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José Guadalupe Ontiveros Guerra
Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México C.P. 25315

Ernesto Cerna Chávez
Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México C.P. 25315

Yisa María Ochoa Fuentes
Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México C.P. 25315

Jerónimo Landeros Flores
Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México C.P. 25315

Luis Alberto Aguirre Uribe
Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México C.P. 25315

Agustín Hernández Juárez
Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México C.P. 25315

Corresponding Author:
José Guadalupe Ontiveros Guerra
Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México C.P. 25315

Insecticidal activity of plant extracts against whitefly nymphs *Bemisia tabaci* (Hemiptera: Aleyrodidae) in laboratory

José Guadalupe Ontiveros Guerra, Ernesto Cerna Chávez, Yisa María Ochoa Fuentes, Jerónimo Landeros Flores, Luis Alberto Aguirre Uribe and Agustín Hernández Juárez

Abstract
21 plant extracts were evaluated against nymphs *Bemisia tabaci* under laboratory conditions. For the establishment of the test, the nymphs (N2) immersion technique was used, using a completely randomized design with 8 concentrations and 10 repetitions evaluating after 72 hours. For the estimation the susceptibility (LC_{50} and LC_{95}) the Probit Analysis was used, the main phytochemical compounds in the extracts were determined using the infrared spectrophotometer. The results show a high susceptibility to cat's claw (*Mimosa zygophylla*), dragon's blood (*Jatropha dioica*), garlic (*Allium sativum*), ribwort (*Plantago lanceolata*) and lechuguilla (*Agave lechuguilla*), with a LC_{50} of 778, 795, 890, 996 and 1035 ppm respectively. The compounds present in the evaluated extracts were different types of acids (benzoic, linoleic, palmitic, shikimic, cinnamic, coumaric and tartaric), 1, 8-cineole and eugenol, possibly responsible for the insecticidal activity.

Keywords: *Bemisia tabaci*, susceptibility, extracts, phytochemical

Introduction
Bemisia tabaci (Gennadius) attacks and damages around 600 species of plants of agricultural importance (Nombela and Muniz 2010; Alemandri *et al.* 2015) [23, 3] in 162 countries (CABI, 2018) [6]. *B. tabaci* causes direct damage by sucking the phloem sap, also causes indirect damage by transmitting different viruses (Singh *et al.* 1994) [27], this whitefly is an important vector of begomovirus (family Geminiviridae), transmitting approximately 111 viruses (Tiwari *et al.* 2013) [28].
The main control strategies for this whitefly contemplate chemical control, with a significant number of substances reported for the manage (Roy *et al.* 2014; Chen *et al.* 2018) [26, 8], but there are disadvantages such as the rapid development of resistance (Kliot *et al.* 2016) [15] also the substances used are dangerous for humans and the environment (Abbassy, 2017) [1]. Different alternatives of plant origin which can contribute in the management strategies, botanical insecticides (BIs) are products derived from plants with many active substances (Isman, 2006) [13]. Historically, the properties and potential of some plants and their secondary metabolites against different insects are known (Jaber *et al.* 2018) [14].
The BIs have been tested for whitefly control with good results, but there is a necessity to explore more about the BIs involved in whitefly control (Ashfaq *et al.* 2019) [5] in order to offer efficient control alternatives and biorational. In the present study, 21 plant extracts were evaluated against whitefly nymphs, with the aim of generating a contribution for their management.

Materials and Methods
The present investigation was carried out in the Entomology and Acarology Laboratory of the Department of Parasitology of the Universidad Autonoma Agraria Antonio Narro (UAAAN).

Extract Preparation
The plants used for the preparation of the extracts were obtained commercially and collected in the experimental field "El Bajio" (Table 1), this vegetal material subsequently individualized and cut into small pieces and then placed on newspaper in a drying oven (weber anhidro

incubator, model; 4252 Arthur H. Thomas Co.) a temperature of 30 ± 1 ºC for a period of 15 days. After the drying period, the vegetal material were ground in two phases; the first in a manual mill (Estrella, Model: 11539) this to avoid problems with the lignified tissues of some of the plants used and the second in an electric mill (Hamilton Beach, Model: 80393), at the end of the milling process the powder was passed through a sieve of 250 µm to homogenize the particle size. For the preparation of the extracts a 1:6 ratio (w / v) was used, the extraction process was carried out with a 250 mL soxhlet (Pyrex® 50X250MM) for a period of 5 hours, the temperature was regulated to prevent the equipment exceed 100 °C. Half of the solvent was removed from the resulting extract using the rotary evaporator (R-205 Base model; Büchi Labortechnik AG, Flawil, Switzerland). The concentrated extracts were stored in 250 mL glass bottles and kept refrigerated at 2 ± 2 °C.

Table 1: Species of plants and solvents used for obtain the extracts evaluated against nymphs of *Bemisia tabaci*.

Family	Common and scientific name	Tissue	Solvent	Place of obtaining the plants
Amaryllidaceae	Garlic (*Allium sativum)*	Bulb	Ethanol/Water (1:1)	Commercial (Saltillo, Coahuila)
Apiaceae	Cumin (*Cuminum cyminum)*	Seed	Ethanol	Commercial (Saltillo, Coahuila)
Asparagaceae	Mojave yucca (*Yucca schidigera)*	Leave	Ethanol/Water (1:1)	Collect (University experimental field "El Bajio")
	Lechuguilla *(Agave lechuguilla)*	Leave	Ethanol/Water (1:1)	Collect (University experimental field "El Bajio")
Asteraceae	Marigold (*Tagetes erecta)*	Complete plant	Ethanol	Collect (University experimental field "El Bajio")
Cucurbitaceae	Buffalo gourd (*Cucurbita foetidissima*)	Leave and stem	Ethanol	Collect (University experimental field "El Bajio")
Euphorbiaceae	Castor bean (*Ricinus communis)*	Seed	Ethanol	Collect (University experimental field "El Bajio")
	Dragon's blood (*Jatropha dioica)*	Complete plant	Ethanol	Collect (University experimental field "El Bajio")
Fabaceae	Jicama (*Pachyrhizus erosus)*	Seed	Ethanol	Commercial (Irapuato, Guanajuato)
	Huizache (*Acacia farnesiana)*	Leave and stem	Ethanol	Collect (University experimental field "El Bajio")
	Mezquite (*Prosopis laevigata*)	Leave and stem	Ethanol	Collect (University experimental field "El Bajio")
	Cat's claw (*Mimosa zygophylla)*	Leave and stem	Ethanol	Collect (University experimental field "El Bajio")
Lamiaceae	Thyme (*Thymus vulgaris*)	Leave	Ethanol	Commercial (Saltillo, Coahuila)
Lauraceae	Cinnamon (*Cinnamomum verum)*	Stem bark	Ethanol	Commercial (Saltillo, Coahuila)
Meliaceae	Lilac (*Melia azedarach)*	seed	Ethanol	Collect (University experimental field "El Bajio")
Myrtaceae	Eucalyptus (*Eucalyptus globulus)*	Leave	Ethanol	Collect (University experimental field "El Bajio")
Piperaceae	Black pepper (*Piper nigrum)*	Seed	Ethanol	Commercial (Saltillo, Coahuila)
Plantaginaceae	Ribwort (P*lantago lanceolata)*	Complete plant	Ethanol	Collect (University experimental field "El Bajio")
Resedaceae	Dyer's Rocket (*Reseda luteola*)	Complete plant	Ethanol	Collect (University experimental field "El Bajio")
Rutaceae	Orange (*Citrus sinensis*)	Orange peel	Ethanol	Commercial (Saltillo, Coahuila)
Solanaceae	Tree tobacco (*Nicotiana glauca)*	Leave and stem	Ethanol	Collect (University experimental field "El Bajio")

Determination of phytochemicals

The concentrated extract was dried in the oven at a temperature of 35 ± 3 °C for 120 hours, completely removing the solvent. The solid fraction was used to determine the phytochemical compounds using the infrared spectrophotometer (Bruke, Model; ALPHA II FTIR), the compounds were corroborated in the database of Duke and Beckstrom (1994) [9].

Bioassays

For the establishment of the bioassays *B. tabaci* was collected from merlot vine leaves, located in the ejido San Juan de la Vaquería, Saltillo Coahuila (25 ° 15'0 "N and 101 ° 13'1" W), the whiteflies were multiplied on bean plants (*Phaseolus vulgaris*) under laboratory conditions inside a bioclimatic chamber (Lab-Line Biotronette Mark III Environmental Chamber. Model: 846) a temperature of 26 ± 2 ºC, relative humidity (70 ± 10%) and photoperiod (12:12).

The whitefly adults from the principal colony were placed inside of entomological cages with bean plants for a period of 24 hours, after this time the adults were withdrawn. The second instar nymphs (N2) were used for the test, the infested leaf were collected for later by the immersion method to perform the evaluations. The treated leaves were placed on a sponge saturated with distilled water, with the abaxial side up. For the evaluation of plant extracts it was used a completely randomized design with eight concentrations and ten repetitions. Distilled water and Tween (0.1%) were used to prepare the concentrations. The death criteria used was total immobilization, dehydration and starvation at 120 hours after application.

Statistical analysis

The results obtained from the different concentrations were corrected by Abbott (1925) [2]. The data were analyzed with the Probit using the maximum likelihood method (Finney, 1971) [11], for the determination of LC_{50} and LC_{95} using the Rstudio program.

Results and Discussion

The different lethal concentrations (LC_{50} and LC_{95}) of the 21 extracts evaluated against *B. tabaci* after 120 hours are shown below.

Table 2: Lethal concentrations, fiducial limits and confidence parameters of plant extracts against *B. tabaci.*

Extract	LC_{50}	Fiducial limits	LC_{95}	Fiducial limits	df	Slope+SE	X^2	P value
R. communis	1257	1058-1455	6042	4622-8996	8	2.41+0.16	17.1	0.029
C. sinensis	2804	2151-3561	19185	12919-35082	7	1.97+ 0.11	21.9	0.003
N. glauca	1260	1035-1497	6498	4646-11154	6	2.31+0.17	12.4	0.054
M. azedarach	1394	1164-1647	8685	6131-14767	7	2.07+ 0.14	13.2	0.067
P. erosus	2026	1773-2303	7519	5877-10796	6	2.89+ 0.19	10.2	0.118
E. globulus	1295	1122-1477	5009	3865-7417	7	2.80+ 0.18	14.6	0.041

T. erecta	2008	1622-2430	11639	8120-20560	6	2.16+ 0.15	13.1	0.042
A. farnesiana	1638	1474-1832	10585	7904-15753	6	2.03+ 0.17	2.57	0.861
P. laevigata	1365	1149-1616	5015	3726-8048	5	2.91+ 0.20	10.9	0.053
P. nigrum	1528	1243-1886	10648	6608-24703	5	1.95+ 0.17	8.44	0.133
M. zygophylla	778	638-924	3885	2782-6705	5	2.36+ 0.19	8.47	0.132
Y. schidigera	1233	1104-1364	7709	6171-10296	7	2.07+ 0.15	8.66	0.278
A. lechuguilla	1035	932-1137	4983	4100-6448	6	2.41+0.19	7.22	0.301
A. sativum	890	823-956	2853	2472-3442	6	3.25+ 0.24	5.22	0.516
C. foetidissima	1656	1325-2005	8266	5504-18022	7	2.36+ 0.18	23.5	0.001
J. dioica	795	698-885	4301	3466-5796	6	2.24+ 0.20	6.96	0.324
T. vulgaris	1808	1415-2281	10422	6636-23733	7	2.16+ 0.15	26.9	0.001
C. cyminum	2803	2221-3398	15740	10209-36640	6	2.19+ 0.19	15.2	0.019
C. verum	1420	1137-1739	6512	4542-11975	6	2.49+ 0.16	18.7	0.005
R. luteola	1948	1777-2142	9294	7502-12243	6	2.42+ 0.17	4.73	0.579
P. lanceolata	996	730-1253	11098	6856-25663	6	1.57+ 0.14	9.97	0.126

CL: Lethal concentrations (ppm). Fiducial limits: Confidence intervals. $P \leq 0.05$.

B. tabaci was susceptible to the extracts *M. zygophylla*, *J. dioica*, *A. sativum*, *P. lanceolata*, *A. lechuguilla*, *Y. schidigera* and *R. communis* with the lowest concentrations (LC_{50} and LC_{95}) of the assay (Figure 1 and Figure 2), the LC_{50} of this group is between the values of 778 to 1257 ppm. The other group of extracts to which *B. tabaci* showed medium susceptibility; *N. glauca*, *E. globulus*, *P. laevigata*, *M. azedarach*, *C. verum*, *P. nigrum* and *A. farnesiana* with LC_{50} values between 1260 and 1638 ppm. The extracts that showed lower susceptibility were *C. foetidissima*, *T. vulgaris*, *R. luteola*, *T. erecta*, *P. erosus*, *C. cyminum* and *C. sinensis* with values from 1656 to 2804 ppm (Table 2). The *M. zygophylla* extract showed the lowest values of LC_{50} and LC_{95} in the bioassay with 778 and 3885 ppm respectively (Figure 1 and Figure 2). *C. sinensis* with LC_{50} and CL_{95} of 2804 and 19185 ppm respectively, showed a low susceptibility against *B. tabaci.*

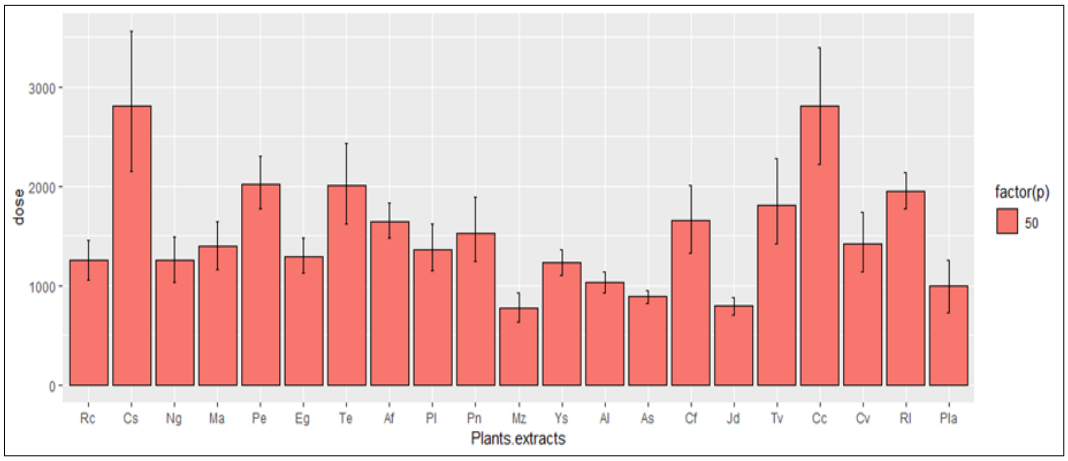

Fig 1: Lethal Concentration 50 (ppm) of plant extracts against nymphs of *B. tabaci*. Rc: *R. comunis*, Cs: *C. sinensis*, Ng: *N. glauca*, Ma: *M. azedarach*, Pe: *P. erosus*, Eg*: E. globulus*, Te: *T. erecta*, Af: *A. farnesiana*, Pl: *P. laevigata*, Pn*: P. nigrum*, Mz: *M. zygophylla*, Ys*: Y. schidigera*, Al*: A. lechuguilla*, As: *A. sativum*, Cf: *C. foetidissima*, Jd: *J. dioica*, Tv: *T. vulgaris*, Cc: *C. cyminum*, Cv: *C. verum*, Rl: *R. luteola*, Pla: *P. lanceolata.*

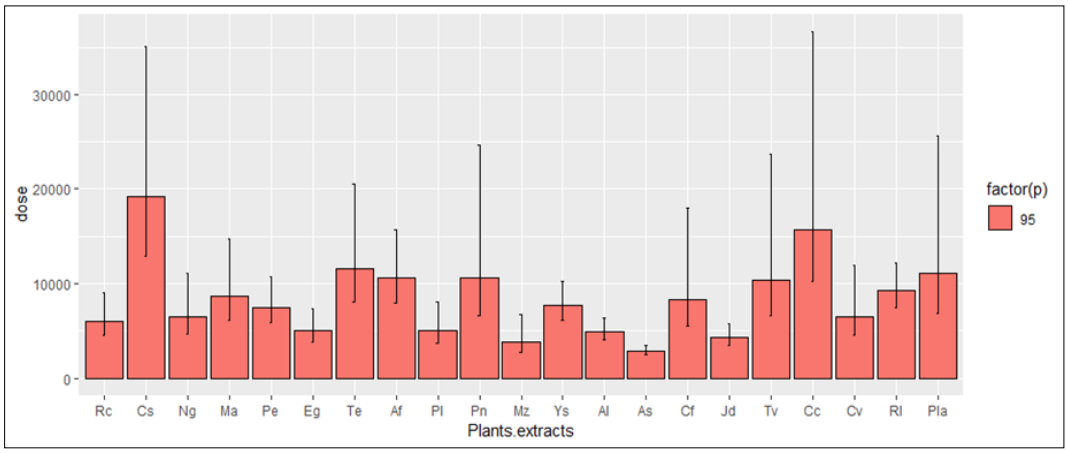

Fig 2: Lethal Concentration 95 (ppm) of plant extracts against nymphs of *B. tabaci*. Rc: *R. comunis*, Cs: *C. sinensis*, Ng: *N. glauca*, Ma: *M. azedarach*, Pe: *P. erosus*, Eg*: E. globulus*, Te: *T. erecta*, Af: *A. farnesiana*, Pl: *P. laevigata*, Pn*: P. nigrum*, Mz: *M. zygophylla*, Ys*: Y. schidigera*, Al*: A. lechuguilla*, As: *A. sativum*, Cf: *C. foetidissima*, Jd: *J. dioica*, Tv: *T. vulgaris*, Cc: *C. cyminum*, Cv: *C. verum*, Rl: *R. luteola*, Pla: *P. lanceolata.*

The main phytochemical compounds present in the extracts evaluated against *B. tabaci* are shown below, by readings in the infrared spectrophotometer with the solid fraction.

Table 3: Main phytochemical compounds present in the solid fraction of the extracts evaluated against *B. tabaci*.

Extract	Phytochemical compounds
R. communis	linoleic acid, palmitic acid, lectins, ricin, shikimic acid
C. sinensis	D-limonene, p-cumaric acid, P-cimeno, terpenes, ascorbic acid
N. glauca	nicotine, rutin, alkaloids, cinnamic acid, phenols
M. azedarach	palmitic acid, cinnamic acid, benzoic acid, 1-terpen-4-ol, 6-hydroxy-7-methoxycoumarin.
P. erosus	tartaric acid, flavonoids, phenols, D-mannose, rotenone
E. globulus	tartaric acid, terpenes, eucalyptol, 1,8-Cineole, chlorogenic acid
T. erecta	1,8-Cineole, D-limonene, beta-sitosterol, p-cumaric acid, kaempferol
A. farnesiana	tannins, methyl eugenol, terpineol, tartaric acid, stigmasterol
P. laevigata	tartaric acid, phenols, D-mannose, 4-5-7-trihydroxy flavone, linoleic acid
P. nigrum	tartaric acid, piperine, benzoic acid, beta-elemene, piperidine
M. zygophylla	tartaric acid, tannins, mimosine, phenols, D-mannose
Y. schidigera	gitogenin, phenols, saponins, yucca-saponin-B-2, flavonoids
A. lechuguilla	2-4-6 trinitrophenol, flavonoids, 2-hydroxy 1-2-3 propanetricarboxylic acid, 2-amino-2 methyl-1- propanol, vasaponins
A. sativum	2-3 dihydroxybutanedioic acid, 2,4,6-trinitrophenol, phenols, flavonoids, chloroquine, lectins, allicin
C. foetidissima	Cucurbitacin D, oleic acid, alanine, glycine, tryptophan
J. dioica	tartaric acid, 4-methylaniline, palmitic acid, alkaloids, flavonoids, saponins
T. vulgaris	ascorbic acid, alpha-terpineol, 1-8-cineol, tartaric acid, eugenol, thymol
C. cyminum	1-8-cineole, cinnamaldehyde, phenols, ascorbic acid, eugenol
C. verum	1-8-cineole, phenols, flavonoids, alpha terpineol, eugenol
R. luteola	kaempferol, quercetin, flavonoids, isoflavones, luteolin
P. lanceolata	coumar R-15, benzoic acid, phenols, apigenina, alkaloids, ascorbic acid

The most abundant compounds were different types of acids; oleic, linoleic, palmitic, tartaric, ascorbic, cinnamic, benzoic and shikimic present in most of the extracts evaluated, these acids are known to have important insecticidal activity (Table 3). The eugenol is a compound with important biological activity, present in cinnamon, cumin, huizache and thyme. In garlic and castor bean we found lectins, another important compound is 1,8-cineole present in cumin, cinnamon, thyme, marigold. D-Limonene, compound with important insecticidal activity is present in orange and calendula. The coumarins are present in ribwort and orange. D-Manosa is present in jicama, mesquite and cat's claw, finally different amino acids; alanine, glycine and tryptophan in buffalo gourd.

B. tabaci shows a high susceptibility (LC_{50} and LC_{95}) to *M. zygophylla*, *J. dioica*, *A. sativum*, *P. lanceolata*, *A. lechuguilla*, *Y. schidigera* and *R. communis.* The effectiveness of this extracts is due to their different secondary metabolites with insecticidal action, Phenolic compounds and mimosine responsible for insecticidal activity against whiteflies are found in cat's claw. (Duke and Beckstrom, 1994; Marimuthu *et al.* 2010) [9, 18]. Dragon's blood has important metabolic composition; alkaloids, flavonoids and saponins (Valenzuela *et al.* 2019) [29], compounds with important effects against insects (Moreno *et al.* 2016) [21]. The garlic is one of the plants with the highest metabolic composition, sulphurous compounds and allyl groups (Farag *et al.* 2017; Misiorek *et al.* 2017) [10, 20], saponins (voghieroside A1, A2, B1 and B2) (Lanzotti *et al.* 2012) [16], allicin, alliin, malic acid, pyroglutamic acid and some amino acids such as proline and isoleucine (Hrbek *et al.* 2018) [12]. Navarrete *et al.* (2016) [22] pointed out the compounds in Ribwort (catalpol, aucubina and verbascoside), Mazzutti *et al.* (2017) [19] reported the presence of polyphenolic compounds (plantamajoside and isobasbasoside). Almaraz *et al.* (2013) [4] described the potential of lechuguilla, phenolic compounds, flavonoids, isoflavonoids, saponins and phenolic acids are responsible for cytotoxic activity (Ramos *et al.* 2012) [25]. Yucca mojave is an important source of steroidal saponins and polyphenols (Piacente *et al.* 2005) [24], resveratrol, stilbenes, for example, yuccaols of different groups (A, B, C, D and E) with important cytotoxic function (Cheeke *et al.* 2006) [7]. Different fatty acids are present in the seed of *R. communis* with multiple applications in pest control, ricinoleic, stearic, linoleic acid (Lin and Arcinas, 2007) [17] and pyrimidine alkaloid (Wachira *et al.* 2014) [30].

Conclusion

The most efficient plants extracts for whitefly control are *M. zygophylla*, *J. dioica*, *A. sativum*, *P. lanceolata*, *A. lechuguilla*, *Y. schidigera* and *R. communis*, this plants extracts presented metabolites such as saponins, fatty acids, flavonoids, terpenes and phenolic compounds, all with reports insecticidal activity.

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