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SPECIAL ISSUE EFFECTS OF EL NIÑO SOUTHERN OSCILLATION ON THE PRIORITY SECTORS OF THE REGION

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MISCELLANEOUS

The El Niño Phenomenon and the Prevalence of Infectious Diseases: A Review

Climate Risk in Local Health Services in Ecuador Impact of Metals on the Physicochemical and Antimicrobial Properties of Arrowroot Starch-Based Films

Changes in the hematology and blood metabolites of Guinea Pigs under intensive Rearing System in Humid Tropical conditions

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IA GRANJA: REVISTA DE CIENCIAS DE LA VIDA

Editorial



Dear reader:

It is an honor to present Volume 40 of the La Granja Journal. This edition features a Special Issue on the "Effects of the El Niño Southern Oscillation on Priority Sectors in the Region", highlighting the efforts of researchers dedicated to understanding the influence of anthropogenic climate change on public health, contributing to the management of the health sector.

From a regional perspective, Molleda & Velásquez Serra offer a review titled "El Niño Phenomenon and the Prevalence of Infectious Diseases", exploring the relationship between changes in precipitation and temperatures during "El Niño" periods and the prevalence of various infectious diseases, including viral, bacterial, parasitic, and fungal illnesses. The authors establish a clear link between extreme climatic events and the increase in diseases such as leptospirosis, dengue, and respiratory illnesses, underscoring the need to enhance epidemiological surveillance protocols and early warning systems to control disease outbreaks.

At a local scale, the study by Vilema-Escudero & Manya, titled "Climatic Risk in Local Health Services in Ecuador", provides an analysis of the impact of climate risk caused by weather events associated with the El Niño phenomenon on public health services in 221 municipalities across Ecuador. The authors reveal that healthcare coverage and community resilience are key factors positively influencing society's capacity to recover from adverse climate effects. One of the most significant findings highlights the need to incorporate local geographical particularities in determining climatic risk and improving the quality of health information in sectoral planning strategies to strengthen decision-making processes.

Continuing with our miscellaneous articles, the work "Ecological, Economic, and Social Impacts of the Colombian Cocoa Sector" by Diego Iván Cavie-

des and Fabián Parra of the Universidad Cooperativa de Colombia, along with Karla Andrade of Kyoto University, Japan, analyzes the effects of cocoa production in Colombia. Using a systematic review based on the Joanna Briggs Institute, the authors identify 59 key impacts in the country's main production regions, emphasizing the relevance of these findings for the sector's sustainable development. From the field of conservation, Ricardo Villalba-Briones and Edwin Jiménez of the Escuela Superior Politécnica del Litoral, Ecuador, Allison Rezabala (Independent Consultant), and Martin Aguirrebengoa of the University of Granada, Spain, examine how conservation temperature influences the viability of Cavanillesia platanifolia seeds, an endangered species. The study found that cold storage improves emergence rates and reduces seedling mortality, suggesting its potential for reforestation plans in the Neotropics.

Similarly, Julia Martínez and her team from the Universidad del Azuay, Ecuador, address the issue of urban noise pollution in Cuenca, analyzing 31 points across the city and revealing that in most of the analyzed areas, noise levels exceed the national regulatory limits. Additionally, they compared data from sound level meters and sensor nodes, showing a high correlation and emphasizing the importance of calibrating instruments to ensure accurate noise measurements. In the biotechnological sciences, Antonio Pereira de Menezes Filho and his team from the Goiano Federal Institute, Brazil, investigate the impact of metal incorporation on the biodegradability and antimicrobial properties of arrowroot starch-based biodegradable films. The study demonstrates that the addition of metal sulfates and chlorides significantly improves biodegradability, UV resistance, and bacterial inhibition, suggesting the potential of these films for applications in biodegradable packaging with enhanced antimicrobial properties.

Omar Malagón and his research team from the Universidad Técnica Particular de Loja, Ecuador, present an exhaustive study on the chemical characterization of the ethanolic extract of Gynoxys cuicochensis Cuatrec, an endemic plant of the Loja páramos. Through advanced techniques such as chromatography and mass spectrometry, they identified two key metabolites: nicotiflorin, a glycosylated flavonoid, and 1,3-di-O-trans-feruloylquinic acid, a phenolic derivative. In the same field, Jessenia Lucero and her team from the Instituto Superior Tecnológico Manuel Encalada Zúñiga, Ecuador, explore the development of biopesticides from Beauveria bassiana, a microorganism with significant entomopathogenic potential. The study highlights the importance of optimizing cultivation and fermentation conditions for large-scale production, emphasizing the relevance of these biotechnologies in reducing synthetic pesticide use and promoting sustainable agricultural practices that ensure toxic residue-free food.

In the agricultural sciences, Peruvian researchers Daniel Paredes-López from the Universidad Nacional Agraria de la Selva, Uriel Aldava-Pardave from the Universidad Nacional Agraria La Molina,

and Miguel Morales-Cauti from the Universidad Nacional Mayor de San Marcos, examine the hematological profiles and blood metabolites in guinea pigs reared under an intensive system in the humid tropics. The study reveals that these parameters show significant age-related variations, providing key information for the management and well-being of guinea pigs in intensive production. In the same field, José Alcívar-Cobeña and his team from the Universidad Estatal del Sur de Manabí. Ecuador, and the Instituto de Ciencia Animal, Cuba, present an experimental study to evaluate digestibility in Cobb 500 chickens fed with different levels of Sacha Inchi cake, suggesting it as a viable supplement in broiler diets, with benefits for feed efficiency and animal welfare.

In conclusion, these scientific articles offer valuable knowledge across various research areas. This issue reflects our continued commitment to integrating relevant topics that serve as a starting point in managing strategic sectors to increase population resilience to the adverse effects of climate change. We invite you to delve into these studies that provide relevant solutions to current challenges.

Sincerely,

PhD. Sheila Serrano Vincenti Universidad Politécnica Salesiana Editor in Chief PhD. Ignacio de los Ríos Universidad Politécnica de Madrid Editor in Chief PhD. Rubén Basantes Universidad Yachay-Tech Guest Editor

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EL NIÑO SOUTHERN OSCILLATION





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EL NIÑO SOUTHERN OSCILLATION AND THE PREVALENCE OF INFECTIOUS DISEASES: REVIEW

EL FENÓMENO DEL NIÑO Y LA PREVALENCIA DE ENFERMEDADES INFECCIOSAS: REVISIÓN

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Abstract

Climate changes caused by the El Niño oscillation southern (ENSO) significantly influence human diseases, because they are related to anomalies in precipitation or periods of drought. The aims of this paper are: to evaluate the anomalies of the average sea surface temperature, to calculate the average sea surface temperature of the Niño and Niño 1+2 regions, to contextualize infectious pathologies according to their classification: viral, bacterial, parasitic and caused by fungi in the geographical areas or countries of the Region where they have been studied in the historical context (1982-1983; 1997-1998 and 2016-2017) periods of the ENSO considered strong. A bibliographic, descriptive, documentary, retrospective and cross-sectional review was carried out during the months of October 2023 to March 2024. Sea Surface Temperatures (SST) are higher than average, observed throughout the equatorial Pacific Ocean, with anomalies being greater in the central and central-eastern Pacific. For ENSO 2023-2024, in the Niño 4 region the last calculated values of the Niño index remained at a temperature of +1.4 °C, in the Niño 3,4 region it was +1.9, in the Niño -3 region it was +2.0 °C while in the Niño-1+2 regions it weakened to +1.0 °C. The highest incidence and prevalence of ENSO infection (1997-1998) in Ecuador stands out, accounting for an increase in 13 infectious pathologies. Attention must be paid and the links between the environment and the risk of infection must be better understood.

Keywords: ENSO, temperature, climate, changes, infectious pathologies.

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Resumen

Los cambios climáticos causados por el Niño oscilación sur (ENOS) influyen significativamente en las enfermedades humanas, debido a que está relacionado con anomalías en las precipitaciones o períodos de sequía. El presente trabajo estableció como objetivos específicos: evaluar las anomalías del promedio de la temperatura de la superficie del mar, calcular el promedio de la temperatura de la superficie del mar de las regiones del Niño y Niño 1+2, contextualizar las patologías infecciosas según su clasificación: virales, bacterianas, parasitarias y causadas por hongos en las áreas geográficas o países de la Región donde han sido más estudiadas en el contexto histórico (1982-1983; 1997-1998 y 2016-2017) períodos de ENOS considerados fuertes. Se realizó una revisión bibliográfica, descriptiva, documental, retrospectiva y de corte transversal durante los meses de octubre 2023 a marzo de 2024. Las temperaturas de la Superficie del Mar (TSM) son superiores al promedio observándose por todo el Océano Pacifico ecuatorial, siendo mayores las anomalías en el Pacifico central y centro-este. Para ENOS 2023-2024, en la región Niño 4 los últimos valores calculados del índice Niño se mantuvieron a una temperatura de +1,4 °C, en la región Niño 3,4 fue de +1.9, en la región Niño -3 fue de +2,0 °C mientras que en la región Niño -1+2 se debilitó a +1,0 °C. Destaca la mayor prevalencia de infección de ENOS (1997-1998) en Ecuador, contabilizando un incremento en 13 patologías infecciosas. Se debe prestar atención y comprender mejor los vínculos que existen entre el medio ambiente y los riesgos de infección.

Palabras clave: ENSO, temperatura, clima, cambios, patologías infecciosas.

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1 Introduction

The El Niño/Southern Oscillation (ENSO) is a climatic phenomenon that modulates climate variability on both global and national scales on an interannual timescale (NOAA, 2024). ENSO causes abnormal warming of the Pacific Ocean waters near the coasts of Peru and Ecuador, impacting the Indian Ocean, equatorial southern Africa, South America, and marginally Europe (Huarcaya Castilla et al., 2004; Rúa-Uribe et al., 2013). ENSO also refers to disruptions in the normal circulation of the ocean and atmosphere (Huarcaya Castilla et al., 2004; Rúa-Uribe et al., 2013; NOAA, 2024). Occurring at intervals of 2 to 7 years, ENSO influences oceanic components associated with changes in Sea Surface Temperature (SST) in the tropical Pacific Ocean and also affects atmospheric components (Kovats, 2000; NOAA, 2024; Latif et al., 2015). It is related to variations in atmospheric pressure between the western and eastern tropical Pacific Ocean, disturbing global climate through atmospheric teleconnections (Huarcaya Castilla et al., 2004; Rúa-Uribe et al., 2013; NOAA, 2024; Carlowicz and Schollaert, 2017; Kovats, 2000; Li et al., 2021).

El Niño/La Niña events are parts of the global climate cycle. El Niño is characterized by the warming of equatorial Pacific waters, while La Niña occurs during the cooling phase. During El Niño, the western coasts and extreme south of South America experience intense rainfall, causing higher water levels, while the northern subcontinent and much of Central America and the Caribbean suffer from reduced precipitation, leading to droughts and a high risk of wildfires. Conversely, La Niña sees the opposite pattern (Marinho et al., 2022; Fleck, 2022; Huarcaya Castilla et al., 2004; Fuller et al., 2009; Moraes et al., 2019; Kovats, 2000; Fan et al., 2017).

Due to the vast size of the Pacific Ocean basin, which spans one-third of the planet, changes in temperature, wind, and humidity are transmitted globally. Therefore, ENSO is an ocean-atmosphere interaction phenomenon related to climate variability (Del Carpio, 2023). Ocean condition variations cause changes in weather patterns and fisheries along the western coasts of the Americas (NOAA, 2024). In South America, ENSO impacts result in flooding along the west coast of Ecuador, Peru, and Colombia and droughts in the Amazon and northeast

of the continent. Additionally, dry areas in countries like Peru, Chile, Mexico, and the southwestern United States often experience flooding from rains and snow, with arid deserts blooming. Meanwhile, the wetter regions of the Brazilian Amazon and northeastern United States frequently suffer from prolonged droughts (Carlowicz and Schollaert, 2017; Huarcaya Castilla et al., 2004; NOAA, 2024; Cai et al., 2020; Del Carpio, 2023; Lam et al., 2019; Da Silva et al., 2020; Yglesias-González et al., 2023).

The climatic changes caused by ENSO significantly influence human diseases. ENSO is associated with precipitation anomalies in many cities worldwide, where excessive rainfall or drought periods lead to an increase in infections with high epidemic risks, such as vector-borne diseases, dermal diseases, diarrheal illnesses, and lower respiratory tract infections (Arbo et al., 2022; Flahault et al., 2016).

These conditions tend to increase infection risks, exacerbated by poor water storage practices, lack of access to clean water, insufficient sanitation facilities, open defecation, unsafe drinking water supply, and poor solid waste management, leading to increased pests and vectors (Loayza-Alarico and De La Cruz-Vargas, 2021; Anderson, 2010; Lam et al., 2019; Da Silva et al., 2020; Kovats, 2000; Molleda and Velásquez, 2022; Arbo et al., 2022; Woyessa et al., 2023).

Malaria is one of the most studied diseases in this context. A study conducted in Piura, Peru, between 1996 and 1997 found that the highest incidence of cases occurred in May, following the April rains (Huarcaya Castilla et al., 2004; Cai et al., 2020). The research revealed that breeding sites and vector development increased, with transmission favored by ambient temperature even at unusual altitudes. This observation was corroborated in western Kenya, where malaria outbreaks were noted at altitudes above 2000 meters if temperatures exceeded 18 °C and precipitation surpassed 15 mm³/month (Huarcaya Castilla et al., 2004). Such climatic changes influence the living conditions, longevity, and dynamics of adult Anopheles mosquitoes, impacting disease transmission (Huarcaya Castilla et al., 2004).

Similarly, a team of researchers found a strong relationship between ENSO-driven weather conditions in the Pacific and dengue epidemics in Sri Lanka. Their findings indicated that the dengue risk increased with weekly rainfall exceeding 50 mm. The strongest correlation between precipitation and dengue occurred six to ten weeks after weekly rainfall exceeded 300 mm, under extremely humid conditions and high flooding probability. They noted that temperatures rising to 30 °C or higher consistently increased the dengue risk, with a delay of four weeks post-event (Liyanage et al., 2016; Fuller et al., 2009).

ENSO's global climatic variability influences social and economic components, but its impact on human health is not well understood (Anttila-Hughes et al., 2021). During ENSO periods in coastal regions near the Pacific Ocean, anomalies in temperature and precipitation often result in landslides, floods, wildfires, droughts, and other natural disasters, affecting community health. It is crucial to identify the prevalence of diseases triggered by these extreme weather events to develop prevention plans to mitigate epidemics or vector-borne infectious, viral, bacterial, parasitic, or fungal diseases.

This literature review was conducted to evaluate sea surface temperature anomalies, calculate the average sea surface temperature in Niño regions, analyze ENSO temperature increases in Niño 1+2 Region (Pacific region affecting Ecuador), based on data from the National Oceanic and Atmospheric Administration (NOAA). The review also examined air temperature and monthly precipitation to contextualize and classify infectious diseases into viral (Dengue, Zika, Chikungunya, Rift Valley Fever, Influenza, Enterovirus), bacterial (Leptospirosis, Shigellosis, Cholera, Salmonellosis, Plague), parasitic (Cryptosporidiosis, Schistosomiasis, Leishmaniasis), and fungal (Mycosis, Coccidioidomycosis, Pityriasis) categories that have affected populations in regions studied during strong ENSO events in 1982-1983, 1997-1998, and 2016-2017.

2 Materials and Methods

A bibliographic, descriptive, documentary, retrospective, and cross-sectional investigation was conducted between October 2023 and March 2024. The study was structured through a literature search in the *Pubmed* database (https:

//pubmed.ncbi.nlm.nih.gov/). Additionally, articles published by the WHO (https://www.paho.org/es, https://www.cdc.gov/), and the National Oceanic and Atmospheric Administration (https://www.noaa.gov/education/resource-collections/weather-atmosphere/el-nino) were considered.

The following search equations were used as filters in the search engine bar, using DeCS/MeSH descriptors: "Infectious Diseases", "Vector-Borne Diseases", "Dengue", "Zika", "Chikungunya", "Rift Valley Fever", "Respiratory Diseases", "Diarrhea", "Cholera", "Salmonellosis", "Shigellosis", "Plague", "Hantavirus", "Cryptosporidiosis", "Leishmaniasis", "Mycosis", "Coccidioidomycosis", and "Pityriasis" for the classification of viral, bacterial, parasitic, and fungal diseases. To associate infectious diseases with climatic factors, the following terms were searched: "ENSO", "ENSO and diseases", all combined with the Boolean operators AND, OR, NOT, and NOR. Figure 1 shows the algorithm used to select articles and the search and selection strategy for carrying out the bibliographic review.

Inclusion criteria were as follows: articles published from 2000 to 2023, focusing on infectious diseases and their relationship with ENSO climatic variations during the strong periods of 1982-1983, 1997-1998, and 2016-2017. Original peer-reviewed articles in indexed journals, comparative studies, evaluation, and meta-analyses published in English, Spanish, and Portuguese were included. These articles provided quantitative associations with epidemiological data between viral, bacterial, parasitic, and fungal diseases in the population and ENSO. Excluded from the research were guidelines, letters to the editor, editorials, theses, and dissertations. Articles in languages other than Spanish, English, or Portuguese, and those not published between 2000 and 2023 were also excluded.

2.1 Data collection

The literature search was verified twice to ensure consistent results. For vector-borne viral diseases, the following articles were found: 429 for dengue, 139 for Zika, 182 for Chikungunya, 156 for Rift Valley Fever, 91 for Leptospirosis, 235 for Influenza, 19 for Enterovirus, 69 for Salmonellosis, 33 for Plague, 3 for Shigellosis, 38 for Cryptosporidiosis, 111 for

Leishmaniasis, 2 for Mycosis, and 3 for Coccidioidomycosis. These articles were evaluated to apply exclusion and inclusion criteria, then organized in an Excel sheet by infectious disease, ENSO year, and geographic area to select those best suited to the study objectives.

Subsequently, the selection of articles was based on the geographic area affected by ENSO, the years in which data were recorded (1982-1983, 1997-1998, 2016-2017), and the type of infectious disease presented (viral, bacterial, parasitic, and fungal).

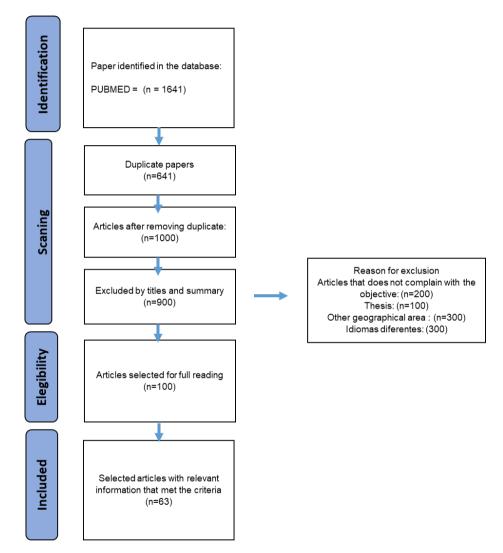


Figure 1. Algorithm Used for Article Selection. Search strategy and selection of scientific articles for conducting the literature review.

3 Results and Discussion

Figure 2 shows that Sea Surface Temperatures (SST) are above average across the equatorial Pacific Ocean, with the greatest anomalies observed in the central and east-central Pacific. According to the

National Oceanic and Atmospheric Administration (NOAA), NOAA's Climate Prediction Center, and the National Weather Service, there is a 73 % probability that the 2023-2024 ENSO will be considered "Neutral"for the months of April to June 2024. In December 2023, positive subsurface temperature

anomalies in the Pacific Ocean decreased, indicating a strengthening and eastward expansion of subsurface temperatures that will be below average in the Western Pacific (NOAA, 2024). Therefore, ENSO in some Niño regions may not cause as much damage as the strong El Niño events.

Due to the vast expanse of the Pacific Ocean,

ENSO has been divided into Niño regions or areas. According to the ENSO regions in the Pacific Ocean (Figure 3), for the 2023-2024 El Niño event, the latest calculated Niño index values were as follows: in the Niño 4 region, the temperature remained at $+1.4\,^{\circ}\text{C}$; in the Niño 3.4 region, it was $+1.9\,^{\circ}\text{C}$; in the Niño 3 region, it was $+2.0\,^{\circ}\text{C}$; while in the Niño 1+2 region, it weakened to $+1.0\,^{\circ}\text{C}$ (NOAA, 2024).

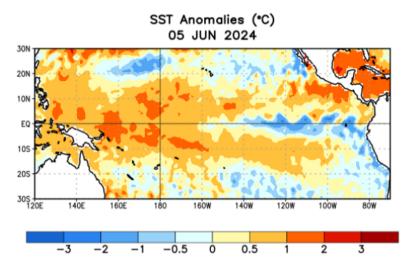


Figure 2. Anomalies of the Average Sea Surface Temperature (SST) °C for the Week Centered on January 3, 2024. The anomalies are calculated relative to the weekly averages of the 1991-2000 base period. Source: NOAA, (2024).

In the Niño 4 and Niño 3.4 regions, SST increased from April, progressively rising from June and peaking in December. The Niño 3 region showed an increase in SST starting in March, with the highest temperatures observed during September, October, November, and December 2023. In the Niño 1+2 region, which affects countries such as Ecuador and Peru, temperatures increased from February, remained high until June, decreased, and then rose again from July through December, starting to decline in December.

Figure 4 shows the evolution of ENSO from 1957-58, 1965-66, 1982-1983, 1991-1992, 1997-1998, 2009-2010, 2015-2016, up to November 2023, with respect to temperature (the years in red are considered strong El Niño events). This graph evidences how the 1982-83 ENSO period evidenced temperatures rise by 4 °C from July to January, and during the 1997-1998 El Niño event, temperatures also reached 4 °C from March to September (NOAA, 2024). The Niño 1+2 region is the area

of the Pacific Ocean used to observe the climatic variations caused by ENSO in Peru and Ecuador. This graph shows sea surface temperature (SST) anomalies in the Niño 1 and Niño 2 regions with coordinates 0-10° South, 90° West, 80° West. The values were calculated from the monthly ERSST V5 by NOAA/CPC (NOAA, 2024).

The El Niño events of 1982-1983 and 1997-1998 are considered the most significant of the 20th century due to the severe catastrophes they caused, especially in South America, which prompted governments and researchers to conduct more studies to understand the nature of ENSO and, thus, predict it to mitigate the impact of climatic disasters caused by this event (Kovats, 2000). The black line represents the 2023-2024 ENSO, where the SST begins to decrease from January 2024. For this reason, the El Niño phenomenon of this period is considered neutral or moderate because temperatures did not exceed 2.5 °C (NOAA, 2024).

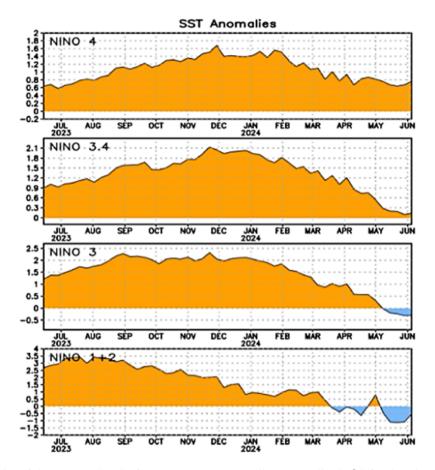


Figure 3. Time Series of the Average Sea Surface Temperature Anomalies. Anomalies in °C for the Niño regions: Niño-1+2 (0°S, 90°W-80°W), Niño-3 (5°S, 150°W-90°W), Niño-3.4 (5°N-5°S, 170°W-120°W), and Niño-4 (5°N-5°S, 150°W-160°E). The anomalies are calculated relative to the weekly averages of the 1991-2000 base period. Source: NOAA ,(2024).

Table 1 shows the prevalence of infectious diseases during the strongest El Niño events of the 20th century (1982-1983, 1994-1995, 1997-1998) reported for South American countries such as Ecuador, Peru, Bolivia, Venezuela, and Colombia. The highest incidence and prevalence of ENSO (1997-1998) was observed in Ecuador, with an increase in 13 infectious diseases. Brazil ranks second, showing an increase in encephalitis cases (Rio, West Nile Virus, and Rocio) during the 1994-1995 El Niño. Thirdly, malaria cases rose significantly in Bolivia, Colombia, Ecuador, Peru, and Venezuela during the 1982-1983 event. Similarly, dengue cases in Ecuador, Peru, and Brazil increased significantly in 2016-2017.

In Table 2, out of a total of 1641 articles found in the consulted database, 63 articles that met the review's objectives and inclusion/exclusion criteria were selected. These articles are classified according to the type of disease: viral, bacterial, parasitic, and fungal. From the 1641 articles, 429 were related to Dengue, of which 10 were selected as they met the study's objectives. For Zika, 6 out of 139 articles were selected. For Rift Valley Fever, 3 out of 156 articles were selected. For Enterovirus, 3 out of 19 articles were selected. For infectious Gastroenteritis, 1 out of 42 articles was selected. For Influenza, 6 out of 235 articles were selected.

Regarding bacterial diseases, 7 out of 91 articles on Leptospirosis were selected. For Cholera, 4 out of 273 articles were selected. For Shigellosis, 2 out of 3 articles were selected. For Plague, 2 out of 33 articles were selected. In the literature search on parasitic diseases, 2 out of 111 articles on Leishmaniasis were selected. For Cryptosporidium, 2 out of 38

articles were selected. For Cyclospora, 1 out of 6 articles was selected. For fungal diseases, 1 out of 2 articles on Mycosis was selected, and 2 out of 3 articles on Coccidioidomycosis were selected. Additio-

nally, 5 articles that dealt with infectious diseases in general and the influence of ENSO were selected, as they were considered relevant and met the study's objectives.

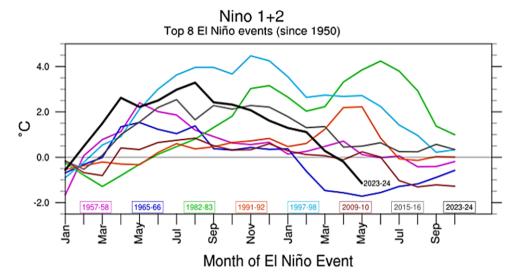


Figure 4. Sea Surface Temperature (SST) Relationship from the 1950 El Niño Event to the 2023 El Niño Event. Source: NOAA, (2024).

Fisman et al. (2016) conducted a study related to ENSO and infectious diseases in the United States. They identified groups of diseases that can cause epidemiological changes due to climate change: vector-borne diseases, viral diseases causing conditions such as pneumonia and influenza, enteric diseases, bacterial, zoonotic, parasitic, and fungal diseases. Evaluations of historical ENSO events did not show the presence of fungal diseases, suggesting that daily morbidity records from health centers and hospitals should be reviewed to determine their occurrence.

Due to the irregular periodicity of ENSO with strong temperature and precipitation variability patterns affecting the risk of infectious diseases, some studies demonstrated a significant impact on vector-borne diseases in the Western United States, without showing morbidity in other regions. In this regard, vectors move according to water availability needed for breeding site survival, which could be influenced by either the lack of water resources or the implementation of permanent vector control strategies in that region.

The study also found an increase in the prevalence of tick-borne diseases due to elevated temperatures and precipitation, which favored the presence of ticks and other rodents that serve as reservoirs for diseases such as Babesiosis, Lyme disease, and Rickettsiosis, among others (Fisman et al., 2016). It has been documented that the transmission rate of microorganisms is higher with shorter tick life cycles due to high temperatures, suggesting that ENSO could increase the number of tick-borne disease cases beyond usual expectations (Rodríguez Arranz and Oteo Revuelta, 2016).

3.1 El Niño Southern Oscillation and viral diseases

Li et al. (2016) evaluated an emerging viral infection caused by *Coxsackievirus 16* and *Enterovirus*, affecting children and infants in China. The clinical presentation includes fever, vesicles, and ulcers in the hands, feet, and mouth. While clinical symptoms can be mild, severe neurological symptoms such as meningitis, encephalitis and polio-like paralysis can occur, as well as pulmonary edema. The study

analyzed how ENSO and climatic variations affect this disease. They determined that high Southern Oscillation Index values were associated with an in-

creased incidence of the disease. The study suggested that meteorological factors predict the occurrence of this infection in China.

Table 1. Infectious Diseases Associated with the El Niño Phenomenon in Latin America (2000-2019).

Disease	Country or region	Year of the El Niño Phenomenon	Reference
Malaria	Ecuador, Peru, Bolivia,	1982-1983	Kovats (2000)
	Venezuela and Colombia	1702-1703	Kovats (2000)
Paludism	Ecuador, Peru, Bolivia,	1982-1983	Huarcaya et al. (2004)
1 arudisiii	Colombia	1702-1703	Truatedya et al. (2004)
West Nile Encephalitis			
Ross River Fever	Brasil	1994-1995	Huarcaya et al. (2004)
Rocio Encephalitis			
Cholera			
Conjunctivitis			
Diarrhea			
Sexually transmitted diseases			
Yellow Fever, Chagas			
Hepatitis	Ecuador	1997-1998	OPS (2000)
Leishmaniasis	Ecuador	1997-1998	OPS (2000)
Leptospirosis			
Marsh fever			
Paludism			
Plague			
Chickenpox			
	Peru	1997-1998	Huarcaya et al. (2004)
Dengue	Ecuador	2010-2011	Stewart et al. (2013); Lipi et al. (2018)
	Brasil	2015-2016	Anyamba (2019)

On the other hand, Oluwole (2017) conducted a study demonstrating that ENSO dynamics drove the incidence of seasonal influenza from 2009 to 2019, a period during which severe seasonal flu epidemics coincided with ENSO dynamics. The authors concluded that the combination of all influenza pandemics over the past 140 years, with chaotic low-transitivity regimes, shows that ENSO dynamics contribute to driving influenza pandemics. Therefore, all models forecasting this event should complement influenza virus surveillance from now on.

Similarly, studies conducted in Japan by Zaraket et al. (2008) found that the highest peak of influenza was related to the warm ENSO period (Xiao et al., 2022; Tang et al., 2022). The warm phase of El Niño, where sea surface temperatures are abnormally high, occurs during the first seven months of the year, followed by a drop in temperature below nor-

mal. This could lead to large-scale environmental changes that alter the trend of flu viruses to redistribute and transmit to the human host.

Latinne and Morand (2022) expressed that climate variability and anomalies are factors driving the emergence of infectious diseases. They highlighted the association between climatic factors like EN-SO, land surface temperature anomalies, and the emergence and transmission of viral diseases from bats to humans and livestock in Asia, the Pacific region, and the Arabian Peninsula. The authors found that ENSO and its climatic anomalies can create opportunities for spreading bat-transmitted rabies viruses to humans and animals. Their results suggest that most of these viral diseases transmitted by bats were likely caused by ENSO climatic anomalies, as 9 out of 12 bat-transmitted viruses emerged in the Asia-Pacific region and the Arabian Peninsula after an El Niño event.

Additionally, the recent emergence of the SARS-CoV-2 virus responsible for COVID-19 in China in 2019 occurred after an ENSO event that impacted China. According to these authors, the battransmitted viruses include the Coronaviridae, Paramyxoviridae, Reoviridae, Rhabdoviridae, Nipah virus, and Hendra virus. Regarding SARS-CoV-2, which appeared in Wuhan, China, it was likely transmitted from bats to humans through an intermediate host believed to be a pangolin. The transmission chain, involving caging wild animals in food markets, subsequent slaughter, and contamination in sales areas, presents high risks for pathogen transmission to both buyers and market workers, leading to the rapid spread of the virus to the broader population (Silva-Jaimes, 2020).

Furthermore, it affected the biodiversity of invasive species, causing loss of local flora and fauna due to aggressive exploitation without adequate biological controls, invading ecosystems unsustainably results in infectious outbreaks by disrupting the balance between predators and prey. However, it is believed that the bat moved unusually from its habitat due to climate change.

Climate change has accelerated the anthropogenic emission of greenhouse gases, increasing zoonosis risks. Additionally, global warming increases the number of infectious disease vectors, such as mosquitoes and ticks. Global warming directly influences pathogens with a higher thermal range than their hosts, including humans, where the balance between the invasive pathogen and the host's immune system shifts in favor of the pathogen. Another important aspect is the migration patterns of wildlife, which could favor the emergence of new diseases through interactions between different animals. Moreover, increased global trade fosters interactions between wild fauna and domestic animals, leading to greater global exposure of humans to pathogenic microorganisms, promoting the transmission of zoonotic diseases, COVID-19 being an example (Valladares, 2020).

3.2 Southern Oscillation El Niño and vector-borne viral diseases

According to Anyamba et al. (2019), the investigation into patterns of interannual climate variability related to ENSO indicates that it results in climatic and environmental anomalies in specific regions worldwide, leading to outbreaks or increases in a wide variety of diseases, notably Dengue, Chikungunya, Zika, Hantavirus, Rift Valley Fever, and plague, among other infectious diseases.

The authors state that these outbreaks, which occurred during the strong ENSO event of 2015-2016, affected regions including Southeast Asia, Tanzania, the western United States, and Brazil. Changes in precipitation, temperature, and vegetation caused excessive droughts and floods, creating favorable ecological conditions for the emergence and spread of pathogenic microorganisms and their vector transmitters in these regions.

The researchers demonstrated that the intensity of diseases in some regions teleconnected with EN-SO doubled compared to years when this climatic event did not occur (Anyamba et al., 2001, 2019). Dengue in Brazil and Southeast Asia is also associated with higher-than-normal land surface temperatures (Anyamba et al., 2001, 2019; Coelho-Cruz et al., 2023). An increase in temperature above the usual levels, closely related to increased humidity, shortens the development cycles of vectors, subsequently increasing vector density. This rise in vector population density leads to greater mosquitohuman interaction, resulting in arboviral diseases such as Dengue, Chikungunya, Zika, and even urban yellow fever, due to the domestic habits of the vector. Consequently, the number of cases could be expected to double.

Table 2. Articles Found in Databases, Classified by Type of Infectious Disease Classified by type of infectious disease: viral, bacterial, parasitic, and fungal. Specified by infectious pathology, article title, author, year of the El Niño-Southern Oscillation (ENSO) event during which the study was conducted, associated causes and effects, and conclusions.

ž	Article title	Author	Diseases	Year of the El Niño Phenomenon	Cause /Effect	Conclusion
			Viral Diseases	es		
H	Spatiotemporal clustering, climate periodicity, and social-ecological risk factors for dengue during an outbreak in Machala, Ecuador, in 2010	Stewart-Ibarra et al. (2014)	Dengue	2009-2010	Precipitation, abnormally high temperatures associated with dengue outbreaks	The presence of the Ae. aegypti mosquito is associated with temperature and abundant rainfall. In 2010, during the peak of the Dengue season (February-March), rainfall was almost double the normal. This increased the availability of habitat for mosquito larvae. Fluctuations in rainfall and temperature influences the rate of mosquito oviposition and virus replication.
74	El Fenómeno ENOS y el dengue, Regiones Pacífico Central y Huetar Atlántico, Costa Rica, 1990 a 2011	Ramírez-Solano et al. (2017)	Dengue	1990-2011	El Niño (warm phase) increased the incidence of dengue in the Pacific and decreased in the Caribbean. La Niña (cold phase) increased the incidence of dengue in the Caribbean and decreased in the Pacific.	The El Niño Southern Oscillation (ENSO) phenomenon affects the increase or decrease of dengue cases. In Costa Rica during the warm season, the cumulative incidence of dengue fever increased in the Pacific Ocean zone and decreased in the Caribbean Sea zone. La Niña has the opposite effect.
т	Effects of local and regional climatic fluctuations on dengue outbreaks in southern Taiwan	Chuang et al. (2017)	Dengue	1998-2015	Temperature, rainfall and high humidity increased dengue transmission	Dengue transmission can be affected by regional and local climatic factors.
4	Climate change and dengue fever transmission in China: Evidences and challenges	Li et al. (2016)	Dengue	2018	Extreme El Niño weather conditions affect the survival, replication and development of dengue virus and mosquito vectors.	Climatic factors temperature, precipitation, humidity, wind speed and atmospheric pressure can affect the spread of dengue because all these factors impact the survival of the virus, the mosquito vector and modify the dengue transmission environment.
ιC	Influencia del evento climático El Niño sobre la dinánica de transmisión de dengue en Medellín, Antioquia, Colombia	Rúa-Uribe et al. (2013)	Dengue	2002-2010	El Niño climate variability influences disease incidence by affecting vector population dynamics and the extrinsic incubation period of the virus.	It has been shown that although the dynamics of dengue transmission is a multicausal event, the potential impact of macro-climatic variables such as the increase in sea level temperature caused by the El Niño phenomena on the incidence of dengue in Medellin Colombia has been demonstrated.

		T	Table 2 - Continuation of the table	n of the table		
$\overset{\circ}{\mathbf{Z}}$	Article title	Author	Diseases	year of the El Niño Phenomenon	Cause /Effect	Conclusion
9	Spatial Hierarchical Analysis of the Temporal Influences of the El Niño-Southern Oscillation and Weather on Dengue in Kalutara District, Sri Lanka.	Liyanage et al. (2016)	Dengue	2009-2013	Increase in temperature has influenced the cases of dengue fever.	Study showed strong association between climate, El Niño-Southern Oscillation and dengue in Sri Lanka.
^	ENSO-driven climate variability promotes periodic major outbreaks of dengue in Venezuela	Vincenti-Gonzalez et al. (2018)	Dengue	1991-2016	High temperatures and low rainfall increase dengue cases.	The study findings provide significant evidence of the relevant climate effect on dengue dynamics. The local and regional climatic factors studied here should be included in an early warning system for dengue and other Ae.eegypti in Venezuela.
∞	Impacts of El Niño Southern Oscillation on the dengue transmission dynamics in the Metropolitan Region of Recife, Brazil	Dos Santos Ferreira et al. (2022)	Dengue	2001-2017	Temperature abnormalities and reduced rainfall favored the mosquito's life cycle.	The dengue epidemic coincided in the Recife region with the El Niño Southern Oscillation (ENSO) phenomenon, spread regionally and was very synchronized.
6	The effect of weather and climate on dengue outbreak risk in Peru, 2000-2018. A timeseries analysis	Dostal et al. (2022)	Dengue	2000-2018	Positive impact between temperature increases and dengue outbreaks in Peru.	The results obtained provide strong evidence that temperature and the El Niño Southern Oscillation (ENSO) have significant effects on dengue.
10	Seasonal patterns of dengue fever in rural Ecuador: 2009-2016	Sippy et al. (2019)	Dengue	2009-2016	High temperatures increase the number of dengue cases. Precipitation and flooding cause mosquito eggs to hatch and periods of drought also favor breeding sites.	This is the first report on the long-term seasonality of dengue in Ecuador, one of the few studies using daily disease reports.
=	Global risk model for vector -borne transmission of Zika virus reveals the role of El Niño 2015	Caminade et al. (2017)	Zika	2015-2016	Warm temperatures associated with the El Niño phenomenon favored mosquito-borne transmission of Zika throughout 2015.	The risk of Zika transmission in South America in 2015 was the highest since 1950. It was found that temperature favors the biting rate and the intrinsic incubation period of the vector. South America and tropical countries present higher level of transmission where Ae. Aegypti is more abundant. There is a risk of Zika transmission in the United States, China and Europe.

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$\overset{\circ}{\mathbf{Z}}$	Article title	Author	Diseases	year of the El Niño Phenomenon	Cause /Effect	Conclusion
12	Climate Variability, Vulnerability, and Natural Disasters: A Case Study of Zika Virus in Manabi, Ecuador Following the 2016 Earthquake	Sorensen et al. (2017)	Zika	2016	Fluctuations in temperature and precipitation. High vector densities during the rainy season.	In Ecuador, after El Niño 2016, the trigger of a natural disaster during abnormal weather and underlying social vulnerabilities multiplied the force that contributed to a dramatic increase in ZIKV cases after the earthquake.
13	Post-earthquake Zika virus surge: Disaster and public health threat amid climatic conduciveness	Reina Ortiz et al. (2017)	Zika	2015-2016	Variations in temperature, changes in precipitation, humidity and atmospheric pressure. Ecological changes caused by the 2016 earthquake in Ecuador.	This study provides critical information to help prevent public health pooing in densely populated areas of North America, Europe and Australia where rising temperatures may be within the range of mosquito vectors of this disease.
14	An Ecological Assessment of the Pandemic Threat of Zika Virus.	Carlson et al. (2016)	Zika	2015- 2016	Precipitation and diurnal fluctuations in temperature may limit transmission of Zika.	Comparison of Zika's habitat with the known distribution of dengue suggests that Zika is more limited by seasonal rainfall and temperature variations which could limit non-sexual indigenous transmission of this virus.
15	Analyzing climate variations at multiple timescales can guide Zika virus response measures	Muñoz et al. (2016)	Zika	2015-2016	Severe droughts and very high temperatures favor the cases of Zika.	High temperatures helped set the climate scenario for Zika virus transmission.
16	Environmental Changes and the Impact on the Human Infections by Dengue, Chikungunya and Zika Viruses in Northern Brazil, 2010–2019	Marinho et al. (2022)	Dengue Chikungunya Zika	2010-2019	High temperatures were correlated with Zika cases in Brazil between 2014 and 2016. The increase in precipitation and temperature caused by La Niña between 2015 and 2016 increased Chikungunya infections.	This study found that deforestation and climate change had a strong influence on infections caused by Dengue, Chikungunya and Zika viruses.
17	Prediction of a Rift Valley fever outbreak	Anyamba et al. (2009)	Rift Valley fever	2006-2007	Heavy rainfall favors vegetation growth, creating an ideal habitat for the mosquito vector of the virus.	The convergence of ENSO conditions in the eastern Pacific and the simultaneous warming of the temperature above sea level in the western equatorial region of the Indian Ocean was the triggering mechanism for the outbreak of Rift Valley fever. In East Africa, ENSO was found to have a major influence on this disease.

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	Article title	Author	Diseases	El Niño Phenomenon	Cause /Effect	Conclusion
• • • • • • • • • • • • • • • • • • • •	Climate Conditions During a Rift Valley Fever Post-epizootic Period in Free State, South Africa, 2014–2019.	Anyamba et al. (2022)	Rift Valley fever	2014-2019	High rainfall periods, cooler than normal conditions and abundant vegetation.	Climatic factors influenced by the El Niño phenomenon, rainfall, humidity, vector detection, surveillance in high-risk areas and vaccination campaigns should be methods for preventing this disease.
	NDVI anomaly patterns over Africa during the 1997/ 98 ENSO warm event	Anyamba et al. (2001)	Rift Valley fever	1997-1998	Extensive flooding created the conditions for the proliferation of this disease.	El Niño recorded between 1997-1998 was the strongest on record in the 20th century. By causing flooding and creating conditions that favored some diseases affecting livestock and humans in East Africa, forecasting tools could provide early warning to these regions to avoid the transmission of infectious diseases.
- Z	Investigation of the Correlation between Enterovirus Infection and the Climate Factor Complex Including the Ping-Year Factor and El Niño-Southern Oscillation in Taiwan	Yu et al. (2024)	Enterovirus	2007-2022	Enterovirus correlates with temperature, humidity, precipitation, wind speed, with temperature being the most important climatic factor affecting the prevalence of this disease.	El Niño significantly impacted the incidence of enterovirus infections in Taiwan.
)	Short-Term Effect of El Niño-Southern Oscillation on Pediatric Hand, Foot and Mouth Disease in Shenzhen, China	Lin et al. (2013)	Enterovirus	2008-2010	Humidity and temperature were associated with an increase in this disease.	Meteorological factors could be important predictors of the occurrence of pediatric hand-footand-mouth disease in Shenzhen.
	Emerging and re-emerging viruses in Malaysia, 1997—2007	Tee et al. (2009)	Enterovirus	1997-1998	Drought conditions, temperature increase due to the El Niño Southern Oscillation (ENSO) phenomenon.	Anthropogenic factors such as a gricultural encroachment or expansion confounded with the temporal and spatial dynamics of both viruses and hosts and their immunity have been linked to outbreaks of viral infections in Malaysia, Bangladesh and India. Therefore, to avoid the re-emergence of viruses or zoonoses, deforestation and agricultural intensification and agricultural intensification activities must be regulated.
.≒	Effect of non- stationary climate on infectious gastroenteritis transmission in Japan	Onozuka (2014)	Infectious gastroenteritis	2000-2012	These infections are strongly associated with changes in temperature, humidity and precipitation.	Quantitative evidence was found that environmental changes caused by the El Niño and Indian Ocean dipole phenomena are associated with the prevalence of infectious gastroenteritis. Early warning systems should be developed for epidemics caused by this disease.

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$\overset{\circ}{\mathbf{Z}}$	Article title	Author	Diseases	year of the El Niño Phenomenon	Cause /Effect	Conclusion
24	Seasonal Influenza Epidemics and El Niños	Oluwole (2015)	Influenza	2000-2015	Seasonal influenza severity increased during El Niño and decreased during La Niña.	The severity of the influenza epidemic with the strength and waveform of the El Niño phenomenon indicates that models forecasting this phenomenon should be integrated into the health surveillance program focused on the prevention of influenza epidemics.
25	Dynamic Regimes of El Niño Southern Oscillation and Influenza Pandemic Timing.	Oluwole (2017)	Influenza	2009-2020	Low temperatures and precipitation favor aerosol transmission of influenza. The virus is sensitive to temperature.	Coupling of all influenza pandemics over the past 140 years to chaotic low-transitivity regimes indicates that ENSO dynamics drive the prevalence of influenza pandemic infection.
26	How do El Niño Southern Oscillation (ENSO) and local meteorological factors affect the incidence of seasonal influenza in New York state?	Xiao et al. (2022)	Influenza	2015-2018	It is highly correlated with temperature and humidity anomalies caused by El Niño.	Low ENSO index, low temperature and absolute humidity may drive influenza epidemics in New York.
27	The El Niño- Southern Oscillation (ENSO) -pandemic Influenza connection: Coincident or causal?	Shaman and Lipsitch (2013)	Pandemic Influenza	1918-1920 1957-1958 1968-1969 2009-2010	Birds are the carriers of the influenza virus, and an association was observed during La Niña between the influenza pandemic and changes in bird migration caused by ENSO.	It was shown that influenza virus can emerge in the human population in other places due to the transport of migratory birds and the movement of the virus should be considered from geographies where the El Niño phenomenon is frequent.
58	Association of early annual peak influenza activity with El Niño southern oscillation in Japan. Influenza Other Respir. Viruses	Zaraket et al. (2008)	Influenza	1983-2007	Evident relationship between peak activity of influenza viruses and the El Niño Southern Oscillation ENSO phenomenon.	There are clear complexities in trying to understand the relationships between climate change and disease patterns such as influenza.
29	Can El Niño-Southern Oscillation Increase Respiratory Infectious Diseases in China? An Empirical Study of 31 Provinces.	Tang et al. (2022)	Influenza	2007-2018	The 2015-2016 El Niño event caused severe drought and extreme temperatures favoring the influenza virus in China.	Improve the climate early warning system on respiratory infectious diseases in China to control the spread of respiratory infectious diseases.
			Bacterial Diseases	1565		
30	La correlación entre tres teleconexiones y la 30 incidencia de leptospirosis en el distrito de Kandy, Sri Lanka, 2004-2019	Ehelepola et al. (2021)	Leptospirosis	2004-2019	La Niña period and high rainfall were associated with the prevalence of leptospirosis.	Monitoring of extreme teleconnection events such as El Niño and La Niña and improvements in flood prevention measures in Sri Lanka could mitigate leptospirosis spikes that may occur.

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$\overset{\circ}{\mathbf{Z}}$	Article title	Author	Diseases	year of the El Niño Phenomenon	Cause /Effect	Conclusion
31	Quantifying the relationship between climatic indicators and leptospirosis incidence in Fiji: A modelling study	Rees et al. (2023)	Leptospirosis	2006-2017	Heavy rainfall leads to increased incidence of leptospirosis.	This study could identify that climatic factors influence the risk of leptospirosis transmission in Fiji.
32	Climatic Variability and Human Leptospirosis Cases in Cartagena, Colombia: A 10-Year Ecological Study	Cano-Pérez et al. (2022)	Leptospirosis	2008-2017	Precipitation and humidity during the La Niña phenomenon were correlated with the increase of cases.	The climate in Cartagena (Colombia) favors the incidence of leptospirosis. Prevention and control of this disease in the city should be promoted and strengthened.
33	Changes in epidemiology of leptospirosis in 2003–2004, a two El Niño Southern Oscillation period, Guadeloupe archipelago, French West Indies	Storck et al. (2008)	Leptospirosis	1994-2001	Relationship between exceptional meteorological events such as El Niño phenomenon and its influence on the population of leptospiriosis-transmitting rodents. There is a positive correlation between precipitation and leptospirosis.	Changes in the epidemiology of leptospirosis in the tropical island of Guadeloupe are highly related to climatic conditions, with a high potential for a rapid outbreak of transmission and a possible impact on the serogroups responsible for the infection, and the clinical characteristics of the disease in the human population.
46	El Niño Southern Oscillation and Leptospirosis Outbreaks in New Caledonia.	Weinberger et al. (2014)	Leptospirosis	2000-2012	There is a significant association between leptospirosis cases and each of the El Niño indices such as rainfall and sea surface temperature anomalies.	Leptospirosis outbreaks should be predicted as of paramount importance for public health decision-makers to implement preventive measures, such as rodent control, riverbank cleanups, and sewage systems to avoid flooding.
35	The interrelationship between meteorological parameters and leptospirosis incidence in Hambantota district, Sri Lanka 2008–2017 and practical implications.	Ehelepola et al. (2021)	Leptospirosis	2008-2017	High soil temperatures, evaporation rate, light duration, and high rainfall coincided with increased prevalence of this disease.	Favorable weather contributed to the to the 2011 leptospirosis outbreak. It represents the first long-term study to show that soil temperature, evaporation rate and climatic phenomenon such as the Indian Ocean dipole rather than El Niño are correlated with leptospirosis in Sri Lanka.
36	Towards a leptospirosis early warning system in northeastern Argentina	Lotto Batista et al. (2023)	Leptospirosis	2009-2020	Floods related to the El Niño phenomenon are associated with leptospirosis.	Climatic events are strong drivers of leptospirosis incidence in Argentina.
37	Extreme water-related weather events and waterborne disease	Cann et al. (2013)	Vibrio cholera	1995-2005	ENSO and extreme temperature changes favor the prevalence of cholera.	Rising global temperature will increase prevalence of waterborne diseases.
38	Cholera forecast for Dhaka, Bangladesh, with the 2015-2016 El Niño: Lessons learned	Martinez et al. (2017)	Cholera	2015-2016	Rainfall and flooding associated with cholera.	There is a teleconnection between the El Niño Southem Oscillation (ENSO) and cholera in Bangladesh.

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$\overset{\circ}{\mathbf{Z}}$	Article title	Author	Diseases	year of the El Niño Phenomenon	Cause /Effect	Conclusion
39	The Impact of El Niño on Diarrheal Disease Incidence: A Systematic Review	Solomon and Bezatu (2017)	Cholera (Diarrhea)	2002-2016	ENSO associated with drought and flooding increase risk of diarrheal diseases worldwide.	A systematic review in which many studies showed a significant association between diarrheal diseases and El Niño. However, research on the impact of El Niño or climatic change in diarrheal diseases are limited.
40	Cholera and Shigellosis: Different Epidemiology but Similar Responses to Climate Variability	Cash et al. (2014)	Cholera and Shigellosis	1985-2005	El Niño has been shown to cause favorable conditions for the increase of Cholera, as well as increased rainfall that causes flooding favorable for the proliferation of Cholera and Shigellosis.	Cholera and Shigellosis are diarrheal diseases whose causative organisms differ in their ecology, transmission routes, dosage and other characteristics. In Bangladesh, inter-annual variations in the outbreaks of infection of both diseases were shown to be related to flooding caused by heavy rainfall due to El Niño phenomenon.
41	Effects of El Niño/La Niña on the Number of Imported Shigellosis Cases in the Republic of Korea, 2004–2017	Kim et al. (2021)	Shigellosis	2004-2017	Shigellosis is considered sensitive to climate; its incidence increases with the onset of droughts and floods.	The incidence of Shigellosis in travelers causes Shigellosis outbreaks in Southeast Asia, so it can be expected that Shigellosis cases will increase significantly among international tourists visiting Korea during the La Niña period.
42	Interannual Variability of Human Plague Occurrence in the Western United States Explained by Tropical and North Pacific Ocean Climate Variability	Ben Ari et al. (2010)	Plague (<i>Yersinia pestis</i>)	1950-2005	Precipitation and high temperatures affect both hosts and vectors transmitting plague. Snow is also key as soil moisture aids the survival and development of plague-transmitting fleas, and vegetation growth favors rodents.	The increase in temperature caused by climate change will decrease soil moisture, which could decrease the survival and development of the flea that transmits plague and could lead to a decrease of this disease in the southwestern United States, but it could increase in New Mexico.
43	A Non-Stationary Relationship between Global Climate Phenomena and Human Plague Incidence in	Kreppel et al. (2014)	Plague	1960-2008	They found a relationship between El Niño, the Indian Ocean dipole phenomenon, temperature, precipitation, and plague incidence.	This study demonstrates the complex and changing relationship between climate factors and plague in Madagascar.
			Parasitic Diseases	səs		

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$\overset{\circ}{\mathbf{Z}}$	Article title	Author	Diseases	year of the El Niño Phenomenon	Cause /Effect	Conclusion
44	Effects of El Niño-Southern Oscillation on human visceral leishmaniasis in the Brazilian State of Mato Grosso do Sul	Da Silva et al. (2020)	Human visceral leishmaniasis	2002-2015	Variations in temperature and the incidence of precipitation on humidity are associated with the incidence of visceral Leishmaniasis in Brazil. Vectors are influenced by temperature, humidity, luminosity, altitude and vegetation, all of which influence parasite transmission.	This study determined that the occurrence of extreme climatic phenomena, such as El Niño and La Niña phases, can significantly influence the incidence of visceral leishmaniasis. The authors demonstrated that El Niño reduces the incidence of Leishmaniasis and La Niña is believed to increase it.
45	Cutaneous Leishmaniasis and Sand Fly Fluctuations Are Associated with El Niño in Panama	Chaves et al. (2014)	Cutaneous leishmaniasis	2000-2010	ENSO precipitation and temperature are associated with this disease.	There is an association between ENSO and cutaneous Leishmaniasis. The variability of temperature and precipitation in Panama is associated with the vector that could cause an epidemiological outbreak.
46	Extreme water -related weather events and waterborne disease	Cann et al. (2013)	Cryptosporidium	1995-2010	Extreme weather conditions, related to contaminated water.	Waterborne pathogen from environmental exposures following extreme climatic conditions.
47	Infectious Disease Sensitivity to Climate and Other Driver- Pressure Changes: Research Effort and Gaps for Lyme Disease and Cryptosporidiosis	Ma et al. (2023)	Cryptosporidiasis	2000-2022	Sensitivity to climate and environmental factors temperature and rainfall.	Improve the availability of data to mitigate infectious diseases associated with climate variability.
84	Effects of the 1997–1998 El Niño Episode on Community Rates of Diarrhea	Bennett et al. (2012)	Cyclospora gayetanensis	1997-1998	High temperatures and low humidity increased the prevalence of diarrhea.	They highlighted the importance of considering pathogens, seasonality, infrastructure, water sanitation and also the effects of climate change in order to predict catastrophic climatic events such as El Niño on public health and thus be able to predict and anticipate in order to mitigate the risks of diarrhea in vulnerable communities.
			Fungal diseases	S		
49	Climate drivers of hospitalizations for mycoses in Brazil	Brito-Silva et al. (2019)	Mycosis	2008-2016	La Niña Phenomenon Climate modulates mycosis hospitalizations with minimum temperatures as a climatic variable.	The influence of the Pacific climatic oscillation was observed, specifically the low temperatures of La Niña, on the prevalence of mycosis in Brazil.
20 C	Expansion of Coccidioidomycosis Endemic Regions in the United States in Response to Climate Change	Gorris et al. (2019)	Coccidiomicosis	2019	Increased temperature and precipitation may alter the endemic regions of this disease.	It was determined that the endemic area of this disease, as well as the number of cases per year, will increase in response to climate change.

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$\overset{\circ}{\mathbf{Z}}$	Article title	Author	Diseases	year of the El Niño Phenomenon	Cause /Effect	Conclusion
51	Coccidioidomycosis (Valley Fever), Soil moisture, and El Niño Southern Oscillation in California and Arizona.	Tobin et al. (2022)	Coccidiomicosis	2009-2012	Fungal infection associated with soil moisture states. There is a moderate but significant connection with El Niño.	This study provides an example of how ocean-atmospheric teleconnections can affect human health.
		Other articles on infectious	Other articles on infectious diseases and the El Niño Southern Oscillation (ENSO) phenomenon	uthern Oscillati	on (ENSO) phenomenon	
52	Infectious Diseases: Research and Treatment	Anderson (2010)	Chikungunya, Dengue, Malaria, Cholera, Rift Valley fever, respiratory diseases, Lyme disease, cutaneous Leishmaniasis.	2005-2006	Drought favored the conditions of the vector causing Chikungunya.	Education focused on environmental health is incorporating community and cultural aspects to reduce the risks of infections caused by mosquito vectors that increase in number during extreme weather events.
			Cooming and the coordinate of			The coastal El Niño of 2017 was
53	Reflections on the impact and response to the Peruvian 2017 Coastal El Niño event: Looking to the past to prepare for the future.	Yglesias-González et al. (2023)	Dengue, Cholera Yellow fever, Malaria, Zika, Leptospirosis, diarrhoeal diseases, respiratory diseases and pneumonia.	2012-2017	The 2017 coastal El Niño in Peru presented conditions called neutral to cold. El Niño caused an increase in rainfall and flooding of rivers, creating strong impacts on health. Increase in infectious and vector-borne diseases.	The coastal El Nino of 2017 was intense and abrupt, which increased heavy rains and floods tenfold, similar to the effects of the 1983 and 1998 El Niño. An increase in infectious and vector-borne diseases was observed, with the largest outbreak of dengue fever ever recorded in Peru and an increase in cases of leptospirosis during the study period.
54	El Niño and human health	Kovats (2000)	Dengue, Australian Encephalitis, Ross River virus, Rift Valley fever, Hantavirus, Cholera, Shigellosis, Typhoid.	1997-1998	Extreme weather conditions causing floods and droughts provoked by the ENSO cycle associated with mosquito and rodent-borne diseases.	There is epidemiological evidence that the El Niño phenomenon is associated with risk of transmission of some diseases in specific geographic areas where climatic anomalies are associated with the El Niño event.
55	Global Disease Outbreaks Associated with the 2015–2016 El Niño Event	Anyamba et al. (2019)	Cholera, Dengue, Chikungunya, Hantavirus, Malaria, Ritk Valley fever, respiratory diseases, Ross River virus disease	2015-2016	The extreme temperature and precipitation caused by El Niño.	It is evident that the extreme weather conditions caused by the El Niño phenomenon are closely associated with a high risk of infectious disease transmission.
26	Multiple impact pathways of the 2015–2016 El Niño in coastal Kenya	Fortnam et al. (2021)	Cholera, Malaria, Rift Valley fever, Dysentery, Diarrhea.	2015-2016	The floods led to conditions suitable for an increase in vector-borne diseases.	Social, ecological and health vulnerability to El Niño and other extreme weather events demonstrated in Kenya.

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$\overset{\circ}{\mathbf{Z}}$	Article title	Author		year of the El Niño Phenomenon	Cause /Effect	Conclusion
22	Influencia de factores climáticos sobre las enfermedades infecciosa	Huarcaya Castilla et al. (2004)	Malaria, Dengue, Bartonelosis, Leishmaniasis, Diarrhea, Cholera, Plague Hantavirus, Ringworm, Pityriasis versicolor, Folliculitis, Pyodermitis, Dermatitis, Lyme disease.	1973-1998	Climate changes caused by El Niño and human migrations have increased the number of cases of these diseases.	Advances in molecular biology and mathematical modeling analysis have been improving our understanding of the biological explanations for infectious diseases, allowing the opportunity to predict outbreaks of infectious disease in areas at risk to obtain the biological explanations for infectious diseases, allowing to predict outbreaks of infectious diseases, allowing to predict outbreaks of infectious diseases at risk to climate variability.
28	Climate and Infectious Diseases	Kelly-Hope and Thomson (2008)	Malaria, Dengue, Meningitis, Meningococcus, Esquistosomiasis, Rotavirus, Leishmaniasis.	1880-1998	Indices that determine El Niño events have been associated with diseases such as Leismaniasis in Brazil, Malaria in Ghana, and Riff Valley fever in Australia.	This review provides a platform from which to launch future research and policy development in relation to climate-sensitive diseases, and suggests that vulnerable countries should be the focus of this research.
59	Climate change and infectious diseases	Flahault et al. (2016)	Rift Valley fever, Cholera, Malaria, Dengue, Chicungunya, Zika, Yellow fever, Flu or influenza.	2004-2016	in East Africa, El Niño- induced excess humidity linked to increase in Rift Valley Fever cases.	Climate scientists have recently observed that climate change is linked to more frequent and intense El Niño events. Increases in the frequency and severity of emerging infectious, vector-borne and airborne diseases in the world can be expected.
09	Climate variability and water-related infectious diseases in Pacific Island Countries and Territories, a systematic review	Hosking et al. (2023)	Cholera, Typhoid fever, Cryptosporidiasis, Dengue, Malaria, Diarrhea, Leptospirosis, Chicungunya, Zika, Hepatitis A.	2022	Temperature, precipitation and humidity as key factors in dengue transmission.	The water supply and hygiene system must be strengthened to reduce infectious diseases during extreme weather events.
61	Impact of El Niño Southern Oscillation on infectious disease hospitalization risk in the United States	Fisman et al. (2016)	Vector-borne diseases, Preumonia and Influenza, enteric diseases, zoonotic bacterial diseases, fungal diseases.	1970-2010	High rainfall and temperatures, very wet or dry conditions increase the risk of enteric diseases.	It is demonstrated the importance of understanding the links between the environment and infection risks when environmental conditions are extreme, such as during El Niño and the need to invest in public health surveillance capable of detecting changes in disease burdens.

3.3 El Niño Phenomenon and bacterial diseases

In the case of leptospirosis, research by Weinberger et al. (2014) on the relationship between EN-SO and leptospirosis in New Caledonia shows that this disease exhibits seasonal outbreaks in the tropics. Using time series data from 2000 to 2012, the authors evaluated whether climatic factors such as ENSO and meteorological conditions can predict leptospirosis outbreaks. They found that periods coinciding with La Niña were associated with abundant precipitation, which in turn was related to outbreaks of this disease. Their study demonstrated a strong association between ENSO and leptospirosis, suggesting that similar studies should be replicated in regions of the South Pacific, Asia, or Latin America where El Niño also induces climatic variability that poses a risk of outbreaks. Leptospirosis cases were above the annual average observed from 2012 to 2016. However, the incidence of other diseases sensitive to ENSO, with shorter incubation periods such as acute respiratory infections, diarrhea, and pneumonia, was lower than the reported during 2012-2016 (Weinberger et al., 2014).

Leptospirosis outbreaks are closely related to climatic events like floods caused by heavy rainfall, considered a risk factor associated with the disease. For example, plague outbreaks in Colorado and New Mexico are linked to abnormal precipitation (Anyamba et al., 2019). Plague is caused by *Yersenia pesti*, a zoonotic bacterium found in small mammals and their parasitic fleas. Abundant winter precipitation followed by hot summers leads to increased flea production, as noted by the authors.

Additionally, heavy rainfall influences the contamination of surface water with sewage, representing common causes of diarrhea associated with contaminated water supplies and floods, directly impacting the transmission of typhoid fever and shigellosis. Typhoid fever is common where sanitation is poor and potable water is unavailable, similar to conditions for shigellosis. In areas without water supply, events like heavy rainfall often lead to unusual outbreaks of these diseases due to contamination of drinking water with sewage (Anyamba et al., 2001, 2019).

Kim et al. (2021) studied the effects of El Niño

and La Niña on the number of shigellosis cases in Korea from 2004 to 2017. They demonstrated that the risk of shigellosis infection increased with the rise in La Niña index, as fluctuations caused by La Niña in South and Southeast Asian countries affect water sanitation.

Floods and ENSO cannot be directly related to disease transmission. However, droughts can be linked to increased pathogens in surface water and hygiene-related diseases. ENSO-induced temperature increases directly influence gastrointestinal infections. For instance, the 1997-1998 El Niño in Peru caused temperature rises that resulted in a significant number of children being hospitalized with diarrhea (Kovats, 2000).

Solomon and Bezatu (2017) conducted a systematic review on the impact of El Niño on the mortality and morbidity of diarrheal diseases. This study found a significant relationship between ENSO and these diseases. Childhood diarrheal diseases cause morbidity and mortality in developing countries, and ENSO has been shown to affect the dynamics of diarrhea incidence in South America and Asia. However, the effects of this event on diarrhea in sub-Saharan Africa, where the burden of diarrheal infections among children under five is highest, are not well understood. Diarrhea is a leading cause of mortality in Africa due to the lack of water and basic services.

Diarrhea usually occurs due to infections caused by rotavirus or bacteria, leading to the deaths of thousands of children due to the loss of salts, electrolytes, and nutrients. Southern Africa and Southwest Asia account for nearly 80% of all diarrhearelated deaths globally, possibly linked to the lack of nutrient-rich water, exacerbating the epidemiological situation in these children. ENSO is characterized by a warming period of sea surface temperatures and the consequent suppression of nutrient-rich cold water currents, which follows the pattern observed off the coasts of Peru and Ecuador and generally lasts between 12 and 18 months.

Regarding enteric diseases in the western United States, a decrease in this pathology was observed, with increased risk in other regions of the country. Thus, both wet and dry conditions increase the risk of enteric diseases (Fisman et al., 2016).

3.4 El Niño Southern Oscillation and parasitic and fungal diseases

Climate change triggers extreme weather events that have been associated with parasitic diseases worldwide, with leishmaniasis being one of the most studied. Da Silva et al. (2020), in their research on the effect of ENSO on human visceral leishmaniasis, mention that the increase in visceral leishmaniasis cases in Panama was associated with the cold phase of ENSO. They also note that factors influencing the variation in the number of visceral leishmaniasis cases in Brazil include environmental variables such as temperature, light, humidity, altitude, and vegetation cover. These factors can influence the transmission of the parasite and the development of the disease.

Regarding *Cryptosporidium*, Cann et al. (2013) mention that most outbreaks of parasitic diseases occurred after extreme water-related weather events, such as severe storms, heavy rains related to ENSO, cyclones, seawater flooding, hurricanes, or tides. After an extreme weather event, the probable causes of parasitic infections could be water contamination, scarcity of potable water, and poor sanitation and hygiene.

In Peru, it was reported that most of the population affected by floods presented skin diseases, mainly among children. The most frequent cases included infections, excoriations or superficial wounds, allergic dermatitis, pyodermatitis, mycoses, among other pathologies (MSP/OPS, 1989). However, there are few studies that reveal the effect of ENSO as a trigger for mycoses. To evaluate coccidioidomycosis in the United States, a soil-borne fungal disease common throughout the southwestern U.S. and related to soil moisture, Tobin et al. (2022) analyzed the correlation between ENSO, soil moisture, and coccidioidomycosis incidence from 2009 to 2012. They observed a moderate and significant connection between ENSO, soil moisture, and coccidioidomycosis. The authors demonstrated that ocean-atmosphere teleconnections could affect human health.

Gorris et al. (2019), in their study on the expansion of coccidioidomycosis in endemic regions of the United States in response to climate change, determined that elevated temperatures could shift the

location of this fungal disease from the southeastern U.S. to also affect the western part of the country. By 2100, the west is expected to become hotter and more affected by rain, conditions that favor the fungus and could double the number of affected individuals in the country. The Coccidioides fungus thrives in areas with little rainfall and high temperatures, so when soil moisture is high, accompanied by high temperatures, it favors the dispersion and transmission of the fungus, as observed between 2009 and 2012, facilitated by ENSO.

As previously noted, ENSO events may become more frequent due to climate change. Although El Niño events are global, the magnitude of their impact varies, with some regions considered teleconnected to ENSO. This means they experience climatic anomalies related to ENSO despite being thousands of kilometers away, while other regions are not teleconnected (Fisman et al., 2016).

3.5 Strengths and Limitations

This study focused on research regarding infectious diseases and the influence of climatic anomalies caused by ENSO, conducted between 2000 and 2023. It included the strong El Niño-Southern Oscillation events of 1982-1983, 1997-1998, and 2016-2017. The geographical areas selected for the research were those vulnerable to El Niño events, where studies on epidemics or infectious diseases had been conducted. The search terms were very broad, and some articles were excluded because they dealt with general infectious diseases, such as diarrhea, which can be caused by bacteria, parasites, or viruses, or respiratory diseases without specifying the causative microorganism.

For viral infectious diseases like Zika and Chikungunya, few studies were found for the years 1982-1983 and 1997-1998. Similarly, few studies on parasitic or fungal diseases met the inclusion criteria. The broad search terms and the search for complete citations may have overlooked important literature. Additionally, possible biases could have arisen from not conducting a more exhaustive meta-analysis. Limited data availability and heterogeneous climatic indicators should also be considered.

Furthermore, the vulnerable geographical areas

are typical in developing countries that do not generate data or research comparing epidemiological cases with environmental or climatic data. More research is recommended on the influence of extreme climatic variables under ENSO, which generally leads to catastrophes like wildfires, floods, and droughts, usually resulting in an increase in viral, bacterial, parasitic, and fungal diseases.

There is a need to determine the nature of ecological mechanisms and their relationship with diseases, analyzing the ecological foundations of diseases. Links should be established between health professionals, policy makers, and meteorological predictors to predict long-term epidemiological risks in areas vulnerable to extreme climatic factors. Additionally, early epidemiological alerts should be created to mitigate the proliferation of diseases caused by viruses, bacteria, parasites, and fungi.

4 Conclusions

The climatic variability caused by ENSO promotes the emergence of imported infectious diseases, which can become endemic rather than being eradicated. The projected consequences of diseases resulting from ENSO will vary depending on how the phenomenon manifests (flooding, drought, temperature increase, or heavy precipitation).

To forecast the repercussions in different endemic areas due to ENSO, it is imperative to consider risk factors, climate variability, and the geographical context. These factors should be integrated with programs that control infectious diseases. Attention should be focused on the climate and the necessity for programs to adapt and identify changes in morbidity and mortality induced by the climate. This will help stratify the risk of these diseases influenced by climatic variations, aiding in decision-making.

It is crucial to better understand the links between the environment and the risk of infection in various regions to establish working groups, perhaps prioritizing the most prevalent infectious diseases in the region.

Author contribution

P.M.: Conceptualization, Data processing, Data curation, Methodology, Visualization, Writing- original draft, Writing- review and Editing; G.V.S.: Conceptualization, Conceptualization, Data processing, Data curation, Methodology, Visualization, Writing-original draft, Writing- review and Editing.

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CLIMATE RISK IN LOCAL HEALTH SERVICES IN ECUADOR

RIESGO CLIMÁTICO EN LOS SERVICIOS DE SALUD LOCAL EN ECUADOR

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Abstract

This work analyzes the interaction between climate risk, caused by extreme events resulting from the El Niño-Southern Oscillation (ENSO) and/or Climate Change, and its impact on local health services in Ecuador. The analysis focuses on Ecuador's growing vulnerability to the effects of climate change, which exacerbates challenges in the delivery of health services, particularly in the context of severe climate events. The main objective is to analyze the relationship between the number of hectares affected by climate events and the availability and effectiveness of local health services, considering community resilience and health coverage as moderating variables. The methodology uses a linear regression analysis using data from 221 Ecuadorian municipalities, covering variables such as climate risk, health services, community resilience, health coverage, and demographic and socioeconomic factors. The results indicate a significant influence of climate risk on the effectiveness of health services, moderated by community resilience and health coverage show a better capacity to respond to extreme weather events. Finally, it is important to integrate climate risk management into health services planning, suggesting that greater community resilience and broad health coverage are essential to mitigate the negative impacts of climate change on public health.

Keywords: Climate Risk; Health services; Resilience, Ecuador.

Resumen

El presente trabajo analiza la interacción entre el riesgo climático, originados por eventos extremos producto del fenómeno El Niño-Oscilación del Sur (ENOS) y/o Cambio Climático, y su impacto en los servicios de salud local en Ecuador. El análisis se centra en la creciente vulnerabilidad de Ecuador a los efectos del cambio climático, que exacerba los desafíos en la prestación de servicios de salud, particularmente en el contexto de fenómenos climáticos severos. El objetivo principal es analizar la relación entre el número de hectáreas afectadas por eventos climáticos y la disponibilidad y eficacia de los servicios de salud locales, considerando la resiliencia comunitaria y la cobertura sanitaria

como variables moderadoras. La metodología emplea un análisis de regresión lineal utilizando datos de 221 municipios ecuatorianos, abarcando variables como riesgo climático, servicios de salud, resiliencia comunitaria, cobertura sanitaria, y factores demográficos y socioeconómicos. Los resultados indican una influencia significativa del riesgo climático en la eficacia de los servicios de salud, moderada por la resiliencia comunitaria y la cobertura sanitaria. Se observa que las áreas con mayor preparación y cobertura sanitaria muestran una mejor capacidad de respuesta ante eventos climáticos extremos. Finalmente, es importante integrar la gestión del riesgo climático en la planificación de servicios de salud, sugiriendo que una mayor resiliencia comunitaria y una amplia cobertura sanitaria son esenciales para mitigar los impactos negativos del cambio climático en la salud pública.

Palabras clave: Riesgo Climático; Servicios de Salud; Resiliencia, Ecuador.

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1 Introduction

Climate change constitutes a significant global challenge. Combined with natural climate variability events, such as the El Niño Southern Oscillation -ENSO-, it has dramatic effects on populations, especially in Latin America and Ecuador (García-Parra et al., 2022). ENSO, characterized by the warming of the Central Pacific Ocean, has important repercussions on global weather patterns, causing extreme meteorological events (Alatrista-Salas et al., 2021). In Ecuador, these events manifest through increased rainfall, floods, and droughts, directly impacting agriculture, healthcare services, and infrastructure. Additionally, indirect effects include injuries, diseases, deaths, significant economic losses, and mental health consequences for affected individuals (Toulkeridis et al., 2020).

Thielen et al. (2023) and Hidalgo et al. (2024) detail the impact of ENSO extreme events on Ecuador's precipitation, highlighting the region's increased vulnerability to such climatic anomalies. Furthermore, Rollenbeck et al. (2022) provide a meteorological radar analysis of the 2017 coastal ENSO event, emphasizing the severe challenges faced by Ecuador and Peru.

For the purposes of this study, climate risk is defined as the likelihood of extreme weather events, such as floods and droughts, negatively impacting natural and human systems, exacerbating existing vulnerabilities (Field and Barros, 2014). These risks are assessed in terms of their frequency, magnitude, and the extent of damage caused.

In this context, healthcare services primarily refer to the healthcare infrastructure, including the availability of health facilities, their operational capacity, and population accessibility (Hahn, 2019). This encompasses hospitals, clinics, and other care centers integral to the local health system and its ability to provide preventive, care, and emergency services (Phillips, 2005). The focus is not on the quality of individual care or specific public health programs but on the overall availability and responsiveness of the healthcare infrastructure (Moyo et al., 2023).

In Ecuador, health facilities are classified into four levels of care and complexity. The first level in-

cludes basic health centers. The second level covers basic hospitals with limited specialized services. The third level consists of general hospitals with a wide range of specialties. The fourth level comprises specialty hospitals and high-complexity centers, offering advanced and specialized care (Ron et al., 2018).

Ecuador's healthcare services face significant challenges due to extreme weather events, exacerbated by the ENSO (Barberán et al., 2019). These events have direct and indirect impacts on public health in Ecuador. For example, increased rainfall and flooding can lead to outbreaks of waterborne diseases, while droughts can affect food security and nutrition (Thielen et al., 2023). Ecuador's healthcare services, already strained by limited resources, struggle to respond to these climate-induced health crises.

Rollenbeck et al. (2022) highlight the profound impact of extreme ENSO events on Ecuador's precipitation patterns, emphasizing the urgency for healthcare services to adapt to these changing climatic conditions. Therefore, it is important to study climate risk in local healthcare services in Ecuador to improve understanding of the implications for health economics and climate risk management (Fernandez et al., 2015).

The intersection between climate risk and healthcare delivery in Ecuador represents a critical yet underexplored research area (Subía-Cabrera and Subía-Cabrera, 2022). Despite acknowledging the broad impacts of climate change on public health, few specific studies examine how these changes affect healthcare delivery in the country (Vaccaro Witt et al., 2023). This gap is significant, given Ecuador's vulnerability to climatic events like ENSO, which can drastically alter disease patterns, healthcare needs, and the overall demand for health services (de Guenni et al., 2017).

This study would not only contribute to the academic knowledge but also provide practical insights for policymakers and health service planners in Ecuador. Therefore, the following research questions arise: How does ENSO specifically alter the demand for health services in different regions of Ecuador? How are the capacity and distribution of health services in Ecuador affected by extreme

weather events associated with ENSO?

The conceptual foundations exploring the nexus between health services and climate risk are based on a multidisciplinary approach, combining insights from environmental health, health economics, and disaster risk management. Environmental health theories elucidate the direct and indirect pathways through which climate change affects health outcomes, emphasizing the role of environmental determinants in shaping health vulnerabilities (McMichael et al., 2003). Health economics models, such as the supply and demand framework, are fundamental for analyzing how climateinduced health risks alter the demand for health services and the corresponding adjustments needed in service delivery (Phillips, 2005). Additionally, disaster risk management theories offer frameworks for understanding the resilience and adaptive capacities of health systems in the face of climate risks, highlighting the importance of preparedness, response, and recovery phases to mitigate the impact of climate-related health emergencies (Paton and Johnston, 2017).

The relationship between health services and climate risk in Ecuador, particularly under the ENSO, has provided valuable insights but also presents several limitations (Arjona et al., 2016). Methodologically, many studies rely on retrospective analyses of health outcomes and climate patterns, which may not adequately capture the complex, bidirectional interactions between climatic events and health service responses (Gutierrez et al., 2020; Sorensen et al., 2017; Thielen et al., 2023). The objective of this study is to evaluate the climate risk on health services in Ecuador, focusing on how the effects of ENSO influence the demand for services in different cities.

2 Materials and Methods

ENSO significantly impacts public health in Ecuador, increasing the prevalence of waterborne diseases, respiratory infections, and vector-borne diseases such as dengue, malaria, and cholera (Sorensen et al., 2017). Vulnerable rural populations face barriers to accessing healthcare due to displacement and economic hardships (Díaz-Vélez et al., 2020). Additionally, there are disparities in the accessibility and quality of health services between urban and rural areas, exacerbated by socioeconomic fac-

tors (Gutierrez et al., 2020; Liu et al., 2023). Therefore, it is necessary to improve emergency preparedness, enhance disease surveillance and response mechanisms, and integrate climate risk assessments into the planning and delivery of healthcare services (Schwartz et al., 2023). In this context, the following hypothesis is proposed: *H1*: Climate risk significantly affects local health services in Ecuador.

2.1 Community Resilience

Community resilience to natural disasters and climate change encompasses a wide range of concepts and measures, focusing on the capacity of communities to anticipate, prepare for, respond to, and recover from adverse situations. This resilience is built on several pillars, including robust infrastructure, effective local governance, social cohesion, and economic diversification (Aldrich and Meyer, 2015). Community resilience measures often involve assessments of these pillars, along with the capacity for adaptive learning and the implementation of sustainable environmental management practices (Patel et al., 2017; Pacheco-Peña et al., 2023).

Examples of municipal strategies to increase resilience to ENSO include the development of early warning systems, the construction of flood-resistant infrastructure, and the establishment of community emergency response teams (Vilema and Mendoza, 2014). In Ecuador, municipalities have engaged in reforestation projects to reduce landslide risks and have implemented water management strategies to address the challenges posed by both excess water during heavy rains and water scarcity during droughts (Vilema and Roman, 2018).

Additionally, the implementation of local public policies on resilience and the effectiveness of health service responses have considered integrating a health approach into broader municipal planning for disaster risk reduction and climate adaptation (Arjona et al., 2016). Studies have shown that municipalities prioritizing the resilience of health services in their planning processes experience fewer disruptions in healthcare delivery and can provide more effective responses to public health emergencies induced by the ENSO (Gutierrez et al., 2020; Sorensen et al., 2017; Vilema et al., 2017; Vilema and Mendoza, 2014). Therefore, the following hypothesis is proposed: *H2*: Community resilience modera-

tes the effect between climate risk and local health services in Ecuador.

2.2 Health Coverage

Comprehensive health coverage plays a fundamental role in enhancing the resilience of vulnerable populations to the impacts of climate change. Effective health coverage can reduce these populations' exposure to climate-related health risks by improving access to preventive care, emergency services, and disease management programs (Moyo et al., 2023). Additionally, integrating climate change considerations into health coverage policies can guide the development of specific interventions aimed at reducing health disparities and improving public health preparedness for the effects of climate change (Ansah et al., 2021).

Comprehensive health coverage ensures timely access to health services, thereby reducing morbidity and mortality associated with climate-induced health problems (Gutierrez et al., 2020). For instance, during the 1997-1998 ENSO episode, areas with extensive health coverage in Ecuador were able to provide more effective disease surveillance, early warnings, and timely interventions, significantly reducing adverse health outcomes associated with the episode (Thielen et al., 2023).

This coverage included the establishment of mobile health units, the stockpiling of essential medications, and the training of healthcare workers on climate-related health risks (Patel et al., 2017; Rollenbeck et al., 2022). Therefore, the following hypothesis is proposed: *H3*: Health coverage moderates the effect between climate risk and local health services in Ecuador.

2.3 Geographic Diversity

The importance of geography in the planning and delivery of health services in the face of climate risks cannot be underestimated. Geographic considerations are crucial for developing tailored health service delivery models that address the specific vulnerabilities of each region (Fernandez et al., 2015; Vilema and Mendoza, 2014). For example, coastal areas may require robust infrastructure to withstand flooding, while mountainous regions might benefit from enhanced food security programs to prevent malnutrition during ENSO events (Gutierrez et al., 2020; Thielen et al., 2023). Thus, incorporating geographic diversity into health service planning allows for the development of adaptive strategies that are sensitive to the distinct needs and vulnerabilities of different regions (Schwartz et al., 2023).

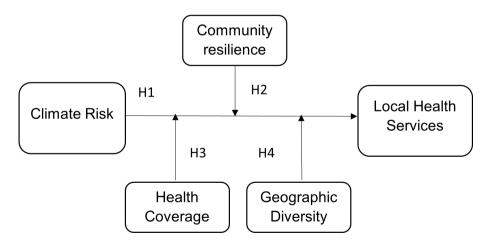


Figure 1. Research model.

Geographic factors in Ecuador significantly influence the relationship between health services and climate risk, highlighting the importance of spatial analysis to understand vulnerability and response capacity (Vilema et al., 2024). Gutierrez et al. (2020) mention that higher altitude areas face different challenges compared to coastal regions, including variability in temperature and precipitation patterns that affect disease vectors differently.

Díaz-Vélez et al. (2020) note the spatial correlation between ENSO-induced climate anomalies and outbreaks of climate-sensitive diseases such as malaria and dengue. Therefore, the following hypothesis is proposed *H4*: Geographic diversity drives the effect between climate risk and local health services in Ecuador.

To analyze the influence of climate risk on local health services in Ecuador, data from the country's 221 municipalities are used, considering various information sources and years. Table 1 presents the study variables used to test the hypotheses, and the following linear regression equation is proposed.

$$services_i = \beta_0 + \beta_1 risk_i + \beta_j \sum_{n=1}^{j=2} \chi_i + \beta_k \sum_{n=1}^{j=2} Z_k + \varepsilon$$
 (1)

In Equation 1, the proposed statistical model is shown, where, services $_i$ is the dependent variable local health services in canton i. risk $_i$ is the independent variable climate risk of city i. $\sum_{n=2}^{j-2} \chi_i$ is a set of moderating variables for city i is (community resilience and health coverage). $\sum_{n=2}^{j-2} Z_k$ is a set of control variables for city i (population, per capita income, coastal, highlands, Amazon). $\beta_0, \beta_1, \beta_j, \beta_k$ are the regression coefficients representing the relationship between the variables. And ϵ the error term, capturing the variation not explained by the independent variables.

The participation of local health services in the city is an indicator that highlights the health sector's contribution to the overall economy, providing information on the importance of resource allocation, efficiency, and the sector's capacity to respond to health crises, including those exacerbated by climate risks (Phillips, 2005). Analyzing investments in the health sector correlates with improvements in accessibility, quality, and distribution of health services, crucial factors for mitigating the impacts of

climate change on public health (Moyo et al., 2023).

On the other hand, the indicator of hectares affected by hazardous events due to increased precipitation and temperature serves as a critical measure for assessing climate risk. This metric quantitatively captures the extent of environmental and agricultural damage, directly reflecting the increased vulnerability and exposure of ecosystems and human settlements to climate-induced hazards (Field and Barros, 2014).

In evaluating the study's hypotheses, the variability in data quality across different municipalities is considered, as it could influence the study's results. This disparity in data quality could be due to differences in monitoring and reporting capacity for climate and health events among municipalities. Municipalities with better recording and monitoring systems may provide more accurate and comprehensive data, while those with limited resources may have less reliable data. This difference in data quality is accounted for in the analyses and mitigated by using multiple data sources and applying robust statistical techniques to control for potential biases and variations.

3 Results and Discussion

Table 2 presents descriptive statistics crucial for understanding the impact of climate risk on the delivery of local health services in the Ecuadorian context. The data reveal that, on average, the gross value added (GVA) of local health services, expressed in thousands of dollars, is 5.078, with a standard deviation of 6.362, indicating significant variability among cities in terms of the economic contribution of health services to the total GVA of the city.

Regarding climate risk, measured by the number of hectares affected by hazardous events related to increased precipitation and temperature between 2010 and 2020, the mean is alarmingly high, with 4 225.756 hectares affected and a standard deviation of 27 179.89, highlighting the extreme variability and significant impact of these events in the country. Community resilience, assessed through the municipal operational capacity index, shows an average of 17.279 with a standard deviation of 3.835, reflecting differences in emergency response capa-

city. Health coverage, measured by the number of standard deviation of 2.034, evidencing discrepanhealth establishments, has a mean of 14.185 and a cies in the availability of health services.

Table 1. Study variables.

CODE	DESCRIPTION	MEASUREMENT	SOURCE	
	Local health services measured by the		Regional	
Services	share of the Gross Value Added (GVA) of	Thousands of	Accounts	
Services	the health economic branch in the Total	dollars	- (Banco Central	
	Gross Value Added of the city in 2020.		del Ecuador, 2022)	
	Climate risk measured by the number		Threat and	
	of hectares affected by reported		Hazardous Event	
Risk	hazardous events due to increased	Hectares	Monitoring Report	
KISK	precipitation and temperature during	ricciares	- Secretaría Nacional	
	2010 to 2020.		de Gestión de	
	2010 to 2020.		Riesgos (2022)	
			GAD monitoring	
	Community resilience Measured by		and evaluation	
Resilience	the municipal operational capacity	Scores	report - Consejo	
	index (2016-2020).		Nacional de	
			Competencias (2022)	
	Health coverage measured by the		Health in figures	
Cover	number of health facilities (2020).	Establishment	- Ministerio de Salud	
	number of health facilities (2020).		Pública (2022)	
			Population	
	Share of the city's population in the total		projections 2010- 2020	
Pop20	population, projection to the year 2020.	Inhabitants	- Instituto Nacional	
			de Estadística y	
			Censos (2022)	
	Per capita income in the city	Thousands of dollars	Regional Accounts	
Ingpc	GVA / Population (2020)	per capita	- Banco Central	
	GVA / Topulation (2020)	рег сариа	del Ecuador (2022)	
Coastline	Coastal region	1 = yes ; 0 = no	Population and	
Highland	Highlands region	1 = yes ; 0 = no	Housing Census	
Amazon	Amazon region	1 = yes; 0 =no	- INEC (2022)	

Table 3 presents a linear regression analysis to evaluate the impact of climate risk, measured by the number of hectares affected by hazardous events, on local health services, represented by the share of the Gross Value Added (GVA) of health in the local economy. The results demonstrate a statistically significant negative relationship between climate risk and local health services, both in the model without control variables (coefficient = $-1.57*10^{-5}$, p < 0.01) and in the model with control variables (coefficient = $-1.36*10^{-5}$, p < 0.01).

This finding suggests that an increase in hectares affected by hazardous climate events is associated with a decrease in the economic contribution of the health sector at the local level. These results provide empirical evidence in support of Hypothesis H1, which postulated that climate risk significantly affects local health services in Ecuador. The statistical significance of the climate risk coefficient in both models underscores the relevance of climate change and its adverse effects as a critical determinant of the capacity and efficiency of local health services.

Table	2	Descri	ntive	Statistics.
Table	4.	Desch	puve	Statistics.

Variable	Mean	Std. Dev.	Units
Services	5.078	6.362	Miles US \$
Risk	4225.756	27179.89	Hectares
Resilience	17.279	3.835	Points
Coverture	14.185	2.034	Establishments
Pop20	0.452	1.534	Inhabitants
Ingpc	3.388	5.094	Miles of US \$
Coastline	0.402	0.4915	%
Highland	0.375	0.485	%
Amazon	0.144	0.352	%

Table 4 presents a detailed analysis investigating the moderating effect of community resilience on the relationship between climate risk and local health services. The results indicate that, both with and without controls, climate risk has a significantly negative impact on local health services (coefficients of $-1.55*10^{-5}$ and $-1.35*10^{-5}$, respectively, p < 0.001), highlighting the vulnerability of health services to extreme climate events. However, community resilience, measured by the municipal operational capacity index, did not show a statistically significant moderating effect on this relationship (coefficients of -0.0191 without controls and -0.00539 with controls, p > 0.05).

These findings provide partial evidence for Hypothesis *H*2, which postulated that community resilience moderates the effect of climate risk on local health services in Ecuador. While climate risk demonstrates a negative effect on health services, the lack of statistical significance in the moderating role of community resilience suggests that other factors may be influencing the capacity of communities to mitigate the impacts of climate risk on health ser-

vices. Table 5 examines the moderating effect of health coverage on the relationship between climate risk and local health services, contributing to the understanding of how extensive health coverage can mitigate the adverse effects of climate change on healthcare delivery.

The results reveal that, without controls, climate risk has a significant negative effect on local health services with a coefficient of $-1.57 * 10^{-5}$ (p < 0.01), while, with controls, this effect intensifies to $-2.14 * 10^{-5}$ (p < 0.01).

Notably, health coverage emerges as a significant moderator in this context, with a positive coefficient of 0.119 (p < 0.05) in the controlled model. This finding indicates that greater health coverage helps mitigate the negative impacts of climate risk on health services. Consequently, these results support Hypothesis H3, suggesting that effective health coverage plays a crucial role in moderating the adverse effect of climate risk on local health services in Ecuador.

Table 3. Linear regression between local health services and climate risk.

Variables	No controls (1)	With controls (2)	
Risk	-1.57*10 ⁻⁵ ***	-1.36*10 ⁻⁵ ***	
NISK	$(4.64*10^{-6})$	$(4.55*10^{-6})$	
Constant	5.145***	5.055***	
Constant	(0.434)	(1.718)	
Observations	221	221	
R-squared	0.004	0.040	

Robust standard errors in parentheses: *** p < 0.01, *** p < 0.05, * p < 0.1

Variables	No controls (1)	With controls (2)
Risk	-1.55*10 ⁻⁵ ***	-1.35*10 ⁻⁵ ***
KISK	$(4.77*10^{-6})$	$(4.71*10^{-6})$
Resilience	-0.0191	-0.00539
Resilience	(0.0938)	(0.0972)
Constant	5.475***	5.148***
Collstant	(1.661)	(2.194)
Observations	221	221
R-squared	0.005	0.040

Table 4. Linear regression of the moderating effect of community resilience between local health services and climate risk.

Robust standard errors in parentheses:

Table 6 presents a pioneering analysis exploring how geographic diversity modulates the impact of climate risk, associated with ENSO, on local health services. The regression model used reveals that climate risk, measured in terms of hectares affected by extreme climatic events, has a significant relationship with the capacity of local health services, adjusted for the geographic influence of the coastal, highlands, and Amazon regions. Specifically, it was found that climate risk increases local health services by 0.0734 units for each unit increase in risk in the coastal region (p < 0.05), while a similar decrease is observed in the highlands and Amazon regions, indicating a negative relationship between climate risk and health services in these areas.

This finding supports Hypothesis *H4*, which postulated that geographic diversity drives the effect between climate risk and local health services

in Ecuador. It indicates that the geographic regions of the country not only differ in terms of their exposure and vulnerability to climate risk but also in how this risk affects the availability and demand for health services.

The results reveal significant interactions between climate risk and the capacity of local health services to respond effectively to emerging needs, particularly in the context of the ENSO. Linear regression analyses indicate that climate risk, measured by the number of hectares affected by hazardous events ($\beta = -1.57*10^{-5}***, p < 0.001$), has a significantly negative impact on local health services, confirming Hypothesis H1. This underscores the vulnerability of the health sector to extreme climatic changes and events, affecting its operational capacity and emergency response.

Table 5. Linear regression of the moderating effect of health coverage between local health services and climate risk.

Variables	No controls (1)	With controls (2)		
Risk	-1.57*10 ⁻⁵ ***	$-2.14*10^{-5}***$		
NISK	$(4.74*10^{-6})$	$(4.77*10^{-6})$		
Coverture	0.000699	0.119**		
Coverture	(0.0166)	(0.0475)		
Constant	5.136***	4.223***		
Constant	(0.511)	(1.735)		
Observations	221	221		
R-squared	0.004	0.063		

Robust standard errors in parentheses:

^{***} p < 0.01, ** p < 0.05, * p < 0.1

^{***} p < 0.01, ** p < 0.05, * p < 0.1

Variables	No controls (1)	With controls (2)
Risk	0.0734*	0.0714*
KISK	(0.0375)	(0.0378)
Risk x Coastal	-0.0735*	-0.0714*
KISK X COASTAI	(0.0375)	(0.0378)
Diels v Highlands	-0.0735*	-0.0714*
Risk x Highlands	(0.0375)	(0.0378)
Risk x Amazon	-0.0741**	-0.0720*
KISK A AIIIaZUII	(0.0375)	(0.0378)
Constant	5.046***	5.648***
Collstallt	(0.434)	(0.532)
Observations	221	221
R-squared	0.039	0.057

Table 6. Linear regression of the geographic diversity effect between local health services and climate risk.

Robust standard errors in parentheses: *** p < 0.01, ** p < 0.05, * p < 0.1

The moderating influence of community resilience on the relationship between climate risk and local health services, although not statistically significant in all models ($\beta = -0.0191, p > 0.05$), suggests a trend towards mitigating the negative impact of climate risk, partially supporting Hypothesis H2. This finding highlights the importance of strengthening community resilience as a strategy to improve the response capacity of health services to extreme climatic events.

On the other hand, health coverage shows a positive and significant moderation ($\beta = 0.119 * *, p <$ 0,01) in the relationship between climate risk and local health services, supporting Hypothesis H3. This result emphasizes the crucial role of extensive and accessible health coverage in mitigating the adverse effects of climate risk on public health. Finally, the effects of geographic diversity ($\beta = 0.0714*, p <$ 0,05 for specific regions) corroborate Hypothesis H4, indicating that geography plays a determining role in how climate risk affects local health services. Specific regions, such as coastal and Amazonian areas, show significant variations in vulnerability and response capacity, highlighting the need for adaptive and customized approaches in health planning.

4 Conclusions

This study evaluated the impact of climate risk, particularly events associated with ENSO, on local

health services in Ecuador. Through a comprehensive analysis of data from 221 municipalities, we revealed how climate variability significantly affects the capacity and distribution of health services. Community resilience and health coverage proved to be significant moderators in this relationship, suggesting that strengthening local response capacity and access to health services can mitigate the negative effects of climate risk. However, geographic differences introduce notable variability in this effect, underscoring the importance of adapting mitigation strategies to the specific characteristics of each region.

Despite these significant findings, the study faced limitations, including variability in data quality among municipalities and the difficulty of capturing the full complexity of health systems and their interaction with climate factors. Future research should explore in greater detail how specific adaptation and mitigation interventions can improve the resilience of health systems to climate change, especially in more vulnerable areas. Additionally, better integration of health and climate data is crucial for developing more accurate predictive models to guide public health planning in the context of climate change.

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Author contribution

S.V.E.: Conceptualization, Data processing, Data curation, Methodology, Visualization, Writing- original draft, Writing- review and Editing; M.M.O.: Conceptualization, Conceptualization, Data processing, Data curation, Methodology, Visualization, Writing- original draft, Writing- review and Editing.

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ECOLOGICAL, ECONOMIC AND SOCIAL IMPACTS OF THE COLOMBIAN COCOA SECTOR

IMPACTOS ECOLÓGICOS, ECONÓMICOS Y SOCIALES DEL SECTOR CACAOTERO COLOMBIANO

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Abstract

Due to its excellent quality, Colombian cocoa has become a product that has made considerable inroads in the national and international market, increasing its demand and the effects inherent to its production practices. This scope review was carried out following the guidelines of the Joanna Briggs Institute (JBI), which allowed the selection of 72 documents, whose information extraction led to the identification and standardization of the economic, ecological and social impacts of the cocoa sector in the different producing cities of Colombia, of which Santander, Antioquia, Arauca, Huila and Tolima stand out since they generate 70% of the cocoa production at the national level. In the review, a total of 13 social, 25 ecological and 21 economic impacts are clearly observed, which correspond to the most persistent impacts evidenced in the different investigations carried out in this country.

Keywords: impact, cocoa, Colombia, producers

Resumen

El cacao colombiano, debido a su excelente calidad, se ha convertido en un producto que ha incursionado de manera considerable en el mercado nacional e internacional, lo que ha incrementado su demanda y con ello los efectos inherentes a sus prácticas productivas. Esta revisión de alcance se realizó siguiendo las orientaciones del Joanna Briggs Institute (JBI), lo que permitió la selección de 72 documentos, cuya extracción de información condujo a la identificación y homologación de los impactos económicos, ecológicos y sociales del sector cacaotero en los diferentes departamentos

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productores de Colombia, de los cuales destacan los departamentos de Santander, Antioquia, Arauca, Huila y Tolima, quienes generan el 70% de la producción de cacao a nivel nacional. En la revisión, se observa claramente un total de 13 impactos sociales, 25 ecológicos y 21 económicos, que corresponden a los impactos más persistentes evidenciados en las diferentes investigaciones desarrolladas en este país.

Palabras clave: impacto, cacao, Colombia, productores

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1 Introduction

Global agriculture is growing with the increasing population, which drives a rise in the demand for products that ensure the availability of safe and nutritious food. Cocoa is a fundamental and extremely important product in global agriculture, due to its numerous derivatives and products manufactured from it. Africa dominates the market with 77% of global production, followed by Latin America, where the global production of cocoa beans is 17% (International Cocoa Organization, 2019). Colombian cocoa stands out for its high quality, aroma, and flavor, ranking tenth in the world after the Dominican Republic, with a production of 63,416 tons in 2020, of which 11,148 tons were exported (Cataño, 2019; Baquero, 2021).

African countries, such as Côte d'Ivoire and Ghana, are the world's leading cocoa producers, followed by South American countries like Ecuador and Brazil (International Cocoa Organization, 2021). However, cocoa production, like any other agricultural production, generates adverse environmental effects. For instance, in Côte d'Ivoire, only 4% of its territory remains covered with tropical rainforest, and in 13 of its 23 protected areas, the populations of primates and elephants have completely disappeared. According to the NGO Mighty Earth, one of the main reasons for this is the expansion of cocoa cultivation fields (Claus et al., 2018; Jagoret et al., 2020). In Ghana, alternatives such as climate-smart agriculture, whose objectives are climate change mitigation and adaptation and food security, pose a threat of inequity to small producers while favoring multinational dominance (Taylor, 2018; Maas et al., 2020; Nasser et al., 2020). These data are truly alarming and require urgent intervention due to the significant impact the cocoa sector is causing not only in Africa but globally.

Garmendia et al. (2005) and Mereddy et al. (2017) define environmental impact as the alteration of environmental quality generated by anthropogenic activities, both directly and indirectly. Generally, these impacts are evaluated from ecological and socioeconomic perspectives, measuring changes in individual well-being as long as these can be attributed to an activity, project, or policy as a causal relationship (Gertler et al., 2017). This makes impact assessment a priority component for the

development and implementation of sustainable agriculture, ensuring food production and distribution, and restoring natural resources (Bergez et al., 2022; Kross et al., 2022).

Studies conducted by Schroth et al. (2011); Braga et al. (2019); Rocha et al. (2019) in Brazil, and Hands (2021) in other Latin American countries indicate that only through production by small farmers with reduced agricultural management in a forest landscape (agroforestry) can the normalcy of ecosystem services and the conservation of wild communities be guaranteed. According to studies conducted by Brito et al. (2018) and Rooduijn et al. (2018) in Mexico, cocoa production in agroecosystems positively involves carbon sequestration, reduction of greenhouse gas emissions, soil fertility and biodiversity, and water quality. Similarly, studies conducted in Bolivia by Armengot et al. (2017) and Chumacero et al. (2018) describe the impacts of cocoa production on biodiversity according to the farming system and diversification of farm production, although also noting adverse socioeconomic aspects such as lack of access to credit, increased labor, and consequently, increased production costs.

Several studies have been conducted on the Colombian cocoa sector in different producing cities, but none provide sufficiently clear national data on the environmental impact of this sector, which is so important to the Colombian economy. The objective of this review is to identify the ecological, economic, and social impacts generated by cocoa cultivation in Colombia, based on the frequency of mention in studies related to cocoa cultivation in this territory.

2 Methodology

The procedure for conducting this review followed the guidelines proposed by the *Joanna Briggs Institute* (JBI) (Peters et al., 2015, 2020) and the *Preferred Reporting Items for Systematic review and Meta-Analyses extension for Scoping Reviews* (PRISMA-ScR) (Tricco et al., 2018).

2.1 Study Selection

The selection process for sources of evidence included three phases that defined the studies to be reviewed for extracting information. These phases co-

rrespond to the identification of sources of evidence and studies, the review and search strategies, and the eligibility of documents for obtaining and analyzing information according to the defined selection criteria.

2.2 Identification of Sources of Evidence

Based on the review question defined as "What is the environmental impact generated by cocoa agricultural production in Colombia?" the objective was to review the positive and negative impacts at the ecological, economic, and social levels of cocoa production in Colombia. Due to its inclusive nature, the review is classified as a scoping review according to Munn et al. (2022), allowing for the extension or broadening of the nature of the documents to be studied in particular contexts, identifying various evidence and limitations of the existing information (Pollock et al., 2022). Given the limited abundance of publications on this topic, the review included scientific articles from specialized journals, undergraduate and postgraduate theses (master's and doctoral), institutional documents regardless of the methodological approach, and conference abstracts.

2.3 Search Strategies

The Colombian cities with significant cocoa influence were identified, and the level of studies conducted in these influential cocoa regions of the country was reviewed, including the applied research approaches and methodologies. During the search and data collection, repositories from different university libraries and documents from the National Federation of Cocoa Growers (FEDECACAO) were consulted, and the following databases were used: *Proquest, IFPRI, Redalyc, Sage, Science Direct, AGRIS, Google Scholar, Springer Journal, Scielo.* The search

2.5 Data Extraction and Processing

The impacts cited in these publications were recorded in a matrix for classification, standardization, and frequency determination of citations. As the process is iterative, the matrix was refined during data extraction. Finally, the matrix for data recording included study characteristics such as source category and origin, publication year, author de-

was conducted using the following keywords and Boolean operators: economic impacts, social impacts, ecological impacts, certification, cocoa culture, cocoa, to obtain documents related to the information analysis. The search equation used was (economic impacts OR social impacts OR ecological impacts) AND (cocoa OR «cocoa culture») AND (Colombia), and searches were also conducted including the keywords in the equation in both English and Spanish followed by "", "\$" and ().tw. This strategy yielded 182 records along with three unpublished institutional documents, from which 109 were filtered out after removing duplicates.

2.4 Eligibility and Inclusion

The potential sources of evidence were fully downloaded and evaluated by at least two reviewers following the inclusion criteria presented below:

- Published as articles in scientific journals, books, undergraduate and postgraduate theses.
- Written in English or Spanish.
- Published between the years 2000 and 2020.
- Studies evaluating the Colombian territory.
- Studies covering topics related to cocoa only.

Disagreements among the reviewers during the selection process were resolved with an additional reviewer. After the evaluation, a total of 72 documents with the necessary characteristics were identified for their inclusion in this study. These included 51 peer-reviewed articles, 17 theses, 1 symposium report, and 3 institutional documents. The selection process is shown in Figure 1 with the records identified at each phase through a PRISMA flow diagram.

tails, title, keywords, study area, sample, research methodological design, environmental impacts by category, and description of the impact. With the data condensed in the table, the most studied regions of the country on this topic were defined, the most frequent methodological designs were identified, and the various impacts were standardized according to the authors' descriptions.

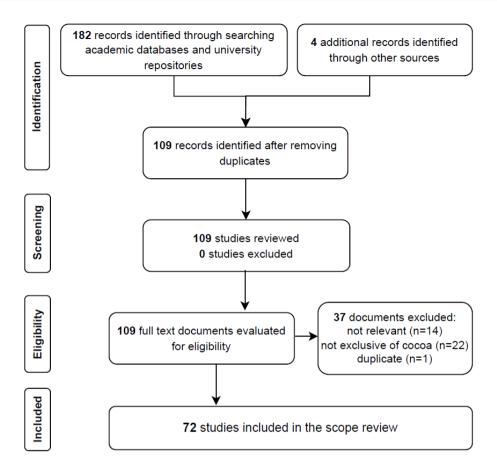


Figure 1. PRISMA flowchart with scoping review study selection process.

3 Results

3.1 Cocoa-Producing states in Colombia

In the review, thirty (30) Colombian cocoaproducing states were identified as having significant participation in the sector. Moreover, it was evident that five (5) of these states have the highest percentage of national production, as highlighted in Table 1: Santander, Antioquia, Arauca, Huila, and Tolima, which collectively account for 72% of the country's cocoa production. Santander stands out as the most influential, contributing 42% of Colombian cocoa production (Fedecacao, 2019).

 Table 1. Major cocoa producers in Colombia, production quantity, cultivated area and percentage share in the Colombian market.

State	Production (t)	Production (%)	Cultivated Area (ha)	National Cultivated Area (%)
Santander	25158	42	73699.20	42.11
Antioquia	5259	9	15406.00	8.80
Arauca	4546	8	13317.30	7.61
Huila	4051	7	11867.22	6.78
Tolima	3928	7	11506.89	6.58

3.2 Most Evaluated Cocoa-Producing states

The 72 studies reviewed in this research are distributed across 18 states in Colombia (Table 2). Nearly 70% of these studies focus on Santander (27.1%), Norte de Santander (17.1%), Antioquia, and Huila (11.4% each). It is noteworthy that several of the selected documents mentioned more than one state within their study areas.

Table 2. Cocoa growing states with the highest frequency of occurrence.

State	Frecuency
Santander	19
Norte de Santander	12
Antioquia	8
Huila	8
Arauca	6
Cundinamarca	6
Nariño	5
Meta	4
Tolima	3
Cesar	3
Boyacá	3
Caquetá	2
Valle del cauca	2
Caldas	1
Bolívar	1
Guaviare	1
Vichada	1
Amazonas	1

3.3 Methodological Approaches Employed in the Analyzed Studies

During the review of the selected documents, information was obtained to determine the methodologies employed by the authors in their investigations (Table 3). It was identified that the predominant methodology in these studies is mixed-methods research, with a frequency of 70% among the selected studies. Additionally, it was noted that some documents did not clearly state their research methodology.

Table 3. Methodologies used in the studies analyzed in this review.

Methodologies used	Frecuency
Qualitative	7
Quantitative	14
Mixed	49
The methodological approach	2
is not indicated or is not clear	2
Total	72

3.4 Ecological Impacts

The impacts most frequently identified in the review were ecological, with 14 positive and 11 negative impacts named in 56 out of the 72 selected documents. It is important to clarify that many studies reported overlapping impacts; thus, Table 4 presents the impact and the frequency with which it was referenced.

The increase in measures aimed at protecting biological diversity is the most frequently mentioned impact by the authors of the selected studies (Ramírez et al., 2014; Gutierrez et al., 2015; Agudelo, 2016; Suárez, 2018; Cubillos, 2017; Hernández et al., 2018; Naranjo et al., 2017; Ordoñez, 2019). These studies indicate the relationship of this impact with environmental awareness within the Colombian cocoa sector and various public and private entities that direct efforts towards sustainable productivity and the protection of the diversity of plant and animal species inhabiting these spaces, especially when cultivation is small-scale and conducted within appropriate agroforestry systems (Zoe et al., 2018).

The second most replicated impact in the review was the increased application of pesticides in cocoa crops (Ortiz et al., 2016; Vásquez et al., 2018; González et al., 2018; Ferro and Meneses, 1996; Sánchez and Gamboa, 2014; García-Cáceres et al., 2014). This can cause health issues for individuals directly and indirectly exposed to such substances, resulting in increased infections and diseases in humans (Montoya et al., 2015; Villamizar et al., 2016). Furthermore, the lack of knowledge among cocoa farmers regarding pesticide application leads to significant crop losses due to inefficient protocols and controls, affecting production yields and increasing soil contamination (Torrente, 2016).

Table 4. Ecological impacts of cocoa farming in Colombia.

Impacts	Type	Frequency	% citations
Increased measures aimed at biodiversity protection	Positive impact	33	22.15
Increased pesticide application on cocoa crops	Negative impact	18	12.08
Decrease in good agricultural practices (GAP)	Negative impact	8	5.37
Increased contamination of water resources	Negative impact	7	4.7
Reduction in carbon dioxide emissions	Positive impact	7	4.7
Increased soil conservation measures	Positive impact	7	4.7
Increased soil yields	Positive impact	7	4.7
Increased air pollution	Negative impact	6	4.03
Decrease in the contamination of water resources	Positive impact	6	4.03
Increased nutrient alteration of soils by using artificial components	Negative impact	5	3.36
Increased investment in the improvement of food safety, phytosanitary management and international certifications.	Positive impact	5	3.36
Increased landscape modification	Negative impact	5	3.36
Increase in the impact on biodiversity	Negative impact	5	3.36
Increased conservation of water resources	Positive impact	4	2.68
Increased use of environmentally friendly fertilizers	Positive impact	4	2.68
Increased adaptability to climate change	Positive impact	4	2.68
Decrease in the use of artificial fertilizers	Positive impact	3	2.01
Decrease in the use of pesticides	Positive impact	3	2.01
Increase in human infections and pests	Negative impact	3	2.01
Increase in shade plantings	Negative impact	2	1.34
Decrease in logging of forests and jungles	Positive impact	2	1.34
Reduction of forest fires	Positive impact	2	1.34
Increase in greenhouse gases	Negative impact	1	0.67
Decrease in water resources	Negative impact	1	0.67
Increased utilization of wastes	Positive impact	1	0.67

The third impact identified was the decline in good agricultural practices (GAP) (Ferro and Meneses, 1996; Sánchez and Gamboa, 2014; Arias et al., 2016; Contreras, 2014; Ramírez et al., 2014). It is noted that the Colombian cocoa sector becomes negligent when their cocoa lacks certification, directly affecting processes related to safety, quality, and production. This impact also leads to an increase in landscape modification (Ruiz, 2014; Ramírez et al., 2014; Espinoza and Ríos, 2016; Carrillo, 2017; Correa, 2017; Caviedes and Olaya, 2020) and biodiversity loss (Montoya et al., 2015; González et al., 2018; Rodríguez et al., 2017; Carrillo, 2017; Correa, 2017) due to non-compliance with necessary protocols for proper agricultural practices. This negligence is also due to the cocoa sector's disinterest, knowing that the lack of certification directly affects their income.

The fourth impact identified during the review is the increase in contamination of water resources (Montoya et al., 2015; Ferro and Meneses, 1996; Ramírez et al., 2014; Ramos et al., 2016; Correa, 2017). This analysis indicates that, in addition to soil impacts, poor agricultural practices are indirectly affecting water sources near the planting areas, causing significant damage to the diversity of species that inhabit these areas and rely on this resource. This can lead to reduced water flow (Montoya et al., 2015).

3.5 Economic Impacts

It was evident that economic impact records were mentioned in more than 70% of the reviewed documents, with 14 positive impacts and 7 negative impacts identified. Their frequency and percentage of occurrence are presented in Table 5.

The most frequently noted impact was the decreased profitability of conventional cocoa due to the expansion of certified cocoa production (Pedroza, 2012; Rangel et al., 2013; Sánchez and Gamboa, 2014; García-Cáceres et al., 2014; Arias et al., 2016;

Ortiz, 2015). Cocoa farmers tend to produce lower-quality cocoa with lower investment without certification to ensure product quality. However, the second most frequently noted impact was the improvement in cocoa quality (Carrillo et al., 2014; Plazas et al., 2017; Machado et al., 2018; Suárez, 2018; Barragán and Rey, 2004).

Table 5. Economic impacts of cocoa farming in Colombia.

Impacts	Type	Frequency	% citations
Decrease in the profitability of conventional cocoa due to the expansion in the generation of certified cocoa.	Negative impact	23	18.55
Cocoa quality improvement	Positive impact	16	12.9
Cocoa sector market development	Positive impact	14	11.29
Increasing programs and project initiatives in the cocoa sector	Positive impact	12	9.68
Decrease in investment interest in the cocoa sector	Negative impact	9	7.26
Increase in cocoa growers' and cocoa entrepreneurs' confederations	Positive impact	6	4.84
Increased production in the cocoa sector	Positive impact	6	4.84
Increase in disputes between certification programs	Positive impact	5	4.03
Increase in the evolution and technological progress of the cocoa sector.	Positive impact	5	4.03
Increased economic progress and development in the region	Positive impact	5	4.03
Decrease in certified cocoa	Negative impact	4	3.23
Expansion of the cocoa sector	Negative impact	3	2.42
Increased economic dependence of the population on the cocoa sector	Negative impact	3	2.42
Increased compliance with domestic regulations	Positive impact	3	2.42
Increased support and sponsorship from national and international organizations	Positive impact	2	1.61
Increase in the price of the product	Positive impact	2	1.61
Decrease in production quotation	Positive impact	2	1.61
Increased access to investment loan securities	Positive impact	1	0.81
Increased production costs	Negative impact	1	0.81
Increase in the replacement of cocoa with other crops	Negative impact	1	0.81
Decrease in consumption and acquisition of external inputs	Positive impact	1	0.81

The third most frequent impact was the increase in the cocoa sector market (Rojas et al., 2008; Jaimes et al., 2011; Arias et al., 2016; Ramírez et al., 2014; Arias et al., 2016; Osorio et al., 2017). This impact is parallel to the increased production in the cocoa sector (Correa et al., 2014; Álvarez et al., 2015; Montoya et al., 2015; Plazas et al., 2017; Cely, 2017), driven by high international recognition and increased market demand, generating profitability and increased trade in the sector (Correa et al., 2014; Ál-

varez et al., 2015; Montoya et al., 2015; Plazas et al., 2017; Cely, 2017). This has led to the formation of cocoa producers and entrepreneurs' confederations (Barragán and Rey, 2004; Unión Europea, Ministerio de Comercio, Industria y Turismo, 2011; Sánchez and Gamboa, 2014; Gutiérrez et al., 2020), granting prestige and credibility to this activity in the region. Generally, this contributes to economic progress and development in the region (Rodríguez, 2011; Unión Europea, Ministerio de Comercio, In-

dustria y Turismo, 2011; Pabón et al., 2016; Ortiz et al., 2016; Cataño, 2019). In some regions, economic dependency on the cocoa sector has been observed (García-Cáceres et al., 2014; Cardona et al., 2016; González et al., 2018).

The fourth most predominant impact in the reviewed studies is the increase in programs and project initiatives in the cocoa sector (Forero et al., 2015; Rodríguez, 2017; Suárez, 2018; Unión Europea, Ministerio de Comercio, Industria y Turismo, 2011; Correa et al., 2014; Ruiz, 2014). These programs leverage acquired knowledge to improve planning and resource management, facilitating the optimal development of projects aimed at increasing cocoa productivity. However, the fifth most referenced impact indicates that investment interest in the cocoa sector has declined (García et al., 2012; Rodríguez et al., 2017; Oliveros, 2013; Contreras, 2014; Morillo et al., 2014; Cardona et al., 2016; Contreras, 2017; Rodríguez, 2017), due to low quality and productivity in some regions.

3.6 Social Impacts

The number of social impacts was the smallest, with 10 positive and 3 negative impacts, appearing in

more than 50% of the reviewed documents. Table 6 presents the frequency and percentage of reference for each impact.

The main social impact identified by the authors of the reviewed documents was the increase in training and educational programs in the cocoa sector (Unión Europea, Ministerio de Comercio, Industria y Turismo, 2011; Sánchez and Gamboa, 2014; Morillo et al., 2014; Montoya et al., 2015; Ortiz, 2015; Pabón et al., 2016). This involves entities sending qualified personnel who share their knowledge with producers to assist in the development and maintenance of crops, thereby revitalizing the regional economy (Barragán and Rey, 2004; Sánchez and Gamboa, 2014; Arias et al., 2016; Vásquez et al., 2018).

Closely related to this, the second most referenced impact was the increase in research alternatives (Cleves et al., 2013; Forero et al., 2015; Ramos, 2014; Morillo et al., 2014; Cubillos, 2017; Plazas et al., 2017; Rodríguez, 2017). These are aimed at improving productivity, working conditions, and agro-industrial processes for cocoa transformation.

Table 6. Social impacts of cocoa farming in Colombia.

Impacts	Type	Frequency	% citations
Increased teaching and training procedures in the cocoa sector	Positive impact	12	23
Increase in research alternatives	Positive impact	7	13
Increased labor conditions for cocoa farmers	Positive impact	7	13
Increase in jobs	Positive impact	6	11
Reduction of poverty in the rural sector	Positive impact	4	8
Decrease in illicit crop production	Positive impact	4	8
Knowledge application and wage improvement	Positive impact	3	6
Consolidation of the cocoa sector culture	Positive impact	3	6
Inequity among cocoa sector producers	Negative impact	2	4
Reduction in the occupation of children in the labor sector	Positive impact	2	4
Increase in school absenteeism	Negative impact	1	2
Decrease in jobs	Negative impact	1	2
Increasing the involvement of women in cocoa sector activities	Positive impact	1	2

The improvement of working conditions for cocoa farmers was the third most frequently recorded social impact (Rodríguez, 2011; Fiallo, 2014; Parrado and Torres, 2017; Diaz and Sierra, 2018; Cataño, 2019; Velásquez, 2019; Vásquez et al., 2018). This not only allows farmers access to the benefits of farming machinery but also supports families by helping them initiate or continue their studies, gradually reducing child labor (Rodríguez, 2011; Rangel et al., 2013).

The fourth most frequently recorded impact in the reviewed documents is the increase in job opportunities due to high market demand, which requires a larger workforce during production (Rangel et al., 2013; Ramos et al., 2016; Vásquez et al., 2018; González et al., 2018; Correa, 2017; Cely, 2017). The increase in employment generated by cocoa cultivation in many regions has led to improved wages, greater purchasing power, and contributed to the reduction of rural poverty (Trujillo and Perdomo, 2016; Ortiz et al., 2016; González et al., 2018; Cataño, 2019; Jaimes et al., 2011; Correa et al., 2014; Suárez, 2018). This aspect even contributes to conflict resolution, as being a legal crop, cocoa has presented as an alternative to illicit crops (Vásquez et al., 2018; Suárez, 2018).

4 Conclusions

The available studies documenting the environmental impacts generated by cocoa farming in Colombia are limited in number. It was found that ecological and economic aspects are the most frequently described, while the social aspect is addressed in only 50% of the available documents. However, regarding the negative impacts generated by this activity, social impacts are significantly less identified than ecological and economic ones.

Considering cocoa as a raw material, the transformation process is basic, and its commercialization is closely tied to market price variations. Therefore, it is imperative that the respective support policies for cocoa farmers encourage the implementation of new strategies to differentiate the product in the market. This can be achieved through origin demarcations, type of cocoa, city and country of production, and quality standards in production processes, given that Colombia is one of the stron-

gest and most significant cocoa-consuming industries. Such support focuses on development within local institutions.

It is evident that an excellent and robust product articulation in the market is necessary, alongside the creation of good business alliances for strategy development and the establishment of strong governmental relationships to secure capital support. Considering that to initiate these activities, it is necessary to commercialize cocoa beans with support and interventions aimed at improving the infrastructure of storage centers, enabling better control of product volume and quality by implementing internal control systems directed at obtaining or adopting certification programs.

Finally, organizational pilot projects should be undertaken to initiate a quality production process, thereby improving the quality of life of cocoa farmers through these new financial models. This approach aims at participation in local and international organizations that support farmer integrity, intervening not only in cultivation but also in economic and social aspects.

The findings of this review provide a master list and baseline available to the scientific community for future environmental impact studies. Additionally, this information can be used in the formulation of evidence-based public policies and as support for planning impact assessments of different programs or policy modalities that are implemented or intended to be implemented, aimed at supporting cocoa farmers in Colombia.

Author contribution

D.I.C.R.: Conceptualization, Research, Writing- reviewing and editing, Supervision; F.E.P.G.: Research, data processing, Writing- original draft; K.C.A.V: Conceptualization, Methodology, Validation, Visualization.

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FORESTRY





EFFECT OF CONSERVATION TEMPERATURE ON CAVANILLESIA PLATANIFOLIA SEED VIABILITY

EL EFECTO DE LA TEMPERATURA DE CONSERVACIÓN SOBRE LA VIABILIDAD DE LAS SEMILLAS DEL PIJÍO CAVANILLESIA PLATANIFOLIA

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Abstract

Cavanillesia platanifolia is a tree species distributed in the tropical dry forest ecosystem threatened by the fragmentation and loss of its habitat. Reforestation is thus to be considered for its conservation. The ex situ production of young individuals is key for any reforestation plan. In this study, we compare the recruitment capacity in nursery of two seed conservation treatments: cold conservation vs. conservation at ambient temperature. The emergency rate increased and the seedlings grew more vigorous in cold-preserved seeds. In addition, seedling mortality was significantly reduced in cold-preserved seeds, which we believe is due to a slowdown in seed ageing and a decrease in pathogenic activity. This easily replicable and economic nursery cultivation methodology can be incorporated into the potential reforestation plans of C. platanifolia, as well as to other threatened plant species of the neotropic with seeds of similar characteristics.

Keywords: Tropical dry forest, seed conservation, Chocó-Darién, seedling recruitment, Malvaceae-Bombacaceae.

Resumen

Cavanillesia platanifolia es una especie de árbol distribuido en el ecosistema de bosque seco tropical que se encuentra amenazado por la fragmentación y pérdida de su hábitat. La reforestación es una actividad a considerar para su

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conservación, y para ello es clave optimizar la producción de individuos jóvenes *ex situ*. En el presente estudio comparamos la capacidad de reclutamiento en vivero de dos tratamientos de conservación de las semillas: conservación en frío vs. conservación a temperatura ambiente. La tasa de emergencia se incrementó y las plántulas crecieron más vigorosas en las semillas conservadas en frío. Además, mediante la conservación en frío de las semillas se redujo notablemente la mortalidad de las plántulas, lo cual creemos que es debido a una ralentización del envejecimiento de las semillas y a la disminución de la actividad patogénica. Esta fácilmente replicable y económica metodología de reproducción en vivero puede ser incorporada a los potenciales planes de reforestación de *C. platanifolia*, así como a otras especies vegetales amenazadas del neotrópico con semillas de similares características.

Palabras clave: Bosque seco tropical, conservación de semillas, Chocó-Darién, germinación de semillas, Malvaceae-Bombacaceae.

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1 Introduction

The Neotropical dry forest stands out for its size, biodiversity, and ecosystem services (Calvo-Rodriguez et al., 2017; Hubbell, 1979). These forests have been subjected to significant anthropogenic impact over the past decades (Aguirre et al., 2017). Particularly in Ecuador, a Neotropical country with the highest number of plant species classified as at least vulnerable (The International Union for Conservation of Nature)(IUCN, 2019), it is estimated that 50% of the dry forest area has disappeared in recent decades due to human activities (Aguirre et al., 2005), mainly as a result of deforestation and land conversion to agricultural and livestock purposes (Madriñán, 2014; Aguirre et al., 2017; Aguirre and Kvist, 2009). The impact these forests are subjected to contrasts with their status as one of the least studied ecosystems across various ecological scales (Aguirre et al., 2017). To identify, establish, and maintain restoration and conservation actions, it is therefore necessary to expand the biological knowledge of at least the most significant plant species (Aguirre et al., 2006).

One of the most significant species in the Ecuadorian dry forest is *Cavanillesia platanifolia* (Humb. Bonpl.) Kunth (Aguirre et al., 2017; Espinosa et al., 2011), commonly known in Central and South America as mocundo de Cartagena, bongo/a, macondo, cuipo, güipo, hameli, hamelí, pretino, pigio, or pijío. Its distribution mainly lies within the tropical dry forests of the Chocó-Darién corridor, from Panama to northern Peru, but it is also present in moist forests.

This tree, belonging to the Malvaceae-Bombacaceae family, can exceed 40 meters in height (Figure 1A), and in Ecuador, it plays a notable role as a habitat generator, among others, for the nesting of the Guayaquil macaw Ara ambigua guayaquilensis (López-Lanús and Socola, 2000), and for the nutritional contribution of its seed, which is edible and a resource for local fauna (Adler, 1995; Madriñán, 2014). Its significance at a landscape and structural level is also noteworthy (Aguirre, 2012). It is utilized as a timber resource by local communities in various countries, such as Panama (Correa et al., 2004), although there is no record of this in Ecuador (Madriñán, 2014). The species has been declared globally threatened in Central America (IUCN,

2019). In Colombia, where it has been indexed as one of the most important species of the tropical dry forest (Ruiz and Saab, 2020), it is considered endangered (IUCN, 2019). Its potential distribution in this country may not be severely threatened by climate change, although it is expected that it could decimate its populations (Aguirre et al., 2017).

Many characteristics are common among the different species of the genus, such as sciophily; the seedlings (Figure 1B) and juveniles grow in places where solar radiation is scarce (Vieira et al., 2007; Montalvo et al., 2013). Generally, these species are characterized by their low density (Vieira and Scariot, 2006b; Vieira et al., 2008; Melo-Júnior et al., 2015), rapid growth (Condit et al., 1993), and drought tolerance during the dry season (Wolfe, 2017). In September-October, they produce their large, fleshy fruits, containing an anemochorous seed with an outer shell that includes soft mucilage (Vieira and Scariot, 2006b). The seed traits have been studied primarily in C. arborea. The seeds of this species weigh around 8g fresh, while the dry weight is about 1g (Vieira and Scariot, 2006b; Romero-Saritama, 2016). Over time, the seeds lose viability (Walters et al., 2005) and are sensitive to desiccation (Vieira and Scariot, 2006b; Vieira et al., 2007, 2008; Lima et al., 2008). As with many species of the tropical dry forest, the low humidity keeps the seeds dormant, which can be interrupted during the rainy season, activating the embryo for germination (Debeaujon et al., 2000).

When individuals of *C. platanifolia* have been sampled in the dry forests of Ecuador for conservation and restoration plans, the most relevant finding is the low presence of seedlings and juveniles, indicating a very low rate of natural regeneration (Villalba-Briones, personal observation). The absence of young individuals of *C. platanifolia* not only occurs near adult individuals, often related to high density-dependent mortality due to pathogens, seed predators, and/or herbivory under the maternal canopy (Alvarez-Loayza and Terborgh, 2011; Comita et al., 2014; Xu et al., 2015), but is consistent across the forests.

In other species of Malvaceae-Bombacaceae from the Neotropics, such as *Pachira quinata* (Jacq.) Alverson, low rates of natural regeneration have also been observed, attributed to decreased fruit

production, intense fruit predation, and seedling mortality due to pathogens (Castellanos and Stevenson, 2011).

Regarding seed predation, it is known that in other species of the genus *Cavanillesia*, such as *C. arborea* (Willd.) Schum. and *C. chicamochae* Alonso, their seeds are intensely predated both predispersively (various insects and parrots of the family Psittacidae) and post-dispersively (rodents and other mammals), and seed predation can significantly increase with silvopastoral activity (Vieira and Scariot, 2006a; Vieira et al., 2007; Díaz-Pérez et al., 2011; Souza-Silva et al., 2015). While silvopas-

toral activity is not as intense in the dry forests of Ecuador (Aguirre, 2012), the rate of seed predation, particularly by rodents may have increased due to the continued loss of carnivorous mammals (Catterall, 2018) and defaunation of large herbivores due to hunting pressure (Rosin and Poulsen, 2016). More importantly, as observed for *C. arborea* and *C. chicamochae*, other anthropogenic activities such as forest fragmentation and land conversion may be intensely reducing the emergence and recruitment of *C. platanifolia* seeds (Vieira and Scariot, 2006b; Vieira et al., 2007; Melo-Júnior et al., 2015; Souza-Silva et al., 2015).

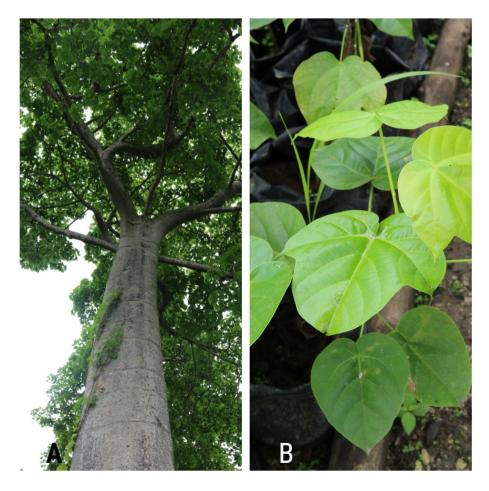


Figure 1. Cavanillesia platanifolia (Humb. & Bonpl.) Kunth (A) Specimen from the sampling point in the Guayas province (Ecuador), with its characteristic transverse rings on the trunk. (B) Seedling in nursery with its simple and trilobed leaves.

Understanding fundamental concepts in biology, such as seed viability and recruitment capacity, represents a crucial area of investigation in the realm of species conservation. Within tropical ecosystems, seeds play a pivotal role in the reproductive process, with their persistence, survival, and emergence serving as key determinants for the generation of seedlings and young trees pivotal to restoration initiatives (Vieira and Scariot, 2006a; Sarmiento et al., 2017). Particularly, the effective conservation of seeds is imperative for the planning and execution of species propagation within a nursery setting, as a strategy for ex situ conservation (Becerra-Vázquez et al., 2018). This study examines the hypothesis that cold storage of C. platanifolia seeds may enhance their germination, survival rates, and overall yield.

2 Materials and Methods

2.1 Study area

The dry forest in Ecuador is located on the western flank of the Andes, in the provinces of Esmeraldas, Manabí, Guayas, El Oro, and Loja (Aguirre et al., 2017). Cavanillesia platanifolia is commonly found in these forests below 800 meters above sea level (Jørgensen and León-Yanes, 1999; Aguirre et al., 2006). According to Espinosa et al. (2011), it is distributed across the provinces of El Oro, Loja, Manabí, Guayas, and in the Tumbesian dry forest, as well as in the province of Esmeraldas (Aguirre et al., 2017).

For this study, seeds of *C. platanifolia* were collected from Hacienda Pabeclar, located in the Chongón Protective Forest (2° 7′ 13.97"S, 80° 4′ 57.78"W; Guayas province, Figure 2). This region, bordering the Chongón-Colonche Mountain range and being part of the Chocó-Darién corridor on the coast of Ecuador, is a priority conservation target (Mittermeier et al., 2011). In the forest area, agricultural activity is increasing, mainly due to the expanding cultivation of corn (Figure 2). The seed collection took place in November 2017, in an area of approximately 6000 m^2 , selecting those from the ground that showed no signs of predation (Bonfil-Sanders et al., 2008; Orantes-García et al., 2013). Within the collection area, the individuals (N=6) of C. platanifolia were characterized, which in this area had a height circumference (CBH) at 1.30 m from the ground of 321 cm to 570 cm and a height of 25 to 37 m, measured using the Range Finder technique (Wing et al., 2004).

2.2 Conservation methodology, nursery methodology, seed sowing, and monitoring of seedling emergence and performance

2.2.1 Pilot test

A greenhouse pilot study with 187 seeds (starting the seed hydration on February 28, 2018) was conducted to verify the viability of the collected seeds. Prior to planting, from the previously mentioned collection, the seeds were stored in a dark, enclosed space at room temperature $(21.3^{\circ}C-26.8^{\circ}C)$ for 3 months. Afterwards, the seeds underwent a physical scarification process, and were then soaked for 48 hours. According to Trujillo (1995), this hydration should not be less than two hours, but also not exceed 48 hours to avoid possible fermentation.

After hydration, seeds were arranged in $1m^2$ x 30 cm high squares with 2 cm between them. Due to the high mortality of *C. platanifolia* seedlings caused by pathogens (Villalba-Briones et al., personal observation), a silica-rich sand substrate with a fungicidal effect was used. The squares were placed in a nursery (greenhouse) covered with a saran mesh, which reduces solar radiation by 80% and favors the emergence of seeds from other species of the genus (Vieira et al., 2008). From that point, the squares were watered in the nursery 5 days a week for 10 minutes by misting. From the time of planting, and over 1 month, we measured the cumulative emergence rate, which was 89.3%.

2.2.2 Response test to conservation conditions

With seeds of the same origin and collection date, we proceeded with the experimental trial, with a longer storage time than in the pilot test. For this experiment, first, the collected seeds of *C. platanifolia* were stored dry in a closed space protected from external environmental elements for a total of 3 months (November 2017 to February 2018), after which they were randomly assigned to 2 treatments with 55 seeds per treatment. For the first treatment, the seeds were stored for an additional 6 months at room temperature (21.3- 26.8°*C*) and in darkness.

For the second treatment, the seeds were stored in a refrigerator $(4.3-7.6^{\circ}C)$ for the same period. Through the cold storage conservation treatment, we aimed to verify if it favors the conservation of seeds maintaining their viability. This has been suggested

by several authors (Becerra-Vázquez et al., 2018; Li and Pritchard, 2009), although high intraspecific variability has also been observed (Posada et al., 2014; Truiillo, 1995).

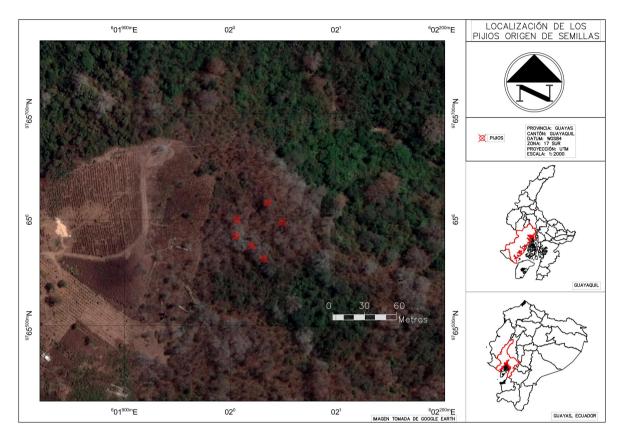


Figure 2. Map representing the seed collection area of *Cavanillesia platanifolia* in the Guayas province (Ecuador), marking the location of the characterized trees. Map developed using ArcGIS 10.3.

This observed intraspecific variability is largely due to the morphological and physiological characteristics of the seeds, such as their water content and thermotolerance (Kranner et al., 2010; Sasaki, 2008). For our experimental trial, we opted for refrigerator conservation as it is cost-effective and easily reproducible, considering that conservation in liquid nitrogen (-196°C) does not significantly increase emergence in other species like C. arborea (Lima et al., 2008). After 6 months of treatment and prior to planting, the seeds underwent a physical scarification process, and were then soaked for 24 hours. After hydration, we proceeded to sow and cultivate in the nursery (greenhouse) in the same

way as described for the pilot trial. At the same time, the seed development progression was photographically recorded in a glass container adapted for image collection (Figure 3).

From the moment of sowing and over 1 month, we measured the cumulative emergence rate and mortality of the emerged seedlings. From these variables, we also estimated recruitment success (emergence + survival). In turn, we measured the growth (height) of the seedlings at 14 and 25 days after sowing (see example of seedling in Figure 4). Throughout the process, environmental parameters of temperature, relative humidity, and dew point

were measured. The measurements were taken with a thermohygrometer, taking 3 measures per square/day to then make the daily average. The measurements were homogeneous over time, with a variation of $3^{\circ}C$ in temperature (27.3-30.3°C) and $2.8^{\circ}C$ in dew point (21.7-24.5°C). Regarding relative humidity, it did not fluctuate beyond 25% between the different measurements (65.1-85.3%).

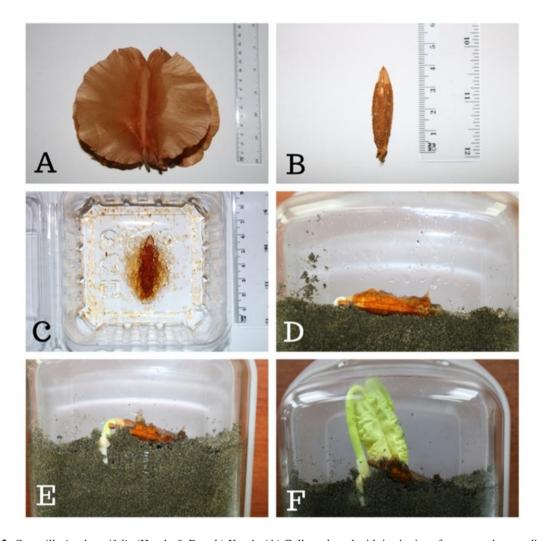


Figure 3. Cavanillesia platanifolia (Humb. & Bonpl.) Kunth. (A) Collected seed with its 4 wings for anemochorous dispersion. (B) Seed once dehulled. (C) Seed hydrated at 24 hours. (D) Positioning in sand of a seed for germination. (E) Seed germinating at 48 hours. (F) Emergence of the seedling.

2.3 Statistical analysis

We analyzed the effect of both treatments (cold storage vs. room temperature storage) on seed emergence, mortality, and recruitment success using generalized linear models with a binomial distribution. Regarding the effect of the treatments on the growth (height) of the seedlings at both temporal

measurements (14 and 25 days after sowing), we evaluated whether the normality parameters were met. Quantile-Quantile plots determined that the data fitted well to a normal distribution, and the Shapiro-Wilk normality test determined that the null hypothesis of normality assumption should not be rejected in both cases. Therefore, the height of the seedlings could be analyzed using a general linear

model (one-way ANOVA). The analyses were performed using the statistical software R 3.5.2 (R Core

Team, 2017), through the nlme (Pinheiro et al., 2011) and lme4 (Bates et al., 2014) packages.



Figure 4. Cavanillesia platanifolia (Humb. & Bonpl.) Kunth. Axonomorphic root and simple cuneate leaves of four-day-old seedling.

3 Results and Discussion

In the experimental trial, seeds preserved in cold storage exhibited a 10% higher emergence rate than those stored at room temperature (98.18 \pm 1.81 vs. 89.09 \pm 4.24%, χ^2 = 4.20, P = 0.040; Figure 5A) and, furthermore, the mortality rate with cold storage was 317% lower once emerged (16.66 \pm 5.11 vs. 71.42 \pm 6.52%, χ^2 = 33.30, P <0.0001; Figure 5B). Mortality was severely influenced by bacterial and especially fungal infections that affected the endosperm and cotyledons. The recruitment success of the seeds, considering both emergence and survival, was 228% higher in seeds preserved in cold compared to those kept at room temperature (81.81 \pm 5.24 vs. 25.45 \pm 5.92%, χ^2 = 37.35, P <0.0001; Figure 5C).

In addition to higher recruitment, seedlings from seeds stored in cold showed greater growth during the follow-up conducted the month after sowing. Thus, seedlings emerged from seeds stored in cold were 45% taller than those stored at room temperature at fourteen days (8.60 \pm 0.42 vs. 5.92 \pm 0.73 cm, $F_{1,58} = 9.53$, P = 0.003; Figure 5D), and 28% taller at twenty-five days (17.10 \pm 0.55 vs. 13.29 \pm

1.28 cm, $F_{1.57} = 9.74$, P = 0.002; Figure 5D).

Various factors are decimating the populations of Cavanillesia platanifolia in the Neotropics. The loss and fragmentation of forest areas and their logging are formed by the recently observed low natural regeneration in the dry forests of Ecuador. This could be due to a decrease in the number of individuals and lower production of fruits and seeds, a higher seed predation rate, an increase in seed desiccation and/or in seedling mortality due to environmental factors. Thus, the establishment of young individuals may be compromised by increasing physiological stress due to the ever-greater seasonal climate fluctuations. This situation could worsen in the future, so it would be advisable to fully study the life cycle of C. platanifolia up to its adult stage and determine the influence of various biotic and abiotic factors on its biological effectiveness. It cannot be ignored that seed viability may also be decreasing due to lower rates of cross-pollination (Hamrick and Murawski, 1990).

This study demonstrates that cold storage of seeds for six months allowed for the maintenance of their viability compared to seeds stored at room temperature, increasing the emergence rate. Cold storage would thus maintain the viability of seeds sensitive to desiccation (Normah et al., 2019), as is presumably the case with *C. platanifolia*. Our study shows that cold storage of the seeds for 6 months significantly maintained the seeds' viability. The emergence of seeds stored in cold for six

months (with three previous months of room temperature storage in a closed space) was similar, and even slightly superior to the emergence observed in three-month seeds at room temperature in the same closed space (pilot trial), an effect possibly mediated by the interruption of dormancy due to cold.

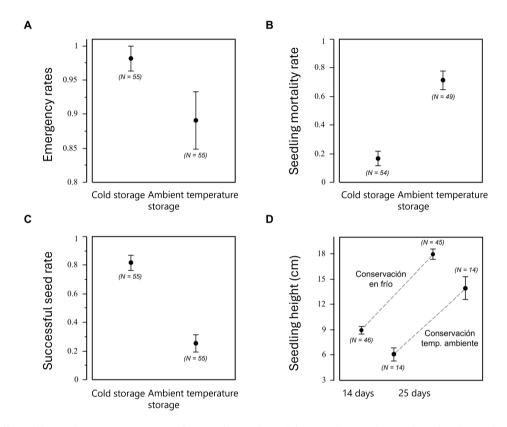


Figure 5. Effect of the seed storage temperature of *Cavanillesia platanifolia* (Humb. Bonpl.) Kunth on the observed variables. (**A**) Emergence rate. (**B**) Seedling mortality rate. (**C**) Recruitment rate (successful seeds). (**D**) Height of the seedlings with different treatments at 14 and 25 days after sowing.

Moreover, it is noteworthy that seedlings from the cold storage treatment grew more vigorously. This type of conservation would limit the adverse effects of high temperatures and water deficit on the seeds, reducing their aging. Through the multicausal phenomenon of aging, the physiological quality of seeds is reduced, inducing structural, compositional, and functional changes, limiting their viability and vigor (Kranner et al., 2010; Li and Pritchard, 2009). However, the most novel and notable result is that the mortality of these seedlings was considerably reduced in seeds stored in cold. Pathogeni-

city is a determining factor in seedling recruitment (Kranner et al., 2010), being one of the major causes of their mortality during their first month of life in tropical forests (Comita et al., 2014).

In these forests, pathogen attack usually has more acute effects on those seedlings that grow in shade (Augspurger and Kelly, 1984), as is the case with *C. platanifolia*. Cold storage, therefore, would not only maintain the vigor of the seeds but also reduce the activity of pathogens, limiting their negative impact on the physiology of the seeds and the es-

tablishment of the seedlings. It is common in tropical forests for seedling mortality due to pathogenic action to also be linked to their density (Alvarez-Loayza and Terborgh, 2011; Augspurger and Kelly, 1984). Our methodology shows that low mortality rates can be achieved even when sowing at high densities ($\sim 83\%$ survival in seeds stored in cold, with 2 cm distance between seedlings).

4 Conclusions

We consider that cold storage could decrease mortality by both limiting the weakening caused by aging and slowing down the growth of pathogens, leading to a lower probability of lethal infection. The easily replicable and cost-effective nursery methodology used in this study can be employed for the production of seedlings in the necessary reforestation plans for *C. platanifolia*. Thus, the nursery can be utilized at different sowing times, planning the activation and sowing of the seed kept in cold storage as convenient, thereby optimizing efficiency in seedling production.

This methodology could also be employed in other species of the Malvaceae-Bombacaceae family, as nearly half of the species in this family are listed as at least vulnerable (IUCN, 2019). The methodology could similarly be tested on other important and threatened species from the Chocó-Darién with seeds of similar characteristics, among which are *Quercus humboldtii* Bonpl. (Fagaceae), *Prioria copaifera* Griseb. (Fabaceae), *Caryocar amygdaliferum* Mutis (Caryocaraceae), and *Anacardium excelsum* (Bertero Balb. ex Kunth) Skeels (Anacardiaceae).

Author contribution

R.V.B.: Conceptualization, visualization, research, resources, methodology, formal analysis, project administration, data collection, data processing, supervision, writing-original draft, writing-review and editing. E.R.J: Visualization, resources, methodology, project administration, data collection, supervision, writing-review and editing. A.R.L.: Research, methodology, resources, validation. M.A.B.: Research, formal analysis, data curation, validation, writing-review and editing.

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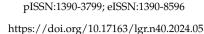
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Scientific paper/ Artículo científico

ENVIRONMENTAL POLLUTION







EVALUATION OF URBAN POLLUTION BY NOISE EMISSIONS REGISTERED WITH A SOUND LEVEL METER AND SENSOR NODES

EVALUACIÓN DE LA CONTAMINACIÓN URBANA POR EMISIONES SONORAS REGISTRADAS CON SONÓMETRO Y NODOS SENSORES

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Abstract

The increase in noise emissions is associated with an increase in the population, with anthropic activities, and it is in urban centers where a series of effects on both the population and the environment occur. The present study covered the evaluation of the noise behavior in the city of Cuenca during 2019, for which monitoring was carried out in 31 points distributed in the city based on the average daily traffic, the ordinance of use and occupation of the land and the dynamics of the population. The national environmental standard TULSMA (Unified Text of Secondary Legislation of the Ministry of the Environment) was taken as a reference. To establish the comparison of the data collected with a (low-cost, noise level) sensor and a sound level meter, measurements were made in 6 monitoring stations established on the basis of noise complaints made by citizens to the autonomous decentralized municipal government of Cuenca (GAD) and the security conditions to place for the placement of the equipment. The results showed that noise, in the city of Cuenca during 2019, noise is above the TULSMA environmental standard in Zone EQ1 (100% of the measurements), Zone R1 (96% of the measurements), Zone CM (100% of the measurements) and in Zone ID3 / ID4 (72% of the measurements). There was a high correlation coefficient (r = 0.8) and determination coefficient ($R^2 > 0.6$), between the data collected with the sensor and the sound level meter. It is worth highlighting the use of the calibrated sound level meter when making sound measurements with various instruments, as it allows the data to be verified and validated.

Keywords: noise, sound level meter, sensor nodes, TULSMA.

Resumen

El incremento de las emisiones sonoras se asocia a la actividad antrópica y es el centro urbano en donde se presentan afectaciones a la población y al entorno. El presente estudio tuvo dos componentes, el primero abarcó la evaluación del comportamiento sonoro en el área urbana de Cuenca-2019, en donde se realizaron monitoreos en 31 puntos distribuidos en la ciudad en función del tráfico promedio diario, uso del suelo y dinámicas de la población; se tomó como referencia la norma ambiental nacional TULSMA (Texto unificado de legislación secundaria del Ministerio del Ambiente). Los resultados mostraron que el ruido sobrepasa los límites de la norma en el 100% de las mediciones realizadas en la zona de equipamientos de servicios sociales (EQ1), 96% de las mediciones en la zona residencial (R1), 100% de las mediciones en la zona comercial (CM) y en el 72% de las mediciones de la zona industrial de mediano y alto impacto (ID3/ID4). En el segundo componente se compararon los datos levantados de manera simultánea con sensor y sonómetro, en seis nuevas estaciones de monitoreo establecidas sobre la base de las denuncias de ruido realizadas por la ciudadanía a la autoridad municipal de Cuenca y las condiciones de seguridad para la colocación de los equipos. Se obtuvo un alto coeficiente de correlación (r = 0.8) y de determinación ($R^2 > 0.6$), entre los datos levantados con sensor y sonómetro. Se resalta el uso del sonómetro calibrado cuando se realizan mediciones sonoras con diversos instrumentos, ya que permite verificar y validar los datos.

Palabras clave: ruido, sonómetro, nodos sensores, TULSMA

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1 Introduction

Sound is defined as a sound wave capable of producing the sensation of sound; and loudness is the subjective sensation generated by pressure variation in the ear (Laforga, 2000). For physics, "it is a sensation perceived by the ear, due to pressure differences produced by the vibration of a body" (Robles and Arias, 2015, p. 10), while noise is associated with annoying, unwanted sounds (García and Garrido, 2003; Robles and Arias, 2015), or "any sound that disturbs human beings and the development of their activities" (Rodríguez, 2015, p. 3).

Since their appearance on the planet, living beings have been receptors of a variety of sounds coming from the environment; however, environmental sound has been associated with anthropic activity. It is currently considered undesirable because it affects the natural balance, harms the population and is presented as an important source of pollution in cities (Alfie and Salinas, 2017) and a public health problem (Fiedler and Zannin, 2015).

The sources of noise range from the use of vehicles at high speeds, without revision or maintenance, raising the voice, among others, which come from the usual actions of the population (García and Garrido, 2003), including factors such as age, stress level or aspects such as air quality can affect positively or negatively the perception of sound and influence the soundscape of a territory (Moraga et al., 2017).

Urban areas present a continuous population growth that propitiates the entry of population for studies, management, work or residence and leads to an abandonment of the peripheries causing imbalances in the territory (Gómez and Vallarino, 2010), resulting in alterations to the urban environment such as vehicular congestion that emits gases, particulate matter and noise into the atmosphere, causing loss of environmental quality of the urban center.

It is necessary to establish the impact of noise in terms of the effects it can cause to society, either physiologically or psychosociologically; for this reason, noise is studied by universities, public and private entities worldwide. According to Romo Orozco and Gómez Sánchez (2013), high noise

levels can affect the auditory organ and low levels can affect psychosomatic health.

As expressed by Burneo (2007a), the continuous and constant exposure to excessive or high levels of noise slowly induces the irreversible loss of hearing (Daiber et al., 2019), increases cardio-metabolic diseases, arrhythmia, diabetes mellitus and, there are cases of stress that can trigger vasoconstriction, heart rate variability and coagulation depending on the emotional conditions of the people exposed (Daiber et al., 2019).

Grass et al. (2017, p. 5) expresses: "When exposed to high levels of noise for a prolonged period of time, the stomach may secrete an acid substance and a quantity of adrenal hormones; these are the first alarm symptoms of acute stress; there may also be difficulty in concentrating, which triggers a decrease in the level of performance and increases the level of anxiety in the professional by feeling uncommunicative with his environment, in addition to irritability, sleep disorder, fatigue and depression", and critical groups such as infants, children, the elderly, the sick and pregnant women are more prone to this (Burneo, 2007b).

The global burden of disease has changed as a consequence of industrialization and modernization, because it incorporates a risk factor such as noise that generates chronic diseases (Daiber et al., 2019); not only physical but also psychological such as stress, interference with speech communication, loss of performance, alterations that grow when the sound increases, affecting the quality of life (Basner et al., 2014; Nazneen et al., 2020).

The sound level is increasing, and it necessary to delimit it, regulate it and combat it with policies and legislation (García and Garrido, 2003). It is not a habitual activity because it has been considered as a common fact product of everyday life, giving little importance to its effects (Bañuelos Castañeda, 2005). For society, pollution is the negative effects on factors such as water, soil, air, while noise is qualified as "not very serious" (Zamorano et al., 2015, p. 2).

Sound was initially quantified with music in a qualitative way, i.e., loud, high, etc., without scientific accuracy, depending on the auditory acuity of the person who perceives it (Long, 2006). After mul-

tiple experimental studies Fletcher Munson (1933), Robinson and Dadson (1956), elaborated the curves with loudness levels based on human judgments about a perceived tone, compared to a reference one (Long, 2006); but they could not be used with an analog sound meter, so electrical weighting filters have been developed, which approximate the Fletcher Munson curves, named with letters of the alphabet A, B, C (Long, 2006).

With the aim of showing the population information related to noise, the University of Azuay (UDA) monitors these emissions in Cuenca since 2009 with certified sound level meter on an annual basis, and since 2018 with sensor nodes in real time on a continuous basis, contributing to compliance with Art. 14 of the Constitution of the Republic of Ecuador which stipulates: "The right of the population to live in a healthy and ecologically balanced environment, which guarantees sustainability and good living, sumak kausay...." (Asamblea Constituyente, 2008, p. 13).

The city under study is home to approximately 66% of the canton's population (INEC, 2010), with a total of 330,000 inhabitants; resulting in areas with widespread noise pollution, a phenomenon that requires attention (García and Garrido, 2003). Along with population growth, technological evolution allows for better development of cities, forming what are called "smart cities" (UNRN, 2010), a concept related to the communication infrastructure for collecting information and distributing it through networks.

Information and communication technologies (ICTs) have improved the quality of life of the population, reducing management and mobilization time and contributing to the reduction of emissions and environmental pollution (Lin et al., 2011).

Wireless sensor networks consist of a data acquisition network and a data distribution network, controlled and monitored by a management center (Lewis, 2004); this network consists of data generator and relay nodes, a microprocessor and a transceiver (Akyildiz and Vuran, 2010). Its applicability is wide, the data captured can be environmental variables such as meteorological data,

sound emissions, water quality, environmental monitoring, with the ability to transmit in real time (Quiñones Cuenca et al., 2017).

Acoustic emissions are monitored in cities of Ecuador; noise levels from fixed and mobile sources have been studied in Guayaquil in the framework of the design of an air quality monitoring network, determining the equivalent sound pressure (NPSeq) in decibels dB (IDYC, 2016). Quito and the Historic Center of Cuenca have used tools such as Cadna A software and the prediction-propagation model "HARMONOISE P2P" respectively, to elaborate maps that represent the noise in areas of the city (Bravo Moncayo, 2019; Armijos-Arcos, 2018).

The research carried out included the evaluation of noise behavior in Cuenca in 2019 in 31 pre-established points, data collected with a sound level meter. Additionally, simultaneous measurements were made with sound level meter and noise sensors in six new monitoring points in order to establish the accuracy degree in data collection; the values allowed the comparative analysis.

2 Materials and methods

The project was developed in the urban area of Cuenca, province of Azuay, located at 2550 m.a.s.l., with 331 888 inhabitants (INEC, 2010), in an area of 79.59 km^2 .

2.1 Equipment

The information was collected with a SOUND-PRO DL-2-1/3 SLM model sound level meter, series BCQ120001, Class 1 integrator and duly certified, which allows obtaining the equivalent average level automatically on the basis of all the samples recorded in the determined period (15 minutes). The data collected for each monitoring point was the equivalent sound level (Leq) with A/C scale, recommended for environmental noise monitoring (Long, 2006). The (Leq) measures "the constant level of noise with the same energy content as the acoustic variation of the calculated sound signal" (Aagesen, 2002, p. 57).

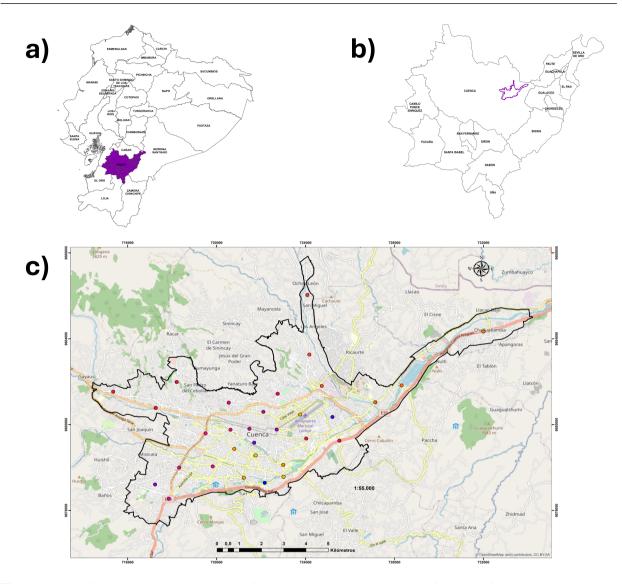


Figure 1. Maps of Project location: a) Location of the Azuay province. b) Location of the city of Cuenca. c) Project area. Preparation based on information from INEC, 2010 and the Institute of Sectional Regime Studies of Ecuador IERSE-UDA, 2019.

The technology used for noise sensors is based on the internet of things model, and is constituted by a set of wireless sensor nodes that form a network; these devices have the capacity of processing, storing and communicating, and it is equipped with a battery that provides them with energy (Salgado and Carranco, 2017).

The referential sensor nodes are called "Wasp-mote plug sense", which consist of a waterproof packaging (IP65) with sockets for sensor connection, solar panel, antenna and a terminal for node

programming. The model used is: *Smart Cities PRO* (*SC_NODE*) (Salgado and Carranco, 2017).

This research is empirical with a quantitative approach and the data were collected between March and April, 2019. The research started collecting the data in situ with both sound level meter and sensor. The information collected was compared with the standards established in the text from the Ministry of the Environment (Ministerio de Ambiente, 2019), then the data obtained with sensor and sound level meter were correlated.

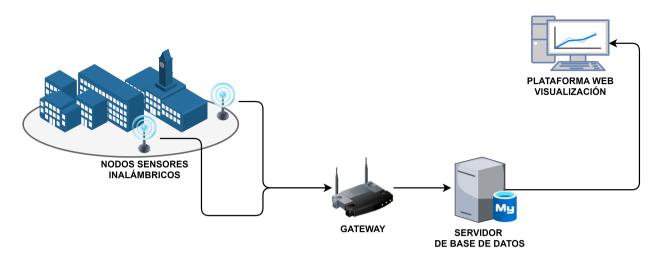


Figure 2. Schematic of the operation of the wireless sensor network and data transmission. Figure made by Darío Espinoza.

2.2 Sound level meter monitoring

There is a network of 31 monitoring points (Figure 3); three criteria were considered for their location: traffic density, land use and occupation, and population dynamics. Measurements were taken at six times, corresponding to the peak hours of vehicular flow in the city of Cuenca: 7:01 a.m., 1:00 p.m. and 6:00 p.m., and off-peak hours 10:00 a.m. and 3:00 p.m.; in addition, the night hours of 9:01 p.m. were included.

These schedules were established based on the study conducted by the GADMCC, which determined the peak and off-peak hours of vehicles from the city center (GAD Cuenca, 2007). The monitoring period per point was 15 minutes in each schedule, complying with the provisions of Annex V of the TULSMA, which recommends a monitoring period of at least 10 minutes (Ministerio de Ambiente, 2019).

2.3 Monitoring with sensor nodes

Six monitoring points were established (Figure 4) and their location was based on noise complaints received in the Municipality of Cuenca, areas reported as noise generators and logistical conditions such as power requirements, video cameras and equipment security.

The information from the sensor nodes is captured continuously and in real time since October 2018; the data are presented in periods of 12.25 min on the UDA website. The information reported between March 18 and April 15, 2019 was used, and the sensor schedule was unified with those of the sound level meter, i.e., the equivalent sound level (Leq) was determined for 7h01, 10h00, 13h00, 15h00, 18h00 and 21h01.

This calculation was performed with the data reported by the sensor for one hour. The values obtained are the result of the automatic average of all the samples captured during one hour. The weighting scale of the sensors is (A) and the averages in this period were arithmetic. The data obtained with a sound level meter were measured for a period of 15 minutes in each hour at each monitoring point.

2.4 Assessment of noise in the city

To establish the sound behavior in 2019, Annex 5 of the Unified Text of Secondary Legislation of the Ministry of Environment (TULSMA, 2019) of Table 2 was used as a reference. The 31 monitoring points with sound level meters are described in Table 3. While, the six points where simultaneous monitoring was performed are described in Table 4.

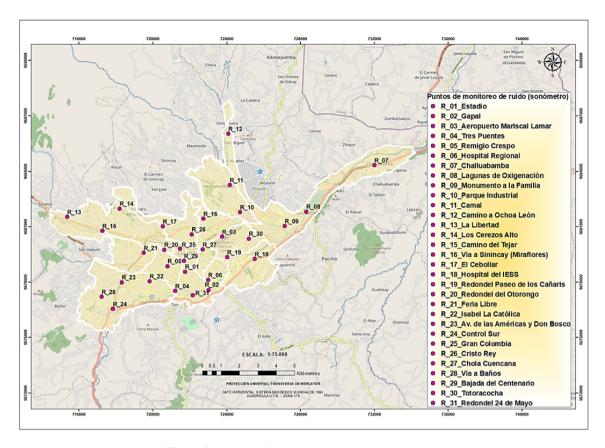


Figure 3. Location of sound level meter monitoring sites.

Table 1. Location of noise sensors in 2019.

Code	Area	Primary Street	Secondary Street
SCP-04	Escalinata Juana de Oro	Calle Larga	Escalinata
SCP-05	Presidente Córdova	Presidente Córdova	Vargas Machuca
SCP-06	Remigio Crespo	Remigio Crespo	Agustín Cueva
SCP-07	Estadio Serrano Aguilar	Av. del Estadio	Av. Manuel J. Calle
SCP-08	Mercado El Arenal	Av. de las Américas	Av. Remigio Crespo
SCP-09	Parque Industrial	Paseo Río Machángara	Av. Octavio Chacón Moscoso

3 Results and discussion

3.1 Noise emissions in Cuenca with sound level meter 2019

The analysis of the data collected with sound level meter and evaluated based on the TULSMA (2019) are represented in the Figure 5. Furthermore, the noise data obtained for all the study zones are above the limits established in the environmental standard -TULSMA, in all sampling hours (See Table 2). In the social services equipment zone (EQ1),

the means at all times are between 68 dB and 72.3 dB. At 7:00 am, 1:00 pm, 3:00 pm and 6:00 pm, most of the data are above 70 dB.

The residential zone (R1) has the highest monitoring number, with mean values at different times ranging from 68.2 dB to 72.4 dB. The lowest value was at 3:00 p.m. with 54.3 dB. The mean values in the commercial zone (CM) are between 68.2 dB and 72.4 dB and at 7:01 am, 1:00 pm, 6:00 pm and 9:01 pm, the data obtained are mostly above the mean and at 10:00 am and 3:00 pm most are below the

mean.

Table 2. Permissible noise limits -TULSMA, (2019).

Land	Lkeq (dB)					
Use	Daytime period	Night period				
	07:01 a 21:00h	21:01 a 07:00h				
R1	55	45				
EQ1	55	45				
CM	60	50				
ID3 / ID4	70	65				

In the industrial zone (ID3/ID4), the mean ranges from 64.7 dB to 70.4 dB, in the hours 7h01, 10h00, 13h00, 18h00 and 21h01; the data obtained are mostly above the mean; there is only one value of 64.3 dB in the 21h01 schedule that is below the TULSMA -2019 standard; all the remaining values in the different schedules are above the mentioned standard.

The noise values in the city of Cuenca for the year 2019 are above the established legislation in all monitored schedules and in all land use and occupancy zones. The surveyed data that are over the TULSMA standard are: Zone EQ1 (100%), Zone R1 (96%), Zone CM (100%) and Zone ID3/ID4 (72%) of the total measurements taken. The WHO indicates that the noise values for vehicular traffic are around 70 dB. Therefore, since the monitoring in Cuenca was carried out at critical points on the roads with the most vehicular traffic, the data obtained are in accordance with the WHO (Berlung et al., 1999).

3.2 Noise emissions with sensor nodes vs. sound level meter

With the aim of conducting a comparison, measurements with sensor and sound meter were done in the six points indicated in Figure 4; and the results are detailed in Table 5.

Table 3. Sound level meter monitoring points by land use.

No.	Measured point	Land use
NO.	(area)	(TULSMA 2019)
R_02	Gapal	
R_04	Tres Puentes	
R_07	Challuabamba	
R_08	Lagunas de	
N_00	oxigenación	
R_09	Monumento a	
K_09	la Familia	Residential
R_12	Camino a Ochoa	
N_12	León	
R_13	La Libertad	
R_15	Camino al Tejar	
R_16	Vía a Sinincay	
	(Miraflores)	
R_17	El Cebollar	
R_26	Cristo Rey	
R_28	Vía a Baños	
R_30	Totoracocha	
R_01	Estadio	
D 02	Aeropuerto Mariscal	
R_03	Lamar	
R_05	Remigio Crespo	
R_19	Redondel Paseo de	Commercial
K_19	los Cañaris	Commerciai
R_21	Feria Libre	
D 22	Av. de las Américas	
R_23	y Don Bosco	
R_24	Control Sur	
R_25	Gran Colombia	
R_27	Chola Cuencana	
R_29	Bajada del Centenario	
R_06	Hospital Regional	
R_18	Hospital del IESS	Social
D 20	Redondel del	services
R_20	Otorongo	facilities
R_22	Isabel La Católica	
R-31	Redondel 24 de Mayo	
R_10	Parque Industrial	
R_11	Camal	Industrial
R_14	Los Cerezos Alto	

Figure 6 shows the differences presented between measurements made with sensor and sound level meter for each of the measurement points. At the "Calle Larga" station, the sound measurement difference between the sound level meter and the sensor is between 2.5 dB and 3 dB. At the "Calle Presidente Córdova" station, the difference of measured value with sensor and sound level meter varies between 2.9 dB and 5.1 dB. The greatest differences are found at the "Remigio Crespo Avenue" station, which vary between 1.3 dB and 9 dB. At the

station "Sector Estadio Serrano Aguilar", the variations range between 0.0 dB and 2.9 dB, which occur between 15h00 and 21h00; while the differences are minimal at the other measurement times. The data collected at "El Arenal Market Sector" station show a variation of less than 1 dB. Noise emissions at "Parque Industrial" station vary between 3 dB and 6.3 dB.

Table 4. Monitoring points with sensor and sound level meter by land use.

No.	Measured point	Land use
INU.	(area)	(TULSMA, 2019)
	Calle Larga	
SCP-04	(Escalinata	
	Juana de Oro)	
SCP-05	Presidente Córdova	Commercial
SCP-06	Remigio Crespo	
SCP-07	Estadio Serrano Aguilar	
SCP-08	Mercado El Arenal	
SCP-09	Parque Industrial	Industrial

The correlation was mainly analyzed from the data obtained with the sensor vs. sound level meter. Pearson's correlation coefficient (r) and Spearman's correlation coefficient (rho) were obtained, in addition to their p-values of significance, where the most representative data are highlighted in Table 6. The r coefficient in the monitoring sites: Calle Larga, Presidente Córdova, El Arenal and the Industrial Park, present values between 0.8 and 0.99, which show a high correlation between the data obtained with the sensor and the sound level meter; in the sector of the Serrano Aguilar Stadium, the rho coefficient applies (due to the non-normality of the data) with a value of 0.81. Remigio Crespo street presents erroneous values, indicating that there is some problem with this sensor node. In general, the statistical indicators show that there is variability in the data in areas such as the Industrial Park and the Serrano Aguilar Stadium, while Remigio Crespo Street showed errors in the measurement, indicating the possibility of failures when using sensor nodes.

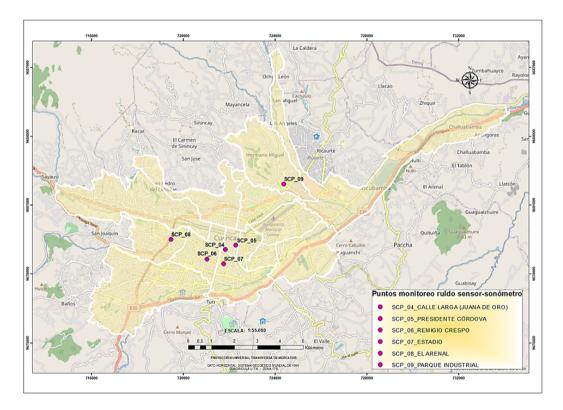


Figure 4. Location of sensor monitoring sites-2019.

As can be seen in this section, the data collected at the six monitoring points with sensor, sites where data were simultaneously collected with the sound level meter were analyzed. The noise data obtained with the two devices are above the limits established in the environmental standard (Ministerio de Ambiente, 2019).

Additionally, it can be observed that there are differences between the data collected at all monitoring points. Among the causes of the difference, it can be mentioned: The weighting filter in which the sensor measures is "A", while with the sound level meter it is A/C; the optimal measurement range of the sensor is "50 dB- 100 dB". The sound level meter, being simultaneously configured in A/C weighting, has a greater measurement range that allows it to perceive low and high frequencies (Salgado and Carranco, 2017).

Another element analyzed is the height of data collection. The sensors were placed on the poles where the necessary logistical conditions were met; but the height of placement depends on the location where the ECU 911 surveillance cameras are located.

The approximate height was 4 m above ground level and each sensor was located at a different

height, making it impossible to establish a correlation that would allow a generalization of behavior. According to the results obtained, the values captured by the sensors are lower than those of the sound level meter, due to the difference in the height of data capture, since the sound level meter monitors noise at 1.80 m above the floor level and the sensors are above 4 m above the floor level.

There are other experiences such as in Quito, which has a noise map for the day and another one for the night (Bravo Moncayo, 2019). The city has been divided by sectors (32) for collecting the data, identifying information such as: sector name, noise emissions (dB), potential and highly annoying population. To determine noise pollution, CadnaA (Computer Aided Noise Abatement) software was used, which models noise as a function of vehicular traffic and road characteristics such as lane width, road surface, traffic speed, IMD, etc. They randomly perform data validation with the use of a calibrated sound level meter (Bravo Moncayo, 2019).

The results show noise levels between 61.90 dB (El Condado) to 72.70 dB (Historic Center). During the day and at night the values decrease from 54.80 dB (El Condado) to 67.40 dB (La Libertad sector) (Bravo Moncayo, 2019).

Table 5. Noise emissions with sensor and sound level meter.

Schedule	07:01	10:00	13:00	15:00	18:00	21:01				
Sensor noise										
SCP-04	68.8	68.2	68.7	68.0	68.5	65.0				
SCP-05	72.6	71.9	73.3	71.7	72.0	66.2				
SCP-06	63.5	66.5	65.9	65.6	66.0	61.3				
SCP-07	66.9	68.0	67.5	68.0	67.9	62.9				
SCP-08	69.9	69.7	69.4	70.0	70.2	67.1				
SCP-09	73.1	72.3	72.3	72.8	71.8	67.6				
	N	loise sou	nd level	meter						
SCP-04	71.8	70.5	71.1	70.7	70.9	67.4				
SCP-05	76.1	75.8	76.2	75.9	75	71.3				
SCP-06	70.4	67.8	70.8	70.7	69.3	70.3				
SCP-07	67.1	68	67.4	68.3	70	65.8				
SCP-08	70.8	70.3	69.6	69.5	70.3	67				
SCP-09	76.1	76.5	78.6	76.2	75	72.6				

Noise in the Historic Center of Cuenca has been studied by the Salesian Polytechnic University (Armijos, 2018), specifically in an area of 250 m, around a monitoring point located in Gran Colombia and

Tarqui streets. Through the use of the prediction-propagation model "HARMONOISE P2P" a map of the noise generated by vehicular traffic is elaborated, using a sound level meter to validate the data.

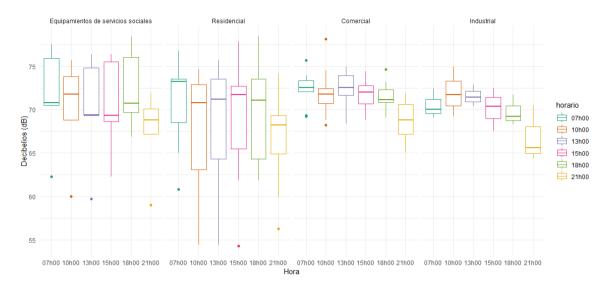


Figure 5. Measurement Graph of sound level—Classified by land use of Cuenca, 2019.

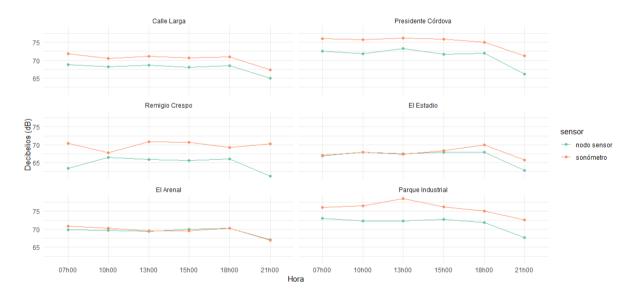


Figure 6. Comparison of the emissions made with sensor and sound level meter.

An average of 68.58 dB is obtained with the prediction model. The value is 71.84 dB with the sound level meter, and the difference is -3.26 dB; hence, it is concluded that the propagation model is adequate (Armijos-Arcos, 2018). Guayaquil monitors noi-

se using a sound level meter that monitors 52 preestablished points based on vehicle traffic, in periods of 12 min. The values obtained are above 70 dB (IDYC, 2016).

Table 6. Correlation of the data

Performance index		Comparison of Sound Level Meter vs. sensor					
Name	Symbol	Calle Larga	Presidente Córdova	Remigio Crespo	Estadio Serrano Aguilar	El Arenal	Parque Industrial
Mean square error	RMSE	2.54	3.84	5.65	1.47	0.5	4.34
Standard deviation	rSD	0.93	1.36	1.74	1.42	0.85	1.03
Pearson correlation	r	0.99 (value-p =	0.98 (value-p =	-0.41 (value-p =	0.77 (value-p =	0.93 (value-p =	0.8 (value-p =
coefficient $(-1 \le r \le 1)$	•	0.0003)	0.0008)	0.6034)	0.0724)	0.0064)	0.0571)
Spearman's correlation coefficient (-1<= rho<=1)	rho	0.94 (value-p = 0.0167)	0.77 (value-p = 0.1028)	-0.43 (value-p = 0.4194)	0.81 (value-p = 0.04989)	0.46 (value-p = 0. 3542)	0.46 (value-p = 0.3542)
Determination coefficient $(0 \le R2 \le 1)$	R2	0.97	0.95	0.17	0.6	0.87	0.64
Regression coefficient $(0 \le bR2 \le 1)$	bR2	0.94	0.91	0.15	0.59	0.87	0.6

4 Conclusions

The proposed methodology allowed to have a set of noise monitoring points distributed in the city and taking as a criterion the density of vehicular traffic. The data collected present greater acoustic intensity, being the most unfavorable for the population. The period of sound emissions survey for 15 minutes allowed to comply with the sampling method in force in the law (Ministerio de Ambiente, 2019) and to establish a diagnosis.

The emissions measured with a sound level meter in the 31 points are above the environmental standard; this is explained because the monitoring was performed in places with high intensity of vehicular traffic and not inside the buildings. The data obtained in six simultaneous monitoring areas with sensor and sound level meter do not comply with the TULSMA. In this case, the points were chosen based on complaints of excessive and periodic noise nuisance.

Other experiences in cities such as Quito and Guayaquil show emission results similar to those obtained in Cuenca and exceed the limits of the legal standard. It is important to highlight the use of the calibrated sound level meter because it is a reference for measurements made with other devices or tools, whether simulation, prediction or sensor nodes that have required random validation.

The data collected with sensor and sound level

meter simultaneously in the following sectors: Calle Larga, Presidente Córdova, Estadio Serrano Aguilar, Mercado El Arenal and Parque Industrial have a correlation between (0.81 to 0.99), and - 0.41 were obtained in the sector Av. Remigio Crespo Toral due to an error in the measurement of the sensor node, making it difficult to determine a correction factor between the sensor and sound level meter for this point. However, it is important to present this result since it shows a risk that can occur when using sensor nodes. Additionally, determination coefficients (R2) and regression coefficients (bR2) were calculated, whose results ratify the alteration of the data captured with the sensor (SCP-06). For subsequent monitoring, the existence of anomalies in the equipment or factors such as the presence of magnetic fields or an electric transformer that disturbs the normal operation of the sensor should be chec-

For analyzing the noise data, the continuous equivalent level (Leq) captured in the different schedules was used. The (Leq) programmed from the sensor was arithmetic and in band (A), and the average is logarithmic and in band (A/C), from the sound level meter factors that influenced the results obtained.

Author Contribution

J.M.G.: Conceptualization, formal analysis, methodology, project administration, supervision, visualization, writing-original draft, writing-review and

editing; O.D.I.:Conceptualization, methodology, validation; I.V.G.: Data curation, formal analysis, research; D.E.S.: Data curation, Software, research; F.S.C.: Formal analysis.

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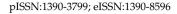
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IMPACT ON THE INCORPORATION OF METALS IN PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES IN FILMS BASED ON ARROWROOT STARCH

IMPACTO EN LA INCORPORACIÓN DE METALES EN LAS PROPIEDADES FISICOQUÍMICAS Y ANTIMICROBIANAS EN PELÍCULAS A BASE DE ALMIDÓN DE ARRURRUZ

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Abstract

New biodegradable packaging has been developed from renewable sources, mainly of vegetable origin. Arrowroot starch has been recently used to produce high-quality biodegradable films capable of behaving well when incorporating oils, extracts, metal, and metal nanocomposites. The study aimed to verify the impact of incorporating metals in the sulfate and chloride forms in a biopolymeric matrix from arrowroot starch in terms of biodegradability, physicochemical and microbiological parameters. Different arrowroot films were produced to incorporate solutions with a concentration of 1 Mol L⁻¹ of sulfate and chloride metals. The action of biodegradability in soil, UV transmittance, and visible light were observed in UV-Vis spectrophotometry and antimicrobial action on *Escherichia coli, Staphylococcus aureus, Salmonella serovar Typhimurium*, and *Salmonella serovar Enteritidis*. Good results were obtained, such as biodegradability time between 81.70 to 100% (30 days), a low transmission rate of UV radiation and visible light between 250 to 890 nm, high capacity for bacterial inhibition between 22.08 to 10.05 mm for *E. coli*, among 25.59 to 11.10 mm for *S. aureus*, between 22.14 to 11.66 mm for *S. serovar Typhimurium* and between 21.11 to 8.26 mm for *S. serovar Enteritidis*. It is concluded that the biodegradable films of arrowroot starch incorporated with metals showed potential in all the evaluated tests, thus characterizing possible new products for different uses, such as low time available in the environment, preservation of the characteristics of special products, and antimicrobial capacity.

Keywords: Escherichia coli, Salmonella, Enterococcus, Biodegradable packaging, Antimicrobial activity.

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Resumen

Se han desarrollado nuevos embalajes biodegradables de fuentes renovables principalmente de origen vegetal. El almidón de arrurruz se ha usado en la producción de películas biodegradables de alta calidad, capaces de tener un buen comportamiento al incorporar aceites, extractos, metales y nanocompuestos metálicos. El estudio tiene como objetivo verificar el impacto de la incorporación de metales en las formas de sulfato y cloruro en una matriz biopolimérica de almidón de arrurruz en términos de biodegradabilidad, parámetros fisicoquímicos y microbiológicos. Se produjeron diferentes películas de arrurruz con soluciones a una concentración de 1 Mol L⁻¹ de metales sulfato y cloruro. La acción de biodegradabilidad en el suelo, transmitancia UV y luz visible se observó en espectrofotometría UV-Vis, y acción antimicrobiana sobre *Escherichia coli, Staphylococcus aureus, Salmonella serovar Typhimurium y Salmonella serovar Enteritidis*. Se obtuvieron buenos resultados, como tiempo de biodegradabilidad entre 81,70 a 100 % (30 días), baja tasa de transmisión de radiación UV y luz visible entre 250 a 890 nm, alta capacidad de inhibición bacteriana entre 22,08 a 10,05 mm para *E. coli*, entre 25,59 a 11,10 mm para *S. aureus*, entre 22,14 a 11,66 mm para *S. serovar Typhimurium* y entre 21,11 a 8,26 mm para *S. serovar Enteritidis*. Se concluye que las películas biodegradables de almidón de arrurruz incorporadas con metales mostraron potencial en todas las pruebas evaluadas, caracterizando así posibles nuevos productos para diferentes usos, tales como bajo tiempo disponible en el ambiente, preservación de las características de productos especiales y capacidad antimicrobiana.

Palabras clave: Escherichia coli, Salmonella, Enterococcus, Embalaje biodegradable, Actividad antibacteriana.

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1 Introduction

The first packages on the market were produced from synthetic polymers of the processed oil. Currently, there is a wide variety of packaging for different purposes, although its use is to preserve the product during transport, during storage and protection against mechanical shocks that may reduce or render the use of the product unfeasible by the consumer (Kubowicz and Booth, 2017; Santos et al., 2021).

The indiscriminate use of plastics with prolonged degradation time, which can reach 500 years, presents serious problems, mainly environmental and human health. Plastic polymers undergo abrasion over time, forming microplastics absorbed during feeding by mainly marine animals and humans (Sobral et al., 2011; Olivatto et al., 2018; Haider et al., 2019).

Countless countries have been promoting the development of means capable of altering this catastrophic scenario caused by the excess of plastic packaging. Several researchers from the green line of research have been developing new biodegradable polymers from naturally renewable sources such as starch, fats, chitosan, and methylcellulose (Brito et al., 2011). According to Cheviron et al. (2014) y Farias et al. (2016), renewable sources of polymers offer an alternative for maintaining the sustainable development of economically and ecologically attractive technologies. Natural polymers have biological, physicochemical, mechanical, and morphological characteristics comparable to synthetic polymers (Pitt et al., 2011; Akter et al., 2012).

A branch of natural polymers focuses on incorporating metals, nanocomposites, plant extracts, fixed and essential oils capable of promoting numerous active and intelligent functions to these biodegradable polymers. Several studies evaluate the structural biodegradability of the natural polymer with incorporated materials, verifying its harmonic capacity to promote a resistant product capable of being used in different production chain processes (Gómez-Estaca et al., 2010; Nor Adilah et al., 2018; Youssef et al., 2019).

Several biodegradable packages of starch from different plant sources such as arrowroot have been tested with surprising results. *Maranta arundinacea* (arrowroot) belonging to the Marantaceae family, which has rhizomes rich in starch (>85%), has specific characteristics that are different from other sources

of natural starches and can also be used for therapeutic purposes (Madineni et al., 2012). Several studies have evaluated this new source of natural starch capable of promoting the development of high-quality biodegradable films, in addition to providing stability of interaction support, for example, with metal solutions (Yin et al., 2009; Cruz et al., 2020; Nogueira et al., 2018; Valadares et al., 2020).

Various metals as sulfates, chlorides, or nanocomposites have structural characteristics with potential activity as an antibacterial agent, in itself, the biopolymer from arrowroot starch has no action capable of inhibiting the development of bacteria, due to being a source of carbohydrates, but when incorporated with metals that present this action, the films promote an active action in the preservation of the product, such as food (Cruz et al., 2020; Shafiei Shafiei et al., 2021).

Thus, this study aimed to produce biodegradable films from arrowroot starch incorporated with metals (sulfates and chlorides), verifying the impact of these metals on some characteristics such as biodegradability time, the transmission of UV radiation and visible light, and aptitude as an antibacterial source.

2 Materials and Methods

2.1 Production of biodegradable films incorporated with metals

Biodegradable films incorporated with metals were obtained using the Casting technique described by Issa et al. (2017), but modified. To produce all films, 5 g of commercial arrowroot starch dissolved in 100 mL of distilled water was used. The film-forming solution was then moderately stirred at an ambient temperature of 25 °C for 5 min.

Then, the solution was heated to 70 °C, with constant mechanical stirring for 30 min. After the gelatinization of the starch, glycerol was added as a plasticizer 30% (w/w), and the emulsion was again stirred for another 10 min. When filmogenic emulsions reached 30 °C, they were incorporated with different metal solutions as described in (Table 1).

All filmogenic solutions were poured on polystyrene slabs and dried in an air circulation oven at 35 $^{\circ}$ C for about 48 h.

Table 1. Formulas (films) of arrowroot starch in different solutions of incorporated metals.

Films	Metals
1 control	5 g starch + 1.5 g glycerol
2	5 g starch + 1.5 g glycerol + 1000 μ L Iron sulfate II 1 Mol L ⁻¹
3	5 g starch + 1.5 g glycerol + 1000 μ L Nickel sulfate 1 Mol L ⁻¹
4	5 g starch + 1.5 g glycerol + 1000 μ L Manganese sulfate 1 Mol L ⁻¹
5	5 g starch + 1.5 glycerol + 1000 μ L Cobalt chloride 1 Mol L ⁻¹
6	5 g starch + 1.5 g glycerol + 1000 μ L Iron chloride II 1 Mol L ⁻¹
7	5 g starch + 1.5 g glycerol + 1000 μ L Cooper chloride II 1 Mol L ⁻¹

Source: Authors, 2021.

2.2 Determination of biodegradability time and light transmission

The biodegradability was carried out by the methodology described by Martucci and Ruseckaite (2009), with modifications. Film samples ($2 \times 2 \text{ cm}^2$) were dried up to constant weight (Mi). Samples were then placed in open polyethylene packaging to enable microorganisms and moisture to gain access to the 40%. After that, they were buried in natural soil, at constant moisture, and room temperature, and natural luminosity. Three, ten, fifteen and thirty days after the experiment installment, the artificial packaging with the samples was removed from the soil, washed with distilled water, and dried up to constant weight (Mf). The time of biodegradability was calculated using equation [1]. Ultravioletvisible (UV-Vis) light transmission of the film samples was placed in a quartz cuvette and transmittance was measured at wavelengths that ranged from 900-200 nm, in agreement with Santos et al. (2021).

Bio(%) =
$$(Mf - Mi)/Mi * 100$$
 (1)

2.3 Antibacterial activity

Antibacterial assay was evaluated *in vitro* against four bacteria: *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Salmonella serovar* Thyphimurium (ATCC 14028), and *Salmonella serovar* Enteritidis (ATCC 13076) commercially acquired and maintained from the bacteriological bank of the first author. Briefly, 150 μ L of bacterial culture 1x10⁴ cells mL⁻¹ were grown

on *Petri* dishes with dextrose tryptone agar (DTA) and 150 μ L of spore suspension 1x10⁸ CFU mL⁻¹ on *Petri* dishes with plant count agar (PCA). Films (7 mm diam.) were then placed on the surface agar and incubated at 36 °C for 36 h. The diameter of the inhibition zone was measured with a digital caliper. As a positive control, Azithromycin (15 μ g disc) and Cephalexin (30 μ g disc) discs were used and opposing control starch metals films disc. The minimum acceptable diameter was 5 mm. The assay was performed in triplicate by described Valadares et al. (2020) modified.

2.4 Statistical analysis

The statistical program used was PAST 3 (version 2019). The data were subjected to analysis of variance (ANAVA), and the averages were compared using the Tukey's test at a 5% significance level.

3 Results

Biodegradable films from biopolymers such as arrowroot showed a high biodegradability rate *in vitro* soil, as seen in (Figure 1). The films incorporated compared to the standard presented statistical difference except for the films incorporated with Iron sulfate II = 94%, Manganese sulfate = 94%, Iron chloride II = 93%, and Copper chloride II = 91%.

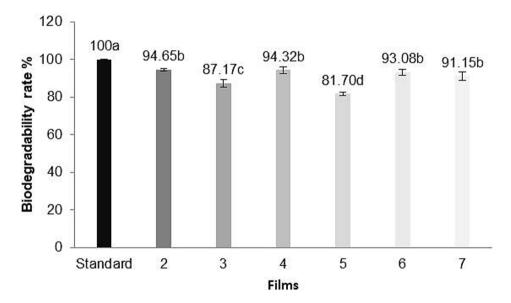


Figure 1. Biodegradability time of arrowroot starch films incorporated with metals. Standard, 2. Iron sulfate II, 3. Nickel sulfate, 4. Manganese sulfate, 5. Cobalt chloride, 6. Iron chloride II, 7. Copper chloride II. Equal letters between the averages for each biodegradable film incorporated with metals do not show a significant difference by the Tukey test 5%. Source: Authors, 2021.

Figure 2 shows seven photographic images of films produced with arrowroot starch incorporated with metals: (A) Standard film, (B) Iron sulfate II, (C) Nickel sulfate film, (D) Manganese sulfate film, (E) Cobalt chloride film, (F) Iron chloride II

film, and (G) Copper chloride II.

Concerning colors of films under development, the results show a decrease in light transmittance rates of films incorporating different metals in the visible region (from 250 to 890 nm). Maximum light transmission rate for Standard film 69.36 T%, Iron sulfate II film 68.22 T%, Nickel sulfate film 59.10 T%, Manganese sulfate, 30.85 T%, Cobalt chloride film 65.68 and 17.45 T%, Iron chloride II film 78.82 T%, and Copper chloride II film 62.94 T% (Figure 3).

The antibacterial activity demonstrated potential inhibitory activity for all films incorporated with metal compared to the reference antibiotics Azithromycin and Cephalexin (Table 1). *E. coli* statistical difference was observed according to Tukey test 5% for all metallic films, except for Nickel sulfate film and Cobalt chloride, both with inhibition zone = 22 mm, and Manganese sulfate = 10 mm, and Iron chloride II = 13 mm. *S. aureus* a difference was observed only for the films Cobalt chloride = 25 mm, and Iron chloride II = 13 mm according to Tukey test 5%. *S. serovar* Typhimurium showed statistical difference according to Tukey test 5% for the metallic films incorporated with Manganese sulfate = 14 mm, and Cobalt chloride = 22 mm, and for *S. serovar* Enteritidis significant difference by Tukey test 5% for the films incorporated

with Manganese sulfate = 8 mm, Iron chloride II = 15 mm, and Copper chloride II = 19 mm. The control films did not show any inhibitory activity for the bacterial strains evaluated.

4 Discussion

In general, the incorporation of metals in a biodegradable polymeric matrix presents satisfactory results, both mechanical, thermal, biodegradability, and visual and ultrastructural morphological aspects. Arrowroot starch in this study demonstrated good aptitude during the incorporation of metals in the form of sulfates and chlorides and plasticity with glycerol. A harmonious interaction between the components in the matrix is observed, promoting resistant films and specific intermolecular interaction. Although this is a pioneering study different from that observed that includes metal nanocomposites, other tests need to evaluate this interaction.

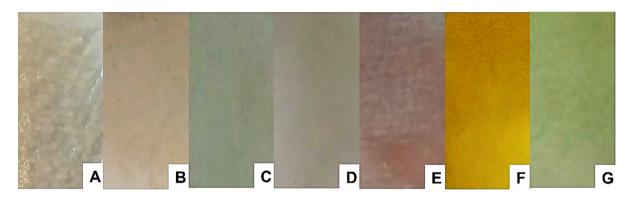


Figure 2. (A) Standard film, (B) Iron sulfate II film, (C) Nickel sulfate film, (D) Manganese sulfate film, (E) Cobalt chloride film, (F) Iron chloride II film and (G) Copper chloride II. Source: Authors, 2021.

Table 2. Antibacterial activity of biodegradable arrowroot films incorporated with different metals. Source: Authors, 2021.

Films	Inhibition zone (mm)								
	E. coli	S. aureus	S. serovar Typhimurium	S. serovar Enteritidis					
1 Control	0.00±0.00f	0.00±0.00e	0.00±0.00f	0.00±0.00g					
2	$19.35 \pm 0.012c$	$20.11 \pm 0.19b$	$17.03\pm0.21c$	$21.11 \pm 0.06b$					
3	$22.06 \pm 0.09 b$	$20.35 \pm 0.10b$	$18.12 \pm 0.33c$	13.67 ± 0.17 ed					
4	$10.05 \pm 0.21e$	11.10±0.30dc	$14.82 \pm 0.05 d$	$8.26 \pm 0.66 f$					
5	$22.08 \pm 1.12b$	$25.59 \pm 0.08a$	$22.14 \pm 0.94b$	$21.02 \pm 1.66b$					
6	$13.07 \pm 0.08e$	13.90±1.14c	11.66 ± 0.05 de	$15.09 \pm 0.96d$					
7 17.33 ± 0		$20.15 \pm 1.02b$	$16.55 \pm 0.18c$	19.13±0.27c					
Antibacterial references ^{a/b}	27.60 ± 0.17^{b} a	23.68 ± 0.06^a b	28.12 ± 0.11^{a} a	28.36 ± 0.83^a a					

^{1.} Standard film, 2. Iron sulfate II film, 3. Nickel sulfate film, 4. Manganese sulfate film,

Same lowercase letters in the same column do not differ statistically by the Tukey test (p < 0.05); equal capital letters on the same line do not differ statistically by the Tukey test 5%.

^{5.} Cobalt chloride film, 6. Iron chloride II film and 7. Copper chloride II.

^aCephalexin and ^bAzithromycin.

The assays evaluated in this study demonstrated that arrowroot starch is a prosperous biodegradable polymer capable of being compared to polymers that have been studied for a long time, as observed below in the discussion of the results obtained in this study for biodegradability, UV and visible light transmission, and antibacterial activity.

Biodegradability is one of the main factors when choosing a biopolymer, where the less it stays in the environment, the more attractive its production source. In this study, it was observed that most arrowroot films incorporated with metals had a high biodegradation rate (90%) in the soil. All metals, except Nickel sulfate and Cobalt chloride, did not interfere negatively during natural microbial activity. Although it was not the objective of

this study to evaluate the behavior of the metal regarding its toxicity in the microbiota, the visual organoleptic analysis was satisfactory.

Thus, this evaluation provides subsidies for further studies evaluating all metals, especially Cobalt, in the bioaccumulation of organisms living in that soil. Some metals are part of developing various forms of life in the soil, supplying the needs throughout the development process. However, some metals have a toxicity degree in some groups of microorganisms. Saral Sarojini et al. (2019) obtained variable time during the biodegradability test of chitosan-based films with Zinc oxide nanoparticle (Zn-NP), although all films had a good degradability rate of 28 days with a maximum median of 95%.

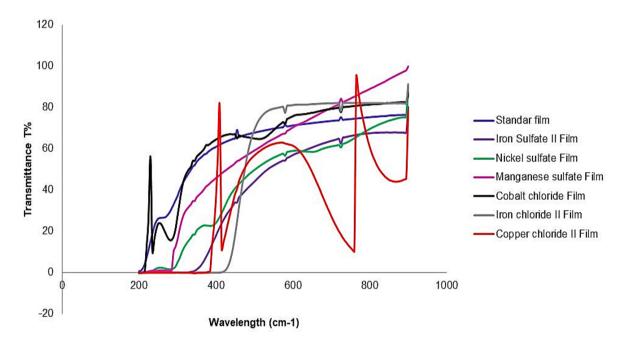


Figure 3. UV-Vis light transmittance rate in arrowroot films incorporating different metals. Source: Authors, 2021.

The transmission of ultraviolet rays and visible light negatively influences products, especially in foods and photosensitive solutions. The action caused by visible and UV light can cause lipid oxidation in foods with high-fat content, such as meat (Hosseini et al., 2015; Fathi et al., 2018). Biodegradable packaging incorporated with metals has a low rate of transmission or absorption of UV and visible light, thus promoting the maintenance of the product to be stored without changing its characteristics. The films incorporated with metals presented in this study demonstrated to be viable alternatives for the maintenance of various products, observing the desired metal and, of course, its concentration for a determined use. All embedded films were shown to promote the low transmission of both UV and visible light.

Several studies using biopolymers incorporated with metals, as in Pagno et al. (2015), evaluated the transmission rate of UV radiation in biodegradable quinoa starch films incorporated with Gold (Au) nanocomposites (NPs), obtaining a high radia-

tion absorption rate. The same was observed by Hasheninya et al. (2018) using kefiran-carboxymethyl cellulose with NPs of Copper oxide (CuO). High concentrations of CuO NPs resulted in a significant decrease in light transmission between the UV (200-400 nm) and visible (400-800 nm) ranges. Similar results were also observed by Arfat et al. (2017) using fish skin gelatin incorporated with NPs Ag-Cu, where the higher the concentration of NPs, the lower the transmission rate of UV and visible light. Semolina films incorporated with ZnO also showed a low UV and visible light transmission in the study by Jafarzadeh et al. (2017).

The metals incorporated in the arrowroot biopolymer matrix demonstrated potential in the capacity of bacterial inhibition against gram-positive and gram-negative bacteria, demonstrating that they are new options for active biodegradable films with antimicrobial capacity. There are several studies with metals, their cations, anions, or oxides incorporated in polymeric matrices (gelatin fibers/ Ce^{3+}) showing bacterial inhibition activity for *S. aureus* and *E. coli* (Yin et al., 2009), a film produced from TiO2 incorporated with CeO2 with 95% inhibition on sulfate-reducing bacteria (Wang et al., 2010).

Films based on carboxymethyl cellulose with modified zeolite incorporating particles of silver (Ag+) and gold (Au+3) exhibited potential bacteriostatic (*S. aureus* and *E. coli*) and fungistatic (*Candida albicans* and *Aspergillus niger*) effects in the study by Youssef et al. (2019). Cruz et al. (2020) found for two Cobalt I/II complexes a high inhibition activity for *E. coli* of 22.66 and 20.66 mm, respectively. These results are similar to that observed in this study for the biodegradable film containing Cobalt chloride on *E. coli*.

5 Conclusions

This study presented a proposal for the development of six new biodegradable packaging's (films) incorporated with different metals in the form of sulfates or chlorides, directly and mainly impacting on biodegradability, the transmission of UV rays and visible light and as an effective antibacterial agent from the biopolymer considered relatively new is arrowroot starch, which has characteristics that make it possible to incorporate materials without losing its capacity to form an active resistant film.

The results obtained demonstrate that arrowroot films incorporated with metals proved to be excellent options aiming at a complex ecological system that aligns biodegradability, with physical-chemical characteristics in decreasing the transmission rate of UV rays or visible light, and also microbiological with characteristic active use of products capable of acting with antibacterial and/or bacteriostatic action.

Finally, this work allows new *in vitro* assays to be produced evaluating both the biopolymer incorporated with metals, having its evaluation in cytotoxic terms the microbial flora of the soil to evaluate possible bioaccumulation of these metals in different types of tissues, organs or even absorption those with development effect and biotransformation.

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Author contribution

A.C.P.M.F.: Conceptualization, data processing, writing of studies, translation, final corrections, correspondence; M.V.A.V.: Data analysis, writing, translation corrections; R.F.: Provision of study materials, reagents, materials, and methodology development or design; M.B.T.: Preparation, creation and/or presentation of the published work; C.F.S.C.: Preparation, creation and/or presentation of published work, and provision of study materials and reagents; F.A.L.S.: Preparation, creation and/or

presentation of published work, and provision of study materials and reagents.

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EVALUATION OF THE CHEMICAL COMPOSITION OF THE ALCOHOLIC EXTRACT OF GYNOXYS CUICOCHENSIS CUATREC: IDENTIFICATION OF METABOLITES AND EXPLORATION OF THEIR PHARMACOLOGICAL PROPERTIES

EVALUACIÓN DE LA COMPOSICIÓN QUÍMICA DEL EXTRACTO ALCOHÓLICO DE GYNOXYS CUICOCHENSIS CUATREC: IDENTIFICACIÓN DE METABOLITOS Y EXPLORACIÓN DE SUS PROPIEDADES FARMACOLÓGICAS

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Abstract

Gynoxys cuicochensis Cuatrec., a member of the Asteraceae family, inhabits the Fierro Urco moor in the province of Loja. Despite not being used in traditional medicine, it possesses significant landscape value due to its striking yellow inflorescence. For investigating its chemical composition and potential pharmacological properties, a phytochemical characterization of the plant's ethanolic extract was conducted. The extract was obtained through static maceration for three days for the initial filtration, followed by one day for the second and third filtrations. Chlorophyll was separated using Diaion HP-20 resin as a solid phase packed in separation funnels, and an ethanol:water liquid phase with a concentration gradient ranging from 6:4 to 9:1 for elution. The dechlorophyllized extract was then freezedried and fractionated using gravity column chromatography. The obtained fractions were further purified through microcolumn and preparative chromatography. To elucidate the chemical structure of the molecules, nuclear magnetic resonance spectroscopy and electrospray ionization mass spectrometry were employed. Two metabolites were isolated for this study. The first one is a known glycosylated flavonoid called Nicotiflorin, while the second one is a phenolic derivative named 1,3-di-O-trans-feruloylquinic acid, which lacks a precise pharmacological description. This discovery represents an interesting and unique finding for this species, suggesting its potential medicinal use.

Keywords: Gynoxys cuicochensis Cuatrec, NMR, ESI, flavonoid, nicotiflorin, feruloylquinic acid.

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Resumen

La especie *Gynoxys cuicochensis Cuatrec.*, es una Asterácea que habita el páramo de Fierro Urco de la provincia de Loja. Si bien no es utilizada dentro de la medicina tradicional, posee un alto valor paisajístico por su llamativa inflorescencia amarilla. Con el objetivo de conocer su composición química y posibles propiedades farmacológicas, se efectuó una caracterización fitoquímica del extracto etanólico de la planta, obtenido mediante maceración estática por el lapso de 3 días para la primera filtración, y un día para la segunda y tercera filtración. La clorofila fue separada utilizando una fase sólida de resina Diaon Hp 20, la misma que fue empacada en embudos de decantación, y una fase líquida de EtOH:*H*₂*O* en gradiente de concentración desde 6:4 hasta 9:1 para la elución. El extracto desclorofilado se liofilizó y posteriormente se fraccionó utilizando cromatografía en columna por gravedad. Las fracciones obtenidas fueron purificadas mediante microcolumna y cromatografía preparativa. Para elucidar la estructura química de las moléculas, se recurrió a la espectrometría de resonancia magnética nuclear y espectrometría de masas de ionización por electrospray. Como resultado, se aislaron dos metabolitos: el primero es un flavonoide glicosilado conocido como Nicotiflorina, mientras que el segundo es un derivado fenólico, denominado ácido 1,3-di-O-trans-feruloilquinico que aún no cuenta con una descripción farmacológica precisa. Este descubrimiento representa un hallazgo interesante y único para esta especie en particular, lo que sugiere un posible uso medicinal.

Palabras clave: Gynoxys cuicochensis Cuatrec, RMN, ESI, flavonoide, nicotiflorina, ácido feruloilquinico.

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1 Introduction

The Asteraceae family is known for its diversity with around 24,000 species worldwide, making it the largest plant family after the orchids (Del Vitto and Petenatti, 2009). They are found in a wide variety of habitats, from the tropics to temperate regions. In Ecuador, this plant family represents approximately 10% of the country's flora.

Ecologically, the Asteraceae have a significant impact as they are important food sources for various animals, including insects, birds, and mammals (Flann et al., 2010). Regarding the chemical composition, the Asteraceae are known for their richness and variety of secondary metabolites, including iso- and chlorogenic acids, sesquiterpene lactones, pentacyclic triterpene alcohols, essential oils, alkaloids, and various acetylenic derivatives. These compounds have a great medical interest due to their cytotoxic and anticancer properties, among others (Figueroa, 2016).

A member of this plant family is the species Gy-

noxys cuicochesis Cuatrec., which features a flexible, striated stem with finely tomentose nodes. Its leaves are opposite, ovate-oblong, with striated petioles and slightly curved edges. The upper surface is glabrous and smooth green, except for the main vein, which is tomentulose, while the underside is densely tomentose-ochraceous, and the main vein is prominent, surrounded by 10-11 secondary veins on each side (Robinson and Cuatrecasas, 1992). The inflorescences are terminal, cymose-paniculate, and tomentose, with lanceolate, short, tomentose bracts. The heads are heterogeneous, with a conicalcampanulate involucre, consisting of 7-8 scarious and glabrous bracts. The female flowers are marginal, with ligulate corollas, and the hermaphroditic flowers are grouped in 8-9, with tubular corollas and deeply dentate campanulate limbs (León-Yánez et al., 2011). These morphological characteristics provide a basis for identifying the species and are important for its botanical description. In the Ecuadorian highlands, this plant is commonly known as "piquiles" or "tunash". It is used both as firewood and to provide structural support in the construction of temporary shelters (Lojan, 1992).



Figure 1. Gynoxys cuicochensis Cuatrec. in its habitat.

Although this species is not used in traditional medicine, this plant serves as an excellent pollinator attractor due to its abundant inflorescence du-

ring the early months of the year, making it an interesting candidate for phytochemical studies. To date, there are no records of scientific studies on the fixed chemical composition of this plant species. For this reason, this research focuses on the isolation of secondary metabolites from the plant using microcolumn gravity liquid chromatography techniques. The structure of the metabolites was elucidated through Nuclear Magnetic Resonance (NMR) and electrospray ionization mass spectrometry (ESI). The objective of this paper is to chemically characterize the species *Gynoxys cuicochesis Cuatrec*. to contribute to its ethnobotanical description.

2 Materials and Methods

2.1 General Information about the Sample

The collection of 25 kg of the aerial parts (leaves) of the plant species Gynoxys cuicochensis Cuatrec. was carried out in the Sebadal - Fierro Urco sector of the San Isidro neighborhood, San Pablo de Tenta parish, Saraguro, Loja province, located at an altitude of 2 990 meters above sea level (m.a.s.l.). The exact coordinates of the collection were S 3°40'47.4816 and W 79°1838.5056, under the collection permit for plant species MAE-DNB-2016-0048 granted by the Ministry of the Environment. For subsequent processing, the plant material was dried for 10 days at a temperature of 40°C, using the dehydrator located in the Chemistry laboratory at Universidad Técnica Particular de Loja (UTPL). As a result, 5 kg of dry plant material was obtained, ready to be used in research.

2.2 Extraction of the Plant Extract

The dry plant sample was manually ground and then macerated in individual containers containing 2 kg of plant material and 20 liters of an ethanol: water (EtOH:*H*₂*O*) solution in a 7:3 ratio, respectively. Three static maceration processes were conducted, the first for 3 days and the subsequent two for one day each. After filtering the extract, the ethanol was removed using a rotary evaporator. Then, the dry extract was lyophilized using a Labconco Corporation model 7754047 freeze-drier. The 100 ml samples were stored in Boeco®jars, and the experimental process lasted 5 days for each sample.

For separating the chlorophyll, two separation funnels of 500 ml and 1,000 ml were used. In the 1,000 ml funnel, 400 g of Diaion®HP-20 resin was

packed in an EtOH: H_2O solution with a 6:4 gradient, and 50 g of the extract diluted in the previously mentioned EtOH: H_2O solution was added. The same procedure was applied in the 500 ml funnel, but with 240 g of Diaion®HP-20 resin and 35 g of the extract. To obtain the solvent-free extract (EtOH), a BUCHI R-220 Pro rotary evaporator was used under the following conditions: cooling temperature of $-10^{\circ}C$, rotation of 40 rpm, bath water temperature of $30^{\circ}C$, steam temperature of $27^{\circ}C$ respectively, and vacuum pressure starting at 120 mbar and gradually increasing to 30 mbar.

Finally, to facilitate the use of the dechlorophyllized extract, it was lyophilized using the Labconco Corporation model 7754047 equipment. The sample was placed in a 100 ml Boeco®jar with a 400 ml volume, and the lyophilization process was conducted over 5 days.

2.3 Fractionation and purification of metabolites

The preparation of a sugar-free extract was carried out using a 1000 ml Butanol:Water (ButOH: H_2O) solution, dividing the solution into equal parts and constantly shaking for half an hour to achieve complete homogenization. After one hour of resting, the water was removed, and 15 grams of lyophilized extract were diluted in 700 ml of butanolic solution, adding an equal volume of distilled water, and set aside for an hour to allow the separation of compounds according to their polarity. This process was repeated twice to obtain the organic phase.

The organic phase was subjected to liquid chromatography on a microcolumn by gravity, using a polarity of Ethyl Acetate, Methanol, and Water (AcOEt, MeOH, and H_2O) in an 8:1:1 ratio, with a load of 800 mg of organic phase extract and 3 grams of direct phase silica gel. The collected fractions were subjected to continuous flow column chromatography (CCF), using Dichloromethane (DCM) as the solvent, and applying a polarity of DCM, AcOEt, MeOH in a 6:2:2, 5:3:2, and 4:5:1 ratio, respectively, adding 5 drops of Acetic Acid (AcOH) to each concentration. Preparative thin layer chromatography was performed on direct phase silica gel plates of 20cm x 20cm, using the same DCM, AcOEt, MeOH solution in a 6:2:2 ratio and 5 drops of AcOH.

The elution process was conducted in a glass chamber, and with the aid of UV light at 254 nm, the compounds to be separated were identified. The silica gel impregnated with the compounds was washed with the solution (DCM, AcOEt, MeOH 6:2:2 and 5 drops of AcOH) which was eluted in the CCF and placed in 10 ml vials for drying using nitrogen.

2.4 Characterization of Secondary Metabolites

The samples containing the separated metabolites were taken to the BRUKER NMR equipment, model MSC 500 MHz, to perform the necessary analyses and obtain spectra of ¹H, ¹³C, DEPT, COSY, HMBC, HSQC, and TOCSY. The equipment operated at a frequency of 500 MHz, and deuterated chloroform (CD*Cl*₃) was used as the solvent. Additionally, an ESI experiment was conducted through direct injection on a Bruker amaZon speed device, using nitrogen during the process.

3 Results and Discussion

Previous studies on various species of the genus Gynoxys, such as Gynoxys Sancto-antonii Cuatrec, Gynoxys psilophylla Klatt. (Bohlmann et al., 1986), Gynoxys dielsiana Domke (Zdero et al., 1980), Gynoxys acostae Cuatr., Gynoxys nitida Mushcl. and Gynoxys buxifolia Cass. (Keriko et al., 1995), have facilitated the isolation of various 3β, 6β 10β-H diacetyloxy furanoeremophilanes and 1β, 6β 10β-H diacetyloxy furanoeremophilanes, primarily modified with tigloyloxy, angeloyloxy, acetyloxy, and senecioyloxy substituents, which have been proposed as potential chemotaxonomic markers of the genus. However, a study on Gynoxys oleifolia Muschl. reports the existence of substituted ent-kaurane diterpenes, generating a debate about the presence of this metabolite in the genus Gynoxys (Beltrán et al., 2006).

It should be noted that, as minority components, 6-acetyl-2,2-dimethyl-chroman-4-one compounds have been isolated in *Gynoxys psilophylla Klatt* (Bohlmann et al., 1986) Germacrene D, bicyogermacrene, spatulenol, oleanolic acid in *Gynoxys nítida* Muschl.

and piceol in *Gynoxys buxifolia* (HBK) Cass (Keriko et al., 1995) Betulin in *Gynoxys cf. pulchella* (Kunth) Cass (Rodriguez, 2016). After conducting various phytochemical analysis tests on ethanolic fractions of *Gynoxys hirsuta* Wedd., the presence of pyrrolizidine alkaloids, furanoeremophilanes, and coumarins has been suggested (Ramírez, 2011).

Similarly, tests carried out on the volatile fraction of the leaves of *Gynoxys meridiana* Cuatrec, obtained through hydrodistillation, show γ -curcumene (31.9%), fukinanolide (22.3%), β -pinene (9.5%), α -phellandrene (7.1%), and α -pinene (5.7%) as main compounds. The most recent study on the chemical composition of the essential oil of *Gynoxys miniphylla* Cuatrec. reports that its major components are α -phellandrene (\sim 17%), α -pinene (\sim 15%), germacrene D (\sim 13,5%), trans-myrtanol acetate (8.8%), δ -cadinene (\sim 4.5%), β -phellandrene (\sim 3,5%), (E)- β -caryophyllene (\sim 2,5%), o-cymene (2.4%), α -cadinol (2.5%), and α -humulene (2%) (Malagón et al., 2022).

In this research, for the first identified fraction, in the proton analysis (Table 1), a doublet at 6.47 ppm and another at 6.73 ppm were detected, corresponding to H-6 and H-8 respectively. Both signals displayed a meta coupling constant J6,8= 2 Hz. Additionally, a doublet at 7.80 ppm integrating for 2H was identified, related to the equivalent positions H-2' and H-6'. Shifted to higher fields due to the influence of a hydroxyl group, there is a doublet at 6.94 ppm that integrates for 2H, corresponding to the equivalent protons H-3' and H-5', which have an ortho coupling constant J2',3'= 8.8 Hz. Furthermore, a singlet at 5.56 ppm corresponding to the anomeric position 1" of glucose and a doublet at 5.38 ppm related to the anomeric position 1" of rhamnose were observed, suggesting the presence of the disaccharide rutinose.

The combination of data derived from proton analysis and ESI mass spectrometry provides significant information about the molecule. The ESI analysis reveals a molecular ion $[M+H]^+$ =595.13, which indicates the presence of nicotiflorin, chemically known as Kaempferol-3-O-rutinoside.

Table 1. ¹H RMN of Nicotiflorin (Kaempferol-3-O-rutinoside).

13C (500 MHz), CD ₃ OD	δ (ppm)	DEPT	1H (500 MHz), CD ₃ OD	δ (ppm)	Mult.	ſ	J (H)	COSY	НМВС	НМQС
C-2			-	-	-	-	-	-		
C-3			-	-	-	-	-	-		
C-4			-	-	-	-	-	-		
C-5	162.2		-	-	-	-	-	-		
C-6	99.30	СН	Н-6	6.47	d	1H	2	H-6 / H-8	162; 94.27; 106.23	H-6 / C-6
C-7			-	-	-	-	-	-		-
C-8	94.27	СН	H-8	6.73	d	1H	2	H-8 / H-6	156.69; 162.2; 99.30; 106.23	H-8 / C-8
C-9	156.69		-	-	-	-	-	-		
C-10	106.23		-	-	-	-	-	-		
C-1'	120.98		-	-	-	-	-	-		
C-2'	130.63	СН	H-2'	7.80	d	2H	8.8	H-2' / H-3'	160.38 130.63	H-2' / C-2'
C-3'	115.32	СН	H-3'	6.94	d	2H	8.8	H-3' / H-2'	160.38; 115.32; 120.8	H-3' / C-3'
C-4'	160.33	С	-	-	-	-	-	-	-	
C-5'	115.32	СН	H-5'	6.94	d	2H	8.8	H-5' / H-6'	160.38 115.32 120.98	H-5' / C-5'
C-6'	130.63	СН	H-6'	7.80	d	2H	8.8	H-6' / H-5'	160.38 130.63	H-6' / C-6'
C-1"	98.4	СН	H-1"	5.56	s	1H	-			H-1" / C-1"
C-2"			H-2"							
C-3"			H-3"							
C-4"			H-4"							
C-5"			H-5"							
C-6"	69.7	CH ₂	H-6"	3.83	dd	2H	3.3; 9.5			
										TT 133 /
C-1"	102.08	СН	H-1"	5.38	d	1H	1.3		70.75	H-1"" / C-1""
C-2""	70.75	CH	H-2""	3.67	m	1H	-	-	69.65; 72.11	
C-3""	72.11	СН	H-3""							
C-4""	-	СН	H-4"	3.33	m	1H	-	-	70.75	
C-5"	69.65	СН	H-5"	3.48	m	1H	-	-	16.71; 70.75	
C-6"	16.71	СН	Н-6"	1.25	m	1H	-	-		

Nicotiflorin (Figure 1) is a flavonoid composed of a glucose molecule and a rutinose molecule. Glucose is a monosaccharide with the chemical formula $C_6H_{12}O_6$, while rutinose is a flavonoid consisting of a flavone structure attached to a glucuronic acid molecule. Furthermore, nicotiflorin possesses a nicotinyl group attached to the C-6 position of glucose, distinguishing it from other flavonoids. The chemical structure of nicotiflorin is complex and features various functional groups, such as hydroxyls, ketones, and ethers.

Glycosylated flavonoids, also known as flavonoid glycosides, are widely distributed in plant species and are known for their diverse pharmacological effects. Most of these compounds contain glucose as the sugar present, although galactose, rhamnose, xylose, and the disaccharide rutinose have also been found. In plants, flavonol glycosides and aglycones have important functions, such as protection against UV radiation, internal physiological regulation, and reproduction. They also act as antioxidants, scavenging free radicals and supporting the plant's immune system (Slámová et al., 2018).

Addressing pharmacodynamics, it is known that glycosylation significantly enhances the water solubility of flavonoids, thereby increasing the bioavailability of the corresponding flavonoid aglycone, depending on the nature of the sugar. Importantly, glycosides are absorbed more rapidly than rhamnosides and rhamnoglucosides due to the availability of hydrolyzing enzymes in the human

gastrointestinal tract. These enzymes, such as intestinal lactase-phlorizin hydrolase or β -glucosidase present in the epithelial cells of the small intestine can metabolize glycosides. Conversely, there are no α -l-rhamnosidase or rutinosidase enzymes in humans, which means that the bioavailability of flavonoids containing rhamnose depends entirely on their cleavage by the intestinal microbiota (Slámová et al., 2018; Khodzhaieva et al., 2021).

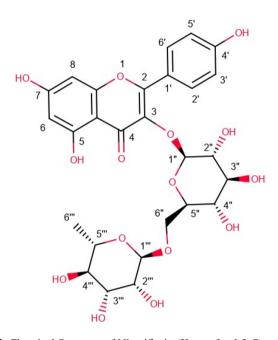


Figure 2. Chemical Structure of Nicotiflorin (Kaempferol-3-O-rutinoside).

The addition of rhamnose to flavonoids can enhance their solubility and stability, as well as confer specific pharmacological properties and selectivity. The sugar used in glycosylation is typically a disaccharide composed of L-rhamnose (also known as 6-deoxy-mannose) and D-glucose, which are linked by an α -(1-2) or α -(1-6) glycosidic bond. While glucosylation or galactosylation increases the water solubility of the flavonoid, the presence of rhamnosyl residues may slightly decrease it. Thus, the presence of a sugar residue in a flavonoid appears to play a significant role in solubility, bioavailability, and biological activity. For example, flavonoid glycosides can act as "prodrugs" to release aglycones into the gastrointestinal tract (Slámová et al., 2018).

Kaempferol is a flavonoid commonly found in the Asteraceae family, and it has been shown to have anticancer and anti-inflammatory effects. Furthermore, kaempferol and its associated compounds have also been found to possess antibacterial, antifungal, and antiprotozoal activities (Adebayo et al., 2010). Recently, kaempferol has been reported to have neuroprotective action in the brain, inhibiting pro-inflammatory cytotoxicity and the activity of significant inflammatory pathways (Silva et al., 2021). Chronic inflammation and free radicals are known risk factors for cancer development. Therefore, active principles capable of inhibiting these factors are useful for inducing cytotoxicity in cancer cells. The anticancer activity observed in so-

me kaempferol derivatives is noteworthy, making plant species that contain it ideal candidates for the search for treatments against various types of cancer.

Numerous derivatives of kaempferol have demonstrated anticancer activity. For instance, trifolin (kaempferol 3-O-D-galactoside) induces apoptosis in lung cancer cells through both intrinsic and extrinsic pathways (Kim et al., 2016). Azfelin (kaempferol-3-O-rhamnoside) has been suggested as a chemotherapeutic therapy for breast cancer due to its antioxidant properties and its ability to prevent oxidative damage to biomolecules (Vellosa et al., 2015). Meanwhile, nicotiflorin (kaempferol-3-O-rutinoside) has been shown to be hepatoprotective, reducing cytokine and serum enzyme levels and restoring antioxidant indicators in liver homogenate (Zhao et al., 2017).

Furthermore, Li et al. (2006) have demonstrated that nicotiflorin significantly reduces neuronal damage and cell death in models of permanent focal cerebral ischemia and in neuronal cultures subjected to oxidative stress. It also decreases the production of reactive oxygen species (ROS) and the activation of inflammatory factors in these models, suggesting a neuroprotective action of kaempferol. Therefore, kaempferol derivatives hold significant potential as therapies against cancer and neurodegenerative diseases.

According to Patel (2022), nicotiflorin has pharmacological properties such as antioxidant, antiinflammatory, cardioprotective, and antidiabetic effects. This compound has traditionally been used in Chinese medicine to treat various conditions, including gastrointestinal disorders, respiratory infections, and cardiovascular diseases. To date, glycosylated flavonoids have not been reported in the literature as present in species of the genus Gynoxys. Therefore, the isolation of nicotiflorin in Gynoxys cuicochensis Cuatrec. represents a valuable contribution to the available chemotaxonomic information. For the second fraction, a structural elucidation has been proposed that suggests the presence of a derivative of quinic acid known as 1,3-di-O-trans-feruloylquinic acid.

NMR analysis reveals distinctive features in the proton signals (Table 2). Firstly, there is an evident

aromatic contribution due to signals corresponding to positions H2 (7.07 ppm, d, J=8Hz, 2H), H5 (6.82 ppm, d, J=8Hz, 2H), and H6 (7.18 ppm, d, J=6Hz, 2H), indicating the presence of a trisubstituted system. Additionally, two signals are highlighted for H7 (7.64 ppm, d, J=16Hz, 2H) and H8 (6.40 ppm, d, J=16Hz, 2H), suggesting the existence of a double bond in a trans configuration. It is important to note that the presence of methoxy groups in the aromatic structure is also observed by the signals for OCH₃ (3.88 ppm, s, 6H).

The 1,3-di-O-trans-feruloylquinic acid (Figure 2) is an ester derivative of dihydroxycinnamoylquinic acid, characterized by the presence of two feruloyl groups attached at positions 1 and 3 of the quinone unit in the central ring. Wenzl et al. (2000) also reported the adoption of a chair conformation with the carboxylic group in an axial position in this molecule, due to steric interactions between the functional groups in its structure.

The axial position of the carboxylic group is attributed to the need to minimize such steric interactions among the present functional groups. In this context, the hydroxyl group at position 5 and the feruloyl groups at positions 1 and 3 are oriented in the opposite direction to the carboxyl group, leading to the minimization of steric interactions.

Additionally, it is important to note that the chair conformation is stable for molecules with six-membered rings, such as the central ring of 1,3-di-O-trans-feruloylquinic acid. In this conformation, the substituent groups on the carbon atoms of the ring are oriented in opposite directions, which contributes to the minimization of steric interactions and, thereby, enhances the overall stability of the molecule.

It has been observed that this metabolite accumulates in the more mature regions of the root system, excluding both root tips and shoots. This metabolite has been attributed with antioxidant activity that regulates blood lipids in rats. Moreover, *in vitro* studies have demonstrated its anti-inflammatory activity, suggesting its potential as a therapeutic agent in inflammatory diseases. Various additional biological activities have been described for this compound, including antimicrobial, antitumor, antidiabetic, and neuroprotective properties (Wenzl et al., 2000).

¹ H (500 MHz), CD ₃ OD	δ (ppm)	m	J (Hz)	ſ	δ (ppm)	m	J (Hz)	ſ
7"	7.54	d	16	2.3	7.52	d	16	1
7'	7.44	d	16	3.5	7.37	d	16	1
2"	7.30	s ancho	-	1.2				
2'	7.27	s ancho	-	1.6	7.24	s	-	2
6"	7.10	d	8	1.4				
6'	7.05	d	8	2.8	7.03	d	8.1	2
	7.01 - 6.90	m	-	2.7				
5' 5"	6.79	d	8	2.9	6.75	d	7.8	1
	0.79	u	O	2.9	6.73	d	7.8	1
	6.78 - 6.67	m	-	4.0				
8"	6.44	d	16	1.5	6.39	d	16	1
8'	6.36	d	16	1.5	6.36	d	16	1
	6.28 - 6.13	m	-	2.3				
	5.74	m	-	0.5				
	5.51	m	-	0.7				
3	5.19 - 5.38	m	-	2.1	5.18	dt	10/4	1
	4.89	m	-	0.8				
	4.11	s ancho	-	0.8				
	4.01	s ancho	-	1.3				
	3.83	S	-	3	3.78	s	-	3
	3.82	S	-	3	3.75	s	-	3
4.5	3.76	m	-	2.2	3.69	m	-	1
					3.64	m	-	1
2	2.35	m	-	1.3	2.28	t	13	3
	2.24 - 2.14	m	-	2.1				
	2.11 - 1.95	m	-	2.1				
6	1.92 - 1.63	m		4.7	2.00	dd	12/3	1
	1.92 - 1.03	111		4./	1.85	d	12	1
	1.48	m	-	1.4				
	1.24	s ancha	-	3.48				

Table 2. ¹H RMN of 1,3-di-O-trans-feruloylquinic Acid.

Given that the bibliographic information available on this metabolite is limited and does not allow for a comprehensive comparison among sources, the biological activity described for its precursor, quinic acid, has also been considered as a reference point.

Quinic acid is a natural hydroxycarboxylic acid with a simple phenolic chemical structure. It consists of a six-carbon ring bearing two hydroxyl groups and one carboxyl group. In plants, it plays several important roles, including regulating growth and development, protecting against environmental stress, and defending against pathogens. Moreover, quinic acid is involved in the synthesis

of other phenolic compounds, such as flavonoids. It has been shown to induce the production of phytoalexins, which are antimicrobial compounds produced by plants in response to pathogen infection (Ma and Ma, 2015).

Although the direct relationship between the metabolite and quinic acid still requires more detailed investigation. The known biological activity of quinic acid provides a solid foundation for exploring the therapeutic potential and biological properties of this understudied metabolite. A comparative approach with quinic acid may aid in gaining a deeper understanding of its biological activity and potential applications.

Figure 3. Chemical Structure of 1,3-di-O-trans-feruloylquinic Acid.

Quinic acid has been extensively researched due to its remarkable biological activity, spanning various areas of interest. Properties such as antioxidant, anti-inflammatory, antihypertensive, antidiabetic, and neuroprotective activities have been related with this compound (Pero et al., 2009; El-Askary et al., 2022). Additionally, it has been demonstrated to exert beneficial effects on cardiovascular health, glucose metabolism, obesity, and cellular damage.

Studies have revealed that quinic acid can regulate gene expression, modify enzymatic activity, and modulate cellular signaling pathways. These actions shape its biological impact on various diseases and pathological conditions. Its antioxidant capacity enables it to neutralize reactive oxygen species, thus protecting cells from oxidative damage. Likewise, its anti-inflammatory activity helps to reduce excessive inflammatory responses in different tissues and biological systems (Heikkilä et al., 2019).

Quinic acid has also shown positive effects on blood pressure regulation, making it a potential agent for controlling hypertension. Additionally, its ability to enhance glucose metabolism is interesting in the context of diabetes and insulin resistance (Singh et al., 2021; Lee et al., 2022). Regarding its neuroprotective action, quinic acid has demonstrated the capacity to protect nerve cells against oxidative stress and other harmful events, suggesting

its potential in the prevention and treatment of neurodegenerative disorders (Zuo et al., 2015; Clifford et al., 2017).

4 Conclusions

The alcoholic extract of *Gynoxys cuicochensis Cuatrec.*, was successfully obtained from a sample collected in the Sebadal-Fierro Urco area in Loja. Using microcolumn liquid chromatography, NMR, and ESI techniques, the fractionation and chemical characterization of the extract were achieved.

Phytochemical screening of the alcoholic extract from the leaves of *Gynoxys cuicochensis Cuatrec*. revealed the presence of a flavonoid compound and a quinic acid derivative, both known for their pharmacological properties. However, it is important to note that this species is not used in traditional medicine.

In the experimental phytochemical study of Gynoxys cuicochensis Cuatrec., the analysis of proton and carbon nuclear magnetic resonance spectra (¹H and ¹³C), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple-bond correlation (HMBC), distortionless enhancement by polarization transfer (DEPT), correlated spectroscopy (COSY), and total correlation spectroscopy (TOCSY) enabled the identification of the first me-

tabolite as nicotiflorin (kaempferol-3-O-rutinoside). It is noteworthy that the presence of this compound in the Gynoxys genus has not been previously reported, opening new doors for further research on this species and contributing to scientific advancement in the region.

Regarding the second isolated metabolite, 1,3-di-O-trans-feruloylquinic acid, the available scientific information is limited. However, a possible antioxidant and anti-inflammatory activity can be proposed due to its derivation from quinic acid, as phenolic compounds are generally known to have these properties. To fully understand the properties and therapeutic potential of this metabolite, further research and more detailed studies are necessary. These results contribute to the scientific knowledge of the region and lay the groundwork for exploring new pharmacological and therapeutic applications in the field of phytotherapy.

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Author Contribution

Conceptualization: A.M.; Data curation: O.M, G.G., S.E., N.C. and A.M.; Formal analysis: O.M., P.C., G.G., S.E. and A.M.; Acquisition Financing: O.M.; Research: O.M., G.G., S.E. and A.M.; Methodology: A.M.; Project administration: O.M.; Resources: O.M.; Supervision: O.M. and G.G.; Validation: O.M. and G.G.; Visualization: O.M. and A.M.; Writing-original draft, review and editing: A.M.

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Review / Reseña bibliográfica







PRODUCTION OF BEAUVERIA BASSIANA FOR THE FORMULATION OF BIOPESTICIDES

Producción de BEAUVERIA BASSIANA para la formulación de **BIOPLAGUICIDAS**

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Abstract

The harmful effects of chemicals in conventional agriculture and the growing demand for food free of toxic residues has developed environmentally sustainable strategies. An effective alternative for integrated pest management in agricultural crops are biopesticides formulated with microorganic structures or from the production of active compounds. This paper describes the production processes of Beauveria bassiana for formulating biopesticides for agricultural use. The information was collected through a systematic search in Research Gate, Google Scholar, Science Direct and PubMed, using keywords such as production, Beauveria bassiana, solid fermentation, liquid fermentation and metabolites. The results affirm that B. bassiana is one of the microorganisms with great potential to produce biopesticides, due to the entomopathogenic mechanism of action and secondary metabolites, which can be used for the biological control of phytophagous insects. Likewise, for the formulation of B. bassiana it should be considered a profitable culture medium for large-scale production, also the control of environmental variables such as temperature at 25 °C, relative humidity 65-70%, pH of 5.4, propagation time between 4 to 8 days, and for the liquid fermentation process, a constant agitation between 200 to 400 rpm must be maintained. Biological products represent an alternative to minimize the use of synthetic pesticides, reduce environmental pollution and ensure food safety and security.

Keywords: Biopesticides, Beauveria bassiana, solid fermentation, liquid fermentation, metabolites.

Resumen

Los efectos nocivos de los productos químicos en la agricultura convencional y la creciente demanda de alimentos libres de residuos tóxicos, ha dado lugar al desarrollo de estrategias sostenibles con el medio ambiente. Una alternativa eficaz para el manejo integrado de plagas en los cultivos agrícolas son los bioplaguicidas formulados con estructuras de microorganismos o a partir de la producción de los compuestos activos. En este contexto, el presente trabajo describe los procesos de producción de *Beauveria bassiana* para la formulación de bioplaguicidas de uso agrícola. La recolección de la información se realizó mediante una búsqueda sistemática en ResearchGate, Google Académico, ScienceDirect y PubMed, empleando palabras claves como producción, *Beauveria bassiana*, fermentación sólida, fermentación líquida y metabolitos. A partir de los resultados de la investigación se afirma que *B. bassiana* es uno de los microorganismos con gran potencial para la producción de bioplaguicidas, por el mecanismo de acción entomopatógeno y los metabolitos secundarios, que pueden ser utilizados para el control biológico de insectos fitófagos. Así mismo, en la producción de *B. bassiana* se debe considerar un medio de cultivo rentable a gran escala, además de controlar las variables ambientales como temperatura a 25 °C, humedad relativa 65-70%, pH de 5.4, tiempo de propagación entre 4 a 8 días, y para el proceso de fermentación líquida agitación constante entre 200 a 400 rpm. Los productos biológicos representan una alternativa para minimizar el uso de plaguicidas sintéticos, reducir la contaminación ambiental y garantizar la seguridad e inocuidad de los alimentos.

Palabras clave: Bioplaguicidas, Beauveria bassiana, fermentación sólida, fermentación líquida, metabolitos.

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1 Introduction

Agricultural crops are affected by bacteria, fungi, weeds, insects and nematodes, causing yield reductions (Thakur et al., 2020). Since 1960, agricultural pest control methods have been carried out by applying synthetic pesticides, such as dichlorodiphenyl-trichloroethane (DDT), and other organophosphate pesticides and carbamates (Kumar, 2012).

Green revolution technologies contributed to increase food production through intensive agriculture by using chemical fertilizers and pesticides (Kumar and Singh, 2015). However, adverse effects such as soil degradation, water pollution, insect resistance, and toxic residues in food (Lengai and Muthomi, 2018), have demanded the production of healthy food, decreasing the use of natural resources, and strengthening sustainable agriculture (Kumar, 2012).

The production of microbial biopesticides has increased due to the demand for chemical-free food, being essential in organic agriculture (Mascarin and Jaronski, 2016). Biopesticides are generally composed of beneficial bacteria, viruses, fungi, and nematodes with chitinolytic, entomopathogenic and antagonistic activities, used as biological controllers of phytopathogens, insects and phytophagous nematodes (Lengai and Muthomi, 2018).

Biochemical and genomic analyses have shown that the metabolites produced by the microorganisms have great potential in biological pest control (Luo et al., 2017). The identified metabolites of *Beauveria bassiana* are beauvericin and bassiacridin with insecticidal action (Al Khoury et al., 2019; Quesada-Moraga and Vey, 2004), are used for controlling *Tetranychus urticae*, *Bemisia tabaci* and *Locusta migratoria*; oosporein with antiviral and antibacterial effects on *Enterococcus faecalis* and *Stenotrophomonas* spp. (Jeffs and Khachatourians, 1997), and bassianin as an ATP inhibitor (Patočka, 2016).

Formulation technologies of biological control agents for commercial scale-up must consider certain criteria for the production process (Ávila-Hernández et al., 2020) such as stabilization of the microorganism in the production; hence, distribution and storage stage conditions conducive to

field application should also be provided (Dannon et al., 2020). Evaluations of persistence after application and adaptation to environmental conditions without altering the physicochemical properties of the microorganism should be carried out (Ávila-Hernández et al., 2020).

Pesticides made from entomopathogenic fungi are frequently used in phytosanitary programs to control populations of phytophagous insects (Luo et al., 2014). One of the most relevant entomopathogenic fungi in the agricultural field is *Beauveria bassiana*, used to control pests such as the coffee berry borer (*Hypothenemus hampei*), black banana weevil (*Cosmopolites sordidus*), aphids and spider mites, among others (Gerónimo et al., 2016; Al Khoury et al., 2019; Ávila-Hernández et al., 2020). This fungus is considered a natural enemy of insects in ecosystems, crop residues and colonized hosts (Marín et al., 2018).

Commercial formulations of Beauveria bassiana include artisanal methods such as fermentation on solid substrates, in which the fungus is inoculated on a substrate and the application is done by filtering the conidia of the microorganism. On the other hand, the most innovative methods consist of developing dry and liquid formulations, boosting the fungal propagule (conidia or blastospores). The drying process is performed by spray drying, air drying, rotary vacuum drying and fluidized bed drying; these techniques are employed to stabilize Beauveria bassiana propagules on a large scale and have a satisfactory shelf life (Mascarin and Jaronski, 2016). Regarding the analysis on the use of chemical pesticides and the need to produce environmentally friendly inputs, a systematic review has been carried out with the aim of describing the production processes of Beauveria bassiana for the formulation of biopesticides for agricultural use.

2 Methodology

A systematic review of 60 manuscripts was carried out in specialized search engines and databases of ResearchGate (18), Semanticscholar (10), Google Scholar (14), Springer (2), SciELO (2), ScienceDirect (6) and PubMed (8), dedicating approximately 1920 hours for the bibliographic search, review and scientific writing. The research focused

on the entomopathogen Beauveria bassiana by its biocidal potential for controlling phytophagous insects, and the in vitro and commercial scale production processes for formulating biopesticides for agricultural use. The search was carried out in journals such as the Journal of Applied Entomology, Plant Protection Science, World Journal of Microbiology and Biotechnology, Biology, Journal of Invertebrate Pathology and others as primary sources; and in institutions such as the National Agricultural Health Service and companies as secondary sources that formulate insecticides containing Beauveria bassiana. The information selected corresponds to articles published during the last 10 years, with some exceptions considered by their relevance to the bibliographic review.

3 Beauveria bassiana as biological controllers

The entomopathogen *B. bassiana* was first observed in silkworms. The larvae had a white outer covering with multiple inflorescences that could infect healthy larvae in a short period of time (Bassi, 1835). In 1954, the first outbreaks of infection in acridids were reported, but it was in 1987 when the pathogenicity of the microorganism in grasshoppers was proven under laboratory conditions (Inglis et al., 1993).

In recent years, about 700 species of insect hosts of *B. bassiana* have been discovered (Xiao et al., 2012). This fungus can infect major insect taxa when it finds the right conditions to inoculate the host. However, studies related to the pathogenicity of *B. bassiana* have focused on insects considered pests (Meyling and Eilenberg, 2007). Genome sequencing of infected hosts showed that *B. bassiana* evolved from insects; it is also assumed that the expression of certain protease and chitinase genes are associated with functions necessary for insect pathogenesis and convergent evolution (Xiao et al., 2012).

4 Biopesticidal potential of *Beauve-ria bassiana*.

Beauveria bassiana is an entomopathogenic fungus used as a biological controller of agricultural crop

pests (Barcenilla, 2021). The pathogen enters insects through conidia that attach to the host cuticle. Formation of the germ tube and appressorium allows attachment to the insect integument by pressure. The action of hydrolytic enzymes such as lipases, proteases and chitinases enter the insect body through the soft parts (Lara-Juache et al., 2021). The hyphae have contact with the hemolymph containing high nutrient initiating a stage of unicellular blastospore budding (Mascarin and Jaronski, 2016). The fungus colonizes the internal tissues of the insect and during this process they release metabolites such as beauvericin and bassiacridin that help inhibit the immune system, facilitating entry into the internal organs of the host, causing its death (Figure 1) (Harith-Fadzilah et al., 2021). Infected insects show a cottony, powdery, creamy-yellow, cottony cover that coats the external part of the host (Barcenilla, 2021).

In the colonization stage, *B. bassiana* secretes proteins and enzymes that can be used as cell factories for producing commercial inputs. The evaluation of the metabolic activity by complete genome sequencing expresses that *B. bassiana* has an open pangenome with the capacity to colonize different hosts (insects, nematodes and plants). In addition, 10366 genes coding for proteins such as proteases and 145 carbohydrate-active enzymes of the chitinases, cellulases and hemicellulases type were identified (Vidal and Jaber, 2015).

Gene expression of *B. bassiana* in the insect inoculation process demonstrates the presence of peroxidase, trehalase, lipase, peptidase, phosphatase and lyase enzymes responsible for host cuticle degradation. The chitinase enzymes of B. bassiana hydrolyze the b-bonds of chitin polymers into Nacetyl b-D-glucosamine monomers present in the arthropod exoskeleton (Amobonye et al., 2020). The cuticle of insects consists of surface lipids that act as a protective barrier to pathogens. However, lipases produced by B. bassiana contribute to the degradation of the insect cuticle (Salazar et al., 2020); these are water-soluble enzymes that act on insoluble substrates that can hydrolyze triglycerides and transform them to fatty acids and glycerol. Entomopathogenic proteases act on the host cuticle and attack weakened tissue by chitinolytic actions (Amobonye et al., 2020).

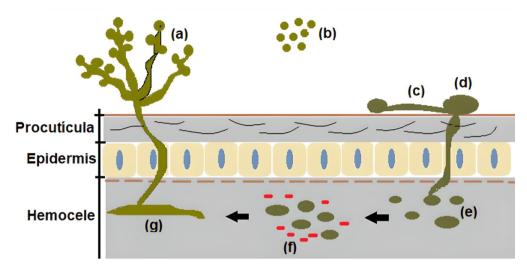


Figure 1. Infection process of *Beauveria bassiana* in the cuticle of insects. (a) Structures of *B. bassiana* (b) Dissemination of *B. bassiana conidia* (c) Formation of the germ tube on the surface of the insect body (d) Formation of the appressorium and entry of the hyphae into the procuticle and epidermis. (e) Production of blastospores and invasion into hemocele. (f) Release of secondary metabolites. (g) Formation of the fungal structures (hyphae, mycelium and conidia) and release into the outer space.

The diversity of toxins produced by B. bassiana can range from simple compounds such as biological macromolecules, e.g. oxalic acid, 2,6- pyridindicarboxylic acid (dipicolinic acid), and compounds with more complex structures of cyclic and linear peptide nature such as beauvericin, bassiacridin, beauverolides and bassianolides (Borges et al., 2010). Toxins alter the natural and artificial permeability of membranes, induce cell fluid loss, produce changes in the nucleus during molting and metamorphosis processes, deform the external structures of insects and interfere with fertilization processes (Patočka, 2016). Finally, lipase, chitinase, protease and amylase enzymes degrade the exoskeleton of the host and allow the fungus to enter its internal tissues (Cortés and Mosqueda, 2013).

5 In vitro production of Beauveria bassiana

The processes for the conidia production of *B. bassiana* can range from very simple methods to processes that involve more technification (Vela et al., 2019). The first step for *B. bassiana* production consists of isolating the strains of the microorganism, for which it is important to determine the composition of the culture medium and the optimal parameters for growth. Greenfield et al. (2016) state that *B. bassiana* strains can be grown on glucose

agar, peptone of soy, potato-dextrose agar (PDA), dextrose-Sabouraud agar (SDA) or oatmeal agar. Optimal temperatures for growth range from 22-26°C, alternating light and dark for eight days. In contrast, Bugti et al. (2018) state that a spore suspension of the microorganism should be made and inoculated on dextrose-Sabouraud agar culture medium (20 g agar, 10 g peptone, 40 g dextrose, 0.5 mg potassium, in 1000 mL of distilled water) and incubated at 24 ± 1 °C for twelve days.

The second step is to test the pathogenicity of *B. bassiana* by inoculating healthy insects by preparing conidial suspensions in 0.05% Tween 80 solution, with constant agitation for five minutes, adjusting the spore concentration and spraying the insects (Bugti et al., 2018). Table 1 details studies conducted to test the pathogenesis of *B. bassiana* on different hosts.

6 Fermentations in solid substrates

The production of *Beauveria bassiana* on solid substrates by aerial conidial reproduction is the most used method at commercial level for small, medium and large companies dedicated to the production of biopesticides (Feng et al., 2008). Culture media for the production of *B. bassiana* must have a nutrient composition and controlled conditions of pH, tem-

perature, light, water availability and atmospheric gas mixture, crucial factors for the growth of the microorganism and sporulation (Patočka, 2016). The production process consists of inoculating *B. bassiana* conidia on nutrient solid substrates. Fermentation on solid substrates, under controlled conditions can achieve yields from 4×10^{12} to 4×10^{13} conidia·kg⁻¹.

The most commonly used materials for solidstate fermentation are rice, barley, wheat, rye, sorghum and corn, (Jaronski, 2014). Likewise, agroindustrial wastes allow reducing production costs and energy consumption. An example of this is potato skin and rice husk, which allow reaching a production of $1.3 \times 10^9 \text{ spores} \cdot \text{g}^{-1}$ of *B. bassiana* under optimal conditions of humidity 65-70%, temperature 25°C and time between 7 to 8 days (Sala et al., 2021).

Another important factor is the fermentation containers, which can be high temperature resistant plastic bags or large chambers, considering aeration since propagules require a balanced gas exchange for mycelial growth. The production of aerial conidia can be done in one or two stages. The first stage consists of inoculating the fungus directly from the solid substrate and the second stage involves the production of the inoculum by liquid fermentation and subsequent inoculation into the solid substrate (Mascarin and Jaronski, 2016).

Table 1. Studies conducted to test the pathogenesis of *Beauveria bassiana* on different hosts and their mortality percentage.

Conidia concentration/ml	Host	Morphological phases	Mortality percentage	Reference
	Lygus lineolaris Anthonomus		77.47	
1×10^8	signatus	Adultos	60.35	Sabbahi et al. (2008)
	Otiorhynchus ovatus		54.50	
1×10^{7}	Bemisia tabaci	Adults	67	Ruiz et al. (2009)
1.7×10^{8}	Ceratitis capitata	Adults	91.90	Muñoz et al. (2009)
1×10^{7}	Bemisia tabaci	Eggs	27	Ruiz et al. (2009)
1 × 10 ⁸	Premnotrypes vorax	Larvae	96	García et al. (2013)
2×10^7	Panonychus citri	Nymphs Adults	94	Alayo and Krugg (2014)
1×10^7	Hypothenemus hampei	Adults	100	Gerónimo et al. (2016)
1×10^{8}	Helicoverpa zea	Larvae	100	Everton et al. (2016)
1×10^{8}	Tenebrio molito	Larvae	100	López-Sosa et al. (2018)
1 × 10 ⁶	Rhynchophorus palmarum L.	Adults	43.33	León et al. (2019)
4×10^{10}	Monalonion velezangeli	Nymphs	84	Góngora et al. (2020)

The substrate must be previously sterilized at 121°C, 15 lb pressure for 30 min, the substrate is inoculated with the suspension of *B. bassiana* conidia or a proportion of mycelium. The inoculated substrates are incubated at 25 °C for 7 days and contaminated substrates are removed (López-Sosa et al., 2018). Consequently, bags are shaken to oxygenate the inoculum and achieve a homogeneous mixture, incubated for 21 days at 24 °C (Monzón,

2001) preserving a relative humidity of 53%. Once the fungus is sporulated throughout the substrate, the drying process is carried out at temperatures between 16-20 °C for a period of 5 to 6 days to reduce the relative humidity to 15% (Gómez et al., 2014).

The main problem of solid fermentation is commercial scale-up, whose focus is the efficient and economic production of a large number of conidia to reduce production costs and be able to compete with traditional pesticides (Rodríguez-Gámez et al., 2017). Therefore, it is important to use good quality substrates and containers, but at the same time cheap; and to constantly monitor each of the production processes to avoid contamination, performing a correct sterilization process and handling of materials. The substrates and environmental conditions for the solid fermentation of *Beauveria bassiana* are shown in Table 2.

Inoculum Substrate		Incubation	Production in the substrate	Reference
1×10^7 conidia·mL ⁻¹	Barley	25 °C/14 days	s/n	Sabbahi et al. (2008)
1×10^{8}	Rice	28 °C/14 days	4.3×10^{8}	Mishra et al. (2016)
conidia⋅mL ⁻¹	husk	20 0,11 days	esporas·g ⁻¹	111151114 et all (2 010)
1×10^{8}	Wheat	28 °C/14 days	2.1×10^{8}	Mishra et al. (2016)
spores⋅mL ⁻¹	bran	20 C/14 days	$spores \cdot g^{-1}$	Misira et al. (2010)
3.5×10^{9}	Torn	25 °C/7 days	109	López-Sosa et al. (2018)
conidia·mL ⁻¹	rice	25 C// days	conidia⋅g ⁻¹	Lopez-30sa et al. (2018)
1×10^{6}	Oatmeal	25 °C/14 days	10^{8}	Rodríguez-Gámez et al. (2017)
blastospores \cdot mL ⁻¹	Oatmear	25 C/14 days	conidia⋅g ⁻¹	Rodriguez-Gamez et al. (2017)
6×10^{6}	Rice	20 °C/5 days	6.2×10^{-10}	Deemals at al. (2010)
$spores \cdot mL^{-1}$	flour	30 °C/5 days	$spores \cdot g^{-1}$	Deepak et al. (2019)
6.6×10^{6}	Rice	25 °C/7 days	1.3×10^{9}	Sala at al. (2021)
$spores \cdot mL^{-1}$	husk	25 °C/7 days	$spores \cdot g^{-1}$	Sala et al. (2021)

Table 2. Substrates used for the solid fermentation of *Beauveria bassiana*.

7 Fermentations on liquid substrates

The liquid fermentation process facilitates massive scale-up for the formulation of biopesticides. This method allows better control of environmental variables and reduces production times (Jaronski, 2014). However, implementation on a commercial scale requires considerable investment in equipment for mass production of mycoinsecticides, and is one of the most widely applied methods on a commercial scale (Mascarin and Jaronski, 2016).

Liquid fermentation can be performed stationary or by submerged fermentation. The former produces mycelia and aerial conidia; on the contrary, the latter produces blastospores, microspore conidia, or microsclerotia in an agitated and aerated liquid medium (Jaronski, 2014). Culture media should be nutrient-rich with high concentrations of carbon and nitrogen to induce the production of blastospores or conidia. An optimal C:N ratio induces fungal growth under controlled conditions (Pham et al., 2009).

According to García et al. (2013), SDA (Sabouraud dextrose agar) culture medium provides adequate nutrients for the development of *B. bassiana* in an incubation period of 15 days at 30 °C. Likewise, Pham et al. (2009) point out that conidial inoculum for liquid culture media should be obtained from two-week-old sporulated cultures on Papa Dextrose Agar (PDA) at a temperature of 25 \pm 1 °C. Conidia are harvested by scraping the inoculum with 0.02% tween 80 solution (Lee et al., 2016). Flasks containing liquid culture medium with molasses as a carbon source (García et al., 2013), yeast broth or glucose are inoculated for 6 days at a temperature of 24-26 °C (Lee et al., 2016) on a rotary shaker at 200 rpm until a stable suspension is obtained (Pham et al., 2009). The suspension obtained is used to inoculate the fermenter, at a concentration of 10% relative to the fermenter volume, with a propagation phase of 4 days until the fungus reaches 80% of the logarithmic growth phase (García et al., 2013).

The substrates used for elaborating liquid culture media should be low cost, and at the same time should provide suitable conditions to produce blastospores or conidia (Mascarin et al., 2015).

In this context, Pham et al. (2009) report that a culture medium with 3% corn flour, 2% corn maceration powder and 2% rice bran yields 8.54×10^8 blastospores·mL $^{-1}$. In contrast, García et al. (2013) report that the optimal medium for blastospore production should have molasses 14.5mL·L $^{-1}$, (NH₄) 2 SO₄ 6 g·L $^{-1}$, KH₂PO₄ 3.5 g·L $^{-1}$, MgSO₄ 0.5 g·L $^{-1}$, NaCl 0.1g·L $^{-1}$, CaCl₂ 0.1g·L $^{-1}$, and a production of 8.40 \times 10 8 blastospores·mL $^{-1}$ can be obtained. On the other hand, Elías-Santos et al. (2021) report that a culture medium with 20 g of peanut pericarp flour and 200 g of corn glucose allows obtaining 5.10 \times

 10^8 blastospores·mL⁻¹.

Similarly, environmental conditions are important for *Beauveria bassiana* production in submerged culture media. The temperature range is between 25-30 °C, pH of the culture medium 5.4, constant agitation from 200 to 400 rpm, until the fungus can reach 80% of the logarithmic growth phase (Pham et al., 2009; García et al., 2013; Elías-Santos et al., 2021). Table 3 shows the culture media and environmental conditions to produce *B. bassiana* by fermentation of liquid substrates.

Strain	Inoculum	Cultivate substrate	Incubation	Production	Reference
GHA	1×10^{7} conidium·mL $^{-1}$	50 g glucose, 50 g sucrose and 20 g corn macerate liquor	26 °C/3 days 300 rpm	6.38×10^9 blastospores·mL ⁻¹	(Chong-Rodríguez et al., 2011)
Iran 441c	1×10^4 conidium·mL ⁻¹	Sugarcane molasse	25 °C/3 days	2.4×10^8 spores·mL ⁻¹	(Latifian et al., 2013)
ATP-02	$\begin{array}{c} 5\times10^4\\ spores\cdot mL^{-1} \end{array}$	5% sugar beet	25 °C/8 days 600 rpm	5.32×10^{10} spores·mL ⁻¹	(Lohse et al., 2014)
GHA	5×10^{6} conidium·mL $^{-1}$	100 g glucose 25 g cotton seed flour	28 °C/3 days 350 rpm	$11.6 \pm 0.6 \times 10^{8}$ blastospores·mL ⁻¹	(Mascarin et al., 2015)
ESALQ1 432	5×10^{6} conidium·mL $^{-1}$	100 g glucose and 25 g cotton seed flour	28 °C/3 days 350 rpm	12.4×10^{8} blastospores·mL $^{-1}$	(Mascarin et al., 2015)
KK5	5×10^{7} conidium·mL $^{-1}$	3% corn flour 2% powder corn 2% rice	25 °C/2 days 200 rpm	$8.54\times10^{8} \\ blastospores \cdot mL^{-1}$	(Atef and Behle, 2017)

Table 3. Fermentation in liquid substrates of *Beauveria bassiana*.

8 Production of secondary metabolites of emphBeauveria bassiana

bran

Beauveria bassiana secretes a wide variety of biologically active enzymes and metabolites (Amobonye et al., 2020), with potential applications in the industrial, agricultural and pharmaceutical sectors, among others (Mancillas-Paredes et al., 2019). The most important enzymes produced by *B. bassiana*

are chitinases, lipases and proteases; although they also produce amylase, asparaginase, cellulase, galactosidase, etc. (Amobonye et al., 2020); and metabolites with insecticidal activity (Table 4) such as beauvericin (Al Khoury et al., 2019), bassiacridin (Quesada-Moraga and Vey, 2004), bassianolide (Patočka, 2016), antiviral and antibacterial as oosporein (Jeffs and Khachatourians, 1997), and bassianin as an ATP inhibitor (Patočka, 2016). The production

mental conditions and nutrients available in the cul-

of secondary metabolites is influenced by environ- ture medium (Ávila-Hernández et al., 2020) (Table

Metabolites	Action mode	Dose	Objective	Mortality percentage	Reference
Bassiacridin	Insecticide	$2.8 \mu\mathrm{g}\cdot\mathrm{g}^{-1}$	Locusta migratoria	50%	(Quesada-Moraga and Vey, 2004)
Oosporein	Antibacterial	$100 \mu\mathrm{g}\cdot\mathrm{mL}^{-1}$	Enterococcus faecalis	81 %	(Fan et al., 2017)
Oosporein	Antibacterial	$10 \mu\mathrm{g}\cdot\mathrm{mL}^{-1}$	Stenotrophomonas sp.	34%	(Fan et al., 2017)
Beauvericina	Insecticide	$100 \mu \mathrm{g} \cdot \mathrm{g}^{-1}$	Tetranychus urticae	100%	(Al Khoury et al., 2019)

Table 4. Metabolites secreted by *Beauveria bassiana* for arthropod control.

Use of biopesticides in the agriculture

Population growth, environmental degradation (Thakur et al., 2020), demand for chemical-free crops and stringent pesticide regulations in European countries and North America (Mascarin and Jaronski, 2016), pose a challenge to food production worldwide. Conventional agriculture relies on pesticides for integrated pest and disease management in agricultural crops.

An alternative that contributes to reducing the incidence of phytophagous or phytopathogens are biopesticides formulated through the reproduction of beneficial microorganisms or the production of metabolites with insecticidal, fungicidal or bactericidal activities (Table 6). The advantages of these formulations are that they degrade naturally in the environment, do not store residues in plant tissues, do not create resistance to the active ingredients they produce, and reduce the presence of natural enemies in crops (Thakur et al., 2020).

The growing acceptance of biological products has allowed the development of formulations from entomopathogenic fungi by solid, liquid, biphasic fermentations and secondary metabolites. They are now frequently used in agricultural crop protection programs to control phytophagous insect populations (Luo et al., 2014). The insecticidal activity of B. bassiana is faster compared to other entomopathogenic microorganisms, and conidia can persist longer in the environment.

In addition, Sabbahi et al. (2008) affirm that the chances of insects acquiring resistance to B. bassiana are null, due to the different modes of action that the fungus uses to invade the host body and it can adapt to host changes by being a living organism.

Table 5. Cul	ture media formulat	ed to produce secondar	ry metabolites of Be	eauveria bassiana.
mentation	Metabolites	Cultive media	Dose	Referenc

Fermentation	Metabolites	Cultive media	Dose	Reference
		Glucose (20 g·L $^{-1}$),		
		Ammonium tartrate		
		$(4.6 \text{ g} \cdot \text{L}^{-1})$,		
		KH_2PO_4		
		$(1 \text{ g} \cdot \text{L}^{-1}),$		
		$MgSO_4.7H_2O$		
		$(0.5 \text{ g} \cdot \text{L}^{-1})$,		
		NaCl $(0.1 \text{ g} \cdot \text{L}^{-1})$,		
		$CaCl_2 (0.1 \text{ g} \cdot \text{L}^{-1}),$		
	Tenellin/	$CuSO_4 + 5H_2O$		
Submerged	Bassianin	$(3.93 \times 10^4 \text{ g} \cdot \text{L}^{-1}),$	$60~\mathrm{mg}\cdot\mathrm{L}^{-1}$	(Basyouni et al., 1968)

	tion			
		H_3BO_3		
		$(5.7 \times 10^{-5} \text{ g} \cdot \text{L}^{-1}),$		
		$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$		
		$(3.68 \times 10^{-5} \text{ g} \cdot \text{L}^{-1}),$		
		MnSO ₄ ·H ₂ O		
		$(6.1 \times 10^{-5} \text{ g} \cdot \text{L}^{-1})$,		
		$ZnSO_4 \cdot 7H_2O$		
		$(8.79 \times 10^{-3} \text{ g} \cdot \text{L}^{-1}),$		
		$FeSO_4 \cdot 7H_2O$		
		$(9.96 \times 10^{-4} \text{ g} \cdot \text{L}^{-1}).$		
		Glucose (20 g·L $^{-1}$),		
		Difco neopeptone		
Codensorad	0	$(20 \text{ g} \cdot \hat{L}^{-1})$,	100 1 -1	(P
Submerged	Oosporein	Glycine (5 g·L $^{-1}$),	$100~{ m mg}{\cdot}{ m L}^{-1}$	(Basyouni et al., 1968)
		$KH_2PO_4 (2 g \cdot L^{-1}),$		
		$MgSO_4.7H_2O(1 g\cdot L^{-1}).$		
		40 g Glucose·L ⁻¹ ,		
		40 g Yeast		
Submerged	Bassiacridin	extract· L^{-1} ,	$2.8 \mu \mathrm{g} \cdot \mathrm{g}^{-1}$	(Quesada-Moraga and Vey, 2004
0		30 g macerated corn	-10 FB B	(2g
		$liquor \cdot L^{-1}$		
		Mannitol (50 g·L ⁻¹),		
		KNO ₃ (5 g·L ⁻¹),		
		$KH_2PO_4 (1 \text{ g} \cdot \text{L}^{-1}),$		
		MgSO ₄ ·7H ₂ O (0.5 g·L ⁻¹),		
Submerged	Tenellin	NaCl $(0.1 \text{ g} \cdot \text{L}^{-1})$,	No information	(Eley et al., 2007)
Submergea	Terterint	CaCl ₂ (0.2 g·L ⁻¹),	140 Information	(Eley et al., 2007)
		FeSO ₄ ·7H ₂ O		
		$(20 \text{ mg} \cdot \text{L}^{-1}),$		
		Mineral ion		
		msolution-2 (10 mL),		
		$ZnSO_4 \cdot 7H_2O$		
		$(880 \text{ mg} \cdot \text{L}^{-1}),$		
		$CuSO_4.5H_2O$		
		$(40 \text{ mg} \cdot \text{L}^{-1}),$		
		$(40 \text{ mg} \cdot \text{L}^{-1})$, MnSO ₄ ·4H ₂ O		
		$(40 \text{ mg} \cdot \text{L}^{-1}),$ $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1}),$		
		$(40 \text{ mg} \cdot \text{L}^{-1}),$ $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1}),$ Boric acid $(6 \text{ mg} \cdot \text{L}^{-1}),$		
		$(40 \text{ mg} \cdot \text{L}^{-1})$, $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1})$, Boric acid (6 mg · L ⁻¹), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$		
		$\begin{array}{c} (40~\text{mg}\cdot\text{L}^{-1}),\\ \text{MnSO}_4\cdot 4\text{H}_2\text{O}\\ (7.5~\text{mg}\cdot\text{L}^{-1}),\\ \text{Boric acid (6 mg}\cdot\text{L}^{-1}),\\ (\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}\\ (4~\text{mg}\cdot\text{L}^{-1}). \end{array}$		
		$(40 \text{ mg} \cdot \text{L}^{-1})$, $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1})$, Boric acid (6 mg · L ⁻¹), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$		
		$\begin{array}{c} (40~\text{mg}\cdot\text{L}^{-1}),\\ \text{MnSO}_4\cdot 4\text{H}_2\text{O}\\ (7.5~\text{mg}\cdot\text{L}^{-1}),\\ \text{Boric acid (6 mg}\cdot\text{L}^{-1}),\\ (\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}\\ (4~\text{mg}\cdot\text{L}^{-1}). \end{array}$		
		$(40 \text{ mg} \cdot \text{L}^{-1}),$ $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1}),$ $Boric acid (6 \text{ mg} \cdot \text{L}^{-1}),$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ $(4 \text{ mg} \cdot \text{L}^{-1}).$ $Glucose (40 \text{ g} \cdot \text{L}^{-1})$		
Submovad	Pod coloring	$(40 \text{ mg} \cdot \text{L}^{-1}),$ $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1}),$ $Boric acid (6 \text{ mg} \cdot \text{L}^{-1}),$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ $(4 \text{ mg} \cdot \text{L}^{-1}).$ $Glucose (40 \text{ g} \cdot \text{L}^{-1})$ $Yeast extract$	490 mg I = 1	(Amin et al. 2010)
Submerged	Red coloring	$(40 \text{ mg} \cdot \text{L}^{-1}),\\ \text{MnSO}_4 \cdot 4\text{H}_2\text{O}\\ (7.5 \text{ mg} \cdot \text{L}^{-1}),\\ \text{Boric acid (6 mg} \cdot \text{L}^{-1}),\\ (\text{NH}_4)_6\text{Mo}_7\text{O}_24 \cdot 4\text{H}_2\text{O}\\ (4 \text{ mg} \cdot \text{L}^{-1}).\\ \hline\\ \text{Glucose (40 g} \cdot \text{L}^{-1})\\ \text{Yeast extract}\\ (5.0 \text{ g} \cdot \text{L}^{-1}),\\ \end{cases}$	480 mg⋅L ⁻¹	(Amin et al., 2010)
Submerged	Red coloring	$(40 \text{ mg} \cdot \text{L}^{-1}),$ $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1}),$ $Boric acid (6 \text{ mg} \cdot \text{L}^{-1}),$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ $(4 \text{ mg} \cdot \text{L}^{-1}).$ $Glucose (40 \text{ g} \cdot \text{L}^{-1})$ $Yeast extract$ $(5.0 \text{ g} \cdot \text{L}^{-1}),$ $NaNO_3 (1,0 \text{ g} \cdot \text{L}^{-1}),$ $KH_2PO_4 (2,0 \text{ g} \cdot \text{L}^{-1}),$	480 mg⋅L ⁻¹	(Amin et al., 2010)
Submerged	Red coloring	$(40 \text{ mg} \cdot \text{L}^{-1}),$ $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1}),$ $Boric acid (6 \text{ mg} \cdot \text{L}^{-1}),$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ $(4 \text{ mg} \cdot \text{L}^{-1}).$ $Glucose (40 \text{ g} \cdot \text{L}^{-1})$ $Yeast extract$ $(5.0 \text{ g} \cdot \text{L}^{-1}),$ $NaNO_3 (1,0 \text{ g} \cdot \text{L}^{-1}),$ $KH_2PO_4 (2,0 \text{ g} \cdot \text{L}^{-1}),$ $KCl (0,5 \text{ g} \cdot \text{L}^{-1}),$	480 mg⋅L ⁻¹	(Amin et al., 2010)
Submerged	Red coloring	$(40 \text{ mg} \cdot \text{L}^{-1}),$ $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1}),$ $Boric acid (6 \text{ mg} \cdot \text{L}^{-1}),$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ $(4 \text{ mg} \cdot \text{L}^{-1}).$ $Glucose (40 \text{ g} \cdot \text{L}^{-1})$ $Yeast extract$ $(5.0 \text{ g} \cdot \text{L}^{-1}),$ $NaNO_3 (1,0 \text{ g} \cdot \text{L}^{-1}),$ $KH_2PO_4 (2,0 \text{ g} \cdot \text{L}^{-1}),$ $KCl (0,5 \text{ g} \cdot \text{L}^{-1}),$ $MgSO_4 \cdot 7H_2O$	480 mg⋅L ⁻¹	(Amin et al., 2010)
Submerged	Red coloring	$(40 \text{ mg} \cdot \text{L}^{-1}),$ $MnSO_4 \cdot 4H_2O$ $(7.5 \text{ mg} \cdot \text{L}^{-1}),$ $Boric acid (6 \text{ mg} \cdot \text{L}^{-1}),$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ $(4 \text{ mg} \cdot \text{L}^{-1}).$ $Glucose (40 \text{ g} \cdot \text{L}^{-1})$ $Yeast extract$ $(5.0 \text{ g} \cdot \text{L}^{-1}),$ $NaNO_3 (1,0 \text{ g} \cdot \text{L}^{-1}),$ $KH_2PO_4 (2,0 \text{ g} \cdot \text{L}^{-1}),$ $KCl (0,5 \text{ g} \cdot \text{L}^{-1}),$	480 mg⋅L ⁻¹	(Amin et al., 2010)

Source: Ávila-Hernández et al. (2020).

The efficacy of the biopesticide *Beauveria bassia*na depends on the integration into integrated pest management programs to enhance efficacy in insect control (Mascarin and Jaronski, 2016). Although *B.* bassiana is an effective microorganism for the biological control of insects, environmental conditions play an important role in the mechanism of action of the entomopathogen, so it is essential to develop new technologies to produce active compounds that enhance biopesticide formulations.

Table 6. Commercial formulations of Beauveria bassiana.

Area of Reference	Foliage (Agroinsumos del Sur, 2021)	Foliage (Invesa, 2021)	Foliage (Certiseurope, 2021)	Foliage	Foliage (FAGRO, 2022) Soil	Foliage
Dose	Scc/L	500 g/ha	1-1.50 L/ha	1-2 L/ha	1-3 L/ha	1-4 L/ha
Pest	Crickets Bedbugs Leafhoppers Bugs	White fly Coffee berry borer Fruit pin Spider mite	White fly	White fly Fruit maggot	Budworm Worm Fruit maggot Blind hen	Fruit maggot Soldier worm
Crop	Cocoa Plantain Corn	Cotton Orange Coffee Avocado Rose	Cotton Horticultural	Solanáceas	Corn	Brosoli Cabbage Cauliflower Brussels
Concentration	1×10^9 conidia·mL $^{-1}$	$\begin{array}{c} 1\times10^9\\ \text{viable spores.g}^{-1} \end{array}$	$2,11 \times 10^{10}$ conidia·mL ⁻¹		1×10^6 spores·mL $^{-1}$	
Active Ingredients	Beauveria bassiana	Beauveria bassiana	Beauveria bassiana cepa GHA		Beauveria bassiana	
Commercial Name	BFICAX	Bovetrópico WP	BOTANIGARD CS		Biotech BMI	

Area of Reference	Foliage (FAGRO, 2022)	Leaf		Leaf	Leaf Leaf	Leaf Leaf Tramps soil	Leaf Tramps soil Leaf (Agroactivo, 2020)						
Dose	1-2 L/ha	1 a 1.5 kg/ha	1 kg/ha		1 kg/ha	1 kg/ha 20 g a 1 kg/ha	1 kg/ha 20 g a 1 kg/ha 1 a 1.5 kg/ha	1 kg/ha 20 g a 1 kg/ha 1 a 1.5 kg/ha 1.5 kg/beds	1 kg/ha 20 g a 1 kg/ha 1 a 1.5 kg/ha 1.5 kg/beds 1 to 1.5 kg/ha	1 kg/ha 20 g a 1 kg/ha 1 a 1.5 kg/ha 1.5 kg/beds 1 to 1.5 kg/ha 1 to 1.5 kg/ha	1 kg/ha 20 g a 1 kg/ha 1 a 1.5 kg/ha 1.5 kg/beds 1 to 1.5 kg/ha 1 kg/ha 1 kg/ha	1 kg/ha 20 g a 1 kg/ha 1 a 1.5 kg/ha 1.5 kg/beds 1 to 1.5 kg/ha 1 kg/ha 1 kg/ha	1 kg/ha 20 g a 1 kg/ha 1 a 1.5 kg/ha 1.5 kg/beds 1 to 1.5 kg/ha 1 kg/ha 1 kg/ha 1 kg/ha 1 kg/ha
Pest	White fly	Coffee berry borer	Citrus weevil Mites	Whitefly	Whitefly Whitefly Mites and thrips	Whitefly Whites and thrips Striped weevil Black Weevil	Whitefly Whitefly Mites and thrips Striped weevil Black Weevil Lace bug Scales	Whitefly Whites and thrips Striped weevil Black Weevil Lace bug Scales Thrips Whitefly	Whitefly Whitefly Whites and thrips Striped weevil Black Weevil Lace bug Scales Thrips Whitefly Loggers White grub Flea beetle	Whitefly Whitefly Whites and thrips Striped weevil Black Weevil Lace bug Scales Thrips Whitefly Loggers White grub Flea beetle Defoliators Mites	Whitefly Whitefly Whites and thrips Striped weevil Black Weevil Lace bug Scales Thrips Whitefly Loggers White grub Flea beetle Defoliators Mites Fruit Scrapers	Whitefly Whitefly Whites and thrips Striped weevil Black Weevil Lace bug Scales Thrips Whitefly Loggers White grub Flea beetle Defoliators Fruit scrapers Weevil Defoliators	Whitefly Whitefly Whites and thrips Striped weevil Black Weevil Lace bug Scales Thrips Whitefly Loggers White grub Flea beetle Defoliators Mites Fruit Scrapers Weevil Defoliators Thrips White
Crop	Cucumber Watermelon, Melon, Pumpkin	Coffee	Citrics		Avocado	Avocado Banana Plantain	Avocado Banana Plantain Soursop	Avocado Banana Plantain Soursop Flowers Foliage	Avocado Banana Plantain Soursop Flowers Foliage	Avocado Banana Plantain Soursop Flowers Foliage Potato	Avocado Banana Plantain Soursop Flowers Foliage Potato Rice Oil	Avocado Banana Plantain Soursop Flowers Foliage Potato Rice Oil palm Forestry	Avocado Banana Plantain Soursop Flowers Foliage Potato Oil palm Forestry Pasifloras
Concentration	1×10^6 spores·mL ⁻¹						$\frac{1\times10^{10}}{\mathrm{spores}\cdot\mathrm{g}^{-1}}$	$\frac{1\times10^{10}}{\text{spores.g}^{-1}}$	1×10^{10} spores·g $^{-1}$	1×10^{10} spores g^{-1}	$\begin{array}{c} 1\times10^{10}\\ \text{spores.g}^{-1} \end{array}$	$\begin{array}{c} 1\times10^{10}\\ \text{spores.g}^{-1} \end{array}$	$\begin{array}{c} 1\times10^{10}\\ \text{spores.g}^{-1} \end{array}$
Active Ingredients	Beauveria bassiana			_			Beauveria bassiana	Beauveria bassiana	Beauveria bassiana	Beauveria bassiana	Beauveria bassiana	Beauveria bassiana	Beauveria bassiana
Commercial Name	Biotech BMI						MICOSIS	MICOSIS	MICOSIS	MICOSIS	MICOSIS	MICOSIS	MICOSIS

10 Conclusions

The production of biopesticides based on *Beauveria bassiana* can be carried out through solid and liquid fermentations. The first protocol has been widely used because it requires a low investment in equipment and does not involve complex protocols in production and formulation. However, it is necessary to use a low-cost substrate and to exhaustively control the phases of each process to avoid contamination events.

On the other hand, liquid fermentation allows more control of environmental variables and reduces production times, but implementation on a commercial scale requires considerable investment in equipment for mass production of mycoinsecticides.

Regarding fermentation processes, it is necessary to control environmental variables since these factors influence the production of spores and blastospores. Optimal conditions consist of maintaining a temperature of 25°C, relative humidity of 65 to 70%, pH of 5.4, propagation time between 4 to 8 days, and the inoculum requires constant agitation between 200 to 400 rpm for the liquid fermentation process.

Author contribution

JRLM; Conceptualization, Data curation, Formal analysis, Research, Methodology, Software, Original draft writing, review and editing. JWMT; Acquisition of financing and supervision. ICLM; Project validation and administration. YAOG; Resources and visualization.

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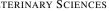
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CHANGES IN THE HEMATOLOGY AND BLOOD METABOLITES OF GUINEA PIGS (CAVIA PORCELLUS) UNDER INTENSIVE REARING SYSTEM IN HUMID TROPICAL CONDITIONS

Cambios relacionados con la edad en la hematología y METABOLITOS SANGUÍNEOS DE CUYES (CAVIA PORCELLUS) EN SISTEMA DE CRIANZA INTENSIVO EN CONDICIONES DE TRÓPICO HÚMEDO

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Abstract

Guinea pigs in intensive production generates an imperative necessity of physio-pathological bases for diagnosing their state of welfare, health, and production; those which are available for this species are referred to as laboratory or companion animal. The aim of this research is to determine changes in hematological and blood metabolites profiles in relation to age of Cavia porcellus reared in intensive system at 660 masl in humid tropic. For this purpose, forty 15-120 days old guinea pig in healthy conditions from the inti x Peru lines born in the humid tropic were used and the evaluation ages were 15-21, 22-35, 36-60 and 61-120 days. Blood samples were obtained by puncture of the cephalic vein; profiles of erythrocytes (RBC), total leucocytes (WBC), lymphocytes, granulocytes, hematocrit, hemoglobin, MCV, MCH and MCHC indices were determined. In serum, profiles of glucose, total protein (TP), albumin, total cholesterol (TC), triglycerides, total bilirubin (TB), and direct bilirubin (DB), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined. Hemoglobin profile, MCH and MCHC indices increased as age increased (p < 0.05) and the erythrocytes, leucocytes, hematocrit, hemoglobin profiles and MCV, MCH, MCHC indices showed the narrower interval ranges in the 15-35- and 61-120-days old guinea pigs. Profiles of TP, DB and AST showed increase with age (p < 0.05) and the DB, AST, ALT, and TC profiles showed the narrower interval range at 36-120 days old. The hematological and blood metabolites profiles of guinea pigs raised in intensive system in humid tropical conditions show significant changes with production age.

Keywords: Welfare, guinea pigs, rearing system, hematology, intensive production

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Resumen

Los cuyes en producción intensiva generan la necesidad de bases fisiopatológicas para diagnosticar su estado de bienestar, salud y producción; las disponibles están referidas a esta especie como animal de laboratorio o de compañía. El objetivo de esta investigación es determinar los perfiles hematológicos y metabolitos sanguíneos en relación con la etapa de producción de cuyes en sistema de crianza intensivo a 660 msnm en trópico húmedo. Cuarenta cuyes clínicamente sanos de las líneas inti x Perú nacidos en trópico de 15-120 días de edad se distribuyeron en cuatro grupos etarios: 15-21, 22-35, 36-60 y 60-120 días. En sangre se determinó los perfiles de eritrocitos, leucocitos, granulocitos, hematocrito y hemoglobina e índices de MCV, MCH y MCHC y en suero sanguíneo los perfiles de glucosa, proteína total (PT), albumina, colesterol total (CT), triglicéridos, ALT, AST y bilirrubina directa (BD). El perfil de hemoglobina y los índices de MCH y MCHC incrementaron con la edad (p < 0.05) y los perfiles de eritrocitos, leucocitos, hemoglobina y hematocrito y los índices de MCV, MCH, MCHC mostraron una menor amplitud de intervalos en los cuyes de 15-35 y 61-120 días de edad. Los perfiles de PT, BD y AST incrementaron con la edad (p < 0.05) y los perfiles de BD, AST, ALT y CT mostraron la menor amplitud de intervalo entre los 36-120 días de edad. Los perfiles hematológicos y de metabolitos sanguíneos en los cuyes criados en sistema de crianza intensivo en condiciones de trópico húmedos muestran cambios significativos con la edad de producción.

Palabras clave: Bienestar, cuy, sistema de crianza, hematología, producción intensiva.

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1 Introduction

The guinea pig (*Cavia porcellus* L.) is an animal that originates in the South American Andes; the breeding in Peru mainly occurs in the Andean regions, the coast (Yamada et al., 2019) and recently in the Amazon region, characterized and reared as a meat producing animal with high nutritional value. Guinea pigs have been bred in the Andes at altitudes of more than 3800 masl for thousands of years, under extreme cold and hypoxia conditions; likewise, at sea level under temperate and normoxia conditions (Lechner et al., 1981, 1980b), having adapted similarly to other animal species (Ding et al., 2014; Al-Sweedan and Alhaj, 2012; Snyder et al., 1982)(Ding et al., 2014; Al-Sweedan and Alhaj, 2012; Snyder et al., 1982).

In Peru, breeding of guinea pigs was originally oriented to self-consumption and food security, generating additional income from the sale of the remnant and more opportunity in the workforce, mainly for women in rural areas (Chauca, 1995). However, it tends to move towards developing into an intensive production (Olazábal et al., 2019; Yamada et al., 2019), generating an increase in the development of pathologies in the specie (Venturo and Morales-Cauti, 2020; Bazán et al., 2019; Paredes-López et al., 2014; Layme et al., 2011); hence, in order to control these pathologies, it is necessary to develop physiopathological bases for diagnosing their health and production.

These conditions, along to those changes in climate and food factors, could lead to adaptive physiological variations and among them, one of the most sensitive is what occurs in blood. In preventative medicine, physiological changes in blood are part of minimum database for any animal species, with the purpose of interpreting its state of health or disease and production (Ramirez-Borda et al., 2019; Luna et al., 2018; Izurieta et al., 2017; Gupta et al., 1999).

In many animal species used for production, as is the guinea pig, normal reference hematological and blood metabolites profiles and their intervals are not available, and those which are available are referred to the guinea pig as laboratory animal (McClure, 1999; Dyer and Cervasio, 2008; Hoffman and

Solter, 2008; Zimmerman et al., 2010; Washington and Van Hoosier, 2012; Zimmerman et al., 2015; Alberton et al., 2019). Therein, obtaining the blood profiles of guinea pigs during the different stages of intensive rearing, in a normal state of health under humid tropical conditions, would contribute to defining the changes in the blood markers and indicators, according to the age, for the purposes of diagnosing the health and production state of this animal species (Lewis, 1992; Doneley, 2015).

The aim of this paper is to study the changes in hematological and blood metabolites profiles at different production ages of guinea pigs in an intensive rearing system under humid tropical conditions.

2 Materials and Methods

2.1 Area of research

The study was carried out on the zootechnics farm at the Universidad Nacional Agraria de la Selva, Tingo María, Peru, located at 09°17′58″S latitude and 76°01′07″ W longitude, with an altitude of 660 masl, presenting an annual pluvial precipitation of 3293 mm, an average annual temperature of 24.85°*C*, a relative humidity of 80% during the season of least precipitation (June-September) and 85% during the greatest precipitation season (October-May) (Servicio Nacional de Meteorología e Hidrología (SENAMHI), 2021). This study was carried out from October to December.

2.2 Animals Studied

Forty guinea pigs from 15-120 days' old obtained from inti x Peru lines adapted to humid tropic for two years were used. The evaluation phases were at 15-21 days old (initial), 22-35 days (growth), 36-60 days (finishing) and 60-120 days old (adults).

Four groups were formed and each one was composed of ten guinea pigs that were placed in separate cages, administering a daily diet of King Grass as *ad libitum* forage and a balanced diet for guinea pigs, according to their production phase (Vergara, 2009). Each group or production phase was independent from the other.

2.3 Immobilization and Blood Sampling

The guinea pigs were taken from below the body trunk with one hand and supported below the hind-quarters with the other hand, trying not compress the thorax or abdomen. The skin was shaved and alcohol was applied for disinfecting and to visualize the cephalic vein, and the blood samples were obtained by puncturing it (Dyer and Cervasio, 2008; Quesenberry et al., 2012).

All the blood was obtained in vacutainers containing 2 mg of EDTA. In order to obtain the serum, the blood was put into clean vials and left to coagulate for 1-2 hours and then was centrifuged at 1500 rpm for three minutes; later the serum was separated into vacutainers to preserve it at $-20^{\circ}C$. Ten whole blood samples and ten blood for serum samples were drawn from each of the groups in the phases 15-21, 15-21

2.4 Hematological assessment and blood metabolites profile

In the whole blood, the number of erythrocytes (ER), leukocytes (LE), lymphocytes (Ly), and granulocytes (Gra) were determined using the Neubauer and Wright-Giemsa techniques (Harvey, 2001) and the hematocrit (Ht) was determined using the microhematocrit technique at 11,000 rpm in a Kert Lab Tom's centrifuge (USA Science Tech Group); the hemoglobin (Hb) was determined using the cyanmethemoglobin method and quantified using a 405 to 530 nm DIALAB DTN spectrophotometer. Mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) indices were determined (Samour et al., 2016; Doneley, 2015).

In the blood serum, the glucose level was determined through the glucose oxidase-peroxidase method; the total protein, using the complex ED-TA/Cu method in sodium hydroxide; the albumin, using the tetrabromide phenolsulfonphthalein method; the total cholesterol, alanine transaminase, aspartate transaminase, total bilirubin, and conjugated bilirubin (Laboratorios QAC, Espain). The

measurements were done using a DIALAB DTN 405 to 515 and 530 nm spectrophotometer.

2.5 Statistical Analysis

The average values and the standard deviation were calculated from the data obtained, and later the ANOVA was done; in a previous step the assumption of the sphericity between the measurements was verified using Mauchly's test. The multiple comparisons test was done using the Bonferroni test; in a previous step the assumption of normality test was done for each measurement using the Shapiro-Wilk test. The Infostat statistical software was used. The differences at $p \leq 0.05$ were significant.

3 Results and discussion

Most of the studies regarding hematology and blood metabolites profiles in *Cavia porcellus* that are found in scientific literature refer to studies as an experimental specie in laboratory, but not as a specie for meat production purpose, and particularly under intensive rearing systems (Paredes-López et al., 2017; Ramirez-Borda et al., 2019). As a laboratory animal, many strains or biotypes of guinea pigs have been obtained, and characteristics of sensibility have been found for different aspects of one specific disease or another (Waner et al., 1996; Kitagaki et al., 2005; Genzer et al., 2019).

Profiles of red blood cells, hemoglobin, hematocrit, MCV, MCHC, MCH indices, white blood cells, granulocytes and lymphocytes percentage for guinea pigs under an intensive rearing system in tropic humid conditions were evaluated at 15-120 days old (Tables 1 and 2). The hemoglobin profiles and MCH and CMCH indices in 61–120 days old guinea pigs show higher levels than the 15–35 days old (p < 0.05); but like the 35-60 days old guinea pigs for the MCHC and MCH indices (p > 0.05). Nonetheless, the hematocrit and red blood cell profiles in the 22–35 days old guinea pigs were lower than those obtained for the 15–21 and 61–120 days old (p < 0.05), but equal to those for the 36 – 60 days old guinea pigs (p > 0.05) (Table 1).

Table 1. Hematological profiles of guinea pigs 15-120 days old under intensive rearing system in humid tropical conditions (95% CI).

			Age (days)						
Hematological	p-	CV	15-21	22-35	36-60	61-120			
Profiles	values	(%)	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$			
HCT (%)	0.004	9.26	37.00 ± 0.49^a	32.10 ± 0.97^b	33.60 ± 1.61^{ab}	36.40 ± 0.60^a			
$Hgb (gdL^{-1})$	0.000	10.49	11.88 ± 0.30^{c}	11.04 ± 0.22^{c}	13.73 ± 0.74^{b}	15.65 ± 0.27^{ba}			
WBC ($\times 10^3$)	0.000	17.94	3.38 ± 0.13^{b}	5.74 ± 0.25^{a}	3.08 ± 0.31^{b}	3.47 ± 0.15^{b}			
RBC ($\times 10^6$)	0.003	9.62	4.03 ± 0.06^{a}	3.43 ± 0.11^{b}	3.70 ± 0.18^{ab}	3.93 ± 0.07^{a}			
$MCHC (gdL^{-1})$	0.000	10.06	32.11 ± 0.73^{b}	34.53 ± 0.68^b	41.23 ± 1.92^a	43.10 ± 1.04^a			
MCH (pg)	0.000	8.86	29.44 ± 0.64^{b}	32.30 ± 0.65^{b}	37.48 ± 1.73^{a}	39.97 ± 0.93^a			
MCV (fl)	0.169	3.08	91.76 ± 0.56^{a}	93.69 ± 1.54^{a}	90.91 ± 0.00^{a}	92.70 ± 0.74^{a}			
GRAN (%)	0.000	33.12	31.30 ± 4.48^{b}	29.80 ± 3.28^{b}	22.20 ± 1.40^{b}	46.30 ± 4.02^{a}			
LYM (%)	0.001	16.95	65.10 ± 4.07^{ab}	68.50 ± 3.47^{a}	74.50 ± 1.99^a	52.80 ± 4.04^{b}			

CV: variation coefficient; abc: Different letters within each variable express significant differences with a 95 \bar{x} : arithmetic average; SE: standard error; HCT: hematocrit, Hgb: hemoglobin, WBC: leukocytes (white blood cells), RBC: red blood cells, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin,

MCV: mean corpuscular volume, GRAN: granulocytes.

The erythrocyte profiles obtained in this study in local guinea pigs with differences in genetics, nutrition, and breeding purposes, follow similar tendencies of those for established references of strains or biotypes of laboratory guinea pigs (Zimmerman et al., 2010; McClure, 1999) the 13/N strain (Genzer et al., 2019), Dunkin-Hartley (Waner et al., 1996), Weiser-Maples (Kitagaki et al., 2005). Nonetheless, these profiles are much lower than those obtained from guinea pigs reared in environments of hypoxia (p O_2 :80 Torr) and a temperature of 22°C(Lechner et al., 1980a), an environment of hypoxia (p O_2 :85 Torr) and a temperature of 6°C; conditions similar to those at more than 4000 masl (Lechner et al., 1981), in which the erythrocyte profiles increased between 13 and 42%, with respect to the referential levels, as well as being lower than the erythrocyte profiles obtained from guinea pigs in normal oxygen environments (p O_2 =133 Torr) and a temperature of $5^{\circ}C$ (Lechner et al., 1980c).

The highest red blood cell profiles showed in guinea pigs reared in an environment of hypoxia rather than those in environments of normoxia, as in the current study, relates to the fact that the low oxygen pressure stimulates an increase in the erythropoietin levels, which at the same time stimulates the erythropoiesis as a compensation mechanism for reestablishing the levels of the oxygen pressure in an organism (Reece, 2015).

In this study it was showed that the hematocrit levels and number of erythrocytes varied with the age or physiological state (p < 0.05). It is worth noting that the hematocrit and the erythrocytes reduced in the 21-35 days old and reestablished their levels in the 61–120 days old guinea pigs, which is similar to those reported by Lechner et al. (1980b). Different tendencies have been reported in guinea pigs of the 13/N strain, in which these parameters increased with age within a six-month period. Notwithstanding, the hemoglobin, MCH and MCHC levels increased with the age of the guinea pigs in the present study (p < 0.05), and these were evident in the 36-120 days old. The results contrast with those obtained in the 13/N strain, in which the levels of these profiles were greater in younger guinea pigs than in adults (Genzer et al., 2019), as well as the results reported by Lechner et al. (1980a).

The white blood cell and lymphocyte profiles in the 22–35 days old guinea pigs showed greater levels than those presented in the 15–21 and 61-120 days old (p < 0.05), notwithstanding, the granulocytes profile was greater in the 61–120 days old than that found for 15-60 days old of production (p < 0.05).

The withe blood cells profile reached a peak in the 22–35 days old, declining and staying steady at the same level in the 36–120 days old guinea pig. The lymphocytes and granulocytes did not vary in the 15-60 days old guinea pigs; later the lymphocytes reduced and the granulocytes increased in the 61–120 days old. Similar tendencies were shown in strain 13/N guinea pigs for the leukocytes and lymphocytes, nonetheless, it was different for the granulocytes, for which the neutrophils also diminished with age (Genzer et al., 2019).

Reference amplitude intervals were established to each of the guinea pig's production phase for these hematologic profiles. These intervals of the means showed different amplitudes in the four production phases and within these, the means of granulocytes and lymphocytes in the 15–21, 61–120 and 22–35 days old guinea pigs show from the greatest to the least, the higher amplitude. On the other

hand, the means of RBC, WBC, hemoglobin and hematocrit profiles and the MCV, MCH and MCHC indices, in the15–21, 22–35 and 61–120 days old guinea pigs show from least to greatest, the lesser amplitude (Table 2).

Publications referring to intervals of hematologic profiles by production phases of guinea pigs are not found, nonetheless, the intervals found in this research follow similar tendencies to those reported as established reference intervals for experimental guinea pigs in laboratory (Zimmerman et al., 2015; Quesenberry et al., 2012; McClure, 1999); strain 13/N (Genzer et al., 2019), Weiser-Maples (Kitagaki et al., 2005).

Table 2. Means amplitude intervals for the hematologic profiles of 15-120 days old guinea pigs under intensive rearing system in humid tropical conditions (95 % CI).

Hematologic		Age (days)								
Profiles	15-21	22-35	36-60	61-120						
Hto (%)	[35.88 – 38.12]	[29.90 – 34.30]	[29.95 – 37.25]	[35.04 – 37.76]						
$Hb (gdL^{-1})$	[11.19 – 12.56]	[10.54 - 11.54]	[12.06 - 15.39]	[15.05 - 16.25]						
WBC ($\times 10^3$)	[03.08 - 03.68]	[05.17 - 06.30]	[02.38 - 03.77]	[03.13 - 03.81]						
RBC ($\times 10^6$)	[03.90 - 04.17]	[03.18 - 03.68]	[03.29 - 04.10]	[03.29 - 04.10]						
$MCHC (gdL^{-1})$	[30.47 - 33.75]	[32.99 - 36.07]	[36.88 - 45.59]	[40.76 - 45.45]						
MCH (pg)	[28.09 - 30.80]	[31.04 - 33.56]	[33.53 - 41.45]	[37.57 - 42.36]						
MCV (fl)	[90.49 – 93.02]	[90.20 - 97.17]	[90.91 - 90.91]	[91.03 - 94.38]						
Gra (%)	[21.16 – 41.44]	[22.37 - 37.23]	[19.04 - 25.36]	[41.21 - 59.39]						
Ly (%)	[55.89 – 74.31]	[60.65 - 76.35]	[70.00 - 79.00]	[46.67 - 61.93]						

Hto: hematocrit, Hb: hemoglobin, WBC: white blood cells, RBC: red blood cells, Gra: granulocytes, Ly: lymphocytes, MCHC: mean corpuscular hemoglobin concentration,

MCH: mean corpuscular hemoglobin, MCV: mean corpuscular volume.

The profiles and intervals of blood metabolites in the guinea pigs at 15–120 days old are shown in table 3 and 4. The total bilirubin and urea profiles showed greater levels in 61–120 days old compared to 15–60 days old guinea pigs (p < 0.05). Similarly, the total protein, BD and AST profiles showed greater levels in the 36–120 days old guinea pigs than in the 15–35 (p < 0.05) days old. Glucose and triglycerides profiles decreased in the 22-35 days old and then they reestablished in the 36-120 days old guinea pigs (p < 0.05).

The ALT profile was greater in the 22–35 days old guinea pigs compared to the 15-21 and 36-120 days old (p < 0.05). On the other hand, the total

cholesterol profile was higher in the 15-21 days old than in the 22-120 days old guinea pigs (p < 0.05) (Table 3).

The blood metabolites profiles obtained in this study as the hematological profiles, follow similar tendencies than those established in strains and biotypes of experimental guinea pigs in laboratories (Washington and Van Hoosier, 2012; Zimmerman et al., 2010; McClure, 1999), strain 13/N (Genzer et al., 2019), Dunkin-Hartley (Waner et al., 1996), Weiser-Maples (Kitagaki et al., 2005); improved criollo (Paredes-López et al., 2017; Ramirez-Borda et al., 2019).

Table 3. Blood metabolites profiles of 15-120 days old guinea pigs under intensive rearing system in humid tropical conditions (95 % CI).

				Age	(days)	
Blood metabolite	p-	CV	15-21	22-35	36-60	61-120
Profile	value	(%)	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
$TP (gdL^{-1})$	0.000	9.50	6.78 ± 0.25^{c}	6.89 ± 0.33^{bc}	7.73 ± 0.14^{ab}	$8.22{\pm}0.10^a$
$AL (gdL^{-1})$	0.001	8.32	3.94 ± 0.04^{ab}	3.57 ± 0.15^{bc}	3.50 ± 0.11^{c}	4.04 ± 0.05^{a}
$TB (mgdL^{-1})$	0.000	14.20	0.58 ± 0.02^{c}	$0.55{\pm}0.02^{c}$	0.71 ± 0.06^{b}	$0.92{\pm}0.02^a$
$DB (mgdL^{-1})$	0.001	18.91	0.15 ± 0.01^{b}	0.15 ± 0.01^{b}	0.20 ± 0.01^{a}	0.20 ± 0.01^{a}
$Glucose(mgdL^{-1})$	0.000	11.09	86.22 ± 4.80^a	59.55 ± 2.62^{b}	87.23 ± 0.71^a	86.00 ± 0.93^a
$TC (mgdL^{-1})$	0.000	11.56	71.64 ± 2.70^a	47.28 ± 1.55^{b}	27.64 ± 0.66^{c}	28.49 ± 0.34^{c}
$TG (mgdL^{-1})$	0.024	31.95	58.08 ± 9.11^a	37.17 ± 1.43^{b}	44.57 ± 0.70^{ab}	44.14 ± 0.93^{ab}
$AST (UIL^{-1})$	0.000	4.90	61.19 ± 0.81^{c}	81.63 ± 2.24^{b}	89.39 ± 0.72^{a}	91.32 ± 0.26^a
$ALT (UIL^{-1})$	0.000	6.93	11.34 ± 0.19^{c}	20.30 ± 0.49^{a}	16.41 ± 0.36^{b}	12.19 ± 0.16^{c}
Urea $(mgdL^{-1})$	0.000	11.61	37.70 ± 1.95^{b}	34.06 ± 1.38^{b}	35.55 ± 0.81^{b}	45.63 ± 1.24^a

CV: Variation Coefficient; abc: Different letters in each row express significant differences with a 95

TP: total protein, AL: albumin, TB: total bilirubin, DB: direct bilirubin, TC: total cholesterol, TG: triglycerides, AST: aspartate transaminase, ALT: alanine transaminase.

The total protein, total bilirubin, direct bilirubin, AST and urea profiles show a tendency to increase with age, showing greater levels between 61–120 days old. These results are supported by physiological mechanisms which make that total protein, albumin, and globulins in serum of all animal species increase as they are getting to the adult phase (Eckersall, 2008).

The total protein and urea profiles are like those reported by Genzer et al. (2019) and (Kitagaki et al., 2005) in Weisser-Maple guinea pigs; studies in which it was show that the profiles of these metabolites are correlated with the age of the guinea pigs. The AST and albumin profiles are different to those found in those studies and as for the total bilirubin profiles, it does not correlate with the age of the guinea pigs.

On the other hand, the TC profiles markedly reduced as age increased, showing the highest levels between 15 – 21 days of age, which contrasts with the results showed by Kitagaki et al. (2005) in Weisser-Maple guinea pigs, in which this metabolite increased in a gradual and continuous form as the age increased.

The glucose, triglyceride and ALT profiles showed homogenous levels in the different stages of

age, with similar results to those reported by Genzer et al. (2019) and Kitagaki et al. (2005) in Weisser-Maple guinea pigs, which have also shown that the levels of these metabolites are not correlated with the age of the guinea pigs. Nonetheless, in the 22–35 days old, glucose and triglycerides markedly reduced, and the ALT increased, and these metabolites reestablished in the 36-120 days old guinea pigs. The variations of these metabolites in this stage could be due to diminishing energy reserves by suppression of easily degraded carbohydrates due to weaning, thus lowering the glucose levels and accelerating the growth of different tissues, and above all, the hepatic tissue, for metabolizing more complex components that come from their new diet, generating an increase in the ALT profiles (Hoffman and Solter, 2008).

The means intervals of the different blood profiles were observed in the 15–35 days old guinea pigs and within them, triglycerides, total bilirubin, glucose, urea and protein showed the greatest amplitude in intervals, from greatest to least, respectively. On the other hand, in the 36–120 days old guinea pigs, intervals of lesser amplitude were found and the direct bilirubin, AST, ALT and TC profiles showed the least amplitudes, from least to greatest, respectively (Table 4).

Table 4. Means amplitude intervals for blood metabolites profiles of 15-120 days old guinea pigs under intensive rearing system in humid tropical conditions (95 % CI).

Blood metabolite	Age (days)						
profile	15-21	22-35	36-60	61-120			
$TP (gdL^{-1})$	[06.22-07.34]	[06.15-07.64]	[07.42-08.04]	[07.98–08.46]			
$AL (gdL^{-1})$	[03.85-04.02]	[03.22-03.91]	[03.25–03.75]	[03.93-04.14]			
$TB (mgdL^{-1})$	[00.54–00.62]	[00.51–00.58]	[00.58-00.83]	[00.89-00.95]			
$DBIL (mgdL^{-1})$	[00.14-00.17]	[00.12-00.18]	[00.17-00.23]	[00.18-00.21]			
$Glucose(gdL^{-1})$	[75.36–97.08]	[53.64–65.47]	[85.61-88.84]	[83.90-88.10]			
$TC (mgdL^{-1})$	[65.74–77.74]	[43.77–50.79]	[26.15–29.14]	[27.72–29.26]			
$TG (mgdL^{-1})$	[43.56–54.93]	[33.52-40.90]	[42.68–46.27]	[41.69–46.46]			
$AST (UIL^{-1})$	[59.36–63.03]	[76.57–86.70]	[87.75–91.03]	[90.72-91.91]			
$ALT (UIL^{-1})$	[10.90–11.77]	[19.19–21.41]	[15.59–17.24]	[11.82–12.56]			
Urea ($mgdL^{-1}$)	[33.29 – 42.11]	[30.95–37.18]	[33.72–37.38]	[42.84–48.43]			

TP: total protein, AL: albumin, TB: total bilirubin, DBIL: direct or conjugated bilirubin, TC: total cholesterol, TG: triglycerides, AST: aspartate transaminase, ALT: alanine transaminase.

Similarly to the intervals for the hematological profiles, publications referring to intervals of blood metabolites profiles by development phase in guinea pigs for production are not found, nonetheless, the mean amplitude intervals found in this research follow similar tendencies to those reported as reference intervals established for laboratory guinea pigs (Quesenberry et al., 2012; McClure, 1999; Ness, 1999; Washington and Van Hoosier, 2012; Williams et al., 2016); strain 13/N (Genzer et al., 2019), Weiser-Maples (Kitagaki et al., 2005).

In guinea pigs for production, due to elevated metabolic needs in order to respond to the pressure for production, the hematological and blood metabolites physiology varies in short periods of age or stages, contrasting with guinea pigs with the purpose of being laboratory animals, and thus it is useful to have profiles and their mean amplitude intervals for these profiles in order to interpret the state of health and production of this animal species more adequately.

The profiles and mean amplitude intervals of the hematologic and blood metabolites profiles obtained in this study, despite the genetic differences, nutrition, and purpose for breeding, differ only slightly from those obtained in the strains or biotypes of guinea pigs for laboratory experiments.

4 Conclusions

The hematologic and blood metabolites profiles obtained from the guinea pigs raised under intensive breeding systems in humid tropical conditions show significant changes with their age of production.

These profiles are close to those reported for guinea pigs used as laboratory animals, raised in environments of normoxia and the hematological profiles are inferior to those reported for the guinea pigs raised in environments of hypoxia, nonetheless, studies in endocrine biochemistry should be carried out with the aim of contributing to the improvements of the welfare, health, and production of this specie.

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Author contribution

D.P.L.: Conceptualization, project administration, methodology, original draft writing; R.R.H.: Formal analysis, investigation, validation, visualization; U.A.P.: Data curation, resources, software;

M.M.C.: Funding acquisition, supervision, writingreview and editing.

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APPARENT ILEAL DIGESTIBILITY OF THE PROTEIN IN BROILERS PARTIALLY FED WITH SACHA INCHI (*PLUKENETIA VOLUBILIS*) CAKE

Digestibilidad ileal aparente de la proteína, en pollos de engorde alimentados parcialmente con torta de Sacha Inchi (PLUKENETIA VOLUBILIS)

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Abstract

This research was conducted at the experimental farm "Andil" of the State University of the South of Manabí, Cantón Jipijapa, Manabí, Ecuador, with the aim of evaluating the ethological behavior of the apparent ileal digestibility of dry matter (DM) and crude protein (CP) of Cobb 500 chickens in individual cages. 36 male chickens were used which were distributed in four treatments with different inclusion levels of sacha Inchi cake (SIC): T1 = control, without SIC, T2 = 10% SIC, T3 = 20% SIC and T4 = 30% SIC. A Completely Randomized Design (DCA) was used for the statistical analysis, where each treatment had 12 repetitions, locating one animal per experimental unit. The results determined that chickens had a good response in quickly adapting to the cages, in addition to not reporting mortality and accepting food. The apparent ileal digestibility of DM did not show a difference between the treatments, while the N differed between the control group and the inclusion of T4 with 30% (SIC), which was higher for the latter (85.20 vs 90.23%). The results allow to conclude that the ethological behavior of the animals is not affected and good indices of ileal digestibility of DM and N are obtained when adding SIC in the diet of broilers.

Keywords: Animal physiology, adaptability, animal nutrition, metabolism.

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Resumen

El presente trabajo experimental se desarrolló en la finca experimental "Andil" de la Universidad Estatal del Sur de Manabí, Cantón Jipijapa, Manabí, Ecuador, con el objetivo de evaluar el comportamiento etológico y la digestibilidad ileal aparente de la materia seca (MS) y la proteína bruta (PB) de pollos Cobb 500 en jaulas individuales. Se utilizaron 36 pollos machos que se distribuyeron en cuatro tratamientos con diferentes niveles de inclusión de torta de sacha Inchi (TSI): T1 = testigo, sin TSI, T2 = 10% TSI, T3 = 20% TSI y T4 = 30% TSI. Para el análisis estadístico se empleó un Diseño Completamente Aleatorizado (DCA), donde cada tratamiento contó con 12 repeticiones, ubicando un animal por unidad experimental. Los resultados determinaron que los pollos tuvieron buena respuesta en adaptarse rápidamente a las jaulas, además de no reportar mortalidad y de aceptar el alimento. La digestibilidad ileal aparente de la MS no mostró diferencia entre los tratamientos, en tanto que el nitrógeno (N) difirió entre el grupo testigo y la inclusión del T4 con 30% (TSI), el cual fue superior para este último (85,20 vs 90,23%). Los resultados permiten concluir que no se afecta el comportamiento etológico de los animales y se obtienen buenos índices de digestibilidad ileal de la MS y el N con la inclusión de TSI en la dieta de pollos de ceba.

Palabras clave: Fisiología animal, adaptabilidad, nutrición animal, metabolismo.

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1 Introduction

The growing food demand requires looking for an appropriate nutritional model, using functional, novel and efficient resources to be applied in livestock production. Poultry farming in Ecuador involves agricultural production to obtain raw materials and/or by-products for poultry feed, for subsequent productive conversion (Vargas González, 2016).

When non-traditional feeds are to be included in animal feed, knowledge of nutrient concentrations is not enough, but it is also necessary to study their availability (Adedokun et al., 2011). According to Atchade et al. (2019) nutrient digestibility refers to bioavailability and depends on the animal, the composition of the feed and the treatment to which it may be subjected. When one wants to study the nutritional value of protein sources for poultry, ileal digestibility is used because these animals have different anatomical characteristics in the large intestine, so the study of fecal digestibility can be influenced by the fermentation of the microorganisms that inhabit this portion (Denbow, 2015).

Soybean (*Glycine max*) provides excellent energy concentration and availability, amino acids and essential fatty acids, fat content (18 to 20%) and protein (37 to 38%), satisfying the nutritional needs of modern poultry (Dei, 2011). It presents high digestibility (82%) and good amino acid balance (García-Rebollar et al., 2016). In broiler feeding, it is used as the main source of protein in diets (Stefanello et al., 2016). However, it has high prices in the international market, hence alternative sources are sought that allow its substitution in rations.

Sacha Inchi (SI) (*Plukenetia volubilis*) is a plant species with excellent yields, crude protein content (29%), amino acids, essential fatty acids (54%) and vitamin A and E (Romero et al., 2019). After oil extraction, its seeds have high protein values (59.13%) and fat of 6.93% (Kodahl and Sørensen, 2021). According to Romero, Valdiviezo and Bonilla (2019) the percentage of oil extraction by extrusion is lower (26.92%) when compared to that obtained in physicochemical analysis (42.03%). The residual product known as Sacha Inchi cake (SIC) has been added in the diet of some monogastrics (Robles-Huaynate et al., 2014). Although its leucine and lysine levels

are lower than those of soybean, they are equal to or higher than in other seeds (Wang et al., 2018). Sacha Inchi is currently an important crop in the Ecuadorian Amazon that could replace traditional crops in the area (Andino Nájera et al., 2018). Sacha Inchi seeds that do not meet the established standards for commercialization could be considered an alternative for animal feed (Viamonte et al., 2020).

SI seeds contain anti-nutritional factors that affect digestibility and inhibit enzymatic activity. These factors could be inactivated by chemical or thermal treatment (Reátegui and Reiner, 2012; Alayón and Echeverri, 2016). Thermally processed SIC is considered a high-quality input, as it has a high level of protein and crude energy (Alcívar-Cobeña et al., 2020a).

From the nutritional point of view, research has emerged on new techniques and food sources with the available resources that can supply the traditional ones, which allows competing in quality/price in the market, being Sacha Inchi by-products a good alternative (Castro et al., 2017). The aim of the paper is to evaluate the ethological behavior in individual cages and the apparent digestibility of dry matter (DM) and crude protein (CP) of male Cobb 500 broilers fed with SIC (*Plukenetia volubilis*) at three levels as a partial replacement of Soy (*Glycine max*).

2 Methodology

2.1 Research methodology

The analytical method was used, which consists of separating the whole, decomposing it into its parts or elements to observe the causes, nature and effects. In this case, the ethological behavior of each chicken per cage was observed. Likewise, the review of scientific literature related to the topic of feeding with cakes of different oilseeds, including *Sacha inchi*, was included. The descriptive method: quantitative and qualitative data (physiological data) were also used.

2.2 Location of the study

The study was carried out at the Andil experimental farm of Universidad Estatal del Sur de Manabí, located in the Andil commune, San Lorenzo parish,

Jipijapa canton, in the province of Manabí. The coordinates of the study site are: North 1° $15^{'}$ $54^{''}$ latitude South and 80° $41^{'}$ $24^{''}$ longitude West and an average annual temperature of $24\text{-}26^{\circ}\text{C}$.

2.3 Sample population

Thirty-six male broilers of the Cobb 500 line from 7 to 42 days of age were used for the study. This line was selected because of its high resistance to diseases in the study area and its rapid conversion into muscle mass.

2.4 Treatments

The treatments consisted of: T1= control without SIC, T2=10% SIC, T3=20% SIC and T4=30% SIC. The diets evaluated in each treatment were offered from day 7 and according to physiological stages such as initial (0-14 days), growth (15-28 days) and fattening (29-42 days). The diets were the same as those used by Alcívar-Cobeña et al. (2020b).

2.5 Experimental design

A Completely Randomized Design (CRD) composed of 4 treatments and 9 replicates was used, where three animals are considered as the experimental unit for analyzing ileal digestibility of DM and N. Tukey's dyzyme at P< 0,05 probability was used for detecting differences where necessary, and the statistical package InfoStat was used for the statistical processing (Di Rienzo et al., 2012).

2.6 Management of the study

2.6.1 Facilities

Prior to the arrival of the birds in the house, the house was cleaned and fumigated, and the floor was disinfected with lime. One day prior to the arrival of the birds, the brooder was turned on, optimizing a temperature between 30 and 32°C. The litter was built with rice shavings, and was removed daily to maintain adequate cleanliness conditions, where the birds remained for 7 days. In the second week, the litter was replaced by 48 galvanized wire cages of $40 \times 40 \times 80$ cm, equipped with their own drinking and feeding troughs, in which each bird was placed individually. Asepsis, washing and disinfection measures were applied daily.

2.6.2 Feed management

During the last week that corresponds to the fattening stage (35-42 days of age), the animals were fed with 200g of feed per day. They were divided into two rations: 100g in the morning and the same amount in the afternoon. Feed consumption was calculated from the supply and refusal.

2.6.3 Sampling

The ethology of the animals was studied from day 7 to day 14 according to (Cebrián et al., 2011). This moment coincided with the adaptation to the change from floor rearing to individual cages, located one meter above ground level together with the supply of feed that was formulated by adding SIC in different percentages. The time spent on food and water consumption and excretion and the manifestations of stress due to the management conditions were recorded. With these data, the percentage dedicated to each activity was calculated considering the total observation time (period between 8:00 am and 6:00 pm), as well as the ingestion-excretion ratio (IER). For the latter, the Equation 1 was used.

$$RIE, \% = \frac{No. \, consuming \, food}{No. \, sometimes \, excreting} * 100 \qquad (1)$$

The scale used in each case was from 1 to 100% where low values represent stress/rejection; medium values neutrality and high values or close to 100 mean good adaptation and acceptance inside the cages. After this time, animals were sacrificed for studying apparent ileal digestibility of dry matter and crude protein. Both components were determined in the diet and ileal content according to AOAC (2019) analytical techniques. Samples were taken by manually extracting the contents in the intestinal section by sliding the index finger and thumb along the ileum and were put on Petri dishes (Sebastian et al., 1997). The ileal contents of three chicks were taken as a replicate. Samples were stored at -20 °C until further processing.

2.6.4 Slaughter of the animals and calculation of apparent ileal digestibility of nutrients

The birds were sacrificed, using electric shock to stun them and reduce the level of stress. Subsequently, the abdominal cavity was opened and the ileum was identified, which is defined as the portion of the small intestine that extends from Meckel's diverticulum to the ileo-cecal junction (Castro et al., 2020). Once the samples were processed, the apparent ileal digestibility of DM and CP was calculated by the direct method according to the Equation 2. Where $(DM \lor CP)$ consumed and $(DM \lor CP)$ ileum represent the content of both nutrients in the feed consumed and in the ileal content, respectively.

3 Results and Discussion

3.1 Ethological analysis of the birds in cages

A high level of stress was observed during the first two days in the ethological descriptive analytical study (Table 1). The birds showed restlessness, so they were orally administered (dissolved in the drinker) a commercial stress regulator "Trolvit", which also contained electrolytes, amino acids and vitamins. This contributed to a faster adaptation to management.

Good responses were obtained in the ethological analysis in all variables between days 6 and 7, valued on a percentage scale from 0 to 100%, which showed a satisfactory adaptation level during the days of the study and a good sanitary control from the cages. The results are recorded in Table 1. The use of individual cages gave good results and the birds began to show their adaptation to this environment two days after their establishment. The results agree with those obtained by Martínez-Pérez et al. (2008), who proposed an adaptation time to the cages and feed of 7 days, when metabolic studies are carried out in individual cages for broilers.

Table 1. Descriptive analysis of chick ethology.

DC	CV	RCM	RIE
1	30%	30%	50%
2	40%	40%	40%
3	60%	60%	60%
4	70%	70%	60%
5	75%	75%	70%
6	90%	95%	80%
7	100%	100%	100%

DC day of rearing, CV voluntary feed and water consumption, RCM response to management conditions, RIE ingestion-excretion ratio.

3.2 Apparent ileal digestibility of nutrients using Sacha inchi cake

Table 2 shows the analysis of variance of the ileal digestibility of DM. The p value was not significant for p < 0.05. The means by treatments are shown in Table 3. The ileal digestibility coefficients were high in all cases, showing good SIC digestion by the animals. Chickens have an anatomically and physiologically adaptable gastrointestinal tract according to the type of food provided (Flórez and Osorio, 2013), which favored the consumption of the cake without causing unfavorable changes for the animal.

Table 2. Analysis of Variance of Apparent Ileal Digestibility of dry matter.

F.V.	SC	Gl	CM	F	p-value
Tratamientos	0.33	3	0.11	0.10	0.9585^{NS}
Error	8.79	8	0.11		
Total	9.11	11			

NS: Not significant.

The analysis of variance for the apparent ileal digestibility of crude protein is shown in Table 4. The p-value in this case was significant, and differences between treatments were found when comparing the means shown in Table 5. The control group and the inclusion of 30% SIC show differences between them, and their coefficients were the lowest and highest, respectively. The 10 and 20% SIC were similar to the rest of the treatments. It seems that the quality of the protein provided by the Sacha inchicake is high, so there is an eminent utilization by the animal, hence it is excreted in lower concentration and therefore it increases the digestibility when

included in the poultry rations.

Despite the differences observed, the coefficients were generally high. These results do not coincide with those observed by Woyengo et al. (2017) when using camelina cake (around 75.30%). The differen-

ces could be explained by the chemical composition of both ingredients after the oil extraction process to which the oilseeds were subjected. These authors reported CP and FDN values of 39.80 and 38.30%, respectively, while in the Sacha inchi cake used in this study.

Table 3. Analysis of means by Tukey's test for Apparent Ileal Digestibility of Dry Matter (DIAMS).

Variable (%)	Treatments	Means	No.	E.E ±
DIAMS	T4	82.59	3	0.61
	T3	82.51	3	0.61
	T2	82.50	3	0.61
	T1	82.16	3	0.61

Alcívar-Cobeña et al. (2020a) obtained values of 41.49 and 16.64%, respectively. The fact of having a higher protein concentration and lower fiber content favors its digestibility; several authors mention that fiber content has a direct incidence on the digestive process (Zając et al., 2020).

On the other hand, according to Woyengo et al. (2017) products resulting from oil extraction in oil-seeds generally contain anti-nutritional factors that can limit the use of nutrients by the animal. Sacha inchi cake shows presence of alkaloids, saponins and α -amino groups (Alcívar-Cobeña et al., 2020a), however, the concentration of these components does not seem to be very high, since they did not unfavorably impact ileal digestibility of DM and CP.

Palpa (2009) determined the metabolizable nutrients and apparent metabolizable energy for precooked and pre-cooked extruded sacha inchi cake in broilers by collecting fecal samples. The metabolizable dry matter was between 73.95% and 74.78%; the metabolizable crude protein between 42.97% and 44.26% and the apparent metabolizable energy between 4570.0 and 4578.91 kcal/kg, respectively. Since the amount of protein and fat in SIC is high, its application could optimize the resources available to producers since soybean cake has high prices in the international market and livestock activity imports approximately one hundred thousand tons per year (Benítez et al., 2018).

Table 4. Analysis of Variance for Apparent Ileal Digestibility of CP.

F.V.	SC	Gl	CM	F	p-value
Treatments	37.96	3	12.65	7.43	0.0107
Error	13.62	8	1.70		
Total	51.58	11			

Table 5. Analysis of means by Tukey's test for Apparent Ileal Digestibility of CP (DIAPB).

Variable (%)	Treatments	Means	No.	$\mathbf{E}.\mathbf{E}\pm$	
DIAPB	T4	90.23	3	0.75	A
	T3	87.79	3	0.75	AB
	T2	87.74	3	0.75	AB
	T1	85.20	3	0.75	В

Means with different letters differ significantly.

4 Conclusions

Ethologically, the Cobb 500 chickens obtained a positive response in adapting satisfactorily to the individual cages. During the process of the study, there was no mortality, and better control of feed and drinking water intake was achieved. Sanitary control was better compared with rearing in litter.

Good indices of ileal digestibility of DM and N were obtained when using SIC the diet of broilers, so it can be used as a partial substitute for soybean in the conventional feed for Cobb 500 broilers. Further research with greater variability in the experiments is recommended for its application in other breeds.

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Author contribution

Conceptualization, methodology and discussion: J.L.A.C.; Experimental design and statistics: M.M.P.; Management of field research, laboratory analysis: M.F.F.T.; Writing and bibliography: L.V.S.S.

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