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| cetlogo ***CHEMICAL ENGINEERING TRANSACTIONS***  ***VOL. , 2024*** | A publication of  aidiclogo_grande |
| The Italian Association  of Chemical Engineering  Online at www.cetjournal.it |
| Guest Editors:  Copyright © 2023, AIDIC Servizi S.r.l. **ISBN** 979-12-81206-XX-X; **ISSN** 2283-9216 | |

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 saponin solution in 75% ethanol at 240 min. In addition, the ratio of waste generated by the scarification process is 6 g per 1 kg, which means that for each ton of *Chenopodium quinoa* processed, 600 kg of residual powder containing saponin would be obtained, which, under the concept of circular economy, would yield 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).

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.

* 1. Methodology

The experimental design and method of the research consisted of extracting the saponin component from the biomass of *Chenopodium quinoa* residues, then elaborating a bioinsecticide and characterizing it, determining above all the level of effectiveness of its behaviour as a bioinsecticide against the potato maggot of the *Premnotrypes vorax* species.

2.1 Obtaining the bioinsecticide: Saponins solution

The bioinsecticide was a solution of saponins extracted from residual biomass of Chenopodium quinoa, following the process shown in Figure 1.

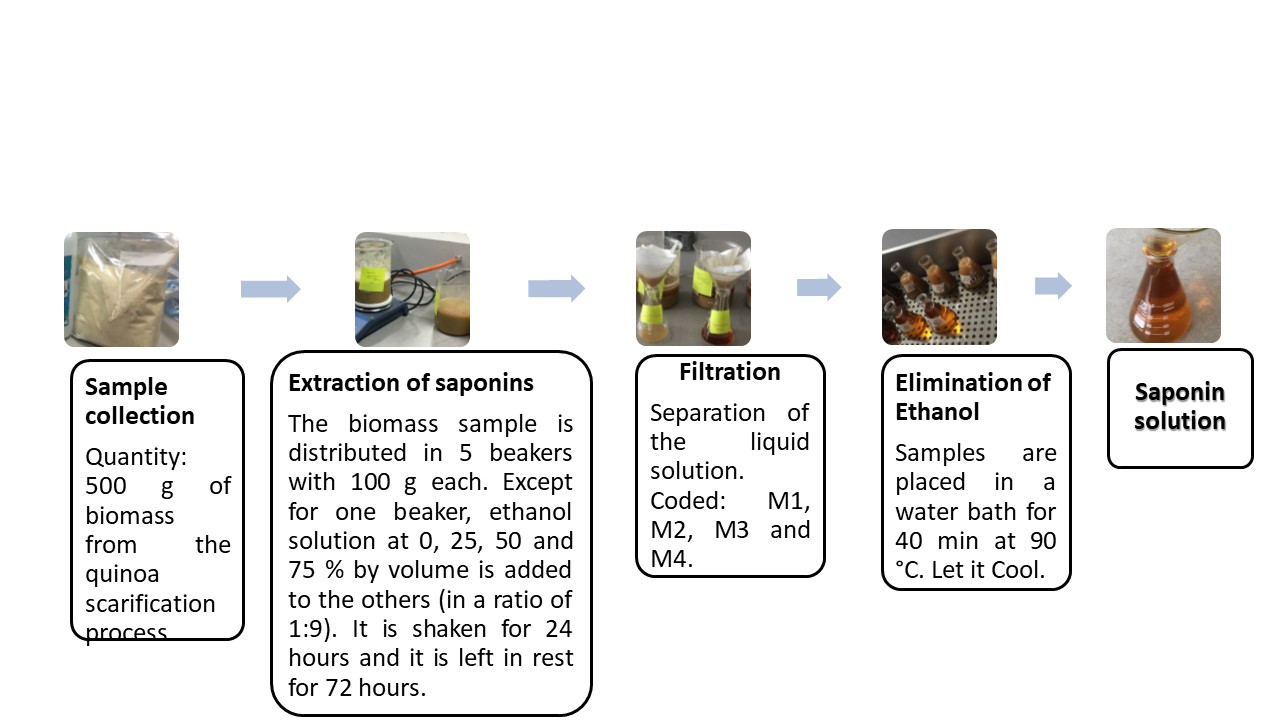


Figure 1: Process for obtaining the biocide saponin

2.2 Identification of saponins

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

* + 1. Afro-metric test for the presence of saponins by means of foam:

This test allows to verify the presence of saponin, a sample of 7 mL was taken in a test tube and shaken vigorously for 10 minutes, then the level of foam formed should be observed with a minimum of 2 mm per and remain for a time greater than 2 minutes, if this happens it indicates the presence of saponin, Figure 2 shows the test tube with foam formation.



Figure 2: Foam test

* + 1. *Other tests performed: To determine the presence of secondary metabolites of the saponin*
* Fehling assay to establish the presence of reducing sugars.
* Shinoda assay to determine the presence of flavonoids.
* Lieberman Buchard assay to identify the presence of steroids and/or triterpenes.
* Bortrager assay to determine the presence of quinones.
* Wagner assay to establish the presence of alkaloids.
  1. Lethality test for bioinsecticide of saponins

To verify the insecticidal behaviour of the saponin biocide, a lethal dose test was carried out on larvae of the potato maggot *Premnotrypes vorax*. The verification of mortality and survival of 5 groups of worms, after being subjected to the saponin biocide, was carried out as follows: Group 1 was subjected to 1 g of the residual saponin powder, group 2 was subjected to 1 mL of the saponin solution; and, the other 3 groups of potato worms were subjected to 1 mL of saponin solution reinforced with 25, 50 and 75 % by volume ethanol solution, respectively. Monitoring was done at 30, 60, 120, 180 and 240 minutes, i.e. for 4 hours, see Table 1.

Table 1: Arrangement of tests for the mortality test

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Repetitions | Witness  (Control) | Saponin powder | Saponin solution | Saponin solution  with 25 % Ethanol | Saponin solution with 50 % Ethanol | Saponin solution with 75 % Ethanol |
| Number of *Premnotrypes vorax* | Cluster 0:  15 | Cluster 1:  15 | Cluster 2:  15 | Cluster 3:  15 | Cluster 4:  15 | Cluster 5:  15 |

In other investigations, quinoa bran has been used, which showed insecticidal activity only against insect species that do not affect the quinoa plantation, such as *Pseudaletia impuncta*, while insects that are pests of quinoa, such as *Trichoplusia* and *Feltia subterranea*, were not affected. (McCartney et al., 2019)

* 1. Results and discussion

Qualitative identification of saponins

To qualitatively determine the presence of saponins in the residual biomass of the quinoa scarification stage, 0.5 g of this biomass was taken and dissolved in 5 mL of distilled water, shaken vigorously for 5 seconds and left to stand for 30 minutes. Then the agitation was repeated for 30 seconds and allowed to stand for 5 minutes. The result to the "foam test" was positive for all 5 samples. See Table 2.

Table 2: Foam testing for initial samples of biomass of Chenopodium quinoa

|  |  |
| --- | --- |
| Samples | Presence of saponins |
| Sample 0 (M0) | Positive |
| Sample 1 (M1) | Positive |
| Sample 2 (M2) | Positive |
| Sample 3 (M3) | Positive |
| Sample 4 (M4) | Positive |

Saponins are secondary metabolites of plants, among them Chenopodium quinoa, are a hydrophilic sugar moiety linked to a lopophilic aglycone with special properties such as antiphilic, which give it surfactant properties, form stable and complex foams with other molecules (Timilsena, et al., 2023).

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 3. The solutions were made in the ratio of: 1 residual biomass / 9 ethanol solvent.

After a maceration time, the presence of saponins in the solutions of samples M1, M2, M3 and M4 was qualitatively evaluated, and saponins were found in these samples, except in sample M4.

A phytochemical screening of the samples was also carried out, determining the presence of secondary metabolites of Chenopodium quinoa. The results presented in Table 3.

Table 3: Phytochemical screening to identify secondary metabolites in bioinsecticide solution of Chenopodium quinoa saponin

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| 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.

Another way of extraction 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). In another investigation, at 45 °C with ethanol-chloroform solution (90:10 ratio) in 60 min and with the sample-solvent ratio of 1:50 g/mL, 10.63 % extract and 0.45 % triterpenoid saponins were obtained (Letchumanan, et al., 2023).

Physicochemical properties of the bioinsecticide saponin solution

Table 4 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 4: 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 |

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 5.

Table 5: 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 powder 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 saponin solution that behaved with good effectiveness as a bioinsecticide was sample M4 (75 % ethanol), since at 120 min it caused the death of 100 % of *Premnotrypes vorax*, i.e., the saponin hydrolytic solution acquired greater insecticidal strength with ethanol. See Figure 3, where the behavior of all the saponin-based bioinsecticide samples can be seen.

Figure 3: Percentage mortality of Premnotrypes vorax with saponin bioinsecticide.

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).

In the research presented, the use of the saponin solution reinforced with ethanol should be observed with some caution, due to the probable damage to the skin by the alcoholic solution; However, these effects are considered insignificant and there is a greater danger to health when ingested (Wolf, 1999). On the contrary, studies have shown that ethanol protects plants from drought and heat (Khurram, et al., 2022)

* 1. Conclusion

It was determined that the saponin extracted from the residual biomass of *Chenopodium quinoa* behaved as a bioinsecticide that can be used to combat the *Premnotrypes vorax* worm pest that attacks potatoes, finding an efficiency of 100% when applied for 120 minutes as a solution of saponin with ethanol at 75% by volume; Therefore, the saponin that is discarded as residue in the peel 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 product, biodegradable that can replace highly dangerous insecticidal chemicals with a negative impact on the environment.

Acknowledgments

The authors and the GITA Group would like to thank the Vice-Rectorate of Research of the Universidad César Vallejo for the financial support for the dissemination and publication of this scientific work.

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