Biological Activity of *Trichilia americana* (Meliaceae) on *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae)[1]

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Abstract The biological activity of extracts from *Trichilia americana* (Sesse and Mocino) T.D. Penn. (rind), *Carica papaya* L., *Jatropha curcas* L., *Ricinus communis* L., and *Lupinus campestris* Schldl. & Cham. (seeds), at 1% and 5%, were evaluated on neonates of *Copitarsia decolora* Guenée in ingestion bioassays. The plants that caused the highest percentage of larval mortality were *T. americana* at 1% and 5%, and *Carica papaya* at 5% with 98% and 100% and 100%, respectively. *Trichilia americana* was the plant with higher toxicological properties against *C. decolora*. Extracts in hexane, ethyl acetate, acetone, and methanol from *T. americana* were then evaluated at 10, 100, 300, and 1,000 ppm also in ingestion bioassays against *C. decolora*. Extracts of ethyl acetate and acetone at 1,000 ppm recorded the highest percentages of larval mortality of *C. decolora* (90% and 55%, respectively). In addition to larval mortality, feeding on *T. americana* extract also decreased mean larval weight, prolonged duration of the larval stage, resulted in malformed pupae and adults, and affected adult fertility and fecundity. Finally, these extracts were evaluated in a toxicity model, for which the crustacean *Artemia salina* Leach was used. The ethyl acetate, acetone, and hexane extracts showed no toxicity to *A. salina*, while the methanol extract caused 30% mortality, which is considered to be slightly toxic. Based on the results obtained, *T. americana* is a species with insecticidal and insectistatic activity against *C. decolora* and can be considered as a potential insecticide for the management of this or other insect pests.

Key Words *Copitarsia decolora,* plant extracts, biopesticides

In Mexico, about 50,000 ha are planted each year with cruciferous plants, mainly broccoli (*Brassica oleracea* L. var. *italica*), cauliflower (*Brassica oleracea* L. var. *botrytis*), and cabbage (*Brassica oleracea* L. var. *capitata*). Mexico is fourth in the world in production of broccoli and cauliflower, with a total production of all three crops valued at approximately US$15,8224.39 (INFOSIAP 2016, SIAP 2016). *Copitarsia decolora* Guenée (Lepidoptera: Noctuidae) is one of the main cruciferous pests in Mexico (Acatitla-Trejo 2010, Barrios-Díaz et al. 2004, Bujanos 2000, Fernández-Cevada and Vázquez-Ortiz 2003, Suárez-Vargas et al. 2006). While feeding, larvae cause deformities of cabbage heads and can even kill the plant (Monge-Villalobos et al. 1984). Population peaks coincide with the period of

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formation of the heads of cabbage, cauliflower, and broccoli (Suárez-Vargas 2006, Tovar-Hernández et al. 2007).

Copitarsia decolora is distributed from Mexico to Argentina (Angulo and Olivares 2003, Angulo et al. 2006). In Mexico, it has been reported in Chiapas, Veracruz, Puebla, State of Mexico, Morelos, Guanajuato, and San Luis Potosi feeding on alfalfa (*Medicago sativa* L.), peas (*Lathyrus sativus* L.), coriander (*Coriandrum sativum* L.), epazote (*Chenopodium ambrosioides* L.), asparagus (*Asparagus officinalis* L.), huauzontle (*Chenopodium berlandier* Moquin-Tandon), and potato (*Solanum tuberosum* L.) among many other host plants (Acatitla-Trejo et al. 2006, Angulo and Olivares 2003, Bautista 2006, Bautista et al. 2003, Castillo and Angulo 1991, Fernández-Cevada and Vázquez-Ortiz 2003, Suárez-Vargas et al. 2006, Torres-Nohra and Rangel-Machain 2003) as well as on cut flowers such as *Alstroemeria* (Moreno and Serna 2006).

This pest is quarantined by the United States, which prevents Mexican producers from exporting cabbage (*Brassica oleracea* var. Capitata L.), broccoli (*Brassica oleracea* var. Italica L.), cauliflower (*Brassica oleracea* var. Brotrytis L.), coriander (*Coriandrum sativum* L.), lettuce (*Lactuca sativa* L.), and some cut flowers (Acatitla-Trejo et al. 2006, Venette and Gould 2006). Some methods of management that have been proposed for this pest are the emission of frequencies (Paz et al. 2008), temperature (Gould et al. 2005), sex pheromones (Barrientos-Hernández et al. 2011, Muñiz-Reyes et al. 2007), and biological control with parasitoids (Díaz et al. 2012). Other alternatives, such as extracts from plants and cells produced in vitro, are being investigated in the laboratory (Pavón-Reyes 2010, Vázquez-Covarrubias et al. 2015). However, the most commonly used method of management is the application of synthetic chemical insecticides, that is, organophosphates, pyrethroids, and carbamates, alone or in mixtures (Bayer Crop Science 2011, Díaz-Gómez et al. 2003, Fernández-Cevada and Vázquez-Ortiz 2003, Pérez et al. 2009). However, in Puebla, Mexico, the application of chemical insecticides in broccoli, cabbage, and cauliflower for the control of *C. decolora*, *Plutella xylostella* L., and *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae) significantly impacts parasitism by *Diadegma insolare* Cresson (Hymenoptera: Ichneumonidae) and *Voria ruralis* Fállen (Diptera: Tachinidae) (Tovar-Hernández et al. 2007). This creates the need for alternative methods of handling this pest that are environmentally friendly. An alternative is the use of plant extracts with insecticidal properties as they are biodegradable and effective for the control of insect pests (Lee et al. 2004, Villavicencio et al. 2010). Therefore, the objective of the present work was to evaluate the biological activity of five plant species with insecticidal properties against *C. decolora*.

Materials and Methods

Insects. Adult *C. decolora* males and females were obtained from a colony maintained at 25 ± 3°C, 60 ± 5% relative humidity (RH) and a photoperiod of 12 h light:12 h dark, in the Laboratory of Chemical Ecology, Department of Interactions Insect Plant, Center for the Development of Biotic Products, National Polytechnic Institute. The original colony was initiated from cabbage crops in Juchitepec, state of Mexico (between N19°01′22″ and 19°10′28″ and between W98°48′92″ and

98°58′46″). Pairs of adults were placed in 20 × 20-cm acrylic cages and fed with a 9:1 water and honey solution in 5-mL Eppendorf tubes. Once eggs were obtained, they were removed and checked daily for eclosion to obtain neonates used in the bioassays.

Plants and extractions. The plant species from which extracts were obtained were collected in four states of Mexico. *Carica papaya* L. (Caricacae) and *Ricinus communis* L. (Euphorbiaceae) were from Tlalnepantla and Tepoztlan (N18°57′00″, W98°14′00″) and Morelos (N18°53′00″, W99°02′00″). *Jatropha curcas* L. (Euphorbiaceae) was from Chiapa de Corzo, Chiapas (N16°42′00″, W93°11′00″); *Lupinus campestris* Schlechtendal & Chamisso (Fabaceae) was from the Izta-Popo National Park, Puebla (N19°24′00″, W98°43′00″); and *Trichilia americana* (Sesse and Mocino) T.D. Penn. (Meliaceae) in the locality "Los Naranjos" Iguala, Guerrero (N18°13′00″, W99°29′00″). Selection of these plants was based on reported insecticidal activity on other noctuid insects (Bermúdez-Torres et al. 2009, Figueroa-Brito et al. 2013, Flores-Macías et al. 2016, Ratnadass and Wink 2012, Vieira et al. 2014).

The rind of *T. americana* was used for extractions, while seeds of the remaining species were used. Plant materials were air-dried in the dark for 15 d at room temperature (25 ± 3°C). They were then ground using an electric mill (Siemens, Ciudad de México, México) and sieved with a 1-mm mesh screen until a fine powder was obtained for each species. The only exception was with the seeds of *R. communis* which, after the milling process, yielded a paste that was used in the assay. The powders of each plant as well as the paste were deposited in plastic bags and stored with their respective collection data at 4°C until used in assays.

Bioassays. The powders of the extracts from *Carica papaya*, *T. americana*, *J. curcas*, and *L. campestris* and the paste extract from *R. communis* were evaluated at concentrations of 1% and 5% (w/w) in 500 g of diet based on corn flour, wheat germ, beer yeast, ascorbic acid, sorbic acid (antibacterial and antifungal), bacteriological agar, and distilled water (modified of Rojas et al. 1995) for an ingestion bioassay. Once the diet was prepared, it was cooled to a temperature of 45 ± 5°C when the powder or paste was incorporated. Once the diet solidified, a 1-cm^3 cube of the appropriate diet treatment was placed in 30-mL plastic cup, after which a single *C. decolora* neonate was placed on the diet cube and the cup was covered. Controls consisted of larvae with untreated diet. Larvae were maintained in an environmental chamber at 25 ± 3°C, 60 ± 5% RH. Each treatment included 100 larvae.

Larvae were observed once a week. Percentage of larval mortality, larval weight (mg), larval development (days), percentage of deformities of larvae, pupae, and/or adults, percentage of pupation, and percentage of adult emergence were recorded. Percentage of fecundity and percentage of fertility were quantified in those adults that emerged. Larval weight was recorded with a digital analytical balance (Explorer E02140, O'Haus Corporation, Gaithersburg, MD, USA).

Pupae that survived were separated by sex using a stereoscope and placed in 20 × 20-cm acrylic cages as appropriate for each treatment until adult emergence. As adults emerged, adults from the same treatment were placed together. Once mating occurred, five couples were selected from each treatment and placed in waxed-paper bags and fed as previously described. The bags were changed daily to count the number of oviposited eggs (fecundity) per female for 10 consecutive

days. Bags with eggs were stored for 7 d, after which numbers of hatched larvae (fertility) were enumerated (Callado-Galindo et al. 2013).

Statistical analyses. A completely randomized design was used, where one larva was the experimental unit and each larva was considered a replicate. Data on larval mortality, and deformities in larvae, pupae, or adults were analyzed using the Chi square test (χ^2), while larval development, number of eggs laid (fecundity), and larval hatching (fertility) were analyzed using a one-way analysis of variance (ANOVA). For the data that did not meet the normality and homogeneity of variance, a nonparametric ANOVA (Kruskal–Wallis test) was performed. In cases where a significant difference was found, a mean separation test (Tukey or Dunn's test according to the nature of the data) was applied. Unless otherwise noted, all values reported are the mean $\pm$ standard error and the probability of rejection was 0.05. The data were analyzed with the statistical program Sigma Plot 11.0 (Systat Software Inc., Chicago, IL).

Comparison of *T. americana* extraction solvents. Based on the results of the previous bioassays, *T. americana* was selected for additional evaluation and assessment. First, solvents selected on the basis of their degree of polarity (hexane, ethyl acetate, acetone, methanol) were assessed for extraction of active ingredient. The powder from the rind of *T. americana* was derived as previously described, and 110 g of the powder was deposited in individual 1-L beakers to which 500 ml of the appropriate solvent was added. Each mixture was filtered at ambient laboratory conditions for 72 h using Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, UK). Each sample was then concentrated in a Rotavapor (Büchi R-114, Büchi Labortechnik AG, Flawil, Switzerland) at 25 $\pm$ 3°C, according to the boiling points of each solvent. The resulting concentrated samples were deposited in 30-ml glass vials and placed in a laminar-flow chamber to remove the remaining solvent. Each sample was placed on 5 mg of silica gel and stored for later use in the bioassays.

These samples were incorporated into *C. decolora* larval diets as previously described, with the exception that concentrations of 10, 100, 300, and 1,000 ppm of each extract preparation were established in 200 g of larval diet. Forty *C. decolora* neonates were used with each treatment, with each larva considered as an experimental unit. Response variables and statistical analyses were performed as previously described.

Toxicity of *T. americana* rind extracts against brine shrimp. Response of brine shrimp, *Artemia salina* Leach, to the *T. americana* rind extracts was determined in the Laboratory of Evaluation of Natural and Pharmacological Products of the Center of Chemical Investigations of the Autonomous University of the State of Morelos. A culture medium, based on sea salt (3.8 g/L water), was prepared in a beaker which served as the incubation chamber for developing brine shrimp. The salt maintained the pH between 7 and 8, and an oxygen pump aerated the solution to saturation during the bioassay. After preparation of the culture medium, 45 mg of *A. salina* eggs were added. The beaker with the *A. salina* eggs was kept in a permanently illuminated area. The light source was a lamp placed at a distance of 40 cm from the beaker, which was illuminated throughout the bioassay to maintain a temperature of 25 $\pm$ 3°C. Brine shrimp larvae (nauplii) were observed hatching 24 h later. Twenty days later, these larvae were used in the bioassays as described by Rahman et al. (2005).

The extracts used were the same ones evaluated in the previous bioassay with *C. decolora*. A saline solution was prepared with 0.9 g of NaCl in 100 ml of distilled water. For the preparation of the initial samples of the extracts, 20 mg of each extract and 10 μl of Tween 20 were weighed to homogenize the sample and then brought to a volume of 1 ml using the saline solution previously prepared. These samples were serially diluted to obtain concentrations of 10, 100, and 1,000 ppm. Only the saline solution (1 ml) was used in the control with Tween 20 (10 μl) but without an extract. Another control consisted of an insecticide of botanical origin (Nimicida®, Biokrone, Celaya, Guanajuato, México) based on the extract of *Azadirachta indica* A. Juss (Meliaceae) at 20 ppm. In all treatments and controls, 100 μl of each treatment solution was placed in individual petri dishes. Ten nauplii of *A. salina* were placed in each petri dish, with each larva considered as an experimental unit and each petri dish a replicate. Counts of living brine shrimp larvae were made 24 h and 48 h after the larvae were added to the extract treatments as per Meyer et al. (1982).

Results

Bioassays of plant powders against *C. decolora*. The powders of the rind of *T. americana* (1% and 5%) and the seeds of *Carica papaya* (5%) caused significantly higher *C. decolora* larval mortality (98%, 100%, and 100%, respectively) than the other powder treatments and the control ($\chi^2 = 509.64$; df = 10; $P < 0.001$; Table 1), but did not differ from each other. Extracts from *T. americana* rinds (5%) and *L. campestris* seeds (1%) reduced mean larval weights to 3.57 mg and 22.7 mg, respectively, which was significantly lower than that measured in the control (336 mg) ($H = 37.03$; df = 9; $P < 0.001$; Table 1). Larval development was significantly prolonged by ingestion of *L. campestris* seed powder (1% and 5%) and *T. americana* rind powder (1%) ($H = 30.20$; df = 9; $P < 0.001$; Table 1) in comparison to the other powder treatments and the control, but there was no significant difference among these three treatments. The *Carica papaya* seed powder (1%) prolonged larval development by 7 d and differed significantly from the control. The other treatments did not differ significantly from the control (Table 1). Powders from *T. americana* (1%) rind and *J. curcas* seeds (5%) were the only treatments that significantly reduced percentage pupation with respect to the control ($\chi^2 = 203.72$; df = 10; $P < 0.001$; Table 1). All other treatments did not differ from the control. Significantly fewer adults emerged from larvae fed on diets containing powders of *J. curcas* seed (5%), *R. communis* seed (1% and 5%), and *L. campestris* seed (1%) with respect to the other treatments and to the control ($\chi^2 = 205.21$; df = 10; $P < 0.001$; Table 1), but these four treatments did not differ significantly from each other. In comparison to the control, all treatments except the *J. curcas* seed powder at 1% yielded a significantly higher percentage of deformed pupae and adults ($\chi^2 = 44.6$; df = 5; $P < 0.001$; Table 2). The highest percentages of deformities followed ingestion of *R. communis* seed powder (1% and 5%) and *L. campestris* seed powder (5%). The only two specimens that survived treatment with *T. americana* rind powder (1%) and successfully pupated, but with deformities, did not emerge as adults.

Table 1. Effect of different plant extract powders at 1% and 5% in artificial diet on *Copitarsia decolora*.[1]

Treatments (%)[2]	Mortality (%) Mean ± SE	Weight (mg) 25 < Median < 75[3]	Development (d) 25 < Median < 75[3]	Pupation (%) Mean ± SE	Adult Emergence Mean ± SE
Trichilia americana 1	98 ± 2.0 g	19.66 < 22.78 < 25.87 b	38.78 < 42.1 < 49.45 d	2 a	—
Trichilia americana 5	100 g	3.02 < 3.57 < 3.59 a	30.03 < 33.7 < 34.93 b	—	—
Carica papaya 1	15 ± 0.2 c	672.95 < 760.32 < 847.68 h	27. 15 < 27.4 < 28.19 a	85 ± 31.8 cd	82 ± 8.0 c
Carica papaya 5	100 g	—	—	—	—
Jatropha curcas 1	11 ± 1.4 b	353.13 < 383.35 < 465.74 g	32.21 < 33 < 33 b	89 ± 6.4 c	89 ± 7.1 c
Jatropha curcas 5	54 ± 5.1 f	415.35 < 422.45 < 440.71 g	15.41 < 30.55 < 33 b	46 ± 5.6 b	46 ± 8.1a
Ricinus communis 1	42 ± 0.6 e	200.49 < 219.54 < 226.79 e	15.41 < 40 < 41.75 bc	58 ± 4.2bc	55 ± 12.5 ab
Ricinus communis 5	47 ± 4.7 ef	138.27 < 163.58 < 182.89 d	31.86 < 39.3 < 43.5 bc	53 ± 10.6 bc	48 ± 8.5a
Lupinus campestris 1	39 ± 5.4 e	19.66 < 22.78 < 25.87 b	43.91 < 47 < 48.31 d	61 ± 10.6 bc	61 ± 16.1 ab
Lupinus campestris 5	25 ± 1.8 d	32.19 < 67.41 < 101.39 c	40.67 < 44.9 < 47.26 d	75 ± 23.3 c	75 ± 11c
Control	8 a[*]	306.63 < 336.84 < 341.39 f	33 b[**]	92 ± 31.1 cd	92 ± 6.4 c

[1] $n = 100$. Values within the same column followed by the same letter are not statistically different ($P < 0.001$).
[2] 1 = 1%, 5 = 5%.
[3] Quartile 25 < Median < Quartile 75.
[*] Unique data.
[**] All the surviving larvae had the same development time.

Table 2. Anatomical deformities in *Copitarsia decolora* caused by different plant extract powders in artificial diet at 1% and 5%.[1]

	Deformities		
Treatments (%)[2]	**Pupae**	**Adults**	**Total**
Trichilia americana 1	2	0	2 b
Jatropha curcas 1	0	0	0 a
Jatropha curcas 5	1	1	2 b
Ricinus communis 1	8	2	10 c
Ricinus communis 5	9	3	12 c
Lupinus campestris 1	5	0	5 b
Lupinus campestris 5	8	1	9 c
Control	0	0	0 a

[1] $n = 100$. Values within the last column followed by the same letter are not statistically different ($P < 0.001$). Treatments not shown had already caused 100% mortality or did not cause abnormalities.
[2] 1 = 1%, 5 = 5%.

None of the treatments significantly impacted *C. decolora* fecundity ($F = 2.35$; df = 7; $P = 0.067$; Table 3). However, ingestion of the powders from the seeds of *R. communis* and *L. campestris* (5%) significantly decreased *C. decolora* fertility in comparison to the control ($F = 3.47$; df = 7; $P < 0.014$).

Table 3. Effect of plant extract powders on the fecundity and fertility of adults of *Copitarsia decolora*.[1]

Treatments (%)[2]	**Eggs/Female/Day Mean ± SE**	**Hatched Larvae (%) Mean ± SE**
Carica papaya 1	14.6 ± 4.6 ab	73.1 ± 23.2 ab
Jatropha curcas 5	63.9*	—
Jatropha curcas 1	14.9 ± 2.4 ab	74.4 ± 11.7 ab
Ricinus communis 5	20.3 ± 4.6 ab	61.2 ± 13.7 a
Ricinus communis 1	20.8 ± 3.9 ab	83.3 ± 15.8 ab
Lupinus campestris 5	12.2 ± 6.5 ab	48.9 ± 25.9 a
Lupinus campestris 1	23.8 ± 5.4 a	71.7 ± 15.6 ab
Control	17.5 ± 1.8 ab	87.7 ± 9.1 b

[1] Values within the same column followed by the same letter are not statistically different ($P < 0.001$). Treatments not shown had already caused 100% mortality.
[2] 1 = 1%, 5 = 5%.
* Unique value was not considered for statistical analysis.

Table 4. Yield of extracts from the rind of *Trichilia americana*.

Extracts[1]	Weight (mg)	Yield (%)
1. Hexane	0.139	0.27
2. Hexane	1.660	1.45
1. Ethyl acetate	0.344	0.68
2. Ethyl acetate	0.856	0.79
1. Acetone	0.544	1.08
2. Acetone	1.132	1.03
1. Methanol	0.770	1.54
2. Methanol	5.578	5.07

[1] 1 = 55 g, 2 = 110 g.

There was no significant difference among the other treatments, including the control (Table 3).

***Trichilia americana* rind extraction and toxicity.** Based on the previous results, the *T. americana* rind powder was determined to possess greater potential for development as an insecticide than the other plant powders evaluated (Table 1). We, therefore, subjected the *T. americana* rind to additional testing.

In comparing four solvents for extraction of the rind, the acetone and methanol extracts yielded the highest percentages of extract with a tendency of greater chemical polarity (Table 4). The ethyl acetate extract followed by the acetone extract, both at 1,000 ppm, caused significantly higher *C. decolora* larval mortality (90% and 55%, respectively) than their respective solvent-only controls, the other extracts, and the absolute control ($\chi^2 = 235.45$; df = 20; $P < 0.001$; Table 5). Ingestion of the diets containing the extracts of ethyl acetate at 1,000 ppm, hexane at 300 and 10 ppm, and the acetone at 10 ppm resulted in significantly reduced larval weights with respect to their respective solvent-only controls, the other extracts, and the absolute control ($H = 261.15$; df = 20; $P < 0.001$; Table 5); however, there were no significant differences among these treatments. The duration of *C. decolora* larval development was significantly longer in treatments with the extracts of ethyl acetate, acetone, methanol (1,000 ppm), and hexane (100 ppm) than their respective solvent-only controls, the other treatments, and the absolute control ($H = 291.35$; df = 20; $P < 0.001$; Table 5); however, there were no significant differences among these treatments.

Extracts of ethyl acetate and acetone at 1,000 ppm resulted in a significantly lower percentage of pupation and emergence of adults with respect to their respective solvent-only controls, the other treatments, and absolute control ($\chi^2 = 33.1$; df = 20; $P = 0.033$ and $\chi^2 = 37.5$; df = 20; $P = 0.010$, respectively; Table 5). Additionally, the ethyl acetate extract at 300 ppm limited total emergence to only 50%. Significantly greater percentages of pupae and adults were deformed in treatments with extracts of ethyl acetate and acetone at 300 ppm, followed by the hexane extract at 300 and 100 ppm in comparison to their respective solvent-only

controls, the other extracts, and the absolute control ($\chi^2 = 43.036$; df = 20; $P = 0.002$; Table 6). Ethyl acetate extracts at 300 and 100 ppm and hexane at 100 ppm significantly affected fecundity ($H = 30.54$; df = 19; $P = 0.04$) and fertility ($H = 43.636$; df = 19; $P = 0.001$) with respect to their respective controls, other treatments, and the absolute control (Table 7).

Toxicity of *T. americana* extracts against *A. salina*. The hexane, ethyl acetate, and acetone extracts from the *T. americana* rind that caused *C. decolora* larval mortality were not toxic to *A. salina* larvae. Mortality following exposure to the concentrations of these extracts did not differ significantly from mortality in the untreated control. However, the methanol extract and the botanical insecticide Nimicida caused significantly higher, but less than 50% and 60%, mortality compared to the other treatments and to the control ($\chi^2 = 4.1215$; df = 4; $P = 0.0422$; and $\chi^2 = 3.9553$; df = 4; $P = 0.0467$).

Discussion

Of the plant species evaluated in this study, powders from *T. americana* rind appear to have the greater potential for development as a bioinsecticide in managing *C. decolora* in that it was the only species that caused a significantly higher larval mortality with the two concentrations tested. Similar results were reported by López-Olguín et al. (1997) with an extract from the fruit of *Trichilia havanensis* Jacquin (Meliaceae) at 5% and 1%, resulting in mortality levels of 100% at 16 and 51 d, respectively, of *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae). In another study, López-Olguín et al. (2002) reported that an extract from *T. havanensis* seed caused 100% mortality of first-instar Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae), larvae at 5% and 1% concentrations, while larvae completed development at lower concentrations of 0.2% to 0.8%. These latter results confirm the importance of the concentrations used against target pest species and further suggests that it is necessary to assess *T. americana* rind powders at concentrations below 1% to determine activity against *C. decolora*. Both *T. havanensis* and *T. americana* could cause significant mortality on other insects than Lepidoptera, and extracts from *T. americana* should be tested on a variety of insects, including tephritids.

Exposure of diets containing 1% and 5% concentrations of *T. americana* rinds, *L. campestris* seeds, and *R. communis* seeds decreased larval weights and extended larval development in comparison to the control. López-Olguín et al. (1997) also found that the extract from fruits of *T. havanensis* incorporated in artificial diet at 1% and 5% reduced the larval weight and delayed larval development of *S. littoralis*. The results of these two studies suggest that the *T. havanensis* fruit extract and the *T. americana* rind powder possess antifeedant properties. Caballero et al. (2008) demonstrated that fifth-instar *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) presented antifeedant activity associated with a toxic mode of action when exposed to a mixture of *T. harvanensis* seeds. Many plant-based substances prevent or reduce insect feeding as a result of prefeeding (deterrent) and postdigestive (toxic) effects (Klocke and Kubo 1991, Schoonhoven 1982). In the case of powders from *T. americana* rinds, *L. campestris* seeds, and *R. communis* seeds, tests must be

Table 5. Effect of plant extracts from the rind of *Trichilia americana* on *Copitarsia decolora*.[1]

Treatments (ppm)	Mortality Mean ± SE	Weight (mg) 25 < Median < 75[2]	Development (d) 25 < Median < 75[2]	Pupation (%) Mean ± SE	Emergence (%) Mean ± SE
Hexane 1,000	2.5 b	208. 5 < 488.9 < 692.7 bcd	33 < 36.5 < 40 ab	97.5 ± 2.4 k	97.5 ± 2.4 k
300	27.5 ± 4.0 g	109.8 < 223 < 350.8 ab	33 a	72.5 ± 1.6 d	72.5 ± 1.6 e
100	27.5 ± 3.5 g	211 < 653.8 < 816.9 bcde	40 b	72.5 ± 1.6 d	72.5 ± 1.6 e
10	32.5 ± 4.0 g	182.7 < 236.8 < 437.9 ab	33 a	67.5 ± 2.2 c	67.5 ± 2.2 d
Control—Hexane	12.5 ± 1.5 e	692.3 < 793. 6 < 869.1 de	33 a	87.5 ± 0.5 g	62.5 ± 1.7 c
Ethyl acetate 1,000	90 ± 13.7 i	57.5 < 111.3 < 187.7 a	40 < 43.5 < 47 bc	10 ± 1.1 a	10 ± 1.1 a
300	7.5 ± 1.0 d	256.4 < 709.2 < 826 bcde	33 < 36.5 < 40 ab	92.5 ± 1.1 i	50 ± 4.4 c
100	12.5 ± 1.5 e	380.4 < 544.6 < 722.5 bcde	33 < 33 < 40 ab	87.5 ± 1.1 g	87.5 ± 1.1 h
10	2.5 b	199.9 < 412.5 < 718. 6 bcd	33 < 33 < 40 ab	97.5 ± 0.9 k	97.5 ± 0.9 k
Control—Ethyl acetate	5 ± 0.6 c	536 < 668.4 < 794 cde	33 a	95 ± 0.7 j	87.5 ± 0.9 h
Acetone 1,000	55 ± 6.1 h	304.8 < 529.7 < 706.4 bcd	40 b	45 ± 2.0 b	45 ± 2.0 b
300	17.5 ± 2.5 f	415.2 < 495 < 589.9 bc	33 < 33 < 40 ab	82.5 ± 0.5 e	82.5 ± 0.5 f
100	15 ± 2.5 f	449.7 < 532.1 < 632.2 bcd	33 < 33 < 40 ab	85 ± 0.7 f	85 ± 0.7 g
10	12.5 ± 2.1 e	177.7 < 212.8 < 488.7 ab	33 < 33 < 40 ab	87.5 ± 0.5 g	62.5 ± 1.1 c
Control—Acetone	12.5 ± 2.0 e	559.0 < 703.8 < 800. 5 cde	33 a	87.5 ± 1.1 g	87.5 ± 1.1 h
Methanol 1,000	17.5 ± 2.5 f	473.8 < 865 < 944.1 bcde	38.2 < 40 < 40 b	82.5 ± 0.9 e	67.5 ± 1.1 d
300	15 ± 1.5 f	397.5 < 681.8 < 876 bcde	33 < 35 < 40 ab	85 ± 1.3 f	82.5 ± 0.5 f
100	5 ± 0.6 c	492.0 < 635.5 < 896.5 cde	33 < 33.7 < 40 ab	95 ± 0.7 j	95 ± 0.7 j

Table 5. Continued.

Treatments (ppm)	Mortality Mean ± SE	Weight (mg) 25 < Median < 75[2]	Development (d) 25 < Median < 75[2]	Pupation (%) Mean ± SE	Emergence (%) Mean ± SE
10	10 ± 1.0 e	373.5 < 614.2 < 811.3 bcde	33 < 33 < 40 ab	90 ± 0.7 h	90 ± 0.7 i
Control—Methanol	0 a	211.9 < 343. 6 < 592.4 bc	33 a	100 l	95 ± 0.7 j
Absolute control	0 a	282.8 < 400.1 < 582.48 b	33 a	100 l	100 l

[1] $n = 100$. Values within the same column followed by the same letter are not statistically different ($P < 0.001$).
[2] Quartile 25 < Median < Quartile 75.

Table 6. Anatomical deformities of *Copitarsia decolora* treated with extracts of the rind of *Trichilia americana*.[1]

	Deformities (%)		
Treatments (ppm)	**Pupae**	**Adults**	**Total**
Hexane 1,000	0	0	0 a
300	2.5	15	17.5 f
100	0	15	15 f
10	0	5	5 c
Control—Hexane	0	5	5 c
Ethyl acetate 1,000	0	5	5 c
300	7.5	17.5	25 g
100	2.5	5	7.5 d
10	0	7.5	7.5 d
Control—Ethyl acetate	0	7.5	7.5 d
Acetone 1,000	2.5	2.5	5 c
300	5	25	30 h
100	2.5	7.5	10 e
10	0	5	5 c
Control—Acetone	0	5	5 c
Methanol 1,000	0	2.5	2.5 b
300	0	5	5 c
100	2.5	10	12.5 e
10	0	7.5	7.5 d
Control—Methanol	0	5	5 c
Absolute control	0	2.5	2.5 b

[1] $n = 100$. Values within the last column followed by the same letter are not statistically different ($P < 0.001$). Treatments not shown had already caused 100% mortality or did not cause abnormalities.

conducted to determine whether they contain antifeedant or food-dissuasive compounds against *C. decolora* larvae.

In addition to the mortality and antifeedant activity of some of these powders, *T. americana* and *J. curcas* powders reduced successful pupation and adult emergence, powders from several plant species caused anatomical deformities in *C. decolora* pupae and adults, and *L. campestris* and *R. communis* seed powders at 5% significantly reduced *C. decolora* fertility. Interference with any or all of these life factors will contribute lack of ability to reproduce and survive.

Table 7. Effect of extracts of the rind of *Trichilia americana* on the fecundity and fertility of adults of *Copitarsia decolora*.[1]

Treatments (ppm)	Eggs/Female/Day Mean ± SE	Hatching Eggs (%) Mean ± SE
Hexane 1,000	98.8 h	98.7 g
300	52.9 f	52.9 c
100	7.2 ± 1.9 b	21.5 ± 5.8 b
10	29.1 ± 3.6 de	87.4 ± 10.7 ef
Control—Hexane	16.2 ± 0.8 c	80.9 ± 4.0 ef
Ethyl acetate 1,000	—	—
300	0.7 a	0.7 a
100	7.5 ± 6.9 bc	15.1 ± 13.8 b
10	31.5 ± 0.2 d	94.5 ± 0.7 f
Control—Ethyl acetate	16.2 ± 0.9 c	80.8 ± 4.4 ef
Acetone 1,000	34.9 ± 11.0 de	69.8 ± 21.9 ef
300	18.8 ± 2.1 cd	75.1 ± 8.3 e
100	15.8 ± 1.7 c	63.1 ± 6.6 e
10	19.1 ± 8.9 d	57.4 ± 26.7 e
Control—Acetone	17.1 ± 0.9 cd	85.5 ± 4.7 ef
Methanol 1,000	36.1 ± 11.5 de	72.3 ± 22.9 ef
300	53.8 g	53.8 d
100	37.7 ± 3.2 de	75.4 ± 6.4 e
10	30.1 ± 1.6 d	90.2 ± 4.9 f
Control—Methanol	15.9 ± 1.6 c	79.6 ± 8.2 ef
Absolute control	17.2 ± 0.9 cd	85.8 ± 4.5 ef

[1] Values within the same column followed by the same letter are not statistically different ($P < 0.001$).

The acetone and ethyl acetate extracts from *T. americana* rind were the only treatments with larvicidal activity against *C. decolora* in these ingestion bioassays. Similar results were reported by Xie et al. (1994), who showed that the extract of *T. americana* rind yielded the highest percentages of *Peridroma saucia* Hübner (Lepidoptera: Noctuidae) mortality when mixing the extract in the artificial diet. Methanol extracts from *T. americana* shoots yielded LC_{50}s of 1,200 ppm and 6,400 ppm with *Trichoplusia ni* in ingestion and spray tests, respectively (Akhtar et al. 2008).

Other species and parts of the plant of the genus *Trichilia* also have shown toxicity against Lepidoptera. For example, seed extracts, rind, and leaves of

Trichilia roka (Forsskål) Chiovenda, *Trichilia connaroides* (Wight & Arnott) Bentvelzen, and *Trichilia hirta* L. (Meliaceae) showed a larvicidal effect on *Spodoptera frugiperda* (J.E. Smith) and *Peridroma saucia* (Lepidoptera: Noctuidae) (Isman et al. 1995). In addition, Bogorni and Vendramim (2003) reported that an aqueous extract of branches and leaves of *Trichilia pallens* Casimir Pyrame de Candolle (Meliaceae) caused 75.5% and 100% mortality in *S. frugiperda*.

The ethyl acetate and acetone extracts caused the lowest percentage of *C. decolora* pupation and adult emergence and, in general terms, most of the extracts evaluated showed concentration-dependent response with a higher concentration of extract causing lower percentages of pupation and adult emergence. Similar results were reported by Wheeler et al. (2001), who determined that the methanol extract concentration of *T. americana* branches prolonged the pupation process and decreased the emergence of adults of *Spodoptera litura* F. (Lepidoptera: Noctuidae). Similarly, fruit extracts of *Melia azedarach* L. (Meliaceae) at 1,000 and 5,000 mg/kg against *S. frugiperda* recorded high percentages of larval mortality (57% and 100%) and low percentages of pupation (0–35%) and adult emergence (0–42%) (Scapinello et al. 2013). Wheeler and Isman (2001) postulated that the methanol extract of the branches of *T. americana* contains substances that cause chronic toxicity, which has only been observed when the extract is ingested, suggesting the possibility that the extract acts on the intestinal tract of the insect or interferes with the digestion of *S. litura*.

Larval weight was reduced with ingestion of ethyl acetate, hexane, and acetone extracts of *T. americana* rind. A similar effect was observed with the extract from *M. azedarach* (Meliaceae) fruit against *S. frugiperda* (Scapinello et al. 2013). Xie et al. (1994) reported that the incorporation of leaf extract and rind extract of *T. havanensis* also reduced larval weight when incorporated into the larval diet of *P. saucia*. Extracts and compounds from other plants also reportedly affect larval weight of *C. decolora*. For example, the dichloromethane extract of the roots of *Aristolochia brevipes* Benth (Aristolochiaceae) and the compound 9-methoxyitariacuripyrone (100 mg/20 g diet) showed inhibitory activity of larval-weight *C. decolora* (Álvarez-Fitz 2014).

Extended the larval development of *C. decolora* following exposure to various extracts agrees with results of studies with other species of *Trichilia* and Lepidoptera. For example, the aqueous extracts of leaves of *Trichilia pallida* Swartz, *Trichilia catigua* Henri de Jussieu, *Trichilia casaretti* Casimir Pyrame de Candolle, and *Trichilia elegans* Henri de Jussieu, and the branches of *Trichilia claussenii* Casimir Pyrame de Candolle and *Trichilia pallens* Casimir Pyrame de Candolle to 1% (= 10,000 ppm) (Meliaceae) also significantly slowed the larval development of *S. frugiperda* (Bogorni and Vendramim 2005). Similarly, Xie et al. (1994) reported that the extract of rind of *T. connaroides* (29.1 and 185.1 ppm, respectively) reduced the larval development of *P. saucia* and *S. litura* by 50%, while Wheeler et al. (2001) reported that the methanol extract of *T. americana* at 1,000 ppm reduced larval growth of *S. litura*. These authors also showed that extract of the branches incorporated in the diet at a concentration of 17.2 ppm was more active than extracts of wood, rind, or leaf in reducing the larval growth of *S. litura* by 50%, while concentrations of 10, 25, 50, and 75 ppm slowed growth. The aforementioned work is related to our results obtained with *T. americana* against *C. decolora*, in that the highest concentrations resulted in the lowest percentages of

larval weight, suggesting that there is an antifeedant effect that affects the weight and larval development of *C. decolora*. For example, López-Olguín et al. (1998), with the acetone extract of *T. havanensis* seeds at 5,000 ppm and its mixture of limonoid compounds 1,7-di-0-acetylhavanensin and 3,7-di-0-acetylhavanensin at 1,000 ppm, showed the highest antifeedant activity with respect to the antiappetitive index, of deterrence and suppression on larvae of *Helicoverpa armigera* Hübner (Noctuidae). Wheeler and Isman (2001) stated that when the fourth-instar larvae of *S. litura* were fed a diet mixed with the methanol extract of *T. americana*, consumption rates and, therefore, growth declined as the extract concentration increased. It is likely that this decrease in the rate of consumption is due to the antifeedant nature of the extract and explains most of the decline in the rate of growth. It is necessary to perform other bioassays with these extracts to determine if their effect is by starvation and/or by postdigestive activity of *C. decolora* larvae.

Herein, the ethyl acetate and acetone extracts at 300 ppm and the hexane extract at 300 and 100 ppm caused the highest percentage of pupal and adult deformities, respectively. Similar results were obtained by Scapinello et al. (2013), who reported that, at a lower concentration (100 and 500 mg/kg) of the extracts of *M. azedarach*, there was a higher percentage of morphological deformities of *S. frugiperda*, such as adults with deformed wings and difficulty of the insects to free themselves from the pupal case. The occurrence of these deformities in *C. decolora* moths resulting from larvae fed diets containing extracts of *T. americana* reaffirm that there is an effect on the morphology of *C. decolora*. However, it is necessary to perform different studies characterize the mode of action.

There are currently few studies on the effect of extracts of the genus *Trichilia* on the fecundity and fertility of *C. decolora*. Rodríguez-Yescas (2015) determined that acetone (500 ppm) and hydroethanolic (500 ppm and 1,000 ppm) extracts of *T. havanensis* seeds decreased the number of oviposited eggs (49.2**%**, 29.9**%**, and 35.5**%**, respectively) and the number of fertile eggs (55.8**%**, 40.3**%**, and 45.6**%**, respectively) by *C. decolora* females. Extracts from other plant species, such as *Tagetes lucida* Cavanilles (Asteraceae) (Barajas 2010), and *Chenopodium ambrosioides* L. and *Beta vulgaris* L. (Chenopodiaceae) (Vázquez-Covarrubias et al. 2015), also affect the fecundity and fertility of *C. decolora*.

And, finally, a salient point in the toxicity of *T. americana* is that the ethyl acetate and acetone extracts that were toxic to *C. decolora* proved to be safe for *A. salina* larvae. Although the methanol extract caused significantly higher mortality of *A. salina* larvae compared to the other treatments, the mortality level observed was less than 50**%** and was below the mortality caused by the botanical insecticide Nimicida (60**%**), indicating that the botanical insecticide is more toxic than the methanol extract against *A. salina*. Mikolajczak and Reed (1987) reported that neem products derived from *Azadirachta indica* and, like the neem Nimicida botanical insecticide evaluated in our study, are toxic to *A. salina*. Although the genera *Trichilia* and *Azadirachta* belong to the family Meliaceae, the *T. americana* is not toxic to *A. salina*; whereas, *Azadirachta indica* products were toxic to this crustacean. *Artemia salina* is an organism used as a model to assess possible nontarget impacts of biocidal products in various stages of development. In our case, the *T. americana* extracts had no deleterious effects on this nontarget. Additional toxicity bioassays with other organisms more closely related to the target

insect and associated predators or parasitoids of that target should be conducted to determine if the low toxicity against *A. salina* extends to beneficial organisms.

Based upon the results of this study, *T. americana* is now considered a plant species with insecticidal activity. Extracts from the plant have the potential for development as plant-derived bioinsecticides. Phytochemical studies of the ethyl acetate extract from the *T. americana* rind should be conducted to identify the compound(s) responsible for the biological activity of this species.

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