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Original Article

Effect of Essential Oils of Wild Herbs on Germination and Seed Bank of a Commercial Lot in Fusagasuga (Colombia)

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Abstract

BACKGROUND: One of the problems encountered in agriculture which affects most crops, is weed control. Herbicide use has created environmental problems, toxicity and resistance of some species. An alternative is the use of essential oils, which can produce allelopathic effects causing inhibition of germination and growth of weeds.

METHODS: The trial was set up in a greenhouse at an Esperanza farm (Fusagasuga, Colombia), consisting of placing soil in trays and apply uniform irrigation with a completely randomized design with nine treatments with five replications. Treatments included a *Bacharis trinervis* and *Lippia alba* extracts in three concentrations of 100, 300 and 500 mg/L, water, water plus coadjuvant and a commercial herbicide as control. Germination rates of different groups of weeds were valued and control rates were determined.

RESULTS AND DISCUSSION: For dicotyledonous treatments *Baccharis trinervis* (100 mg/L) and L. alba (500 mg/L) showed the best values controls 59.9 and 54.9% against uncontrolled treatment. A second group includes all treatments of *L. alba* with values showing a regular control against uncontrolled treatment. The herbicide atrazine showed a percentage of control to 37.3%) is a poor control as measured for scale Association Latinoamerican weed management (ALAM). To treat grass weeds with atrazine worth 76.6%, within the range that ALAM is a good control. *Baccharis s trinervis* (500 mg/L) with 59.8% and *B. trinervis* (300 mg/L) with 50.9% in the scale that appears as a regular ALAM control. For sedges, weeds show that better controls corresponded to *L. alba* (500 mg/L) and the commercial herbicide with values of 59.7 and 54.0%, respectively. Continue treatments *B. trinervis* (100 mg/L and 300 mg/L) controls 50.7 and 43.6%. Controls obtained are largely due to the metabolites present as sesquiterpenes Essential Oils (EO) as acting as inhibitors of seed germination.

CONCLUSION: The EO of *B. trinervis* and *L. alba* showed better control of dicotyledonous weeds and sedges that the herbicide. Grass weeds in herbicide outperformed the other treatments but showed percentages of acceptable control.

Keywords: *Baccharis trinervis; Lippia alba;* escale ALAM; Percent control.

Introduction

Overuse of synthetic chemical products for weed control in crops, has created environmental problems, toxicity to human health and has generated genetic resistance of some weed species, to such an extent that it has become necessary to increase dose and spectrum of these products, increasing production costs and decreasing revenue for the farmer. [1]

A trial was established in order to evaluate the herbicidal action of essential oils of *Lippia alba* and *Baccharis trinervis* on weeds farm La Esperanza (Guavio bajo) and seeds of two weeds limitations in warm area as Cartagena (*Stenotaphrum secundatum*) and canary seed (*Phalaris minor*), was designed and an assay using as seed crop phytotoxicity indicator of pea (*Pisum sativum M.*).

Essential oils are obtained primarily from non-woody plant parts, especially the leaves, by stripping by steam or hydro-distillation.[2] They are a mixture derived terpenoids particularly monoterpenes (C10), and sesquiterpenes (C15), and a variety of aromatic compounds, oxides, ethers, alcohols, esters, aldehydes and ketones, which determine the characteristic flavor and odor of the plant that produces.[2] The volatile terpenoids also play an important role in plant-plant and act as an attractant of pollinators[3] interactions.

Lippia alba (Mill), Pronto alivio: This plant is native to America. In Colombia it is distributed in almost all the territory up to 1800m altitude and with greater presence in the regions of Valle del Cauca, Bolivar, Amazonas, Guajira,

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Magdalena, Atlántico, Cundinamarca, Meta and Quindío. It takes place in regions without excessive heat or cold, with temperatures up to 32° C, with high light intensity. [4]
Phytochemical screening shows the presence of alkaloids, diterpene derivatives, tannins, essential oil, and resins.[5] The essential oil (1.2%) is composed of geraniol (34%), neral (23%), β-caryophyllene (6%), metilheptona (5.8%), citronellal (5.2%), borneol (2.6%), Cariofileno oxide (2.5%), allo-aromadendreno (2.4%), α-bisabolene (2.1%) cis, germacrene D (2%), nerol (1.6 %), linalol (1.1%) , limonene (0.4%), geranyl isobutyrate (0.2%) and eugenol (0.2%). [6]
Baccharis trinervis (Lam), ragwort, varejón: The genus *Baccharis* is the richest in species in the Asteraceae tribe. They are between 400 and 500 species. It has a unique geographical distribution in America, from the southern United States, to the southern tip of Argentina and Chile. In Colombia it is in open forest and scrub, stubble, mountain edges and flat road. [7]
Through hydrodistillation they obtained, from the aerial parts of the plant, volatile constituents 26 representing 95.8 % of the same oils. Older volatile components were: thjene α-, α-pinene, β-pinene, β- feladreno, methyl (Z) dec-2 -en-4, 6 dinonato [(E) -ácidolachno - phyllummetilester. The leaves have saponins and diterpenes: ácidokaurenoico and labdenoico. The branches contain saponins and tannins. [8]
Robayo and Rodriguez [9] conducted trials to test the inhibitory effect of extracts of decomposition of the fruit of *Swinglia glutinosa* and *Piper aduncum*, on the germination of seeds of five weeds: *Echinochloa colona, Rotboellia cochichinensis, Leptochloa filiformis, Ipomea tiliacea* and *Senna obtusifolia*. In bioassays they found that doses of the extracts used inhibit seed germination of weed species highly significant statistically. In greenhouse trials also they reported severe phytotoxicity and death of leaves and tillers in the species reported.
Arminante et al [10] proved essential oils *Hyssopus officinalis* L., *Lavandula angustifolia* Miller, *Majorana hortensis* L., *Melissa officinalis* L., *Ocimum basilicum* L., *Origanum vulgare* L., *Salvia officinalis* and *Thymus vulgaris* L., showing having inhibitory activity on the germination of *Raphanus sativus* L. and garden cress.
Chavez and Perez [11] evaluated the effect of five ethanol extracts of piperaceas on germination and seedling stage of five crops and weeds. They found that a reduction in germination and seedling weeds. The cultures particularly affected are tomato, carrot and lettuce. Barrera and Martinez [12] studied the effect of extracts of plants belonging to the Piperacea family on the seed bank Soil Farm Esperanza and reported no effect of inhibiting germination of weed especially the group of broad leaves. They also found that the inhibitory effect of extracts was not as efficient as commercial herbicides.
Urueña and Rodriguez [13] evaluated the effect of extracts of *Lantana camara*, *Petiveria alliacea* and *Swinglia glutinosa* Murray, weed seed and crop phytotoxicity indicators to determine the inhibitory effect of plant extracts from these species, tests of pre-emergence and post-emergence were performed, with applications four concentrations (0, 0.5, 1, 1.5% V/V) to five species of weeds (chilinchil, sorguillo, frijolillo and nit pork) and five crops (rice, corn, carrot, lettuce and tomato). In pre-emergence tests it was observed that *L. camara* extract inhibited germination and root length decreased weeds when seed was applied with the lowest concentration (0.5%). For extracts of *Lantana camara*, it required an average rate of 2% to observe the effect of control variables evaluated.
The use of essential oils for weed control seems promising in organic farming, but they act very fast and its activity is limited because most rapidly volatilized. [14]
Verdaguer [15] evaluated the potential herbicide essential oil of Cistus L. was tested in vitro against *Amaranthus hybridus* L., *Portulaca oleracea* L., *Chenopodium album* L., *Conyza canadensis* (L.) Cronq. and *Parietaria judaica* L. Its activity differed according to arvense on which acted: completely inhibited germination of *A. hybridus,* and controlled almost completely the germination of *C. canadensis* and *P. judaica* all concentrations tested, with no differences between them.
Gil et al [16] determined the inhibitory effect of plant extracts of the species *Swinglia glutinosa* and *Lantana camara*, performing tests pre-emergence and post-emergence. In pre-emergence tests it found that S. glutinosa extract inhibited germination and root length decreased weeds when seed was applied with the lowest concentration (0.5%). For extracts of *Lantana camara*, it required an average rate of 2% to observe the effect of control variables evaluated.
Santana et al [17] carried out a study with aromatic plants from Castilla, La Mancha, and verified the phytotoxic activity of their essential oils in *Lactuca sativa* L. and *Lolium perenne* L., giving good results in all cases, although some oils showed more activity than others against L. sativa and against L. perenne.
Ismail et al [18] evaluated the effect of the essential oils of *Juniperus phoenicea* L, *Pistacia vera* L. and *Pistacia terebinthus* L., which had a strong inhibitory effects on the germination of the tested weeds, being more effective at equal doses in dicotyledons than monocotyledons.

Materials and Methods

Aromatic native species contents important of essential oils (EO) were selected from the existing clonal garden at the La Esperanza farm (Guavio bajo) those who have a mayor quantity and quality of essential oils. From selected plants, samples of fresh stems and leaves were taken, which were taken to the laboratory of *Plant Natural Products Chemistry Department of the Universidad Nacional de Colombia* in Bogotá. These facilities in plant material having a distillation processes submitted by steam in order to get their essential oils, which are used for testing biological activity.

Methodology

The trial was conducted under controlled conditions at home or greenhouse mesh consisted of placing a layer of soil, which already Seed Bank (BS) was known in trays and apply uniform watering every 48 hours. Each Essential Oils (EO) was applied and commercial control, which corresponds to a product of chemical synthesis and recommended included for each crop. The same work was performed with the EO, but postemergence i.e. when germinated weeds soil had two true leaves, also a commercial control, which corresponds to a product of chemical synthesis and recommended for each crop are included.

Experimental Design

Completely randomized design was used. Nine experimental units each with five repetitions, which consisted of aluminum trays filled with soil from the farm La Esperanza for cases of emergency pre settled. For laboratory tests nine experimental units each with four petri dishes as a repetition for each species, in total there were 108 Petri dishes each with 10 seeds were taken. A design of each of the pre- and post-emergence applications and for laboratory test was implemented. The data were tabulated and analyzed using statistical software version Cropstat 2008, supplemented with Tukey contrast test.

Treatments

Table 1 shows the treatments which were applied to the the corresponding nine tests, including a control without application, a control with only water application and a commercial herbicide according to whether the application was in pre or post-emergence occur.

Soil samples from a commercial lot of farm La Esperanza (Guavio bajo) were taken. The test was conducted with soils belonging to the farm of the university. The samples were taken as indicated, [19] digging a hole forming a cairn 12.5cm long x 12.5cm wide and 20cm depth The test was conducted in controlled conditions at home or greenhouse mesh, which consisted of placing soil in trays from the commercial lot, which already he knew the seed bank (SB). Tests were conducted to evaluate the preemergence ie seeds had not emerged weeds.

Parameters evaluated: Percentage of emerged weeds classified as monocots, dicotyledonous forbs and commelináceas. Percentage control with respect to absolute control, and percentage of control with respect to the commercial control.

Results and Discussion

Effects of Essential Oils Preemergence

These bioassays were used EO of *Lipia alba* and *Bacharis trinervis* applied on a soil of a commercial lot of farm la Esperanza, before the emergence of weeds. The evaluation was performed at eight and fifteen days after the application taking into account the number of emerged seedlings to estimate an average effective control compared to the absolute control.

Control Efficacy

Data on the number of weeds emerged by treatment and repetition, were estimated by counting for each of the groups, using data from the absolute control for each case, the control rates according ALAM (1974) were estimated

Table 1. Treatments used in the tests.

Treatment	Name	Description
	PREEMERGENCE	
T_1	Witness	Applying distilled water to the trays
T_2	Witness + Tween	Application of Tween solution + distilled water
T_3	Commercial Witness	Application of solution Atrazine WP + water
T_4	*Lippia alba* 100mg. L^{-1}	Solution of the mixture of EO *Lippia alba* 100mg. L^{-1} and Tween + water
T_5	*Lippia alba* 300mg. L^{-1}	Solution of the mixture of EO *Lippia alba* 300 mg. L^{-1} and Tween + water
T_6	*Lippia alba* 500mg. L^{-1}	Solution of the mixture of EO *Lippia alba* 500 mg. L - ^{1} and Tween + water
T_7	*Baccharis trinervis* 100mg. L^{-1}	Solution of the mixture of EO *Baccharis trinervis* 100 mg. L^{-1} and Tween + water
T_8	*Baccharis trinervis* 300mg. L^{-1}	Solution of the mixture of EO *Baccharis trinervis* 300 mg. L^{-1} and Tween + water
T_9	*Baccharis trinervis* 500mg. L^{-1}	Solution of the mixture of EO *Baccharis trinervis* 500 mg. L^{-1} and Tween + water

and subjected to one ANAVA using the program CropStat version 2008.

The metabolites present in EO are molecules having electric charges and therefore, when deposited on the ground, are attracted by the micelles or soil colloids, represented by organic matter and clays.

Therefore soil more or less organic content and more or less percentage of clays will influence the activity of herbicides, affecting their efficiency and persistence. [20] Physical analyzes of soil pH and indicate that the soil is classified as frank, pH values are located near neutrality and should not be a differentiating factor in weed control by metabolites present in the extracts evaluated. Counting weeds present at 8 days per group were subjected to ANAVA.

Weeds Dicotyledonous

In the study of weeds and their control specifically it recommended for group study. The respective Anava for these weeds which are also known as broadleaf shows highly significant differences between treatments. This indicates that there are differences in the control of these weeds by the different treatments used to determine which of the treatments were more effective in controlling a Tukey test presented in Table 2 was established.

The test displays five different groups and statistically the first group including T7 which is the application of *B. trinervis* 100mg L^{-1} and *L. alba* 500mg L^{-1} the corresponding values were 59.87% and 54.87%. These percentages in the table control efficiency according ALAM, listed as a regular control. A second group includes treatments: *Lippia alba* 500mg. L^{-1}, *Lippia alba* 300mg. L^{-1} and Lippia alba 100mg L-1 according to the scale values that show regular control of this group of weeds. It should be noted that

Table 2. Tukey test for the application of nine treatments on weed control Dicots under house conditions. Fusagasugá 2014.

Number	Treatments	Percent Control
T_7	*Baccharis trinervis* 100mg. L^{-1}	59.87 a
T_6	*Lippia alba* 500mg. L^{-1}	54.87 ab
T_5	*Lippia alba* 300mg. L^{-1}	50.93 b
T_4	*Lippia alba* 100mg. L^{-1}	49.00 b
T_3	Commercial Herbicide	37.33 c
T_8	*Baccharis trinervis* 300mg. L^{-1}	34.67 c
T_2	Witness + Tween	19.73 d
T_9	*Baccharis trinervis 500mg. L^{-1}*	6.60 d
T_1	Absolute control	3.00 e

Means followed by the same letter no statistical differences
Significance level = 0.05
Tukey = 6.413

Table 3. Tukey test for the application of nine treatments on weed control Monocots under house conditions mesh. Fusagasugá 2014.

Number	Treatments	Percent Control
T_3	Commercial Herbicide	76.57 a
T_9	*Baccharis trinervis* 500mg. L^{-1}	59.83 b
T_8	*Baccharis trinervis* 300mg. L^{-1}	50.93 c
T_4	*Lippia alba* 100mg. L^{-1}	28.77 d
T_7	*Baccharis trinervis* 100mg. L^{-1}	22.17 de
T_2	Witness + Tween	17.30 ef
T_5	*Lippia alba* 300mg. L^{-1}	14.60 fg
T_6	*Lippia alba* 500mg. L^{-1}	9.33 gh
T_1	Absolute control	4.00 h

Means followed by the same letter no statistical differences
Significance level = 0.05
Tukey = 7.4873

the commercial herbicide selected as witness atrazine corresponded to a percentage of 37.33% control by pointing ALAM scale is a poor control. In reviewing the record of the commercial herbicide it is observed to be recommended for weed control some broadleaf called as *Ipomoea sp.* and other weeds of this type, but present in warm weather. Because the type of weeds present in the Granja La Esperanza possibly the herbicide did not have an efficient control action. Due to the type of weeds present at the La Esperanza farm possibly the herbicide did not have an efficient control action.

Weeds Monocots

The respective Anava for these weeds are known as broadleaf shows highly significant differences between treatments. This indicates that there are differences in the control of these weeds by the different treatments used to determine which treatment was more effective in controlling a Tukey test presented in Table 3 was established. The test displays eight different groups and projects statistically the first group corresponding to T3 one Atrazine commercial herbicide present a value of 76.57%, which in the ALAM scale corresponds to a good control. Continue *Baccharis trinervis* 500mg. L^{-1} with a percentage of 59.83% and *Baccharis trinervis* 300mg. L^{-1} with 50.93% in the scale that appears as a regular ALAM control such weeds . The other treatments listed with percentages lower control 30% pointing classified as poor or no control controls.

Weeds Sedges

The respective Anava for these weeds showing highly significant differences between treatments. This indicates that there are differences in the control of these weeds by the different treatments. In order to determine which of the treatments were more effective in controlling a Tukey test presented in Table 4 was established.
Tukey test indicates that six groups differ statistically were formed, T6 and T3 being presented higher percentages of control 59.7 and 54.03%, that within the scale ALAM appear as a regular control. T7 and T8 *Baccharis trinervis* 100mg. L^{-1} and *Baccharis trinervis* 300mg. L^{-1} with values of 50.73 and 43.63% are other treatments with control regular. The checks show percentages of poor control. Apparently EO of *B. trinervis* in middle and low concentrations have inhibitory effect germination of weed sedges, even surpassing control obtained with the commercial herbicide atrazine.

Table 4. Tukey test for the application of nine treatments in controlling weeds sedges under house conditions .Fusagasugá 2014.

Number	Treatments	Percent Control
T_6	*Lippia alba* 500mg. L^{-1}	59.70 a
T_3	Commercial Herbicide	54.03 ab
T_7	*Baccharis trinervis* 500mg. L^{-1}	50.73 bc
T_8	*Baccharis trinervis* 300mg. L^{-1}	43.63 c
T_9	*Baccharis trinervis* 500mg. L^{-1}	33.23 d
T_5	*Lippia alba* 300mg. L^{-1}	32.50 d
T_4	*Lippia alba* 100mg. L^{-1}	19.43 e
T_2	Witness + Tween	14.67 e
T_1	Witness	2.67 f

Means followed by the same letter no statistical differences
Significance level = 0.05
Tukey = 7.7151

Many plants are important for the production of essential oils, which are rich in compounds with a structure isoprene, terpenes and terpenoids secondary metabolites. The controls obtained are due largely to the metabolites present in the EO as sesquiterpenes which act as inhibitors of seed germination, considering that are precursors of abscisic acid (ABA) and phenols (germination inhibitors) of according to the noted by Rice. [21]
The possible mechanism of action of the precursor terpenes, corresponding to abscisic acid (ABA), would be antagonized with the gibberellins in the seeds, inducing the formation of certain compounds acting as sprout inhibitor, in accordance with scored by Salisbury and Ross.[22]

Conclusions

The results obtained in tests preemergence in the seed bank, showed differences in the control rates for dicotyledonous noting that the inhibitory effects on germination may be due to secondary metabolites present in essential oils from aromatic plants, especially sesquiterpenos (precursors of abscisic acid) and phenols, which act preventing root protrusion.

Application of *B. trinervis* at concentrations of 100mg L^{-1} and *L. alba* 500mg L^{-1} had a regular control dicotyledonous ALAM scale. It is indicating that for best results dicots and monocots low concentrations of *B. trinervis* are preferred *L. alba* whereas high concentrations are preferred.

The application of essential oils of seeds of weeds and applications preemergence and postemergence showed that some treatments showed some degree of control in all three groups of weeds, which may be of interest as these essential oils have no formulation as herbicides help improve the spectrum of control also compounds such as resins, tannins and lignin you tend to remain in the soil in original state or are slowly decomposed.

These results may be promising from the point of view that EO are not formulated products and only contain secondary metabolites, but do not have dispersants, adhesion promoters and other substances in herbicides are formulated to improve the spectrum control weeds.

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