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Authors: Valdés-Estrada, Ma. Elena, Aldana-Llanos, Lucila, Salinas-Sánchez, David Osvaldo, Figueroa-Brito, Rodolfo, Hernández-Reyes, María C., et al.

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Toxicity of plant extracts to *Scyphophorus acupunctatus* (Coleoptera: Curculionidae)

Ma. Elena Valdés-Estrada[1], Lucila Aldana-Llanos[1], David Osvaldo Salinas-Sánchez[2], Rodolfo Figueroa-Brito[1], María C. Hernández-Reyes[1], and María Guadalupe Valladares-Cisneros[3,]*

Abstract

We studied the insecticidal effect of plant extracts against larvae of *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae), an important pest of Agavaceae. In the bioassays, the organic extracts of *Argemone mexicana* L. (Ranunculales: Papaveraceae), *Allium sativum* L. (Asparagales: Amaryllidaceae), *Bursera grandifolia* (Schltdl.) Engl. (Sapindales: Burseraceae), *Chenopodium ambrosioides* L. (Caryophyllales: Amaranthaceae), *Prosopis laevigata* (Willd.) M. C. Johnst. (Fabales: Fabaceae), and *Tagetes erecta* L. (Asterales: Asteraceae), which were individually incorporated into artificial diet at 500 ppm, were fed to 1st instars. The variables studied were larval, pupal, and adult weight, and larval and pupal mortality. The extracts of *A. sativum*, *A. mexicana*, *P. laevigata*, *T. erecta*, and *B. grandifolia* reduced larval weight by at least 50%. The greatest larval mortality was caused by the extracts of *A. mexicana* (53%), *A. sativum* (43%), and *T. erecta* (43%), whereas the greatest mortality of pupae was caused by extracts of *T. erecta*. The results suggest that *A. mexicana*, *A. sativum*, and *T. erecta* extracts have the greatest potential for insecticidal activity against *S. acupunctatus*.

Key Words: *Allium sativum*; *Argemone mexicana*; bioinsecticide; *Tagetes erecta*

Resumen

Se estudió el efecto insecticida de extractos vegetales en contra de larvas de *Scyphophorus acupunctatus* Gyllenhal (Coleoptera: Curculionidae), plaga importante de Agaváceas. Se realizaron bioensayos de ingestión empleando individualmente los extractos orgánicos de *Argemone mexicana* L. (Ranunculales: Papaveraceae), *Allium sativum* L. (Asparagales: Amaryllidaceae), *Bursera grandifolia* (Schltdl.) Engl. (Sapindales: Burseraceae), *Chenopodium ambrosioides* L. (Caryophyllales: Amaranthaceae), *Prosopis laevigata* (Willd.) M. C. Johnst. (Fabales: Fabaceae) y *Tagetes erecta* L. (Asterales: Asteraceae), todos ellos a una concentración de 500 ppm. Los extractos se incorporaron en una dieta artificial, sobre la cual se colocaron larvas del primer estadio del insecto. Las variables evaluadas fueron peso y mortalidad del insecto. Los extractos de *A. sativum*, *A. mexicana*, *P. laevigata*, *T. erecta* y *B. grandifolia*, inhibieron en un 50 por ciento el peso larval con respecto del testigo. Los extractos que provocaron mortalidad en larvas fueron: *A. mexicana* (53%), *A. sativum* (43%) y *T. erecta* (43%). Así mismo, *T. erecta* fue la especie que produjo la más alta mortalidad de pupas. Estos resultados permiten posicionar a *A. sativum y a T. erecta* como las especies más promisorias con actividad bioinsecticida en contra de *S. acupunctatus*.

Palabras Clave: *Allium sativum*; *Argemone mexicana*; bioinsecticide; *Tagetes erecta*

Scyphophorus acupunctatus Gyllenhal (Coleoptera: Curculionidae), also known in Mexico as "picudo negro" (black weevil), is becoming a major pest of Agavaceae, Asparagaceae, and Dracaenaceae worldwide. Native to Mexico, it has decimated populations of agave crops, in particular the economically important species used in industries such as tequila (*Agave tequilana*, F. A. C. Weber 'Azul'), henequen (*Agave fourcroydes* Lem.), and maguey (*Agave salmiana* Otto ex Salm-Dyck subsp. *crassispina* 'Cultra') (Asparagales: Asparagaceae) (Figueroa-Castro et al. 2013).

The importation of ornamental agave plants worldwide has allowed *S. acupunctatus* to establish in many parts of the world, particularly in Central America and the Caribbean but also in Africa, Asia, and South America. This species infests other economically important spice plants in the genera *Beaucarnea*, *Dasylirion*, and *Yucca* as well as tuberose, *Polianthes tuberosa* L. (all: Asparagales: Asparagaceae) (Camino Lavin et al. 2002). Moreover, it is a vector of the plant pathogens *Erwinia cacticida* corrig. (Enterobacteriales: Enterobacteriaceae) (Rodríguez 1999) and *Pseudomonas* sp. (Pseudomonadales: Pseudomonadaceae) (Ruíz-Montiel et al 2008), and these microorganisms kill *P. tuberosa* (Molina 2013).

In Mexico, the control of *S. acupunctatus* mainly relies on the synthetic organophosphates tebupirimfos and terbufos (De Liñán 2009). The use of synthetic insecticides reduces the damage caused by this insect, but these insecticides can be dangerous and unsafe for the tuberose producers. Terán-Vargas et al. (2012) evaluated the effect of other synthetic insecticides in Tamaulipas, Mexico. The study included the pyrethroids cypermethrin, lambda-cyhalothrin, and beta-cyfluthrin. Some insecticides evaluated in that study effectively controlled the agave weevil. Therefore, it was recommended that insecticides with differing modes of action be rotated or used in combination with other control methods.

[1]Centro de Desarrollo de Productos Bióticos del Instituto Politécnico Nacional, Carretera Yautepec-Jojutla Km. 6, AP 24, 62731 San Isidro, Yautepec, Morelos, México
[2]Centro de Investigación en Biodiversidad y Conservación (CIByC), Universidad Autónoma del Estado de Morelos, México
[3]Laboratorio de Principios Fitoquímicos Bioactivos de la Facultad de Ciencias Químicas e Ingeniería, Universidad Autónoma del Estado de Morelos, México
*Corresponding author; E-mail: dra.mg.valladares.cisneros@gmail.com

Over the past decade, research has focused on developing environmentally friendly strategies for the control of *S. acupunctatus*, including pheromone-baited traps (Ruíz-Montiel et al. 2008; López-Martínez et al. 2011; Azuara-Domínguez et al. 2012) or botanical insecticides known as green insecticides, which in many cases were compatible with other pest management methods (García-Ramírez et al. 2014). Use of such methods may enable sustainable agroecosystems, because they meet internationally established standards for organic farming and sustainable agriculture.

Powders, extracts, essential oils, and mixtures of purified compounds obtained from various parts of plant, such as roots, seeds, and stem and leaf (leaves, fruits, or flowers) were shown to have insecticidal activity and reduce populations of economically important curculionids (Tinzaara et al. 2006; Girma et al. 2008; Abdul Majeed & Abidunnisa 2011; Suthisut et al. 2011; Wang et al. 2011). An extract of leaves and seeds from *Ricinus communis* L. 'Mirante' (Malpighiales: Euphorbiaceae) applied against *S. acupunctatus* caused repellency and weight reduction of adult weevils (Pacheco-Sánchez et al. 2012). Several spice plants exhibited insecticidal activity against other important insect pests. These included *Allium sativum* L. (Asparagales: Amaryllidaceae) (Arannilewa et al. 2006), *Argemone mexicana* L. (Ranunculales: Papaveraceae) (Abdul Majeed & Abidunnisa 2011), *Bursera grandifolia* (Schltdl.) Engl. (Sapindales: Burseraceae), *Tagetes erecta* L. (Asterales: Asteraceae), *Chenopodium ambrosioides* L. (Caryophyllales: Amaranthaceae) (Tapondjou et al. 2002), and *Prosopis juliflora* (Sw.) D. C. (Fabales: Fabaceae) (Oliveira et al. 2002).

The objective of this study was to evaluate the insecticidal activity of organic extracts from selected plants against *S. acupunctatus*. Such green insecticides may allow development of sustainable alternatives to control this pest insect.

Materials and Methods

INSECTS

Larvae of *S. acupunctatus* were collected in damaged *P. tuberosa* commercial cultures located in Emiliano Zapata (22.1750000°N, 98.0000000°W), Morelos, Mexico, between Jan 2010 and Mar 2011. Damaged tuberose bulbs containing *S. acupunctatus* larvae were kept individually in plastic containers (5.8 cm diameter ´ 2.7 cm height) with small perforations for air circulation and moist filter paper on the bottom, and fresh tuberose bulbs (15 g) were added as necessary. The containers were placed in a bioclimatic chamber (model 818; Precision Scientific, Winchester, Virginia) at 27 ± 1 °C, 60.0 to 70.5% RH, and a photoperiod of 12:12 h L:D until adults emerged.

The species identity (sample of 30 adult specimens, 15 ♂ and 15 ♀) was confirmed by Dr. Héctor González-Hernández from the Entomology Department of the Phytosanitary Institute of Postgraduate College, Montecillos, Mexico. The sex of the adults was determined using a stereomicroscope (model SMZ 800; Nikon, Tokyo, Japan) as described by Ramírez-Choza (1993).

Paired weevils were placed in plastic containers with small tuberose bulbs as food and as an oviposition substrate. After 3 d, each tuberose bulb was replaced with a new one. The removed bulbs were dissected to recover eggs, which were placed on moist filter paper in Petri dishes (6 cm diameter × 1.5 cm height) and kept at the above described controlled conditions. The hatching 1st instar larvae were used in bioassays.

THE ARTIFICIAL DIET

Thirty g of tuberose bulb, 1.45 g vitamins (Centrum; Wyeth, S. A. de C. V., Mexico), 15.62 g sucrose, 6.25 g brewer's yeast, 19.86 g wheat bran, 0.50 g cholesterol, and 1.00 g Wesson salt mixture were blended with 56 mL distilled water, and another 180 mL to dissolve the agar (6.25 g agar; Bioxon). The amount of antimicrobials was 0.35 g sorbic acid, 1.02 g ascorbic acid, and 0.60 g methyl *p*-hydroxybenzoate.

PLANTS AND PLANT EXTRACTS

Six plants known to possess insect-repellent or insect-deterrent properties were collected in the summer from 3 places in central Mexico, namely: Sierra de Puebla in Puebla, and Sierra de Huautla in Morelos, and Tetela del Volcán in Morelos (see Table 1). The botanical material collected was identified by Dr. Rolando Ramírez from the herbarium of the Autonomous University of Morelos State.

The plant tissue was dried individually at room temperature (26–28 °C) in darkness for 1 or 2 mo, the time differing for each species. The individual plant extracts were obtained by maceration of the dried tissue (500 g) with 1 L of the respective solvent (purity 99.8%; J. T. Baker, Mexico) that was known to extract the biologically active compounds (Kuklinski 2000; Table 1). The plant–solvent mixtures were kept in an amber glass container for 72 h at room temperature in darkness. Then the solvent, containing the soluble chemical components, was filtered and transferred into a 1,000 mL round bottom flask, from which the solvent was removed by reduced pressure distillation with a rotary evaporator (R-205 Base model; Büchi Labortechnik AG, Flawil, Switzerland). The extraction from the macerated plant material was repeated 3 times, each time adding the plant–solvent mixture to the same flask. All extracts were weighed to calculate the yield (Table 1) and stored at 4 °C in the previously used amber glass flask.

BIOASSAYS

To evaluate toxic effects in an ingestion assay, each plant organic extract (TeFH, TeLE, AmSLH, PlLK, BgLM, CaSLE, and AsBK; see Table 1) was incorporated individually into the artificial weevil diet, at 500 ppm, according to the method described by Valdés et al. (2014), and the

Table 1. Sources, plants, and percentage of recovery of extracts evaluated on *Scyphophorus acupunctatus* larvae.

Site collected	Scientific name (family)	Tissue of plant	Solvent employed	Code	Recovery (%)
Sierra de Puebla	*Argemone mexicana* (Papaveraceae)	stem and leaf	hexane	AmSLH	3.95
	Tagetes erecta (Asteraceae)	flowers	hexane	TeFH	2.86
		leaves	ethanol	TeLE	1.16
Sierra de Huautla, Morelos	*Bursera grandifolia* (Burseraceae)	leaves	methanol	BgLM	2.36
	Prosopis laevigata (Fabaceae)	leaves	ketone	PlLK	1.60
Local Yautepec Morelos Market	*Allium sativum* (Amaryllidaceae)	bulbs	ketone	AsBK	2.55
	Chenopodium ambrosioides (Amaranthaceae)	stem and leaf	ethanol	CaSLE	3.40

artificial diet with less than 1.0% of each individual solvent (ethanol, hexane, ketone, or methanol, depending the solvent used in the extraction) was used as control. Plastic containers (4 cm diameter ´ 1.5 cm height) were filled with 8.5 mL of the artificial diet and sealed with plastic film (Kleen Pack, Mexico). Once the diet had cooled and solidified, 1 *S. acupunctatus* neonate larva was placed in each container with the aid of a fine camel hair brush. The larvae were incubated at controlled conditions in a bioclimatic chamber (27 ± 1 °C, 60 to 70% RH, and 12:12 h L:D photoperiod).

Response variables included larval weight at 12 and 24 d, pupal and adult weights, and percentage of mortality among larvae and pupae. The experimental design was completely random with 3 repetitions (n = 90 neonatal larvae). We applied an analysis of variance (ANOVA) and the Tukey test for statistical separation of means. Percentage of mortality was calculated according to the formula of Abbott (1925). Prior to ANOVA, the normality and homoscedasticity of the data were verified by the Shapiro–Wilk and Levene's tests, respectively (SigmaPlot 12.5).

Results

Results from the no-choice feeding bioassays with ketone, hexane, ethanol, and methanol extracts of *P. laevigata*, *B. grandifolia*, *C. ambrosioides*, *T. erecta* (flowers and leaves), *A. mexicana*, and *A. sativum* at 500 ppm are shown in Tables 2 and 3. Table 2 presents larval mortality rates and the weights of larvae at 12 and 24 d. All larvae fed diet with plant extracts suffered mortality, and the average weights of these larvae were statistically significantly lower than of those fed control diet.

Extracts of *A. mexicana*, *A. sativum*, and *T. erecta* (leaves) caused 43 to 53% mortality in *S. acupunctatus* larvae, whereas extracts of *P. laevigata, B. grandifolia, C. ambrosioides*, and *T. erecta* (flowers) caused less than 26% larval mortality (Table 2). The 3 extracts that produced the greatest weight reduction in larvae at 12 d were from *A. sativum*, *A. mexicana*, and *P. laevigata*, with larvae weighing less than 11 mg, whereas control larvae had reached an average weight of 192.0 mg (Table 2). The lowest mean larval weights at 12 and 24 d were associated with *A. sativum* extract, 7.3 and 29.3 mg, respectively (Table 2). Larvae fed *A. mexicana* extract had a statistically similar low mean weight (56.8 g) at 24 d, whereas larvae fed other plant extracts weighed on average 123.0 to 246.3 mg, and control larvae weighed 336.9 mg (Table 2).

Table 3 presents the mortality of pupae and the mean weights of pupae and adults of *S. acupunctatus*. Significant effects of plant extracts on weight of both pupae and adults were observed for nearly all extracts. Extracts from some of the plant species also caused mortality of pupae (3–15%). The extracts affecting survival of larvae were usually the same ones affecting pupae (Table 3).

Discussion

Our results demonstrated that organic extracts of bulbs, flowers, or leaves of 6 plant species had effects on the development and survival of *S. acupunctatus* in no-choice feeding assays. Silva et al. (2003) established criteria to identify promising botanical insecticides and proposed to select plant extracts that produced mortality rates greater than 40% for further evaluation. Three of the extracts tested in this study fell into this category: the ethanol extract of *T. erecta* leaves, the hexane extract of *A. mexicana* stem and leaf, and the ketone extract of *A. sativum* bulbs.

The extracts of *A. sativum*, *A. mexicana*, and *P. laevigata* disrupted growth, as demonstrated by the low larval weights after 12 and 24 d, and had insecticidal activity, as shown by a larval mortality of 16 to 53%. These species contain characteristic secondary metabolites to which low larval weights and insecticidal activities have been attributed. *Allium sativum* contains sulfur compounds such as allicine (Nwachukwu & Asawalam 2014) and flavonoids such as rutin. It was studied by Hoffman-Campo et al. (2013), who reported high toxicity of rutin against *Trichoplusia ni* Hübner (Lepidoptera: Noctuidae), and Simmonds (2003) mentioned effects of rutin on the feeding behavior of a range of noctuid larvae.

For *A. mexicana*, the alkaloids berberine and argemonine in a lipophilic mixture were shown to have antifeedant effects (Brahmachari et al. 2013). Another study demonstrated repellent effects of the aqueous extract of *A. mexicana* against the rice weevil, *Sitophilus oryzae* Hustache (Coleoptera: Dryophthoridae), and the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Abdul Majeed & Abidunnisa 2011). Gallegos-Infante et al. (2013) reported that *P. laevigata* contains polyphenol compounds, which are known as effective insect feeding inhibitors (Pavela 2010). The 43% larval mortality produced by the ethanol extract of *T. erecta* leaves could be attributed to the natural chemical substances present in this species, which include the monoterpene ketone piperitone and, in relatively lower amounts, some sesquiterpenes such as β-caryophyllene and caryophyllene oxide (Marques et al. 2011). Although *C. ambrosioides* extract caused statistically significant weight loss in larvae, pupae, and adults, it caused only intermediate levels of larval and pupal mortality (20 and 6%, respectively).

In summary, 4 of the plant extracts tested in this study showed promising pest control activity against the weevil *S. acupunctatus*, because ingestion ultimately reduced the number of emerging adults. Botanical insecticides present advantages over synthetic chemical insecticides because they are compatible with other biological control options, as such pheromones, oils, soap, entomopathogenic fungi, predators, and parasites. They have been applied in the control of pest insects as such fall armyworm (*Spodoptera frugiperda* Smith & Abbot;

Table 2. Effects of diet containing plant extracts on *Scyphophorus acupunctatus* larvae.

Organic extract of plant (500 ppm)	Weight of larvae (mg)[a] 12 d	Weight of larvae (mg)[a] 24 d	Mortality of larvae (%)
Argemone mexicana (AmSLH)	9.0 ± 0.06c	56.8 ± 0.05d	53 ± 4.1
Tagetes erecta (TeLE)	80.5 ± 0.08b	147.3 ± 0.11c	43 ± 0.8
Allium sativum (AsBK)	7.3 ± 0.02c	29.3 ± 0.02d	43 ± 1.3
Tagetes erecta (TeFH)	15.8 ± 0.01c	151.0 ± 0.11c	26 ± 1.0
Chenopodium ambrosioides (CaSLE)	106.1 ± 0.08b	123.0 ± 0.06c	20 ± 8.1
Prosopis laevigata (PlLK)	10.6 ± 0.03c	217.0 ± 0.08b	16 ± 5.7
Bursera grandifolia (BgLM)	21.8 ± 0.09c	246.3 ± 0.08b	10 ± 7.0
Control	192.0 ± 0.10a	336.9 ± 0.10a	0

[a]Means followed by the same letter in each column are not significantly different ($P > 0.05$).

Table 3. Effects of diet containing plant extracts on *Scyphophorus acupunctatus* pupae and adults.

	Weight (mg)[a]		
Organic extract of plant (500 ppm)	Pupae	Adults	Mortality of pupae (%)
Tagetes erecta (TeLE)	123.8 ± 0.03c	63.8 ± 0.2d	15 ± 2.1
Prosopis laevigata (PlLK)	135.5 ± 0.03bc	78.2 ± 0.03cd	13 ± 7.7
Allium sativum (AsBK)	128.8 ± 0.03c	62.9 ± 0.01d	10 ± 1.3
Bursera grandifolia (BgLM)	131.3 ± 0.03bc	75.0 ± 0.02cd	10 ± 6.0
Chenopodium ambrosioides (CaSLE)	118.0 ± 0.04c	70.0 ± 0.03d	6 ± 1.5
Tagetes erecta (TeFH)	160.0 ± 0.02b	100.5 ± 0.08ab	5 ± 0.0
Argemone mexicana (AmSLH)	120.0 ± 0.03c	70.7 ± 0.02d	3 ± 0.5
Control	198.8 ± 0.05a	114.6 ± 0.03a	3 ± 0.4

[a]Means followed by the same letter in each column are not significantly different ($P > 0.05$).

Lepidoptera: Noctuidae), whiteflies (e.g., *Trialeurodes vaporariorum* [Westwood]; Hemiptera: Aleyrodidae), aphids (e.g., *Aphis gossypii* Glover; Hemiptera: Aphididae), and many others as part of integrated pest management programs (Jain & Tripathi 2006; Enyiukwu et al. 2014).

The toxicity associated with extracts of *A. sativum*, *A. mexicana*, *P. laevigata*, *T. erecta*, and *C. ambrosioides* may prove useful for the disruption of growth and development in *S. acupunctatus*. Thus, it may support the goal of integrated pest management programs to reduce environmental contamination with synthetic pesticides while suppressing pest insect populations to levels below economic thresholds.

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