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POTENCIAL ALELOPÁTICO DE EXTRACTOS ACUOSOS DE PLANTAS MEXICANAS SOBRE *LACTUCA SATIVA* L

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RESUMEN

Se estudió la toxicidad de extractos acuosos de *Ocimum basilicum*, *Cnidoscolus chayamansa*, *Spondias purpurea*, *Artemisia ludoviciana*, *Punica granatum*, *Piper auritum*, *Hamelia patentes*, *Justicia spicigera*, *Azadirachta indica* y *Porophyllum macrocephalum* sobre semillas *Lactuca sativa*. Se ensayó la toxicidad estática con semillas de L. sativa a diferentes concentraciones de las diez plantas de prueba. Tres de los extractos inhibieron la germinación de las semillas de *L. sativa* y nueve

de ellos inhibieron la elongación tanto del hipocótilo, como de la radícula en las semillas germinadas. Por el contrario, los extractos en las concentraciones más bajas mostraron actividad estimulante (respuesta bifásica: hormesis). Las semillas de *L*. *sativa* respondieron a los extractos de las plantas, ya que éstas ejercieron efectos inhibidores y estimuladores sobre las semillas, dependiendo de la concentración. Estos efectos sugieren actividad alelopática de los extractos; por lo que podrían considerarse para obtener de fertilizantes naturales o plaguicidas como una alternativa sostenible.

ABSTRACT

ALLELOPATHIC POTENTIAL OF AQUEOUS EXTRACTS OF MEXICAN PLANTS ON *LACTUCA SATIVA* L.

The toxicity of aqueous extracts of *Ocimum basilicum*, *Cnidoscolus chayamansa*, *Spondias purpurea*, *Artemisia ludoviciana*, *Punica granatum*, *Piper auritum*, *Hamelia patents*, *Justicia spicigera*, *Azadirachta indica* and *Porophyllum macrocephalum* on seeds *Lactuca sativa* was studied. Static toxicity was tested with *L*. *sativa* seeds at different concentrations of the ten test plants. Three of the extracts inhibited the germination of the seeds of *L*. *sativa* and nine of them inhibited the elongation of both the hypocotyl and the radicle in the germinated seeds. In contrast, the extracts in the lower concentrations showed stimulating activity (biphasic response: hormesis). The seeds of *L*. *sativa* responded to the extracts of the plants, since these exerted inhibiting and stimulating effects on the seeds, depending on the concentration. These effects suggest allelopathic activity

of the extracts; so they could be considered to obtain natural fertilizers or pesticides as a sustainable alternative.

KEYWORDS: Allelopathic potential – aqueous extract – *Lactuca sativa* – Mexican plants – toxic effect.

PALABRAS CLAVE: Potencial alelopático - extracto acuoso - *Lactuca sativa* - plantas mexicanas - efecto tóxico.

INTRODUCTION

Plants, like all living organisms, are a laboratory where substances are produced; when the substances are released from the plant cells, they are capable of producing effects in the surrounding environment. It is a well-known fact that many plants inhibit the growth of other species in a competition where only the strongest survive; likewise, the effects produced by some plants' secondary metabolites over other plants have been reported. All these effects as a whole are known as allelopathy; this is a phenomenon that involves direct or indirect effects, adverse or beneficial, of a plant over another via the release of chemicals into the environment (An *et al.,* 1993; Li *et al.* 2010). The allelopathic responses which have been most studied are stimulation or attraction to low concentrations and the inhibition or repellence as the concentrations increase (An *et al.,* 1993). This double-effect phenomenon is known in toxicology as hormesis, which can be defined as "a process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses induces an adaptive beneficial effect on the

cell or organism" (Matson, 2008; López-Diazguerrero *et al.*, 2013). Allelopathic effects of diverse plant extracts over germination and radicular elongation of several species of seed have been studied in the laboratory. Some examples are *Bidens pilosa* in *Lactuca sativa, Solanum lycopersicum* and *Daucus carota* (Arowosegbe and Afolayan, 2013); *Hyptis suaveolens* in *Lepidum stivum*, *Lactuca sativa*, *Medicago sativa*, *Brassica napus*, *Phleum pratense*, *Digitaria sanguinalis*, *Echinochloa crus-galli* and *Lolium multiflorum* (Mominul-Islam and Kato-Noguchi, 2013); *Varthemia iphionoides* in *Triticum durum*, *Hordeum vulgare*, *Cicer arietinum, Lens culinaris*, *Solanum lycopersicum* and *Capsicum annuum* (Abbu-Roman *et al.,* 2015); *Tripleurospermum inodorum* L on *Daucus carota* (Balicevic and Ravlic, 2015). In all these works, the allelopathic potential of chemical compounds of the studied plants have been shown. Therefore, it can be expected that nature has the most efficient strategies for sustaining and controlling life on the planet.

A niche that exemplifies what has been said is Huasteca Potosina, located in San Luis Potosi State, Mexico; it is zone rich in natural resources amidst a great diversity of plants. Many of these plants have been used by the inhabitants of the region since ancient times for various purposes, either for food preparation or as medical aid for the treatment of illness, among others. Likewise, many of the inhabitants of the disadvantaged and indigenous communities grow their own vegetables in their homes in what is called backyard gardens or backyard orchards (Rivera-Lozoya, 2012; Lárraga-Lara, 2014). In order to sustain these orchards, it is necessary the use synthetic chemical compounds such as fertilizers or herbicides; these substances represent a great investment for people; in addition, the constant use of them can lead to environmental toxicity and health problems as well as

resistance to target species in the case of the herbicides. Thus, it is necessary to develop environment-friendly alternatives that are ecologically acceptable, economically affordable and relatively safe for people's health (Mominul-Islam y Kato-Noguchi, 2013). By the above, the objective of this research was to explore the allelopathic activity of aqueous extracts of regional common plants, with the purpose of generating knowledge for developing more alternatives to the use of herbicides or fertilizers in the backyard gardens.

Materials and methods

Species used. Ten species of plants were used in this research and they were collected in different places of Valles City, San Luis Potosi, Mexico. The names of the species, their common names in Spanish and English, their scientific names, as well as the parts of them that were used, are shown in Table 1.

Preparation of the extracts. Leaves and peel of the fruits (Table 1) were dried in a convection stove (Lindberg/blue ®, Model UT150) to 45 °C, 24 h; later the materials were pulverized by means of a porcelain mortar. Two grams of each species were mixed with 50 mL of purified water in 250 mL glasses; these were covered with watch glasses, and the mixtures were boiled on a hotplate (Corning ® PC-4200) for 15 min to obtain concentrated extracts (40 000 ppm). Then, each extract was filtered and the liquid diluted to 1:10, 1:100, 1:1000 and 1:10 000, concentrations being expressed in percentage as follows: 100, 10, 1, 0.1 and 0.01.

Experimental design. The determination of the extract activities was based on the method of Sobrero and Ronco (2004). A random experimental design by means a static essay was used, with *Lactuca sativa* L. seeds as a test organism. The seeds, free of fertilizers or pesticides, were of the romana variety, resistant to high temperatures. The final points evaluated were seed germination effect, hypocotyl and radicle elongation effect on the germinated seeds. Each treatment was in triplicate (with the concentrated extract and their corresponding dilutions). Simultaneously, a negative control was treated, using reconstituted hard water, as well as a positive control with a reference toxicant (1% $ZnSO_4$).

Test performance. The experiment was carried out in sterile conditions. 100 mm dishes of filter paper (Whatman® # 3) were placed into Petri dishes. Each Petri dish was marked with the corresponding treatment as well as the date and hour of start and end of the test. Each filter paper was saturated with 5 mL of their corresponding extract concentration and positive and negative control, avoiding the formation of airbags; then, 20 seeds were placed with a dissection clamp in each Petri dish, leaving between them 10-15 mm for the elongation of radicles; then the dishes were covered and placed individually in dark plastic bags and were carried to the environmental chamber (PRENDO®) to 22 ± 2 °C, in the dark, 120 h. At the end of this time and as a first final point, data of seeds germinated or not germinated were obtained. Next, all the dishes were frozen all night in order to easily take the measurements of hypocotyls and radicles the next day (second final point). These measurements were made with a rule marked with millimeters.

Calculation of final point's percentages. To get the inhibition percentage in the seed germination, equation (1) was applied. The percentages of the effects of both hypocotyl and radicle elongation were obtained with equation (2).

$$\% IG = [(C - T)/20]100 \quad (1)$$

Where

%IG: germination inhibition percentage

C: Number of germinated seeds in the negative control

T: Number of germinated seeds at a certain concentration

$$\% E = [(LT - LC)/LC]100 \quad (2)$$

Where

$\%E_E$: effect percentage on hypocotyl and radicle elongation

LC: average length (mm) of hypocotyl and radicle in control seeds

LT: average length (mm) of hypocotyl and radicle in treated seeds

Statistical analyses. For analyzing results, the GraphPad Prism version 6.0 ® software was used. The effects of the extracts on final points were analyzed with a single ANOVA when the data were parametric. In this case, the Mann Whitney method was used for comparing means. In the case of parametric data, Kuskall Wallis test and *t* unpaired test for verifying averages differences were applied. To calculate the percentages of stimulation and processing concentration-response graph, Microsoft™ Excel 2010 was used.

Results

Inhibition of L. sativa seeds germination. The concentrated extracts of *S. purpurea, P. auritum* and *J. spicigera* exerted a significant inhibitory effect (> 50%) with respect to the control (Table 2).

Effects in elongation of both hypocotyl and radicle of L. sativa seeds. All the concentrated extracts, except *H. patens*, showed significant inhibition on elongation with respect to control (Tables 3 and 4). The inhibition percentages of both hypocotyl and radicle are shown in Table 5. All the extracts in the lowest concentrations, except *P. granatum*, exerted stimulatory effect (Tables 3 and 4). Likewise, the behavior of this stimulatory effect in relation to the different extract concentrations are shown (concentration-response curves, Figures 1 and 2).

Discussion

Germination inhibition of L. sativa seeds. Only three of the concentrated extracts, *S. purpurea*, *P. auritum* y *J. spicigera* (Table 2), exerted a significant inhibitory effect in *L. sativa* seeds germination; this effect is a manifestation of acute toxicity. For calculating a CL_{50} value (concentration of substance that causes 50% of inhibition in germination), which is a useful parameter in the acute toxicity measurements, it is necessary to test a range of concentrations between 10 and 100%, since in other dilutions (1, 0.1 y 0.01%), inhibition in germination was not

observed. It has been reported that in the chemical composition of mohintli (*J. spicigera*) there are some flavonoids, such as kaempheritrin, which have been related with a cytotoxicity response in cell cultures (Table 6). Purple mombin peel (*S. purpurea*) has flavonoids as quercitin, rhamnetin, kaempherol and kaempheride. In a bioassay with a purple mombin peel extract to 100 ppm, cytotoxic activity was observed in epithelial cell of corneal sheep (Marisco and Pungartnik, 2015). These findings could explain the inhibitory effect of the germination that showed the mohintli and purple mombin extracts: it is probably that their compounds, very similar in structure, had exerted cellular damage in the seed embryos such as in the other experimental models.

Some secondary metabolites extracted from *P. auritum* leaves (vera cruz pepper) are alkaloids, safrole (dioxolane), amines, butenolides, flavonoids, terpenes, among others; some properties that extracts had shown included those antifeeding, fungicides, bactericides and cytotoxic, and have been attributed to these compounds (Mendoza-García *et al.*, 2014). Of all these chemicals, flavonoids are well-known as allelopathic agents (Li *et al.,* 2010).

Effects on elongation of both L. sativa hypocotyl and radicle. Germination bioassay is the most widely used method to determine the phytotoxic activity; however, early seedling growth is considered the most sensitive parameter for testing the phytotoxicity (Mominul-Islam and Kato-Noguchi, 2014). The inhibition in elongation of both hypocotyls and radicles constitute a sub lethal effect, since the mechanisms involved in the seedlings development are affected (Sobrero and

Ronco, 2004). In this work, inhibition in both hypocotyl and radicle elongation of *L. sativa* by concentrated extracts were observed (Tables 3 and 4).

Analyzing the percentages of inhibition in elongation, Table 5 shows that radicles were more affected that hypocotyls; this may be explained with the fact of that during the germination, radicles tend to express one or two days before hypocotyls appear (Falguenbaum and Mouat, 2016). *J. spicigera* (mohintli)*, P. auritum* (vera cruz pepper)*, S. purpurea* (purple mombin) and *A. indica* (neem) extracts were those that caused a greater effect on both radicles and hypocotyls. Fujii *et al*. (2003) determined the allelopathic effect of *P. auritum* leaves on *L. sativa* seeds by the sandwich method, finding 73.5 % of inhibition in radicle elongation when they used 50 mg of dried leaves. In our research, the amount of concentrated extract was obtained from 200 mg of *P. auritum* dried leaves; comparing the results, it can be seen that with the sandwich method, greater inhibition was obtained in the elongation of the radicle. It is probable that with the use of a specific media for growing *L. sativa*, as they did in that research, other factors that promote inhibition in elongation were introduced; in addition, the contact of the allelopathic agents with the *L. sativa* seeds in this and the other experiment occurred in a different form.

In contrast, at low concentrations of the extracts, stimulatory effects in the elongation of both radicles and hypocotyls were evident. Figures 1 and 2 shows a trend in the mentioned response: the lower the concentration of the extracts, the higher the stimulation observed. This trend (estimated as *r*, lineal correlation coefficient) was observed with *A. ludoviciana* (r = 0.91), *P. auritum* (r = 0.86) and *C. chayamansa* (r = 0.84), in the elongation of hypocotyls (Figure 1). It was also

observed with *A. ludoviciana* (r = 0.96), *C. chayamansa* (r = 0.95), *A. indica* (r = 0.93), *P. auritum* (r = 0.90), *J. spicigera* (r = 0.88) and *O. basilicum* (r = 0.82), in the elongation of radicles (Figure 2). The observed trends that have high to very high negative correlations could be fitted to a mathematical model that describes the hormesis, in which it is assumed that both stimulation and inhibition have a sigmoidal response curve to the different concentration of an allelochemical compound (Ann *et al.,* 1993; Liu *et al.,* 2011). The graphs of the Figures 1 and 2 would correspond to the lineal part of their sigmoidal curves since the concentrations were converted to logarithms.

On the other hand, using the equations of the concentration-response curves of the Figures 1 and 2, CE_{50} (concentrations that cause a stimulatory response of 50% in the elongation of both hypocotyls and radicles) was calculated. These values were used for comparing the extracts by their capacity to provoke a stimulatory response in the elongation of both hypocotyl and radicles in *L. sativa* seeds. Hypocotyl elongation: *A. ludoviciana* > *P. auritum* > *C. chayamansa*. With respect to radicle elongation: *C. chayamansa* > *O. basilicum* = *A. ludoviciana* > *J. spicigera* > *A. indica* > *P. auritum.*

Both allelopathic and hormetic effects are the result of the presence of diverse types of substances in the extracts of the plants: organic acids, alcohols, aldehydes, ketones and phenolic compounds; these compounds are polar or partially polar in nature and for this reason they can be found in the infusions of the studied plants. In fact, allelopathy in the strictest sense occurs in nature only when the substances are released to the environment. One of the mechanisms implies a dissolution and drag of the compounds, water being practically the only one solvent

involved (Li *et al.,* 2010). The action mechanism of some allelopathic substances have been studied. For example, it is well known that phenolic compounds (*e.g.* flavonoids) may reduce or increase the production of indol acetic acid (IAA), a hormone involved in the growing of plants. Monophenols, as p-hidroxybenzoic, vanillic, *p*-cumaric and siryngic acids, reduce IAA availability, promoting its decarboxylation. In contrast, many di and polyphenols (*e.g.* clorogenic, caffeic, pherulic and protocatechuic acids) synergize IAA-induced growing, inhibiting hormone degradation (Sampietro, 2016).

The inhibitory effects of the germination of *L. sativa* seeds, as well as of the elongation of both hypocotyls and radicles of these organisms, suggest that the extracts of the studied plants (with exception of *H. patens*) have potential for use in backyard gardens as natural herbicides in form of concentrated infusions. In relation to the stimulatory effects of the extracts, the most diluted (0.1 and 0.01 %) of *C. chayamansa* (chaya)*, O. basilicum* (sweet basil)*, A. ludoviciana* (white sagebrush)*, J. spicigera* (mohintli)*, A. indica* (neem) and *P. auritum* (vera cruz pepper) may be used as promoters of growth in these plants. However, it is necessary to perform more research in crops in order to prove the allelopathic potential of the extracts since the influence of other factors, such as the soil composition, pH and weather, among others, should be considered.

CONCLUSIONS

Of all the extracts studied, only concentrated purple mombin (*S. purpure*a), vera cruz pepper (*P. auritum* Kunth) and mohintli (*J. spicigera* Schechtendal) showed

inhibition on germination of *L. sativa* seeds. Also concentrated extracts, except for scarletbusch (*H. patens*), inhibited the elongation of both hypocotyl and radicle in germinated seeds. In contrast, diluted extracts of *C. chayamansa* (chaya), *O. basilicum* (sweet basil), *A. ludoviciana* (white sagebrush), *J. spicigera* (mohintli), *A. indica* (neem) and *P. auritum* (vera cruz pepper) all stimulated significantly the elongation of radicles, showing a hormetic phenomenon. All those findings suggest that the extracts have allelopathic potential. This opens the possibility that the extracts of the studied plants have similar effects on other species of seeds, including those of undesirable plants, in crops of agronomic interest as well as in the backyard gardens of Huasteca Potosina inhabitants. Nevertheless, it is necessary to conduct field tests with diverse species of crops in order to reach definitive conclusions.

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Conflicts of interest

The authors declare no conflicts of interest.

References

Abu-Romman, S.M., Haddad, M.A. & Al-Hadid, K.J. (2015). The Potential Allelopathic Effects of *Varthemia iphionoides* and the Identification of Phenolic Allelochemicals. *Journal of Biological Sciences,* 8(4): 301-306.

Ann, M., Jhonson, I.R. & Lovet, J.V. (1993). Mathematical modeling of allelopathy: Biological response to allelochemicals and its interpretation. *Journal of Chemical Ecology,* 19 (10): 2379-2388.

Arowosegbe, S. & Afolayan, A.J. (2013). Germination and Growth Inhibitory Effects of Aqueous Extracts of Different Parts of *Bidens Pilosa* L. Var Radiata on Some Vegetable Crops. *Bulletin of Pure & Applied Sciences,* 32(1): 11-20.

Corrêa, G.M. & Alcântara, A.F.C. (2011). Chemical constituents and biological activities of species of *Justicia* - a review. *Brazilian Journal of Pharmacognosy,* 22(1): 220-238.

Espinosa, J.B. & Uy, M.M. (2015). Evaluation of toxicity and antioxidant activities of the crude leaf extracts of *Cnidoscolus chayamansa*. *Advances in Agriculture & Botanics,* 7(2): 109-114.

Falguenbaum, H. & Mouat, P. Biología de cultivos anuales. Retrieved on April 5th, 2016 fromPontificia Universidad Católica de Chile. http://www7.uc.cl/sw_educ/cultivos/index3.htm

Fujii, Y., Parvez, S.S., Parvez, M.M., Ohmae, Y. & Iida, O. (2003). Screening of 239 medicinal plant species for allelopathic activity using the sandwich method. *Weed Biology and Management*, 3:233–241.

Ivanescu, B., Miron, A. & Corciova, A. (2015). Sesquiterpene Lactones from *Artemisia* Genus: Biological Activities and Methods of Analysis. Retrieved April 11th, 2016 from *Journal of Analytical Methods in Chemistry,* http://dx.doi.org/10.1155/2015/247685

Justino-Jácomo, A.C., De Andrade-Velozo, K., Gabilan-Lotti, R., Grosso-Neves, L.M., De Gaspari-de Gaspi, F.O., Marreto-Esquisatto, M.A., Corezola de Amaral, M.E., Sampaio-Mendonca, F.A. & Tech-Dos Santos, G.M. (2015). Activity of *Porophyllum ruderale* leaf extract and 670-nm InGaP laser during burns repair in rats. *BMC Complementary and Alternatve Medicine,* 15: 274. DOI 10.1186/s12906-015-0805-2

Krishnamoorthy, M. & Balakrishnan, R. (2014). Docking studies for screening anticancer compounds of *Azadirachta indica* using *Saccharomyces cerevisiae* as model system. *Journal of Natural Science, Biology and Medicine,* 5 (1): 108-111.

Lárraga-Lara, R. (2014). El traspatio: auto dependencia alimentaria en la vivienda tradicional entre Nahuas y Teneeks en la Huasteca Potosina. Retrieved November 17, 2015 from *Revista Caribeña de Ciencias Sociales.* http://caribeña.eumed.net/traspatio/

Li, Z.H., Wang, Q., Ruan, X., Pan, C.D. & Jiang, D.A. (2010). Phenolics and Plant Allelopathy. *Molecules,* 15:8933-8952.

Liu, Y., Chen, X., Duan, S., Feng, Y. & An, M. (2011). Mathematical Modeling of Plant Allelopathic Hormesis Based on Ecological-limiting-factor Models. *Dose-Response,* 9 (1): 117-129.

López-Diaz-Guerrero, N.E., González-Puertos, V.Y., Hernández-Bautista, R.J., Alarcón-Aguilar, A., Luna-López, A. & Königsberg-Fainstein, M. (2013). Hormesis, lo que no mata fortalece. *Gaceta Médica de México,* 149: 438-447.

Marisco, G. & Pungartnik, C. (2015). *Spondias purpurea* L. (Anacardiaceae): traditional uses, chemical composition and biological activities. *Scientia Amazonia*, 4(2).

Mattson, M.P., (2008). Hormesis defined. *Ageing Research Reviews,* 7(1): 1–7.

Mendoza-García, E.E., Ortega-Arenas, L.D., Pérez-Pacheco, R. & Rodríguez-Hernández, C. (2014). Repellency, toxicity, and oviposition inhibition of vegetable extracts against greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). *Chilean Journal of Agricultural Research,* 74 (1): 41-48.

Mominul- Islam, A.K.M. & Kato-Noguchi, H. (2013). Plant growth inhibitory activity of medicinal plant *Hyptis suaveolens*: could allelopathy be a cause? *Emirates Journal of Food and Agriculture,* 25(9): 692-701.

Mominul-Islam, A.K.M. & Kato-Noguchi, H. (2014). Phytotoxic activity of *Ocimum tenuiflorum* extracts on germination and seedling growth of different plant species. Retrieved March 18th, 2016 from *Indawi Publishing Corporation. The Scientific World Journal.* http://dx.doi.org/10.1155/2014/676242

Plants Database. Natural Resource Conservation Service (NRCS). Retrieved June 26th, 2015 from United States Department of Agriculture (USDA). http://plants.usda.gov/java/

Rivera-Lozoya, E. (2012). Las plantas de los solares en una comunidad nahua de la Huasteca potosina. En Anuschka van ´t Hooft (prod.), Lengua y Cultura Nahua de la Huasteca [DVD Multimedia]. México D.F., CCSYH-UASLP/Linguapax/CIGA-UNAM, 24 pp.

Sampietro, D.A. Alelopatía: Concepto, características, metodología de estudio e importancia. Retrieved February 23th, 2016 from Hipertextos del área de la Biología. Universidad Nacional del Nordeste. Argentina. http://www.biologia.edu.ar/plantas/alelopatia.htm#_3._Naturaleza_química.

Sobrero, M. y Ronco, A. (2004). Ensayo de toxicidad aguda con semillas de lechuga (*Lactuca sativa L.*). En Castillo, G. Ed. *Ensayos toxicológicos y métodos de evaluación de calidad de aguas. Estandarización, intercalibración, resultados y aplicaciones,* pp 71-79, Instituto Mexicano de Tecnología Del Agua, México.

Table 1. Tested plant species

Common name		Scientific name	Part of the plant
English	Spanish (regional)		
Sweet basil	Albahaca	*Ocimum basilicum* L.	Leaves
Chaya	Chaya	*Cnidoscolus chayamansa* Mc. Vaugh	
Purple mombin	Ciruela	*Spondias purpurea* L.	Peel of the fruits
White sagebrush	Estafiate	*Artemisia ludoviciana* Nutt. subsp. *mexicana* (Willd. ex Spreng.) D.D. Keck	Leaves
Pomegranate	Granada	*Punica granatum* L.	Peel of the fruits
Vera cruz pepper	Hoja santa	*Piper auritum* Kunth	Leaves
Scarletbush	Madura plátano	*Hamelia patents* Jacq.	Leaves
Mohintli	Mohuite	*Justicia spicigera* Schltdl.	Leaves
Neem	Neem	*Azadirachta indica* A. Juss	Leaves
Yerba porosa	Tepehua, pápaloquelite	*Porophyllum ruderale* (Jacq.) Cass. subsp. *macrocephalum* (DC.) R.R. Johnson	Leaves

Source: Prepared with data of USDA, 2015

Table 2. Inhibition caused for plant extracts on *Lactuca sativa* seeds germination.

% of Inhibition	***Spondias purpurea***	***Piper auritum***	***Justicia spicigera***
Average	78	83	77
Standard deviation	5.8	2.9	10.4

Table 3. Effects of the plant extracts on hypocotyl elongation (mm) of *L. sativa* seeds

Species/Extract concentration (%)	***100***	***10***	***1***	***0.1***	***0.01***
Ocimum basilicum	8.17±6.45****(I)	13.48±7.42	15.15±9.06*(S)	13.53±7.96	15.89±6.20***(S)

Cnidoscolus chayamansa	7.60±3.60****(I)	18.97±5.55****(S)	19.32±4.54****(S)	20.83±4.16****(S)	22.22±3.93****(S)
Spondias purpurea	1.05±2.09****(I)	17.97±7.97****(S)	15.38±3.88**(S)	15.23±1.75****(S)	16.92±2.27****(S)
Artemisia ludoviciana	10.10±7.51*(I)	16.92±8.07***(S)	17.62±6.45****(S)	19.25±5.50****(S)	20.58±5.30****(S)
Punica granatum	7.55±5.73****(I)	12.67±7.25	12.20±7.18	12.98±7.42	13.23±8.11
Piper auritum	1.45±3.20****(I)	17.62±5.88****(S)	19.28±6.21****(S)	18.92±8.07****(S)	21.9±5.57****(S)
Hamelia patens	13.77±10.48	15.52±6.87**(I)	12.60±7.45	16.70±7.97*(S)	16.18±10.25*(S)
Justicia spicigera	1.15±2.18****(I)	13.17±9.51	19.73±8.48****(S)	17.82±7.07****(S)	18.10±9.86***(S)
Azaridachta indica	1.75±1.35****(I)	12.47±5.53	17.20±6.37****(S)	17.60±6.33****(S)	19.13±6.16****(S)
Porophyllum ruderale	8.2±5.31**** (I)	14.52±8.94	14.97±6.16** (S)	15.57±6.84*** (S)	11.92±7.12

Elongation of control (mm): 13.78 ± 1.47

Stars indicate level of significance between treatments and control: ****($p \leq 0.001$); *** and ** ($p \leq 0.005$); *($p \leq 0.05$).

(I): Inhibition of elongation; (S): Stimulation of elongation

Table 4. Effects of the plant extracts on radicle elongation (mm) of *L. sativa* seeds

Species/Extract concentration (%)	***100***	***10***	***1***	***0.1***	***0.01***
Ocimum basilicum	9.83±6.85	18.53±9.08****(S)	20.17±11.53****(S)	19.78±11.18****(S)	21.1±7.95****(S)
Cnidoscolus chayamansa	5.83±2.80****(I)	14.07±3.21	16.95±3.07****(S)	19.9±4.18****(S)	20.38±4.54****(S)
Spondias purpurea	0.80±1.79****(I)	7.50±3.86****(I)	12.28 ± 3.00	14.45±1.89****(S)	16.50±2.27****(S)
Artemisia ludoviciana	5.95±4.85****(I)	13.57±4.98	18.43±7.03****(S)	2.23±6.17****(S)	22.7±6.98****(S)

Punica granatum	8.83±6.25****(I)	12.18±5.87	13.23±5.98*(S)	12.70±5.17	12.65±5.27
Piper auritum	0.60±1.37****(I)	17.57±5.58****(S)	22.88±9.20****(S)	23.22±9.56****(S)	27.9±5.80****(S)
Hamelia patens	13.77±10.48	15.52±6.87****(S)	12.60±7.45	16.70±7.97***(S)	16.18±10.25**(S)
Justicia spicigera	0.53±1.05****(I)	14.10±9.41**(S)	22.42±9.11****(S)	21.08±8.65****(S)	23.70±11.69****S
Azaridachta indica	1.32±1.03****(I)	5.52±2.02****(I)	21.15±5.74****(S)	21.95±6.87****(S)	25.07±8.82****(S)
Porophyllum ruderale	5.58±4.77****(I)	5.45±4.60****(I)	10.78±4.85***(I)	10.57±5.68***(I)	11.80±5.95

Elongation of control (mm): 13.78 ± 1.47

Stars indicate level of significance between treatments and control: ****($p \leq 0.001$); *** and ** ($p \leq 0.005$); *($p \leq 0.05$).

(I): Inhibition of elongation; (S): Stimulation of elongation

Table 5. Inhibition (%) caused by concentrated plant extracts on the hypocotyls and radicles elongation of *L. sativa* seeds

Species	*Radicle*	*Hypocotyl*
J. spicigera	96	92
P. auritum	95	89
S. purpurea	94	92
A. indica	90	87
P. ruderale	57	40
A. ludoviciana	55	27
C. chayamansa	55	45
P. granatum	33	45
O. basilicum	29	41
H. patens	0	0

Table 6. Water soluble allelochemicals found in some of the studied plants

Species	Allelochemicals	Authors, year of publication
J. spicigera	Kaempheritrin (flavonoid)	Corrêa and Alcântara, 2011
P. auritum	Alkaloids, butenolides (lactones), amines, flavonoids	Mendoza-García *et al*., 2014
S. purpurea	Quercetin, rhamnetin, Kaempherol y Kaempheride (flavonoids)	Marisco and Pungartnik, 2015
A. indica	Meliantriol (steroid), Quercetrin (flavonoid), Ruthin (flavonoid), Miricetrina (flavonoide), Scopoletin (cumarin), Azadirachtin (limonoid)	Krishnamoorthy and Balakrishnan, 2014
P. ruderale	Flavonoids, tanins, saponins, alkaloids	Justino Jácomo *et al.,* 2015
A. ludoviciana	Sesquiterpenlactones	Ivanescu *et al.,* 2015
C. chayamansa	Alkaloids, flavonoids and saponins	Espinosa and Uy, 2015

Source: Prepared with data of the mentioned articles

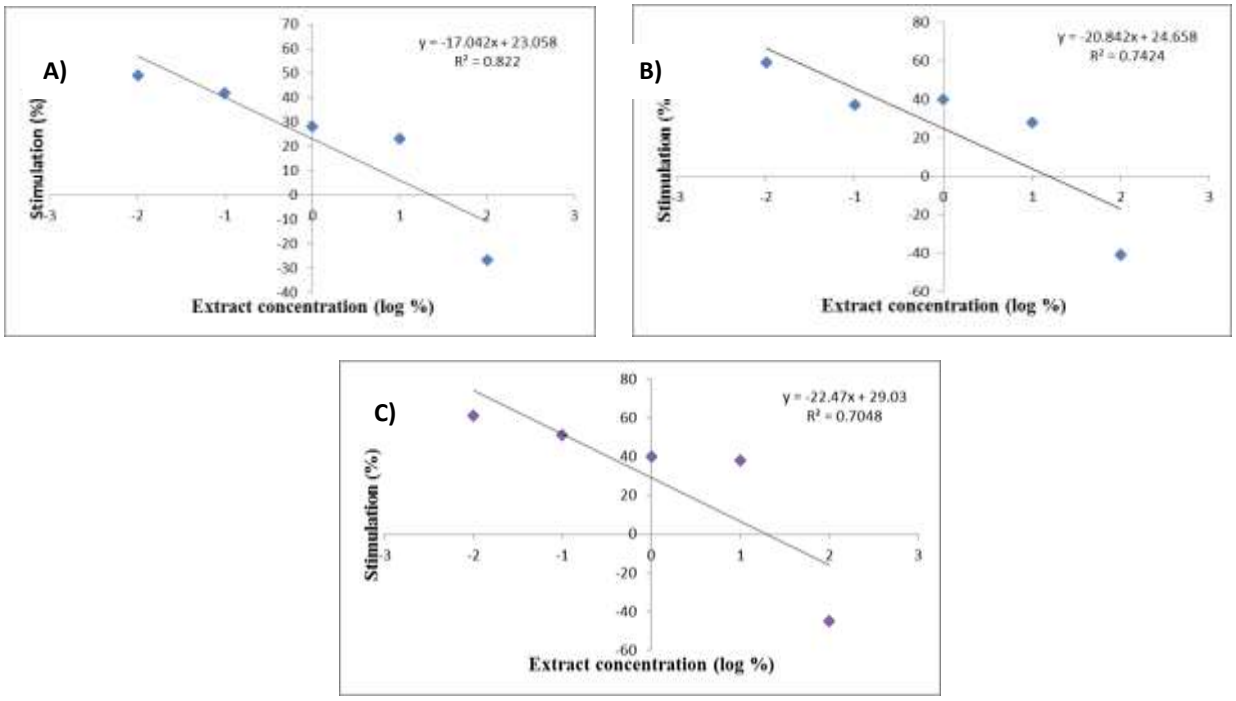

Figure 1. Stimulation behavior on hypocotyls elongation of *L. sativa* seeds with low concentrations of aqueous extracts: A) *Artemisia ludoviciana* CE_{50} = 0.03 %, B) *Piper auritum* CE_{50} = 0.06 %, C) *Cnidoscolus chayamansa* CE_{50} = 0.12 % (CE_{50}: concentration that causes response of 50 %)

Source: Own elaboration.

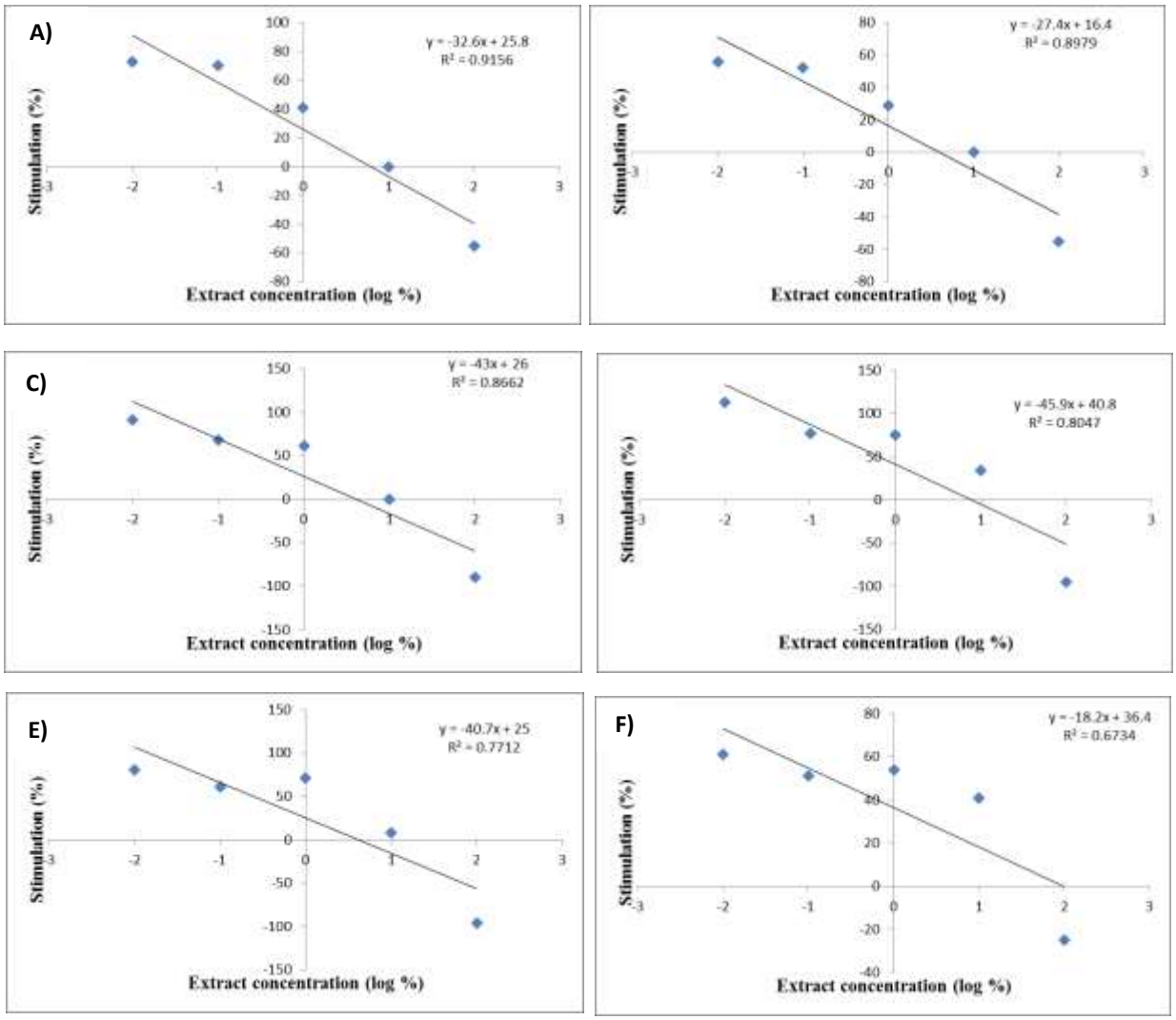
A)
y = -32.6x + 25.8
R² = 0.9156
Stimulation (%)
Extract concentration (log %)
y = -27.4x + 16.4
R² = 0.8979
Stimulation (%)
Extract concentration (log %)
C)
y = -43x + 26
R² = 0.8662
Stimulation (%)
Extract concentration (log %)
y = -45.9x + 40.8
R² = 0.8047
Stimulation (%)
Extract concentration (log %)
E)
y = -40.7x + 25
R² = 0.7712
Stimulation (%)
Extract concentration (log %)
F)
y = -18.2x + 36.4
R² = 0.6734
Stimulation (%)
Extract concentration (log %)

Figure 2. Stimulation behavior on radicles elongation of *L. sativa* seeds with low concentrations of aqueous extracts: A) *Artemisia ludoviciana* CE_{50} = 0.18 %, B) *Cnidoscolus chayamansa* CE_{50} = 0.06 %, C) *Azadirachta indica* CE_{50} = 0.28 %, D) *Piper auritum* CE_{50} = 0.63 %, E) *Justicia spicigera* CE_{50} = 0.24 % and F) *Ocimum basilicum* CE_{50} = 0.18 % (CE_{50}: concentration that causes response of 50 %). Source: Own elaboration.