Trichilia (Meliaceae) Plants: an Important Source of Biomolecules with Insecticidal Properties

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Trichilia (Meliaceae) plants: an important source of biomolecules with insecticidal properties

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Abstract

The repeated application of synthetic insecticides has the potential to induce insecticide resistance. Plant extracts are a good source of bioinsecticides, as these extracts often have several bioactive compounds, thus reducing the occurrence of resistance. The genus *Trichilia* (Meliaceae) is distributed widely in Mexico and it is a sustainable source of bioactive compounds because their bark is shed annually and may be collected without harming the tree. This research evaluated the effect on mortality, weight, larval and pupal duration, fecundity, and fertility of *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae) when fed with a diet supplemented with different concentrations of hexane, ethyl acetate, acetone, methanol, and aqueous extracts of the *Trichilia americana* Sessé & Moc., *Trichilia hirta* L., and *Trichilia havanensis* Jacq. (Meliaceae) bark. All the extracts provoked a dose-response effect on the variables evaluated. The hexane extract of the 3 species was lethal to the larval stage, with the hexane extract of *T. americana* being the most toxic of the 3 species, followed by that of *T. hirta*. The aqueous extract of *T. hirta* displayed an insecticidal effect on the early instars. The ethyl acetate extracts of *T. americana* and hexane extracts of *T. hirta* inhibited growth and induced mortality. The extracts reduced fecundity and fertility of the insect. Extracts of *T. americana* reduced reproductive potential to a greater extent than did *T. hirta* and *T. havanensis*. This research suggests that bark extracts of *T. americana*, *T. hirta*, and *T. havanensis* are a sustainable source of biomolecules.

Key Words: bark; bioinsecticide; sequential extraction; fertility

Resumen

La aplicación repetida de insecticidas sintéticos tiene el potencial de inducir resistencia a los insecticidad. Los extractos de plantas son una buena fuente de bioinsecticidas, ya que estos extractos a menudo tienen varios compuestos bioactivos, lo que reduce la apariencia de resistencia. El género *Trichilia* (Meliaceae) está ampliamente distribuido en México y es una fuente sustentable de compuestos bioactivos ya que su corteza se desprende anualmente y puede recolectarse sin dañar el árbol. Este trabajo evaluó el efecto sobre la mortalidad, peso, duración de larvas y pupas, fecundidad y fertilidad de *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae) al ser alimentadas con una dieta suplementada a diferentes concentraciones de extracto hexánico, acetato de etilo, acetónico, metanólico y acuoso de corteza de *Trichilia americana* Sessé & Moc., *Trichilia hirta* L., y *Trichilia havanensis* Jacq. (Meliaceae). Todos los extractos provocaron un efecto de dosis respuesta sobre las variables evaluadas. El extracto de hexano de las 3 especies fue letal para el estadio larval, siendo el extracto de *T. americana* el más tóxico de las 3 especies seguido por el de *T. hirta*. El extracto acuoso de *T. hirta* ocasionó un efecto insecticida en los primeros instares. Los extractos de acetato de etilo de *T. americana* y hexano de *T. hirta* inhibieron el crecimiento y provocaron mortalidad. Los extractos redujeron la fecundidad y la fertilidad del insecto. Los extractos de *T. americana* redujeron el potencial reproductivo en mayor medida que *T. hirta* y *T. havanensis*. Esta investigación sugiere que los extractos de corteza de *T. americana*, *T. hirta*, y *T. havanensis* son una fuente sustentable de biomoléculas.

Palabras Clave: corteza; bioinsecticida; extracción secuencial; fertilidad

The search for equilibrium between environmental conservation and food production has promoted the implementation of alternative control methods, such as the use of plant extracts (Wink 1993; Celis et al. 2009). These extracts are biodegradable and contain several active compounds; therefore, they slow down the appearance of resistance in pests (Rattan 2010). The fractionation of a plant extract using an ascending/descending polarity gradient may yield different fractions with different compounds (Baskar et al. 2010) that may reduce the reproductive potential, food consumption, or development rate, or may be toxic (Sengottayan 2013).

The plant family Meliaceae is relevant to insect control because a limonoid called azadirachtin is obtained from the neem tree, *Azadirachta indica* A. Juss. (Meliaceae). This compound is a growth disruptor, antifeedant, and is toxic to many insects (Isman et al. 2002). Limonoids, monoterpenes, sesquiterpenes, diterpenes, triterpenes, steroids, coumarins, flavonoids, and glycosylated lignans, among others (Tan & Luo 2011; Curcino-Vieira et al. 2014), have been obtained from the *Trichilia* spp. (Meliaceae). These compounds may affect the normal feeding and physiology of the insect (Li 1999; Ramírez et al. 2000; Simmonds et al. 2001), may kill the insect (Matos et al. 2009; Liu et al. 2017), inhibit growth (Kubo & Klocke, 1982), reduce reproductive potential (Freitas et al. 2014), and cause adult malformation (Cunha et al. 2008).

The methanol extract of the bark of *T. americana* had an antifeedant effect on *Spodoptera litura* F. (Lepidoptera: Noctuidae) larvae (Wheeler & Isman 2001) and the methanol extract from the twigs of *T. hirta* and the bark of *T. americana* inhibited the larval growth of *S. litura* (Wheeler et al. 2001). The acetone, ethanol, and hydroethanolic extract of seeds and pericarp of *T. havanensis* had antifeeding activity on the last instar of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae); fractions containing different limonoids were more active on the larvae than the limonoids alone, indicating a synergetic effect (López-

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Olguín 1998). Acetone extract of *T. connaroides* leaves inhibit growth, cause malformations, and reduce fertility of *Spilosoma obliqua* Walker (Lepidoptera: Arctiidae) (Tandon et al. 2009).

The family Meliaceae consists of small to large trees and, rarely, shrubs. The 52 genera including 61 species are distributed in the tropical and subtropical regions (Stevens 2001). Large populations of *T. havanensis*, *T. hirta*, and *T. americana* are distributed widely in Mexico (Calderón & German 1993) and they shed a large quantity of bark annually when the new growing season begins (Jiménez-Saa 1967), so the bark is readily available and its use sustainable as the tree does not have to be harvested. Our study reports the effect on mortality, development time, fecundity, and fertility of *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae) when fed on an artificial diet supplemented with different extracts from the bark of these *Trichilia* spp.

Materials and Methods

INSECTS

Insects were obtained from the colony at the Chemical Ecology Laboratory at Ceprobi, Instituto Politécnico Nacional, Yautepec, Morelos, Mexico. They were reared on an artificial diet (Callado-Galindo et al. 2013) at 22 ± 3 °C, 60 ± 3% RH, and a 12:12 h (L:D) photoperiod. All experiments were carried out under these conditions.

PLANT MATERIAL

The bark of *T. americana* trees was collected on 17 Apr 2015 at Iguala (18.13000°N, 99.29000°W), Guerrero, Mexico. *Trichilia hirta* bark was obtained at Tepoztlán (18.95943°N, 99.11327°W), Morelos, Mexico on 28 Jul 2015, and *T. havanensis* bark at Nauzontla (18.55460°N, 99.041290°W), Puebla, Mexico on 31 Jul 2015. Dr. Cesario Catalán-Heverástico (Guerrero State University, Iguala de la Independencia, Guerrero, Mexico) identified the plant material. The bark was dried in the shade for 30 d at 26 ± 5 °C and 75 ± 2% RH before passing through a grinder (MM 200, Pulvex, Mexico City, Mexico) and a 1.5 mm mesh.

PLANT EXTRACTS

The same bark sample was extracted sequentially with hexane (HPLC, JT Baker® Chemical Company, Phillipsburg, New Jersey, USA), then ethyl acetate (HPLC, JT Baker® Chemical Company), then acetone (HPLC, JT Baker® Chemical Company), then methanol (HPLC, JT Baker® Chemical Company), and finally distilled water (conductivity of 2.0 μΩ, pH 5-7, MILAB Distribuidora, Mexico City, Mexico) (Fig. 1). In all cases, the plant material:solvent ratio was 1:5 w:v and the bark was extracted for 48 h in each solvent. A rotary evaporator (R-200 Büchi, Zurich, Switzerland) was used for evaporation of the solvents, and concentrated extracts were stored in brown bottles at 4 °C until needed.

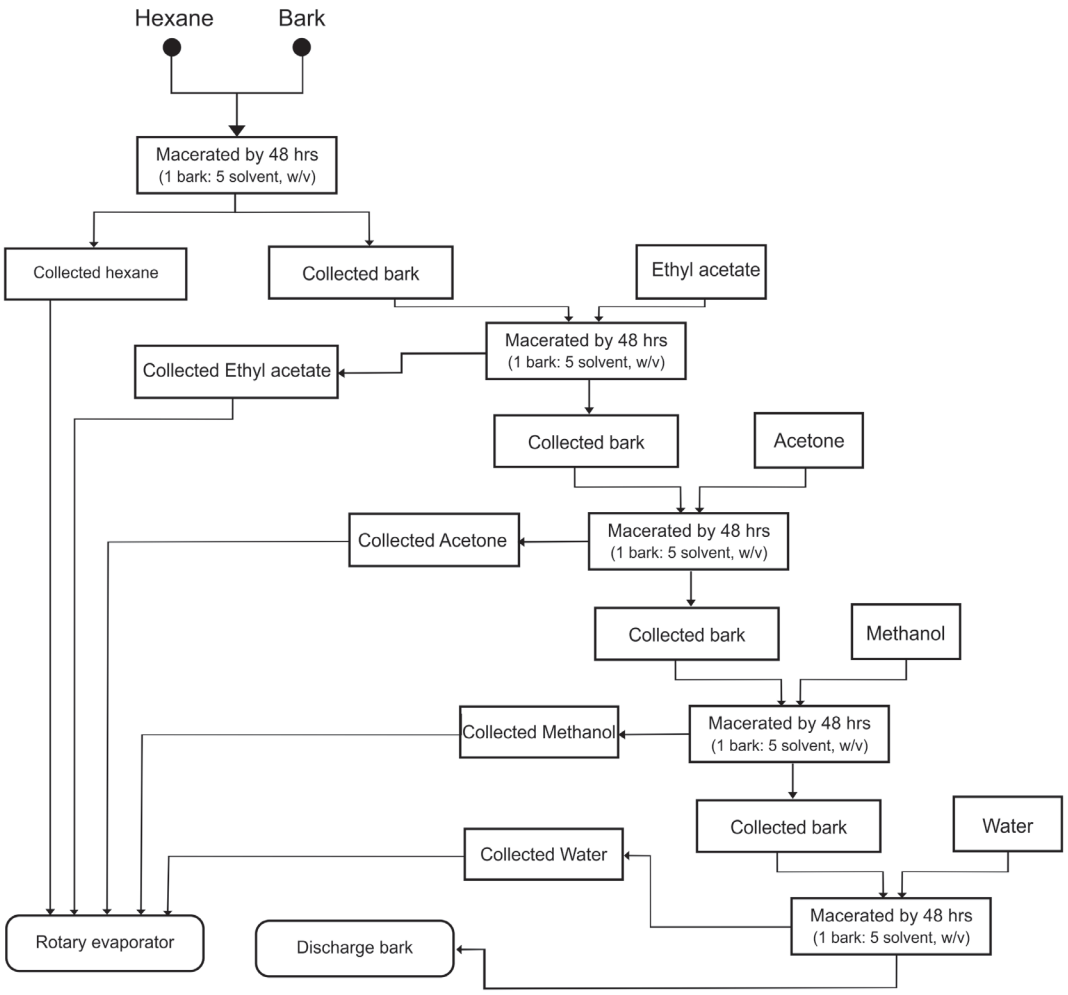

Fig 1. Extraction procedure. Each *Trichilia* spp. bark was extracted sequentially using organic solvents (hexane, ethyl acetate, acetone, methanol, and water) with increasing polarity.

EFFECT OF EXTRACTS ON LARVAL SURVIVAL, WEIGHT, AND DEVELOPMENT TIME

Four concentrations (100, 500, 1,000, and 1,500 ppm) of each extract were added to the aforementioned artificial diet; the experimental design included 2 controls: diet only and diet + 0.5% solvent. A total of 25 newly hatched larvae were used for each treatment; each larva was considered a replicate. A neonate larva was placed in a 30 mL plastic container with each diet to be tested. The diet was replaced every 7 d and survival and insect weight recorded (Explorer Ohaus scale, Parsippany, New Jersey, USA, accuracy 0.0001 g). According to Angulo et al. (2007) pupae were sexed under a Nikon SMZ 1500 stereomicroscope (Melville, New York, USA).

EFFECT OF EXTRACTS ON FECUNDITY AND FERTILITY

Adult males and females from the same treatment and 24 h old were confined in pairs in a wax paper bag (20 ×20 cm) and were fed honey from a 1.5 mL Eppendorf tube with a hole at the tip. If mating occurred, eggs were collected from the bag for 10 d, as more than 80% of the total eggs are laid during that time (Callado-Galindo et al. 2013). Eggs were incubated for 9 d, then the total number of eggs (fecundity) and the number of eggs with a viable larva (fertility) were counted.

STATISTICAL ANALYSIS

Kaplan-Meier (Log-Rank Test) curves followed by a Holm-Sidak (Rich et al. 2010) multiple mean comparison test was used to assess the effect of the extracts on larval survival. Larvae weight at 14 d was compared with a 2-way ANOVA (solvent and plant as main factors) followed by a LSMEANS procedure. Larval weight at 21 and 28 d was analyzed by a Kruskal-Wallis (nonparametric ANOVA) followed by a Dunn's test (Hollander et al. 2013). ANCOVA was used to analyze fecundity and fertility data, where the female and male weights (as mature larvae) were used as concomitant variables, followed by an LSD test. All reported values are mean ± SEM and the rejection probability was $P = 0.05$.

Results

A comparison of the survival, fecundity, and fertility data between the diet-only and diet + solvent treatments failed to show any difference (for survival: Log-Rank Test = 1.553; df = 4; $P < 0.817$; fecundity: $F = 0.46$; df = 6,18; $P > 0.05$; and fertility: $F = 0.11$; df = 6,18; $P > 0.05$). Therefore, only the diet-only treatment was included in the subsequent analysis. Larvae fed readily on the diets supplemented with the plant extracts; feces were observed in the plastic containers and larvae continued feeding on the diet until they pupated or died.

EFFECT OF THE EXTRACTS OF *TRICHILIA AMERICANA* ON *COPITARSIA DECOLORA* SURVIVAL

Copitarsia decolora larvae fed on a diet supplemented with hexane extracts of *T. americana* displayed significantly reduced mean survival times (Log-Rank Test = 48.33; df = 4; $P < 0.001$; Table 1; Fig. 2a). Larvae fed the 1,500 ppm treatment had a significantly shorter mean survival time than the others, and all of them died as larvae. Larvae on the 100, 500, and 1,000 ppm treatments had similar survival times, but they were significantly shorter than the diet-only control treatment. Larval mortality was over 70% in all treatments except at 100, where larval mortality was 40%, and very few casualties were observed at the pupal stage. The cumulative mortality for the control diet treatment was 8%.

Larvae fed the 1,500 ppm ethyl acetate-extract treatment had the shortest mean survival time (Log-Rank Test = 42.61; df = 4; $P < 0.001$;

Table 1. Mean (± SEM) survival time, and larval, pupal, and cumulative mortality of *Copitarsia decolora* larvae fed with artificial diet supplemented with hexane, ethyl acetate, acetone, methanol, and aqueous extracts of *Trichilia americana* bark at 100, 500, 1,000, and 1,500 ppm.

Treatment	Concentration ppm	Mean larval survival time (d)	Larval mortality (%)	Pupal mortality (%)	Cumulative mortality (%)
Control diet*	0	33.6 ± 1.6 a	8	0	8
Hexane	100	25.2 ± 2.6 b	40	4	44
	500	23.8 ± 3.6 b	72	4	76
	1,000	19.6 ± 3.4 b	76	0	76
	1,500	9.2 ± 1.1 c	100	**	100
Ethyl acetate	100	33.6 ± 3.4 b	56	20	76
	500	29.9 ± 3.9 bc	80	20	100
	1,000	26.2 ± 4.2 bc	80	20	100
	1,500	20.2 ± 4.3 bc	92	8	100
Acetone	100	44.8 ± 2.5 a	24	12	36
	500	43.9 ± 2.6 a	24	20	44
	1,000	43.1 ± 2.7 a	24	48	72
	1,500	39.5 ± 2.9 a	44	40	84
Methanol	100	30.5 ± 1.9 a	36	12	48
	500	29.1 ± 2.1 a	36	12	48
	1,000	28.0 ± 2.3 a	40	8	48
	1,500	26.1 ± 2.4 a	44	8	52
Water	100	31.9 ± 1.4 a	36	12	48
	500	30.2 ± 2.0 a	36	12	48
	1,000	28.6 ± 2.3 a	40	8	48
	1,500	26.3 ± 2.7 a	40	8	48

Means within the same solvent followed by the same letter are not significantly different ($P > 0.05$; Holm-Sidak test). *Control diet is the same for all the solvents. **No larvae reached the pupal stage.

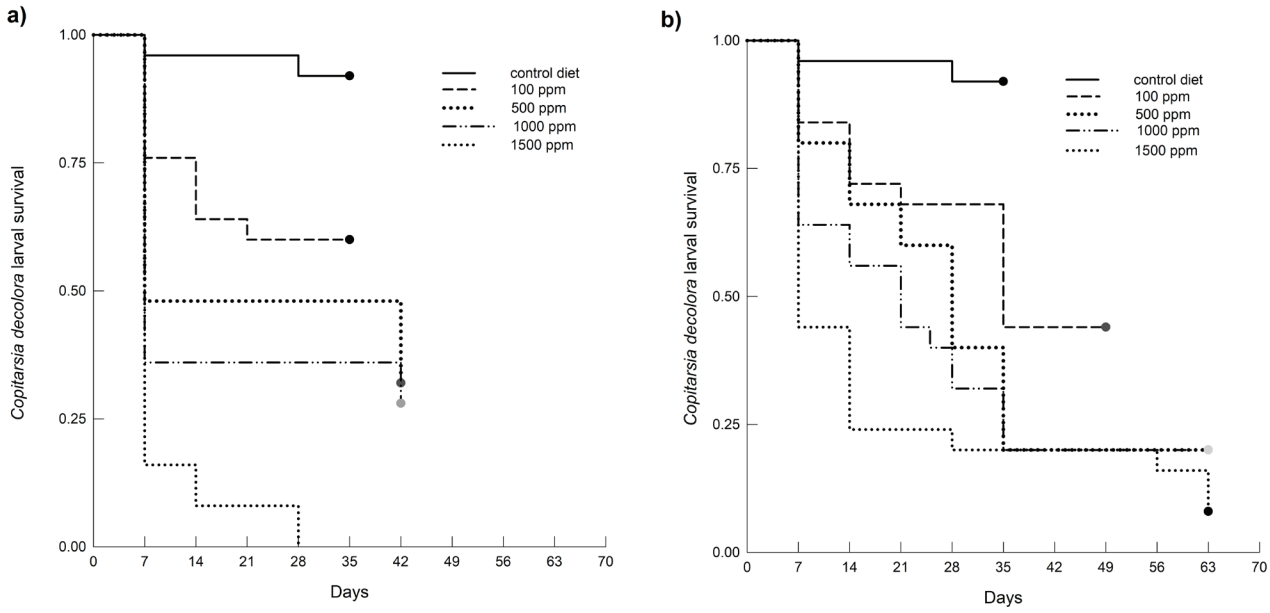

Fig. 2. Survival curves of *Copitarsia decolora* larvae fed on an artificial diet supplemented with extracts of *Trichilia americana* bark at 100, 500, 1,000, and 1,500 ppm: (a) hexane, (b) ethyl acetate.

Table 1; Fig. 2b). All treatments had significantly shorter mean survival times than the control diet treatment. Larval mortality increased as concentration increased. No larvae from the 500, 1,000, and 1,500 ppm treatments reached the adult stage, and at 100 ppm, cumulative mortality was 76%.

Mean larval survival times were similar in all the concentrations of acetone extract (Log-Rank Test = 6.26; df = 4; $P < 0.18$; Table 1) and mean survival time for larvae fed acetone extract was the same as the control diet treatment. The highest cumulative mortality was obtained in the 1,500 ppm treatment.

Mean survival times of *C. decolora* larvae were similar across all the concentrations of methanol extract (Log-Rank Test = 9.35; df = 4; $P < 0.053$) and aqueous extract (Log-Rank Test = 7.93; df = 4; $P < 0.094$). This varied from 26.1 ± 2.4 d for methanol extract at 1,500 ppm, to 30.5 ± 1.9 d at 100 ppm treatment. The highest level of mortality was 44% at 1,500 ppm and the shortest was 36% at 100 ppm. The highest cumulative mortality was 52% in the 1,500 ppm treatment. Mean larval survival time of the aqueous extracts ranged from 26.3 ± 2.7 d in the 1,500 ppm treatment to 31.9 ± 1.4 d in the 100 ppm treatment. The highest mortality was 40% in the 1,500 ppm treatment and the lowest mortality was 36% in the 100 ppm treatment. The highest level of cumulative mortality was 48% in the 1,500 ppm treatment.

EFFECT OF THE EXTRACTS OF *TRICHILIA HIRTA* ON *COPITARSIA DECOLORA* SURVIVAL

Mean larval survival time was significantly affected by *T. hirta* bark hexane extracts (Log-Rank Test = 33.43; df = 4; $P < 0.001$; Table 2; Fig. 3a). Larvae fed the 1,500 ppm treatment had the shortest mean survival time; however, this was not significantly different from those fed the 1,000 and 500 ppm treatments. Larval mortality was over 50% except at 100 ppm. The highest cumulative mortality occurred in the 1,500 ppm diet, and no adults were obtained from this treatment.

Mean survival time of *C. decolora* larvae was not affected by *T. hirta* bark ethyl acetate extract (Log-Rank Test = 7.227; df = 4; $P < 0.124$). This varied from 28.8 ± 3.4 d at 1,500 ppm treatment to 33.6 ± 1.6 d for the control diet treatment. The highest larval and cumulative mortalities were recorded in the 1,500 ppm treatment, and exceeded 60%.

Trichilia hirta bark acetone extracts significantly affected the mean survival time of *C. decolora* larvae (Log-Rank Test = 13.886; df = 4; $P < 0.008$; Table 2; Fig. 3b). The shortest mean survival time was recorded in the 1,500 ppm concentration; however, this treatment was significantly different only from the control diet treatment. The highest larval, pupal, and cumulative mortalities were recorded in the 1,000 ppm treatment.

Methanol extract significantly reduced mean larvae survival time (Log-Rank Test = 17.523; df = 4; $P < 0.002$; Table 2; Fig. 3c). Larvae on the 1,500 and 1,000 ppm concentration had the shortest mean survival times and differed only as compared to the control diet treatment. The highest larval, pupal, and cumulative mortalities were recorded in 1,500 ppm treatment.

Aqueous extracts had a significant reduction in mean larvae survival times in all the concentrations as compared to the control diet treatment (Log-Rank Test = 76.205; df = 4; $P < 0.001$; Table 2; Fig. 3d), with the 1,500 ppm treatment the shortest of all. All insects died as larvae before 21 d of age (Fig. 3d).

EFFECT OF THE EXTRACTS OF *TRICHILIA HAVANENSIS* ON *COPITARSIA DECOLORA* SURVIVAL

Mean survival times of larvae fed with 1,000 and 1,500 ppm hexane extracts were significantly shorter than on the control diet treatment (Log-Rank Test = 19.26; df = 4; $P < 0.001$; Table 3; Fig. 4). Larval and cumulative mortality increased along with concentration.

None of the ethyl acetate, acetone, methanol, and aqueous extracts of *T. havanensis* affected the mean larval survival times of *C. decolora* (Log-Rank Test = 7.819; df = 4; $P < 0.098$; Log-Rank Test = 4.917; df = 4; $P < 0.296$; Log-Rank Test = 7.157; df = 4; $P < 0.128$; and Log-Rank Test = 3.895; df = 4; $P < 0.42$ for ethyl acetate, acetone, methanol, and aqueous extracts, respectively; Table 3). Mean larval survival time varied from 26.9 ± 2.4 to 34.4 ± 0.5 d. Larval mortality oscillated from 16 to 32% and cumulative mortality from 28 to 56%.

Table 2. Mean (± SEM) survival time, and larval, pupal, and cumulative mortality of *Copitarsia decolora* larvae fed with artificial diet supplemented with hexane, ethyl acetate, acetone, methanol, and aqueous extracts of *Trichilia hirta* bark at 100, 500, 1,000, and 1,500 ppm.

Treatment	Concentration (ppm)	Mean larval survival time (d)	Larval mortality (%)	Pupal mortality (%)	Cumulative mortality (%)
Control diet*	0	33.6 ± 1.6 a	8	0	8
Hexane	100	36.9 ± 2.1 a	32	24	56
	500	31.6 ± 2.9 bc	52	4	56
	1,000	36.1 ± 4.1 bc	72	12	84
	1,500	22.7 ± 3.7 c	88	12	100
Ethyl acetate	100	36.9 ± 2.5 a	28	20	48
	500	35.3 ± 2.6 a	32	16	48
	1,000	32.2 ± 3.1 a	32	12	44
	1,500	28.8 ± 3.4 a	44	16	60
Acetone	100	31.6 ± 1.8 ab	28	16	44
	500	31.4 ± 1.8 b	48	16	64
	1,000	30.5 ± 1.9 b	52	16	68
	1,500	28.6 ± 2.4 b	52	12	64
Methanol	100	32.8 ± 1.2 ab	32	4	36
	500	31.1 ± 1.6 ab	40	36	76
	1,000	26.1 ± 2.2 b	48	24	72
	1,500	24.9 ± 2.3 b	60	16	76
Water	100	19.6 ± 0.6 b	100	**	100
	500	18.5 ± 0.7 bc	100	**	100
	1,000	18.2 ± 0.7 bc	100	**	100
	1,500	16.2 ± 0.9 c	100	**	100

Means within the same solvent followed by the same lowercase letter are not significantly different ($P > 0.05$; Holm-Sidak test). *Control diet is the same for all the solvents. **No larvae reached the pupal stage.

EFFECT OF EXTRACTS OF *TRICHILIA AMERICANA*, *TRICHILIA HIRTA*, AND *TRICHILIA HAVANENSIS* AT 1,500 PPM ON *COPITARSIA DECOLORA* LARVAL SURVIVAL

The mean larval survival time of the larvae fed with the hexane extract of *T. americana* bark was significantly shorter than the other treatments (Log-Rank Test = 151.205; df = 15; $P < 0.001$; Table 4). No differences in mean larval survival time were detected among those containing hexane or aqueous *T. hirta* extracts or the ethyl acetate *T. americana* extract, but all those were different from the control diet treatment. All other treatments were not significantly different from the control diet treatment. The reduction in survival time, relative to the control diet treatment, was 72 and 52% for *T. americana* hexane and *T. hirta* aqueous extracts treatments, respectively.

EFFECT OF EXTRACTS OF *TRICHILIA AMERICANA*, *TRICHILIA HIRTA*, AND *TRICHILIA HAVANENSIS* AT 1,500 PPM ON THE WEIGHT OF *COPITARSIA DECOLORA* LARVAE AND PUPAE

The weights of 7-d-old larvae were not analyzed because the larvae were too small to weigh accurately. Larval weights at 14 d were significantly affected by the species ($F = 143.61$; df = 3,265; $P < 0.0001$), the solvent ($F = 5.77$; df = 4,265; $P < 0.0002$), and the species-solvent interaction ($F = 2.93$; df = 8,265; $P < 0.003$). The largest weight inhibition was recorded with ethyl acetate extraction of *T. americana*, a reduction in weight of 99% relative to the control diet treatment. This was followed by hexane and aqueous extracts of *T. hirta* treatments and the hexane extract of *T. americana*, with a 98, 97, and 97% reduction, respectively, in relation to the control diet treatment. All other treatments were significantly different from the control diet treatment, with about 64% larval weight inhibition (Table 5).

At 21 d, larvae fed with the *T. americana* ethyl acetate extract displayed a 99% reduction in weight in relation to the control diet treatment, and weighed the least of all the treatments (Table 5). A reduction of 98 and 96% in relation to the control diet treatment was recorded for larvae with the *T. hirta* and *T. americana* hexane treatments. Only the *T. hirta* hexane treatment and *T. americana* ethyl acetate extract were significantly lighter than the control ($H = 201.330$; df = 14; $P < 0.001$).

At 28 d, statistically significant 99 and 98% reductions in weight were recorded for the larvae fed with ethyl acetate extract of *T. americana* and hexane extract of *T. hirta* ($H = 240.041$; df = 13; $P < 0.001$) (Table 5). The methanol extract of *T. hirta* and the ethyl acetate, methanol, and aqueous extracts of *T. havanensis* were similar to the control diet treatment. A 25 to 65% significant reduction in larval weight related to the control diet treatment were obtained with the acetone, methanol, and aqueous extract of *T. americana*, the ethyl acetate and acetone extract of *T. hirta*, and the hexane and acetone extract of *T. havanensis*.

The greatest reduction in pupal weight (21%) was recorded for the larvae fed with *T. havanensis* acetone extract, and this value was significantly different from the other treatments ($F = 210.61$; df = 3,137; $P < 0.0001$ for species; $F = 308.18$; df = 4,137; $P < 0.0001$ for solvent; and $F = 74.63$; df = 4,137; $P < 0.0001$ for the species-solvent interaction). Larvae fed with *T. havanensis* hexane extract and *T. hirta* acetone extract had significant 19% reductions in pupal weights in relation to the control diet treatment (Table 5).

EFFECT OF BARK EXTRACTS OF *TRICHILIA AMERICANA*, *TRICHILIA HIRTA*, AND *TRICHILIA HAVANENSIS* APPLIED AT 1,500 PPM ON FERTILITY AND FECUNDITY OF *COPITARSIA DECOLORA*

Because adults were not obtained from all the treatments, the fecundity and fertility were recorded from pairs of the aqueous and methanol

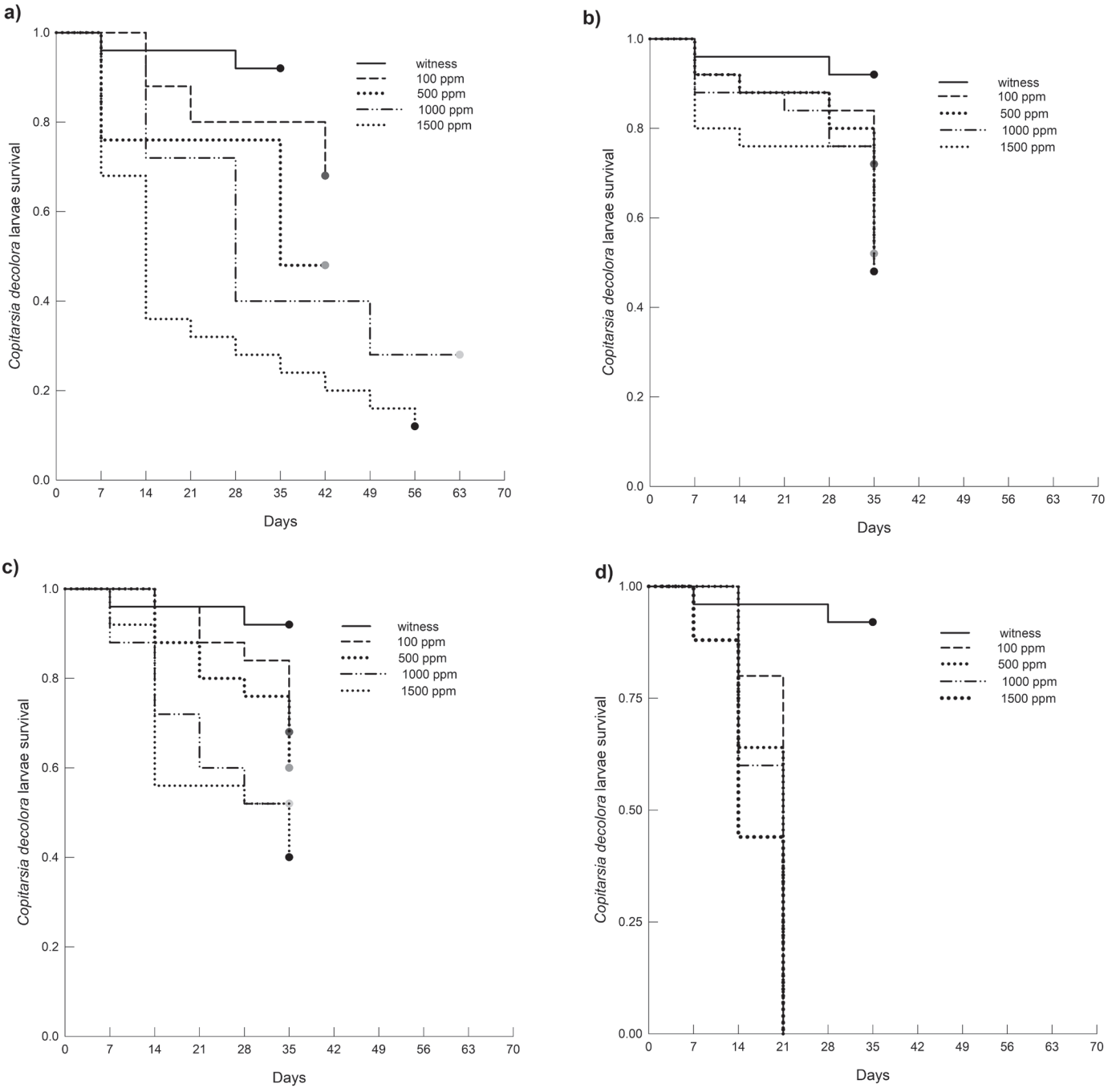

Fig. 3. Survival curves of *Copitarsia decolora* larvae fed on an artificial diet supplemented with extracts of *Trichilia hirta* bark at 100, 500, 1,000, and 1,500 ppm: (a) hexane, (b) acetone, (c) methanol, and (d) aqueous.

extracts of *T. americana*, the acetone, aqueous, and methanol extracts of *T. havanensis*, and the ethyl acetate and acetone extracts of *T. hirta*. Fecundity was significantly affected by the treatments ($F = 55.54$; df = 7,22; $P < 0.0001$), but female ($F = 0.08$; df = 1,22; $P > 0.05$) and male weights ($F = 1.06$; df = 1,22; $P > 0.05$, data not shown) were not significantly affected. Fecundity from the controls was highest of the treatments (Fig. 5). Significant reductions of 62 and 57% in fecundity, in relation to the control diet treatment, were recorded from insects fed the aqueous and methanol extracts of *T. americana*, respectively. The other treatments significantly reduced fecundity by 32 to 44%.

Fertility was significantly affected by the extracts ($F = 194.84$; df = 7,22; $P < 0.001$), but female ($F = 0.51$; df = 1,22; $P > 0.05$) and male weights ($F = 0.47$; df = 1,22; $P > 0.05$, data not shown) were not. The highest fecundity was obtained from the controls and was significantly greater than all other treatments (Fig. 5). Aqueous and methanol extracts of *T. americana* significantly reduced fertility by 90 and 86%, respectively. All other treatments reduced fertility by 47 to 70%.

Discussion

The genus *Trichilia* is a good source of biomolecules, with potential use in many fields. They have been shown to have antibacterial, anticarcinogenic, and insecticidal properties, among others (Sengottayan

Table 3. Mean (± SEM) survival time, and larval, pupal, and cumulative mortality of *Copitarsia decolora* larvae fed with artificial diet supplemented with hexane, ethyl acetate, acetone, methanol, and aqueous extracts of *Trichilia havanensis* bark at 100, 500, 1,000, and 1,500 ppm.

Treatment	Concentration ppm	Mean larval survival time (d)	Larval mortality (%)	Pupal mortality (%)	Cumulative mortality (%)
Control diet	0	33.6 ± 1.6 a	8	0	8
Hexane	100	33.3 ± 1.1 a	12	8	20
	500	31.6 ± 1.7 ab	20	24	44
	1,000	29.7 ± 2.1 b	48	16	64
	1,500	28.0 ± 2.4 b	52	12	64
Ethyl acetate	100	32.5 ± 1.6 a	16	12	28
	500	29.7 ± 1.9 a	32	8	40
	1,000	28.8 ± 2.1 a	32	8	40
	1,500	26.9 ± 2.4 a	36	20	56
Acetone	100	31.6 ± 1.5 a	24	12	36
	500	31.4 ± 1.6 a	28	8	36
	1,000	31.1 ± 1.7 a	32	4	36
	1,500	29.7 ± 1.9 a	32	4	36
Methanol	100	34.2 ± 0.9 a	8	24	32
	500	32.2 ± 1.4 a	20	12	32
	1,000	29.7 ± 2.0 a	28	8	36
	1,500	28.6 ± 2.3 a	28	16	44
Water	100	34.4 ± 0.5 a	12	20	32
	500	32.5 ± 1.4 a	20	12	32
	1,000	31.1 ± 1.8 a	24	12	36
	1,500	29.7 ± 2.2 a	24	12	36

Means followed by the same letter are not significantly different ($P > 0.05$; Holm-Sidak test).

2013; Curcino-Vieira et al. 2014), and this is the first report of its effects on *C. decolora*. The bark extracts from the 3 *Trichilia* species showed strong insecticidal activity against *C. decolora*. These effects can be related to the different molecules extracted by each solvent.

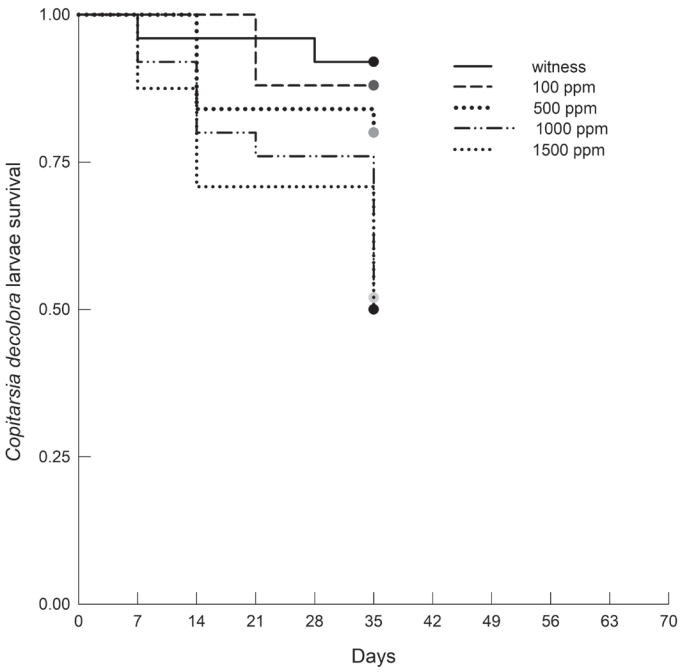

Fig. 4. Survival curve of *Copitarsia decolora* larvae fed on an artificial diet supplemented with hexane extract of *Trichilia havanensis* bark at 100, 500, 1,000, and 1,500 ppm.

Single solvent extractions with hexane, acetone, ethanol, methanol, and water are used for obtaining terpenes (Castillo-Sánchez et al. 2010). Monoterpenes, sesquiterpenes (Kumar et al. 2011), limonoids (Okorie & Taylor 1967), steroids (Wang et al. 2008), coumarine, and lignans (Curcino-Vieira et al. 2014) have been identified in polar extracts from bark of *Trichilia* spp. On the other hand, diterpenes (Ramírez et al. 2000), protolimonoids (Garcez et al. 1996), limonoids (Nakatani et al. 1981), flavonoids (Castro et al. 1996), lactones, phytosterols, and fatty

Table 4. Mean (± SEM) survival time of larvae of *Copitarsia decolora* fed on an artificial diet supplemented with bark extracts of *Trichilia americana*, *Trichilia hirta*, and *Trichilia havanensis* at 1,500 ppm.

Treatment source	Extract	Mean survival time of larvae (d)
Trichilia americana	Acetone	39.5 ± 2.9 a
Control diet		33.6 ± 1.6 a
Trichilia havanensis	Acetone	29.7 ± 1.9 a
Trichilia havanensis	Aqueous	29.7 ± 2.2 a
Trichilia hirta	Ethyl acetate	28.8 ± 3.4 a
Trichilia havanensis	Methanol	28.6 ± 2.3 a
Trichilia hirta	Acetone	28.6 ± 2.4 a
Trichilia havanensis	Hexane	28.0 ± 2.4 a
Trichilia havanensis	Ethyl acetate	26.9 ± 2.4 a
Trichilia americana	Aqueous	26.3 ± 2.7 ab
Trichilia americana	Methanol	26.1 ± 2.4 ab
Trichilia hirta	Methanol	24.9 ± 2.3 abc
Trichilia hirta	Hexane	22.7 ± 3.7 bc
Trichilia americana	Ethyl acetate	20.2 ± 4.3 bc
Trichilia hirta	Aqueous	16.2 ± 0.9 c
Trichilia americana	Hexane	9.2 ± 1.1 d

Means followed by the same letter are not significantly different ($P > 0.05$; Holm-Sidak test).

Table 5. Mean (± SEM) weights of *Copitarsia decolora* larvae when fed for 14, 21, and 28 d with artificial diet supplemented with bark extracts of *Trichilia americana*, *Trichilia hirta*, and *Trichilia havanensis* at 1,500 ppm. Also shown are subsequent pupal weights.

		Larval weight (mg)		Larval weight (mg)		Larval weight (mg)		Pupal weight	
Treatment		at 14 d	n	at 21 d	n	at 28 d	n	(mg)	n
Control diet		127.4 ± 4.7 a	24	214.7 ± 2.5 ab	24	600.4 ± 6.8 a	23	392.8 ± 1.9 a	23
Trichilia americana	Hexane	3.1 ± 0.6 cd	2	8.3 ± 0.2 bc	2	*		*	
	Ethyl acetate	0.8 ± 0.1 d	6	1.3 ± 0.1 c	6	4.1 ± 0.9 e	6	*	
	Acetone	34.1 ± 11.2 bc	18	47.4 ± 1.6 bc	18	211.0 ± 7.7 e	18	356.2 ± 0.9 d	4
	Methanol	46.1 ± 13.1 b	20	167.0 ± 7.5 b	20	443.4 ± 5.5 bcd	20	334.6 ± 1.8 f	12
	Aqueous	14.2 ± 0.5 cd	23	148.6 ± 4.9 bc	23	337.1 ± 3.6 cde	22	368.3 ± 1.3 bc	15
Trichilia hirta	Hexane	1.5 ± 0.1 d	9	2.4 ± 0.1 c	8	7.2 ± 0.1 e	7	*	
	Ethyl acetate	13.7 ± 0.4 cd	15	150.6 ± 4.2 bc	15	255.2 ± 1.3 e	15	363.9 ± 0.9 cd	10
	Acetone	21.8 ± 6.4 cd	19	237.5 ± 5.1 a	19	452.3 ± 1.9 bcd	19	319.3 ± 6.8 g	10
	Methanol	17.9 ± 0.3 cd	14	246.9 ± 4.2 a	14	520.3 ± 1.3 abc	13	363.8 ± 0.9 cd	6
	Aqueous	2.6 ± 0.1 d	23	*		*		*	
Trichilia havanensis	Hexane	35.5 ± 0.8 bc	17	162.5 ± 3.0 bc	17	304.0 ± 1.2 de	17	317.8 ± 1.6 g	10
	Ethyl acetate	21.3 ± 2.0 cd	21	146.6 ± 5.9 bc	20	515.6 ± 5.3 abc	20	343.2 ± 0.7 e	12
	Acetone	16.6 ± 1.2 cd	24	123.1 ± 6.4 bc	24	286.7 ± 1.6 de	23	309.1 ± 1.6 h	16
	Methanol	28.1 ± 7.7 c	22	209.9 ± 9.1 ab	22	563.9 ± 3.3 ab	21	371.5 ± 1.2 b	15
	Aqueous	15.8 ± 0.9 cd	24	209.3 ± 6.5 ab	24	602.5 ± 2.9 a	23	390.3 ± 0.4 a	16

Means in the same column followed by the same letter are not significantly different ($P > 0.05$; Holm-Sidak test). *No insects alive at this stage. n = number of living larvae.

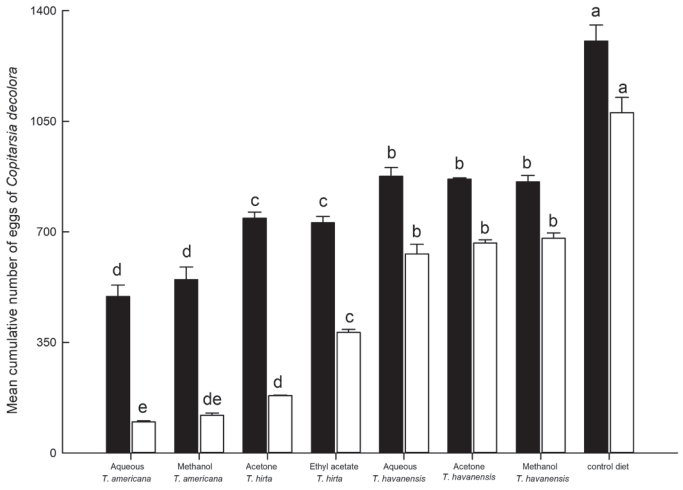

Fig. 5. Mean **(± S**EM) fecundity (black) and fertility (white) of pairs of *Copitarsia decolora* fed with artificial diet supplemented with aqueous and methanol extracts of *Trichilia americana* (n = 5), acetone (n = 2), and ethyl acetate (n = 3) of *Trichilia hirta*; aqueous, acetone, and methanol of *Trichilia havanensis* (n = 4); and the control diet (n = 5). Same color bars topped by the same letter are not significantly different ($P > 0.05$; LSD test). Error bars are Standard Error of the Mean.

acids (Pizzolatti et al. 2004) have been identified in non-polar extracts of bark from the *Trichilia* species.

We used a sequence of solvents (from non-polar to polar) to extract the different molecules from the plant. This method extracts more compounds due to the different polarities used (Harborne 1998). Matos et al. (2009), using the aforementioned extraction method, found that the hexane and methanol extracts at 1,000 ppm of *Trichilia elegans* A. Juss. (Meliaceae) fruits killed all *Spodoptera frugiperda* JE Smith (Lepidoptera: Noctuidae) larvae within 10 d. From these extracts, a limonoid and mostly coumarines and steroids were isolated; these authors reported similar effects to those produced by azadirachtin because larvae could not shed the exuviae and died. Essoung et al. (2017) using a sequential extraction, found that the methanolic extract of *Turraea abyssinica* (Meliaceae) leaves possess "good toxic potential" (LD_{50} = 270.7 ppm) on second instar larvae of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). The fractionation of this extract led to the isolation of 4 limonoids with $LD_{50} < 7.0$ ppm. In our experiment, the methanol extracts of the 3 species had no significant effects on larvae or pupae of *C. decolora*, whereas the methanolic extract of *T. americana* reduced the reproductive potential by more than 50%.

Giongo et al. (2016) identified the limonoid cedrelone from the hexane extract of *Toona ciliate* M. Roem. (Meliaceae) stems, which killed all the *S. frugiperda* larvae 7 d after intake. It is possible that a limonoid, coumarin, or steroid is responsible for the mortality of *C. decolora* reported herein, because the hexane extract at 1,500 ppm *T. americana* bark killed more than 80% of *C. decolora* larvae 7 d after consumption. As reported by Roel et al. (2000) for *S. frugiperda* larvae, 500 ppm of ethyl acetate extract of *T. americana* bark caused high mortality of *C. decolora* larvae, with a reduction in weight and an increase of the larval stage length, and many died during metamorphosis.

Lauric, palmitic and stearic acid, sesquiterpenes, and steroids have been identified from a dichloromethane extract of *Trichilia lepidota* Mart. subsp., *schumanniana* Harms (Meliaceae) leaves (Pupo et al. 2002) and with the same solvent, steroids and fatty acid esters have been identified from the stem of *Trichilia casarettii* C. DC. (Meliaceae) (Curcino-Vieira et al. 2010). Fatty acids can be isolated using solvents such as hexane and ethyl acetate (Abay et al. 2013), so it is feasible that these molecules might occur in our hexane and ethyl acetate extracts from *T. americana* bark as well as in the hexane extract from *T. hirta*.

Only the aqueous extract from *T. hirta* showed a strong insecticidal effect on the first stages of *C. decolora*, a result similar to that obtained with the hexane extract from *T. americana* bark, making them powerful insecticides against *C. decolora*. Bogorni and Vendramim (2003), working with *S. frugiperda*, reported that maize leaves treated with an aqueous extract from *Trichilia pallens* C. DC. (Meliaceae) leaves at 5,000 ppm caused 98% larval mortality, which is comparable to the aqueous extract from branches (100% mortality) and leaves (98% mortality) of *A. indica* against *S. frugiperda*. The effect on larval development of the aqueous extract of *T. hirta* may be due to hormone alterations interfering with the ecdysis process, as already observed with *A. indica* extracts (Mordue & Blackwell 1993).

Our results show that all evaluated extracts at 1,500 ppm reduced fecundity at least by one-third and fertility by almost 50%. Even though *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) larvae treated with methanol extracts from *Melia azedarach* L. (Meliaceae) fruit can reach the adult stage, their reproductive potential and longevity is reduced, and damage to the midgut epithelial cells was observed in the treated insects (Schmidt et al. 1997). Freitas et al. (2014) reported an 87 and 100% decrease in fecundity and fertility of *S. frugiperda* when larvae were fed with methanol extract at 1% (10,000 ppm) of *Trichilia silvatica* C. DC. (Meliaceae) bark; these values are similar to those reported in our work for *C. decolora* using methanol and aqueous extracts of *T. americana* at 1,500 ppm.

It takes longer for an insect to create resistance to a mixture of natural active ingredients than to a single component, because it is more difficult to detoxify a mixture of molecules than a single one (Isman 1997); synergy between the components of the extract could maximize the effect of each of its components (Isman 2006). In summary, the insecticide potential of extracts of *T. americana* and *T. hirta* may be due to the presence of limonoids and fatty acids, and any synergy between them.

A mixture of biomolecules producing mortality in the early stages of the insect may prevent resistance, reduce damage, and reduce the use of synthetic insecticides. The identification of the molecules from the naturally available bark of the 3 *Trichilia* species is relevant, and it is worth identifying their mode of action.

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