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Residual Biomass of *Chenopodium Quinoa* to Obtain an Eco-Friendly Product as a Biocide under the Concept of Circular Economy

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The use of industrial insecticides is of growing concern in today's world due to their chemical composition and significant negative impacts on the environment and human health. These products contain toxic chemical compounds that can contaminate soil, water and air, affecting biodiversity and ecosystems in general. Faced with this problem, there is an urgent need to produce and use eco-friendly biocides as an alternative to industrial insecticides. Eco-friendly biocides are products that, instead of relying on harmful chemicals, are based on natural ingredients and environmentally friendly processes, are effective in controlling pests, and minimize risks to human health and the environment. The objective of the research was to take advantage of the residual biomass of *Chenopodium quinoa* in its scarification process, to extract the saponins and elaborate a natural biocide. The method consisted of a phytochemical screening for secondary metabolites to identify the saponins after a hydroalcoholic maceration process, at concentrations of 0, 25, 50 and 75 % ethanol To verify the biocidal property of the product, lethal dose tests were carried out on *Premnotrypes vorax* species, obtaining 100% with a solution of saponin and secondary metabolites in 75% ethanol at 240 min. Furthermore, the ratio of waste generated by the scarification process is 6 g per 1 kg, meaning that for every ton of Chenopodium quinoa processed, 600 kg of residual dust containing saponin would be obtained, which, if used under the concept of circular economy, would be obtained. 46.8 Liters per ton of natural bioinsecticide, environmentally friendly by avoiding chemical contamination and promoting sustainable agricultural practices.

1. Introduction

Due to the negative impacts that have been verified over time by the use of insecticides developed by the chemical industry (Benites, et al., 2023), in recent years various types of insecticides of a vegetable nature or called ecological have been developed, which have different mechanisms of action, efficacy and safety of use (Lopez-Ruiz, et al., 2023). In agriculture is where insecticide products are used with more emphasis, and their efficiency in pest control is valued, for example in horticulture, determining that ecological insecticides are effective in controlling a wide range of pests, their positive result depends on the type of pest, as well as the crop (Rodríguez-Gonzales, 2022). Plant extract insecticides are the best-known option; they are obtained from different types of plants and are of interest for their development because they are products with lower environmental impact and greater safety for human and animal health (Vásquez-Carmona, 2021). Biopesticides, products of natural origin, such as plant extracts, microorganisms or biological products, used to control pests have the advantage of reducing dependence on conventional chemical pesticides, which would improve sustainability in pest management (Gupta and Dubey, 2023). In other investigations, quinoa bran has been used, which showed insecticidal activity only against the insect species that do not affect the quinoa plantation,

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such as *Pseudaletia impuncta*, but against insects that are pests of quinoa, such as *Trichoplusia* and *Feltia subterranea* were not affected (McCartney et al., 2019).Given this reality, there is a great potential for ecological insecticides to be used in pest control and the like by nature to offer more sustainable and safer alternatives; therefore, there is a need for further research and development in this field, with the challenge of its higher cost compared to industrial chemical products, but rewarded by the environmental and health benefits (Akhtar and Akhtar, 2022); also Dwivedi and Singh (2021), highlight that in the face of the potential diverse use of green insecticides, more research is required to overcome some challenges, evaluate the environmental and health benefits of green insecticides to become an attractive option for a more sustainable future.
Therefore, the objective of the research was to determine the effectiveness of a bioinsecticide made from the saponin extracted from the biomass of *Chenopodium quinoa* residues, residues that are generated in the scarification stage of this food product and that could be used and incorporated into the life cycle of this cereal, as another benefit, even used in agriculture to combat pests in the crops of the same species.

2. Methodology

The experimental design and method of the research consisted of extracting the saponin component from the biomass of *Chenopodium quinoa* residues; then, to elaborate a bioinsecticide containing Chenopodium quinoa saponins and to characterize it by determining the level of effectiveness of its behaviour as a bioinsecticide against the potato maggot of the species *Premnotrypes vorax*.

2.1 Obtaining the bioinsecticide: Saponins solution

The bioinsecticide was a solution of saponins extracted from residual biomass of *Chenopodium quinoa*, for which the following process was followed:

- A sample of 5 kg of quinoa husk residue biomass was obtained from a quinoa processing company, which is collected in the scarification (husking) process.
- 500 g of waste biomass was taken from the collected sample and distributed in 5 equal parts in beakers as shown in Figure 1. Then to the samples coded as M1, M2, M3 and M4, a solution with the percentage of ethanol indicated was added and shaken for 24 hours, then left to stand for 72 hours.

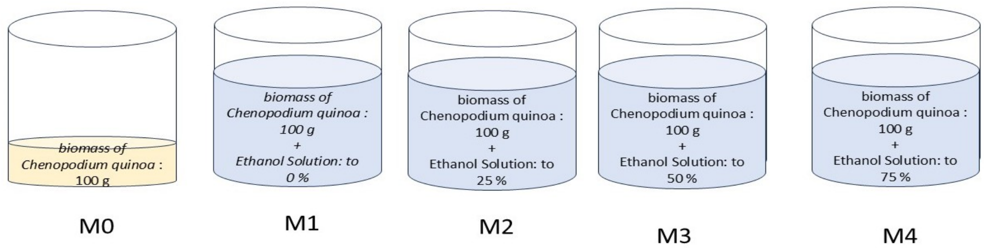

Figure 1: Samples for saponin biocide extraction process

- The rested samples are subjected to a filtration process.
- Ethanol is removed from the filtered saponin solution (by water bath for 40 min at 90 °C).
- The final solutions of saponins will be used in the tests as biocides.

2.2 Identification of saponins

To verify the presence of saponins in the solution obtained, a photochemical screening of the solution samples *obtained was performed to identify secondary metabolites of the saponins. These tests were as follows:*

2.2.1 Afrosimetric assay to determine the presence of saponins by means of foam:

This method also known as Afrosimetric Test or foam method is widely used for its simplicity, low cost and correlates very well with other methods. It is based on the property of saponins to decrease the surface tension of water, forming stable foam whose height is related to the saponin content (Bonilla et al., 2019; Valencia et al., 2005). For the investigation, a sample solution of 7 mL was taken in a test tube and shaken vigorously for 10 minutes, then the level of foam formed with a minimum of 2 mm and with permanence of a time greater than 2 minutes should be observed; if this happens it is an indicator of the presence of saponin.

2.2.2 Other tests performed: For the presence of secondary metabolites

- **Fehling assay to establish the presence of reducing sugars**: It consists of mixing Fehling A (copper sulfate) and Fehling B (sodium and potassium tartrate in sodium hydroxide) solutions, adding the sample to be analysed and heating the mixture. The formation of a brick red precipitate indicates the presence of reducing sugars (Abril, et al., n.d.).

- **Shinoda assay to determine the presence of flavonoids in plant extracts**: Dissolve the sample in alcohol, add magnesium filings and add concentrated hydrochloric acid. A positive result is manifested by the appearance of a red, pink or orange coloration, indicating the presence of flavonoids (Monedero J., 2016).
- **Lieberman Buchard assay to identify presence of steroids and/or triterpenes**: The sample is dissolved in chloroform, acetic anhydride and sulfuric acid are added. The colour change to green or blue suggests the presence of steroids; to red or purple indicates the presence of triterpenes (Dueñas et al., 2020).
- **Bortrager test for quinones**: The sample is extracted with chloroform and shaken with sodium hydroxide. A pink or red coloration in the aqueous phase indicates quinones, (Soto, 2015).
- **Wagner assay to establish the presence of alkaloids:** Alkaloids are detected by the addition of potassium iodide-iodine to an acidified sample. The formation of a brown precipitate indicates alkaloids, which are present when forming insoluble complexes (Soto, 2015).

2.3 Lethality test for saponin bioinsecticide: experimental design

Taking into account *that Premnotrypes vorax*, known as “white potato worm”, is a very important pest that attacks potato crops in the Andean region causing great economic losses, it was chosen to test the effectiveness of a natural biocide based on saponins obtained from the residues of the scarified quinoa (*Chenopodium quinoa*). The result will give indications to propose a method of ecological control of this worm.

The research was carried out to determine the insecticidal behaviour of the saponin biocide by means of the lethal dose test on larvae of the worm *Premnotrypes vorax*. The method consisted of verifying the mortality and survival of 5 groups of 15 worms exposed to the biocide together with a control group, after being subjected to the saponin biocide; the treatment was carried out as follows: Group 1 was subjected to 1 g of the residual saponin powder, group 2 was subjected with 1 mL of the saponin solution; group 3 was subjected to 1 mL of saponin reinforced with 25% ethanol, group 4 was subjected to 1 mL of saponin reinforced with 50% ethanol, and group 5 was subjected to 1 mL of saponin reinforced with 75% ethanol. Monitoring was done at 30, 60, 120, 180 and 240 minutes, with the results shown below.

3. Results and discussion

3.1 Qualitative identification of saponins

The result in the qualitative determination for the presence of saponins in the residual biomass of the quinoa scarification stage was determined by taking 0.5 g of said biomass and dissolving it in 5 mL of distilled water, shaking vigorously for 5 seconds and then leaving it to rest for 30 minutes. Then the agitation was repeated for 30 seconds and allowed to stand for 5 minutes. The result of the “foam test” was positive for all 5 samples. Labelled as M0, M1, M2, M3, M4. Thus, the solutions to be used as biocides were found to contain saponins and secondary metabolites.

Saponins are secondary metabolites of plants, in this research their presence was verified in Chenopodium quinoa, these are a hydrophilic sugar fraction linked to a lopophilic aglycone with special properties such as antiphilic, which give it surfactant properties by forming stable and complex foams with other molecules (Timilsena, et al... 2023), 2023). In quinoa, saponins are found mainly in the husk and is responsible for the bitter taste and which allows the classification of quinoa varieties into sweet (<0.11 %) or bitter (>0.11 %) (Ahumada et all., 2016).

3.2 Extraction of saponin from the biomass of Chenopodium quinoa: bioinsecticide solution

The biomass samples M1, M2, M3 and M4 were subjected to a saponin extraction process using ethanol solution at different concentrations as polar solvent, as shown in Table 1. The solutions were made in the ratio of: 1 residual biomass / 9 ethanol solvent. After a maceration time (72 hours), the presence of saponins was qualitatively evaluated in the solutions of samples M1, M2, M3 and M4, and saponins were found in these samples, except in sample M4 where it was positive for secondary metabolites of reducing sugars and quinones. The secondary metabolites of Chenopodium quinoa found in the samples after phytochemical screening and applying the indicated identification methods are presented in Table 1.

Table 1: Phytochemical screening to identify saponin and secondary metabolites in Chenopodium quinoa saponin boinsecticide solutions

Samples	Solvent concentration: Ethanol Solution, % by volume	Foam test (presence of saponins)	Fehling test (presence of reducing sugars)	Shinoda assay (presence of flavonoids)	Lieberman Buchard Test (presence of triterpenes and/or steroids)	Bortrager assay (presence of quinones)	Wagner Assay (Presence of Alkaloids)
M0	-	+++	+++	+++	+++	+++	+++
M1	0	+++	+++	+++	+++	+	+++
M2	25	++	+++	+++	++	+	++
M3	50	++	+++	++	++	++	+
M4	75	-	+	-	-	+	-

Note: The Table considers the scale of +++ = Very Positive for saponin, ++ = Somewhat Positive for saponin, += Slightly positive for saponin and sign "–" Negative for saponin.

When 75% ethanol was used, no saponins were found, this may have happened for several reasons such as: there may have been interferences when extracting ethanol to other components (Bergesse, et al., 2019), in this case reducing sugars and quinones as shown in Table 1; another reason may be the inadequate temperature in the test, which affected the extraction efficiency since some studies suggest that the best extraction occurs at 50 °C (Coila F, 2019). The scientific literature indicates that it could be by thermostatic bath using some enzyme such as alpha-amylase as was done to extract saponin from roots of Codonopsis javanica (CJR) (To and Vu, 2022). En otra investigación, a 45 °C con solución de etanol-cloroform (proporción de 90:10) en 60 min y con la relación muestra-disolvente de 1:50 g/mL se obtuvo 10.63 % de extracto y 0.45 % de saponinas triterpenoides (Letchumanan, et al., 2023).

3.3 Physicochemical properties of the bioinsecticide saponin solution

Table 2 shows the physicochemical properties of the samples of bioinsecticide solutions of saponin from biomass of Chenopodium quinoa, carried out in the laboratory of Universidad Nacional La Molina

Table 2 Physicochemical parameters of Chenopodium quinoa saponin bioinsecticide solution

Samples	pH	Grade Brix	Refractive index	Proteins (g/100 g sample)	Ash (g/100 g sample)
M0	-	19.0	-	4.9	14.8
M1	5.7	8.0	1.3	0.5	1.5
M2	6.2	14.0	1.3	0.2	1.4
M3	6.5	22.0	1.3	0.3	1.3
M4	6.4	23.3	0.3	0.2	0.5

3.4 Mortality test of *Premnotrypes vorax* to saponin bioinsecticide

A sample of 15 individuals of *Premnotrypes vorax* larvae was subjected to bioinsecticide as biomass powder and in solution with ethanol (0, 25, 50 and 75 %), and progressive mortality of the larvae was obtained as time passed. See Table 3.

Table 3: Amount of mortality of the Premnotrypes vorax

Bioinsecticide	Initial number of *Premnotrypes vorax*	Mortality (Number of individuals dead)				
		30 min	60 min	120 min	180 min	240 min
biomass containing saponin	15	2	3	4	6	7
M1 at 0% ethanol (mL)	15	2	5	8	9	11
M2 at 25% ethanol (mL)	15	5	8	10	13	15
M3 at 50% ethanol (mL)	15	6	8	11	13	15
M4 at 75% ethanol (mL)	15	9	11	15	15	15

The sample solution that behaves with good effectiveness as a bioinsecticide is sample M4 (75 % ethanol) since at 120 min it causes the death of 100 % of Premnotrypes vorax; that is, the saponin hydrolic solution acquired greater insecticidal strength with ethanol. See Figure 2, where the behaviour of all the samples of bioinsecticide based on saponin and its secondary metabolites can be seen.

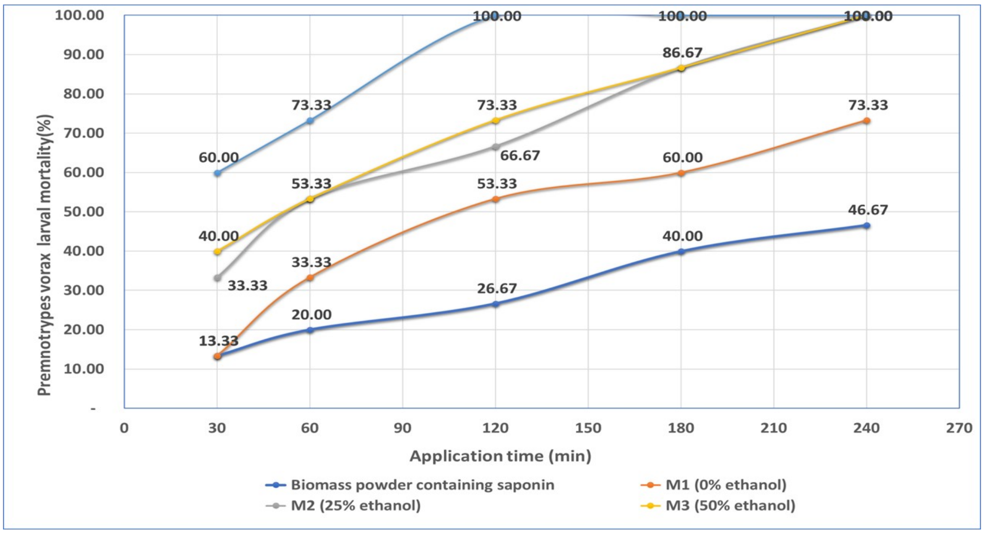

Figure 2: Porcentaje de mortalidad de los Premnotrypes vorax con el bioinsecticida de saponina.

These results ratify the potential insecticidal application of saponins that act by interfering with the biological cycle of insects with lethal effect, altering their growth, development and reproduction, due to the fact that juvenile hormones of these insects have a terpenoids structure similar to the saponins of quinoa, so these compounds could act as growth regulators, for example Bonilla et al., (2019), evaluated the saponin extract in control of *Drosophil melanogaster* at concentrations of 0.1, 0.4, 0.7 and 0.9 % obtaining up to 40 % lethality with the highest concentration (Bonilla et al., 2019). The insecticidal activity of hydrolyzed and non-hydrolyzed saponins is also effective on *Drosophila melanogaster* which after 25 days of treatment obtained an LD50 of 0.08 % for hydrolyzed saponins and 0.58 % with non-hydrolyzed saponins (Bonifaz, 2010). Also, saponin serves as control in pest of corn caterpillars (*Spodoptera frugiperda*) research obtained 83 % mortality (Garófalo, K. 2018), it can also be used in the control of *Fusarium* wilt of tomato when seeds and seedlings are treated with 0.5 and 1.0 % saponin solution (Zhou, et al., 2023). The saponin in alfalfa and quinoa plants has been used as an ecological insecticide due to its effectiveness against insects and mites, as demonstrated by studies where these plants are protected from predation; Furthermore, saponins from *Medicago sativa* (alfalfa) have been observed to inhibit the growth of beetle larvae and have toxic effects on various species, such as *Lobe-sia botrana* and *Tetranychus urticae*. Saponins have also shown lethal effects on brine shrimp and phytopathogenic fungi. In summary, saponin stands out as a botanical pesticide with potential in integrated pest management in organic agriculture and that can be scaled to industrial use with a circular economy approach by using waste (husk) as a source when processing grains. of quinoa (Huaman, et al., 2022).

4. Conclusion

It was determined that the saponin and secondary metabolites extracted from the residual biomass of Chenopodium quinoa behaved as an efficient bioinsecticide in the control of the worm Premnotrypes vorax that attacks potatoes, finding an efficiency of 100% when applied for 120 minutes as a solution reinforced with ethanol at 75% by volume, acquiring bioinsecticide behaviour that can be used in the combat of the worm Premnotrypes vorax; Therefore, the saponin and metabolites that are discarded as residue in the husk and washing of Chenopodium quinoa, can be incorporated into the life cycle of the product, being used to control insect pests, larvae, and others, with the advantage of being a natural, biodegradable product and can replace highly hazardous chemical insecticides with negative impact on the environment.

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