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ANALYSIS OF THE FUNGICIDE AND BACTERICIDE ACTIVITY OF *MORINGA OLEIFERA* AND *PETIVERIA ALLIACEA* EXTRACT ON MICROBIAL CROPS FROM PESTS INFECTING *MANGIFERA INDICA*

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Abstract

The fungicidal and bactericidal activity of *Moringa oleifera* and *Petiveria alliacea* leaf extract against pests affecting *Mangifera indica* was tested in vitro. The process of obtaining the extracts was carried out by maceration with ethanolic solvent followed by distillation with rotavapor at low temperatures to better preserve the plant's active principles, whose final product was packaged in amber bottles isolated from light. To identify mango pests, samples were taken with Stuart transport media followed by seeding and subsequent replication on nutrient agar in the case of bacteria using the streak seeding technique and Sabouraud agar for fungi using the puncture seeding technique. Three different concentrations of extract were made, varying between the weight of solute and the volume of solvent, being added to the agar using the disc-plate method, in which the extract was added to a filter paper disc in direct contact with the agar previously inoculated with the strain of interest. The diameter of the inhibition halo, if formed, was analyzed. Favorable results were obtained in the case of the 1:5 concentration, which formed inhibition halos in the two bacterial strains of interest. It is recommended to deepen the different extraction methods to determine the optimal process to obtain plant extracts to promote the use of fungicides and bactericides of organic origin.

Keywords: <MANGO (*Manguifera indica*)>, <*Moringa oleifera*>, <*Petiveria alliacea*>, <PLANT EXTRACT>, <MICROBIOLOGY>, <BIOTECHNOLOGY>, <BIOCIDE>.

1. Introduction

The mango tree is native to the Indian region, although cultivated in countries with warm or temperate climates. It has proven to be a resistant plant since it does not require constant irrigation and can withstand fires. However, in times of drought, it is complicated for a mango plantation to wither because this is when there is greater biomass growth and an increase in photosynthesis activity due to less cloudiness (Guerrero 2018, p.9). The arrival of this tree to America took place in the 18th century, thanks to ships from Portugal, which introduced it to the current Brazilian territory. On the other hand, the Spaniards contributed to its diffusion in America by transporting these trees from the Philippines to Ecuador (Lucero, 2011, p.8).

In Ecuador, mango production began in colonial times, as a highly appreciated and desired fruit, with yellow tones and sweet flavor. Especially the typical variety of the banks of the rivers of the coast. Currently, several hectares of crops are dedicated to producing different varieties of mango, which are appreciated in the international market, having their greatest boom between October and January. According to Guerrero (2018, p.9), approximately 18,000 hectares of the coastal region are dedicated to mango cultivation, of which around 82,246 tons of mango are produced.

Due to the presence of pests such as insects, fungi and bacteria, a high percentage of the cultivated land is affected, being commonly treated with agrochemicals which alter the composition of the soil and, therefore, the environment, besides being toxic to humans. Agrochemicals have been used as the main strategy to reduce diseases and pests in plants and animals in recent decades. However, the indiscriminate use of these products to avoid pests and thus avoid economic losses has caused contamination of nearby ecosystems, soil erosion, decrease in biodiversity and generates resistance in phytopathogens, indirectly affecting the applicator and the consumer of the products, causing health problems for them. Therefore, obtaining products with low toxicity, minimum cost, and being environmentally friendly is unpredictable. Therefore, obtaining low-toxicity, low cost and eco-friendly products are essential by taking advantage of natural resources through alternative technologies for pest control, progressively reducing the use of chemical pesticides (García, 2010, p.31).

The effectiveness of some plant extracts as an alternative for treating phytopathological pests has contributed to the identification of different secondary metabolites with fungicidal and bactericidal activity. The different effects of these phytochemicals have led to an in-depth investigation of their different effects on plants, emphasizing their ecological interaction and the development of defense mechanisms against pests. However, several secondary metabolites have not yet been studied in depth. The coastal zone of Ecuador has a great variety of flora biodiversity, of which a percentage can be used as biocides; despite this, scientific knowledge is very limited since most of them have not yet been scientifically validated (Gañán, 2014, p.15).

The present research will evaluate the bactericidal and fungicidal activity of two plants commonly used to treat various diseases. The plants to be used will be Moringa Oleifera and Petiveria Alliacea.

1.1 Justification

Current pesticides and chemical pesticides are harmful to human health and pollute the environment, thus affecting future plantations. In addition, several studies have shown that the components of these products used in agriculture produce a wide variety of affections on people, such as respiratory problems, memory disorders, skin diseases, depression, miscarriages, congenital disabilities, cancer, neurological disorders, among others (Pacheco et al., 2013, p.2).

Phytopathogenic diseases are one of the main factors affecting the sustainability of mango production in tropical areas worldwide. Globally, approximately 400 phytopathogenic species have been identified as dangerous for mango production. In addition, the phytosanitary situation of this species is becoming more complex with the expansion of the crop into new areas, changes in crop management, varietal renewal or increased chemical interventions (Gonzales & Hormaza, 2020, p.1).

Undoubtedly, contamination caused by pesticides and chemical pesticides is a major concern for the agricultural sector and government agencies in charge of environmental control. In the face of this, the use of organic pesticides. Faced with this, the use of efficient biocides as extracts of medicinal plants from rural areas of the Ecuadorian coast emerges as a promising alternative to be able to control the use of chemicals in agricultural soils; being necessary to establish a methodological strategy that evidences the potential (Pacheco, 2019, pp.7-8).

The present study seeks to develop a biocide from a mixture of extracts obtained from *Moringa Oleifera* and *Petiveria alliacea*. To demonstrate this product's bactericidal and fungicidal efficacy, a series of microbiological analyses will be carried out on samples of cultures obtained from pests in mango plantations. In addition, these pests are faced with an environmentally friendly alternative without altering the surrounding habitat through green alternatives with low manufacturing costs that will reduce the use of chemical pesticides.

2. Objectives

2.1 General Objective

To test the fungicidal and bactericidal activity of *Moringa oleifera* and *Petiveria alliacea* leaf extract against pests affecting *Mangifera indica*, under in vitro conditions.

2.2 Specific objectives

- For subsequent purification and identification, isolate fungal and bacterial pests from infected *Manguifera indica* cultures.
- To obtain extracts from *Moringa Oleifera* and *Petiveria alliacea* plants.
- To evaluate the inhibitory activity of extracts obtained from *Moringa oleifera* and *Petiveria alliacea* against fungal and bacterial pests.

3. Methodology

3.1 Location of the study

The present research work was carried out in the Biotechnology and Organic Chemistry laboratories of the Faculty of Sciences of the Escuela Superior Politécnica de Chimborazo.

3.2 Study factors

3.2.1 Study population

The study population comprises fungal and bacterial samples from the Mangifera indica tree. These were isolated from samples taken from infected Mangifera indica leaves originating from trees in the urban area of the city of Portoviejo, Manabí.

3.3 Data collection techniques

3.3.1 Fungal and bacterial sample collection

Mangifera indica fungal and bacterial samples were taken from trees in Andrés de Vera's parish in Portoviejo, Manabí. The samples were taken with Stuart transportation means, labeled and transported in a cooler with a controlled temperature of approximately 25oC to the Biotechnology laboratory of the Faculty of Sciences of the Escuela Superior Politécnica de Chimborazo, for subsequent microbiological analysis.

3.3.2 Initial mixed cultivation

Mixed cultures were obtained from the different samples obtained from the Stuart transport medium. Seeding was carried out on surface nutrient agar and incubated for 48 to 96 hours at 25oC until the presence of fungal and bacterial colonies was noted.

3.3.3 Isolation of bacteria.

Bacteria were isolated from the aforementioned mixed cultures, using the streaking or depletion seeding technique and incubated at a temperature of 37oC for 24 to 48 hours until pure cultures were obtained. From each isolate, colonies with macroscopic characteristics typical of common bacterial pests in the Mangifera indica tree were selected, and each colony was identified to genus level with the help of Gram stain, oxidase and catalase tests.

3.3.4 Fungal isolation

To obtain the fungi, fungal colonies were identified in the initial mixed cultures, inoculated in Sabouraud culture medium using the sowing technique by puncture, and incubated for 7 days at a temperature of 25ºC. Each isolate obtained was stained with lactophenol blue for its characterization by microscopic observation of the arrangement of its cellular structures according to the colony.

4. Results and discussion

4.1 Sampling and microbiological analysis

The initial samples were taken with Stuart transport media, which were taken in triplicate, resulting in a total of 9 samples taken, as shown in Figure 1; these samples were sown on starter agar for subsequent isolation.

Figure 1. Samples with Stuart transport media.
Source: Valdiviezo, 2022.

Figure 2 shows the mixed cultures obtained from the samples taken with the Stuart transport media, resulting in a total of 18 inoculated Petri dishes, resulting from the triplicate sowing, in which we can observe both bacterial and fungal strains, which were subjected to analysis to determine those strains that are considered phytopathogenic pests of interest for this study before a bibliographic review

Figure 2. Initial culture of samples with Stuart transport medium on nutrient agar.
Source: Valdiviezo, 2022.

4.1.1 Bacterial isolation

From the initial mixed cultures, 27 bacteria were selected according to their morphological differences, as seen in Figure 3. To obtain pure cultures, 13 replicates of each of the 27 strains to be analyzed were made.

Figure 3. Replicate 13 of the selected bacterial strains.
Source: Valdiviezo, 2022.

4.1.2 Fungal isolation

Thirteen fungal strains observed in the first sowing inoculated on nutrient agar were selected, choosing them according to their morphological differences, as shown in Figure 4. Subsequently, the respective replication was performed through the puncture technique to obtain pure strains, of which 3 replicates were made.

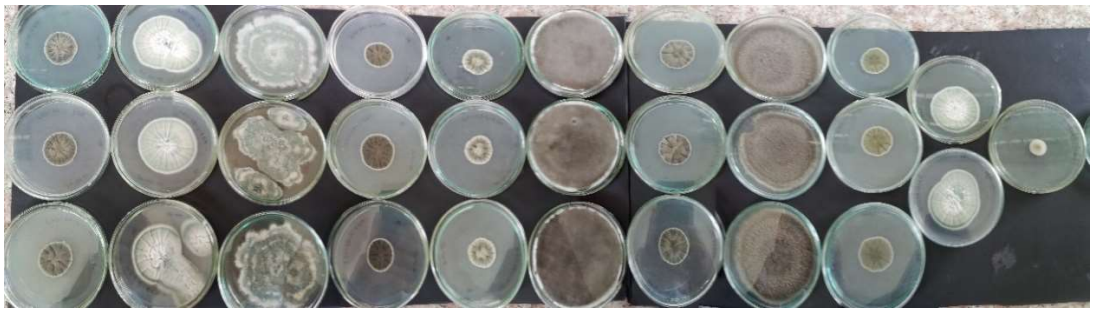


Figure 4. Replicate 3 of the selected fungal strains.
Source: Valdiviezo, 2022.

4.1.3 Pest identification

Among the different pests in the samples taken from *Manguifera indica*, the pest classes specified in Table 1 were found. They were identified by morphological observation of the colonies, Gram staining of samples taken from the colonies, oxidase test, catalase test (in the case of bacterial samples) as shown in Figure 5 and with staining with lactophenol blue shown in Figure 6 (fungal samples).

Figure 5. Biochemical tests performed on the bacterial strains.
Source: Valdiviezo, 2022.

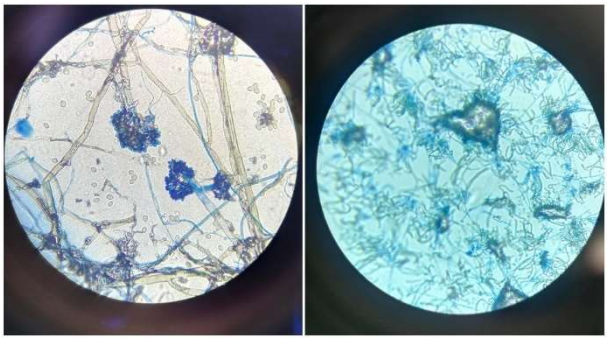


Figure 6. Biochemical tests performed on fungal strains (staining with lactophenol).
Performed by: Valdiviezo, Fabian, 2022.

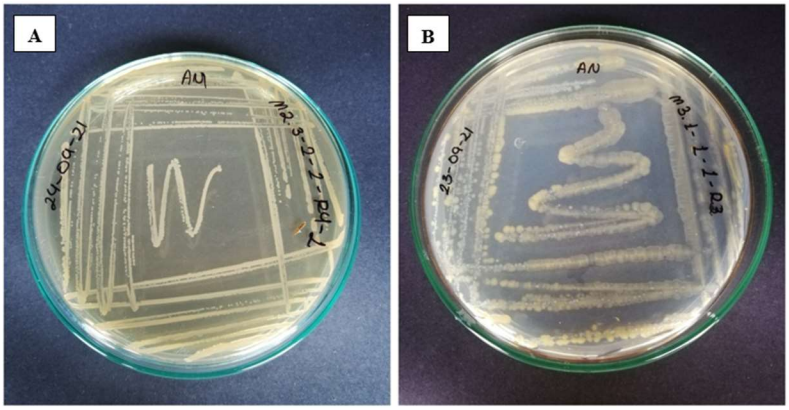
	Code	Name of the microorganism
Bacterial Pests	M 2.3-2-2	*Pseudomonas spp*
	M 3.1-1-1	*Xanthomonas spp*
Fungal Pests	M 2.2-1-C.1.1	*Alternaria spp*
	M 2.1-2-C.1.2	*Cladosporium spp*
	M 3.3-1-C.1.2	*Cladophialophora spp.*
	M 1.2-2-C.5.3	*Brotrytis cinerae.*

Table 1. Fungal and bacterial pests obtained in the sampling of *Manguifera indica*.
Source: Valdiviezo, 2022.

The bacterial pests obtained in the sampling were identified by bacterial standards, using gran staining, oxidase test, catalase test and characterization by morphology. Two were identified as pests: *Pseudomonas spp* (M 2.3-2-2). B: *Xanthomonas spp* (M 3.1-1-1) (Figure 7). The identification of the fungal pests was based on their morphological characterization, which was

determined according to a review of the literature, and staining with lactophenol blue was performed to identify the structures of the fungi to be studied.

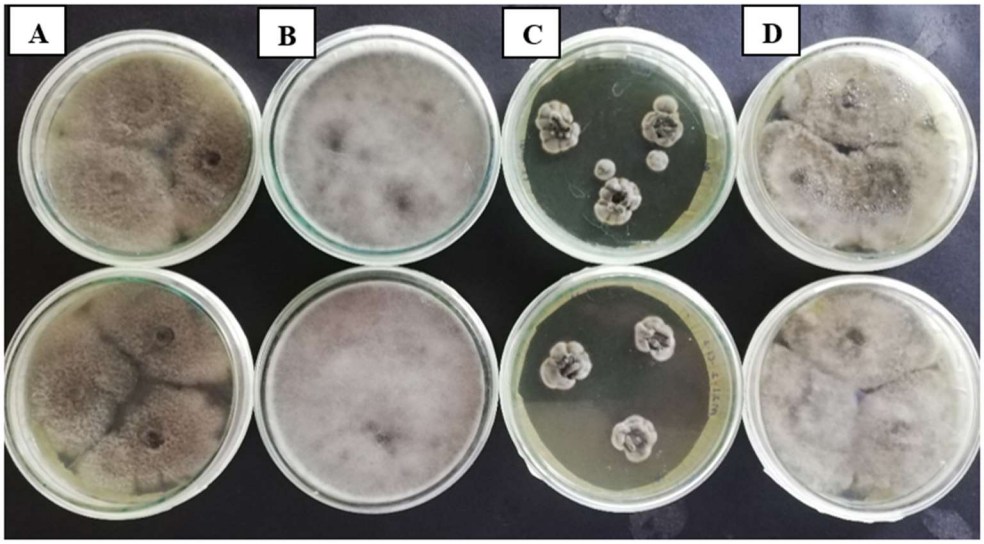
Among the fungal pests encountered were: *Alternaria spp (*M 2.2-1-C.1.1.1), *Cladosporium spp* (M 2.1-2-C.1.2)*, Cladophialophora spp* (M 3.3-1-C.1.2) *and Brotrytis cinerae* (M 1.2-2-C.5.3) as seen in Figure 8-3. The above characteristics are in agreement with those described by (Lucero 2011, p.18), which indicates that, among the most important microbial diseases of the mango crop, due to their high rate of damage, are *Colletotrichum gloeosporioides*, *Brotrytis cinerae, Fusarium spp, Alternaria spp, Cladosporium spp, Rhizhopus stolonifer L*, *Cladophialophora spp*, *E. coli* and bacteria of the genus *Pseudomonas;* in which he describes the damage to fruits and leaves with the appearance of black spots of various shapes, which cause necrosis in the fruits, spoiling them and affecting their commercialization.



A: Pseudomonas spp *(M 2.3-2-2).* ***B:*** Xanthomonas spp *(M 3.1-1-1).*

Figure 7. Bacterial pests identified.

Source: Valdiviezo, 2022.



A: Alternaria spp *(M 2.2-1-C.1.1.1),* ***B:*** Cladosporium spp *(M 2.1-2-C.1.2)*, ***C:*** Cladophialophora spp *(M 3.3-1-C.1.2)* and ***D:*** Brotrytis cinerae (*M 1.2-2-C.5.3).*

Figure 8. Fungal pests identified.

Source: Valdiviezo, 2022.

4.2 Obtaining extracts

The extracts of *Moringa oleifera* and *Petiveria alliacea* were obtained through a series of stages and processes, starting with the collection of fresh plant material as shown in Figure 9, 202, in the area of La Cuesta, canton Santa Ana in the case of the leaves of *Petiveria alliacea* and in the rural parish of Crucita, Los Arenales sector, canton Portoviejo, in the case of the leaves of *Moringa oleifera*, both areas belonging to the province of Manabí.

Figure 9. Collection of plant material.
Source: Valdiviezo, 2022.

Figure 10 shows the initial stages before obtaining the final extract. Initially, the plant material was dried in an oven at a temperature of 35oC for 24 hours, or until it reached a constant humidity of 20% (A). After this process, the previously dried leaves were crushed with the help of a blender until a fine powder was obtained (B), then the leaves were stored in vacuum bags suitable for the process to preserve better the leaf powder obtained (C) to proceed to the next process to obtain the plant extracts.

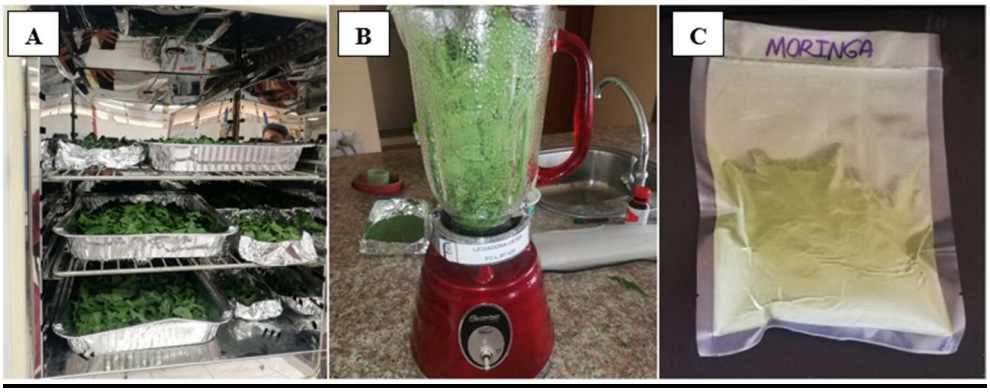


Figure 10. A: Drying of *Moringa Oleifera* and *Petiveria alliacea* leaves. B: Grinding of leaves. C: Packaging of leaf powder.
Source: Valdiviezo, 2022.

Figure 11 shows the process of maceration in alcohol in the previously established proportions [1:3], [1:4], [1:5] for a maximum of 24 hours without the presence of light, stirring every hour during the first 20 hours, leaving the mixture to rest for the remaining 4 hours so that the suspended solids of the mixture settle at the base of the bottle (A) to facilitate the following process, which is vacuum filtration for greater extraction of the alcohol used in the maceration (B). Finally, the distillation of the previously filtered alcohol was carried out with the help of a rotary evaporator at 35ºC and 115 rpm, until a concentrated extract was obtained since working at low temperatures does not alter the active components of the extracts (C).

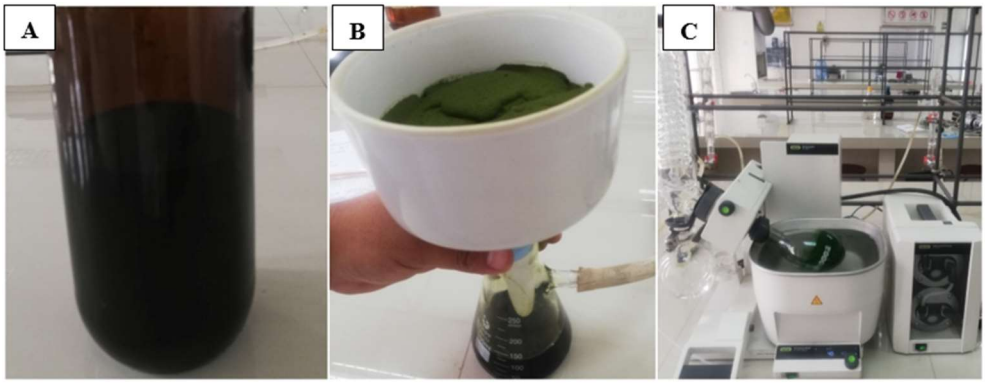


Figure 11. A: Macerated with 75% ethanol, B: Vacuum filtration of macerated samples for 24 hours, C: Distillation by Rotavapor at 35oC.
Source: Valdiviezo, 2022.

4.2.1 Mass balance flow diagrams

4.2.1.1 Mass balance flow diagrams of the ethanolic extract of *Moringa oleifera* at various concentrations

- **Ratio 1:5**

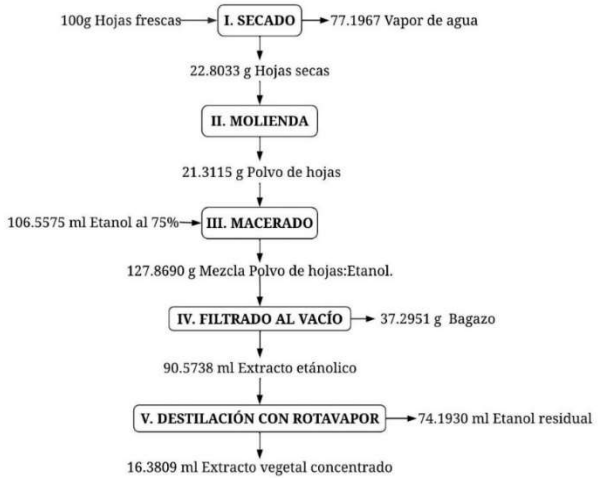


Figure 12. Mass balance diagram of the process of preparing extracts at a ratio of 1:5.

Source: Valdiviezo, 2022.

- **Ratio 1:4**

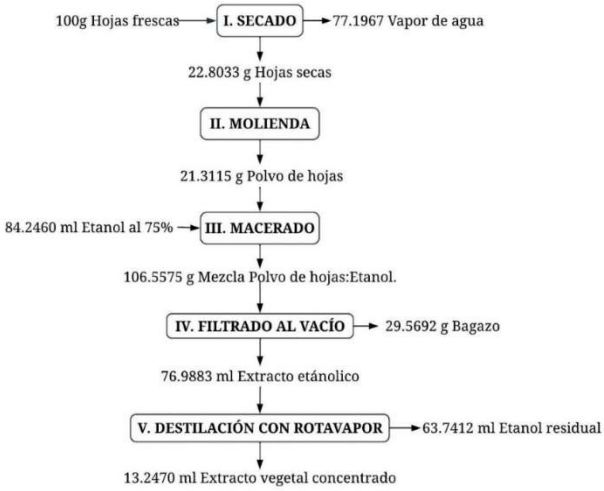


Figure 13. Mass balance diagram of the process of preparing extracts in a 1:4 ratio.
Source: Valdiviezo, 2022.

- **Ratio 1:3**

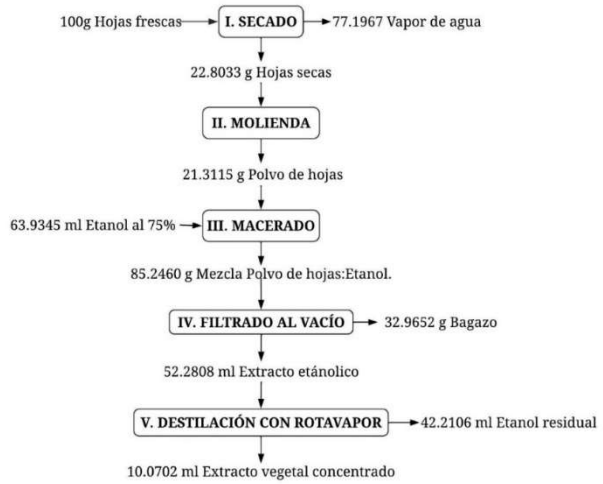


Figure 14. Mass balance diagram of the process of preparing extracts in a 1:3 ratio.
Source: Valdiviezo, 2022.

Flow diagrams of the mass balance of the ethanolic extract of *Petiveria alliacea* at different concentrations.

- **Ratio 1:5**

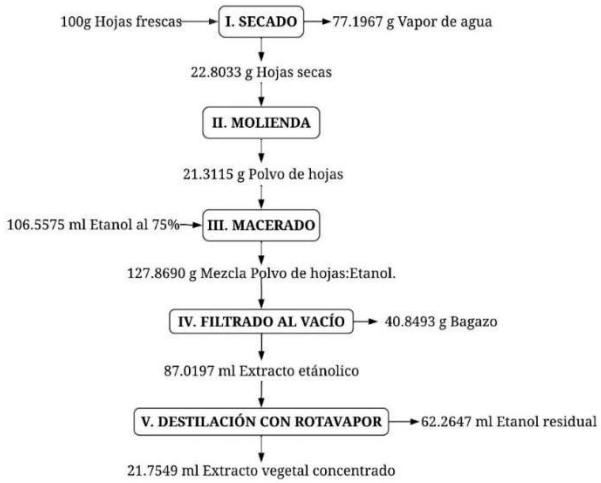


Figure 15. Mass balance diagram of the process of preparing extracts in a 1:5 ratio.
Source: Valdiviezo, 2022.

- **Ratio 1:4**

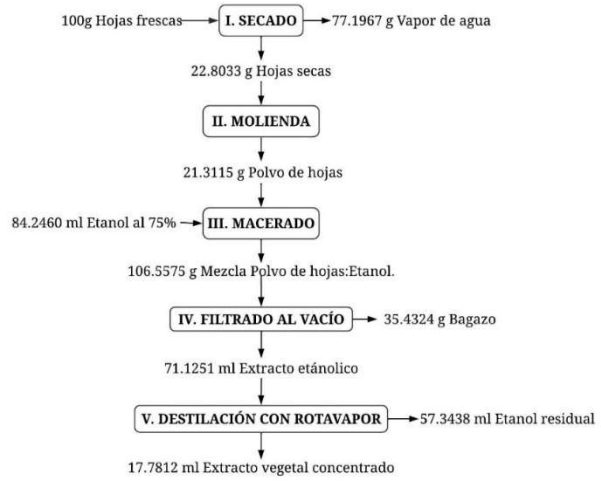


Figure 16. Mass balance diagram of the extract preparation process, ratio 1:4.
Source: Valdiviezo, 2022.

- **Ratio 1:3**

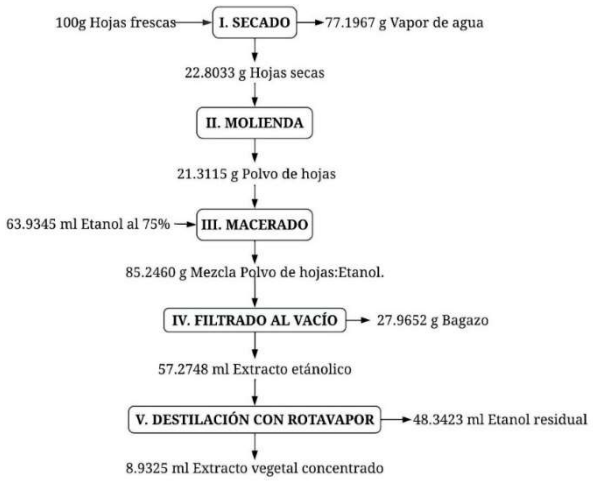
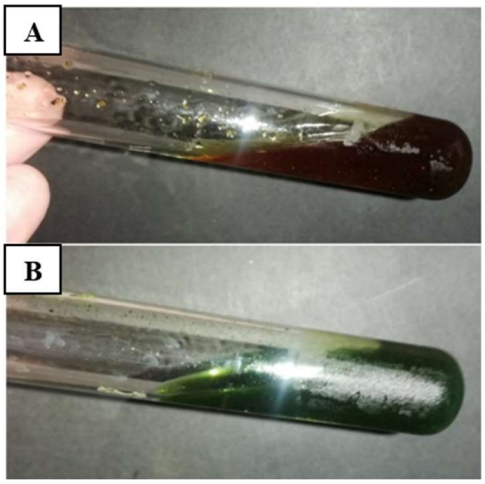


Figure 17. Mass balance diagram of the process of preparing extracts in a 1:3 ratio.
Source: Valdiviezo, 2022.

4.2.2 Physical properties of plant extracts

The ethanolic extracts obtained by rotavapor distillation show a dark amber color in the case of the Moringa Oleifera extract (A) with aromas related to the leaves of the same plant. In the case of the extract of *Petiveria alliacea* (B), a dark green color is identified with a characteristic odor of the plant perceptible to the naked eye, as shown in Figure 18.



A: Moringa Oleifera *extract*. ***B:*** Petiveria Alliacea.
Figure 18. Final ethanolic extracts.
Source: Valdiviezo, 2022.

4.3 Inhibition tests

Inhibition and sensitivity tests against *Moringa oleifera* and *Petiveria alliacea* extracts were carried out at 3 different concentrations. The results obtained from the inhibition tests are detailed in Table 2 and Table 3:

4.3.1 Moringa oleifera Extract

	Sample (code)	[1:3]	[1:4]	[1:5]
Pests Bacteria	*Pseudomonas spp* (M 2.3-2-2)	-	-	30.7 mm
	Xanthomonas spp (M 3.1-1-1)	-	-	42.9 mm
Fungal Pests	*Alternaria spp* (M 2.2-1-C.1.1.1)	-	-	-
	Cladosporium spp (M 2.1-2-C.1.2)	-	-	28.5 mm
	Cladophialophora spp (M 3.3-1-C.1.2)	-	-	-
	Brotrytis cinerae (M 1.2-2-C.5.3)	-	-	>1mm

(-): Negative inhibition*.

Table 2. Inhibition results at different concentrations of *Moringa oleifera* extract.
Source: Valdiviezo, 2022.

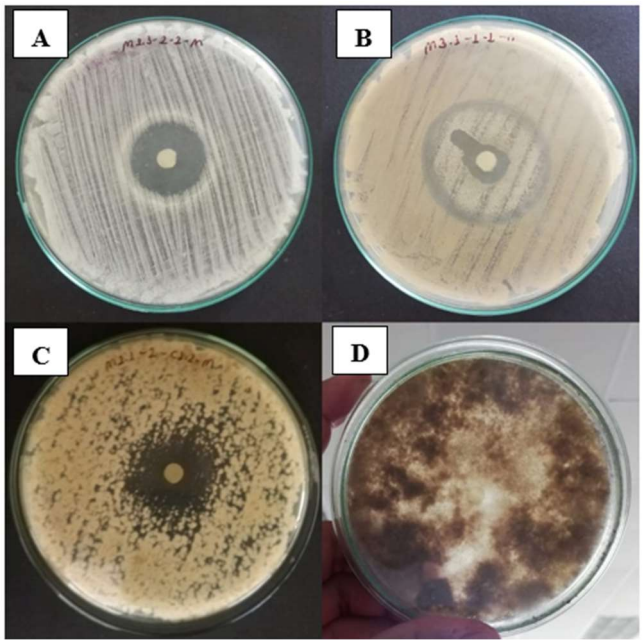
The microbial strains that showed sensitivity to the *Moringa oleifera* extract at a concentration of 1:5 were *Pseudomonas spp* (M 2.3-2-2), achieving an inhibition halo of 30.7 mm, 42.9 mm in the case of the *Xanthomonas spp* strain (M 3.1-1-1) and 28.5 mm in the fungal strain *Cladosporium spp* (M 2.1-2-C.1.2). While the fungal strain *Brotrytis cinerae* (M 1.2-2-C.5.3) presented an inhibition halo >1 mm to the active principles of the extract at the concentration indicated above (Figure 13-3). However, at higher concentrations such as 1:3 and 1:4, no sensitivity to the extracts could be observed in any of the samples, observing that the strains have consumed the entire spot on the plate, including the discs containing the ethanolic extract of *Moringa oleifera* (Figure 14).

According to Mahamadou, in his research work in which he used aqueous extract of *Moringa oleifera,* the strain used in this study, *R solani*, did not present an inhibition halo against the extract (Mahamadou, 2014, p.47), while Goun, E and collaborators, in 2003 conducted a similar study in which they used methanolic and ethanol hydrochloride extracts of this plant, in which their results were negative, because neither of the two extracts could inhibit the growth of *E. coli* (Goun et al., 2003, p.74).

However, a comparative study of the antimicrobial activity of *Moringa oleifera* seeds by Jabeen et al. in 2008 showed that strains *Fusariam solani, Bacillus subtilis and Staphlocuccus aureus* showed high sensitivity to the extract due to the presence of cations such as sodium, potassium or magnesium, while strains such as *Pasturella multocida, Aspergillus niger, Metarhisium aniscoplae and Escherichia coli* showed lower antagonistic activity and strains such as *Rhizopus solani and Pasturella multocida* showed no inhibitory activity against the extract (Jabeen et al., 2008, pp. 2-3).

Considering the above, the hypothesis is accepted in the case of inhibition with bacteria, since, after performing the inhibition tests and analysis of the corresponding results on the different bacterial strains, significant inhibitory activity was demonstrated at a concentration of [1:5]. In the case of the fungal strains, the hypothesis is rejected due to the low or null inhibitory activity they

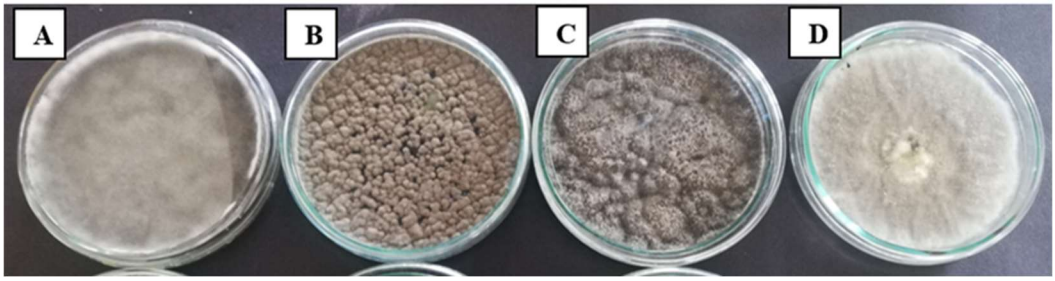
presented. In spite of this, for the use of an ethanolic extract of *Moringa oleifera* leaves to be considered an acceptable biocide for the use and control of pests in agriculture, the use of different extraction methods with which the active principles of the plant can be conserved and obtained in a better way, thus achieving a possible improvement in subsequent trials, is not ruled out.



A: *Pseudomonas* spp (M 2.3-2-2), B: *Xanthomonas* spp (M 3.1-1-1), C: *Cladosporium* spp (M 2.1-2-C.1.2), D: *Brotrytis cinerae* (M 1.2-2-C.5.3).

Figure 19. Fungal and bacterial pest sensitivity tests against *Moringa oleifera* extract at 1:5 concentration.

Source: Valdiviezo, 2022.



A: *Cladophialophora* spp (M 3.3-1-C.1.2), B: *Cladosporium* spp (M 2.1-2-C.1.2), C: *Alternaria* spp (M 2.2-1-C.1.1), D: *Brotrytis cinerae* (M 1.2-2-C.5.3).

Figure 20. Fungal pest sensitivity tests against *Moringa oleifera* extract at 1:4 concentration.

Source: Valdiviezo, 2022.

4.3.2 *Petiveria alliacea* Extract

	Sample	[1:3]	[1:4]	[1:5]
Pests Bacteria	*Pseudomonas spp* (M 2.3-2-2)	-	-	24.7 mm
	Xanthomonas spp (M 3.1-1-1)	-	-	12.2 mm
Fungal Pests	*Alternaria spp* (M 2.2-1-C.1.1.1)	-	-	-
	Cladosporium spp (M 2.1-2-C.1.2)	-	-	-
	Cladophialophora spp (M 3.3-1-C.1.2)	-	-	-
	Brotrytis cinerae (M 1.2-2-C.5.3)	-	-	-

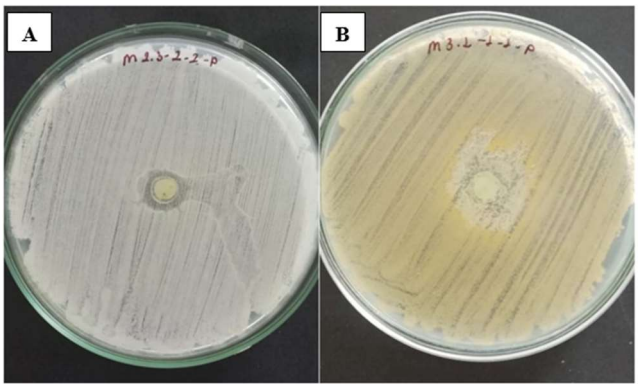
(-): Negative inhibition*.

Table 3. Inhibition results at different concentrations of *Petiveria alliacea* extract.
Source: Valdiviezo, 2022.

As shown in Figure 15, the bacterial strains *Pseudomonas spp* (M 2.3-2-2), *Xanthomonas spp* (M 3.1-1-1) showed sensitivity to the extract of *Petiveria alliacea* at 1:5 concentration, achieving a diameter in its inhibition halo of 24.7 mm and 12.2 mm respectively, results that coincide with a similar study by Pinargote et al. (2019), which proved that the extract showed inhibitory activity against coffee pests, obtaining positive results at laboratory and field levels. While the fungal strains obtained did not show sensitivity to the extracts (Figure 16).

In the case of higher concentrations, there was also no inhibition halo or sensitivity to the Petiveria alliacea extract, as seen in Figure 17. Therefore, according to Illnait et al. (2010), the ethanolic extract of *Petiveria alliacea* at a concentration of 5% can inhibit strains such as *C. parapsilosis, S. cerevisiae, R. mucilaginosa* and *C. grubii,* having null growth at higher concentrations. However, for strains such as *C. albicans and T. asahii* it reached its MIC at a concentration higher than 7.5% or even 10%.

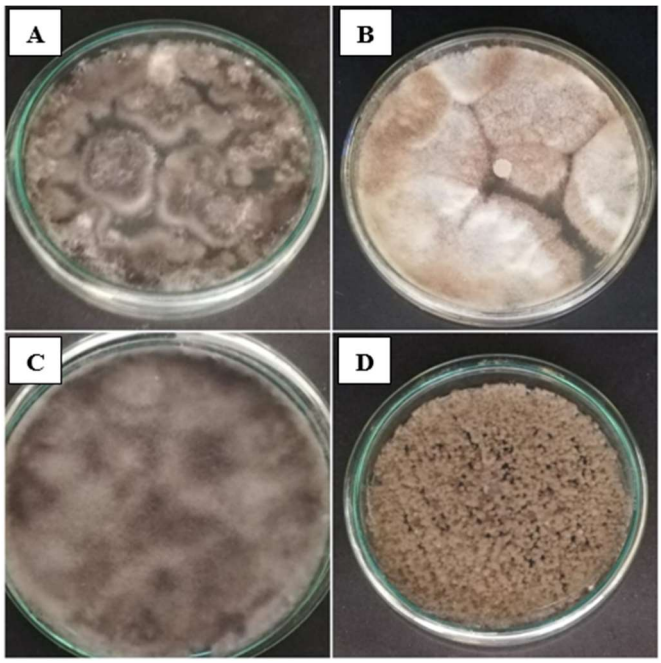
On the other hand, in a study conducted in 2003 at the Research Institute of the Universidad de Los Andes. Mérida. Venezuela, it was demonstrated that the strains of interest presented turbid or null halos before aqueous extracts of *Petiveria alliacea*, showing a minor biocidal activity; on the other hand, with the use of methanolic extracts, they presented medium inhibition halos (Lapenna et al., 2003, p.3). Due to the above, the hypothesis is rejected because it did not present a strong inhibitory activity against the exposed pests in any of the concentrations of the study. However, using other extraction methods with different solvents, there is a possibility that the results may be favorable for possible use in agriculture at the field level, making it a more economically and environmentally viable alternative.



A: Pseudomonas spp (M 2.3-2-2), B: Xanthomonas spp (M 3.1-1-1).

Figure 21. Positive sensitivity tests of bacterial pests against *Petiveria alliacea* extract at 1:5 concentration.

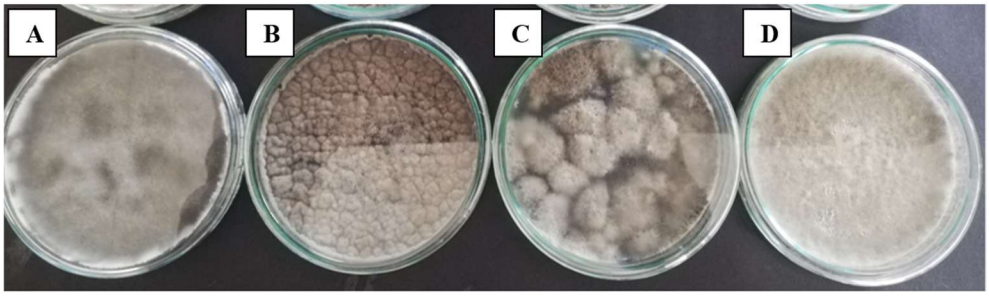
Source: Valdiviezo, 2022.



A: Alternaria spp (M 2.2-1-C.1.1.1), B: Brotrytis cinerae (M 1.2-2-C.5.3), C: Cladophialophora spp (M 3.3-1-C.1.2) and D: Cladosporium spp (M 2.1-2-C.1.2).

Figure 22. Negative sensitivity tests of fungal pests against *Moringa oleifera* extract at 1:5 concentration.

Source: Valdiviezo, 2022.



A: Cladophialophora spp (M 3.3-1-C.1.2), B: Brotrytis cinerae (M 1.2-2-C.5.3), C: Alternaria spp (M2.2-1-C.1.1.1), D: Cladosporium spp (M 2.1-2-C.1.2).

Figure 23. Negative sensitivity tests of fungal pests against *Moringa oleifera* extract at 1:5 concentration.

Source: Valdiviezo, 2022.

5. Conclusions

- It was determined that, although some of the ethanolic plant extracts from *Moringa oleifera* and *Petiveria alliacea* obtained by the rotavapor distillation process showed inhibitory activity at a lower concentration, as in the case of concentration [1:5], compared to lower concentrations such as [1:4] and [1:3], in which they did not show any inhibition against fungal and bacterial pests obtained from the sampling of *Mangifera indica* trees.
- Initially, strains were isolated on solid nutrient agar from samples from *Manguifera indica* trees that presented pests, starting from the first isolation, in which the seeding was performed by streaking on nutrient agar in the case of bacterial strains, identifying 27 strains, respectively. These were replicated 13 times to obtain pure cultures, which were analyzed using a bacterial pattern that included gram staining, catalase test, oxidase test and morphology, in which 2 bacterial strains considered pests were detected *Pseudomonas spp.* and *Xanthomonas spp. In the* case of fungal strains, isolation was carried out through the puncture sowing technique, in which 16 fungal strains were isolated. Two fungal strains were identified as phytopathogenic pests: *Cladophialophora spp, Brotrytis cinerae, Alternaria spp and Cladosporium spp with the* help of the lactophenol blue staining test; in both cases, previous bibliographic research was carried out to identify them.
- The ethanolic extraction of *Moringa Oleifera* and *Petiveria alliacea* was successfully carried out through maceration and distillation from dried leaves and 75% ethanol. These extracts were made at different concentrations to evaluate the inhibitory activity better, obtaining a final volume of between 16.3 ml, 13.2 ml, 10.0 ml of concentrated extract of *Moringa oleifera* and 21.7 ml, 17.7 ml, 8.9 ml in the case of concentrated extract of *Petiveria alliacea,* each in their respective concentrations [1:5], [1:4] and [1:3].
- In response to the inhibitory tests of the concentrated extracts of *Moringa oleifera* and *Petiveria alliacea* against microbiological pests, the bacterial strains showed greater sensitivity to both extracts, compared to the fungal strains in which one of the four strains (*Cladosporium spp*) showed sensitivity to the *Moringa oleifera* extract.

6. Recommendations

Perform correct disinfection to purify the strains of interest at the laboratory level to avoid cross-contamination that could affect the research.

It is proposed to deepen the different extraction methods to determine the most optimal and financially accessible process to obtain plant extracts to promote the use of fungicides and bactericides of organic origin.

It is suggested that the continuous study of different plants with a similar active principle so that it can be corroborated that there is a greater inhibitory activity of the same to have a wide variety of biocides and thus support the reduction of the use of fungicides and bactericides of chemical origin.

It is recommended that the study be complemented with inhibition trials at the field level in different crops with phytopathogenic problems and under different environmental conditions to establish adequate concentrations for their use and obtain favorable results.

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