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## In vitro evaluation with essential oils in watermelon (*Citrullus lanatus*) and its biological optimum, Guayas province.

Evaluación in vitro con aceites esenciales en sandía (*Citrullus lanatus*) y su óptimo biológico, provincia del Guayas

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### Abstract

The purpose of this study was to evaluate in vitro the essential oils of *Equisetum arvense* and *Urtica dioica* as fungicides at different concentrations on phytopathogenic fungi in watermelon. Samples were collected in Empalme, Balzar and Pedro Carbo, in established crops that presented diseases. These samples were taken to the phytopathology laboratory of the Agrarian University of Ecuador where the samples were identified and isolated, duly disinfected in Petri boxes with PDA culture medium, and the different concentrations were applied for each treatment. For this research work, two statistical designs were used, under controlled conditions (laboratory), the complete randomized design with factorial arrangement (A\*B\*C) 3 replicates, consisting of 3 pathogens, 3 doses, 2 treatments with 3 replicates, and for the trial conducted with watermelon seedlings, the complete randomized design (CRD) was used, which consisted of 3 phytopathogens (treatments) and 10 plants for each pathogen including the control (replicates). According to the statistics, the treatment with the highest percentage of inhibition was *Urtica dioica* essential oil with 36.19% and a concentration of 300 microliters with 34.38%. To see the effect of the treatment on the watermelon seedlings, pathogenicity tests were carried out, where the plants were inoculated with the pathogens found, then the best treatment was applied on the seedlings, which resulted that the pathogen *Curvularia sp* and *Colletotrichum sp* had less severity with 2.7%.

**Keywords:** Equisetum arvense, oleomacerate, pathogens, watermelon, Urtica dioica

### Resumen

El presente estudio tuvo como finalidad evaluar de forma in vitro los aceites esenciales de *Equisetum arvense* y *Urtica dioica* como fungicida en distintas concentraciones sobre hongos fitopatógenos en sandía. Las muestras fueron recolectadas En el Empalme, Balzar y Pedro Carbo, en cultivos establecidos que presentaron enfermedades, dichas muestras se llevaron al laboratorio de fitopatología de la Universidad Agraria del Ecuador donde se identificaron y se aislaron las muestras debidamente desinfectadas en cajas Petri con medio de cultivo PDA, y se aplicó las diferentes concentraciones por cada tratamiento. Para este trabajo de investigación se emplearon dos diseños estadísticos, bajo condiciones controladas (laboratorio), se realizó el diseño completo al azar con arreglo factorial (A\*B\*C) 3 repeticiones, que consta de 3 patógenos, 3 dosis, 2 tratamientos con 3 repeticiones, y para el ensayo realizado con plántulas de sandía, se utilizó el diseño completo al azar (DCA), el cual constó de 3 fitopatógenos (tratamientos) y 10 plantas por cada patógenos incluyendo las del testigo (repeticiones). Según la estadística se reflejó que el tratamiento que tuvo mayor porcentaje de inhibición fue el aceite esencial de *Urtica dioica* con el 36,19% y una concentración de 300 microlitros con 34,38%. Para ver el efecto del tratamiento en las plántulas de sandía se realizaron pruebas de patogenicidad, donde se inocularon a las plantas con los patógenos encontrados, luego se aplicó el mejor tratamiento sobre las plántulas, las cuales dieron como resultado que el patógeno de *Curvularia sp* y *Colletotrichum sp* tuvieron menos afectación de severidad con un 2,7%.

**Palabras clave:** Equisetum arvense, oleomacerado, patógenos, sandía, Urtica dioica

### Introduction

In Ecuador in 2010, 1908 ha of watermelon were planted with a production of 25818 tons. In addition, about 378 ha were planted in association with other crops with a production of 383 t, with Guayas being the province with the highest production 49 %, followed by Manabí 44 %, Los Ríos 3 % and Galápagos 1 % (Beltrán, 2015, p. 6).

Watermelon has been identified as a nutritious product for human consumption so it provides nutrients such as lycopene, it also provides few calories, vitamins and minerals so it is good for health (Sylvain and Enoch, 2019, p. 9).

Diseases in cucurbits are caused by microorganisms (fungi, bacteria, viruses, etc.) that are dispersed mainly through water, air, insects and seeds. These microorganisms need certain environmental conditions to develop and cause damage to plants. Among the main diseases affecting the Cucurbitaceae family are leaf spot (*Curvularia spp*), Fusarium (*Fusarium oxysporum*), Alternaria (*Alternaria cucumerina*) (González, et al., 2010).

One of the controls used to prevent fungal diseases is the use of natural compounds such as essential oils, which are acquired from different plant organs such as flowers, roots, leaves, stems, fruits and seeds, which have properties that are used to prevent or control the occurrence of diseases in plants. These can be obtained by pressure, fermentation, or extraction, but the most common method for commercial production is steam distillation (Mendoza, 2017).

One of the most important decisions in the phytosanitary management of the crop is the control of pests and diseases to reduce the incidence and severity of pathogens, monitoring and analysis. On the other hand, the analysis of statistical variables Analysis of Variance, Fisher's test and Tukey's comparative test is important for the rational and economic use of essential oils has acquired special importance within the technology for crop production. The present study will be carried out in ("SPSS Statistics - Overview | IBM," 2020), ("Herramientas estadísticas, de análisis de datos y de mejora de procesos | Minitab," 2020)

On the other hand, crop production is the result of a series of activities that transform inputs into performance. Therefore, a production function represents the amount of product that could be obtained using different doses or amounts of inputs, in this case oils for pathogen control. The most commonly used response functions to explain plant response to growth inhibitors: quadratic, cubic, Cobb Douglas, etc. Mention should also be made of the discontinuous rectilinear model approach, which also allows economic interpretations. However, the most commonly used are the quadratic or second degree functions, describing a curvilinear relationship between the expected production and the amount of essential oil applied.

## **Materials and methods**

For this type of study we will use linear regression, which is basically the simplest design and is used when the experimental units are homogeneous or when the variation between them is very small; such is the case of laboratory experiments, greenhouses, etc. where environmental conditions are controlled. This is a test with only one classification criterion (Segura, 2000)

Experimental design is the arrangement of experimental units used to control experimental error by adapting to the treatment. There are several arrangements in the literature aimed at controlling experimental error, and there is a natural tendency to design experiments based on existing designs. However, it is a more appropriate approach to develop an experimental design that meets the needs of the ongoing experiments.(Kuehl, 2001)

### **Independent Variable**

Evaluation of essential oils, with the concentrations of 100, 200, 300  $\mu$ L in each experimental unit (Pathogens).

### **Dependent Variable**

Response of watermelon crop to the application of essential oils.

### **Variables To Be Evaluated**

#### ***Mycelial growth of pathogens (%)***

It was performed with the help of a millimeter ruler, the diameter of the colonies was measured at intervals of 24 hours during 35 7 days. The formula for measuring mycelial inhibition growth according to Sandoval (2016), is:

$$PIC = \frac{(\text{Crec. del testigo} - \text{crec. del tratamiento})}{\text{crecimiento del testigo}} \times 100$$

### **Pathogenicity Tests:**

For these tests, 10 plants were inoculated for each pathogen (*Fusarium sp.* was inoculated by spraying directly to the soil, while *Curvularia sp.* and *Colletotrichum sp.* were inoculated by foliar spraying). These pathogenicity tests are effective and rapid since they facilitate the detection of the infectious agent.

### **Symptom Assessment (%):**

To determine the effect of diseases on plants, the percentage of severity was calculated using the formula according to (Barea, 2006).

$$\% = \frac{\text{área de tejido vegetal afectado}}{\text{total de tejidos}} \times 100$$

**Table 1. Treatments**

<b>N. Treatments</b>	<b>Oils (A)</b>	<b>Pathogens (B)</b>	<b>Dose( µL/ml) (C)</b>
1	<i>E. arvense</i>	<i>Fusarium sp.</i>	100 µL
2	<i>E. arvense</i>	<i>Fusarium sp.</i>	200 µL
3	<i>E. arvense</i>	<i>Fusarium sp.</i>	300 µL
4	<i>U. dioica L</i>	<i>Fusarium sp.</i>	100 µL
5	<i>U. dioica L</i>	<i>Fusarium sp.</i>	200 µL
6	<i>U. dioica L</i>	<i>Fusarium sp.</i>	300 µL
7	<i>E. arvense</i>	<i>Colletotrichum sp.</i>	100 µL
8	<i>E. arvense</i>	<i>Colletotrichum sp.</i>	200 µL
9	<i>E. arvense</i>	<i>Colletotrichum sp.</i>	300 µL
10	<i>U. dioica L</i>	<i>Colletotrichum sp.</i>	100 µL
11	<i>U. dioica L</i>	<i>Colletotrichum sp.</i>	200 µL
12	<i>U. dioica L</i>	<i>Colletotrichum sp.</i>	300 µL
13	<i>E. arvense</i>	<i>Curvularia sp</i>	100 µL
14	<i>E. arvense</i>	<i>Curvularia sp</i>	200 µL
15	<i>E. arvense</i>	<i>Curvularia sp</i>	300 µL
16	<i>U. dioica L</i>	<i>Curvularia sp</i>	100 µL
17	<i>U. dioica L</i>	<i>Curvularia sp</i>	200 µL
18	<i>U. dioica L</i>	<i>P. cubensis</i>	300 µL
19	Witness	No application	

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**Table 2.** ANDEVA analysis for laboratory

Source of variation	Formula	Development	g.l
<b>A (Essential oils)</b>	(A - 1)	(2 - 1)	1
<b>B (Dosage)</b>	(B - 1)	(3 - 1)	2
<b>C (Pathogenic)</b>	(C - 1)	(3 - 1)	2
<b>AXB</b>	(A - 1)(B - 1)	(2 - 1) (3 - 1)	2
<b>AXC</b>	(A - 1)(C - 1)	(2 - 1) (3 - 1)	2
<b>BXC</b>	(B - 1)(C - 1)	(3 - 1) (3 - 1)	4
<b>AXBXC</b>	(A - 1)(B - 1)(C - 1)	(2 - 1) (3 - 1)(3 - 1)	4
<b>Experimental error</b>	(T - 1) (R - 1)	(18 - 1) (3 - 1)	34
<b>Total</b>	(N - 1)	(54 - 1)	53

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For the seedling trial, a complete randomized design (CRD) will be used, consisting of 3 phytopathogens (treatments) and 10 plants for each pathogen, including the control (replicates).

**Table 3.** ANDEVA analysis scheme for testing

Source of variation	Formula	Development	g.l
<b>Treatments</b>	(T - 1)	(4 - 1)	3
<b>Experimental error</b>	T (R - 1)	4 (10 - 1)	36
<b>Total</b>	(T * R) - 1	(4 * 10) - 1	39

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## Result

Isolation and identification of phytopathogenic agents associated with the watermelon crop *Citrullus lanatus* L. Plant material (leaves, fruits, roots) was collected from established watermelon crops showing symptoms of phytopathogenic agents by cluster or area sampling. Samples were collected in the cantons of El Empalme, Balzar and Pedro Carbo. These samples were identified and isolated in the Phytopathology laboratory of the Agrarian University of Ecuador. The phytopathogenic agents found in the samples of vegetative material.

### Model Summary

S R<sup>2</sup> R<sup>2</sup>(adjusted) R<sup>2</sup> (prediction)

0.348807 99.84% 99.77% 99.64%

**Biological Optimum.** To estimate it, it is necessary to calculate the dose of oils in microliters to generate the maximum physical or biological production. In this case, we take the function obtained and find the first order derivative which is also equivalent to the marginal product of the function resulting in:

$$\% \text{Inhibition} = 30.6574 + 5.5352 \text{ Oils\_T1} + 3.7204 \text{ Dose\_300} + 1.7926 \text{ Pathogens\_Curvularia sp.} + 0.5870 \text{ Oils*Dose\_T1 300} - 0.1630 \text{ Oils*Pathogens\_T1 Curvularia sp.}$$

$$+ 1.2630 \text{ Dose*Dose*Pathogens\_300 Curvularia sp.} + 2.2407 \text{ Oils*Dose*Dose*Pathogens\_T1 300 Curvularia sp.}$$

### Evaluation of fungicide effectiveness on watermelon plants inoculated with pathogens.

To evaluate the effectiveness of the fungicide, watermelon plants of 7 weeks of age were used for the test, inoculating the pathogen and showing the symptoms of each disease to later apply the dosage with the best results in the laboratory, the formula was used to calculate the severity according to (Barea, 2006).

Severity of fungi (%) The data collected in the trial with watermelon seedlings of 7 weeks of age, the oil of *Urtica dioica* was applied especially with the dose in 300 49 microliters where it caused the results shown in table 10 which shows all averages, a coefficient of variation of 28.47% was obtained and according to the variance if significance was found, between treatment the highest average of severity category was obtained by the plant inoculated with *Fusarium* with 4, It should be noted that the watermelon variety used has a property of tolerance to the pathogen, while *Colletotrichum* sp with 2.7% equivalent on the severity scale has 25% of affectation, that is, it shows resistance to the pathogen since the genetic material implemented also shows tolerance to this disease. It should be noted

It should be noted that the laboratory test indicates that *Curvularia* sp has a percentage of 32.42 in PCIM, since the laboratory has controlled environmental conditions.

**Table 4. Percentage of severity in seedlings.**

Treatments	Stockings	n	E.E	
T1 ( <i>Fusarium</i> sp)	4.8	10	0.27	A
T2 ( <i>Colletotrichum</i> sp)	2.7	10	0.27	B
T3 ( <i>Curvularia</i> sp)	2.7	10	0.27	B
T4 (Witness)	1.9	10	0.27	B

Means with a common letter are not significantly different ( $p > 0.05$ ).

The present research work evaluated the in vitro effect of essential oils of *Equisetum arvense* and *Urtica dioica* for the management of phytopathogenic fungi in *Citrullus lanatus* L. Essential oils stand out for their multiple possibilities of application in agriculture, thus providing an environmentally friendly alternative, according to (Pino and Sanchez, 2011).

The study conducted by Mendoza (2017), showed that essential oils obtained from dehydrated leaves, have better results since the plant component is in excellent condition at the time of extracting the oil, it is a method currently used to control pests and diseases in order to minimize the incidence of diseases that occur daily in crops, For this reason, the extraction of essential oils as a treatment limits the use of agrochemicals, which bring with them the consequences of excessive use. For this reason, we agree with Hernández (2013), who indicates in a research he carried out, applying essential oils to control diseases and pests, since with this new alternative he seeks not to alter the environment.

After having carried out the analysis and interpretation of the mycelial inhibition growth data obtained using the PCIM formula presented by Sandoval (2016), thus determining that the highest averages were those of Treatment 1 (*Urtica dioica*) with 36.19%, since the nettle *Urtica dioica* contains properties among them is silicon which is used as a fungicide since it possesses beneficial properties that can be used in organic farming and to the defense of plants, so it agrees with Abad (2011), 51 in a research where it indicates the properties that Nettle possesses as a product or fungicidal substance.

The percentage of severity was also evaluated in watermelon seedlings of 6 weeks of age, where it could be deduced that essential oils do have fungicidal effect as indicated by Flores (2017), in a research where he applied essential oils to control anthracnose in cucurbits. Inoculations of isolated diseases were carried out on seedlings, and the essential oil *U. dioica* was applied, which resulted in a higher inhibitory percentage, thus agreeing with the study conducted by Duarte (2013), where he mentions the use of oils to combat *Fusarium* in inoculated seedlings. According to the research of the aforementioned authors, the hypothesis that essential oils reduce the incidence of phytopathogenic fungi in watermelon *Citrullus lanatus* is accepted.

## Conclusions

Once the in vitro experimental research project was completed, where essential oils of *Equisetum arvense* and *Urtica dioica* were evaluated in different concentrations, after the analysis study it was concluded that when evaluating the inhibitory percentage on phytopathogenic fungi found in El Empalme, Balzar and Pedro Carbo, the pathogens *Colletotrichum sp*, *Fusarium sp*, *Curvularia sp*.

It was determined that the essential oil of *U. dioica* obtained the highest percentage of inhibition of mycelial growth with 36.19% with a dose of 300 microliters, these doses were evaluated on phytopathogenic fungi (*Fusarium sp*, *Colletotrichum sp*, *Curvularia sp*), which resulted that *Curvularia sp* has a percentage of 32.45%, pathogen that was more susceptible to the treatment and the lowest average is *Colletotrichum sp* with 28.21%. It was also evidenced that when evaluating the severity, pathogenicity tests were carried out on watermelon seedlings of 7 weeks of age, which were affected with the pathogens found, statistically it was found that *Curvularia sp* together with *Colletotrichum sp* are resistant to the pathogen, while *Fusarium sp* was considerably affected with 25% of affectation according to the scale proposed, therefore it is concluded that the use of essential oils does reduce the incidence of phytopathogenic fungi in the watermelon crop *Citrullus lanatus*.

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