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Environmental, Economic and Social Perceptions of Community Members on the Role of Water, Soil and Natural Grasslands as a Basis for Local Development in Acopalca, Peru

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Abstract:
The concept of ecosystem services has gained popularity among academics, researchers and policymakers to support environmental management and biodiversity conservation, so that many development projects in rural areas have merited investment for restoration and improvement of grassland ecosystems accompanied by training programs for the beneficiaries, With this criterion in mind, the study investigated the perception of puna pastoralists in Acopalca, Peru, regarding the degree of knowledge about the significance of the ecosystem services provided by soil-water-grasslands, with the objective of characterizing the environmental, social and economic dimensions of this local perception, through the application of a survey to the representative of the livestock family affiliated to two producers' associations. It was evidenced that cattle-raising families have a limited understanding of the role of the natural resources they directly access and little clarity on the relationship between natural pastures, family income and access to basic services. The results revealed limitations in environmental perception, evidencing a lack of knowledge about the multifaceted contribution of pastures. Social perception showed neutrality in the relationship between pastures and family income, and a discrepancy in access to basic services. The comparison between associations highlighted significant differences, indicating the need for training strategies adapted to the local idiosyncrasies of the beneficiaries. In conclusion, addressing the deficiencies identified in community understanding was essential to strengthening sustainable natural resource management in Acopalca. It highlights the importance of designing specific training programs, considering the particularities of each group, to promote self-management and community participation and thus achieve more comprehensive and sustainable local development.

Keywords: Environmental perception, puna pastoralists, soil-water-grassland relationship, community participation

INTRODUCTION

Andean grassland ecosystems play a crucial role in providing a variety of ecosystem services that are beneficial to human populations, as noted by Qian et al. (2021) and Shi et al. (2023). These services include the provision of essential forage for livestock production, as well as the contribution to freshwater supply for downstream communities, as evidenced in the research of Qian et al. (2021). In this context, numerous investment projects have been implemented by public and private entities in the headwaters, where herbaceous vegetation predominates. These projects are aimed at improving ecosystem service provision, with the objective of improving the basic conditions of local development for rural inhabitants. This is achieved through the conservation or restoration of grasslands, with an integrated approach ranging from improving livestock production to the implementation of practices for planting and harvesting water for human consumption and agricultural irrigation

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This specific approach highlights the importance of managing rainwater as an integral part of the management strategy, as emphasized by Deng et al. (2022) and Mosquera et al. (2022). Linking rangeland conservation, livestock production, and sustainable water management practices represents a significant step toward sustainable development and prosperity for rural communities in these Andean areas. The Shullcas River sub-basin, located in the central region of Peru, encompasses diverse agrosystems, forest ecosystems and forest patches, as well as grassland and bofedal ecosystems (Mosquera et al., 2022) and glaciers, with a total area of 4 466 029.38 hectares (ha). Of this area, the vegetation cover corresponding to grassland and bofedal ecosystems covers 900 544,313 ha, equivalent to 20.16% of the sub-basin area (Gore-Junn, 2015).

In this context, environmental investment projects have been implemented, among the most prominent is the project "Conservation of natural grasslands in high Andean areas of the Shullcas River sub-basin - Annexes: Acopalca, Cullpa Alta and Cochas Chico", implemented by Agro rural of the Ministry of Agriculture between 2010 and 2012 with funding from the World Bank (PRAA Peru, 2013). Another relevant project is the "Climate-Smart Territories Project", carried out by the Tropical Agricultural Research and Higher Education Center (CATIE), with the support of the United States Agency for International Development (USAID) between 2015 and 2016 (Minam, 2017). In these initiatives, as well as in other projects of smaller scope, local beneficiaries received environmental training focused on pasture recovery and rainwater regulation, with a focus on improving the quality and productivity of products such as meat, milk, fiber and wool, which constitute the main forage resource for livestock and generate significant family economic income (Guo et al., 2021).

Guo et al. (2021) highlight the importance of addressing the need for water for human activities, industry and agriculture, as well as the risks associated with human activities and ecological security. Furthermore, they underline the fundamental condition that stakeholders are organized and act on the basis of a planned program (Richter et al., 2021). In this way, social actors understand the economic benefits derived from managing the sustainability of the ecosystem in the Shullcas River sub-basin.

Within this framework, the training focused on understanding the fundamental role of grassland ecosystems in water provision. This is achieved through the infiltration of rainwater, its storage in the subsoil and its gradual release at the surface through water eyes, springs and the recharge of wetlands (Mosquera et al., 2022; Monge-Salazar et al., 2022). These processes have been highlighted as one of the main ecosystem services demanded by downstream communities (Richter et al., 2021; Zhang et al., 2021; Monge-Salazar et al., 2022).

On the other hand, the presence of anthropogenic factors that threaten the sustainability of this ecosystem service has been identified. These factors include overgrazing, fires, land use change and natural elements such as climate change (Chen et al., 2019; Jimoh et al., 2020; Piipponen et al., 2022; Couvreur et al., 2019). In addition, the crucial role of soil moisture and weather phenomena in regulating aerial primary productivity of grasslands, which constitute the main feed in extensive livestock farming in Andean ecosystems, has been addressed (Chen et al., 2019; Dong et al., 2022; Guo et al., 2021).

Consequently, the projects have had an impact on the understanding of the responsibility of local stakeholders in the implementation of moderate grazing as well as in the maintenance and restoration of grassland ecosystems. These actions are fundamental to conserving or enhancing ecosystem services, thus contributing to ecosystem sustainability (Couvreur et al., 2019; Wang et al., 2021).

Understanding the relationship between local inhabitants and ecosystems, as well as appreciating the services resulting from these interactions, becomes essential to developing management strategies that benefit both local livelihoods and environmental conservation. The ecosystem services that are most commonly socially perceived include the provision of water for consumption, the provision of raw materials, and food production (Villamagua, 2017).

On the other hand, the concept of ecosystem services has gained relevance among academics, researchers and policymakers as a support for environmental management and biodiversity conservation. Although most studies have focused on the physical or economic measurement of these services, few have explored social preferences towards them from a non-economic perspective, which encompasses human values, attitudes, and beliefs. This non-economic approach reveals underlying motivations and values that are often overshadowed by the monetary approach.

In analyzing how ecosystems and biodiversity influence human well-being, it is crucial to understand how society benefits from nature and why it places value on ecosystem services. Identifying the reasons for protecting these services contributes to understanding their importance to various stakeholders and the trade-offs needed when making decisions about land, soil and water use. Different groups value ecosystem services differently, depending on their individual needs and perceptions (Martín-López et al., 2012). Human actions have modified the use and cover of natural grasslands, affecting the provision of ecological services linked to water. Despite this, there is scarce information on the nature and extent of these impacts, as well as on the effect of restoration practices on the water cycle. The lack of understanding of the eco hydrology of Andean grasslands, especially in situations of degradation and changes in use, hinders sustainable management efforts by the institutions involved. This knowledge is essential for water supply companies seeking to maintain a constant downstream flow (Mosquera et al., 2022).

Hence, Andean grasslands play a vital role in national livestock farming, providing feed for the vast majority of cattle, sheep and camelids livestock. This form of livestock raising is rooted in the social life of the peasants and is key to their economy, complementing their agricultural income.

These pastures supply a significant part of the livestock's food needs, but it is projected that improving their capacity and productivity could double this contribution. However, the lack of implementation of improvement strategies foresees a 20% increase in low-quality pastures by 2070, which will make it difficult to meet the demand for milk and meat (Valverde et al., 2022).

This study focuses on examining social and economic perceptions of ecosystem services, addressing the valuation of the capacity of ecosystems to provide services to society. It also explores the priorities assigned to different categories of these services and investigates the factors that influence these preferences. In addition, the possible mix of services is examined in terms of these divergent preferences. In this context, the question is raised about the level of empowerment achieved by the beneficiaries of the projects developed in associations of agricultural producers in the Acopalca Community. Is there uniformity of criteria among both beneficiaries and producer associations regarding the sustainable management of Andean grassland ecosystems? The purpose of this is to evaluate the effectiveness of the training and determine if there are still limitations that require additional actions to promote sustainability in the use and exploitation of the ecosystem. Therefore, the objective of the research was to interpret the environmental, economic and social perceptions of the community members of the Acopalca Community in relation to the role of water, soil and natural grasslands as the basis for local development.

MATERIAL AND METHODS

Study Area

The research was carried out in a farming community in central Peru, located in the micro-watershed of the Shullcas River. The main population is composed mainly of Andean cattle herders who own small herds of cattle, sheep and alpacas. The communal area ranges from coordinates 482754.42 m E, 8672906.21 m S at 3551 m altitude in its lowest part, to coordinates 494237.50 m E, 8683487.59 m S at 5506 m altitude in the highest part of the sub-basin, according to Google Earth measurements (Figure 1). After the dissolution of the agricultural social interest societies (SAIS), the community received a total of 27,062.30 ha (120.15 ha for rain-fed cultivation; 18,245.65 ha of natural grasslands; and 8,475.69 ha of non-agricultural land) (Diez-Hurtado, 2020).

Subsequently, these lands were divided into plots and allocated to the families of the community, who currently have grazing plots managed by the communal organization. These areas exhibit predominantly herbaceous vegetation, with the prominent presence of families such as Poaceae, Asteraceae, Plantaginaceae and Rosaceae, while the middle areas are interspersed with pine forests (Pinus radiata) reforested as part of a grassland conservation project (PRAA Peru, 2013; Yaranga et al., 2023) in a heterogeneous and rugged terrain.

The population of the Centro Poblado de Acopalca is currently located in the territory of the Huaytapallana Regional Conservation Area, a protected area under the administration of the regional government of Junín (Gobierno Regional de Junín, 2014). The population amounts to 394 inhabitants (INEI, 2023), who have been beneficiaries of several projects related to soil management, water and reforestation. This territory is home to the Huaytapallana glacier, playing a crucial role in the provision of water for the city of Huancayo.

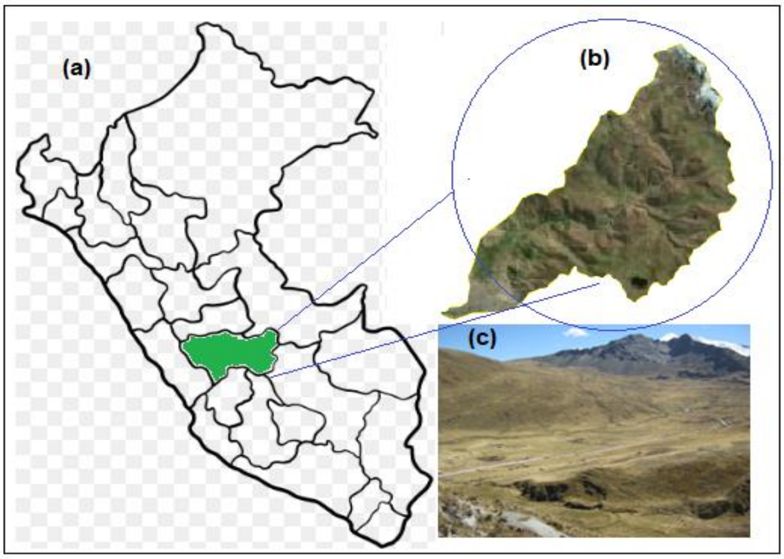

Figure 1: Profile of the Shullcas sub-basin (a) in relation to the Junín region and Peru (b), (c) photo of the grassland ecosystem in the upper part of the sub-basin.

Data Collection

Data were collected from pastoralists affiliated with two organized groups in the rural community of Acopalca: the "Asociación de Productores Agropecuarios Cunyas" and the "Empress de productores Tauribamba Acopalca SAC". A structured survey was carried out in different sessions and at different times between May and September 2023.

This survey was designed with questions aimed at understanding the community members' knowledge of how the environment (water, soil, pastures) relates to the economic and social aspects of the population. Training provided by various entities, both public and private, in environmental projects within the rural community of Acopalca was considered. To measure perception, questions were divided into three areas: environmental, economic and social. The survey was structured so that responses were given on a Likert scale, which has five levels: (1) strongly disagree, (2) disagree, (3) neutral, (4) agree and (5) strongly agree. This scale is commonly used to assess attitudes, assessments, preferences or perceptions (Morales et al., 2016).

The survey was designed to be easy and quick to answer, presenting questions that assess attitudes within the context of pastoralists. These questions address aspects such as information received and the influence of intervention projects. In addition, they explore personal level factors, such as moral obligation or individual norms, as well as personal assessments of the importance of water, soil and rangelands (Moreno et al., 2005).

The purpose was to measure the degree of awareness of environmental, social and economic issues, especially in an area where there is a high level of project intervention. In addition, it was determined whether respondents had internalized the benefits of these initiatives. This approach in the survey allowed to capture both the knowledge acquired and the sense of personal responsibility and perception about the seriousness of environmental problems or the benefits of programs and projects implemented in the area. The survey was designed to be easy and quick to answer, presenting questions that assess attitudes within the context of pastoralists. These questions address aspects such as information received and the influence of intervention projects. In addition, they explore personal-level factors, such as moral obligation or individual norms, as well as personal assessments of the importance of water, soil and rangelands (Moreno et al., 2005).

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Data Analysis

The completed survey forms were reviewed in advance to ensure the clarity of the responses before being digitized. They were then converted into digital format using an Excel spreadsheet. At this stage, the data were organized in a double-entry matrix, where the rows represent the respondents and the columns correspond to the responses to the different items, organized in blocks of perception: environmental, economic and social.

Data analysis began by converting the Excel sheet into a pivot table, following the methodology proposed by Moore (2013). This allowed us to calculate the sum (horizontally) and the average (vertically) of the responses. These calculations were fundamental to identifying critical responses or responses that were poorly aligned with the expected knowledge. In addition, a second table was generated from the first one, counting the responses on the five Likert scales established, with the purpose of elaborating bar charts for each perception block.

Finally, the data collected in the Excel table was used as input to carry out a Non-Metric Multidimensional Scaling (NMDS) analysis using Rstudio software version 4.2.3. This analysis was carried out with the support of the "ordiplot" and "ordihull" extensions (Cuadras, 2007 and Bocard et al., 2018). This process, from survey form review to advanced statistical analysis, provided a detailed understanding of the responses, enabling patterns and relationships between respondents' perceptions of environmental, economic and social issues to be identified. Robust methods supported by academic literature were used.

RESULTS

Integrated Perception

The analysis of the villagers' environmental perceptions, based on the survey results, reveals notable limitations. The mean values of 2.63 and 2.51 for items 2.3 and 2.4, respectively, indicate a position close to neutrality on the Likert scale. These scores suggest a lack of conviction on the part of respondents about the ability of grasslands to provide ecosystem services beyond their function as a source of food for livestock. This lack of conviction is evidence of a significant lack of knowledge about the integral value of grasslands in the ecosystem and points to a lack of awareness, both among authorities and community members, of the responsibility to conserve and preserve these ecosystems. The lack of understanding of the multifaceted contribution of grasslands indicates a profound lack of knowledge about their role in the balance and health of the natural environment.

In terms of social perception, the survey results indicate a neutral stance on the relationship between natural pasture and household income (item 3.1), with an average Likert scale score of 2.91. This neutrality suggests a lack of clarity or awareness of how pasture could influence household income. This neutrality suggests a lack of clarity or awareness of how rangelands might influence household income. Furthermore, in item 3.5 (social perception), the average of 1.43 shows a marked discrepancy in perceptions of access to basic services such as electricity, drinking water and drainage. These results reflect a lack of familiarity with or knowledge about modern technologies such as solar panels, water purification methods and sewage systems such as septic tanks. These findings suggest a significant information gap in the surveyed community regarding the connection between rangeland management and its influence on household income. Furthermore, the discrepancy in perceptions of access to basic services indicates the need for educational programmes that address these knowledge gaps to improve understanding of technologies critical to community well-being and sustainable development. Comparing the overall perception among the respondents, 28% of the participants of the Cunyas Agricultural Producers' Association and 50% of those affiliated with the Tauribamba Producers' Association Acopalca SAC scored below average. The latter group seems to be less convinced of the ecosystem benefit (Table 1), which could be attributed to various factors, such as inadequate implementation of trainings or low interest of beneficiaries in attending trainings.

Table 1: Pivot table database of the analysis of the perceptions of the interviewed community members

	Environmental perception					Economic perception					Social perception				
Inter-viewed	1.1	1.2	1.3	1.4	1.5	2.1	2.2	2.3	2.4	3.1	3.2	3.3	3.4	3.5	Total
E1	4	4	4	4	4	4	4	2	4	4	4	4	2	2	50
E2	2	3	3	4	4	2	4	4	4	4	4	4	4	2	48
E3	2	4	5	4	4	4	4	2	3	2	5	2	3	2	46
E4	4	4	4	5	2	4	5	2	2	3	4	4	4	1	48
E5	4	5	4	5	5	5	5	4	5	5	5	5	3	3	63
E6	4	5	5	5	4	3	5	4	4	3	4	4	3	3	56
E7	5	4	5	5	5	5	5	4	4	5	5	5	4	3	64
E8	4	5	5	5	5	5	5	5	5	5	5	5	5	1	65
E9	4	5	5	5	4	3	5	4	4	5	5	5	3	1	58
E10	2	4	5	2	5	5	5	4	4	5	2	5	4	1	53
E11	4	5	5	4	2	4	5	4	4	4	5	5	2	1	54
E12	4	4	5	4	4	4	5	4	4	4	5	5	4	1	57
E13	4	4	5	3	4	5	5	3	4	4	4	5	4	1	55
E14	4	4	4	4	5	5	5	3	3	3	4	4	1	1	50
E15	4	4	5	4	5	5	5	2	3	3	4	4	2	1	51
E16	5	4	5	3	2	4	5	2	1	2	4	5	2	1	45
E17	5	4	5	3	3	4	4	3	2	2	4	4	2	2	47
E18	5	5	5	4	1	4	4	1	1	2	4	4	4	1	45
E19	5	5	5	4	3	4	5	2	2	2	5	5	3	1	51
E20	4	3	5	3	2	4	5	2	1	2	5	4	4	1	45
E21	5	4	4	5	2	4	4	2	2	3	4	5	3	2	49
E22	5	4	4	4	3	4	4	2	2	3	4	4	4	1	48
E23	5	4	5	4	3	4	4	3	3	1	4	4	4	2	50
E24	3	4	3	2	4	4	2	2	2	4	4	4	4	1	43
E25	4	4	5	4	3	4	5	2	2	2	4	5	4	2	50
E26	5	5	4	4	2	4	4	3	1	2	5	4	3	1	47
E27	3	4	5	4	3	5	3	2	2	3	4	4	3	2	47
E28	4	3	3	3	4	4	4	2	2	2	4	4	3	1	43
E29	5	4	4	3	2	4	4	2	2	3	4	4	3	1	45
E30	4	5	4	5	3	4	4	2	2	2	4	5	3	1	48
E31	4	5	5	5	4	4	4	2	2	2	4	4	3	1	49
E32	4	5	5	4	3	4	3	2	2	2	4	5	2	1	46
E33	4	5	4	4	3	4	4	2	2	2	4	4	3	1	46
E34	4	5	4	4	3	4	5	6	2	2	5	3	3	1	51
E35	4	3	5	3	2	4	4	2	1	2	5	5	3	1	44
E36	5	4	4	3	3	3	4	1	2	4	3	3	3	2	44

E37	4	4	4	3	4	4	4	2	2	4	4	4	4	1	48
E38	5	5	5	4	4	4	4	3	3	3	4	4	3	2	53
E39	5	5	5	5	5	4	4	2	2	3	4	4	3	1	52
E40	5	5	5	5	4	4	5	4	1	1	4	5	3	1	52
E41	5	4	4	3	3	4	3	2	2	2	5	4	2	1	44
E42	5	4	4	4	4	4	4	2	2	3	4	4	3	2	49
E43	5	3	5	3	4	4	4	2	2	3	4	4	4	2	49
E44	5	5	4	4	2	4	4	2	2	3	4	4	3	2	48
E45	4	3	4	3	2	4	4	2	2	2	4	4	4	1	43
E46	4	3	4	3	2	4	4	2	2	2	4	4	3	2	43
Average	4.22	4.22	4.48	3.87	3.35	4.07	4.28	2.63	2.50	2.91	4.22	4.26	3.17	1.43	

When analyzing perceptions in the two beneficiary associations of the investment projects, it stands out that only 22% of the trainees achieved a deeper understanding, followed by 40% who showed some degree of agreement. However, this reveals that a considerable 38% of the beneficiaries were not able to fully develop their capacities to understand the interrelationship between the three essential pillars of the ecosystem: water, soil and grassland (Fig. 2). These findings highlight the urgent need to review the strategies used in the capacity-building process. It is important to look for more effective mechanisms that encourage greater participation and the development of local skills, ensuring that the actions taken are comprehensive and involve the community in the process of sustainable natural resource management. This becomes especially relevant in the implementation of new projects of environmental interest. The detailed analysis (Figure 2) reveals that, despite training efforts, a significant proportion of the beneficiaries have not been able to fully internalize the complex interaction between water, soil and grasslands in the study ecosystem. This challenge can be attributed to possible shortcomings in the training methodology or in adapting the content to the specific needs of the community.

These results underline the importance of addressing identified gaps in the understanding of the interdependence of ecosystem components. Improving the training and involvement of beneficiaries in future projects is essential to strengthening sustainable natural resource management and promoting more effective participation in environmental initiatives. Adapting educational approaches to meet local characteristics and requirements can be key to maximizing the impact of training and ensuring an in-depth understanding of local ecological complexities.

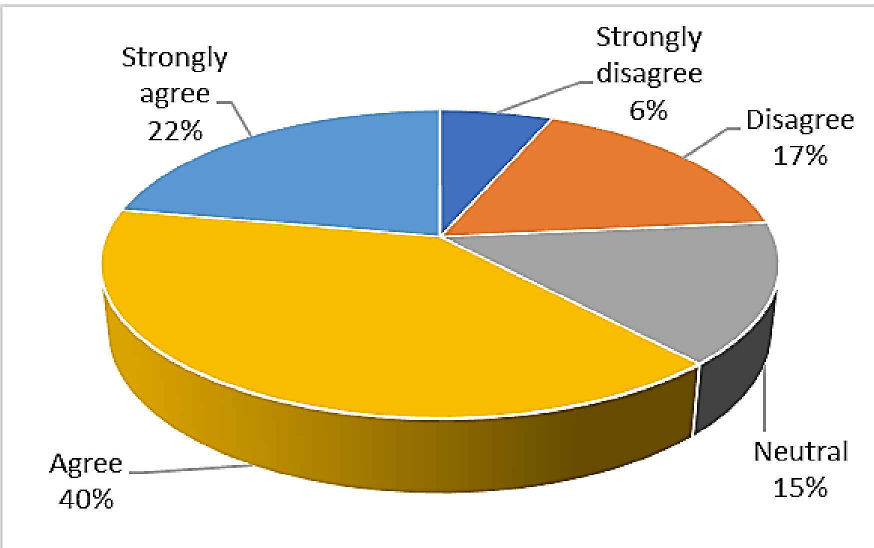


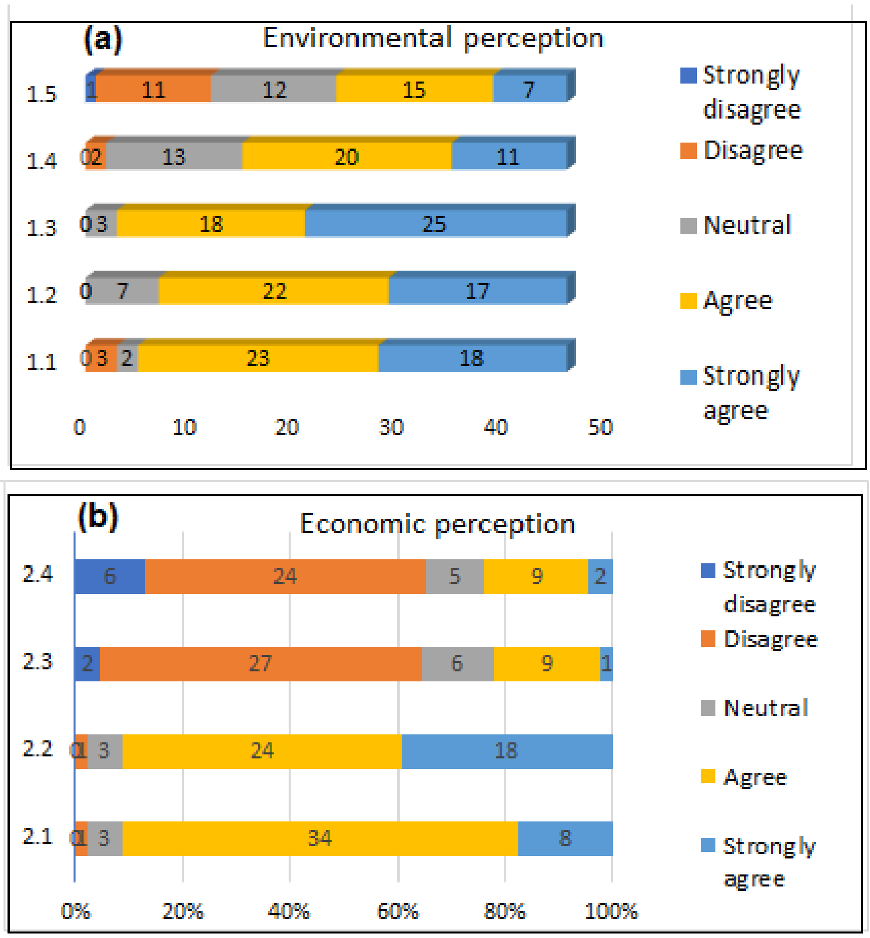
Figure 2: Distribution of the integrated perception between the environmental, economic and social criteria of two producer associations in the rural community of Acopalca

Environmental, Economic and Social Perceptions of Two Producer Associations in The Rural Community of Acopalca

The analysis of environmental, economic and social perceptions in the two producer associations of the Acopalca farming community reveals a variety of opinions and understandings. In relation to environmental perceptions (Figure 3a), a significant percentage, between 36.96% and 39.13%, showed high agreement on the concept that pasture facilitates water infiltration into the soil, releasing it gradually even in dry periods. They also pointed out their usefulness for the storage of nutrients carried by runoff from higher elevations. This aspect, according to 54.35% of those interviewed, benefits fodder production, which is fundamental for pastoralist families.

However, some disparity in perceptions is evident, with between 32.61% and 50% showing moderate agreement with these criteria, while between 0.4% and 26.08% revealed a degree of ignorance regarding the ecosystem services linked to water, soil and grasslands. These data confirm that at least one third of the participants were not completely convinced about the interrelationship between water, soil and grassland.

The results underline the need for greater clarity and education on the ecosystemic relationship between the key components. It is important to address these discrepancies in perceptions to ensure a comprehensive understanding of the importance of sustainable grassland management in the farming community of Acopalca. This educational approach could include strategies that highlight the tangible benefits of grasslands in terms of water infiltration, nutrient retention and their essential contribution to fodder production, thus strengthening the connection between environmental aspects and livestock activity in the locality.



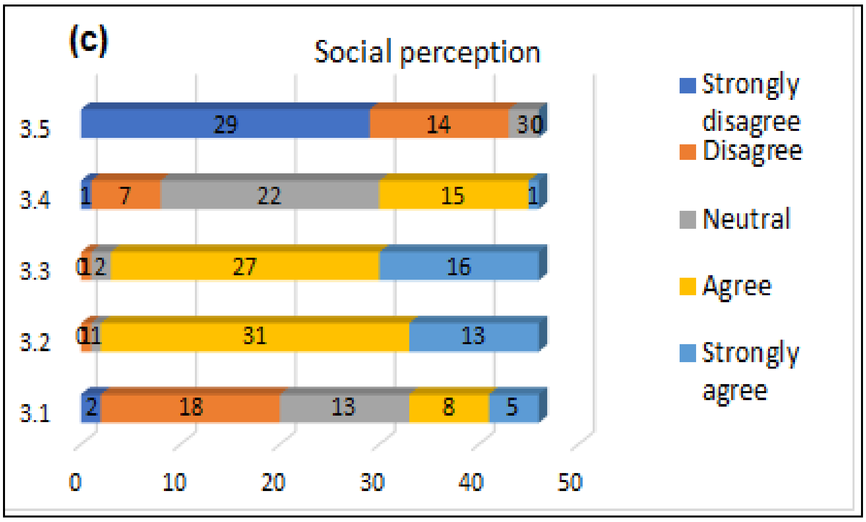


Figure 3: Percepción ambiental, económica y social de los comuneros asociados de Acopalca
Note: The numbers indicate the number of responses found, distributed according to the likert scale applied.

In the analysis of the economic perception with respect to ecosystem services (Figure 3b), 73.91% of the associated community members agreed that these services are intrinsically linked to the prosperity of livestock activity. Furthermore, 52.17% recognized that this relationship regulates the production of fundamental elements such as fiber, wool, meat, milk and cheese, which are essential elements for the family economy. However, a significant discrepancy emerged: 63.04% disagreed that local actors, such as community members and authorities, have a responsibility to conserve and improve grassland ecosystems. This suggests a perceived dependence in the Acopalca community on external interventions for conservation, indicating a lack of confidence in local capacity to care for these ecosystems. Similarly, 65.23% expressed that past implementation of investment projects did not produce visible results in the environmental relationship with the change or improvement of livestock production.

In terms of social perception, specifically the achievement of quality of life influenced by projects implemented by different entities (Figure 3c), different perspectives are revealed. Only 28.26% agreed with the positive effects on quality of life. In contrast, 43.48% disagreed, and 28.26% did not express a clear position. Regarding the technical assistance provided by the project executing agencies, 67.39% agreed and 28.26% strongly agreed with the conviction on the importance of proper management of grassland and wetland ecosystems through human intervention. However, this contrasts with the perception of meat and milk production as the basis for food security, where only 34.78% strongly agreed, stating that the sale of live cattle is fundamental to acquiring basic foodstuffs in the market. Regarding the provision of food through social programmes such as the glass of milk and pension 65, 47.83% adopted a neutral position, and only 34.78% agreed. Finally, 93.48% disagreed with respect to access to basic services.

These data reflect a diversity of opinions and perceptions, highlighting a lack of consensus on a number of issues, from local responsibility for conservation to assessing the effectiveness of past projects in relation to quality of life and food security. These divergences underline the complexity of social, economic and environmental factors in the Acopalca community, indicating the need to address these variations for more effective and sustainable interventions.

Multivariate Analysis of The Response Between the Two Associations

The multivariate analysis of the responses provides a revealing insight into the heterogeneity of the criteria of the community members of the two associations studied. We observed the presence of isolated responses, both within and between the two associations, as well as the identification of a small group whose responses coincide in both associations (Fig. 4).

When examining the disparity in the knowledge or empowerment of the community members with respect to the training received, significant differences can be seen. In the blue frame, corresponding to the Cunyas Agricultural Producers' Association, 88.89% show different criteria from the rest of the group, indicating considerable variability in their perceptions. On the other hand, in the red frame, which represents the Asociación de Productores Tauribamba Acopalca SAC, only 3.85% show isolated criteria compared to their corresponding group. This pattern suggests that the members of the second association have more homogeneous and precise knowledge about the role and importance of ecosystem services in relation to their economic and social interests in order to address the particularities of each group, as recommended by Villamagua (2017).

In general terms, considering all the partners of both associations, only 39.13% show shared criteria and knowledge. These results emphasize the need to design more specific training strategies tailored to the particularities of each group. They also highlight the importance of addressing the identified discrepancies in perceptions in order to achieve a more uniform and consolidated understanding of key concepts related to ecosystem services.

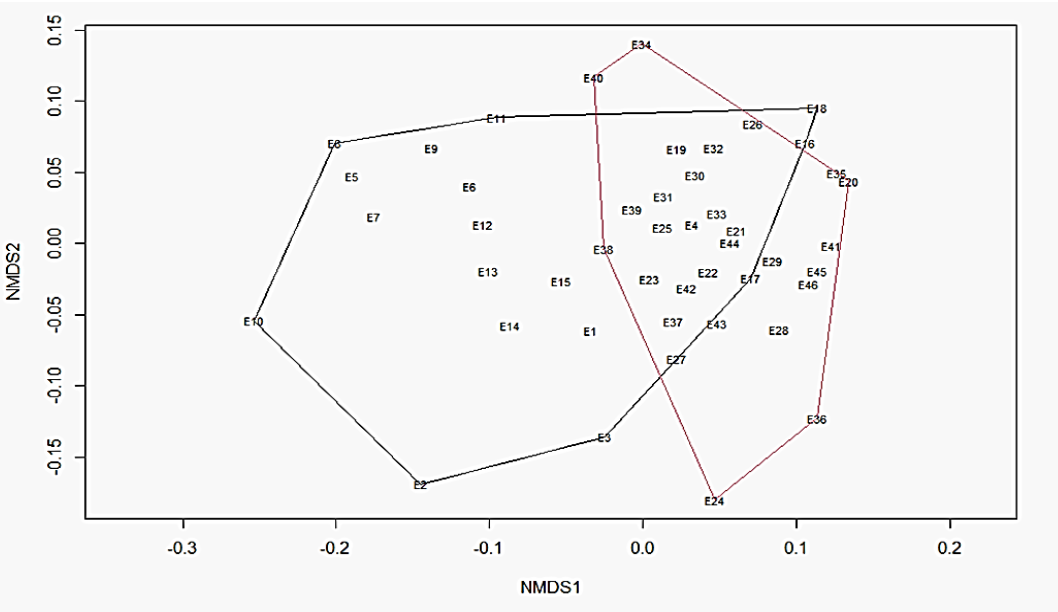


Fig. 4: Multivariate NMDS analysis of the environmental, economic and social perception of the community members in relation to the association they belong to and level of personal response (where "E1-46" is the identity of the respondent)

DISCUSSION

Integrated Perception

According to the results (Table 1) the beneficiaries showed to have internalized more the environmental trainings with respect to the water-soil-grassland relationship; however, it was not so evident in the relationship of the grassland with the level of income, this leads to understand that the trainings did not articulate the forage supply of the ecosystem with the level of livestock production (Rothwell & Whiteford, 2020) for this reason they did not give importance to the maintenance, conservation and improvement of the grasslands by direct action of the associations or the beneficiaries themselves (Zawilińska et al., 2023), in addition to the fact that the associations also do not have funding to develop conservation actions, which reveals that the social actors are always waiting for the intervention of external entities to assume this responsibility (Everard, 2021), Meanwhile, they refuse to recognize the goodness of external investment projects in the quality of life of themselves. On the other hand, table 1 reveals that there are differences in understanding between the members of the two associations, as the first association (E1 to E18) is composed of more educated people and some who are outgoing as workers of livestock companies in the United States, which helps in corporate understanding between them, in contrast to the second association (E19 to E46) which has members with a lower level of education and no person who has gone abroad.

However, of both associations (Figure 2), approximately one third of the associates still carry the shadow of total or partial ignorance of the interdependence between the ecosystem and the individual, family and communal life of Acopalca's associated community members (Everard, 2021); Rothwell & Whiteford, 2020), which could be associated with the low efficiency of the training strategies used by the technical promoters of the projects (Everard, 2021) and the low participation of the beneficiaries due to the scheduling of the training sessions at times that are not in accordance with the beneficiary's time availability (Zawilińska et al., 2023) as commented by some beneficiaries, on the other hand, most of the projects have a purely technical focus, so there is little or no participation of professionals from the social area in the implementation of the project, which raises the need to place special emphasis on the participatory inclusion of the actors involved (Baca-Tavira & Herrera-Tapia, 2016).

Environmental Perception

The environmental perception within the Acopalca Community, according to the results obtained (Table 3a), reflects a moderate understanding of the integral role of grasslands, water and soil in the Andean ecosystem. However, there is a need to strengthen knowledge of hydrological ecosystem services, such as the process of water infiltration into the soil, the crucial role of herbaceous vegetation cover in rainwater infiltration, and the transport of natural nutrients and their deposition in flatter places or hollows. These processes favor higher forage productivity for livestock and soil protection against the effects of erosion, as well as carbon sequestration and fixation in plants and soil (Monge-Salazar et al., 2022; Richter et al., 2021).

It is essential to highlight that, through educational efforts, community perceptions are aligned with the research of Qian et al. (2021) and Shi et al. (2023), who stress the importance of environmental awareness in the sustainable management of Andean grassland ecosystems. The need to bridge the gap in understanding the interdependence between water, soil and grasslands aligns with concerns raised by Deng et al. (2022) and Mosquera et al. (2022).

The diversity of opinions on the importance of grasslands in water infiltration and forage production, as well as the degree of ignorance among some respondents, highlights the need for more clarity and education on the ecosystem relationship. This finding aligns with the recommendations of Martín-López et al. (2012). Furthermore, the perceived reliance on external interventions for conservation, evidenced in the discrepancy about local responsibility, is in line with the concerns raised by Guo et al. (2021). The lack of confidence in local capacity for rangeland care underlines the need for strategies that encourage greater community participation and empowerment, as indicated by Wang et al. (2021). It highlights the importance of addressing not only technical knowledge, but also community perceptions and trust in order to achieve sustainable natural resource management in the Acopalca farming community.

Economic Perception

The majority of the members of the two producer associations have a good understanding of the interrelationship between the ecosystem and their livestock activity, recognizing the importance of a healthy environment to ensure sufficient feed for livestock, which has a direct connection to the economic income generated by the sale of their products (Bitana et al., 2023). However, the majority perception of hesitation or disagreement about the responsibility of local stakeholders, as confirmed in Table 1, highlights the need for further education and awareness raising of local stakeholders on how rangelands can influence local livelihoods, according to the findings of Monge-Salazar et al. (2022). Furthermore, the importance of more effective and tailored training strategies to improve local participation and understanding is supported by Richter et al. (2021). Also, the perceived reliance on external interventions for conservation, evidenced in the discrepancy over local ownership, is in line with the concerns raised by Guo et al. (2021). The lack of confidence in local capacity for rangeland care underscores the need for strategies that foster greater community participation and empowerment, as suggested by Wang et al. (2021).

At the economic level, the perception results highlight the intrinsic connection between ecosystem services and the prosperity of livestock activity, aligning with research by Valverde et al. (2022). However, the discrepancy over local responsibility for conservation reflects the need to address the perception of dependency in the community, as also noted by Couvreur et al. (2019). The low acceptance of the effectiveness of past projects and the perception that they did not produce visible results indicate the need to critically evaluate the implementation and outcomes of such projects, in line with the recommendations of Deng et al. (2022) and Mosquera et al. (2022). The diversity of opinions on the importance of grasslands in water infiltration and forage production, as well as the degree of ignorance among some respondents, highlights the need for more clarity and education on the ecosystem relationship, in line with the recommendations of Martín-López et al. (2012). The results suggest that social perception is influenced by a lack of knowledge about the interrelationship between water, soil and grassland.

Social Perception

The various projects implemented in the community of Acopalca did not show their influence in improving the quality of life of the local people (Figure 3c), as two-thirds of the respondents disagreed or were unclear about the benefit generated by these projects because they did not feel that the activities carried out, or the results obtained were related to their multiple needs and objectives in different areas of their productive and economic lives (Galer et al., 2023). Certainly, the projects implemented had a greater emphasis on reforestation activities with infiltration ditches or the temporary closure of grazing areas and environmental trainings of mostly communal interest (PRAA Peru, 2013; Minam 2017) that did not relate to activities of family interest such as livestock, despite recognizing the importance of technical assistance for the improvement of pastures and wetlands. Apparently, the social programmes granted by the central government, such as the glass of milk program, pension 65 and others, have not contributed to guaranteeing family food security in contrast to the condition of the population settled in the cities, as is the case of the studies by Juárez (2020) who concluded that the glass of milk program has a significant impact on the quality of life of the beneficiary of the Hijos de

Ventanilla Human Settlement, Callao; but it does coincide with the conclusions referred to by Núñez et al. (2019), that the subsidy to the elderly in Peru has not improved their social welfare and living conditions, as the rural population diversifies their income according to their livelihoods with which they reduce the threat of food shortage (Bitana et al., 2023).

In general, the scenario presented in the multivariate analysis highlights that the impact of the trainings carried out by the project-executing entities differs between both associations, which suggests the need to adjust the training strategies to address the particularities of each group as recommended by Villamagua (2017). The low coincidence in the perceptions revealed by the respondents indicates the existence of a group of community members with more advanced knowledge and commitment who could become potential leaders to strengthen future intervention processes aimed at conserving the sustainability of natural resources and the Andean grassland ecosystem (Villamagua, 2017). The diversity in perceptions underlines the importance of differentiated and personalized strategies to maximize the impact of intervention initiatives.

CONCLUSION

The families associated with productive organizations in the community of Acopalca have shown very heterogeneous criteria regarding the soil-water-grassland relationship and the importance of self-management for the conservation of the Andean grassland ecosystem. There is still a low level of understanding of the benefits they obtain directly from it for their livestock activity and the viability of their family income. There is no evidence of a general conviction of their responsibility for the conservation or improvement of the ecosystem; on the contrary, they maintain the hope that the intervention of external entities is crucial for the conservation of the ecosystem. Therefore, each future investment project should focus on strengthening the capacity for self-management of ecosystem sustainability based on an understanding of the interdependence between water-soil-grasslands with livestock activity and family income through the design of more effective training programmes adapted to the needs of each group, inspired by dynamic and joint participation. The discrepancy about the effectiveness of past projects is based on the inadequate training strategies carried out by the promoters of the projects implemented and the little or no participation of professionals from the social area, so that it has not been possible to adequately integrate environmental knowledge, livestock production and family income, as well as the level of commitment to care for and conserve the ecosystem.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest either at the information or economic level.

REFERENCES

Baca-Tavira, N., & Herrera-Tapia, F. (2016). Proyectos sociales. Notas sobre su diseño y gestión en territorios rurales. *Convergencia Revista de Ciencias Sociales*, *72*, 69–87. https://www.scielo.org.mx/pdf/conver/v23n72/1405-1435-conver-23-72-00069.pdf

Borcard, D., Gillet, F. y Legendre, P. (2018). Numerical Ecology with R. ¡Use R! *Springer*, Second Edition. doi:10.1007/978-3-319-71404-2

Bitana E.B., Lachore S.T. & Utallo A.U. (2023). Rural farm households' food security and the role of livelihood diversification in enhancing food security in Damot Woyde District, Southern Ethiopia. Cogent Food & Agriculture, 9(1), ISSN 2331-1932, Informa UK Limited, https://doi.org/10.1080/23311932.2023.2238460

Cuadras C.M. (2007). Nuevos métodos de Análisis Multivariante. *CMC editions*, Barcelona, p:249

Chen, T., Bao, A., Jiapaer, G., Guo, H., Zheng, G., Jiang, L., Chang, C., & Tuerhanjiang, L. (2019). Disentangling the relative impacts of climate change and human activities on arid and semiarid grasslands in Central Asia during 1982–2015. *Science of the Total Environment*, *653*, 1311–1325. https://doi.org/10.1016/j.scitotenv.2018.11.058

Couvreur, S., Petit, T., Guen, R. L. E., Arfa, N. BEN, Jacquerie, V., Sigwalt, A., Haimoud-Lekhal, D. A., Chaib, K., Defois, J., & Martel, G. (2019). Technical and sociological analysis of grassland maintenance in lowland dairy cattle areas. *Inra Productions Animales*, *32*(3). https://doi.org/10.20870/productions-animales.2019.32.3.2940

Deng, W., Chen, M., Zhao, Y., Yan, L., Wang, Y., & Zhou, F. (2022). The role of groundwater depth in semiarid grassland restoration to increase the resilience to drought events: A lesson from Horqin Grassland, China. *Ecological Indicators*, *141*(March), 109122. https://doi.org/10.1016/j.ecolind.2022.109122

Dong, S., Shang, Z., Gao, J., & Boone, R. (2022). Enhancing the ecological services of the Qinghai-Tibetan Plateau's grasslands through sustainable restoration and management in era of global change. *Agriculture, Ecosystems and Environment*, *326*(September 2021), 107756. https://doi.org/10.1016/j.agee.2021.107756

Everard, Mark (2021). Conservation of ecosystems and their services. Ecosystem Services, 52-66, Routledge, https://doi.org/10.4324/9781003182313-3

INEI (2023). Población por distritos 2023. Instituto Nacional de Estadística e informática en línea: https://www.google.com/search?q=inei+poblaci%C3%B3n+por+distritos+2023&sca_esv=583650474&hl=es&sxsrf=AM9HkKlD2x80S8nx6M7mb-bxtegYL2wGtg%3A1700331562408&ei=KgBZZdXBGP3e1sQPh9y-sAQ&oq=INEI&gs_lp=Egxnd3Mtd2l6LXNlcnAiBElORUkqAggEMg4QLhjHARixAxjRAxiABDIKEAAYgAQYigUYQzIIEAAYgAQYsQMyChAAGIAEGIoFGEMyChAAGIAEGBQYhwIyChAAGIAEGIoFGEMyChAAGIAEGIoFGEMyChAAGIAEGIoFGEMyChAAGIAEGIoFGEMyCBAAGIAEGLEDSPeeBlAAWPIFcAJ4AZABAJgBkAGgAd8DqgEDMC40uAEByAEA-AEBwgIKECMYgAQYigUYJ8ICBBAjGCfCAgsQABiABBixAxiDAcICDhAAGIAEGIoFGLEDGIMBwgIFEAAYgATCAgUQLhiABMICEBAuGIAEGIoFGMcBGNEDGEPCAhMQLhiABBiKBRixAxjHARjRAxhDwgIWEC4YgAQYigUYsQMYgwEYxwEYoQMYQ8ICIhAuGIAEGIoFGLEDGMcBGNEDGEMYlwUY3AQY3gQY4ATYAQHCAhoQLhjHARixAxjRAxiABBiXBRjcBBjeBBjgBNgBAeIDBBgAIEGIBgG6BgYIARABGBQ&sclient=gws-wiz-serp

Galer A.P., Ejarque M., Mathey D., y Muscio L. (2023). *Arraigo Rural. Condiciones de vida, políticas y estrategias de las familias productoras en Argentina*. teseo press, INTA, Buenos Aires, Argentina, 340 p. DOI: 10.55778/ts877233681

Gobierno Regional de Junín. (2014). *Plan Maestro del Área de Conservación Regional Huaytapallana (versión ejecutiva)*. https://idoc.pub/documents/plan-maestro-huaytapallana-1-version...

Guo, X., Zhou, H., Dai, L., Li, J., Zhang, F., Li, Y., Lin, L., Li, Q., Qian, D., Fan, B., Lan, Y., Si, M., Li, B., Cao, G., Du, Y., & Wang, B. (2021). Restoration of Degraded Grassland Significantly Improves Water Storage in Alpine Grasslands in the Qinghai-Tibet Plateau. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/fpls.2021.778656

Juárez Amanda Nieves (2020). Programa Vaso de Leche en la calidad de vida del beneficiario del Asentamiento Humano Hijos de Ventanilla, Callao, 2019. Tesis de grado Maestría en Gestión Pública. Universidad Cesar Vallejo, 64 p.

Jimoh, S. O., Feng, X., Li, P., Hou, Y., & Hou, X. (2020). Risk-Overgrazing Relationship Model: An Empirical Analysis of Grassland Farms in Northern China. *Rangeland Ecology and Management*, *73*(4), 463–472. https://doi.org/10.1016/j.rama.2020.03.006

Martín-López, B., Iniesta-Arandia, I., García-Llorente, M., Palomo, I., Casado-Arzuaga, I., Del Amo, D. G., Gómez-Baggethun, E., Oteros-Rozas, E., Palacios-Agundez, I., Willaarts, B., González, J. A., Santos-Martín, F., Onaindia, M., López-Santiago, C., & Montes, C. (2012). Uncovering ecosystem service bundles through social preferences. *PLoS ONE*, *7*(6). https://doi.org/10.1371/journal.pone.0038970

Monge-Salazar, M. J., Tovar, C., Cuadros-Adriazola, J., Baiker, J. R., Montesinos-Tubée, D. B., Bonnesoeur, V., Antiporta, J., Román-Dañobeytia, F., Fuentealba, B., Ochoa-Tocachi, B. F., & Buytaert, W. (2022). Ecohydrology and ecosystem services of a natural and an artificial bofedal wetland in the central Andes. *Science of the Total Environment*, *838*(April), 155968. https://doi.org/10.1016/j.scitotenv.2022.155968

Moore, M. (2013). Dominando Excel: Tablas Dinamicas. (n.p.): CreateSpace Independent Publishing Platform. https://www.google.com.pe/books/edition/Dominando_Excel/8XjLnQEACAAJ?hl=es

Morales, N., Sequeira, N., Prendas, T., & Zúñiga, K. (2016). Escala de Likert una herramienta económica. *Admón. y Gestión En Recursos Humanos*, 6. https://www.academia.edu/30246173/ESCALA_DE_LIKERT_UNA_HERRAMIENTA_ECON%C3%93MICA_Contenido.

Moreno, M., Corraliza, A., & Ruiz, J. (2005). Escala de actitudes ambientales hacia problemas específicos. *Psicothema*, *17*(3), 5002–5508. https://www.psicothema.com/pdf/3136.pdf

Mosquera, G. M., Marín, F., Stern, M., Bonnesoeur, V., Ochoa-Tocachi, B. F., Román-Dañobeytia, F., & Crespo, P. (2022). Progress in understanding the hydrology of high-elevation Andean grasslands under changing land use. *Science of the Total Environment*, *804*. https://doi.org/10.1016/j.scitotenv.2021.150112

Núñez L.A, Ruiz j., Núñez J., Rengifo R., Vigo E., y Díaz J. (2019). Impacto de las políticas sociales en la calidad de vida del adulto mayor. Revista Gestión I+D, 5(1): 121-143. ISSN-e 2542-3142

PRAA Perú (2013). Estudio de praderas en Shullcas PRAA. SCRIBD. En linea https://es.scribd.com/document/291169580/Estudio-Praderas-en-Shullcas-PRAA.

Piipponen, J., Jalava, M., de Leeuw, J., Rizayeva, A., Godde, C., Cramer, G., Herrero, M., & Kummu, M. (2022). Global trends in grassland carrying capacity and relative stocking density of livestock. *Global Change Biology*, *28*(12), 3902–3919. https://doi.org/10.1111/gcb.16174

Qian, D., Du, Y., Li, Q., Guo, X., & Cao, G. (2021). Alpine grassland management based on ecosystem service relationships on the southern slopes of the Qilian Mountains, China. *Journal of Environmental Management*, *288*(23), 112447. https://doi.org/10.1016/j.jenvman.2021.112447

Rothwell W.J., & Whiteford A.P. (2020). Corporate Employee Training and Development Strategies. The Oxford Handbook of Lifelong Learning, Second Edition, Oxford University Press, https://doi.org/10.1093/oxfordhb/9780197506707.013.10

Richter, F., Jan, P., El Benni, N., Lüscher, A., Buchmann, N., & Klaus, V. H. (2021). A guide to assess and value ecosystem services of grasslands. *Ecosystem Services*, *52*(April), 101376. https://doi.org/10.1016/j.ecoser.2021.101376

Valverde, H., Fuentealba, B., Blas, L., & Oropeza Editado, T. (2022). *La importancia de los pastizales altoandinos peruanos*. https://repositorio.inaigem.gob.pe/server/api/core/bitstreams/8f8bf505-e241-4af0-91ae-4c9e9033ee15/content

Villamagua, G. (2017). Percepción social de los servicios ecosistémicos en la microcuenca El Padmi, Ecuador. *Revista Iberoamericana de Economía Ecológica*, *27*, 102–114.

https://www.researchgate.net/publication/328064046_Percepcion_social_de_los_servicios_ecosistemicos_en_la_microcuenca_El_Padmi_Ecuador

Wang, Y., Pei, W., Cao, G., Guo, X., Zhou, H., & Du, Y. (2021). Moderate Grazing Increases Water Use Efficiency for Environmental Health in Alpine Meadows of the Tibetan Plateau. *Frontiers in Ecology and Evolution*, *9*. https://doi.org/10.3389/fevo.2021.684321

Yaranga R, Pizarro S., Cano D., Chanamé F., Orellana J. (2023). Composition, Diversity, and Value of Ecological Importance in Andean Grassland Ecosystems according to the Altitudinal Gradient in the Huacracocha Micro-Watershed, Peru. Annual Research & Review in Biology, 38(5): 43-56. https://doi.org/10.9734/ARRB/2023/v38i530587

Zawilińska B., Dická J.N., Matei E., Švajda J., Łapczyński M., Majewski K.M., Balázs C.A., & Gessert A. (2023). Applying Q-methodology to investigate the perception of the social and economic role of the national park by local stakeholders. Cases of national parks in the Carpathians. Journal for Nature Conservation, 75, 126459, ISSN 1617-1381, Elsevier BV, https://doi.org/10.1016/j.jnc.2023.126459

Zhang, T., Chen, Z., Zhang, W., Jiao, C., Yang, M., Wang, Q., Han, L., Fu, Z., Sun, Z., Li, W., & Yu, G. (2021). Long-term trend and interannual variability of precipitation-use efficiency in Eurasian grasslands. *Ecological Indicators*, *130*, 108091. https://doi.org/10.1016/j.ecolind.2021.108091

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Validation of Commercial Formulation of Difenoconazole Using HPLC Equipped with Dad

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Abstract:
Effective, selective, precise, and accurate liquid chromatographic analytical methods for the determination of difenoconazole fungicide in the formulation have been optimized and validated to meet the accreditation requirements, the performance characteristics of the analytical method were validated which is also one of the basic requirements of ISO Standard 17025:2017, for the rapid determination of difenoconazole by HPLC-DAD. The used method involves the extraction of the substances by sonication of the sample with acetonitrile, followed by dilution in acetonitrile, and direct injection on a liquid chromatography system. For the analysis LC system from Agilent Technologies 1290 Infinity II was used. Good separation was achieved on a Luna C18 column (3µm, 100°A, 3x150mm) with a guard column (C18 4x2.0mm I.D), an isocratic mobile elution consisting of acetonitrile and water acidified 0.075% with formic acid (85:15, v/v), at a flow rate of 0.7 ml/minute and UV detection at 220 nm. The column temperature was 25^{o}C, injected volume was 1 µL. The analysis duration was 10 minutes (the retention time of difenoconazole and 4 – Hydroxybenzoic acid – methyl ester was 1.846 and 1.135 minutes, respectively. The linearity within the concentration range of 750-125 µg mL-1, with the internal standard at a concentration of 250 µg mL-1 with an average correlation coefficient (R2) of 0.9998. We have considered precision, repeatability, and selectivity in the validation.

Keywords: Difenoconazole, HPLC Equipped with DAD, Commercial Formulations, Validation

INTRODUCTION

Crop protection products are essential for reducing crop damage and ensuring a plentiful and high-quality food supply [1-6]. Fungicides from various groups, such as strobilurin, triazole, oximinoacetate strobilurin, and phenylurea, are widely used to control fungal diseases. These fungicides are broad-spectrum with systemic modes of action, meaning they can be absorbed through the leaves, stems, or roots to provide effective control. It's worth noting that only pencycuron is a non-systemic fungicide.

Difenoconazole (Chemical Abstracts Service, CAS name, 1-[[2-[2-chloro-4-(4-chlorophenoxy) phenyl]-4-methyl-1,3-dioxolan-2-yl] methyl]-1H-1,2,4-triazole, CAS No. 119446-68-3) is a systemic triazole fungicide registered for the control of a broad spectrum of foliar, seed and soil-borne diseases caused by Ascomycetes, Basidiomycetes and Deuteromycetes in cereals, soya, rice, grapes, pome fruit, stone fruit, potatoes, sugar beet and several vegetable and ornamental crops [7]. It acts by interfering with the synthesis of ergosterol in the target fungi by inhibiting the 14α-demethylation of sterols, which leads to morphological and functional changes in the fungal cell membrane [8,9]. Due to its broad-spectrum action with preventive and curative properties, and compatibility with other fungicides, difenoconazole is valued by various agricultural sectors for use as a seed treatment and as a foliar spray. The chemical structures of difenoconazole are shown in Figure 1.

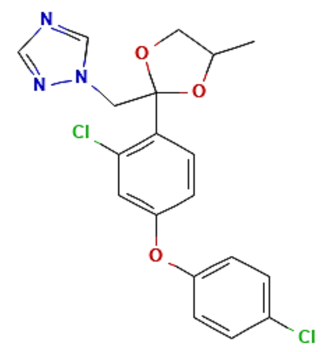

Figure 1: Difenoconazole Structure

In 1994, the EPA first registered the fungicide Difenoconazole. Its toxicological properties were later assessed by the JMPR in 2007. During the evaluation, an Acceptable Daily Intake (ADI) of 0-0.01 mg/kg body weight and an Acute Reference Dose (ARfD) of 0.3 mg/kg body weight were established. Detection, characterization, and quantitation of the active ingredient(s) in agrochemical formulation products are necessary to support product quality control and registration. Pesticide Quality Control Laboratories play an important role in ensuring that pesticide formulation products available in the market are safe to use by farmers and comply with the required quality standards for registration.

Pesticide testing laboratories mainly focus on testing different pesticides that are registered as technical and formulated for quality control. It's important to have comprehensive pesticide formulation analysis protocols that cover methods for determining the concentrations of active ingredients. The use of advanced analytical techniques such as chromatography for pesticide formulation analysis helps to ensure the quality of pesticides for effective pest management. Liquid chromatography (LC) techniques with diode array detector (DAD) are commonly used for determining the active ingredient in routine analysis of formulation samples.

Chromatographic techniques have been widely used for determining trace levels of Difenoconazole residues. A literature review revealed various analytical methods for detecting difenoconazole, either alone or in combination with other pesticides, using high-performance liquid chromatography with a diode array detector (HPLC-DAD) [10], liquid chromatography coupled to tandem mass spectrometry with atmospheric pressure electrospray ionization (LC–ESI-MS/MS) [11,12], and LC-MS/MS [13,14].

Validation data are generally required for analytical methods used for pre-registration and post-registration control and monitoring purposes. The application of validated methods is also one of the basic requirements of ISO Standard 17025 [15].

The present study aims to validate a new, rapid, sensitive, affordable, user-friendly, and simple reverse-phase high-performance liquid chromatographic (HPLC-DAD) analytical method for quantifying difenoconazole in pesticide formulations. The validation includes assessing statistical parameters such as precision, accuracy-recovery, and linearity. After validation, the method was found to meet the referenced guidelines.

MATERIALS AND METHOD

The method used complies with the requirements for analytical methods of chemicals that support all submissions under Regulation (EC) No 1107/2009 [16]. This includes chemical plant protection products for pre-registration, post-registration control, and monitoring purposes, as outlined in Regulations (EU) No. 283/2013 and 284/2013 [17,18]. These regulations address the development of analytical methods required for pre-registration and post-registration control and monitoring purposes for active substances and plant protection products under regulation (EC) N 1107/2009. Additionally, this method has been adopted by UKAS, "The United Kingdom Accreditation Service." The validated method has determined certain method performance requirements, such as the linearity of calibration, system suitability, and analyte stability

Chemicals and Reagents

Certified reference materials (CRM) including difenoconazole with a purity of 98.58% and 4-Hydroxybenzoic acid – methyl ester internal standard with a purity of 99.77% were purchased from Dr Ehrenstofer GmbH (Germany). HPLC-grade acetonitrile was provided by Fisher Scientific. Formic acid of analytical reagent grade was obtained from Honeywell, Germany, and ultra-pure deionized water was sourced from Thermo Fisher Scientific, Hungary. The label claim of commercially formulated difenoconazole is 21.9% (w/w).

Preparation of Reference Solutions

To prepare reference solutions, we first created a stock solution of difenoconazole analytical standard at a concentration of 1000 µg mL-1. We then sequentially diluted the stock solution with acetonitrile to produce concentrations of 750 µg mL-1, 500 µg mL-1, 250 µg mL-1, and 125 µg mL-1 of difenoconazole. At each concentration level, the internal standard was maintained at a concentration of 250 µg mL-1 for the linearity study. These reference solutions were filtered using 0.22 µm polytetrafluoroethylene (PTFE) filters.

Preparation of Internal Standard

Weighed accurately 0.1 g of 4 – Hydroxybenzoic acid – methyl ester (IS) into 100 ml volumetric flask and diluted up to the volume with acetonitrile and it corresponds to 1000 µg mL^{-1}.

Preparation of Internal Standard

To prepare the working standard solution (500µg mL^-1), start by transferring 5.0 ml of Difenoconazole reference solution (1000 µg mL^-1) into a 10 mL volumetric flask. Next, add 2.5 ml of 4-Hydroxybenzoic acid-methyl ester (ISTD) solution (1000 µg mL^-1), then fill the flask to volume with acetonitrile. Mix well and filter the working standard solution using a 0.22 µm polytetrafluoroethylene (PTFE) filter.2-5 Preparation of Sample Solution.

Preparation of Sample Solution

Difenoconazole is available in the form of SC commercial formulations, some of which are mixtures with other fungicides. We used the formulation with a label claim of 21.9% (w/w). We weighed an appropriate amount (114.1mg) to achieve a concentration of approximately 500 µg mL-1 of the active substance in the solution. We added 12.5 ml of 4-Hydroxybenzoic acid-methyl ester internal standard solution with a concentration of 1000 µg/ml, partially filled the volumetric flask with acetonitrile, sonicated for 10 minutes, allowed it to cool to room temperature, and then diluted it with acetonitrile to the final volume (50 mL). We filtered the sample solution with polytetrafluoroethylene (PTFE) filters (0.22 µm). We prepared six separate formulations for method repeatability tests and conducted the analysis of each sample in duplicate."

Instrumentation and Conditions

The separation, identification, and quantification were performed using the Agilent 1260 Infinity II liquid chromatography system (Agilent Technologies, USA) with a DAD detector. The analyte was separated using a Luna C18 column (3µm, 100°A, 3x150 mm) with a guard column (C18 4x2.0mm I.D). An isocratic mobile phase eluent consisting of acetonitrile and water acidified to 0.075% with formic acid (85:15, v/v) was used at a flow rate of 0.7 mL/minute, with UV detection at 220 nm. The column temperature was maintained at 25⁰C, and the injected volume was 1 µL. The analysis duration was 10 minutes, with the retention time of difenoconazole and 4-Hydroxybenzoic acid methyl ester being 1.846 and 1.135 minutes, respectively. Instrument control and data processing were carried out using Agilent OpenLAB software CDS 2.3.

RESULTS AND DISCUSSION

Method validation was performed following ISO Standard 17025:2017 guidelines, with a focus on specificity, linearity, precision (repeatability), accuracy, and reproducibility. Additional details can be found in the subsequent sections.

Method Validation

The HPLC chromatograms of Difenoconazole in the standard and sample solutions (Figures 2 and 3) showed a retention time of approximately 1.846 minutes, while the retention time of the internal standard (Figure 4) was around 1.135 minutes.

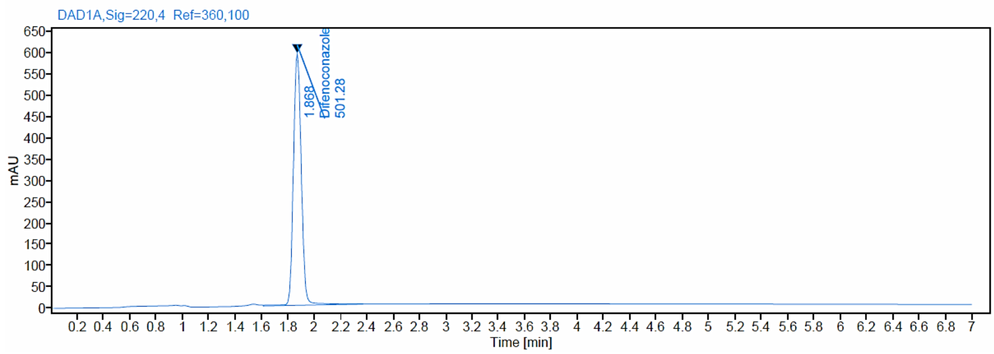

Figure 2: HPLC chromatogram of the Difenoconazole standard solution

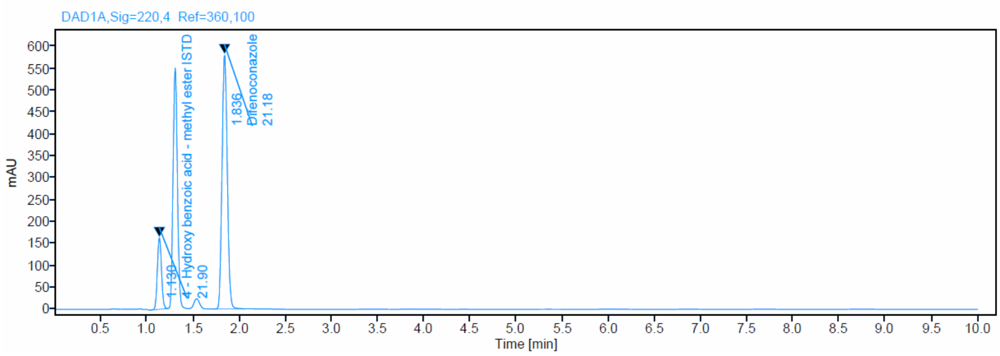

Figure 3: HPLC Chromatogram of the Difenoconazole sample solution

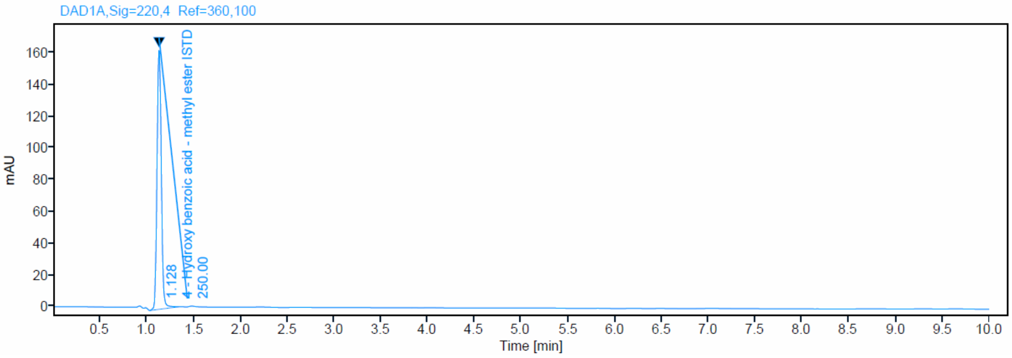

Figure 4: HPLC Chromatogram of internal standard

Specificity:

To assess the specificity of the analysis of pesticide formulations, we conducted a test since a placebo formulation was not available. We evaluated the specificity by comparing the presence of peaks at the same retention times of difenoconazole using a blank extraction. The blank extraction simulated the extraction of a sample that did not contain the formulation. The comparison showed that no interferences occurred at the same retention times of 1.846 min for difenoconazole and 1.135 min for IS (Figure 5). Therefore, we concluded that the validated method was specific to the studied compound. zole and 1.135 min for IS (Figure 5). As such, we found that the validated method was specific to the studied compound.

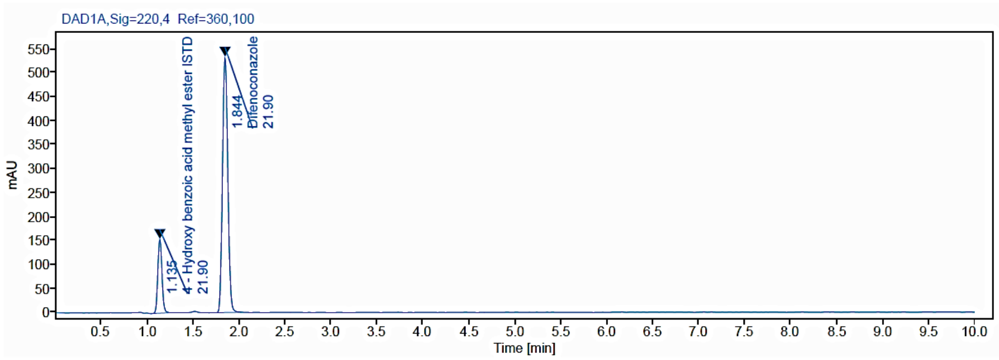

Figure 5: shows a typical result of the separation of detected compounds

Linearity:

Linearity refers to the ability of a detection system to produce a consistent linear relationship between test results and the concentration of analyte within a specific range. To assess this, a set of reference solutions containing difenoconazole was prepared at concentrations of 750, 500, 250, and 125 µg mL-1, with an internal standard at a concentration of 250 µg mL-1. The data was collected from duplicate injections at each calibration level. Linear regression was performed using the least squares method, which showed a strong linear relationship of the analyte response with an average correlation coefficient (R2) of 0.9998, as shown in Figure 6. This R2 value confirms that the extraction/solubility is validated by the HPLC method for difenoconazole pure active ingredient contents. The parameters of linearity obtained are summarized in Table 1.

Table 1: Linearity parameters obtained for HPLC-DAD analysis of Difenoconazole formulations

Substance	Calibration levels	Slope	intercept	Correlation Coefficient	Concentration range µg mL^{-1}
Difenoconazole	4	0.01	0.20	0.9991	125-750

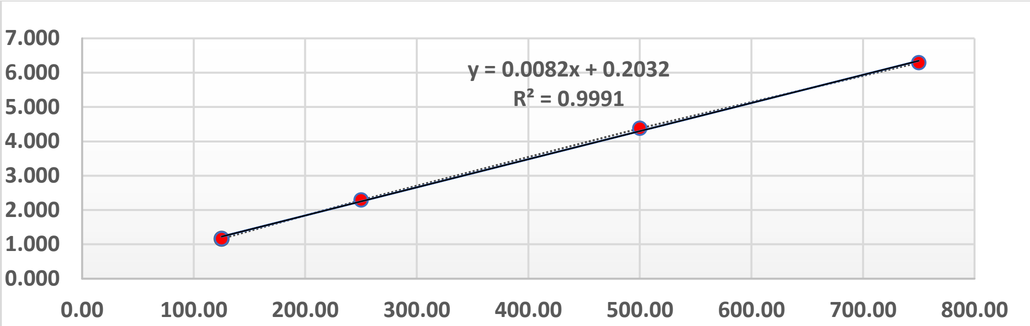

Figure 6: Calibration curve of Difenoconazole by the proposed method

Trueness and Precision (Repeatability):

The precision of the method was assessed by analyzing six sample solutions prepared from the same pesticide formulation within one day. The criteria for precision were based on the modified Horwitz equation [19]. The experimental Relative Standard Deviation (RSDr) value obtained was compared with the RSDr value calculated from the modified Horwitz equation.

$$RSD_R\% = 2^{*}C^{(-0.1505)}$$

The concentration of an analyte in a sample is represented as a decimal mass fraction (C) and the inter-laboratory relative standard deviation is denoted as RSDR. The data indicates that at a concentration of 500 µg mL-1(21.9%), the relative standard deviation was 0.573% and the Horwits RSDr was 2.525%. The experimental RSDR value was lower than the calculated RSDR value, demonstrating acceptable precision for difenoconazole determinations in commercial formulation products as shown in Table 2. These results validate the suitability of the proposed method for the intended application.

Table 2: Precision for difenoconazole in tested commercial formulation product

Compound	Declared concentration of difenoconazole [%]	% $RSD_{r\ theoretical}$	% $RSD_{r\ experimental}$
Difenoconazole	21.9	2.525	0.573

Accuracy:

The accuracy of a measurement refers to how closely the determined concentration of a substance in a sample matches its true value. In this study, since we didn't have analyte-free samples, we calculated recovery rates by comparing the mean quantity of active substances found in the sample to the label content. The accuracy was evaluated by calculating the mean recoveries from 12 replicates at one spiking level, which depended on the amount of the active substance present in the formulation. We found that all the recovery values fell within the respective acceptance ranges as shown in Table 3.

Table 3: Accuracy expressed as the percent recovery of a spike

Label Content (%)	Amount Measured (%)	Intraday Accuracy [%] n=6	Inter day Accuracy [%] n=12
21.9	**21.403**	**98.153**	**98.569**

We analyzed real samples of commercial pesticide formulations containing 21.9% (w/w) of difenoconazole to determine the concentration of the active substance. The results showed that the active substance was within the acceptable range for all tested samples.

Reproducibility:

Twelve replicate commercial samples and calibration standards were prepared on different working days as per the method and obtained 1.843% RSD (Target 2.522%, RSDr = $2\ X\ C^{(-0.1505)}$) for % of active ingredient Difenoconazole contained in the formulation. Table 4 and Figure 7 show that the method is reproducible and selective when applied to different formulations during different intervals of time.

Table 4: Reproducibility of Difenoconazole during different intervals of time

Sl no.	ID	ISTD Area mAU	Difenoconazole Area mAU	Response ratio	Detected concentration (w/w) %	Difference (Absolute Range)	Average of Difenoconazole (w/w) % calculated
1	Std 1inj	482.287	2135.643	4.215			**21.155**
	Std 2 inj	481.947	2127.873	4.201			
	Sample 1inj	475.073	2030.676	4.201	21.171	**0.032**	
	Sample 2 inj	477.635	2038.590	4.201	21.139		
	Std 3 inj	490.917	2145.122	4.370			
2	Std 1inj	482.287	2135.643	4.215			**21.332**
	Std 2 inj	481.947	2127.873	4.201			
	Sample 1inj	493.650	2122.734	4.300	21.298	**0.068**	
	Sample 2 inj	493.640	2129.470	4.314	21.366		
	Std 3 inj	490.917	2145.122	4.370			
3	Std 1inj	482.287	2135.643	4.428			**21.359**
	Std 2 inj	481.947	2127.873	4.415			
	Sample 1inj	497.183	2147.794	4.320	21.396	**0.075**	
	Sample 2 inj	500.208	2153.295	4.305	21.321		
	Std 3 inj	490.917	2145.122	4.370			
4	Std 1inj	482.287	2135.643	4.428			**21.264**
	Std 2 inj	481.947	2127.873	4.415			
	Sample 1inj	489.850	2106.264	4.300	21.296	**0.064**	
	Sample 2 inj	491.140	2105.378	4.287	21.232		
	Std 3 inj	490.917	2145.122	4.370			
5	Std 1inj	482.287	2135.643	4.428			**21.031**
	Std 2 inj	481.947	2127.873	4.415			
	Sample 1inj	507.078	2150.641	4.241	21.006	**0.05**	
	Sample 2 inj	505.121	2147.369	4.251	21.056		
	Std 3 inj	490.917	2145.122	4.370			
6	Std 1inj	482.287	2135.643	4.428			**21.268**
	Std 2 inj	481.947	2127.873	4.415			
	Sample 1inj	490.686	2107.169	4.294	21.269	**0.002**	
	Sample 2 inj	488.533	2097.644	4.294	21.267		
	Std 3 inj	490.917	2145.122	4.370			

7	Std 1inj	511.867	2081.522	4.067			**21.939**
	Std 2 inj	511.771	2079.509	4.063			
	Sample 1inj	512.458	2089.023	4.076	21.962	**0.047**	
	Sample 2 inj	512.308	2083.911	4.068	21.915		
	Std 3 inj	513.174	2077.633	4.049			
8	Std 1inj	501.477	2331.333	4.649			**21.050**
	Std 2 inj	496.391	2303.234	4.640			
	Sample 1inj	495.840	2213.613	4.464	21.051	**0.002**	
	Sample 2 inj	499.363	2229.145	4.464	21.049		
	Std 3 inj	513.174	2077.633	4.049			
9	Std 1inj	490.976	2201.498	4.484			**22.399**
	Std 2 inj	496.905	2217.013	4.462			
	Sample 1inj	476.193	2180.349	4.579	22.419	**0.040**	
	Sample 2 inj	474.817	2170.219	4.571	22.379		
	Std 3 inj	497.448	2243.079	4.509			
10	Std 1inj	504.109	2334.849	4.632			**21.401**
	Std 2 inj	504.736	2319.557	4.596			
	Sample 1inj	505.941	2283.233	4.513	21.422	**0.043**	
	Sample 2 inj	512.453	2307.976	4.504	21.379		
	Std 3 inj	509.192	2346.427	4.608			
11	Std 1inj	506.171	2328.467	4.600			**21.475**
	Std 2 inj	504.065	2310.007	4.583			
	Sample 1inj	510.667	2298.698	4.501	21.470	**0.010**	
	Sample 2 inj	516.915	2327.887	4.503	21.480		
	Std 3 inj	511.589	2345.458	4.585			
12	Std 1inj	515.969	2403.877	4.659			**21.160**
	Std 2 inj	516.689	2408.076	4.661			
	Sample 1inj	510.713	2301.607	4.507	21.180	**0.040**	

	Sample 2 inj	492.832	2216.747	4.498	21.140		
	Std 3 inj	511.589	2345.458	4.585			
Mean			21.425				
STD			0.406				
RSD			1.895				
Uncertainty			3.789				

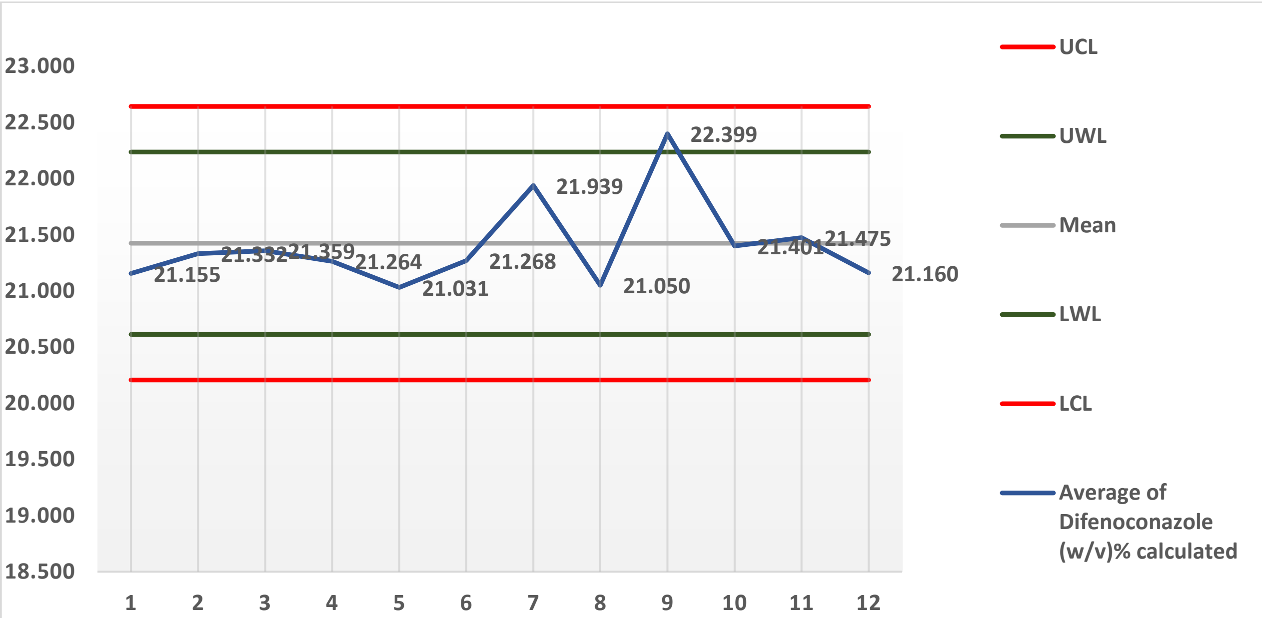

Figure 7: Difenoconazole Reproducibility

Selectivity/Specificity:

The method is specific and selective for monitoring Difenoconazole active ingredient contents. This was achieved by using a blank sample and analyte standard solution separately. No peak was observed or detected near the peak of the desired analytes, demonstrating that the method is highly specific and selective (refer to Figures 8 and 9).

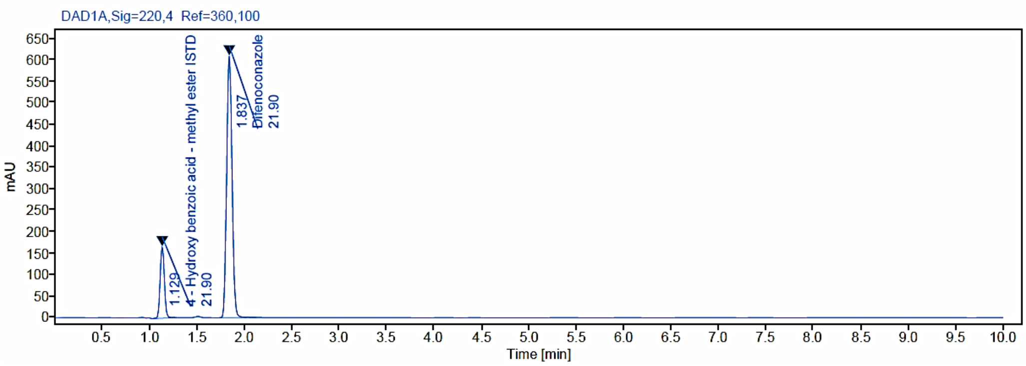

Figure 8: Representative chromatogram demonstrating the method's specificity and selectivity (absence of interfering peaks between internal standard and standard solution.

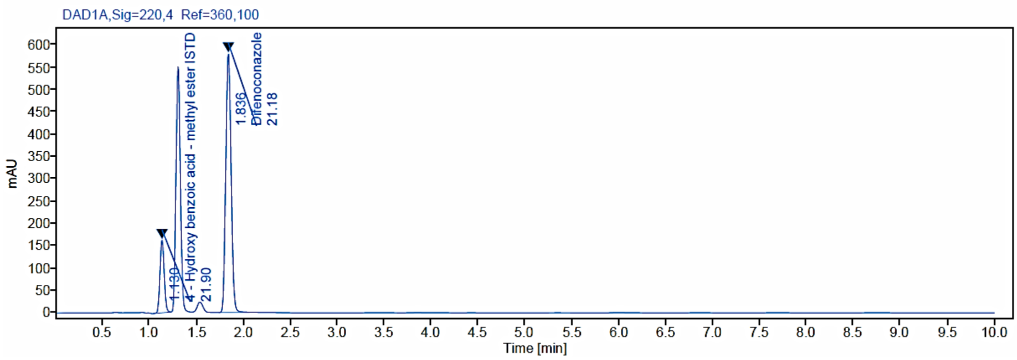

Figure 9: Representative chromatogram showing the specificity and selectivity of the method (no interfering peaks between internal standard and real sample.

System Suitability Parameters

The system suitability test is an important tool in liquid chromatography, used to improve the resolution of a chromatographic system. During the method validation, critical performance parameters are documented and reported along with the validation data. If the results of the performance parameters do not meet the required limits, the operating conditions must be adjusted before analysis. In this study, key performance parameters such as Asymmetry Factor, Resolution, Theoretical Plate Numbers, and Retention Time were all found to be within the specified limits, as shown in Table 6.

Table 6: System suitability parameters

Parameters	SYSTEM SUITABILITY SPECIFICATION			
	Mean		**Limit**	**Status**
	Difenoconazole	4- Hydroxybenzoic acid – methyl ester (IS)		
Asymmetry factor (EP)	1.069	1.092	0.8-1.2	**Pass**
Resolution (EP)	2.721	NA	>2	**Pass**
Theoretical plates (EP)	4545.561	2821.946	>2000	**Pass**
Repeatability retention time	1.846	1.135	1 to 2%	**Pass**

Before implementing the method, we conducted a system suitability test using a standard solution containing Difenoconazole and (IS). This involved injecting the standard solution six times to assess the consistency of retention time and area. We calculated the mean value, standard deviation, and %RSD. The acceptance criteria for peak areas and retention times required a %RSD of less than 1%, and we found that all compounds met this criterion with %RSD values of less than 1%, which we deemed acceptable.

Summary of the Validation Parameters of the Developed Method for Difenoconazole

Table 7 provides a summary of the parameters for validating Difenoconazole in commercial formulations. In analytical research, it is crucial to develop methods for determining analytes. Developing easy, efficient, low-cost, repeatable, and reproducible analytical methods for drugs and pesticides using HPLC is always in demand in industrial research [20,22]. This study proposes a simple analytical method for determining and quantifying Difenoconazole content in commercial formulations using HPLC. The analytical standard solution of Difenoconazole content was analyzed. Optimization of parameters for the solvents used in the mobile phase and sample & standard solutions has resulted in excellent recovery results for the analytes. The chromatograms showed a similar retention time for both the sample and standard, indicating the effectiveness of the analytical method. After initial optimization of parameters, the method was successfully validated based on considerations such as specificity, linearity, precision (repeatability), accuracy, reproducibility, selectivity (specificity), and system suitability. In analytical method validation, linearity is the first parameter considered.

In this study, the precision range was within acceptable limits and better for this analyte compared to previously reported methods. The validated method exhibited excellent accuracy. The percentage recovery of Difenoconazole was calculated by comparing the area under the peak of the standard solution with the sample solution concentration. It has been demonstrated that the developed HPLC method for Difenoconazole is accurate and reproducible for various sample types with excellent recoveries under optimized conditions. Based on the results obtained for different parameters, the developed method for Difenoconazole analysis is found to be fast, efficient, cost-effective, repeatable, and reproducible with excellent recoveries. It is equally valid for the analysis of Difenoconazole in commercial formulation.

Table 7: Summary of the validation parameters of the developed method for the determination of Difenoconazole

Validation Parameters	results		Acceptance Criteria
Linearity	Correlation Coefficient (average) 0.9998		Correlation Coefficient NLT* 0.97
Precision	0.573% RSD		% RSD NMT** 2.0
	Concentration (μg mL^{-1})	% Recovered	
Accuracy	500	98.569	% Recovery within 80% - 120%
Reproducibility	1.843 % RSD		2.522%

* Not Less than in accordance with the ICH Analytical Procedures Developments Guidelines (Guideline, 2022) [17]

** Not More than in accordance with the ICH Analytical Procedures Developments Guidelines (Guideline, 2022) [17]

CONCLUSIONS

A new HPLC-DAD method has been developed and validated for the quality control of commercial formulations containing difenoconazole as the active ingredient. This method involves preparing analyte samples and analyzing them using HPLC in isocratic elution mode.

The method's repeatability, expressed as the percent relative standard deviation (%RSD), was 0.573%. The Horwitz RSDr was 2.525 at a concentration of 21.243%. The obtained HoRat value was 0.226%, which was lower than 1.3%. The RSD for the reproducibility study was 1.843%, and the Horwitz RSDr was 2.522 at a concentration of 21.403%. The obtained HoRat value was 0.731%, which was lower than 2%.

The method's recovery rate was 98.568%. The method demonstrated good performance for specificity, linearity, precision, and repeatability for difenoconazole validation in commercial formulations using HPLC-DAD. Difenoconazole was detected and quantified with a high recovery percentage, excellent linearity, and low standard deviation values (RSD%). The developed method for determining difenoconazole has proven to be more accurate, precise, specific, and reproducible, and can be efficiently used in analyzing difenoconazole contents in commercial formulations. This approach can be easily used in control laboratories.

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REFERENCES

[1]. Ulrich, E. M., Morrison, C. N., Goldsmith, M. R. and Foreman, W. T. (2012). Chiral Pesticides: Identification, Description and Environmental Implications. Reviews of Environmental Contamination and Toxicology. Springer US. 217: 1–74.

[2]. Nasra [M]. Abdelmagid, Ideisan I. Abu-Abdoun, Kaltham A.H. Kayaf, and Abdulla S.A. Janaan. "Monitoring of Pesticide contamination of selected Legume Crops Imported in UAE". European Journal of Nutrition and Food Safety, Vol. 14 (12), 24-38, 2022.

[3]. Nasra M. Abdelmagid, Ideisan I. Abu-Abdoun, Kaltham A.H. Kayaf, Malik M. Alamin, Aisha O. Al Jabri, and Amna K. Alshamsi, "Monitoring of Pesticide Multi Residues in Selected Cereals Crop Grains in the United Arab Emirates". Journal of Agriculture and Ecology Research International, Vol. 23 (6), 199-212, 2022.

[4]. Nasra M. Abd El-Mageed, Ideisan I. Abu-Abdoun, and Kaltham A. H. Kayaf, (2022) "Detection and Optimization of Multiple Pesticides Residues in Honey Using Liquid Chromatography-Tandem Mass Spectrometry", European Journal of Nutrition & Food Safety, 2347-5641. ISSN: 2347-5641.

[5]. D. Tsochatzis, Roxani Tzimou-Tsitouridou, Urania Menkissoglu-Spiroudi, Dimitrios G. Karpouzas & Maria, Papageorgiou. "Development and validation of an HPLC-DAD method for the simultaneous determination of most common rice pesticides in paddy water systems". Journal of Environmental Analytical Chemistry, 2011, 92:5, 548-560.

[6]. Munkvold, G. P., Martinson, C. A., Shriver, J. M. and Dixon, P.M. (2001). Probabilities for profitable fungicide use against gray leaf spot in hybrid maize. Phytopathology, 91 (5), 477–484.

[7]. Jie Liu, Xinxin Xu, Aihong Wu, Shanshan Song, Liguang Xu, Chuanlai Xu, Liqiang Liu, Hua Kuang (2022). Rapid and sensitive determination of difenoconazole in cucumber and pear samples using an immunochromatographic assay, Food Bioscience, volume 47.

[8]. Wang. Z. H., Yang, T., Qin, D. M., Gong, Y. and Ji, Y. (2008). Determination and dynamics of difenoconazole residues in Chinese cabbage and soil. *Chinese Chemical Letters*, vol. 19(8): 969–972.

[9]. EFSA (European Food Safety Authority), 2014. Scientific support for preparing an EU position in the 46th Session of the Codex Committee on Pesticide Residues (CCPR). EFSA Journal 2014;12(7):3737, 182 pp. doi: 10.2903/j.efsa.2014.3737.

[10]. Lazić, S. and Šunjka, D. (2015). Determination of azoxystrobin and difenoconazole in pesticide products. Commun Agric Appl Biol Sci; 80(3):375-80.

[11]. Carpinteiro, I., Ramil, M., Rodríguez, I. and Cela, R. (2010). Determination of fungicides in wine by mixed-mode solid phase extraction and liquid chromatography coupled to tandem mass spectrometry, Journal of Chromatography A, Volume 1217, Issue 48, Pages 7484-7492.

[12]. Sherol Acosta Rodrigues, Sergiane Souza Caldas and Ednei Gilberto Primel (2010). A simple; efficient and environmentally friendly method for the extraction of pesticides from onion by matrix solid-phase dispersion with liquid chromatography-tandem mass spectrometric detection. Analytica Chimica Acta, Volume 678, Issue 1, Pages 82-89.

[13]. Hingmire S, Oulkar DP, Utture SC, Shabeer TA, Banerjee K (2015) Residue analysis of fipronil and difenoconazole in okra by liquid chromatography-tandem mass spectrometry and their food safety evaluation. Food Chemistry 176: 145-151.

[14]. Meenakshi SN, Jeyaramraja PR, Manian R (2007) Pest Technol 2: 133.

[15]. INTERNATIONAL ORGANIZATION FOR STANDARDIZATION, ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories, Geneva (2005).

[16]. Technical Active Substance and Plant Protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013.

[17]. Commission Regulation (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, by Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market Text with EEA relevance.

[18]. Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, by Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market Text with EEA relevance.

[19]. WILLIAM HORWITZ1 and RICHARD ALBERT (2006). The Horwitz Ratio (HorRat): A Useful Index of Method Performance concerning Precision JOURNAL OF AOAC INTERNATIONAL VOL. 89, NO. 4, 2006.

[20]. Hajare, A. A., Hajare, A., Powar, T., Bhatia, N. and Harinath, N. M. (2016). Development and Validation of RP-HPLC Method for Determination of Doxorubicin Hydrochloride from Vacuum Foam Dried Formulation Research Journal of Pharmacy and Technology 9(9):1352-1356.

[21]. ICH Q2(R2) Validation of analytical procedures - Scientific guideline 2024. Reference Number: EMA/CHMP/ICH/82072/2006.

[22]. Georgios Kamaris, Antonia Dalavitsou and Catherine K. Markopoulou "Development and Validation of an HPLC-DAD Method for the Determination of Seven Antioxidants in a Nano-Emulsion: Formulation and Stability Study" Separations 2024, 11(2), 43-55.