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Determinación de la actividad antimicótica de los aceites esenciales de eucalipto, molle y romero sobre (*Fusarium sp*; *Pythium sp*; *Rhizoctonia sp y Sclerotium sp.*), agentes causales del mal de almacigo en condiciones de laboratorio

*Determination of the antifungal activity of the essential oils of Eucalyptus, Molle and Rosemary on (Fusarium sp; Pythium sp; Rhizoctonia sp and y Sclerotium sp.),*causal agents of almacigo disease under laboratory conditions

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Palabras claves:
Agente patógeno, crecimiento radial, extracción de aceites, determinación antifúngica, capacidad de inhibición, dosis eficaz 50%.

Resumen

Introducción: La proliferación de un gran número de hongos, enfermedades y plagas, a causa del desequilibrio de la micostasis del suelo, se debe principalmente al uso irracional de pesticidas, fungicidas y al empleo de prácticas agronómicas ineficientes, como el monocultivo, siembras tradicionales y la deforestación. Factores que han causado serios problemas en el equilibrio ambiental y la salud humana. Una de las posibles alternativas para regular este desequilibrio, es la aplicación los extractos de origen vegetal para el control de los hongos, plagas y enfermedades en los cultivos. **Objetivos:** "El objetivo de esta investigación fue determinar la actividad antimicótica y la capacidad de inhibición del proceso de extracción de los aceites esenciales de Eucalipto, molle y romero, sobre los hongos (Fusarium sp. Pythium sp. Rhizoctonia sp. y Sclerotium sp), agentes causales del mal de almacigo Damping off. Aplicando la **Metodología:** de aislamiento, análisis de varianza, Diseño Completamente al Azar (DCA)", separación de medias y prueba de Tukey al 5%, Determinación de dosis eficaz 50, Capacidad de inhibición, Análisis de regresión lineal; obteniendo los siguientes. **Resultados:** "Los hongos en estudio son de lento crecimiento, ningún patógeno pudo poblar la superficie de la caja Petri desde las 24 a las 120 horas, presentándose el ritmo de crecimiento radial en el siguiente orden: *Pythium sp.* con 32mm, *Rhizoctonia sp.* con 30.82 mm, *Fusarium sp.* con 23.42mm y finalmente *Sclerotium sp con* 19 mm. Los mejores tratamientos fueron los destilados de Eucalipto, molle y romero y la fracción del agua residual de Romero que lograron inhibir el 91,46%; 93,48% y 94.26% sobre Fusarium Sp., Phytium Sp., Rhizoctonia sp. y Sclerotium sp. respectivamente. **Conclusiones:** "La utilización de los aceites esenciales son eficientes para el control de plagas, por lo que haciendo una comparación con el fungicida Benomil mediante la determinación de la dosis eficaz 50% es recomendable utilizar los extractos y destilados para no causar problemas de salud, y afectaciones ambientales. **Área de estudio general:** Fitopatología. **Área de estudio Específico:** Actividad Antifúngica.

Keywords:
Pathogenic agent; Radial growth; Oil

Abstract

Introduction: The proliferation of a large number of fungi, diseases, and pests, due to the imbalance of soil mycostasis, is due to the irrational use of pesticides, fungicides, and the use of

extraction; Antifungal determination; Inhibition capacity; Effective dose 50%.

inefficient agronomic practices, such as monoculture, traditional sowing, and deforestation. Factors that have caused serious problems in environmental balance and human health. One of the possible alternatives to regulate this imbalance is the application of extracts of plant origin to control fungi, pests, and diseases in crops. Objectives: "The objective of this research was to determine the antifungal activity and the inhibition capacity of the extraction process of the essential oils of Eucalyptus, molle and rosemary, on fungi (Fusarium sp. Pythium sp. Rhizoctonia sp. and Sclerotium sp.) , causal agents of almacigo disease Damping off. Applying the Methodology: isolation, analysis of variance, Completely Randomized Design (DCA), separation of means and 5% Tukey test, Determination of effective dose 50, Inhibition capacity, Linear regression analysis; obtaining the following. Results: "The fungi under study are slow growing, no pathogen was able to populate the surface of the Petri dish from 24 to 120 hours, the radial growth rate being presented in the following order: Pythium sp. with 32mm, Rhizoctonia sp. with 30.82 mm, Fusarium sp. with 23.42mm and finally Sclerotium sp with 19 mm. The best treatments were the distillates of Eucalyptus, Molle and Rosemary and the wastewater fraction of Rosemary, which managed to inhibit 91.46%; 93.48% and 94.26% on Fusarium Sp., Phytium Sp., Rhizoctonia sp. and Sclerotium sp. respectively. Conclusions: "The use of essential oils is efficient for pest control, so making a comparison with the Benomyl fungicide by determining the effective dose of 50%, it is advisable to use extracts and distillates to avoid causing health problems. and environmental effects. General study area: Phytopathology. Specific study area: Antifungal Activity.

Introduction

"Essential oils or essences are odoriferous substances that are stored in small quantities in the bark, roots, leaves, seeds of wild plants, shrubs and forest trees" (Gonzales, 2022, p. 19). "Each oil has a different composition and aromatic property, and also presents a variation in the intensity of its tonality, presenting a range of different colors; they have antibiotic, antiseptic, anti-inflammatory, antiviral and antifungal properties to a greater or lesser degree" (Valle et al., 2023, p. 82). "Limitations in the use of antibiotics in public health are promoting the search for new antimicrobial compounds. Essential oils are a

natural source of active compounds and represent an effective alternative as antibiotics to treat bacterial infections" (García & Latorre, 2018, p. 15).

In Ecuador, no study has been carried out on the industrial use of essential oils, they have not been exploited or tested against various types of fungal or bacterial populations, "as potential insecticides and as ecological alternatives in pest control processes" (Montenegro, 2022, p. 3). "The most widely used method for the control of pests and diseases of different crops is chemical control, whose main disadvantage is producing high residual toxicity in consumer products and also the deterioration of the soil due to the decrease in beneficial microorganisms. The present research describes the antifungal activity of the essential oils of the forest species: Eucalyptus (Eucaliptus Glóbulos); Molle (Schinus molle) and the shrub species: Rosemary (Rosmarinus Oficinalis), on the pathogens that cause damping off. "By applying the technique of isolation, reproduction, mycelial development and sporulation of fungi of the Fusarium moniliforme genus, on which the antifungal activity of the active components of essential oils (eugenol, cinnamic aldehyde, thymol, linalool and cineole) will be determined, mixed with PDA, it will be verified whether or not there is inhibition, both the mycelium and the sporulation" (Barrera & García, 2008), it will be determined whether or not it is an alternative for the antifungal combat against pathogens that produce mastic disease or Damping off, for which the following objectives were raised:

Goals

To determine the antifungal activity of essential oils of Eucalyptus, molle and rosemary on the causal agents of mastitis under laboratory conditions, considering the following specific objectives:

1. To measure the radial growth rate of the four strains of the pathogens: Fusarium sp., Rhizoctonia sp., Pythium sp. and Sclerotium sp.

2. To determine the antifungal activity and inhibition capacity of essential oils of Eucalyptus, molle and rosemary on: Fusarium sp., Rhizoctonia sp., Pythium sp. and Sclerotium sp.

Methodology

Isolation of pathogens

- Collection of diseased vegetable specimens (potatoes, lettuce, babaco, etc.)
- select the parts of the affected plant.
- Cleaning with running tap water for 10 minutes
- Disinfection with 5% sodium hypochlorite for 3 minutes, cleaning three times with sterile distilled water.

- Cutting smaller tissue sections and entering the isolation chamber.
- Plate tissue sections in Petri dishes with 15 ml of PDA until colonies appear, incubating them at 25 + 1oC.
- Reseed a portion of young mycelium of each pathogen (Fusarium sp., Rhizoctonia sp., Pythium sp. and Sclerotium sp.), in Petri dishes with PDA, incubating them at 25 + 1oC.

Radial growth rate

- Inoculation of reactivated pathogen strains (Fusarium sp., Rhizoctonia sp., Pythium sp. and Sclerotium sp.) in Petri dishes containing 15 ml of PDA at pH 5.
- Growth measurements were assessed at 12-hour intervals for 120 hours, with each test being recorded for three replicates.
- The radial growth of the different concentrations and the control was measured.
- The inhibition capacity of fungi was calculated using the formula below:

$$\text{CAP. INHIB} = \frac{\text{mm growth of solution dose}}{\text{mm of normal growth}} \times 100 \quad (1)$$

Determination of the antifungal activity of oils (Eucalyptus, Molle, Rosemary)

- Obtain essential oils of Eucalyptus, Molle, Rosemary through the steam extraction technique, using 600 gr of fresh leaves and 3000 ml of water, for each extraction respectively.
- Obtain 5 distillate fractions of 400 ml; fractions 1,3,5, and the residual water, with which the solutions designated as D1, D3, D5, AR and the control with no distillate fraction were prepared.
- "Each of the Petri dishes was inoculated with the reactivated pathogen strains (Fusarium sp., Rhizoctonia sp., Pythium sp. and Sclerotium sp.) on the PDA medium with the corresponding fractions D1, D3, D5, AR of the distillates of Eucalyptus, molle and rosemary respectively" (Valle, 2003, p. 23).

Determination of the effective dose 50 of a fungicide

- For this determination, the fungicide Benomyl was applied at concentrations of 125, 250, 500, 750, 1000 ppm corresponding to solutions A, B, C, D, E, F. Each mixture was distributed in 12 Petri dishes on which rings of 4 mm diameter strains of (Fusarium sp., Rhizoctonia sp., Pythium sp. and Sclerotium sp.) were inoculated; having as an absolute control a Petri dish without any fungicide for each pathogen under study.

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- The radial growth of the mycelium was subsequently evaluated at different concentrations, as well as in the case of the control, every three days over a period of nine days.
- The effective dose 50 was calculated, which indicates the level and capacity to inhibit pathogenic fungi by 50%.

Treatments under study

Table 1

Treatment and dosage table

ESSENTIAL OIL	Eucalyptus				Molle				Rosemary			
Treatments	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0
Dose	D1	D2	D3	AR	D1	D2	D3	AR	D1	D2	D3	AR
Pathogen	*Fusarium sp.*				*Fusarium sp.*				*Fusarium sp.*			
Treatments	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0
Dose	D1	D2	D3	AR	D1	D2	D3	AR	D1	D2	D3	AR
Pathogen	*Phytium. Sp.*				*Phytium. Sp.*				*Phytium. Sp.*			
Treatments	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0
Dose	D1	D2	D3	AR	D1	D2	D3	AR	D1	D2	D3	AR
Pathogen	*Rhizoctonia. Sp.*				*Rhizoctonia. Sp.*				*Rhizoctonia. Sp.*			
Treatments	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0
Dose	D1	D2	D3	AR	D1	D2	D3	AR	D1	D2	D3	AR
Pathogen	*Sclerotium. Sp.*				*Sclerotium. Sp.*				*Sclerotium. Sp.*			

Fountain: Valley (2003)

Study factors

For this research the following study factors were considered

- Fractions 1,3,5 and a fraction of AR (waste water) from the distillates of the extraction of essential oils of Eucalyptus (Eucalyptus Globulus); Molle (Schinus Molle) and Rosemary (Rosmarinus Oficinalis).
- The 4 pathogenic agents: Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.
- Benomyl chemical fungicide, dosage in ppm.

Experimental design

Radial growth

"To determine the "In Vitro" radial growth of pathogens, the experimental method Completely Randomized Design (CRD) was used. With four treatments and three repetitions" (Valle, 2003, p. 20).

Antifungal Activity Tests

- "It was determined by a completely randomized design (DCA) with sixty treatments and three repetitions according to table 1, table of treatments and doses, considering treatments to fractions D1, D3, D5 and a fraction of residual water from the extraction of essential oils of Eucalyptus Molle and Rosemary, against pathogens (Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.) and an absolute witness.
- For the effective dose test 50, six doses were used (1125,250,500,600,750,1000 ppm) with three repetitions and an absolute witness without substance" (Valle, 2003, p. 24).

Functional analysis

- Variance analyses (ADEVA or ANOVA) were used, with a completely randomized design for all treatments applied, for the variable antifungal activity. If there were highly significant differences.

Table 2

Variance analysis scheme (ADEVA) for radial growth tests and antifungal determination

ADEVA For Radial Growth		ADVAFor antifungal determination (Eucalyptus, Molle, Rosemary)
SOURCES OF VARIATION	gl	gl
Treatments	3	59
Repetitions	2	2
Mistake	6	118
TOTAL	11	179

Fountain: Valley (2003)

Regression analysis

"A linear regression analysis was performed to determine the in vitro growth of phytopathogenic fungi against Benomyl concentrations" (Pesántez, 2000).

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Separation of stockings

"A mean separation test was performed using Tukey at 5% to determine the ranges and differences between means of the applied treatments" (Pesántez, 2000).

Results

Radial growth rate

"The analysis of variance of the radial growth presented highly significant differences both at 24H00 and at 120H00, obtaining the following average results: 3.58 and 23.42 mm for Fusarium sp; 4.83 and 32 mm for Phytium sp; 4.50 and 30.82 mm for Rhizoctonia sp; 5.75 and 19 mm for Sclerotium sp.; the coefficients of variation for the pathogens were: 11.88% and 3.93% respectively" (Valle, 2003, p. 38).

Table 3

Analysis of variance for radial growth of: Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp. at 24 and 120 hours

Sources of variation	GL	Sum of Squares		Sum of Mean Squares		F. Calculated	
		24H00	120H00	24H00	120H00	24H00	120H00
Treatments	3	7,208	488,432	2,403	162,811	7,819**	141,446**
Mistake	8	2,450	9,208	0.307	1,151		
TOTAL	11	9,666	497,641				

Fountain: Valley (2003)

Table 4

Tukey test at 5% for radial growth of: Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp. at 24 and 120 hours

Radial growth rate	24H00			120H00	
Pathogen	Socks	Levels	Pathogen	Socks	Levels
Sclerotium sp.	5.75	TO	*Phytium sp.*	32,00	TO
Phytium sp.	4,833	AB	*Rhizoctonia sp.*	30.82	B
Rhizoctonia sp.	4.5	AB	*Fusarium sp.*	23.42	C
Fusarium sp.	3,583	B	*Sclerotium sp.*	19.00	D

Fountain: Valley (2003)

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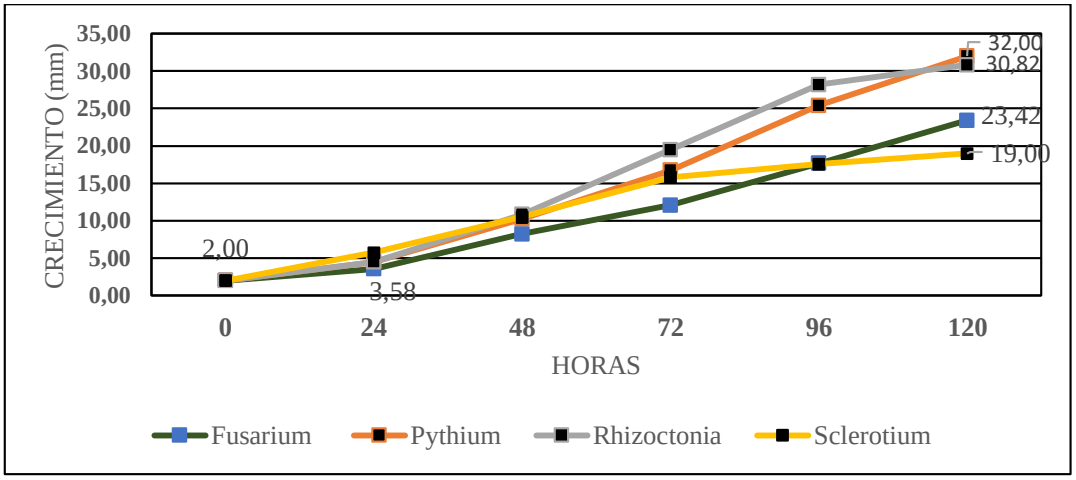
Figure 1

Tukey 5% Test Chart

Radial growth Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.



Fountain: Valley (2003)

"Considering the results of the fungal growth under study, it can be observed that each pathogen has a different radial growth; as shown in table 4, the growth of Phytium sp. at 120 hours reached an average of 32mm; similar to the study by Benavides (2001), who reports that Phytium sp. presents an average in vitro growth of 3.56 mm per day, and that at 120 hours it covered 31.06% of the Petri dish. The Fusarium sp. fungus is slow growing, reaching 23.42mm of the Petri dish, a result that differs from the research carried out by Gallegos (2016), in whose study it reached colonizing 45mm in 36H00" (Arcos, 2017).

"The Rhizoctonia fungus had a radial growth of 30.42 mm at 120 H00, being a slow-growing fungus, a result that agrees with the results reported by Arcos (2017), who obtained 35.75 mm at 120 hours, and differs with the study carried out by RIVAS (1994) who presented 74.44 mm at 120 hours and Benavides who indicates that Rhizoctonia has an aggressive growth" (Arcos, 2017).

"The pathogen Sclerotium sp. reached a radial growth of 19 mm at 120:00, also considered a slow-growing fungus, a result that differs from Gallegos (2016), who reports aggressive growth colonizing the box 100% in 60 hours" (Arcos, 2017).

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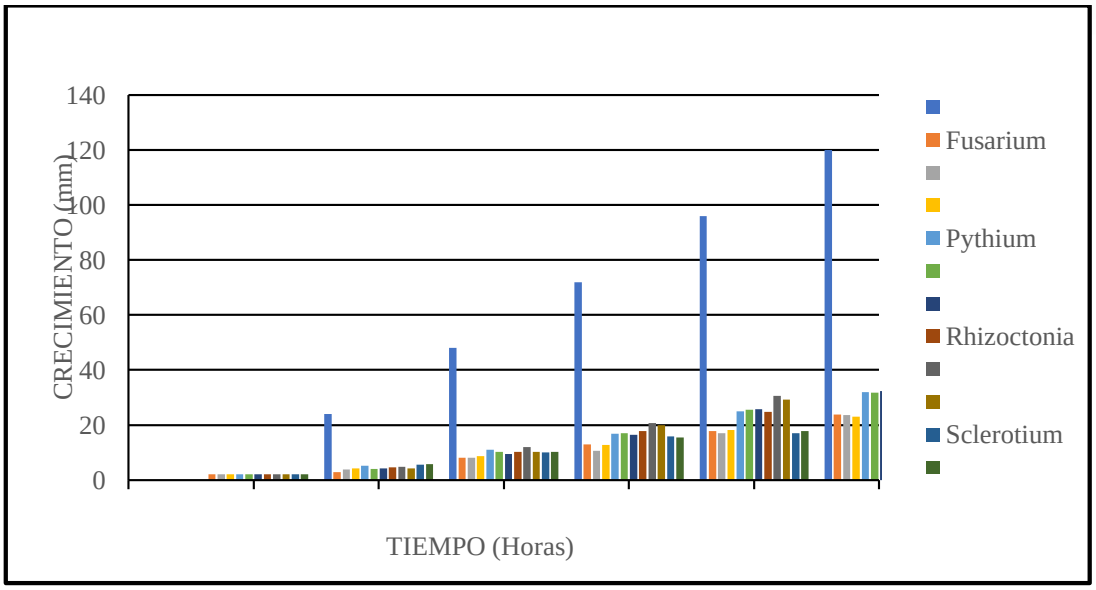
Figure 2

Radial growth rate of*Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp*.



Fountain: Valley (2003)

"Considering the results of the fungi under study, it was observed that each pathogen has a different radial growth, with marked differences; thus, "first of all, the fungus Phytium sp. had a rapid growth rate of 6.4 mm every 24 hours, reaching a radial growth of 32 mm at 120 hours" (Valle, 2003, p. 41).

"In second place, followed by Fusarium sp. With a growth rate of 6.16 mm every 24 hours, reaching a radial growth of 30.83 mm at 120 hours" (Valle, 2003, p. 29).

"In third place, followed by Fusarium sp. With a growth rate of 4.69 mm every 24 hours reaching a radial growth of 23.47 mm at 120 hours" (Valle, 2003, p. 30).

"Finally, the Sclerotium fungus, with a slow growth rate of 3.8 mm every 24 hours, barely reaching a growth rate of 19 mm after 120 hours" (Valle, 2003, p. 31).

Antifungal determination of the extraction process of essential oils of Eucalyptus, molle and rosemary on. Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.

The results of the analysis of variance of the antifungal activity of the extraction process of the essential oils of Eucalyptus, molle and rosemary on Fusarium sp. Pythium sp. Rhizoctonia sp. Sclerotium sp. were the following:

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Table 5

Analysis of variance for antifungal determination. From the extraction process of essential oils of Eucalyptus, molle and rosemary on: Fusarium sp. Pythium sp. Rhizoctonia sp. Sclerotium sp.

Name of the mushroom	Incubation hours	Analysis of Variance Fc.	Description	Coefficient of Variation
Fusarium SP.	24	12,893**	Highly significant	9.80%
	72	107,563**	Highly significant	11.90%
	120	442,820**	Highly significant	6.38%
Pythium sp.	24	28,073**	Highly significant	12.31%
	72	970,232**	Highly significant	4.68%
	120	2446,345**	Highly significant	3.44%
Rhizoctonia sp	24	300,00**	Highly significant	3.16%
	72	211,014**	Highly significant	15.86%
	120	549,922**	Highly significant	10.82%
Sclerotium sp.	24	77,071**	Highly significant	6.10%
	72	538,623**	Highly significant	7.07%
	120	335,595**	Highly significant	7.94%

Fountain: Valley (2003)

The results obtained from the 5% Tukey test at 120 hours of the antifungal activity of the extraction process of the essential oils of Eucalyptus, molle and rosemary, on the pathogens: Fusarium sp., Pythiumsp., Rhizoctonia sp., Sclerotium sp.It presents several ranges of significance. “The treatments with level A are those with high radial growth, which corresponds to the test witnesses and the treatments that are in levels B, C, D, E, F, H, I are the treatments of the distillation fractions of the essential oils that presented a medium antifungal capacity, while the treatments J, G, E, and F were presented as the best treatments for the growth control of the pathogens Fusarium sp., Pythium, Rhizoctonia and Sclerotium, resulting in a high antifungal capacity with a growth of 2 mm” (Valle, 2003, p. 38).

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Table 6

5% TUKEY test for the antifungal determination of the essential oil extraction process on pathogens: Fusarium sp., Pythium sp., Rhizoctonia sp. and Sclerotium sp.

Tukey test at 5% for Fusarium sp. 120 hours

TREATMENTS No	CODES	Average (mm)	LEVELS
13	Witness	23,420	TO
11	D3-Ro-Fus	15,750	B
4	Ar-Eu-Fus	14,580	C
10	D2-Ro-Fus	8,917	D
8	Ar-Mo-Fus	8,500	AND
3	D5-Eu-Fus	7,833	F
7	D3-Mo-Fus	8,500	G
6	D2-Mo-Fus	7,583	H
5	D1-Mo-Fus	6,750	Yo
9	D1-Ro-Fus	2,000	J
2	D3-Eu-Fus	2,000	J
1	D1-Eu-Fus	2,000	J
12	Ar-Ro-Fus	2,000	J

Tukey test at 5% for Pythium sp. 120 Hours

TREATMENTS No	CODES	Average (mm)	LEVELS
13	Witness	31,670	TO
11	D5-Ro-Pyth	18,670	B
8	Ar-Mo-Pyth	11,580	C
4	Ar-EU-Pyth	10,500	D
7	D5-Mo-Pyth	10,250	D
10	D3-Ro-Pyth	8,083	AND
3	D5-EU-Pyth	6,500	F
6	D3-Mo-Pyth	5,917	F
2	D3-EU-Pyth	2,000	G
9	DI-Ro-Pyth	2,000	G
5	DI-Mo-Pyth	2,000	G
1	DI-EU-Pyth	2,000	G
12	Ar-Ro-Pyth	2,000	G

Tukey test at 5% for Rhizoctonia sp. 120 Hours

TREATMENTS No	CODES	Average (mm)	LEVELS
13	Witness	34.83	TO
8	Ar-Mo-Rhizo	17,330	B
11	D5-Ro-Rhizo	10,920	C
7	D5-Mo-Rhizo	4,833	D
1	DI-EU-Rhizo	2,000	AND
2	D3-EU-Rhizo	2,000	AND
3	D5-EU-Rhizo	2,000	AND
4	Ar-EU-Rhizo	2,000	AND
5	DI-Mo-Rhizo	2,000	AND
6	D3-Mo-Rhizo	2,000	AND
9	DI-Ro-Rhizo	2,000	AND
10	D3-Ro-Rhizo	2,000	AND
12	Ar-Ro-Rhizo	2,000	AND

Tukey test at 5% for Sclerotium sp. 120 Hours

TREATMENTS No	CODES	Average (mm)	LEVELS
13	Witness	13.00	TO
8	Ar-Mo-Scler	14.33	B
11	D5-Ro-Scler	13.00	BC
4	Ar-EU-Scler	11.50	C
3	D5-EU-Scler	6,583	D
5	DI-Mo-Scler	5,750	D
6	D3-Mo-Scler	6,333	D
7	D5-Mo-Scler	7,167	D
10	D3-Ro-Scler	4.00	AND
1	DI-EU-Scler	2.00	F
2	D3-EU-Scler	2.00	F
9	DI-Ro-Scler	2.00	F
12	Ar-Ro-Scler	2.00	F

Fountain: Valley (2003)

Inhibition capacity

To calculate the fungus inhibition capacity, the following formula was applied:

CAP. INHIB= Growth using the solution dose (mm) x 100 (2)

Normal growth (mm)

Table 7

Inhibitory capacity of the extraction process of essential oils of Eucalyptus, molle and rosemary on. Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.

Tukey test at 5% for Fusarium sp. 120 hours

No	Treatments Codes	Growth with a solution (mm)	Growth without solution (mm)	Capacity of Inhibition
1	D1-Eu-Fus	2,000	23.42	91.46%
2	D3-Eu-Fus	2,000	23.42	91.46%
9	D1-Ro-Fus	2,000	23.42	91.46%
12	Ar-Ro-Fus	2,000	23.42	91.46%
5	D1-Mo-Fus	6,750	23.42	74.38%
6	D2-Mo-Fus	7,583	23.42	71.18%
7	D3-Mo-Fus	8,500	23.42	67.63%
3	D5-Eu-Fus	7,833	23.42	66.57%
8	Ar-Mo-Fus	8,500	23.42	63.71%
10	D2-Ro-Fus	8,917	23.42	61.91%
4	Ar-Eu-Fus	14,580	23.42	37.75%
11	D3-Ro-Fus	15,750	23.42	32.75%

Tukey test at 5% for Pythium sp. 120 Hours

No	Treatments Codes	Growth with a solution (mm)	Growth without solution (mm)	Capacity of Inhibition
1	DI-EU-Pyth	2,000	31.67	93.68%
2	D3-EU-Pyth	2,000	31.67	93.68%
5	DI-Mo-Pyth	2,000	31.67	93.68%
9	DI-Ro-Pyth	2,000	31.67	93.68%
12	Ar-Ro-Pyth	2,000	31.67	93.68%
6	D3-Mo-Pyth	5,917	31.67	81.31%
3	D5-EU-Pyth	6,500	31.67	79.48%
10	D3-Ro-Pyth	8,083	31.67	74.49%
7	D5-Mo-Pyth	10,250	31.67	67.63%
4	Ar-EU-Pyth	10,500	31.67	66.85%
8	Ar-Mo-Pyth	11,580	31.67	63.44%
11	D5-Ro-Pyth	18,670	31.67	41.05%

Tukey test at 5% for Rhizoctonia sp. 120 Hours

No	TREATMENTS CODES	Growth with a solution (mm)	Growth without solution (mm)	CAPACITY OF INHIBITION
1	DI-EU-Rhizo	2,000	34,830	94.26%
2	D3-EU-Rhizo	2,000	34,830	94.26%

Tukey test at 5% for Sclerotium sp. 120 Hours

No	TREATMENTS CODES	Growth with a solution (mm)	Growth without solution (mm)	CAPACITY OF INHIBITION
1	DI-EU-Scler	2.00	19	89.87%
2	D3-EU-Scler	2.00	19	89.87%

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Table 7

Inhibition capacity of the extraction process of essential oils of Eucalyptus, molle and rosemary on. Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp. (continued)

Tukey test at 5% for Rhizoctonia sp. 120 Hours				
TREATMENTS		Growth with a solution	Growth without solution	CAPACITY OF
No	CODES	(mm)	(mm)	INHIBITION
3	D5-EU-Rhizo	2,000	34,830	94.26%
4	Ar-EU-Rhizo	2,000	34,830	94.26%
5	DI-Mo-Rhizo	2,000	34,830	94.26%
6	D3-Mo-Rhizo	2,000	34,830	94.26%
9	DI-Ro-Rhizo	2,000	34,830	94.26%
10	D3-Ro-Rhizo	2,000	34,830	94.26%
12	Ar-Ro-Rhizo	2,000	34,830	94.26%
7	D5-Mo-Rhizo	4,833	34,830	86.13%
11	D5-Ro-Rhizo	10,920	34,830	68.68%
8	Ar-Mo-Rhizo	17,330	34,830	50.24%

Tukey test at 5% for Sclerotium sp. 120 Hours				
TREATMENTS		Growth with a solution	Growth without solution	CAPACITY OF
No	CODES	(mm)	(mm)	INHIBITION
9	DI-Ro-Scler	2.00	19	89.47%
12	Ar-Ro-Scler	2.00	19	89.47%
10	D3-Ro-Scler	4.00	19	78.94%
5	DI-Mo-Scler	5,750	19	69.74%
6	D3-Mo-Scler	6,333	19	66.68%
3	D5-EU-Scler	6,583	19	65.37%
7	D5-Mo-Scler	7,167	19	62.32%
4	Ar-EU-Scler	11.50	19	39.47%
11	D5-Ro-Scler	13.00	19	31.58%
8	Ar-Mo-Scler	14.33	19	24.58%

Fountain: Valley (2003)

"The data obtained from the inhibition capacity of the extraction process of essential oils of eucalyptus, molle and rosemary on Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp. the treatments that have the greatest inhibition capacity Based on the relationship of radial growth to the dose of the solution (DI, D3, D5) on radial growth without using the treatment dose (Control)" (Valle, 2003, p. 52). "The best treatments to combat the pathogens under study are those that presented a high percentage equal to: 91.46%, 93.68%, 94.36%, 89.47%. That is, the distillation fractions of the essential oils of Eucalyptus, molle and rosemary; Also considering that the treatments that have a strong inhibition capacity are the treatments that present an intermediate percentage equal to or greater than 60%, that is, the residual water (Ar) of the essential oils from the extraction of the essential oils of Eucalyptus, molle and rosemary" (Valle, 2003, p. 54).

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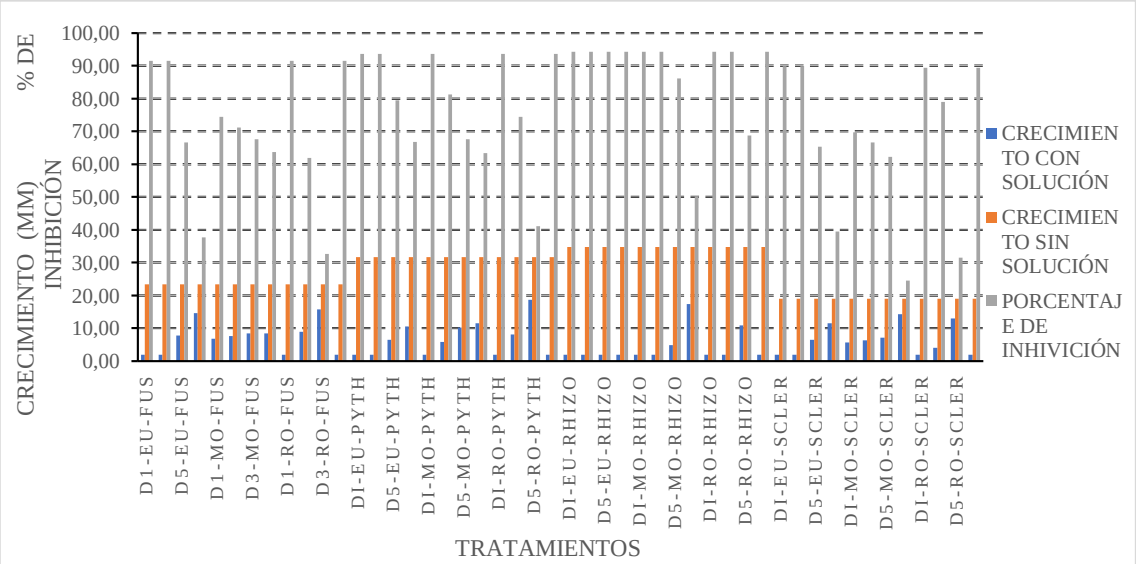
Figure 3

Inhibitory capacity of eucalyptus molle and rosemary on: fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.



Fountain: Valley (2003)

"The results obtained in this research agree with the studies carried out with the inhibition of Fusarium moniliforme, through the application of vegetable powders and some of its chemical components (Cineol, Linalool, Eugeniol, Aldehyde), existing inhibition both in the mycelium and sporulation with the following species: Eucalyptus, Rosemary, Guava, Walnut. The active ingredients such as eugeniol, aldehyde and thymol caused total inhibition in the development of the mycelium" (Bravo et al., 2000, p. 29).

According to the results obtained, the most effective and active treatments that have a high inhibition capacity are: D1-Eu, D3-Eu, D1-Ro, Ar-Ro, D1-Mo. "The essential oils of the forest species of Eucalyptus, molle and rosemary, are made up of a high content of Cineole, Eugeniol, aldehydes, alcoholic and ketone compounds" (Bautista & Leiva, 2019), which have antifungal, antiseptic, antibacterial properties, for this reason that essential oils have managed to effectively inhibit the growth of pathogens (Fusarium sp, Pythium sp, Rhizoctonia sp. Sclerotium sp.) that cause mastic disease or Damping off "(Valle, 2003, p. 50).

"The treatments of molle and rosemary show that there is a marked difference in the inhibition capacity between fractions D1, D3 and D5, "which proves that the oils in their chemical composition have highly volatile components" (Véliz-Jaime et al, 2019, p. 208), and are easily distilled in the first fractions, this is possibly due to the structure of the leaves and seeds (Valle, 2003).

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"However, we can observe that the residual water from the treatments: Ar-Mo and Ar-Ro, has an inhibitory capacity, with the presence of polar compounds, such as flavonoids, tannins, phenolic and glycosidic compounds. The inhibitory capacity of the Romero distillate is due to the presence of diterpenes, tricyclics, Rosmari, diphenol rosmarol, rosmarine acid and likewise in the residual water there is the presence of αPinene, 12-25% camphor, 15-30% borneol" (Valle, 2003, p. 54).

Determination of the effectiveness of the fungicide Benomyl on: Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.

For the determination of the capacity to inhibit the pathogens causing mastic disease (Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.), linear regression analysis was used, relating the application of Benomyl doses of 125, 250, 375, 500, 625, 750, 875, 1000 ppm on the radial growth of Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.

Table 8

Table of inhibition capacity of effective dose 50 of Benomyl on Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.

Pathogen	Normal Radial Growth (mm)	Growth 50% (Mm)	Effective Dose 50 (Ppm)
Fusarium sp.	24.75	12.38	558.57
Pythium sp.	43.11	21.55	531.41
Rhizoctonia sp.	36.75	18.38	543.08
Sclerotium sp.	27.41	13.71	548.92

Fountain: Valley (2003)

Benomyl on Fusarium Sp.

- The results of the inhibition capacity of the fungicide Benomyl on Fusarium sp., showed that the relationship that existed between growth vs. concentration was inversely proportional, whose equation was Y = 24.641-0.2016X with an r = 0.99878. The result of the effective dose 50 for the fungus Fusarium sp. was 558.50 ppm, which corresponds to 12.37 mm, "framed within the commercial recommendation of 500 to 1000 ppm" (Vega & Granados, 2023, p. 485). The results obtained in the present investigation differ from those obtained by (Pesántez, 2000, p. 47), by 16.38%, their recommendation being the use of 668 ppm in PDA, this is because this investigation tested other pathogens in other culture media.

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Benomyl on Pythium sp.

- The inhibition capacity of Benomyl on Pythium sp. has shown that the most concentrated doses effectively inhibit the growth of the fungus, that is, the relationship that existed between growth and concentration was inversely proportional, whose equation was Y = 42.135-0.039X with r = 0.987. corresponding to the value obtained of 531.407 ppm, corresponding to 321.55 mm of radial growth which constitutes 50% of the growth of the fungus; "a dose that is within the framework of what is recommended by the commercial house, which is 500 to 1000 ppm" (Vega & Granados, 2023, p. 489).

Benomyl on Rhizoctonia sp.

- The inhibition capacity of Benomyl on the fungus Rhizoctonia sp. according to linear regression, the most concentrated doses are those that have an efficient inhibition on the fungus according to the data obtained; The relationship between growth vs. concentration was inversely proportional, through the equation Y = 36.501-0.0335 X with an r = 0.992, it was shown that the effective dose 50 for the inhibition capacity of the fungicide Benomyl on Rhizoctonia sp. Was 543.08 ppm. Which corresponds to 18.37 mm, which constitutes 50% of the growth of the fungus, a parameter that is framed in the recommendation of the commercial house that is 500 to 1000 ppm.

- *Benomyl on Sclerotium sp.*

  The results obtained from the regression analysis of the inhibition capacity of the fungicide Benomyl on the fungus Sclerotium sp. showed that doses with high concentrations are those that present the greatest inhibition.

  "The relationship that existed between growth vs. concentration was inversely proportional, whose equation was Y=19.498 - 0.0188X with an r = 0.9641; the effective dose 50 for Benomyl on Sclerotium sp. was 548.92 ppm, which corresponded to 13.70 m, constituting 50% of the radial growth of the fungus, falling within the parameters recommended by the commercial house, which is 500 to 1000 ppm" (Valle, 2003, p. 55).

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Table 9

Dose of the effective dose 50 fungicide Benomyl on fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.

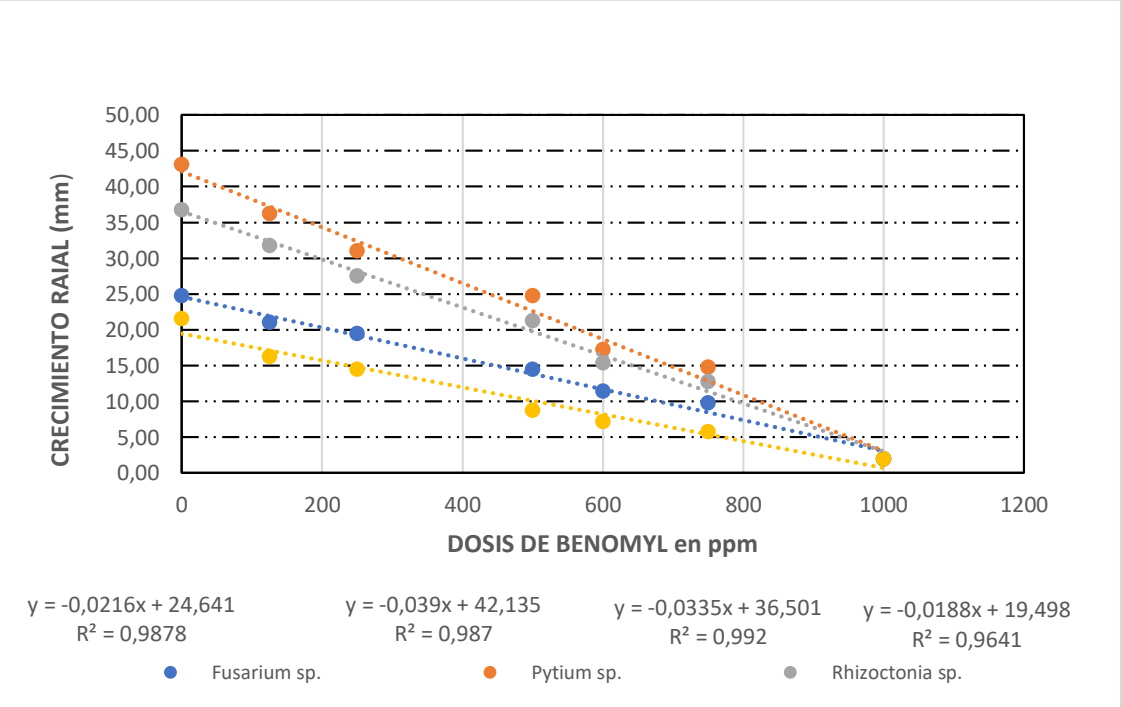
Dose (ppm) growth (mm) PATHOGEN	0	125	250	500	600	750	1000
Fusarium sp.	24.75	21.00	19.50	14.50	11.40	9.80	2.00
Phytium sp.	43.11	36.25	31,00	24.75	17.25	14.75	2.00
Rhizoctonia sp.	36.75	31.75	27.50	21.25	15.40	12.75	2.00
Sclerotium	21.58	16.25	14.45	8.75	7.15	5.75	2.00

Source: Valle (2003)

The values obtained in the calculation of the effective dose 50 for each of the fungi under study fluctuate between 531.407 - 558.57 ppm, which are within the parameters recommended by the commercial house.

Figure 4

Regression analysis of the effective dose 50 of the fungicide Benomyl on Fusarium sp; Phytium sp; Rhizoctonia sp; Sclerotium sp.



Fountain: Valley (2003)

Conclusions

- The pathogens under study, Fusarium sp. Phytium sp. Rhizoctonia sp. and Sclerotium sp. are slow-growing fungi; after 120 hours of incubation, none of the pathogens was able to completely populate the culture medium. The result was the following order: first, Phytium sp. with a diameter of 32 mm, second place Rhizoctonia sp. with 30.82, third place Fusarium with 23.42 mm and lastly Sclerotium with 19 mm of radial growth.

- The best treatments obtained from the extraction process of essential oils and waste water from Eucalyptus, Molle, Rosemary, were the doses of fractions D1, D3, Ar that demonstrated to have inhibition capacity and antifungal activity, achieving the highest levels of growth blocking of the pathogens subjected in the laboratory in the following order Rhizoctonia 94.26%; Phytium 93.48%; Fusarium 91.46% and Sclerotium 89.47% inhibition.

- According to the results obtained in this research, it is shown that it is positive to control the growth of mastic disease or Damping off, with the fractions of the distillates and the residual water from the extraction process of the essential oils of the forest species Eucalyptus, molle and rosemary, since they reached a high level and capacity of inhibition on the pathogenic agents Fusarium sp., Rhizoctonia Sp. Phytium sp. and Sclerotium sp. The antifungal activity is comparable with the effectiveness of the Fungicide Benomyl, in the range that is presented as an effective dose at 50% that fluctuates between 500 and 600 ppm, since they present the same antifungal capacity, but with the advantage that the extracts of the essential oils of the forest species are cheaper, are more degradable, have more active ingredients since they are a complex mixture of active components that do not cause alteration or imbalance in the environment and human health.

- To complement the study of the antifungal, antimycotic and bactericidal properties of the extracts of essential oils of eucalyptus, molle and rosemary, it is necessary to check the treatments in open fields, that is, in nurseries, seedbeds, plantations, where the main problem is affected by the disease of the mal de almacigo or Damping Off.

Conflict of interest

The authors declare that they have no conflicts of interest.

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