Reception: **June 17, 2022** | Acceptance: **November 14, 2021** | Publication: **February 1, 2023**

Phytotoxicity of plant extracts on seed germination and initial development of mono and dicotyledonous plants

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To quote this article:

Anza Cruz, H. G., Ramírez González, S. I., López Báez, O., & Espinoza Zaragoza, S. Fitotoxicidad de extractos vegetales en la germinación de semillas y desarrollo inicial de plantas mono y dicotiledóneas. *Espacio I+D, Innovación más Desarrollo, 12*(32). https://doi.org/10.31644/IMASD.32.2023.a07

— Abstract—

Weed control is an important factor in crop production, but it is necessary to generate less polluting and more sustainable alternatives, for which exploratory research was proposed to determine the phytotoxic activity of plant extracts of *Rosmarinus officinalis, Raphanus sativus, Origanum vulgare, Capsicum annuum* and *Allium sativum* in seed and plant germination of monocots (*Zea mays*) and dicots (*Phaseolus vulgaris*) species; two forms of extraction (liquefied and pressurized) were used with each plant. In the first stage, all the extracts were evaluated at concentrations of 100% and 50% v/v, in *Z. mays* and *P. vulgaris*, and the percentage of germination, healthy seeds, and seedling growth were quantified. The best treatments were evaluated in a second stage on plants, and the following were quantified: degree of phytotoxicity (Rochecouste scale), fresh and dry weight, and root and foliar growth. The design was completely randomized, and a control (water) was included in both stages, analysis of variance and comparisons of Tukey's means (P≤0.05) were performed. The results indicate that the extract of R. officinalis liquefied at 100% achieved nine on the Rochecouste phytotoxicity scale, as well as the lowest value of dry weight in *Z. mays,* and *R. officinalis* pressurized at 100% and 50%, *C. annuum* liquefied and pressurized at 100% and *O. vulgare* pressurized at 100%, also had a phytotoxic effect greater than 4.8 and a reduction in the dry weight of plants by more than 50%, being potential herbicide products in monocots plants. The extract of *A. sativum* liquefied at 100% achieved ten on the Rochecouste scale and the lowest weight in *P. vulgare* plants, being *A. sativum* pressurized at 100 and 50%, and *O. vulgare* liquefied at 100%, extracts with herbicide potential in dicots plants.

Keywords:

Weeds, sustainable agriculture, Allium sativum, Origanum vulgare, Rosmarinus officinalis, Capsicum annum.

For years the use of chemical or synthetic herbicides has been part of the work routine of many producers in the agricultural sector, mainly when carrying out cleaning activities or preparing the land to combat arvenses (weeds). According to García (2013), synthetic herbicides have been used indiscriminately in 47 countries for 50 years for weed control, resulting in the development of resistance in more than 235 arvenses species.

This type of vegetation represents various problems for crops, affecting the development of seedlings by the competition for water, light, territory, and nutrients, decreasing the production capacity, while they can behave as hosts of pests and diseases. In addition, the inappropriate and irrational use of herbicides to combat arvenses has caused environmental impacts such as the loss of soil fertility, contamination of surface and groundwater, causing the decrease of species such as fish, birds, insects, and even human losses due to intoxication in their mismanagement.

Méndez (2019) states that the biggest challenge for organic agriculture is weed management due to the lack of effective natural herbicide products for its control. The allelopathic characteristics exhibited by some plant species could become an important tool to combat the challenges of environmental pollution and the development of herbicide resistance in weeds. In this regard, a relatively unexplored alternative is the use of allelopathic plants. Allelopathy is the science that studies the interrelationships between plants, through the relationships of regulation or repulsion between them and other organisms, having established that many plants produce chemicals capable of repelling other plants, fungi, bacteria, nematodes, viruses, and insects, so they represent a very effective natural control that would avoid the use of pesticides, herbicides, or fungicides (Ormaza, 2017).

In a search for more sustainable solutions, "extracts derived from vegetables have turned out to be an interesting alternative. That is why its use has gained relevance in recent times since they are an economic, renewable, and safer resource for the environment." (Abdullah, 2011; Delbianco & collaborators, 2020) This is because the bioactive compounds extracted from plant organs (leaves, roots, flowers, stems, and seeds) present a phytotoxic potential, which makes them candidates for bioherbicides (Cruz & Flores, 2021). Mexico has a great deal of unexplored plant diversity; according to Diaz *et al.* (2017) research on plants with phytotoxic (*in vitro*) and allelopathic (in soil) properties has been poorly developed due to the small number of plant species that have been studied.

The present exploratory research aimed to: determine the phytotoxic activity of plant extracts of *Rosmarinus officinalis L., Raphanus sativus, Origanum vulgare L., Capsicum annuum L.* and *Allium sativum* in the germination of seeds and plants of monocotyledonous (*Zea mays*) and dicotyledonous (*Phaselolus vulgaris*) species; to explore sustainable alternatives in

the management of arvense plants in crops, which allow local resources to be used and which can contribute to the reduction of the negative effects of herbicides on the environment.

MATERIALS AND METHODS

Collection of plant material

For the two stages of development of this research work, plant material was collected from five species: rosemary (*Rosmarinus officinalis L.*), oregano (*Origanum vulgare L.*), white chili (*Capsicum annuum L.*), radish (*Raphanus sativus L.*), and garlic (*Allium sativum L.*) in March 2021, in the municipality of Berriozábal, state of Chiapas, Mexico, which was free of pests, diseases, and without physical damage.

Extracts preparation

The plant extracts were prepared by two extraction means, hot and cold, through the methods of liquefaction and pressurization, according to the methodology described by Ramírez (2013), for which *R. officinalis* and *O. vulgare* leaves, *C. annuum* fruits, *A. sativum* bulbs, and *R. sativus* root were weighed and crushed.

The pressurization method (P) is an extraction process that consists of cooking the plant material in a pressure cooker to obtain vegetable broth. So 300 g of fresh plant material finely chopped in 1 liter of solvent consisting of a distilled water solution placed into a pressure cooker. It was sealed and subjected to heat for 15 minutes without allowing steam to escape, allowed to cool without removing the lid, and subsequently filtered. For the liquefaction method (L), the plant material was subjected in the same proportion as the previous extraction method to a cold extraction process using a blender for this purpose, leaving the plant material well crushed, to then be filtered; the two processes were carried out under aseptic conditions to avoid contamination.

PHYTOTOXICITY BIOASSAY

Stage 1. Germination tests

In an attempt to have models representing families of the two taxonomic classes of plants (mono- and dicotyledonous) for the development of a reproducible bioassay, the use of seeds from cultivated species was chosen for plants monocotyledonous corn seeds were used (*Zea mays*) provided

by the ejido San Isidro in the municipality of Berriozábal, Chiapas and for dicotyledonous: bean seeds variety "verdín" (line SEN-70) (*Phaselolus vulgaris*), obtained from the National Institute of Agricultural and Livestock Forestry Research (INIFAP) headquarters Ocozocoautla, Chiapas. For this test, a completely randomized experimental design was used, using the five plant extracts with the two forms of extraction (pressurization and blending), each at two concentrations: 100 and 50% volume/volume (v/v), and control with water was counted for a total of 21 treatments, and five repetitions; for each species. The experimental unit consisted of ten seeds placed in a 20 x 15 cm unicel tray, in which an absorbent paper base was placed, and 10 ml of a solution of the plant extracts at the respective concentrations was added (Table 1), subsequently covered with a vinyl paper film.

Table 1
Treatments evaluated in seed germination

No.	Treatments	Extraction method	Concentration
1	*Raphanus sativus*	Liquefied	100% (v:v)
2	*Raphanus sativus*	Liquefied	50% (v: v)
3	*Raphanus sativus*	Pressurized	100% (v:v)
4	*Raphanus sativus*	Pressurized	50% (v:v)
5	*Capsicum annuum*	Liquefied	100% (v:v)
6	*Capsicum annuum*	Liquefied	50% (v:v)
7	*Capsicum annuum*	Pressurized	100% (v:v)
8	*Capsicum annuum*	Pressurized	50% (v:v)
9	*Rosmarinus officinalis*	Liquefied	100% (v:v)
10	*Rosmarinus officinalis*	Liquefied	50% (v:v)
11	*Rosmarinus officinalis*	Pressurized	100% (v:v)
12	*Rosmarinus officinalis*	Pressurized	50% (v:v)
13	*Origanum vulgare*	Liquefied	100% (v:v)
14	*Origanum vulgare*	Liquefied	50% (v:v)
15	*Origanum vulgare*	Pressurized	100% (v:v)
16	*Origanum vulgare*	Pressurized	50% (v:v)
17	*Allium sativum*	Liquefied	100% (v:v)
18	*Allium sativum*	Liquefied	50% (v:v)
19	*Allium sativum*	Pressurized	100% (v:v)
20	*Allium sativum*	Pressurized	50% (v:v)
21	*Water indicator*	N/A	N/A

Source: Own elaboration

For the quantification of the *germination percentage* variable, the data were recorded every 24 hours, counting the number of germinated seeds per

experimental unit, as well as the number of healthy seeds, eleven days for *Z. mays* and nine for *P. vulgaris*.

To evaluate the vigor of the seedlings, the root length and leaf development of those from the seeds that managed to germinate were measured. For both variables a single measurement was performed, expressing the results in millimeters (mm) on day eleven for *Z. mays* seeds and the ninth day for *P. vulgaris*' seeds.

Stage 2. Phytotoxic effect on plants

Based on the results of the previous trial, the treatments that were most effective in reducing the germination and development of *Z. mays* and *P. vulgaris* were evaluated in the second stage. For this, plastic cups with a capacity of 3 ounces perforated at the bottom were used, filled with a substrate (sand:earth), previously solarized for ten days, and a seed was placed in the center.

A completely random design was employed, with nine treatments for *Z. mays* and eight for *P. vulgaris* (see Tables 2 and 3), 12 replicates were counted for each treatment, and one control with water was contemplated for each. In the case of Z. mays was counted with 108 experimental units and P. vulgaris of 96. For the application of treatments, a manual atomizer was used, performing a foliar spray on each plant on day 23 of planting, after five days the first evaluation was carried out, and on the tenth day, the second, applying the Rochecouste phytotoxicity scale (table 4) according to Chaila (1986).

Table 2
Treatments evaluated in Z. mays plants

No.	Treatments for *Z. mays*	Concentration
1	*Capsicum annuum* L. Liquefied	100% (v:v)
2	*Capsicum annuum* L. Liquefied	50% (v:v)
3	*Capsicum annuum* L. Pressurized	100% (v:v)
4	*Capsicum annuum* L. Pressurized	50% (v:v)
5	*Rosmarinus officinalis* L. Liquefied	100% (v:v)
6	*Rosmarinus officinalis* L. Pressurized	100% (v:v)
7	*Rosmarinus officinalis* L. Pressurized	50% (v:v)
8	*Origanum vulgare* L. Pressurized	100% (v:v)
9	Control - Water	NA

Source: Own elaboration

Table 3
Treatments evaluated in P. vulgaris plants vulgaris

No.	Tratamientos for *P. vulgaris*	Concentration
1	*Rosmarinus officinalis* Pressurized	100% (v:v)
2	*Rosmarinus officinalis* L. Pressurized	50% (v:v)
3	*Allium sativum* Liquefied	100% (v:v)
4	*Allium sativum* Liquefied	50% (v:v)
5	*Allium sativum* Pressurized	100% (v:v)
6	*Allium sativum* Pressurized	50% (v:v)
7	*Origanum vulgare* Pressurized	100% (v:v)
8	Control - water	NA

Source: Own elaboration

Table 4
Escala Rochecouste para la evaluación de fitotoxicidad

Rochecouste Phytotoxicity Scale	
Effects	**Score**
No visible effect	0
Slight caustic action on leaves	1
Chlorotic Leaves	2
Moderate caustic action on leaves	3
Moderate caustic action on leaves and stems	4
Slight damage to leaves and stems	5
Slight damage, and death in young outbreaks	6
Dead stems 25%	7
Dead stems 50%	8
Dead stems 75%	9
Total Death	10

Source: Chaila, 1986

In addition, at the end of the phytotoxicity evaluations, fresh and dry weights per plant were determined as response variables. For fresh weight determination, it was recorded by fully weighing the plant on a gram scale. To determine the dry weight of each treatment, the plants were exposed to the sun for 15 days until a constant weight was obtained with these values determining the percentage reduction of dry weight, relative to the control with water.

Statistical analysis

In both trials, a completely randomized experimental design was performed, with five repetitions for the first stage and 12 repetitions for the second stage, for each of the treatments. The data obtained were processed through an analysis of variance (ANOVA), and for those who presented significant differences, the Tukey means comparison test ($P \leq 0.05$) was performed with the SPSS version 17 program.

RESULTS AND DISCUSSION

Extracts effects on Z. mays seeds germination

The results of the treatments' effect on *Z. mays* seeds germination can be seen in Table 5, in which the daily number of germinated seeds for 11 days is presented; the ANOVA performed for each day indicated the existence of statistical differences between the treatments for every day, it is appreciated as from day 2, the control treatment, to which only water was applied, recorded the highest values starting with 4.4 and at the end of day 11 was 9, recording according to the Tukey test difference with the other treatments. It is appreciated that from day 2 to 11 of treatment with *R. officinalis* 100% pressurized, its germination values were low (0.2 to 3), followed by the same plant and extraction form but at an application concentration of 50% (v:v), with values of 0.8 to 6.6 of germinated seeds, followed by the treatment of *C. annuum* 100% pressurized with values ranging from 1.2 to 6; for evaluation day 11 only treatment *R. officinalis* 100% pressurized, with 30% germination, registered statistical difference with the water control, which presented 90% germination.

Table 6 presents the data on the number of healthy seeds for each of the treatments, as well as their germination percentage. The ANOVA practice indicated statistical differences between the treatments in the first days of evaluation, being *R. sativus* 100% pressurized and *A. sativus* 100% liquefied, the only treatments that recorded 100% healthy seeds at the end of the evaluation days, the water control recorded 90% healthy seeds at the end of the 11 days of evaluation.

In the case of the dicotyledonous (*P. vulgaris*), the ANOVA practiced at the number of germinated seeds, indicates differences between treatments on each of the nine days of evaluation, the data are presented in Table 7, where it can be seen that *A. sativum* 100% pressurized, recorded the values of zero germinated seeds until day 7, and ended on day 9 with 1.4, recording statistical differences with the water control, which recorded the highest value with 60% germination. Other treatments that allowed low germination

were *A. sativum* 100% liquefied, *R. officinalis* 100 and 50% pressurized, *O. vulgare* 100% liquefied, *R. officinalis* 100% liquefied, with values between 24 and 30% of germination, at the end of the evaluations.

Regarding the number of healthy seeds, it can be seen in Table 8, when practicing the ANOVA only recorded differences between the treatments for day 3; at the end of the evaluations, the percentage of healthy seeds was between 52 and 72%, with 60% water control, the lowest values being those recorded in the treatments with *R. officinalis* 50% Liquefied with 46%, *O. vulgare* 100% Liquefied and *C. annuum* pressurized to 50% with 48% healthy seeds.

The results allowed us to observe the gradual decrease in the percentage of germination in corn and bean seeds. For the case of pressurized extract *R. officinalis* at a concentration of 100% could be observed to act in both seeds as a major growth inhibitor in monocots (narrow leaf) and dicotyledonous (broad leaves). According to research conducted by Sancho (2011), the herbicidal potential of *R. officinalis* essential oil was evaluated, and tested *in vitro* on *P. oleracea* (purslane) and *C. canadensis* (horsetail). It could be concluded that *C. canadensis* revealed increased phytotoxic activity, as the three highest concentrations (0.25, 0.5, and 1 µl/ml) significantly inhibited its germination, by 40.4, 70.2, and 97.9% respectively. So, it might be possible to use extract *R. officinalis* as a natural herbicide in arvenses in activities before crop preparation.

Table 5
Plant extracts application effects on the Z. mays germination

Treatments	Number of sprouted seeds of *Zea mays* Germination days										Percentage germination day 11
	2	3	4	5	6	7	8	9	10	11	
R. sativus 100% Liquefied	0.2a	0.8a	5.6c	8.8e	9e	9.8h	9.8d	9.8b	9.8b	9.8b	98
R. sativus 50% Liquefied	0a	0.6a	2.4ab	3.8cd	4.6bc	7.2fgh	9cd	9.2b	9.2b	9.2b	92
R. sativus 100% Pressurized	0a	0a	0a	1.2abc	1.2ab	3.8abcdefg	6.2abcd	8.8b	8.8b	9.6b	96
R. sativus 50% Pressurized	0.4a	1a	1ab	0.8abc	0.8a	3abcdef	6.6abcd	8.6b	8.6b	8.6b	86
C. annuum 100% Liquefied	0a	0a	0a	0a	0a	2.8abcdef	6.2abcd	8.6b	8.6b	9b	90
C. annuum 50% Liquefied	0a	0a	0a	0a	0a	0.8ab	3.4ab	7ab	7ab	7.4b	74
C. annuum 100% Pressurized	1.2a	1.2a	1.2ab	1.2abc	1.2ab	1.6abc	4.4abc	6ab	6ab	6ab	60
C. annuum 50% Pressurized	1a	1.6a	1.6ab	1.6abc	1.6ab	3abcdef	5.6abcd	7.2ab	7.2ab	7.8b	78
R. officinalis 100% Liquefied	1.4a	1.4a	1.8ab	2abc	2.2abc	3.2abcdef	6.4abcd	7.2ab	7.2ab	7.4b	74
R. officinalis 50% Liquefied	2ab	2a	2ab	2abc	2.4abc	5bcdefg	5.8abcd	8b	8b	8.2b	82
R. officinalis 100% Pressurized	0.2a	0.2a	0.2a	0.2ab	0.2a	0.2a	2a	3a	3a	3a	30
R. officinalis 50% Pressurized	0.8a	0.8a	0.8ab	0.8abc	0.8a	1ab	4.6abc	6.6ab	6.6ab	6.6ab	66
O. vulgare 100% Liquefied	1a	1a	1ab	1abc	4.4bc	7efgh	7.4bcd	9.4b	9.4b	9.4b	94
O. vulgare 50% Liquefied	0.4a	0.4a	0.4a	0.4abc	1.4ab	2.4abcd	6.4abcd	8.6b	8.6b	8.6b	86
O. vulgare 100% Pressurized	0.8a	0.8a	3.6bc	3.6bcd	5.2cd	6.6defgh	7bcd	8.6b	8.6b	8.6b	86
O. vulgare 50% Pressurized	1a	1a	1ab	1abc	1.6ab	4abcdefg	7.6bcd	8.2b	8.2b	8.6b	86
A. sativum 100% Liquefied	0a	0a	0a	0a	0.6a	3.2abcdef	7.4bcd	8.6b	8.6b	8.8b	88
A.sativum 50% Liquefied	0.8a	0.8a	0.4a	0.4abc	0.6a	2.6abcde	4.4abc	9.4b	9.4b	9.4b	94
A. sativum 100% Pressurized	0.4a	0.4a	0.4a	0.4abc	0.6a	2.8abcdef	5.4abcd	8b	8b	8.4b	84
A. sativum 50% Pressurized	0.8a	0.8a	0.8ab	0.8abc	1.6ab	5.8cdefgh	7.8bcd	9.6b	9.6b	9.6b	96
Water indicator	4.4b	5.6b	6c	6de	8.6de	8.2gh	8.8cd	9b	9b	9b	90

Averages with the same letter in the same column present no statistically significant difference for the Tukey test ($P \leq 0.05$).

Source: Own elaboration

Table 6

Plant extracts application effects on the number of Z. mays healthy seeds

Treatments	Number of healthy *Zea mays* seeds Days										Percentage of healthy seeds day 11
	2	3	4	5	6	7	8	9	10	11	
R. sativus 100% Liquefied	10c	10c	9.8c	9.8bc	9.8b	9.8b	9.8b	9.6b	9.6b	9.6bc	96
R. sativus 50% Liquefied	10c	9.8c	9.8c	9.6bc	9.4ab	9.4b	9.4b	9.2b	9.2b	8.6abc	86
R. sativus 100% Pressurized	10c	10c	10c	10c	10b	10b	10b	19b	10b	10c	100
R. sativus 50% Pressurized	10c	10c	9.8c	9.2abc	9.2ab	9.2ab	9ab	9ab	9ab	9abc	90
C. annuum 100% Liquefied	10c	10c	9.8c	9.8bc	9.8b	9.4b	9.4b	9.4b	9.4b	9.2bc	92
C. annuum 50% Liquefied	10c	10c	10c	9.2abc	9ab	9ab	9ab	8.8ab	8.8ab	8.8abc	88
C. annuum 100% Pressurized	9.4bc	9.4bc	9.4abc	9.4bc	8.2ab	8.2ab	8.2ab	7.8ab	7.8ab	7.8abc	78
C. annuum 50% Pressurized	8abc	8.2abc	8.2abc	8.2abc	8.2ab	8.2ab	8.2ab	8ab	8ab	8abc	80
R. officinalis 100% Liquefied	8.6abc	8.6abc	8.4abc	8.4abc	8.4ab	8.2ab	8.2ab	8.2ab	8.2ab	8.2abc	82
R. officinalis 50% Liquefied	8.8abc	8.8abc	8.8abc	8.8abc	8.4ab	8.4ab	8.4ab	8.4ab	8.4ab	8.2abc	82
R. officinalis 100% Pressurized	6.8a	6.8a	6.8a	6.4a	6.4a	6a	6a	5.8a	5.8a	5.8a	58
R. officinalis 50% Pressurized	7ab	7ab	7ab	7ab	7ab	7ab	7ab	7ab	7ab	6.4ab	64
O. vulgare 100% Liquefied	9.6c	9.6c	9.6bc	9.6bc	9.6b	9.6b	9.6b	9.6b	9.6b	9.6bc	96
O. vulgare 50% Liquefied	9.6c	9.6c	9.6bc	9.6bc	9.6b	9.6b	9.6b	9.6b	9.6b	9.6bc	96
O. vulgare 100% Pressurized	9.8c	9.8c	9.8c	9.8bc	9.8b	9.8b	9.8b	9.6b	9.6b	9.2bc	92
O.vulgare 50% Pressurized	10c	10c	10c	10c	9.8b	9.8b	9.8b	9.8b	9.8b	9.6bc	96
A. sativum 100% Liquefied	10c	10c	10c	10c	10b	10b	10b	10b	10b	10c	100
A.sativum 50% Liquefied	9.6c	9.6c	9.6bc	9.6bc	9.6b	9.6b	9.6b	9.6b	9.6b	9.2bc	92
A. sativum 100% Pressurized	9.2abc	9.2abc	9.2abc	9.2abc	9.2ab	9ab	9ab	8.8ab	8.8ab	8.8abc	88
A. sativum 50% Pressurized	9.8c	9.8c	9.8c	9.8bc	9.8b	9.8b	9.8b	9.6b	9.6b	9.4bc	94
Water indicator	9.2abc	9.2abc	9.2abc	9.2abc	9.2ab	9.2ab	9.2b	9.2b	9.2b	9abc	90

Averages with the same letter in the same column present no statistically significant difference for the Tukey test ($P \leq 0.05$).

Source: Own elaboration

Table 7
Plant extracts application effect on P. vulgaris germination

Treatments	Days Number of sprouted *Phaselolus vulgaris* seeds							Percentage germination day 11
	3	4	5	6	7	8	9	
R. sativus 100% Liquefied	0a	0a	1.8abc	2.2abcd	2.6abcd	3.8ab	4.2ab	42
R. sativus 50% Liquefied	0.6abc	1.8ab	4.4bcd	6cd	5.8cd	5.8b	5.4ab	54
R. sativus 100% Pressurized	0a	3b	4.4bcd	5.4bcd	5.6cd	5.8b	5.6ab	56
R. sativus 50% Pressurized	1.4abc	1.8ab	2.6abc	3.2abcd	3.6abcd	4.2ab	4.4ab	44
C. annuum 100% Liquefied	0a	2ab	3abcd	4.2abcd	4.8bcd	5.6b	5.6ab	56
C. annuum 50% Liquefied	2c	3.2b	4.6cd	6.2cd	6.4cd	5.8b	6.2b	62
C. annuum 100% Pressurized	0a	2.2ab	3.4abcd	4.6abcd	5.2cd	5ab	5.4ab	54
C. annuum 50% Pressurized	0.6abc	1.4ab	2.4abc	3.8abcd	4abcd	4.4ab	4.8ab	48
R. officinalis 100% Liquefied	0a	0.8ab	1abc	1.2ab	2.4abcd	3ab	3ab	30
R. officinalis 50% Liquefied	0a	1ab	1.4abc	2.6abcd	3abcd	3.6ab	3.6ab	36
R. officinalis 100% Pressurized	0a	0a	0a	0.4a	0.4ab	0.8a	2.6ab	26
R. officinalis 50% Pressurized	1.6bc	1.6ab	1.8abc	2.2abcd	2abc	2.2ab	2.8ab	28
O. vulgare 100% Liquefied	0a	0.8ab	0.8ab	1.8abc	2.4abcd	2.2ab	3ab	30
O. vulgare 50% Liquefied	0a	1.8ab	3abcd	4.2abcd	4.2abcd	3.8ab	4.2ab	42
O. vulgare 100% Pressurized	0a	0.6ab	1.6abc	2.8abcd	3.4abcd	3.4ab	4.6ab	46
O.vulgare 50% Pressurized	0a	0.6ab	1abc	4.2abcd	4.4abcd	3.6ab	4ab	40
A. sativum 100% Liquefied	0a	0a*	0.4a	1.6abc	2.4abcd	2.4ab	2.4ab	24
A.sativum 50% Liquefied	0a	0.8ab	2abc	3.2abcd	3.6abcd	3.4ab	4.4ab	44
A. sativum 100% Pressurized	0a	0a	0a	0a	0a	1.4ab	1.4a	14
A. sativum 50% Pressurized	0.2ab	1.8ab	3.2abcd	4.6abcd	4.8bcd	3.8ab	4ab	40
Water indicator	5d	6c	6.4d	6.8d	6.8d	5.4b	6b	60

Averages with the same letter in the same column present no statistically significant difference for the Tukey test ($P \leq 0.05$).

Source: own elaboration

Table 8

Plant extracts application effects on the number of healthy P. vulgaris seeds

Treatments	Number of healthy *P. vulgaris* seeds Days							Percentage of healthy seeds day 11
	3	4	5	6	7	8	9	
R. sativus 100% Liquefied	8.6ab	8.6a	7.4a	7.2a	7.2a	7.2a	6.4a	64
R. sativus 50% Liquefied	8.8ab	8.4a	7.2a	7.2a	7.2a	7.2a	6.4a	64
R. sativus 100% Pressurized	7.8ab	6.8a	6.8a	6.8a	6.8a	6.8a	6a	60
R. sativus 50% Pressurized	7.2ab	7a	5.8a	5.8a	5.8a	5.8a	5.8a	58
C. annuum 100% Liquefied	8ab	7.2a	7.2a	6.6a	6.6a	6.6a	5.6a	56
C. annuum 50% Liquefied	7.6ab	7a	7a	7a	7a	7a	6.4a	64
C. annuum 100% Pressurized	8.8ab	7.4a	7.2a	6.6a	6.6a	6.6a	6a	60
C. annuum 50% Pressurized	7a	5.8a	5.8a	5.8a	5.8a	5.8a	4.8a	48
R. officinalis 100% Liquefied	7.6ab	6.2a	6a	5.8a	5.4a	5.4a	5a	50
R. officinalis 50% Liquefied	7.8ab	6.6a	6.2a	4.6a	4.6a	4.6a	4.6a	46
R. officinalis 100% Pressurized	8ab	7.4a	7a	6.6a	6.4a	6a	5.2a	52
R. officinalis 50% Pressurized	7.8ab	7.8a	7.8a	7.8a	7.8a	7.8a	6.8a	68
O. vulgare 100% Liquefied	7.6ab	6.6a	6.4a	5.8a	5.8a	5.8a	4.8a	48
O. vulgare 50% Liquefied	9.2ab	8.6a	8.6a	8.6a	8.6a	8.6a	8.4a	84
O. vulgare 100% Pressurized	9ab	8a	8a	7.8a	7.6a	7.6a	6.6a	66
O.vulgare 50% Pressurized	8ab	7.4a	7.4a	6.4a	6.4a	6.4a	6a	60
A. sativum 100% Liquefied	10b	8.2a	7.8a	7.6a	7.6a	7.6a	6.4a	64
A.sativum 50% Liquefied	10b	9a	8a	8a	7.8a	7.6a	7.2a	72
A. sativum 100% Pressurized	9.4ab	8a	6.8a	6.4a	6.4a	6.4a	6a	60
A. sativum 50% Pressurized	9ab	6.6a	6.4a	6a	6a	6a	5.8a	58
Water indicator	6.8a	6.8a	6.8a	6.8a	6.8a	6.8a	6a	60

Averages with the same letter in the same column present no statistically significant difference for the Tukey test ($P \leq 0.05$).

Source: Own elaboration

Extracts effects on seedling growth

Table 9 shows the effect of plant extracts on root length growth and foliar development of seeds that managed to germinate in *Z. mays*; we can see that all extracts inhibited the development of both the radicle and the hypocotyl, which according to the ANOVA practiced there are statistical differences between treatments. The highest root growth values were for the control (water) with 133.67 mm, which according to the Tukey test recorded differences with almost all treatments, except with *R. sativus* 100% liquefied, achieving the highest percentage of inhibition extracts: *R. officinalis* pressurized to 100%, *R. officinalis* 100% liquefied, and *C. annuum* liquefied to 50% with

84.69, 73.44 and 72.37 %, respectively, concerning the control water; the other treatments also inhibited the development of the root between 67.88 and 9.08%.

Table 9
Plant extracts effects on Z. mays seedling growth

TREATMENT	Growth (mm)		Inhibition percentage for control	
	Root	Hypocotyl	Root	Hypocotyl
R. officinalis P 100%	20.47a	11.7a	84.69	90.10
R. officinalis L 100%	35.5ab	25.6abc	73.44	78.35
C. annuum L 50%	36.93ab	11.43a	72.37	90.33
R. officinalis P 50%	42.93abc	23.07abc	67.88	80.49
O. vulgare P 100%	45.37abc	20.2abc	66.06	82.91
C. annuum P 50%	45.97abc	21.87abc	65.61	81.50
C. annuum P 100%	52.43bcd	19.77ab	60.78	83.28
R. sativus P 50%	52.5bcd	21.73abc	60.72	81.62
A. sativum L 100%	53.8bcd	30.93abc	59.75	73.84
A. sativum P 100%	54.87bcde	32.33abcd	58.95	72.65
C. annuum L 100%	58.07bcde	24.7abc	56.56	79.11
O. vulgare P 50%	58.57bcde	25.97abc	56.18	78.03
A. sativum L 50%	58.83bcde	39bcde	55.99	67.01
O. vulgare L 100%	66.8cde	38.43bcde	50.03	67.50
R. officinalis L 50%	69.67cdef	44.73bcde	47.88	62.17
A. sativum P 50%	77.87def	59.13e	41.74	49.99
R. sativus P 100%	81.22ef	45.47cde	39.24	61.54
O. vulgare L 50%	81.37ef	40.2bcde	39.13	66.00
R. sativus L 50%	95.3fg	56.77	28.71	51.98
R. sativus L 100%	121.53gh	98.1f	9.08	17.03
Water indicator	133.67h	118.23f	0.00	0.00

Averages with the same letter in the same column present no statistically significant difference for the Tukey test ($P \leq 0.05$).

Source: Own elaboration

Regarding the development of the hypocotyl, the highest value was presented with the control (water) with 118.23 mm, being the extract of *R. sativus* liquefied to 100% which allowed the greatest development, while the rest of the treatments achieved an inhibition in their development of more than 51%; the ANOVA practiced recorded statistical differences between the treatments. *C. officinalis* 50% liquefied, and *R. savitus* pressurized to 100%, recorded the lowest growths with 11.43 and 11.7 mm respectively, which according to the Tukey test, recorded statistical differences with the control (water), being the greatest inhibitions with 90.33 and 90.10%, regarding the witness.

The effect of the treatments application on the *P. vulgaris* seedlings growth, presented in Table 10, shows that the highest root growth occurred in the control (water), with 140.4 mm; according to the ANOVA carried out, differences were recorded between the treatments being *A. sativum* pressurized at 100%, which presented the lowest value with 3.93 mm, recording, according to the Tukey test, differences with all the treatments including the control (water); followed by treatments *R. officinalis* 50% pressurized, *A. sativum* 100% liquefied, *R. oficinalis* 100% pressurized; *A. sativus* liquefied at 50%, *O. vulga* 100% liquefied, *R. oficinalis* 50% liquefied; *C. annuum* 50% liquefied with a root growth inhibition range between 95.04 and 84.49%.

The growth of the hypocotyl, presented in Table 10, shows that the highest value was 8.3 mm in the control (water), and the vast majority of treatments completely inhibited its development. The ANOVA study showed statistical differences between treatments. The Tukey test points to statistical differences between the 12 treatments that inhibited the complement of the growth of the hypocotyl with the other treatments including the control (water), the treatment of *R. sativus* liquefied at 50% was the one that registered the highest growth value within the extracts evaluated with 5.77 mm.

There are several reports of the *A. sativum* effect on various organisms, such as fungicide on the growth of *Aspergillus parasitics* and *Aspergillus niger* (Juárez *et al*., 2019); as an insecticide, on *Aphis gossypii*, Glover (Peña, *et al*., 2013) and *Spodoptera frugiperda* (Figeroa, *et al*., 2019), as well as a bactericide (García & Herrera 2007); however, no report was found on its possible use as an herbicide and that for the case of this research it resulted with potential for the control of dicotyledonous plants, possibly due to the presence of compounds which in the form of extraction (pressurized) and concentration used, resulted with a high potential to be used for this purpose.

Table 10
Plant extracts effects on the growth of P. vulgaris seedlings

TREATMENT	Growth (mm)		Inhibition percentage for control	
	Root	Hypocotyl	Root	Hypocotyl
A. sativum P 100%	3.93a	0a	97.20	100.00
R. officinalis P 50%	6.97ab	0a	95.04	100.00
A. sativum L 100%	8.73ab	0a	93.78	100.00
R. officinalis P 100%	10.17ab	0a	92.76	100.00
A. sativum L 50%	12.3ab	0a	91.24	100.00
O. vulgare L 100%	15.8ab	0a	88.75	100.00
R. officinalis L 50%	21.07ab	0a	84.99	100.00
C. annuum L 50%	21.77ab	0a	84.49	100.00
A. sativum P 50%	22.63ab	0a	83.88	100.00
R. officinalis L 100%	22.8ab	0a	83.76	100.00
O. vulgare P 100%	27.97abc	2.23ab	80.08	73.13
O. vulgare L 50%	28.23abc	1.37ab	79.89	83.49
R. sativus L 100%	33.57abc	0.37a	76.09	95.54
R. sativus P 50%	38.47abcd	1.03ab	72.60	87.59
C. annuum P 100%	38.77abcd	0a	72.39	100.00
C. annuum P 50%	39.2abcd	0a	72.08	100.00
R. sativus P 100%	48.53bcd	0a	65.43	100.00
O. vulgare P 50%	48.63bcd	3.1ab	65.36	62.65
C. annuum L 100%	68.03cd	1.7ab	51.55	79.52
R. sativus L 50%	79d	5.77bc	43.73	30.48
Water indicator	140.4e	8.3c	0.00	0.00

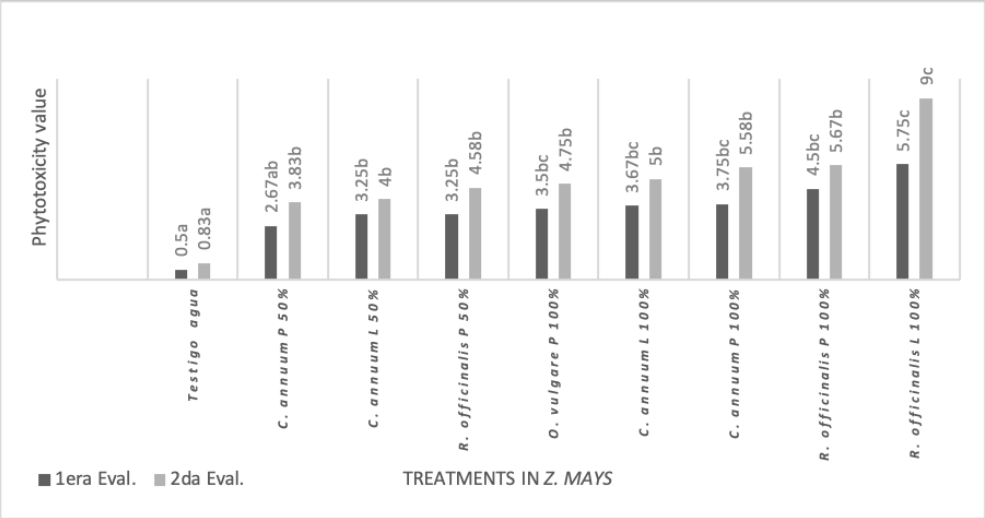
Averages with the same letter in the same column present no statistically significant difference for the Tukey test ($P \leq 0.05$).

Source: Own elaboration

Stage 2. Phytotoxic effect on plants

As can be seen in Figure 1, all the extracts presented different levels of phytotoxicity for both the first and second evaluation, the control water recorded the lowest value with 0.5 for the first and 0.83 for the second evaluation. According to the ANOVA practiced for the two evaluations, there is a statistical difference between the treatments. The treatments that in the first evaluation recorded the highest levels of phytotoxicity were *R. officinalis* 100% liquefied, *R. officinalis* 100% pressurized, *C. annuum* 100% pressurized, and *O. vulgare* 100% pressurized in a range of 5.75 and 3.7, which recorded according to Tukey's test statistical differences with the water witness.

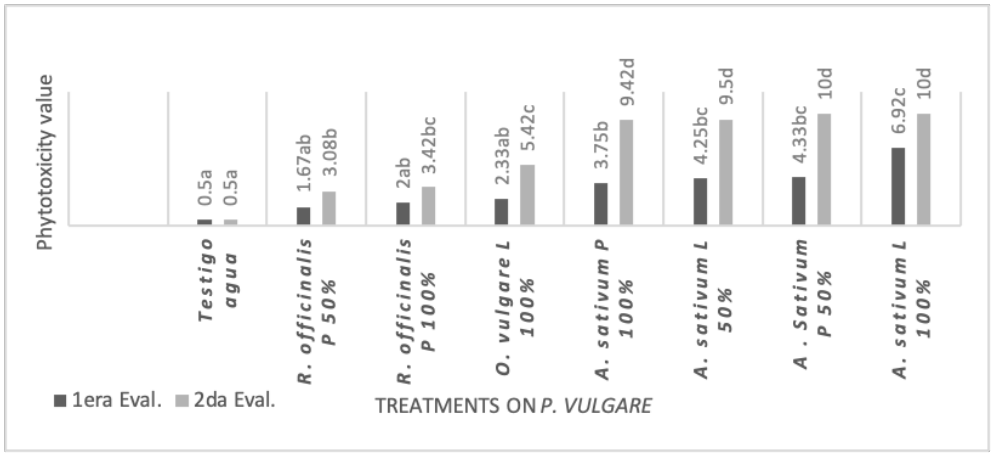
In the second evaluation, the phytotoxicity values increased for all treatments reaching 9 for *R. officinalis* 100% liquefied, which recorded statistical differences with all treatments, including the control with water, which had the lowest value of 0.83, likewise, the rest of the treatments recorded differences with the Control – water.



Averages with the same letter in the same column present no statistically significant difference for the Tukey test (P ≤ 0.05).

Figure 1. Effect of the application of extracts on the phytotoxicity of *Z. mays* - Rochecouste scale. Source: Own elaboration

Regarding the evaluation of dicotyledons (*P. vulgaris*), all extracts for both the first and second evaluations registered statistical differences, according to the ANOVA practice, the highest phytotoxicity was presented for *A. sativum* 100% liquefied *A. sativum* 50% pressurized with 10; *O. vulgare* 100% liquefied with 9.5 and *A. sativum* 100% pressurized with 9.42 on the Rochecouste scale, the other treatments also registered statistical difference according to the Tukey's Test with the water control, which registered the lowest value in the two evaluations with 0.5.



Averages with the same letter in the same column present no statistically significant difference for the Tukey test (P ≤ 0.05).

Figure 2. Application of extracts effect on the phytotoxicity of *P. vulgaris* - Rochecouste scale.
Source: Own elaboration

EFFECTS OF EXTRACTS ON PLANT WEIGHT

Table 11 shows the fresh and dry weight results of the *Z. mays* plants sprinkled with the treatments, it is observed that the lowest weight in fresh and dry was for the extract of *R. officinalis* 100% liquefied, and dry weight was for *C. annuum* 100% liquefied, which recorded significant differences with all treatments including the water control, which managed to reduce dry weight by 47.5% compared to the control.

In the case of the dicotyledonous *P. vulgaris* used, extracts of *A. sativum* and *O. vulgare* 100% liquefied, reduced dry weight by 76.9 and 64.61% concerning the control (water) and recorded statistical differences, according to the Tukey test practiced (Table 12).

Table 11
Influence of treatments on the weight of Z. mays plants mays

Treatments	Fresh weight (g)	Dry weight (g)
Rosmarinus officinalis Liquefied-100%	2.33a	0.53a
Origanum vulgare Pressurized-100%	3.83ab	0.89bc
Capsicum annuum Pressurized-50%	4bc	0.82abc
Rosmarinus officinalis Pressurized-100%	4bc	0.98c
Capsicum annuum Liquefied-100%	4.08bc	0.53a
Capsicum annuum Liquefied-50%	4.08bc	0.62ab
Rosmarinus officinalis Pressurized-50%	4.08bc	0.9bc
Capsicum annuum Pressurized-100%	5.58c	0.93bc
Water indicator	4.75bc	1.01c

Averages with the same letter in the same column present no statistically significant difference for the Tukey test ($P \leq 0.05$).

Source: Own elaboration

Table 12
Treatments' influence on the weight of P. vulgaris plants

Treatments	Fresh weight (g)	Dry weight (g)
Allium sativum Liquefied-100%	0.71a	0.15a
Origanum vulgare Liquefied-100%	1.04a	0.23a
Allium sativum Pressurized-50%	1.32ab	0.27a
Rosmarinus officinalis Pressurized-100%	1.87abc	0.51b
Rosmarinus officinalis Pressurized-50%	2.4bcd	0.61b
Allium sativum Liquefied-50%	2.46bcd	0.52b
Allium sativum Pressurized-100%	3.17d	0.51b
Water indicator	2.87cd	0.65b

Averages with the same letter in the same column present no statistically significant difference for the Tukey test ($P \leq 0.05$).

Source: Own elaboration

A phytotoxic effect is observed in the weight loss of all the extracts used, however, it is differential according to whether it is a monocotyledonous or dicotyledonous plant, which coincides with the higher phytotoxicity values evaluated with the Rochecouste scale, however, in the case of *O. vulgare* a phytotoxic effect is observed for the two species, but because the extraction form is different, the metabolites that exert the phytotoxic action may be different or in different concentrations, in this regard works carried out by Ramírez (2013), Tamayo *et al.* (2016), indicate that due to the amount and

form of extraction, the effect exerted on the target organisms varies, given the amount and diversity of metabolites extracted in the various extraction forms and the solvents used.

On the other hand, Joya *et al*. (2019) report that inhibition of maize seeds in plant extracts of *C. zeylanicum*, *A. indica* and *Z. officinale* to low concentrations stimulate root and aerial growth, and inhibit the growth of phytopathogenic fungi on seeds and roots at the time of sowing, while at 100% concentrations they inhibit the germination of *Z. mays* seeds, coinciding with the phytotoxic effect found in the present investigation with concentrations of the extracts at 100%, both for *Z. mays*, as for *P. vulgaris*, although differential, either for mono- or dicotyledonous plants and Duarte (2020), reports the acute toxicity of anise and rosemary essential oils in onion bulbs, producing a delay in the process of root elongation that affects the cell division of the root system, because the hydration of the onion bulbs was carried out in the presence of the essential oils in whose composition there is a strong presence of allelochemicals, causing an inhibitory effect on the normal growth of the roots with a genotoxicity effect against the cells of onion bulbs roots, being that of rosemary the one that presented a greater phytotoxic effect, however it did not present the same effect on other plants evaluated as those of tomato and cabbage.

The phytotoxic effect found both in the reduction of seed germination and in the initial development of *Z. mays* and *P. vulgare* plants, using local plants and simple forms of extraction, allows for to generate of a possible alternative for the control of arvenses plants, however, this study is exploratory and other research is required.

CONCLUSION

R. officinalis, C. annuum, and *O. vulgare* extractors possess metabolites of high phytotoxicity in monocotyledonous plants and *A. sativum*, *R. officinalis*, and *O. vulgare*, in dicotyledons, which through the appropriate form and concentration can exert an inhibitory effect on the germination and development of plants.

R. officinalis extracts, 100% liquefied, *R. officinalis* pressurized to 100% and 50%, *C. annuum* liquefied and pressurized to 100%, and *O. vulgare* 100% pressurized, achieved higher phytotoxicity, as well as the lowest value of dry weight in *Z. mays*, being potential herbicidal products in monocotyledonous plants.

Extracts with herbicidal potential in dicotyledonous plants are the extracts of *A. sativum* 100% liquefied *A. sativum* pressurized to 100 and 50%, and *O. vulgare* liquefied to 100% (v/v), by achieving high levels of phytotoxicity and low weight in *P. vulgare* plants.

It is possible to reduce the germination of monocotyledonous *Z. mays* seeds between the 66.6% and 26.6%, with the application of extracts of *R. officinalis* pressurized to 100% and 50% (v/v) and *C. annuum* pressurized to 100%; as well as in dicotyledons (*P. vulgaris*) between 76.6 to 50%, with the application of *A. stavius* pressurized and 100% liquefied sativus, *R. officinalis* pressurized and liquefied to 100% and *O. vulgare* liquefied to 100% (v/v), extracts that also inhibit the development of hypocotyl roots.

REFERENCES

Ardila, M., Vargas, A., Pérez, J., Mejía, L. (2009). Ensayo preliminar de la actividad bacteriana de extractos de *Allium sativum, Coriandrum sativum, Eugenia caryophyllata, Origanum vulgare, Rosmarinus officinalis* y *Thymus vulgaris* frente a *Clostrdium perfringens*. *Biosalud*, Volumen 8, 47-57.

Chaila, S. (1986). Métodos de evaluación de malezas para estudios de población y de control. *Revista de la Asociación Argentina para el control de Malezas. ASAM 14*(2):79.

Delbianco, N., Pérez, M., Flamini, D., Priano, C., Ortega, N. (2020). Extractos orgánicos de plantas como inhibidores de corrosión del acero. *Revista argentina de ingeniería*, 72.

Díaz-Mota , M. de los Ángeles, García-Mateos , M. R., Martínez-Solís, J., Acosta-Ramos, M., Serrato-Cruz, M. Á., Colinas-León, . M. T., & Magdaleno-Villar, J. (2017). Fitotoxicidad de los extractos de Dieffenbachia amoena, Nerium oleander, Raphanus sativus y Brassica napobrassica. *Revista De La Facultad De Ciencias Agrarias UNCuyo, 49*(2), 303–318. Recuperado a partir de https://revistas.uncu.edu.ar/ojs3/index.php/RFCA/article/view/3087

Duarte, L. (2020). *Determinación de la actividad antibacteriana y fitotoxicidad de los aceites esenciales de Anís (Pimpinella anisum) y Romero (Rosmarinus officinalis)*. [Tesis de licenciatura Químico ambiental. Facultad de Química ambiental. Universidad Santo Tomás, Bucaramanga, Colombia]

Joya-Dávila, J., Ramírez, S., López, O., Jiménez, S., Alvarado, Á & Espinosa-Zaragoza, S. (2021). Osmoacondicionamiento de semillas de Zea mays con extractos vegetales para aumentar el vigor de establecimiento. *Cien. Agri. 18*(1): 21-35. https://doi.org/10.19053/01228420. v18.n1.2021.12019

Figueroa, A., Castro, E. & Castro, H. (2019). Efecto bioplaguicida de extractos vegetales para el control de Spodoptera frugiperda en el cultivo de maíz (Zea mays). *Acta biol. Colomb., 24*(1):58-66, Enero- Abril 2019. DOI: http://dx.doi.org/10.15446/abc.v24n1.69333

Flores, X. M., Torres, G., Castañeda, D., & Durán, C. (2019). *Efecto fitotóxico de los extractos etanólicos de dos especies del género tagetes*. Colección memorias de los congresos de la sociedad química de México, 40.

García, R., Herrera, F. (2007). Evaluación de la inhibición del crecimiento de cinco cepas bacterianas patógenas por extractos acuosos de *Allium sativum, Allium fistulosum* y *Allium cepa*: estudio preliminar *in vitro*. *Bistua: Revista de la Facultad de Ciencias Básicas*, vol. 5, núm. 2, julio-diciembre, 2007, pp. 68-79.

García-Mateos, M., Claudia Sánchez-Navarro., Juan Martínez- Solís., & Mario Pérez-Grajales. (2013.). Actividad fitotóxica de los extractos de chile manzano (*Capsicum pubescens R & P*). *Rev. Chapingo Ser.Hortic* vol.19 no.4.

Juárez-Segovia, K.G., Díaz-Darcía, E.J., Méndez-López, M.D., Pina-Canseco, M.S., Pérez-Santiago, A.D. y Sánchez-Medina, M.A. (2019). Efecto de extractos crudos de ajo (*Allium sativum*) sobre el desarrollo *in vitro* de *Aspergillus parasiticus* y *Aspergillus niger*. *Instituto Politecnico Nacional*, 103.

Mendez-Navarrete, G. (2019). *Evaluación de extractos vegetales con potencial para el control de malezas en agricultura orgánica*. [Tesis Licenciatura, Universidad Técnica Estatal de Quevedo, Facultad de Ciencias Agrarias. Quevedo-Los Ríos-Ecuador].

Ormaza-Molina, A. (2017). *Capacidad de inhibición de cuatro extractos de arvenses y mucílago de cacao Theobroma cacao, sobre la Rottboellia exaltata y Eleusine indica*. [Tesis Licenciatura, Universidad Técnica Estatal de Quevedo, Facultad de Ciencias Agrarias. Quevedo-Los Ríos-Ecuador].

Peña, M., Castro, J., & Soto, A. (2013). Evaluación de insecticidas no convencionales para el control de Aphis gossypii Glover (Hemiptera: Aphididae) en fríjol. *Revista U.D.C.A Actualidad & Divulgación Científica, 16*(1), 131–138. https://doi.org/10.31910/rudca.v16.n1.2013.867

Ramírez, S. (2013). *Efectividad de extractos vegetales para el manejo de la moniliasis del cacao (Moniliophthora roreri) del cacao (Theobroma cacao)*. [Tesis Doctoral, Universidad Nacional de Costa Rica. Heredia, Alajuela - Costa Rica].

Ramírez, S., López, O., Espinosa, S. & Wong, A. (2016). Actividad antifúngica de hidrodestilados y aceites sobre *Alternaria solani, Fusarium oxysporum* y *Colletotrichum gloesporioides*. *Rev. Mex. Cienc. Agríc* [online]. 2016, vol. 7, n. 8, pp. 1879-1891.

Sancho, M. V. (2011). *Fitotoxicidad de aceites esenciales y extractos acuosos de plantas*. [Tesis Doctoral, Universidad Politecnica de Valencia].

SAS, Institute. (2002). *Statiscal Analysis System User´s Guide*. SAS Institute. Cary USA. 956.

Tamayo, L., Ramírez, S., López, O., Quiroga, R. y Esponisa, S. (2016). Extractos por destilación de *Origanum vulgare, Tradescantia spathacea* y *Zingiber offinale* para el manejo de *Moniliophthora roreri* de *Theobroma cacao*. *Revista Mexicana de Ciencias Agricolas*, Vol.7. Núm 5. 1065-1076