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EFFECT OF AQUEOUS EXTRACTS OF *Helianthus annuus* Lin. ON *Solanum lycopersicum* Lin. GROWTH

Efecto de extractos acuosos de *Helianthus annuus* Lin. sobre el crecimiento de *Solanum lycopersicum* Lin.

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ABSTRACT. The study was developed in order to determine the effect of aqueous extracts of *Helianthus annuus* Lin on *Solanum lycopersicum* Lin growth. Roots and leaves samples of sunflower plants were collected from a policulture system located in a mountainous agroecosystem and their effects. These effects were evaluated on certified tomato seeds about germination, radicle and hypocotyl length. Nine treatments with four replicates were established in Petri plates. The extracts of sunflower from roots, and from leaves, at 15, 30 and 45 and at 75 and 90 days of development, respectively, inhibited tomato seed germination. Sunflower root extracts at 75 and 90 days of development stimulated tomato radical and hypocotyl growth. These results showed that sunflower plant produces chemical substances inhibiting tomato growth specifically the radicle and the hypocotyl in the earlier 45 days of growth after germination, and it is not recommended the association of these two crops simultaneously.

Key words: sunflower, tomato, radicle, hypocotyl, inhibition

RESUMEN. Con el objetivo de determinar el efecto de extractos acuosos de *Helianthus annuus* Lin. sobre el crecimiento de *Solanum lycopersicum* Lin, se tomaron muestras de raíz y hojas de plantas recolectadas de un sistema de policultivo ubicado en un agroecosistema montañoso. Los extractos acuosos de girasol se obtuvieron a partir de raíz y hojas de plantas recolectadas durante dos años. Se evaluaron los efectos de estos extractos sobre la germinación, longitud de la radícula y del hipocótilo de semillas de tomate certificadas, para ello se ejecutaron nueve tratamientos con cuatro repeticiones dispuestos en placas Petri. Los extractos de raíz de girasol a los 15, 30 y 45 días y de hojas a los 75 y 90 días de desarrollo inhibieron la germinación de las semillas de tomate y el crecimiento de la radícula y el hipocótilo. Los extractos de raíz de girasol con 75 y 90 días, estimularon el crecimiento de la radícula y del hipocótilo del tomate. Estos resultados demuestran que el girasol produce sustancias químicas que inhiben el crecimiento del tomate y no se recomienda asociar estos dos cultivos de forma simultánea.

Palabras clave: girasol, tomate, radícula, hipocótilo, inhibición

INTRODUCTION

Suinflower growing (*Helianthus annuus* L), belonging to the *Asteraceae*, family is original of North America; at present it expands to all continents and cover an area of approximately 18 million hectares according to the quality of its oil.

This crop is considered among the four of higher production of edible plant oil, and sunflower oil runs second in world consumption (1). Sunflower plants also have potential as fodder for milk cattle feeding in low rainy periods[A].

The intensification of this crop is related to its use as raw material for the production of biofuel. In Brazil it is driven by the creation of the “National Biodiessel

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[A] Penichet, C.; Carballo, G.; Guerra, G. y Alemán, P. *El cultivo del girasol como alternativa forrajera viable para la alimentación del ganado vacuno lechero*, [95], Observatorio de la Economía Latinoamericana, 2008.

Program" whose target is the insertion of family agriculture into the production of agroenergy[B] (2).

Under the conditions of Cuba's mountainous agroecosystems, sunflower growing is being promoted in polycrops systems. The presence of this specie in the agroecosystem contribute to human feeding with an oil of food value and the enrichment of animal feeding with the residues of the oil extraction process. However, the introduction of sunflower in association with tomato (*Solanum lycopersicum* Lin) affected tomato development when they were mainly associated simultaneously (3).

Previous studies show that sunflower can interfere in the development of neighbor plants (4), though mechanisms responsible for such effects are well known; they probably occur because sunflower is a source of sesquiterpenoids and other compounds with variable biological among genotypes (5). Therefore, it is required to deepen on the knowledge of ecological relations of the introduced specie and its potential as source of bioactive compounds, especially with allelopathic activity.

Research works related to allelopathic effects have gained importance after the boom of ecological agriculture. Nevertheless, results in this field still run short to consider them as an immediate solution to problems that tropical agriculture has historically faced with due to a high specie diversity under interspecific competition in any economic crop[C]. Allelochemicals have multiple functions in the biotic structure of the original distribution area: allelopathic, defense against gass-feeding organisms, metal transporting agents or symbiosis agents between soil microorganisms and the plant (6, 7).

An effect can be identified as allelopathic when it is proven to be the cause of biochemical actions and not of edaphic and climatic factors or competition for water, light and nutrients (8). Therefore, the objective of this research has been determining the effect of sunflower extracts on tomato plants growth, to contribute to make clear the chemical interactions related to the marked negative effect produced in tomato development by the simultaneous association of these crops.

[B] Silva, H.P.; Neves, J.M.G.; Reis, A.P.D.; Brandão Junior, D.S.; Sampaio, R.A. y Colen, F. ''Desenvolvimento da Cultura do Girassol em Diferentes Doses de Lodo de Esgoto e Silicato de Cálcio e Magnésio'', *Congresso Brasileiro de Agrobioenergiae Simposio Internacional de Biocombustiveis*, Anais, Uberlândia, MG, Brasil, 2008.

[C] Leyva, G.A.; Beltrán, R.L. y Falcón, A. ''Alelopatía. Diversidad de plantas potencialmente útil en los agroecosistema'', *Conferencia en Taller Nacional de productores destacados*, edit. Instituto Nacional de Ciencias Agrícolas, La Habana, Cuba, 2005.

MATERIALS AND METHODS

Collection and processing of plant material

Collections were done in September and December 2009 and from September to December 2010 in polycrop systems within the mountainous agroecosystem of La Loma Farm, locality of Limonar de Monte Rous, Guantánamo province, Cuba. Sunflower roots (Caburet variety) were collected at 15, 30, 45, 60, 75 and 90 days from the emergence; leaves were collected at 75 and 90 days. Plant material was dried at room temperature for 15 days and then ground on a knife mill.

Procurement of the extracts

The dried and ground plant material was extracted by maceration with water for 4 hours (2 g in 100 mL of distilled water) (9). Each extract was filtered with Whatman 1 paper and it was stored at a temperature of 4 °C till its evaluation. The total soild content of 1 mL of the extract was determined at the electronic moisture analyzer Santorius MA-45, 3 replicates were made for each extract. Mean values were calculated and analyzed by Tukey's ($p<0,05$).

Bioessay to determine the effect on growth

The effect of water extracts from roots and leaves collected at different times of crop development was determined in Petri dishes (11 cm of diameter) with Whatman No.1 filter paper placed at the bottom. Tomato seeds from the Vita variety were used.

The evaluated treatments were the following:

- Distilled water (Control).
- Sunflower plant extracts 15 days after emergence.
- Sunflower plant extracts 30 days after emergence.
- Sunflower plant extracts 45 days after emergence.
- Sunflower plant extracts 60 days after emergence.
- Sunflower plant extracts 75 days after emergence.
- Sunflower plant extracts 90 days after emergence.
- Sunflower plant extracts 75 days after emergence.
- Sunflower plant extracts 90 days after emergence.

In every Petri dish, 30 seeds were dipped in 2 mL of each treatment (distilled water or extract) for 1 minute. Then, they were placed over the Whatman 1 filter paper previously wet with 5 mL of the corresponding extract (water in the control). Four replicates per treatment were used arranged on a random block design (9). Petri dishes were incubated at room temperature. The number of germinated seeds was determined every 24 hours and the length of the radicle and the hypocotyl after 7 days. Radice and hypocotyl length data are presented as percentage of difference with the control; so cero accounts for the control, positive values represent stimulation of the studied parameter and negative values are equivalent to inhibition (10).

Statistical analysis

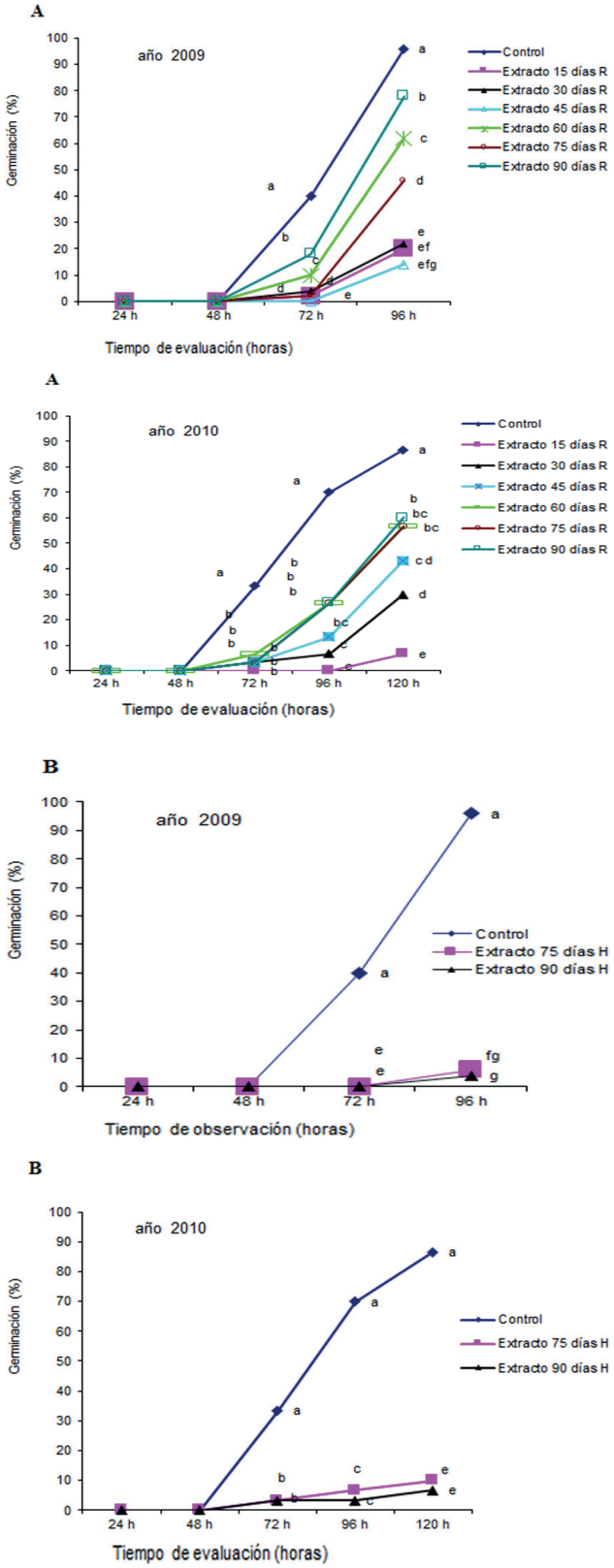
For differences among means, the mean comparison with Tukey's Test at $p < 0.05$ was used, through the statistical package STATGRAPHICS Plus 5.0 (11).

RESULTS AND DISCUSSION

Graphic 1 shows the results of evaluating the effect of root and sunflower leaf extracts collected at different stages of crop development, on the germination of tomato seeds. The start of germination occured at 72 hours in the control treatment, 40 % and 33 % for evaluations of 2009 and 2010 respectively, but the percentages were very low for all extracts in time.

For the bioassay done in 2009, germination reached the highest percentage (96 %) in the control treatment at 96 hours while the rest of the treatments significantly inhibited this parameter. For the second year, germination was monitored till 120 hours when the control treatment reached the maximum percentage (87 %) and again, the number of germinated seeds was lower in all treatments than in the control throughout the evaluation period since germination started.

In both years, it was evident that root extracts collected at 15, 30 andy 45 days produced a more marked inhibitory effect with percentages below 45 %. When leaf extracts are applied, percentages are less than 10 %, lower to those reached in root treatments at the same stage of sunflower phenological development. These results coincide with previous studies showing that water extracts of the upper part of the plants, mainly leaves, produce a higher inhibition for tomato plants that root extracts (12).



Germination percentage values at the same evaluation time with equal letters do not differ for $p < 0.05$.

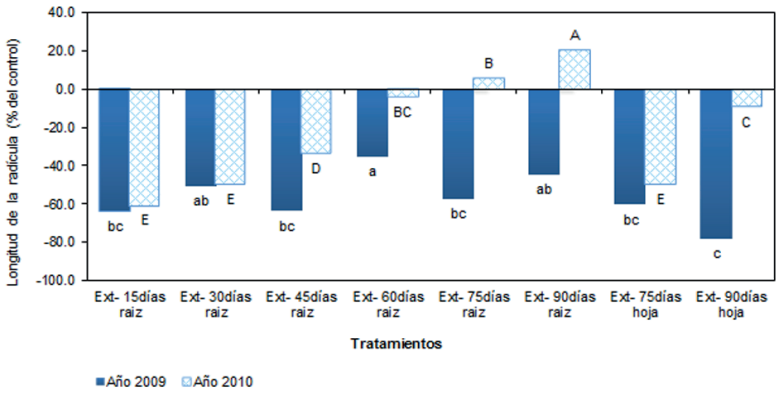
Figure 1. Effect of sunflower root extracts (A) and leaf extracts (B) on the germination of tomato seeds in 2009 and 2010

Different concentration of water extracts from sunflower leaves reduced the germination of some species like mustard (*Brassica nigra* (L.) Koch), radish (*Raphanus sativus* Lin.), wheat (*Triticum vulgare* Willd.) and cucumberpino (*Cucumis sativus* Lin.) (4). Previous research has reported the inhibited germination of other cotyledoneous plants, *Lactuca sativa* Lin., *Lepidium sativum* Lin. and *Allium cepa* Lin, by compounds produced from water extracts of leaves collected one month before harvest (it coincides with 75 days after emergence) (13). Also, different concentrations of water extracts from sunflower leaves inhibited the germination and development of wheat seedlings (*Triticum aestivum*) and mustard (*Sinapis alba*), though mustard was more sensitive[D].

Graphic 2 shows the results of evaluating the effect of sunflower roots and leaves extracts on the radicle growth. All treatments from 2009 inhibited this parameter regardless the part and stage of development of the plant. This behavior repeated with the samples from 2010, except for root extracts of 75 and 90 days that stimulated root development.

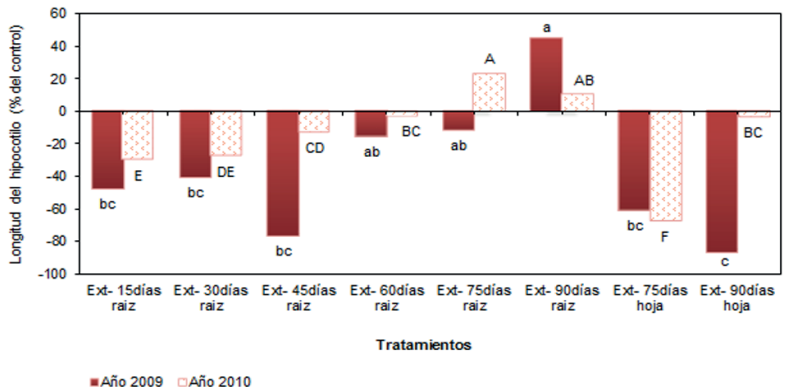
Graphic 3 shows the result of determining the effect sunflower roots and leaves extracts on hypocotyl growth. The lengths of the hypotocyl were lower than the control for the variants used: from root extracts at 15, 30, 45 and 60 days, and the leaf extracts at 75 and 90 days in both evaluated years together with the root extract at 75 days of the first year. A higher hypocotyl growth compared to the control was true for the root extract treatment at 90 days when samples from 2009 were evaluated; in 2010 growth was also stimulated with the root extract corresponding to this time and with the one of 75 days.

[D] Gawronska, H.; Bernat, W.; Janowiak, F. y Gawronski, S.W. "Comparative studies on wheat and mustard responses to allelochemicals of sunflower origin", *European Allelopathy Symposium "Allelopathy - From Understanding to Application*, Poland, 3 de junio de 2004, 28 p.



Germination percentage values at the same evaluation time with equal letters do not differ for p<0.05

Figure 2. Effect of sunflower root and leaf extracts on radicle growth in 2009 and 2010



Germination percentage values at the same evaluation time with equal letters do not differ for p<0.05

Figure 3. Effect of sunflower root and leaf extracts on the growth of tomato hypocotyl in 2009 and 2010

Sunflower is a known specie with a high production of secondary metabolites; however, the great variability of the allelopathic potential on germination, root growth and the aereal part of the plant is recognized among sunflower genotypes (4, 13). The effect of its metabolites can be either stimulant or inhibitory, depending on the indicator specie used, the different compounds isolated from its extracts and its concentrationslos (13). In this study, the effects of the evaluated treatments on the three parameters show the presence of bioactive metabolites in the grown sunflower variety (Caburet) under the conditions of a mountainous ecosystem.

Tomato germination reduced with the sunflower extracts during growth and results indicate that substances are produced at the beginning of its growth, on the roots (till 45 days) and on the leaves in the most advanced stages (75 and 90 days). Such substances affect this parameter, either chemically or for its concentration in the evaluated samples. In addition to this, chemical compounds extracted from the roots at early crop stages, from 15 to 60 days in the leaves and from 75 to 90 days inhibit not only tomato germination, but also radicle and hypocotyl growth and development. During the two years of the trial, the tendency of results kept on, as to the inhibitory effect of the extracts at the start of radicle development and of the leaves at the end of the cycle. It could be due to the fact that metabolites produced by sunflower plants in these development states were similar in both years.

On the other hand, the stimulant effect on growth produced by root samples of 75 and 95 days, mainly at the second evaluated year, indicates the presence of changes in the metabolite profile (qualitative and/or quantitative) in roots as plant develops. These results confirm the theory that allelochemicals can influence by having a stimulant or inhibitory effect (14).

Bioassays to determine the effect of released allelopathic compounds, frequently use extracts from the donor developed plant or in decomposition process (10, 15, 16, 17, 18). The experimental design has the advantage of simulating the interference sunflower-tomato in the different development stages of sunflower plants. In order to achieve the greatest approximation to the situation of plants under field conditions, it is very important to represent the interference among plants at the initial development stages, a time in which the allelopathic process can be determinant to establish seedlings as shown by the inhibitory effect of root extracts (from 15 to 60 days of development) on the three evaluated parameters simultaneously with sunflower plants (3).

When comparing the results of two years regarding the type of effect (inhibitory or stimulant) the only differences show up with root extracts at 75 days (radicle and hypocotyl length) and aty 90 days (radicle length) that could be associated to variations in the biosynthesis of bioactive metabolites, caused b y changes in environmental factors like climate, soil, etc., from one year to another. The allelopathic effects of chemical compounds can increase in nutrient poor soils; for example, it was shown that high levels of capheinquinic acids in sunflower plants are caused by nitrogen, potassium and sulphur deficiencies (19). The changes of the effect on growth by comparing the results of the two years under study could, therefore, be due to variations in the organic matter content and in the content of some of these elements in growing areas.

The mechanism responsible for sunflower effects on the different target species are not totally clear, but they are asociated to the biosynthesis of some metabolities among which some sesquiterpenic lactones are found (5). It is said that most of these compounds make their effects through a common action mechanism, the renting of organic molecules.

Activity levels can be correlated to quantitative and/or qualitative composition of the extracts. The variability of the target plant response can be attributed to substances present on sunflower extracts. Therefore, during the second year of the trial, total solids in each of them were determined (Table I).

Table I. Concentration of total solids of water extracts from evaluated sunflower roots and leaves

Extracts	Concentration (mg mL^{-1})
Sunflower root extract 15 days after emergence	5,7 a
Sunflower root extract 30 days after emergence gencia	2,7 ab
Sunflower root extract 45 days after emergence	4,7 ab
Sunflower root extract 60 days after emergence	3,3 ab
Sunflower root extract 75 days after emergence	2,3 ab
Sunflower root extract 90 days after emergence	1,3 b
Sunflower root extract 75 days after emergence	3,3 ab
Sunflower root extract 90 days after emergence	1,3 b
Standard error	**0,00050**

Only root extracts at 15 and 90 days show significantly different total solid values; the first one with a higher concentration inhibits both radicle and hypocotyl growth, while the second one stimulates both parameters. The total solid values from root extracts are not directly correlated with the effect on the growth because the inhibiting extracts (15, 30 and 45 days) and others that stimulate (75 and 90 days) do not differ statistically in their total solids concentration. Leaf extracts do not differ among themselves as to the concentration of total dissolved solids.

Though total solid values remained within a range in which no statistical differences were found, the variations of root extracts effect on growth can be due to a different qualitative composition, or because the quantity of some bioactive compound significantly varied, though total value did not. Extracts with greater effects on tomato plants can have a higher concentration of the compounds responsible for the biological effect. For allelopathic substances be able to influence target plants, different stages should occur. Compounds should be released from donor plants in adequate quantities and reach enough concentrations to be uptaken, translocated and act on some relevant biochemical mechanisms for the development of the target specie (19).

This research provides very important information that helps explain the marked negative effect of the simultaneous association of tomato plants with sunflower plants. It makes evident the presence of allelochemicals that can be halfway through ecological relations in the agroecosystem and confirms the importance of considering the environmental impact of introducing a new specie into the system.

CONCLUSIONS

Sunflower plants produce chemical substances that inhibit tomato plants growth that can be responsible for the negative effect on the development of this crop when associated to others.

BIBLIOGRAPHY

1. Curti, G.L.; Martin, T.N.; Ferronato, M. de L. y Benin, G. ‘‘Girassol ornamental: caracterização, pós-colheita e escala de senescência’’, *Revista de Ciências Agrárias*, vol. 35, no. 1, junio de 2012, pp. 240-250, ISSN 0871-018X.
2. Pereira, da S.H.; Carvalho, M.G.J. de.; Gomes, N.J.M.; da Silva, B.D.J. y Karam, D. “Levantamento das plantas espontâneas na cultura do girassol’’, *Revista Verde de Agroecologia e Desenvolvimento Sustentável*, vol. 5, no. 1, 2010, p. 167, ISSN 1981-8203.
3. González, P.Y.; Leyva Galán, A. y Pino Pérez, O. ‘‘Competencia por interferencia de *Helianthus annuus* L., asociado a *Solanum lycopersicum* L. bajo condiciones de campo’’, *Cultivos Tropicales*, vol. 35, no. 4, diciembre de 2014, pp. 28-35, ISSN 0258-5936.
4. Silva, H.L.; Trezzi, M.M.; Marchese, J.A.; Buzzello, G.; Miotto, J.E.; Patel, F.; Debastiani, F. y Fiorese, J. “Determinação de espécie indicadora e comparação de genótipos de girassol quanto ao potencial alelopático”, *Planta Daninha*, vol. 27, no. 4, 2009, pp. 655-663, ISSN 0100-8358.
5. Macías, F.A.; Marín, D.; Oliveros-Bastidas, A.; Varela, R.M.; Simonet, A.M.; Carrera, C. y Molinillo, J.M. ‘‘Allelopathy as a new strategy for sustainable ecosystems development’’, *Biological Sciences in Space*, vol. 17, no. 1, 2003, pp. 18–23, ISSN 1349-967X.
6. Oliveros-Bastidas, A. de J.; Macías, F.A.; Fernández, C.C.; Marín, D. y Molinillo, J.M.G. ‘‘Root exudates and their relevance to the allelopatic interactions’’, *Química Nova*, vol. 32, no. 1, 2009, pp. 198-213, ISSN 0100-4042, DOI 10.1590/S0100-40422009000100035.
7. Vázquez, M.L.L. “Manejo de plagas en la agricultura ecológica’’, *Boletín Fitosanitario*, vol. 15, no. 1, 2010, p. 38, ISSN 1816-8604.
8. Lorenzo, P. y González, L. ‘‘Alelopatía: una característica ecofisiológica que favorece la capacidad invasora de las especies vegetales”, *Revista Ecosistemas*, vol. 19, no. 1, 2010, ISSN 1697-2473, [Consultado: 15 de junio de 2015], Disponible en: <http://revistaecosistemas.net/index.php/ecosistemas/article/view/57>.
9. Caamal-Maldonado, J.A.; Jiménez-Osornio, J.J.; Torres-Barragán, A. y Anaya, A.L. ‘‘The Use of Allelopathic Legume Cover and Mulch Species for Weed Control in Cropping Systems’’, *Agronomy Journal*, vol. 93, no. 1, 2001, p. 27, ISSN 1435-0645, DOI 10.2134/agronj2001.93127x.
10. Macías, F.A.; Lacret, R.; Varela, R.M.; Nogueiras, C. y Molinillo, J.M.G. ‘‘Isolation and Phytotoxicity of Terpenes from Tectona grandis”, *Journal of Chemical Ecology*, vol. 36, no. 4, 18 de marzo de 2010, pp. 396-404, ISSN 0098-0331, 1573-1561, DOI 10.1007/s10886-010-9769-3.
11. Statistical Graphics Crop *STATGRAPHICS® Plus* [en línea], versión 5.1, [Windows], 2000, (ser. Profesional), Disponible en: <http://www.statgraphics.com/statgraphics/statgraphics.nsf/pd/pdpricing>.
12. Chon, S.-U. y Nelson, C.J. ‘‘Allelopathy in Compositae plants. A review’’, *Agronomy for Sustainable Development*, vol. 30, no. 2, abril de 2010, pp. 349-358, ISSN 1774-0746, 1773-0155, DOI 10.1051/agro/2009027.
13. Macías, F.A.; Varela, R.M.; Torres, A. y Molinillo, J.M.G. ‘‘Potential Allelopathic Activity of Natural Plant Heliannanes: A Proposal of Absolute Configuration and Nomenclature”, *Journal of Chemical Ecology*, vol. 26, no. 9, 1 de septiembre de 2000, pp. 2173-2186, ISSN 0098-0331, 1573-1561, DOI 10.1023/A:1005524601019.
14. Hernández Escobar, I.; Urra Zayas, I.; Díaz Viruliche, L.; Pérez Petitón, J. y Hernández Cuello, G. ‘‘Labranza mínima y efecto alelopático en la producción de frijol común en la Empresa Agropecuaria 19 de Abril de la provincia Mayabeque’’, *Revista Ciencias Técnicas Agropecuarias*, vol. 22, no. 3, septiembre de 2013, pp. 46-48, ISSN 2071-0054.

15. Macías-Rubalcava, M.L.; Hernández-Bautista, B.E.; Oropeza, F.; Duarte, G.; González, M.C.; Glenn, A.E.; Hanlin, R.T. y Anaya, A.L. ''Allelochemical Effects of Volatile Compounds and Organic Extracts from *Muscodor yucatanensis*, a Tropical Endophytic Fungus from *Bursera simaruba*'', *Journal of Chemical Ecology*, vol. 36, no. 10, 31 de agosto de 2010, pp. 1122-1131, ISSN 0098-0331, 1573-1561, DOI 10.1007/s10886-010-9848-5.
16. Rodrigues, I.M.C.; Souza Filho, A.P.S.; Ferreira, F.A. y Demuner, A.J. ''Chemical prospecting of compounds produced by *Senna alata* with allelopathic activity'', *Planta Daninha*, vol. 28, no. 1, 2010, pp. 1-12, ISSN 0100-8358, DOI 10.1590/S0100-83582010000100001.
17. Casas, L.; Mantell, C.; Rodríguez, M.; Torres, A.; Macías, F.A. y Ossa, E.J.M.D.L. ''Identification of Major Compounds Extracted by Supercritical Fluids from *Helianthus annuus* L. Leaves'', *Solvent Extraction Research and Development, Japan*, vol. 18, 2011, pp. 55-68, ISSN 2188-4765, DOI 10.15261/serdj.18.55.
18. Imatomi, M.; Novaes, P.; Matos, A.P.; Gualtieri, S.C.J.; Molinillo, J.M.G.; Lacret, R.; Varela, R.M. y Macías, F.A. ''Phytotoxic effect of bioactive compounds isolated from *Myrcia tomentosa* (Myrtaceae) leaves'', *Biochemical Systematics and Ecology*, vol. 46, febrero de 2013, pp. 29-35, ISSN 0305-1978, DOI 10.1016/j.bse.2012.09.005.
19. Vyvyan, J.R. ''Allelochemicals as leads for new herbicides and agrochemicals'', *Tetrahedron*, vol. 58, no. 9, 25 de febrero de 2002, pp. 1631-1646, ISSN 0040-4020, DOI 10.1016/S0040-4020(02)00052-2.

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