

Evaluation Of Herbicidal and Insecticidal Properties of Avocado Seed Extract for Application as a Biopesticide

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Currently, due to the environmental and human health implications of the use of conventional pesticides in agricultural crops, it has become necessary to search for new alternatives to commercially available synthetic pesticides, such as organic products with herbicide and insecticide activity due to their biodegradability. Specificity and the ability to alter the behavior of pests in crops. However, the use of residual biomass such as avocado seeds has been studied due to its potential as biopesticides and its application for the mitigation of environmental pollution. Considering the above, this review sought to compare the compounds in different avocado seed extracts, with potential insecticide and herbicide activity.

The species *Drosophila melanogaster* and *Lemna minor* are considered biological models to evaluate the insecticidal and herbicidal properties, respectively, due to their easy handling in the laboratory. The literature consulted shows that the avocado seed is a biomass that can be used as a strategy to control pests and diseases in crops, contributing to its application as an organic pesticide in agribusiness.

1. Introduction

The avocado is the drupe-shaped fruit with the highest oil content, after the olive. This fruit grows on native Mesoamerican trees, with the largest production areas in Central and South American countries such as Mexico, the Dominican Republic, Colombia, Peru, the United States, Brazil, Chile and Guatemala, while its consumption is increasing throughout the world. worldwide (Salazar-López et al., 2020). In 2019, the Food and Agriculture Organization of the United Nations (FAO) reported a global production of 7.0 million tons, of which about half was lost along the production chain (Mora-sand et al., 2021).

The approximate weight of an avocado fruit is between 150 and 400 g. This fruit is made up of the exocarp (skin), mesocarp (pulp), endocarp and seed. The mesocarp is the most abundant, representing between 52.9 and 81.3% of the fruit mass, highly demanded for its biological properties and high nutritional value (Mora-sand et al., 2021).

However, the shell and seed are not frequently used in the food industry, so they become an excessive amount of waste. These by-products represent between 21 and 30% of the avocado mass (Mora-sand et al., 2021). However, the composition and characteristics of the avocado seed have drawn the attention of researchers, which is why today it is proposed as a by-product with high potential for recovery in the textile industry, biopolymer development, pharmaceutical industry and in the development of biopesticides (Bahru et al., 2019). Pesticides currently used to control pests in crops are related to human health problems such as allergic diseases in children and adolescents (Barros et al., 2022), decreased sperm count and damage to the ecosystem due to the persistence of many of the pesticide compounds (Knapke et al., 2022). Some of these persistent compounds enter water bodies and go through biotic and abiotic transformation processes that result in products sometimes even more toxic than the original compounds, so it is necessary to explore green alternatives that reduce risks to human health. in the environment. (Anagnostopoulou et al., 2022).

In this review, we will expose the properties of the avocado seed that make it attractive for developing biopesticides and the future perspectives that can guarantee the use of these agro-industrial residues.

1.1 Composition of avocado seed

The avocado seed constitutes 13 and 18% of the part of the fruit, it is discarded as a waste product incurring waste disposal cost through on-site incineration, disposal in landfills and, in addition, they will pollute the environment and cause health problems (Tesfaye et al., 2022).

Table 1: Avocado seed compounds

Solvent	Analysis Method	Compounds	Reference
Chloroform /Methanol (2:1, v/v)	Gas Chromatographic	<ul style="list-style-type: none"> • Acylsterylglucoside • Monogalactosyl-diacylglycerol • Sterylglucoside • Cerobroside • Diagalactosyl-Diacylglycerol • Phosphatidic acid • Phosphatidylethanolamine • Phosphatidylcholine • Phosphatidylinositol 	(Takenaga et al., 2008)
Hexane	Gas chromatography coupled to mass spectrometry	<ul style="list-style-type: none"> • 1,2,4-trihydroxy-nonadecane • β-sitosterol 	(Leite et al., 2009)
Acetone Methanol	Ultra-performance liquid chromatography Tandem mass spectrometry	<ul style="list-style-type: none"> • Quinic acid, Citric acid, Procyanidin dimer A, Procyanidin trimer, B-isomer 1, Procyanidin dimer B1 • Procyanidin trimer B-isomer 2, Syringic acid • Procyanidin dimer B-isomer 2, Catechin • Procyanidin trimer A, Procyanidin dimer B2 • Procyanidin dimer B2, 5-O-caffeoyl-quinic acid, Caffeic acid, Epicatechin, Vanillin, p-Coumaric acid • Ferulic acid, Sinapic acid, Procyanidin dimer B-isomer 4 • Quercetin diglucoside, Quercetin 3-O-arabinosylglucoside, Quercetin-3-O-glucoside, Quercetin-3-O-rutinoside (rutin) 	(Rosero et al., 2019)
Acetonic Ethanol	Gas chromatography coupled to mass spectrometry	<ul style="list-style-type: none"> • Isoestragole, Estragole, α-Cubebene, α-Cubebene, α-Caryophyllene, α-Farnesene, Germacrened D • Palmitaldehyde, 11-Dodecen-2 one, 9,12-Octadienal • Tridecanoic acid, methyl ester, Linoleic acid, methyl ester, Linolenic acid, methyl ester, Linolelaidic acid, methyl ester, 9,12-Octadecadien-1-ol, 9,12,15-Octadecatrien-1-ol 	(Soledad et al., 2021)

Regarding its composition, the avocado seed constitutes around 66.3% starch, 4.9% protein, 4.12% arabinose and 3.3% pentosans. The predominant bioactive compounds are polyphenols, to which the benefits of the seed for human health are attributed (Dabas et al., 2013). Advances in analytical chemistry and the use of liquid and gas chromatography coupled with components such as mass spectrometry have made it possible to clarify the presence of metabolites in avocado seeds. Some of these bioactive compounds have biocidal activity against bacteria, fungi and some insects, which has allowed us to consider the potential of avocado seed extracts as a biopesticide; however, it is important to continue investigating these properties and correlating them with the identified metabolites.

Most of the compounds with biocidal activity are found in lipid fractions, which has led to the belief that the compounds responsible for these properties are of a moderately polar or apolar nature (Soledad et al., 2021). Table 1 mentions some studies carried out to identify avocado seed metabolites with their respective solvent, analytical method and results. Some of these metabolites are indicated to being responsible for biocidal activities, however, the development of new research aimed at clarifying this activity is necessary.

2. Insecticidal activity of avocado seed

Some insects feed on plant species of relevance to agriculture, causing crop failure. Therefore, the use of insecticides has been implemented to eliminate insects and their larvae from crops. It is essential that the composition of these insecticides does not alter the crop, avoid damage to the environment and human health (Narciso et al., 2013). Recently, plant extracts present bioactive with insecticidal activity, which has drawn the attention of researchers investigating their potential use as alternatives to replace conventional insecticides (Pavela et al., 2019).

The insecticidal activity of avocado seed extracts has been evaluated on different occasions in various studies, both for insect species with ecological relevance, and for arthropod vectors of microorganisms. Leite et al. evaluated the larvicidal activity against *Aedes aegypti* of hexane and methanol extracts from avocado seeds. The results showed an LC50 of 16.7 mg mL⁻¹ for the hexane extract and 8.87 mg mL⁻¹ for the methanol extract, indicating a better insecticidal activity of the hexane extract. This activity is attributed to β -sitosterol, a compound present mainly in the hexane extract of avocado seeds (Leite et al., 2009).

Abe and colleagues (2005) tested crude extracts of Mexican medicinal plants for trypanocidal activity against *Trypanosoma cruzi*, which is the etiologic agent of Chagas disease, one of the most serious protozoal diseases in Latin America. The results indicated that the ethanolic extracts of avocado seed, at doses higher than 500 μ g/mL, presented a moderate activity against epimastigotes. (Abe et al., 2005).

Carvalho et al. (2021) evaluated the insecticidal activity of avocado seed extracts and fractions from two avocado cultivars on *Bemisia tabaci* (Gennadius) (Hemiptera: *Aleyrodidae*) biotype B, an important pest species under tropical conditions. Results indicated that ethanolic and aqueous extracts prepared from P. americana seeds, regardless of the plant cultivar used, showed promising insecticidal activity against whitefly nymphs. However, the bioassays carried out with ethanolic extracts were superior, so the researchers carried out a fractionation using the liquid-liquid partitioning technique. The hexane and dichloromethane fractions of this extract caused significant mortality of the nymphs. Ultraviolet (UV) and hydrogen nuclear magnetic resonance (¹H NMR) analysis showed long-chain aliphatic compounds (alkanols or acetogenins from Lauraceae), alkylfurans (or avocado furans), and unsaturated fatty acids in these fractions, which are possibly related to the observed bioactivity. in *B. tabaci*, in addition to saccharides. The results show that avocado seeds are a promising source of compounds with insecticide action for the control of *B. tabaci* biotype B, a great opportunity to transform environmental problems into eco-friendly solutions for agriculture (Carvalho et al., 2021).

The insecticidal activity of the avocado seed on the insect *Drosophila melanogaster* has not been evaluated, despite being a model that is easy to handle and maintain in the laboratory. *D. melanogaster* has been used as a model to understand the processes that determine insecticide toxicity, including unbiased approaches such as direct genetic screens, population genetics methods, and candidate gene approaches. The above approaches can help understand the penetration, distribution, metabolism and excretion of new insecticides, and thus estimate the efficacy of these substances and their possible impact on the environment. *D. melanogaster* has been used for toxicological assays that have allowed the discovery of target sites of toxic substances and the identification of genes/mutations responsible for altering the toxicity of insecticides (Scott and Buchon, 2019).

Additionally, insects of the *Drosophila* genus are considered an emerging and invasive pest that mainly affects fruit crops. Therefore, the use of this model for the analysis of avocado seed extracts as a potential insecticide would allow evaluating the effect of these extracts on different endpoints of *Drosophila* and will allow continuing with the identification of bioactive that allow the consolidation of avocado extracts as biopesticides in a short time (Brunner et al., 2007).

3. Herbicidal activity of avocado seed

Agriculture is also affected by the plants known as weeds, which prevent the maximal development of a crop. This weed can be controlled with herbicides, many of which cause imbalances in the environment and human health (Cruz-ortiz, 2022). Some plant extracts contain phytotoxic bioactive, causing weed growth inhibition. Elmergawi and Alhumaid (2019) studied the phytotoxic effects of methanol extracts obtained from the plants *Tamarix mannifera*, *Alhagi maurorum*, *Echinops spinosissimus*, *Haloxylon salicórnicum*, *Lactuca virosa*, *Neurada procumbens*, *Ochradenus bacctus*, and *Cyperus* on the germination and growth of *Phalaris minor*, *Echinochloa crusgalli*, *Portulaca oleracea* and *Lactuca sativa*. In tests, they found that extracts of *T. mannifera* at a concentration of 40 g/L completely inhibited the germination and growth of *P. minor* seedlings and the length of *P. oleracea* shoots, as well as the length of shoots. buds. the roots of *E. crusgalli* and *L. sativa*. At this same concentration, *L. virosa* almost completely inhibited the germination and growth of *P. minor* and *P. oleracea* seedlings. Therefore, they concluded that *T. mannifera* and *L. virosa* could be used to develop new natural herbicides (El-mergawi and Al-humaid, 2019).

Kaab et al. (2020) evaluated the herbicidal activity of ten crude extracts obtained from aerial parts of volunteer plants from Tunisia against *Trifolium incarnatum*, *Silybum marianum* and *Phalaris minor*. This confirmed that the methanolic extract of *Cynara cardunculus* better inhibited weed germination and seedling growth and caused necrosis or chlorosis. After bioassay-guided fractionation, five major phenolic compounds, syringic acid, p-coumaric acid, myricitrin, quercetin, and naringenin, were identified in the most active crude methanolic extract. Then, only 3 of the flavonoids contained in the most active fraction were tested on *Trifolium incarnatum*. All 3 compounds had a significant phytotoxic effect and therefore could be used in a new composition of botanical herbicides (Kaab et al., 2020).

The common duckweed (*Lemna minor*) is one of the smallest known flowering plants and can be found growing on the surface of freshwater bodies throughout the world. The size and easy maintenance in the laboratory are the most relevant characteristics of this plant, it also shares taxonomic characteristics with plants of higher orders that are usually a problem for agricultural crops (Thomson and Dennis, 2013). Duckweed has shown great potential for the phytoremediation of organic contaminants, heavy metals, agrochemicals, pharmaceutical and personal care products, radioactive waste, nanomaterials, petroleum hydrocarbons, dyes, toxins, and related contaminants. *L. minor* has been widely applied in different areas of modern society, such as agriculture, pharmaceuticals, biofuels, toxicity testing, environmental monitoring, and bioremediation of a wide range of chemical contaminants in sewage effluents and aquatic environment. The wide application of the plant is due to its ubiquitous nature, invasive mechanism, sporadic reproductive capacity, bioaccumulation potential and resilience in polluted environments (Ekperusi et al., 2019).

Therefore, the use of this plant species is considered to initiate studies of herbicidal properties of avocado seed extracts, since its size and characteristics allow results to be obtained in short time (Thomson and Dennis, 2013).

4. Conclusions

Avocado seed extracts can develop biopesticides with insecticidal activity, due to the bioactive present mainly in ethanolic extracts. The use of biological models such as *Drosophila melanogaster* is necessary to continue evaluating the insecticidal activity of these extracts, reducing time and laboratory materials. The herbicidal activity of avocado seed extracts has not yet been explored, however, the bioactive present in the seed identified to date are associated with herbicidal activities, so it is important to conduct bioassays for the identification of phytotoxic bioactive with seed of avocado. The *Lemna minor* model is suggested for its versatility and easy handling in the laboratory. The insecticidal and boiling activities of avocado seed can be used to develop eco-friendly organic biopesticides.

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References

- Abe, F., Nagafuji, S., Okawa, M., Kinjo, J., Akahane, H., Ogura, T., Martinez-Alfaro, M. A., & Reyes-Chilpa, R. (2005). Trypanocidal Constituents in Plants 5 . 1) Evaluation of Some Mexican Plants for Their Trypanocidal Activity and Active Constituents in the Seeds of *Persea americana*. *Pharmaceutical Society of Japan*, 28(7), 1314–1317.
- Anagnostopoulou, K., Nannou, C., Evgenidou, E., & Lambropoulou, D. (2022). Science of the Total Environment Overarching issues on relevant pesticide transformation products in the aquatic environment : A review. *Science of the Total Environment*, 815, 152863. <https://doi.org/10.1016/j.scitotenv.2021.152863>
- Bahru, T. B., Tadele, Z. H., & Ajebe, E. G. (2019). A Review on Avocado Seed : Functionality , Composition , Antioxidant and Antimicrobial Properties. *Chemical Science International Journal*, 27(2), 1–10. <https://doi.org/10.9734/CSJI/2019/v27i230112>
- Barros, M. De, Siqueira, D., & Carvalho, D. (2022). Association between exposure to pesticides and allergic diseases in children and adolescents : a systematic review with meta-analysis. *Jornal de Pediatria*, 17(33), 1–14. <https://doi.org/10.1016/j.jpmed.2021.10.007>
- Brunner, E., Ahrens, C. H., Mohanty, S., Baetschmann, H., Loevenich, S., Potthast, F., Deutsch, E. W., Panse, C., Lichtenberg, U. De, Rinner, O., Lee, H., Pedrioli, P. G. A., Malmstrom, J., Koehler, K., Schrimpf, S., Krijgsveld, J., Kregenow, F., Heck, A. J. R., Hafen, E., ... Aebersold, R. (2007). A high-quality catalog of the *Drosophila melanogaster* proteome. *Nature Biotechnology*, 25(5), 576–583. <https://doi.org/10.1038/nbt1300>
- Carvalho, S. S. De, Ribeiro, P., & Forim, M. R. (2021). Avocado kernels , an industrial residue : a source of compounds with insecticidal activity against silverleaf whitefly. *Environmental Science and Pollution Research*, 28, 2260–2268.
- Cruz-ortiz, L. (2022). Avances en el desarrollo de nuevos herbicidas biológicos a partir de extractos vegetales fitotóxicos aplicados in vitro. *Informador Tecnico*, 86(1), 34–45.
- Dabas, D., Shegog, R., Ziegler, G., & Lambert, J. (2013). Avocado (*Persea americana*) Seed as a Source of Bioactive Phytochemicals. *Current Pharmaceutical Design*. <https://doi.org/10.2174/1381612811319340007>
- Ekperusi, A. O., Sikoki, F. D., & Nwachukwu, E. O. (2019). Application of common duckweed (*Lemna minor*) in phytoremediation of chemicals in the environment: State and future perspective. *Chemosphere*, 223, 285–309. <https://doi.org/10.1016/j.chemosphere.2019.02.025>
- El-mergawi, R. A., & Al-humaid, A. I. (2019). Searching for natural herbicides in methanol extracts of eight plant species. *Bulletin of the National Research Centre*, 43(22).
- Kaab, S. B., Rebey, I. B., Hana, M., Hammi, K. M., Smaoui, A., Fauconnier, M. L., Clerck, C. De, Jijakli, M. H., & Ksouri, R. (2020). Screening of Tunisian plant extracts for herbicidal activity and formulation of a bioherbicide based on *Cynara cardunculus*. *South African Journal of Botany*, 128, 67–76. <https://doi.org/10.1016/j.sajb.2019.10.018>
- Knapke, E. T., Magalhaes, D. D. P., Aqiel, M., Mandrioli, D., & Perry, M. J. (2022). Environmental and occupational pesticide exposure and human sperm parameters : A Navigation Guide review. *Toxicology*, 465(April 2021), 153017. <https://doi.org/10.1016/j.tox.2021.153017>
- Leite, J. J. G., Brito, É. H. S., Cordeiro, R. A., Brilhante, R. S. N., Sidrim, J. J. C., Bertini, L. M., De Moraes, S. M., & Rocha, M. F. G. (2009). Chemical composition, toxicity and larvicidal and antifungal activities of *Persea americana* (avocado) seed extracts. *Revista Da Sociedade Brasileira de Medicina Tropical*, 42(2), 110–113. <https://doi.org/10.1590/S0037-86822009000200003>
- Mora-sand, A., Ram, A., Castillo-henr, L., Lopretti-correa, M., & Vega-baudrit, J. R. (2021). *Persea Americana* Agro-Industrial Waste Biorefinery for Sustainable High-Value-Added Products. *Polymers*, 1–15.
- Narciso, R., Guedes, C., & Cutler, G. C. (2013). Insecticide-induced hormesis and arthropod pest management. *Society of Chemical Industry*, 70(October), 690–697. <https://doi.org/10.1002/ps.3669>
- Pavela, R., Maggi, F., Iannarelli, R., & Benelli, G. (2019). Plant extracts for developing mosquito larvicides : From laboratory to the field , with insights on the modes of action. *Acta Tropica*, 193(January), 236–271. <https://doi.org/10.1016/j.actatropica.2019.01.019>

- Rosero, J. C., Cruz, S., & Osorio, C. (2019). Analysis of Phenolic Composition of Byproducts (Seeds and Peels) of Avocado (*Persea americana* Mill .) Cultivated in Colombia. *Molecules*, 24(3209).
- Salazar-López, N. J., Domínguez-Avila, J. A., Yahia, E. M., Belmonte-Herrera, B. H., Wall-Medrano, A., Montalvo-González, E., & González-Aguilar, G. A. (2020). Avocado fruit and by-products as potential sources of bioactive compounds. *Food Research International*, 138, 109774. <https://doi.org/10.1016/j.foodres.2020.109774>
- Scott, Jeffry & Buchon, Nicolas. (2019). *Drosophila melanogaster* as a powerful tool for studying insect toxicology. *Pesticide Biochemistry and Physiology*. 161(48),95-103. <https://doi.org/10.1016/j.pestbp.2019.09.006>.
- Soledad, C. P. T., Paola, H. C., Carlos Enrique, O. V., Israel, R. L. I., Guadalupe Virginia, N. M., & Raúl, Á. S. (2021). Avocado seeds (*Persea americana* cv. Criollo sp.): Lipophilic compounds profile and biological activities. *Saudi Journal of Biological Sciences*, 28(6), 3384–3390. <https://doi.org/10.1016/j.sjbs.2021.02.087>
- Takenaga, F., Matsuyama, K., Abe, S., Torii, Y., & Itoh, S. (2008). Lipid and Fatty Acid Composition of Mesocarp and Seed of Avocado Fruits Harvested at Northern Range in Japan. 597(11), 591–597.
- Tesfaye, T., Ayele, M., Gibril, M., Ferede, E., & Yilie, D. (2022). Beneficiation of avocado processing industry by-product: A review on future prospect. *Current Research in Green and Sustainable Chemistry*, 5(December 2021), 100253. <https://doi.org/10.1016/j.crgsc.2021.100253>
- Thomson, E. L. S., & Dennis, J. J. (2013). Common Duckweed (*Lemna minor*) Is a Versatile High- Throughput Infection Model For the *Burkholderia cepacia* Complex and Other Pathogenic Bacteria. 8(11), 1–14. <https://doi.org/10.1371/journal.pone.0080102>