100.0 and 125.0 µg) using quercetin as the standard. We used these data to generate a linear regression model, and an equation for the line was obtained and used for the calculation of the experimental samples. The results are expressed in milligrams of quercetin equivalents per gram of extract. The equation of the quercetin curve was y= 0.04372x + 11.8202 with a correlation coefficient of R = 0.9989. All of the assays were carried out in triplicate.

Insects

Larvae of *S. frugiperda* were obtained by rearing on an artificial diet in the Laboratory of Entomology of Faculdade de Ciências Biológicas e Ambientais (FCBA), according to the methods described by Parra (2001).

Effect of extracts on the development of *S. frugiperda*

Aqueous solutions (1%) were prepared and used in assays according to the method of Roel and Vendramim (1999). For better solubilization, 9.4 µl of Tween 80, per 100 ml of distilled water, was added to the solution. Hybrid maize leaves XB 6012 (about 28 cm^2) aged 50 to 60 days were immersed in the prepared solution for approximately 2 s and then maintained under ambient conditions for the evaporation of excess fluid, per the method used by Bogorni and Vendramim (2003). Corn leaves treated with extracts and distilled water were placed in Petri dishes (90 × 15 mm) containing a second instar larvae of *S. frugiperda* per plate. We opted for the second instar because the first instar was more sensitive to a variety of factors that caused mortality. The leaves were replaced daily with new leaves treated with 1% aqueous solution per the instructions of Roel and Vendramim (1999). The larval viability (percentage of larvae that reached the pupal stage), larval duration (duration of the larval stage in days), pupal viability (percentage of pupae that reached adulthood), pupal duration (duration of the pupal phase in days), and pupal biomass (weight in milligrams) were evaluated in the bioassays. The experimental design was completely randomized with four treatments (three plant species and the control) and five replications, each consisting of 10 larvae, totaling 50 larvae per treatment (Figure 3).

Effect on the reproductive stage

Adults from larvae previously treated during the larval stage were used to assess the following parameters: pre-oviposition period (duration in days from adult emergence of the female until the first day of oviposition), oviposition period (duration in days from the first day until the last day of oviposition), post-oviposition period (duration in days of the last day of oviposition until mortality of the female), total number of eggs per female (number of eggs laid during the oviposition period of a female), egg viability (percentage of hatched larvae), incubation period (number of days from egg laying to hatching of eggs), and adult longevity (duration in days from emergence to death for males and females). The experimental design was completely randomized with four treatments (three plant species and the control) and five replications per treatment, each mating pair of *S. frugiperda* was considered as a replication.

Data analyses

The data were expressed as means ± standard error of mean (SEM). Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by the *Tukey's* test. The differences were considered statistically significant when $P< 0.05$.

RESULTS

Crude extracts, total phenolic and flavonoids content

By maceration, the leaves, bark and flowers of *T. silvatica* produced the highest yield with 27.60, 44.34, and 15.33% of efficiency, respectively. Figure 1 shows the total phenolic and flavonoid methanolic extracts of *T. silvatica*. The extracts of the leaves and bark, on analysis, showed the highest total phenolic content with values of 233.37 and 177.62 mg gallic acid/ g of extract, respectively (Figure 1). By comparison, the extracts also presented a higher content of flavonoids (leaves, 53.17 mg quercetin/ g from the extract; bark, quercetin/41.13 mg/ g from the extract).

Isolation of the constituents of active extract

The chemical study of the hexane, chloroform and hydro-methanol fraction, resulting to liquid-liquid partitioning of methanolic extract of leaves at the time it resulted in α-tocopherol, sitosterol 3-*O*-glucopyranoside, mustakone and N- methylproline. The compounds were identified by comparison of their NMR data with those reported in the literature (Alam et al., 1996; Ayres et al., 2009; Daniewski et al., 1996; Nyasse et al., 1988). α-Tocopherol: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.62 (t, J=6.7 Hz, 2H), 2.20 (s, 3H), 2.15 (s, 6H); ^{13}C NMR (75.5 MHz): δ146.5, 143.5, 122.9, 121.6, 118.4, 117.4, 74.8, 39.8, 39.4, 37.5, 32.8, 31.5, 28.0, 24.8, 24.4, 23.9, 22.7, 21.5, 20.6, 19.7, 12.5, 11.8, 11.3. sitosterol 3-*O*-glucopyranoside: ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.37 (d, J=5.1Hz, 1H); 4.41 (d, J=7,5Hz, 1H), 2.41 (dd, J=12.3 e 4.1Hz), 1.02 (s, 3H), 0.69 (s, 3H). ^{13}C NMR (75.5 MHz): δ 140.1, 122.0, 100.9, 78.9, 77.9, 77.1, 75.6, 69.8, 61.5, 56.5, 55.8, 50.0, 45.6, 42.1, 39.5, 38.4, 37.0, 36.5, 35.9, 33.7, 31.6, 31.4, 29.3, 28.9, 28.0, 25.8, 24.0, 22.9, 20.9, 19.5, 19.0, 18.7, 18.5, 11.6, 11.4. mustakone: ^{1}H NMR (300 MHz, $CDCl_3$): δ2.63 (dd, J=6.5 and 1.5, 1H), 5.76 (m, J=1.5, 1H), 1.94 (d, J=6,5 e 1.5, 1H), 2.60 (s, 1H), 1.64 (m, 1H), 1.50 (m, 1H), 1.73 (m, 2H), 1.85 (m, 1H), 1.73 (m, 1H), 1.54 (dd, J=6.5 and 6.5, 1H), 0.86 (d, J=6.5, 3H), 0.85 (d, J=6.5, 3H), 2.00 (d, J=1.5, 3H), 0.97 (s, 3H). ^{13}C NMR (75.5 MHz): δ56.9, 203.4, 120.8, 171.4, 55.8, 54.7, 45.6, 22.8, 35.9, 57.8, 32.4, 19.6, 20.6, 20.5, 24.3. N-methylproline: ^{1}H NMR (300 MHz, D_2O): δ 2.93, s, CH_3N+; 3.91 (dd, J= 11:5 and 7.5 Hz, 1H), 2.55 (m, 1H), 1.94-2.24 (m, 3H), 3.74 (m, 1H), 3.16 (m, 1H). ^{3}C NMR (75.5 MHz): δ 43.9, CH_3N+; 73.6, 25.9, 31.8, 58.8,

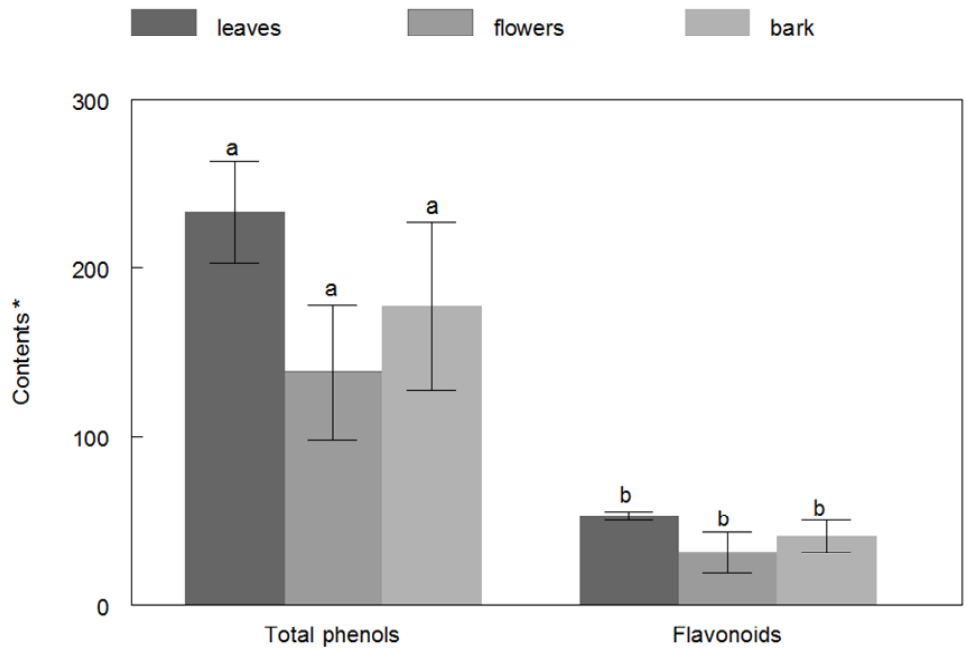

Figure 1. Total phenoic and flavonoid contents of different *T. silvatica* parts. *Total phenolic were expressed by mg GAE/g of extract and flavonoid contents were expressed by mg QE/g of extract. Data are given as mean ± SD (n=3). The data marked with the different lower case letters in the histograms of each category share significant differences at $p < 0.05$ (Tukey test).

Table 1. Mean (+SE) biomass of pupae, duration and viability of the larval and pupal stages of *S. frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) fed on corn leaves treated with methanol extracts of medicinal plants of the family meliaceae. temp.: 25 ± 1°C, RH: 70 ± 10% and photoperiod: 14 h.

Parameter	Pupal biomass (mg)	Larval duration (day)	Larval viability (%)	Pupalduration (day)	Pupalviability (%)
Control	198.80±2.67^{a} *n*=47	16.57±0.13^{b} *n*=50	94.50±0.93^{a} *n*=50	10.11±0.11^{ab} *n*=47	93.77±2.46^{a} *n*=47
LTS	104.00±6.95^{b} *n*=12	20.34±0.49^{a} *n*=50	24.00±6.78^{c} *n*=50	10.90±0.61^{ab} *n*=12	60.00±18.70^{a} *n*=12
BTS	183.13±11.90^{a} *n*=16	17.04±0.72^{b} *n*=50	32.00±4.89^{bc} *n*=50	9.30±0.91^{b} *n*=16	61.99±3.26^{a} *n*=16
FTS	201.78±7.31^{a} *n*=27	16.74±1.07^{b} *n*=50	54.00±10.77^{b} *n*=50	11.90±0.63^{a} *n*=27	86.06±11.08^{a} *n*=27

Different letters in the same column differ at 5% probability by *Tukey* test. SE = standard error. *N* = number of insects.

177.5. However, the isolation of N-metilproline is reported for the first time in *T. silvatica*.

Development and reproductive stage of the *S. frugiperda*

Larval viability was affected in all the treatments (LTS 24.00±6.78, BTS 32.00±4.89 and FTS 54.00±10.77%) when compared with the control (94.50±0.93%). The LTS also prolonged the duration of the larval stage (Table 1). The duration of the pupal phase and viability revealed no significant difference between the treatments and the control (Table 1). The *S. frugiperda* larvae fed on the corn leaves dipped in the LTS solution showed a lower average pupal biomass (104.00±6.95 mg) when compared with the control (198.80±2.67 mg). The other treatments

Table 2. Mean (+ SE) of pre-oviposition, oviposition and post-oviposition, number of eggs, period of incubation and egg viability and longevity of females and males of adult *S. frugiperda* (J.E Smith) (Lepidoptera: Noctuidae). temp.: 25 ± 1°C, RH: 70 ± 10% and photoperiod: 14 h.

	Pre-oviposition (day)	Oviposition (day)	Post-oviposition (day)	Number of eggs (unit)	Incubation period (day)	Eggviability (%)	Longevity of females (day)	Longevityof male (days)
Control (*n*=5)	4.00±0.54^{a}	6.80±0.40^{a}	2.10±0.71^{a}	939.99±214.75^{a}	3.24±0.18^{a}	87.79±2.57^{a}	10.99±0.59^{a}	9.33±0.69^{a}
LTS (*n*=4)	4.00±1.00^{a}	3.50±1.50^{b}	1.50±0.50^{a}	87.50±50.51^{b}	3.30±0.30^{a}	6.25±6.25^{b}	7.25±1.03^{a}	6.00±0.81^{a}
BTS (*n*=3)	6.00±0.00^{a}	1.50±0.76^{b}	1.33±0.33^{a}	124.66±32.63^{b}	3.75±0.38^{a}	-	8.33±0.88^{a}	8.00±1.52^{a}
FTS (*n*=5)	7.00±1.00^{a}	1.37±0.55^{b}	2.00±0.00^{a}	358.80±110.44^{ab}	4.33±0.46^{a}	46.59±22.09^{ab}	9.20±1.35^{a}	7.00±0.94^{a}

Different letters in the same column differ at 5% probability by *Tukey* test. SE = standard error. *n*= number of couples of *S. frugiperda*. -, Data were not sufficient to conduct the analysis due to insect mortality.

Figure 2. Effect of extract of *T. silvatica* on adults of *S. frugiperda.*

showed no statistical difference with respect to the biomass of the pupae compared with the control (Table 1). The leaf, bark and flower extracts of *T. silvatica* also adversely affected the biological characteristics of the *S. frugiperda* adults. The oviposition period was reduced in length (LTS 3.50±1.50; BTS 1.50±0.76; FTS 1.37±0.55 days), when compared with the control (6.80±0.40 days). The periods of pre-oviposition and post-oviposition, however, were not affected by the extracts (Table 2). The *S. frugiperda* females oviposited less numbers of eggs in the BTS and LTS treatments (Table 2). These eggs revealed reduced viability when compared with the control (Table 2). Female and male longevity as well as the incubition period of the eggs was statistically similar in all the treatments compared with the control (Table 2). In adulthood, deformation was also observed in moths, especially in their wings (Figure 2).

DISCUSSION

Effect of the *T. silvatica* extracts on development and reproductive stage of the *S. frugiperda*

The extracts of the different plant parts of *T. silvatica* reduced the larval viability of *S. frugiperda*. Such results have to confirm the insecticidal activity of this genus ever reported for *S. frugiperda* and other insects (Bogorni and Vendramim, 2003; Bogorni and Vendramim, 2005; Conceschi et al., 2011; Cunha et al., 2006; 2008; Mikolajczak and Reed, 1987; Nebo et al., 2010; Roel et al., 2000b; Thomazini et al., 2000; Wheeler and Isman, 2000). Reports regarding the biological activities of the substances and / or extracts from plants of this genus, such as "antifeedant" and insecticidal properties, comparable to azadirachtin are available (Bogorni and Vendramim, 2005; Cunha et al., 2006; Mikolajczak and Reed, 1987).

The LTS was found to affect not only the survival of *S. frugiperda*, but also other biological characteristics, such as the prolonged duration of the larval phase (20.34±0.49 days) when compared with the control (16.57±0.13 days). This could be related to the presence of the compounds that interfered in metamorphosis, not only causing mortality but also hindering the development of the surviving larvae (Bogorni and Vendramim, 2005). The *Trichilia T. pallens*, *T. casaretti* and *Trichilia pallida* leaves and *T. pallid* branches also induced significant lengthening of the larval stage of *S. frugiperda* (Bogorni and Vendramim, 2005). The prolonged stage of the larval may be associated with the effect antifeedant reported in the

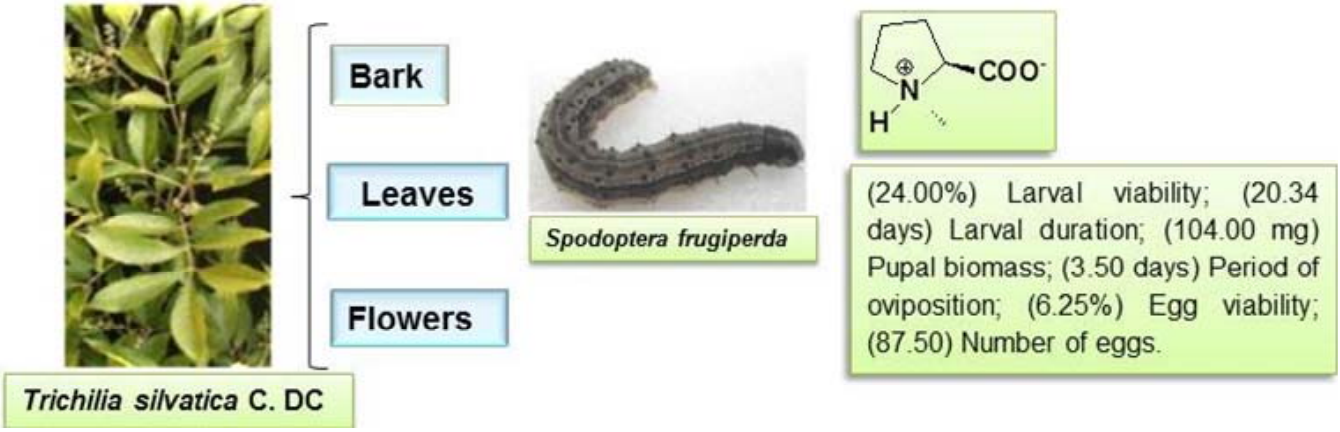

Figure 3. The larval viability (percentage of larvae that reached the pupal stage), larval duration (duration of the larval stage in days), pupal viability (percentage of pupae that reached adulthood), pupal duration (duration of the pupal phase in days), and pupal biomass (weight in milligrams) of *Spodoptera frugiperda.*

Trichilia genus (Mikolajczak and Reed, 1987), and in compounds family Meliaceae (Mossini and Kemmelmeier, 2005). Antifeedants can be described as allomone substances which inhibit feeding and do not kill the insect pests directly, but rather limit its developmental potential considerably (Gokulakrishnan et al., 2012).

Torrecillas and Vendramim (2001) also reported that the branches of *T. pallida* 0.1% affected not only the survival but also the duration of the larval stage of *S. frugiperda.* This fact is attributed to the presence of the growth inhibitors or toxic substances in the extract and emphasize that theelongation of the larval stage is important in the field, as it can increase the exposure time of the insect to natural mortality factors (Torres et al., 2001; Alves et al., 2012). McMillian et al. (1969) observed growth inhibition in *S. frugiperda* by the use of plant extracts as well as reported the prolongation of the larval stage on an artificial diet containing achloroform extract from the *Melia azedarach* leaves. The pupal viability and duration of the stage were not affected by the extracts (LTS, BTS and FTS). Other authors attribute the lower toxicity of chemical agents to the pupa stage to the lower activity of the insect in such stage (Jbilou et al., 2006). Probably, the effect of the plant extracts is felt more intensely in the larval stage because it is at this stage that the insect ingests the chemicals present in the food (Rodríguez and Vendramim, 1996). Maroneze and Gallegos (2009) also found no negative effects of *M. azedarach* on the duration and viability of the pupal stage of *S. frugiperda*. The results concur well with those obtained by Bogorni and Vendramim (2005); Rodríguez and Vendramim (1996, 1997), working with the *Trichilia* species, also found no negative effect of the extracts on the pupal stage of *S. frugiperda*. The biomass of the pupae was reduced (104.00±6.95 mg) in the LTS treatment when compared with the control (198.80±2.67 mg). The biomass of the pupae is directly related to the performance of the insect in the larval stage, increasing the leaf consumption by the larvae, the pupae also increases biomass (Lima et al., 2006); however, in some extracts, the feed inhibitor compounds are present which limit the food intake during the larval stage and thus reduce the pupal biomass (Carpinella et al., 2002; Huang et al., 1996).

The *T. silvatica* extracts also interfere with the biological characteristics of the *S. frugiperda* female adults. The oviposition period was reduced in all treatments (with LTS, BTS and FTS). Probably, the plant species possess substances capable of interfering in the reproduction of insects. A similar result was described for the extract of *M. azedarach*, which reduced the oviposition period and the fertility of *S. littoralis* females (Schmidt et al., 1997). *Azadirachta indica*, considered the most promising plant species in pest control, has substances capable of interfering with the reproduction of insects, either by reduction of the fecundity or complete sterilization (Schmutterer, 1990).

The number of eggs per female was also reduced by treatment with LTS and BTS. According to Costa et al. (2004), the quantity and quality of the nutrients obtained during larval feeding can influence the number of ovarioles per ovary and, by extension, reduce the potential for egg production, that is, larvae which consume protein-rich diets produce heavier pupae and adults, which in turn produce more eggs than those insects which fed on poor diets. The results of the LTS concur with this fact, the larvae fed on treated corn leaves, if fed less than the control, would yield smaller pupae and adults which produce fewer eggs. Reducing the number of eggs and inhibiting oviposition are important effects that plant extracts exert on insect reproduction (Costa et al., 2004). The reduction in the egg viability induced by LTS and BTS demonstrates that the extracts may have a transovarial action (Pratissoli et al., 2004). In the field, reduced egg

viability results in a significant reduction in the damage to the maize crop (Maroneze and Gallegos, 2009).

The malformation in adults was also observed with others species of the *Trichilia*. Bogorni and Vendramim (2005) found that when the aqueous extracts of the *Trichilia* branches and leaves were applied to the corn leaves fed to the larvae, they affected not only the larval and pupal stages, but also the emergence of adults. The main defect observed by them in the *S. frugiperda* adults was the malformation of the wings and antennae. Considering the parameters evaluated during the various developmental stages of the pest (Tables 1 and 2), all the extracts (LTS, BTS and FTS) were seen to affect the parameters of the juvenile and adult stages of *S. frugiperda*. However, based on the results obtained, it was concluded that the methanolic extract from the *T. silvatica* leaves is the most promising for use in the control of *S. frugiperda*. The effect of the extracts varies according to the plant part used in their preparation (Alves et al., 2012). This result is in accordance with the described metabolic variation between plant parts (François et al., 2009). Gobbo-neto and Lopes (2007) reported that plant parts can not only influence the total quantity of the metabolites produced, but also the relative proportions of the components of the mixture.

Constituents of the *T. silvatica*

The chemical composition of the most active extract of *S. frugiperda* was exploited in order to elucidate the main secondary metabolites present in the extract of *T. silvatica* collected in Dourados-MS and correlate with the effect observed from *T. silvatica*. Future studies to report the effect of sitosterol 3-O-glucopyranoside, tocopherol, mustakone and N-metilproline isolated compounds in development and reproductive stage of the *S. frugiperda* is in process. In the literature, the sitosterol 3-*O*-glucopyranoside, tocopherol, mustakone and N-metilproline isolated compounds were not evaluated with insecticidal activity. Sterols and triterpenes have an important biological function as key compounds in the acquirement of cholesterol by insects. Mammals obtain cholesterol either by dietary absorption or by biosynthesis from mevalonate. Based on the fact that insects have no capacity for de novo sterol synthesis, they rely exclusively on exogenous sources for their normal growth, development and reproduction (Ikekawa et al., 1993).

Flavonoids and phenolic compounds in plants exert protection against ultraviolet rays, and protection against insects, viruses and bacteria, while they attract the pollinators, reveal antioxidant activity, are allelopathic agents and express enzyme inhibition (Zuanazzi and Montanha, 2004). In insects, the flavonoids interfere in reproduction; feeding and behavior stimulate oviposition or act as feeding deterrents (Bernays et al., 1991; Harborne and Grayer, 1994; Matsuda, 1978; Morimoto et al., 2000; Musabyimana et al., 2001; Reyes-Chilpaet al., 1995; Simmonds, 2001). The contents of flavonoids and phenolic compounds reported in the leaves from *T. silvatica* may have promoted the insect growth inhibition. Several studies have highlighted the dual nature of specific flavonoids as both pest-feeding deterrents and stimulants.

Our study demonstrates the effect of the *T. silvatica* on growth inhibition of *S. frugiperda* and the presence of the metabolites in leaves extract, for the moment demonstrate the possible metabolites responsible for activity. Based on the results, we suggest that insect growth inhibition caused of leaves extract could be attributed mainly to flavonoids and phenolic compounds. However, studies of the isolated compounds and of polar fractions (ethyl acetate and hydromethanolic) are in progress to verify development of the *S. frugiperda*.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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