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Research Article

Phytotoxic and dissuasive activity of Chihuahua desert plants

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Abstract

With the purpose of finding plant compounds with the potential use as herbicides and insecticides, a research was realized with the objective of evaluate the phytotoxic and dissuasive activity of four Chihuahua desert plants. The phytotoxic activity evaluation was tested on *Lactuca sativa* and *Lolium perenne*, while the dissuasive activity was realized on three species of phytophagous insects: *Myzus persicae*, *Rhopalosiphum padi* and *Spodoptera littoralis*. Raw extracts were used, the solvents hexane, methanol and ethanol of different plants' organs (root, steam, leaf and flower) of four species: *Fouquieria splendens* (ocotillo), *Larrea tridentate* (governor), *Astragalus mollissimus* (wild grass) and *Pachycereus pecten-aboriginum* (echo), by the establishment of *in vitro* bioassays at a concentration of 10 mg/ml extract/solvent. In the toxicity bioassay, the percentage of germination, root and leaf length were measured. The results showed that the leaf extract of *L. tridentata* had phytotoxic activity for *L. sativa*, while for *L. perenne* the phytotoxicity was observed within the ocotillo, governor and echo extracts. In the dissuasive bioassay, each treatment had 20 repetitions with 10 adult insects per repetition. The methanolic extracts of *F. splendens* leaf and root, ethanolic extract of *A. mollisimus* sheet and the ethanolic extract of *P. pecten-aboriginum* stems showed moderate dissuasive response of feeding against *M. persicae*, presenting a settlement inhibition index of 53.53, 54.35, 60.00 and 48.84% respectively. Nevertheless, the results indicated that none of the 10 extracts tested on *S. littoralis* showed significant dissuasive properties for this Lepidoptera, while for *R. padi* all the tested extracts presented dissuasive properties. The treatments of the four vegetable species evaluated showed defensive or dissuasive properties of moderate to strong feeding against the insects *M. persicae* and *R. padi*, presenting interesting potential for being used as insecticides, while the tested extracts that presented phytotoxicity for both lettuce and ryegrass present possibilities for the realization of herbicides.

Keywords: antifeedant; natural insecticides; phytotoxicity; vegetable extracts

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Introduction

The botanical compounds constitute an old alternative for the control of agricultural pests (Perez, 2012). It is currently known that secondary metabolites have an important role in the defensive mechanism of plants. Therefore, in recent years, the use of such components as a source of biopesticide preparation has been returning (Celis *et al*., 2008), since these metabolites can be isolated and used in agriculture as an alternative for the integrated control of pests and diseases (Emilia *et al*., 2013). Synthetic insecticides have been the most used elements for the control of harmful organisms, causing serious consequences to the ecosystem and generating the development of resistance in pests that were intended to be controlled (Perez *et al*., 2013). The aforementioned, together with the recognition of the biological properties of numerous plant species, has led to the search of new natural compounds extracted from plants with the potential use as insecticides and herbicides with less impact on the environment (Rodriguez and Barreto, 2015). Currently there is a certain number of plant-based insecticides that are being marketed worldwide, such as azadirachtin, rotenone, nicotine and sabadilla (Mendoza *et al*., 2007, Perez, 2012). Likewise, botanical pesticides include nematicides that inhibit germination, fungicides and new herbicides (Nava-Perez *et al*., 2012).

The majority of plant species have some type of phytochemical study. However, there are many aspects that remain empirical, which is a limitation to know the bioactive components and their diverse applications in the agronomic field (Celis, 2008). Monreal-Garcia *et al*. (2014) evaluated phenolic compounds of foliar tissues of extracts of *Fouqueiria splendens*, whereas they mention that the presence of these compounds can serve as an indicator of the attack of some pathogenic agent, since these are secreted as a defense mechanism. Likewise, Salas (2013) reports a work done by Fimbres and Garcia (1998) in a combination of extracts of *P. pecten-aboriginum* and *Lphocerus sch*ottii reporting bactericidal and antifungal activity. Flores *et al*. (2015) evaluated the allelopathic activity of *Astragalus mollissimus* in *Lactuca vitreous* seeds, *Halepense sorghum*, *Lolium multiflorum*, *Arundo donax* and *Medicago sativa* detecting allelopathic activity as a possible natural herbicide, with respect to *L. tridentate*. There are scientific investigations that mention the biological properties that this shrub possesses; it has been reported that its components have antifungal, antibacterial, nematicidal, phytotoxic and insect repellent effects (Moreno-Limon *et al*., 2011; Peñuelas-Rubio *et al*., 2017), as well as being used for its medicinal properties (Lambert, 2004).

Therefore, the objective of the present work was to evaluate the phytotoxic and dissuasive activity of the Chihuahua desert plants *Fouquieria splendens* (ocotillo), *Larrea tridentata* (governor), *Astragalus mollissimus* (wild grass) and *Pachycereus pecten-aboriginum* (echo), on *Lactuca sativa* and *Lolium perenne* and the dissuasive effect of feeding on three phytophagous insects of worldwide economic importance, *Myzus persicae*, *Rhopalosiphum padi* and *Spodoptera littoralis*, using raw extracts obtained of the four plant species mentioned.

Materials and Methods

Preparation of raw extracts

The collected plant material (root, stem, leaf and flower) was washed and dried at room temperature, after which was cut and placed in paper bags, dried in a Tork-type stove (Felisa) for five days at 40 °C and milled to obtain a fine powder. For the maceration, the solvents hexane, methanol and ethanol were separately used for each plant organ for 48 hours. The extracts were concentrated under reduced pressure. The extracts were stored at -22 °C; the solutions of each extract to be tested were prepared in the bioassays, at a concentration of 10 mg ml^{-1} extract/solvent. Juglone was used as a positive control and pure methanol as a negative control (Table 1).

Phytotoxic bioassay

The bioassay was carried out in vegetable cultivation chambers with a photoperiod of 16:8 (L:O) at 23-24 °C, according to the methodology proposed by Moiteiro *et al*. (2006). The seeds used were from *Lactuca sativa* 'Carrasco' variety (certified, Arnedo, La Rioja) and *Lolium perenne* variety 'Nui', lot 3881157. Variables regarding the germination percentage and radicle length were evaluated for the two species, while, in addition, leaf length was measured for *L. perenne*.

Table 1. Evaluated treatments for phytotoxic bioassay using Chihuahua desert species

Plant species	Common name	Organ and solvent	Treatment
Fouquieria splendens	Ocotillo	Hexane sheet	ohh
		Methanol sheet	ohm
		Methanol root	orm
Larrea tridentata	Governor	Ethanol root	gre
		Ethanol sheet	ghe
		Methanol sheet	ghm
Astragallus mollissimus	Crazy grass	Ethanol flower	hlfe
		Ethanol sheet	hlhe
Paquicereus pecten-aboriginum	Echo	Stem 10 r. ethanol	ht10e
		Stem 12 r. ethanol	ht12e
Control	Positive control	Juglone	tju
	Negative control	Methanol	tdi

Establishment of the bioassay on L. sativa and L. perenne

The seeds were hydrated for 12 hours before the test. Later, a dose of 20 µl of each treatment was added to 2.5 cm diameter filter paper discs; once dried, they were placed in plastic boxes and 10 seeds per disc were placed and hydrated with 500 µl of distilled water; the boxes were sealed with parafilm and placed in the chamber for germination. The negative control (only with solvent) was prepared in the same manner and with the same dose, plus water; the control with juglone used as reference for phytotoxicity, was prepared likewise, in a concentration of 5 mg ml^{-1} (juglone/acetone) (Kocacaliskan, 2001). The readings were carried out every 24 hours, for six days (144 hours), counting the number of germinated seeds. At the end of the test, the root and leaf length were measured using the program Image J version 1.37 r. 2006 (Rodilla *et al*., 2008). The effect of the treatments was verified by an ANOVA analysis of the results obtained, whereas the design used was a completely randomized factorial arrangement with 4 repetitions per treatment and comparison of Tukey means with an $\alpha = 0.05$.

Dissuasive bioassay of feeding of phytophagous insects

The species used (*Myzus persicae*, *Rhopalosiphum padi*) were raised in secondary host plants *Capsicum annuum* and *Hordeum vulgare*, while *Spodoptera littoralis* was fed a general diet for noctuid's, in chambers heated at a temperature of 22±1 °C, with a relative humidity of 60-70% and photoperiod 16:8 (L:O) (Rodilla *et al*., 2008; Burgueño-Tapia *et al*., 2008).

Establishment of the bioassay on S. littoralis

The dissuasive effects of the feeding of the evaluated extracts were carried out through tests with possibility of choice where larvae of the fifth and sixth stage (L5, L6) of *S. littoralis* were used. Each treatment had 5 repetitions, with completely random experimental design, which consisted of 5 Petri dishes with a layer of insect agar (2.5%), with 4 holes with equivalent distances where discs of leaves of *C. annuum* of 1 cm^2 were placed; in two discs, the extract to be tested was applied (10 µl), while the other two discs served as controls where only the solvent was applied; two larvae of *S. littoralis* were deposited in each box (Gonzalez-Coloma *et*

al., 1995, 1996 and 2008). Once 75% of the surface of the discs was consumed (control or treatment), the larvae were removed. The unconsumed foliar surfaces were measured with the image analysis program Image J. 1.37 r, 2006 (Rodilla *et al*., 2008). The consumption index (FI) was calculated: FI = 1 – (T/C) × 100%, where T: Consumption of the treatment, C: Consumption of the control or witness. It was considered that if FI ≥ 75, it corresponds to a highly active extract, while 50 < FI <75) indicate a moderately active extract (Gonzalez-Coloma *et al*., 2008; Rodilla *et al*., 2008).

Establishment of the bioassay for M. persicae and R. padi

Each treatment consisted of 20 repetitions with 10 adult insects per repetition. Plastic boxes of 3 × 3 × 1.5 cm, covered and ventilated, in an inverted position, with a surface of 2.5% insect agar were used. For *M. persicae* assay, discs of *C. annum* leaves of 1 cm^2 in diameter were prepared, while for *R. padi* two fragments of *H. vulgare* foliole, with a surface area of 2 cm^2 , were used (Gutierrez *et al*., 1997), whereas half disc or foliole fragment was treated with the solution of the extract to be tested, with a dose of 10 µl in a concentration of 10 mg ml^{-1} (extract/solvent), and the other half of the disc or fragment was treated with pure solvent with the same dose as the treatment (Reina *et al*., 2001). Each treatment was subsequently incubated in chambers under the same climatic conditions mentioned for the maintenance of these insects, by a completely random design. After 24 hours, the aphids were counted in the treatment and in the control, calculating the settlement inhibition index (SI) for each treatment: SI = [1 – (% T/% C)] × 100, where T: percentage of aphids on the treated surface, % C: percentage of aphids on the surface of the sample or control. It was considered that a SI ≥ 60 represents a highly active extract and 45 < SI <60 is considered as a moderately active extract (Gonzalez-Coloma *et al*., 2008; Rodilla *et al*., 2008).

Results and Discussion

Phytotoxic effect on Lactuca sativa

In regard with the inhibition of germination of *L. sativa* seeds, of the 12 treatments tested only the ethanolic extract of *L. tridentata* leaf showed germination delay compared to the control, observing 45% germination after 24 hours, reaching 92% after 120 hours and 95% after 144 hours (Figure 1). In the rest of the treatments there were no significant differences in this variable. Regarding the radical length, only two of the evaluated extracts showed activity, significantly inhibiting the growth of the root (Figure 2). The shortest length, in addition to causing deformations, was obtained with the ethanolic treatment of *L. tridentata* leaf. Reported results from similar studies mention that researchers found inhibition of radicular and hypocotyl growth of *L. sativa* with extracts of NDGA (Nordihydroguaiaretic acid), the main component of *L. tridentata* leaf (Delgado *et al*., 2014). On the other hand, the hereby results differ from those reported by Lira-Saldivar *et al*. (2008) who mentioned that when testing extracts of said species, they showed a bio stimulating effect on the germination and growth of *L. sativa*. In the present study, the second treatment that showed phytotoxic activity in root growth was the methanolic extract of ocotillo root, unlike the percentage of germination where it showed no inhibition. At the moment, there are no scientific publications that mention phytotoxic properties of *F. splendens* (ocotillo).

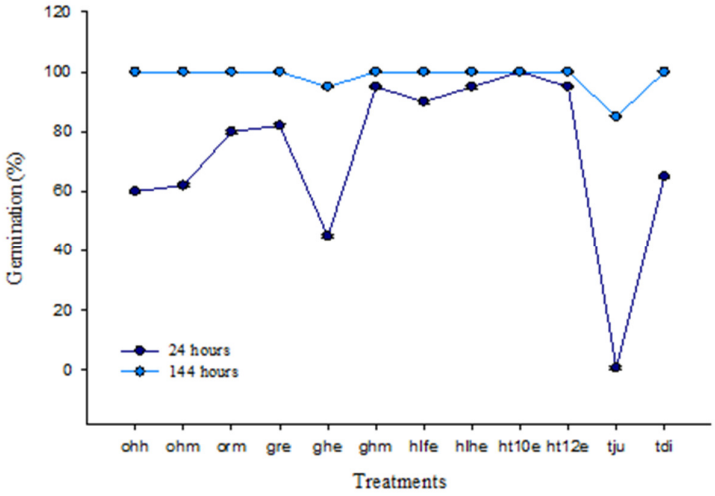

Figure 1. Phytotoxic effects of the applied treatments on germination percentage of *L. sativa* at 24 and 144 hours

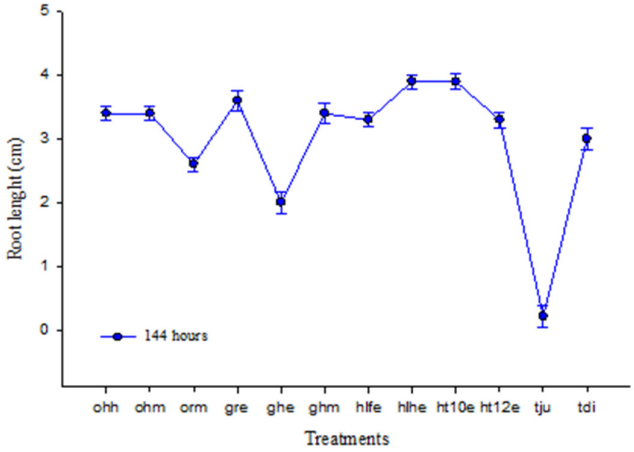

Figure 2. Phytotoxic effects of the applied treatments on the seedling root length of *L. sativa* at 144 hours

Phytotoxic effect on Lolium perenne

The germination percentage for *L. perenne* species was evaluated (Figure 3). The treatments with the methanol extract of the ocotillo and governor leaves, as well as the ethanolic extract of the stem of 10 echo ribs, showed significant phytotoxic activity at the end of the study (144 hours). Regarding the inhibition of root growth of *L. perenne* seedlings, the extracts that showed inhibition were: methanolic from the root of *F. splendens* and stem ethanolic from 10 ribs of *P. pecten-aboriginum* (Figure 4). For leaf length, none of the treatments showed significant inhibition of growth except for the methanolic extract of ocotillo root,and the leaf ethanolic extract of *L. tridentata*, which showed stimulating effects on the leaf length. Zarate-Hernandez *et al*. (2008) found phytotoxic effects on germination, leaf and root length in *L. perenne* when testing aqueous extracts of *Calia secundiflora* leaf at 5% concentration. Young and Bush (2009) reported significant inhibition in germination of grass *Bouteloua curtipendula monocotyledonea* belonging to the family Poaceae, due to the action of leaf extracts of *Juniperus ashei* Buchh.

Dissuasive bioassays of the tested insects feeding

Dissuasive effect of feeding of Spodoptera littoralis

The results obtained in this bioassay indicated that none of the 10 extracts tested on *S. littoralis* showed significant dissuasive properties for this lepidoptera (Table 2). Likewise, no scientific evidence of these plant extracts or the dissuasive properties of this insect have been found in other works previously done. For example, Gonzalez-Coloma *et al.* (2006) reported the low effect (FI of 33%) of essential oils extracted from leaves and flowers of *Lavandula luisieri* against *S. littoralis*. In 2008, the same author and collaborators mentioned that after evaluating diterpene neo-clerodane isolated from parts of the species *Linaria saxactilis*, with a dose of 50 g/cm^2, the results showed no toxic effect for *S. littoralis.* However, there are other works carried out with *S. littoralis* and similar species that show contrary results, such as Rodilla *et al.* (2008) report that noted the essential oils extracted from *L. novocanariensis* showed a moderate effect of 55% of FI against *S. littoralis*.

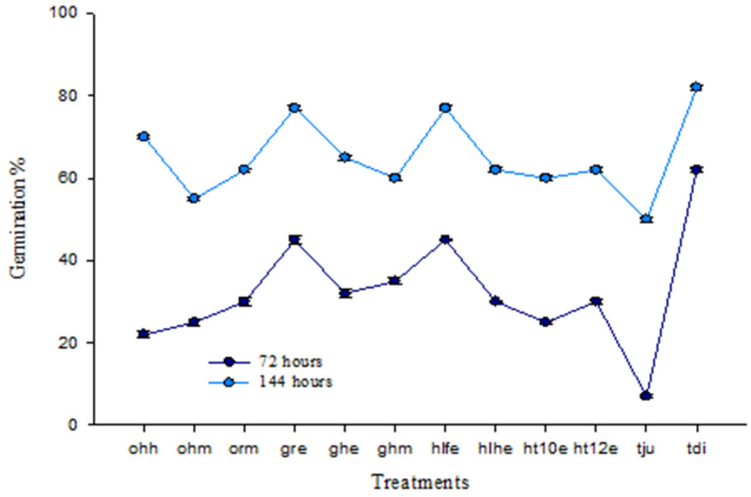

Figure 3. Phytotoxic effects of the applied treatments on germination percentage of *L. perenne* at 72 and 144 hours

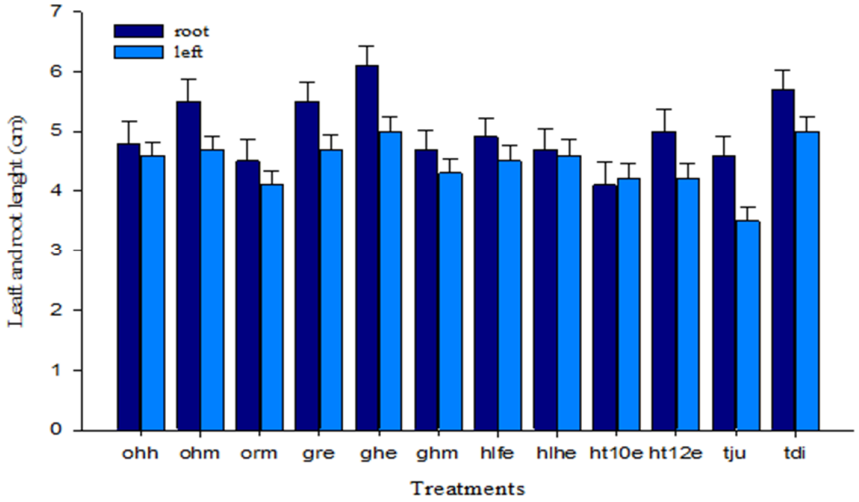

Figure 4. Phytotoxic effects of the applied treatments on the root and leaf length of *L. perenne* at 144 hours

Table 2. Dissuasive activity of four plant species extracts against *Spodopera littoralis*, *Myzus persicae* and *Rophalosiphum padi*

S. littoralis		*M. persicae*				*R. padi*			
Treat.	% FI	Treat.	% C	% T	% SI	Treat.	% C	% T	% SI
ohh	22.13	ohm	70	29	55.53	orm	78	21	70.30
ohm	11.70	orm	69	30	54.35	ghe	72	27	54.80
orm	12.25	ghe	31	68	6.31	ht10e	71	28	54.50
gre	28.14	hlfe	59	40	31.33	ht12e	82	13	76.10
ghe	12.72	hlhe	72	28	60.00				
ghm	20.83	ht10e	62	38	38.60				
hlfe	8.0	ht12e	68	31	48.84				
hlhe	12.24								
ht10e	40.93								
ht10e	46.59								

Treat = Treatment; %FI = dissuasive efficiency of treatment on the feeding; %SI = inhibition of aphid settling; %T = percentage of aphids on the treatment surface; %C = percentage of aphids on the control surface or control

Dissuasive effect of feeding of Myzus persicae

As shown in Table 2, the methanolic extracts of leaf and root of *Fouquieria splendens*, ethanolic extract of *Astragalus mollisimus* sheet and the ethanolic extract of stem of 12 ribs of *P. pecten-aboriginum* showed moderate dissuasive response of feeding against *Myzus persicae*, presenting a settlement inhibition index of 53.53, 54.35, 60.00 and 48.84% respectively, unlike the other extracts tested, with low rates of settlement inhibition, below 40%. In similar works, defensive properties of other plant species were sought against this species of aphid or similar species were noted. It has been found that there was dissuasive activity in *M. persicae* when evaluating neo-clerodane diterpenes of the plant species *Linaria saxactilis*, with a dose of 50 g/cm^2 (Gonzalez-Coloma *et al*., 2008). Likewise, Ricci *et al*. (2006) reported that the essential oils of lemongrass possess a repellent activity between 65 and 80% against Russian aphid (*Diuraphis noxia Kurdj*). Comparing with the results of the hereby bioassay, it can be concluded that the proven concentration of 10 mg ml^{-1} of the raw extracts mentioned have potential as a dissuasive of the feeding for *M. persicae*, taking into account that other works have used isolated and identified substances, unlike the raw extracts that were used in the current work.

Dissuasive effect of feeding of Rophalosiphum padi

All the tested extracts showed dissuasive activity against *R. padi*. It was observed that the methanol extract of the root of *F. splendens* and the ethanolic extract of the stem of 12 ribs of *P. pecten-aboriginum* showed a higher inhibition activity, with settlement percentages of 70.3% and 76.1% (Table 2). The ethanol extracts of *L. tridentata* leaf and the ethanolic extract of the stem of 10 ribs of *P. pecten aboriginum* showed moderate inhibition activity, with settlement percentages of 54.8% and 54.5%. There is evidence of similar research carried out with other plant species on this aphid. Such as the works of Mazoir *et al*. (2008), which mention the strong dissociative effect against this insect of the polygodial derivatives obtained from two species of the Euphorbiaceae family with an SI up to 98%. Moreno-Osorio *et al*. (2008) tested polygodial derivatives of the raw hexanic extract of bark from *Drimys winteri*, finding 98% of SI for the aphid *R. padi*. More recently, it was reported that *Lycium centroides* leaf extract presented dissuasive activity against *R. padi* of 52% of SI (Castillo *et al*., 2009).

Conclusions

The ethanolic leaf extract of *Larrea tridentata* had phytotoxic properties on the root growth of the *L. sativa* species. The extracts of the ocotillo, governor and echo of 10 ribs species showed significant phytotoxicity in germination of *L. perenne*, as well as extracts of ocotillo and echo which inhibited the root length, while the foliar growth was affected by the phytotoxic inhibition of the methanolic extract of ocotillo root. The treatments of the four vegetable species evaluated showed defensive or dissuasive properties of moderate to strong feeding against the insects *M. persicae* and *R. padi*, presenting interesting potential in regard with future possibilities for the realization of insecticides. The tested extracts that presented phytotoxicity for both lettuce and ryegrass present possibilities for the realization of herbicides. It is necessary for the elucidation and identification of the compounds responsible for the phytotoxic and dissuasive activity of the feeding of said treatments to be further reached, since those evaluated in the present work were raw extracts under *in vitro* conditions; respective tests under greenhouse and field conditions are to be followed.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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