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Sustainable Plant-Based Protein Supply Chain for an Ecological Transition Pathway

Action IV.5 PhDs on Green topics

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Summary

Grain legumes a key resource for sustainable agriculture. These crops have significant agronomic and ecological value due to their ability to fix atmospheric nitrogen via symbiotic bacteria in the roots. This natural process significantly reduces the need for chemical nitrogen fertilisers, helping to improve soil fertility, lower production costs and reduce environmental impact. Leguminous plants are also crucial for crop rotation systems by increasing biodiversity. Nutritionally, they offer a high-quality source of protein, representing a key solution to promoting more sustainable diets and reducing dependence on animal protein.

Furthermore, leguminous crops that are well adapted to low environmental impact farming systems combined with the benefits of sod seeding allow for reduced CO_2 emissions, increased organic matter, and reduced soil erosion. The sod seeding technique not only improves crop productivity and quality but also reduces production costs, offering an ecologically and economically sustainable alternative to traditional practices.

A further aspect of recent interest is the use of biostimulants. These products, of natural or synthetic origin, stimulate the ability of plants to overcome abiotic and biotic stresses. Biostimulants have a smaller environmental footprint than chemical fertilisers. Due to their efficacy at low doses and their ability to improve the nutritional efficiency of plants, biostimulants reduce the risk of polluting groundwater and agricultural areas. In leguminous crops in particular, biostimulants have been shown to increase yield and crop quality and improve resistance to climatic stresses. The combined use of dryland sowing and biostimulants can create a synergetic agricultural system that can maximize the benefits of leguminous crops in terms of productivity and sustainability.

To ensure that the farming practices adopted are truly sustainable, it is essential to measure the environmental impact throughout the life cycle of the farming system. The Life Cycle Assessment (LCA) methodology is a key tool for this analysis, allowing the environmental impacts associated with each stage of the production process to be assessed: from sowing to harvesting, through to processing. LCA can also highlight critical issues, such as the energy cost of producing certain biostimulants or the risks associated with different management practices. However, these aspects offer opportunities to further improve agricultural systems, making them more efficient and sustainable.

The aim of the thesis was to contribute to advancing the knowledge on the benefits of grain legumes for the recovery of agrobiodiversity and the promotion of vegetable proteins, with a multidisciplinary approach aimed at improving sustainability, productivity and quality. The research objectives of action concern the adaptability to Apulian soil and climatic conditions, the identification of cultivation systems suitable for the sustainable production of vegetable proteins and the associated environmental impact assessment.

Among the legumes studied in the doctoral programme, chickpea was the first crop to receive attention, given the growing interest of the agro-industrial sector and the consequent increase in area under cultivation. Therefore, a detailed bibliometric analysis was carried out in order to assess the main issues related to chickpea cultivation and to identify the most relevant scientific and agronomic topics related to this crop over the last 45 years (Chapter I).

In parallel, a field study on lentil cultivation was carried out, testing two biostimulants in combination with two different soil management systems, highlighting their interactions and effects on yield and crop sustainability (Chapter II).

Finally, the analysis of the environmental impact of the cropping systems studied revealed a gap in the scientific literature regarding specific models for the production and environmental impact of biostimulants, highlighting the need to develop appropriate tools to assess their effects in a more systematic and comprehensive manner (Chapter III).

Sintesi

Le leguminose da granella rappresentano una risorsa fondamentale per l'agricoltura sostenibile. Queste colture hanno un valore agronomico ed ecologico rilevante, grazie alla loro capacità di fissare l'azoto atmosferico tramite i batteri simbionti nelle radici. Questo processo naturale riduce significativamente la necessità di fertilizzanti chimici azotati, contribuendo a migliorare la fertilità del suolo, ridurre i costi produttivi e ridurre l'impatto ambientale. Le leguminose sono inoltre cruciali per l'adozione di pratiche di avvicendamento colturale aumentando la biodiversità. Dal punto di vista nutrizionale, offrono una fonte proteica di alta qualità, rappresentando una soluzione chiave per promuovere diete più sostenibili e ridurre la dipendenza da proteine animali.

Inoltre, le leguminose che si adattano bene a sistemi di coltivazione a basso impatto ambientale unite ai benefici della semina su sodo permettono la riduzione dell'emissioni di CO_2, aumento della sostanza organica e riduzione dell'erosione del suolo. La tecnica della semina su sodo non solo migliora la produttività e la qualità del raccolto, ma riduce i costi di produzione, offrendo un'alternativa ecologicamente ed economicamente sostenibile rispetto alle pratiche tradizionali.

Un ulteriore aspetto di recente interesse è legato all'uso dei biostimolanti. Questi prodotti, di origine naturale o sintetica, stimolano la capacità delle piante di superare stress abiotici e biotici. I biostimolanti rappresentano un'impronta ambientale ridotta rispetto ai fertilizzanti chimici. Grazie alla loro efficacia a basse dosi e alla capacità di migliorare l'efficienza nutrizionale delle piante, i biostimolanti riducono il rischio di inquinamento delle falde acquifere e delle superfici agricole. In particolare, nelle leguminose, i biostimolanti hanno dimostrato di migliorare la resa e la qualità del raccolto, così come la resistenza agli stress climatici. L'uso combinato della semina su sodo e biostimolanti può creare un sistema agricolo sinergico, capace di massimizzare i benefici delle leguminose in termini di produttività e sostenibilità.

Per garantire che le pratiche agricole adottate siano realmente sostenibili, è essenziale misurare l'impatto ambientale lungo tutto il ciclo di vita del sistema agricolo. La metodologia LCA (Life Cycle Assessment) rappresenta uno strumento chiave per questa analisi, consentendo di valutare gli impatti ambientali associati a ogni fase del processo produttivo: dalla semina alla raccolta, fino alla lavorazione dei prodotti. L'LCA può anche evidenziare criticità, come il costo energetico della produzione di alcuni biostimolanti o i rischi legati a pratiche di gestione inadeguate. Tuttavia, questi aspetti offrono opportunità per migliorare ulteriormente i sistemi agricoli, rendendoli più efficienti e sostenibili.

L'obiettivo della tesi è stato quello di migliorare le leguminose da granella per il recupero dell'agrobiodiversità e la promozione delle proteine vegetali, con un approccio multidisciplinare volto a migliorare la sostenibilità, la produttività e la qualità. Le principali linee di intervento riguardano l'adattabilità alle condizioni pedoclimatiche pugliesi, l'individuazione di sistemi colturali idonei alla produzione sostenibile di proteine vegetali e la relativa valutazione dell'impatto ambientale.

Tra le leguminose studiate nel corso del dottorato, il cece è stato il primo a ricevere attenzione, vista la crescente attenzione del settore agroindustriale e il conseguente aumento delle superfici coltivate. Pertanto, è stata condotta un'analisi bibliometrica dettagliata per valutare le principali tematiche relative alla coltivazione del cece e per identificare gli argomenti scientifici e agronomici più rilevanti relativi a questa coltura negli ultimi 45 anni (Capitolo I).

Parallelamente, è stato condotto uno studio in campo sulla coltivazione della lenticchia, testando due biostimolanti in combinazione con due diversi sistemi di gestione del suolo, al fine di evidenziarne le interazioni e gli effetti sulla resa e sulla sostenibilità della coltura (Capitolo II)

Infine, l'analisi dell'impatto ambientale dei sistemi colturali esaminati ha rivelato una lacuna nella letteratura scientifica riguardante modelli specifici per la produzione e l'impatto ambientale dei biostimolanti, sottolineando la necessità di sviluppare strumenti adeguati a valutarne gli effetti in maniera più sistematica e completa (Capitolo III).

Introduction

1.1 The Origin of Legumes

Legumes (*Fabaceae* or *Leguminosae*) are one of the oldest and most important families of plants cultivated by man, characterized by their ability to fix atmospheric nitrogen through symbiosis with rhizobial bacteria present in their roots. These plants played a crucial role in the development of agriculture due to their high protein content and ability to enrich agricultural soils, improving their fertility. Among the first species domesticated by humans are the lentils, the chickpea, and the pea dating back some 10,000 years(Gepts & Papa, 2003; Smýkal et al., 2015; Zohary, Hopf, et al., 2012). Domestication began during the Neolithic period, at the same time with the domestication of cereals. Humans selected wild plants with useful characteristics for food and agriculture, such as ease of harvesting (no or reduced dehiscence), larger seeds, and nutritional value. The domestication of legumes has been a long and complex process, but many wild species and local varieties remain under-utilized or not fully studied. The rediscovery and utilization of these genetic resources could be useful in developing more sustainable agricultural systems and adapting to climate change and the needs of a growing global population(Graham & Vance, 2003; Zohary, Weiss, et al., 2012).

1.2 The role of legumes in agriculture

1.2.1 Perspectives for sustainable agriculture

Over the past fifty years, agriculture has made remarkable progress in improving crop yields and optimizing production practices, significantly contributing to the reduction of hunger, increased life expectancy, and enhanced global food security. However, these developments have come at a significant environmental and social cost. Modern agriculture finds itself at the heart of a global crisis, as climate change and the adoption of intensive farming practices have made the sector one of the main contributors to greenhouse gas (GHG) emissions. About one-third of global CO_2 emissions come from agriculture and livestock(Stagnari et al., 2017; van Loon et al., 2023). The rise in global temperatures and the consequent desertification of soils are already negatively impacting agricultural productivity and threatening food security. Additionally, the global population, expected to reach 10 billion by 2050, is placing added pressure on agricultural systems, with an increased demand for food and natural resources. This demographic growth is accompanied by a higher demand for animal-based foods, which have an even greater environmental impact, considering that producing 1 kg of animal protein requires up to 6 kg of plant protein(Deolu-Ajayi et al., 2022). However, due to the limitations of agricultural production, this increase could lead to severe nutritional shortages in several developing regions, urging the international community to focus on sustainable solutions for boosting food production. In this context, legumes (*Fabaceae*), the second most important family of cultivated plants after Poaceae, play a crucial role. This family, the third largest among flowering plants, includes approximately 18,000 species and 650 genera. Grain legumes account for nearly 27% of global crop production and provide 33% of the dietary protein consumed by humans. To increase the consumption of plant-based proteins, raising consumer awareness about the nutritional importance of legumes is essential, while also addressing two primary concerns: (1) long preparation times and (2) the presence of antinutritional compounds, along with other compounds that, although not classified as antinutritional, may still raise concerns(EUROLEGUME, 2018).

The European Union's "Farm to Fork" strategy, launched in 2020, represents a decisive step toward agricultural sustainability. This plan aims to reduce the use of chemical pesticides by 50% by 2030,

decrease nutrient losses by 50%, and cut fertilizer use by 20%. Additionally, it envisions the expansion of organic farming to 25% of cultivated land (EUROLEGUME, 2018; Rouphael & Colla, 2018). These goals are essential to promote ecologically compatible agriculture that can ensure future food security.

Although plant proteins are gaining prominence, it is unrealistic to expect a complete substitution of animal proteins. Instead, the goal should be to promote a general increase in the consumption of plant-based proteins, which are also of high biological value. Over the past decades, the growing number of vegetarians, meat avoiders, and meat reducers has encouraged the adoption of plant-based foods and high-protein ingredients as alternatives to meat products. This trend has renewed interest in legumes, not only as a sustainable and nutritious source of protein but also as an opportunity to achieve a more balanced diet and improved health outcomes. Expanding the use of legumes beyond dry seeds to other plant parts, such as leaves, flowers, and transitional stages like immature seeds and pods, opens countless possibilities, making this plant family increasingly attractive to consumers and markets. Despite their potential, the use of legumes for developing novel food products or as ingredients in other food items remains largely underexploited. This requires the development of innovative preparation techniques and food processing methods beyond conventional heat treatments(EUROLEGUME, 2018).

1.2.2 Legumes in agriculture

Legumes are a fundamental resource for sustainable agriculture, thanks to their ability to improve soil fertility, reduce dependence on chemical fertilisers, and promote biodiversity(Kehoe et al., 2017). These benefits make them particularly relevant in a global context where agriculture is called upon to respond to climate change and the rational use of natural resources. One of the distinctive features of legumes is their ability to fix atmospheric nitrogen through symbiosis with *Rhizobium* bacteria, which settle in root nodules. This process allows the conversion of atmospheric nitrogen (N_2) into a form that plants can assimilate, improving soil fertility and reducing the need for chemical nitrogen fertilisers(Stagnari et al., 2017). Additionally, legumes play a crucial role in diversifying agroecosystems through crop rotations, helping to break disease and pest cycles. This approach increases the resilience of agricultural systems, reducing the need for chemical pesticides and enhancing overall sustainability. Many species within this family adapt well to challenging environmental conditions, such as poor soils and dry climates, making them ideal for areas facing water stress or low fertility. Their ability to protect the soil from erosion and increase the resilience of agricultural systems makes them valuable tools for addressing the challenges posed by climate change(Chakraborty & Newton, 2011; Rani et al., 2020). From a nutritional point of view, legumes are an essential source of plant-based proteins, complex carbohydrates, fiber, vitamins, and minerals(Wang & Daun, 2006). This nutritional richness makes them crucial for food security, especially in regions where access to animal proteins is limited. By promoting legume consumption, the environmental impact of animal protein production can also be reduced.

1.2.3 Innovative agricultural practices and biofertilisers

Conservative agriculture offers a promising solution, emphasizing practices such as sod seeding, minimal tillage, and intercropping(Morugán-Coronado et al., 2020; Mukherjee et al., 2023; Rühlemann & Schmidtke, 2015). These methods improve carbon sequestration in the soil, reduce greenhouse gas emissions, and increase soil resilience to drought while optimizing water and nutrient

use. For example, no-till practices in crops like corn, wheat, and lentils have enhanced water retention capacity, root development, and soil aggregate distribution. Additionally, the slower decomposition of plant residues promotes organic matter stabilization and nutrient availability. Integrating legumes into traditional cropping systems has proven advantageous for agricultural sustainability(Alarcón et al., 2018; Dai et al., 2024; Ghimire et al., 2017).

Sod seeding is a soil management technique that involves direct seeding without tilling the soil, placing seeds within the crop residue left by the previous harvest. This method stands out from conventional practices due to its numerous environmental and agronomic benefits. By not disturbing the soil, sod seeding helps preserve its structure, reduces erosion, and improves water retention. Moreover, it maintains vegetative cover throughout the year, fostering soil biodiversity and reducing organic carbon loss. Another significant benefit is the considerable reduction of CO_2 emissions associated with traditional agricultural tilling, as it limits the use of heavy machinery and, consequently, fossil fuel consumption(Stagnari et al., 2017). Sod seeding is especially advantageous in the context of climate change, where water resources are poor, and the soil is more vulnerable to desertification. Besides environmental benefits, this technique has proven effective in containing production costs by reducing the time and labour required for land preparation(van Loon et al., 2023).

Among the tools to address these challenges are biostimulants. These are natural substances or microorganisms used in agriculture to stimulate natural processes in plants and improve their growth, yield, and resistance to abiotic stresses(Rouphael & Colla, 2018; Singh et al., 2018). The term was first coined by Zhang and Schmidt in 1997 to describe substances that, even in small quantities, enhance plant growth. These products do not act as traditional fertilisers but instead optimize nutrient efficiency and improve overall plant conditions, increasing crop adaptability to unfavourable environmental conditions such as drought, salinity, and extreme temperatures(Rojo et al., 2024). Biostimulants include a wide range of compounds, such as seaweed extracts, amino acids, humic and fulvic acids, as well as beneficial microorganisms like bacteria and fungi(Chalk et al., 2006; Rouphael et al., 2015; Zhou et al., 2024). Their use has a dual advantage: on one hand, it promotes soil health by enhancing its fertility and promoting a biological balance; on the other hand, it reduces dependence on chemical inputs like synthetic fertilisers and pesticides, contributing to more sustainable agriculture. These products are used at various stages of the crop cycle, both during sowing and growth phases, and can improve crop quality in terms of yield, nutritional characteristics, and organoleptic properties. The global biostimulant market is expected to grow significantly, reaching an estimated value of 7.6 billion dollars by 2029, with a compound annual growth rate of 12%(marketsandmarkets, 2024). Biostimulants have gained popularity in organic farming, especially in the legume sector, where nitrogen-fixing biofertilisers are widely used. In 2021, legumes represented 46% of the market, with soybean cultivation covering 34% of the total(DunhamTrimmer®, 2020). Field applications of biostimulants have resulted in grain yield increases ranging from 8.5% to 30.8%, with foliar treatments and soil applications showing significant effectiveness(marketsandmarkets, 2023). Similarly, biostimulants derived from microalgae, known for their adaptability and high photosynthetic efficiency, improve soil fertility and nutrient uptake, including phosphorus, potassium, and nitrogen. Extreme events such as rising temperatures in southern Europe, demands the development of innovative crop management techniques. In this context, inoculation with specific *Rhizobium* strains and arbuscular mycorrhizal fungi (AMF) plays a vital role in improving water use efficiency (WUE) and nutrient use efficiency

(NUE). These factors are critical for overcoming challenges related to environmental impacts and ensuring sustainable crop production. The adoption of advanced agricultural practices, including the use of *Rhizobium*, AMF, and other plant growth-promoting rhizobacteria (PGPR), is essential for supporting agriculture, preventing desertification, and promoting rural development(EUROLEGUME, 2018).

1.2.4 Life Cycle Assessment

The Life Cycle Assessment (LCA) is a standardized methodology used to evaluate the overall environmental impact of a product, process, or system throughout its entire life cycle. In agriculture, LCA is applied to identify and quantify the environmental burdens associated with agricultural production, from the cultivation phase to product distribution and the disposal of residues. Life Cycle Assessment (LCA) in agriculture has several key objectives aimed at evaluating and improving the environmental sustainability of agricultural practices(González-García et al., 2021; Ingrao & Wojnarowska, 2023; Zingale et al., 2022). A central goal is the analysis of environmental impacts, which allows for a detailed examination of greenhouse gas emissions, the consumption of vital resources such as water and soil, biodiversity loss, and pollution caused by nutrient excess and many other impacts. LCA also aims to identify the stages of agricultural production that generate the greatest impacts by analyzing specific practices such as intensive use of chemical fertilisers, pesticides, irrigation, and mechanization. Another goal is to provide decision-making support to make agriculture more sustainable. The LCA process consists of several phases. The first phase deserves a more detailed description of functional unit, system boundaries (spatial and temporal), allocation procedures, audience of the study. The second phase is data collection, also known as life cycle inventory (LCI), where information is gathered on resources used, such as energy, water, and fertilisers, and emissions produced during each phase but also computing mass, energy, waste input and output flows per functional unit, and calculating direct field emissions of the agricultural process. Subsequently, the collected data is translated into environmental impact categories during the impact assessment phase (LCIA)(ISO, 2006a; 2006b). This translation allows for the quantification of climate change, eutrophication, soil acidification, consumption of natural resources and many other impacts, depending on the assessment method adopted. Finally, the results of the analysis are interpreted to provide concrete recommendations on how to improve the environmental performance of the analyzed agricultural system. LCA has numerous applications in the agricultural sector. One of the main applications is comparing different agricultural systems, such as conventional, organic, or conservative, to evaluate which is least impactful on the environment. Additionally, LCA can be used to optimize resource use by identifying strategies to reduce the consumption of fertilisers and pesticides, improve water efficiency, and increase biodiversity within agroecosystems. Despite its potential, LCA in agriculture faces some challenges and limitations, often due to multifunctionality in agronomy e.g. food production, income for producers, land management, nutrition. This means that a single functional unit is often not sufficient (Parmar et al., 2023; Rojo et al., 2023). The complexity of agricultural systems, characterized by a great heterogeneity of practices and climatic conditions, makes it difficult to collect accurate and representative data. Furthermore, establishing the temporal and spatial boundaries of the analysis can significantly influence the results, introducing variability in comparisons between systems. Finally, some indirect impacts, such as changes in land use or the loss of ecosystem services, are difficult to fully integrate into the LCA, limiting the

completeness of the assessment. Despite these difficulties, LCA remains a fundamental tool for promoting more sustainable and environmentally friendly agriculture.

1.3 Research Aims

The experimental study conducted over three years had the primary objective of evaluating innovative and sustainable agricultural practices aimed at revolutionizing legumes productivity and quality, while significantly reducing environmental impact. A fundamental first step was an in-depth bibliometric analysis to identify the most advanced research lines in legume cultivation, with a particular focus on chickpeas. This allowed for mapping the key developments in the field, opening the way to new solutions for more efficient and sustainable agriculture. The next step involved adopting cutting-edge agricultural practices with the aim of improving crop yield and quality, using environmentally friendly techniques such as the use of biostimulants.

The definition of innovative and sustainable cultivation methods required validation through the measurement of environmental impacts.

The life cycle assessment (LCA) approach highlighted the lack of data on the material and energy flows associated with a microbial-based biostimulant product. This limited the possibility of analysing its impact within the cultivation system under study.

To this end, a modelling study was carried out for the Trichoderma-based biostimulant used in the field trial. This allowed us to estimate and propose significant improvements in the production cycle of this widely used agricultural product

In summary, the key objectives of research have been to improve agricultural productivity, promote higher nutritional quality, reduce the environmental impact of agricultural practices and make agriculture more resilient to climate change, all through the adoption of sustainable technologies and practices.

These objectives are critical to ensuring long-term food security and promoting global sustainability, while addressing the environmental and food challenges of the future in innovative and practical ways.

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Chapter I:

A Bibliometric Analysis of Chickpea Agronomic Practices in the World During 45 Years of Scientific Research

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A Bibliometric Analysis of Chickpea Agronomic Practices in the World During 45 Years of Scientific Research

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ABSTRACT

Chickpea (*Cicer arietinum* L.) cultivation practices underwent significant transformation in recent decades due to advancements in scientific knowledge and the need for sustainable, productive farming systems. In this study, a bibliometric analysis of scientific publications from 1977 to 2023 on chickpea agronomic practices was conducted, revealing critical insights. India, as the world's leading chickpea producer, plays a pivotal role, not only in production but also as a significant contributor to scholarly research and international collaborations. The choice of journals for publication is found to influence research impact.

Analysis of research trends using co-occurrence networks of keywords reveals evolving focuses, with a recent shift towards qualitative aspects, such as protein content and nutritional quality, as well as sustainable agricultural practices. The study also emphasizes the necessity for further research on chickpea quality characteristics, strategies to mitigate antinutritional factors, yield optimization, and the impact of climate change on chickpea cultivation. Ultimately, chickpea cultivation research holds great promise in contributing to global food security and environmental sustainability. This bibliometric analysis provides a comprehensive overview of chickpea cultivation research and offers valuable insights for researchers, policymakers, and stakeholders as they navigate the future of sustainable agriculture and the quest for protein-rich food production while minimizing the environmental footprint.

1 | Introduction

Today, a third of the world's soils is considered to be degraded due to a number of factors, such as erosion, salinization, nutrient depletion, urbanization, and pollution that threaten the long-term sustainability of agriculture (FAO 2016). The incorporation of pulses into cover crop, intercropping, and crop rotation practices can help in restoring soil health by increasing organic matter content, enhancing soil structure, and promoting beneficial microbial activity (De Mastro et al. 2022; Vidigal, Manuel Romeiras, and Monteiro 2020; Xue et al. 2016). These benefits translate into improved long-term soil fertility and reduced erosion risks (Phiri, Njira, and Chitedze 2023).

The introduction of legumes, of which pulses are a part, into traditional cropping systems holds great potential for making agricultural practices more sustainable (Stagnari et al. 2017). In the family of Fabaceae, there are species able to thrive with limited nutrient availability than many other plant families yet concurrently enriches soil with essential compounds such as nitrogen and soluble phosphates (Mulissa et al. 2016; Stagnari et al. 2017; Xue et al. 2016). Pulses, such as chickpeas, possess specific soil bacteria that enable biological nitrogen fixation (Hirsch, Lum, and Downie 2001; Sellami, Lavini, and Pulvento 2021). Many scientific studies (Fikre 2016; Yirga & Rashid 2010) show that the use of leguminous plants allows a

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saving of nitrogen for the next crop in a range from 30% to 60%, also in different tillage system (Tedone, Alhajj Ali, and De Mastro 2022). Some scholars estimate that globally, legumes contribute about 5–7 million tons of nitrogen that crops can use in the following season (Kebede 2020; Vidigal, Manuel Romeiras, and Monteiro 2020). In addition, the deep root systems of legumes also improve water infiltration and enhance drought tolerance, making them suitable for areas with limited water availability (Tar'an 2015). On the other hand, they are considered as inherently climate-smart since they adapt to climate change and help mitigate its effects by increasing soil carbon sequestration capacities (Jensen et al. 2012). Moreover, the inclusion of legumes in crop rotations helps break disease and pest cycles, reducing the need for chemical pesticides and fostering natural pest control (Idate et al. 2021; Kroeck 2011; Yigezu et al. 2019).

Chickpeas (*Cicer arietinum* L.) also known as garbanzo beans is already cultivated in ancient times by the Egyptians, and they are widely consumed around the world (Sharma et al. 2013). They are the second most common legume species globally after the common bean (*Phaseolus vulgaris* L) and constitute ~18% of the global legumes production (FAO 2021). Chickpea-cultivated area accounts 15 Mha, with an annual production of ~16 Mt (FAO 2021). It is estimated that approximately 80% of the world's chickpea production is concentrated in Asia. Countries such as India, Pakistan, Myanmar, and Türkiye have been major contributors to chickpea production in the region (FAO 2021). World average production per hectare is about 1 $t\,ha^{-1}$, which is much lower than the theoretical yield of 6 $t\,ha^{-1}$ (Varshney et al. 2013; Zwart et al. 2019). This difference between the effective and theoretical yield is due to biotic and abiotic stress that affect chickpea production in different cultivated areas (Roorkiwal et al. 2016; Vidigal, Manuel Romeiras, and Monteiro 2020; Xue et al. 2016).

In recent years, especially after the 68th United Nations General Assembly designated 2016 as the International Year of Pulses (A/RES/68/231; United Nations 2014), there has been an increase in interest in the cultivation of legume crops in order to meet the growing global demand for high-quality, protein-rich foods while reducing those of animal origin in the human diet (Alandia et al. 2020; Sellami et al. 2019). This can help meet the needs of a growing global population (Chojnacka, Moustakas, and Witek-Krowiak 2020) while maintaining environmental sustainability, compatible land use practices, and food security (Abhilash et al. 2016; L. Dubey, Dubey, and Jain 2015).

Chickpeas are considered a highly nutritious food due to their rich content of protein, folate, vitamins, and various minerals such as calcium, phosphorus, zinc, iron, and magnesium (Diapari et al. 2014; Jha 2015; Tripathi et al. 2012). Due to its high protein content (Boukid 2021; Sellami, Lavini, and Pulvento 2021) and low costs of production, the chickpeas are considered an excellent source of livelihood in low-income countries.

Chickpeas can be used in various culinary creations, such as hummus, salads, soups, and curries. Its taste is similar to nut flavor and if combined in food preparations with cereals give a complete and rich protein food (Yigezu et al. 2019). Additionally, ground chickpeas, in the form of chickpea flour or besan, are used in gluten-free and vegan baking, as well as savory dishes (Guimarães et al. 2022).

They come in different varieties, including Desi and Kabuli (Ruta et al. 2020), each with its own characteristics. Consumption of chickpeas has been associated with digestive health, blood sugar regulation (Zafar et al. 2015; Zafar & Kabir 2017), heart health, and weight management (Harini et al. 2015). They are particularly favored by those following vegetarian and vegan diets due to their high protein content (Boukid 2021; Sellami, Lavini, Calandrelli, et al. 2021).

The cultivation of chickpeas has undergone substantial changes in agronomic practices over time, driven by advancements in scientific knowledge and the need for sustainable and productive farming systems. Conventional practices have been replaced by enhanced varieties (Sellami, Pulvento, and Lavini 2021), precise planting methods (Thenkabail 2003), and refined nutrient management practices (Begna Sisay et al. 2023; S. Dubey, Raghav, and Singh 2017). Disease and pest management systems have evolved, using integrating cultural practices as well as biological control methods (Aljuboori, Ibrahim, and Mohamed 2022). The adoption of conservation agricultural concepts (Mishra, Singh, and Kumar 2012), as well as the investigation of digital technology (Jat et al. 2020), contributes to the evolution of chickpea agronomic practices. Through the integration of these technological and methodological developments, farmers can enhance productivity, mitigate risks, and ensure the long-term sustainability of chickpea cultivation.

Therefore, it has been useful to examine the evolution of scientific production on chickpea cropping practices from 1977 to 2022 in the Scopus database and to determine the significance of this subject as a research domain. Consequently, there are posed the following main research questions: What are the major areas of research based on the input dataset? Has there been a significant shift in chickpea agronomic practices during the period analyzed? Are there critical transitions in the history of the development of the research field? Where are the "turning points"?

For this purpose, a bibliometric approach has been employed to synthesize the knowledge base concerning the various chickpea cropping methods used on a global scale. The bibliometric method is a valuable tool for analyzing and understanding scientific literature. By quantitatively examining factors such as citations, authorship patterns, and journal impact factors, researchers can gain insights into research productivity, impact, and collaboration within a field. Bibliometric analysis helps identify influential researchers and journals, reveals emerging trends, and provides a historical perspective on the development of scientific fields (Glänzel & Moed 2002; Glänzel & Schubert 2004; Joshi 2015; Stea et al. 2023).

The findings of this investigation provide valuable insights for agricultural practitioners, academics, and other relevant stakeholders to discern research trend, knowledge gaps as well as potential new lines of research.

2 | Materials and Methods

2.1 | Methodology

A bibliometric analysis was conducted to evaluate the scientific literature related to the field of study. Bibliometric analysis is a powerful tool that can be used to gain insights into the scientific

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literature, by using statistical techniques to analyze significant amount of scientific data. It can be used to track the evolution of a field of study, identify emerging trends, and measure the impact of individual researchers or publications (Donthu et al. 2021). Regardless of its merits, bibliometric analysis remains relatively new in Agricultural and Biological research, and according to the Web of Science database, only 276 research papers on bibliometric analysis were published in Agriculture science over a last 45-year period (1977–2022).

2.2 | Data Source and Search Criteria

The bibliometric analysis was conducted using Scopus database, to identify studies related to the chickpea cropping practices under the world and that were written in English. Scopus is a large abstract and citation database of peer-reviewed literature and quality research from across the sciences, social sciences, and arts and humanities (Baas et al. 2020). Scopus enables users to calculate bibliometric indicators such as the number of publications per scholar, number of citations, number of institutions and journals, and H-index as well as the journal impact factors (Haeffner-Cavaillon & Graillot-Gak 2009). The bibliometric indicators take into account various factors to evaluate the impact and importance of scholarly publications. These indicators are commonly used to assess the influence and productivity of researchers, institutions, or scientific journals.

A search was conducted on August 1, 2022 using the following search string:

TITLE-ABS-KEY ({FIELD} AND ((RHIZOB* OR INOCUL*) OR (FUNGI OR MYCORRIZ* OR {PGPR} OR {PLANT GROWTH PROMOT*} OR BIOSTIMULANT* OR FERTIL* OR FULVI* OR HUMI*) OR (TILL* OR {CONSERVATION AGRICULTURE}) OR (SOWING AND (DATE* OR DENSIT*)) OR INTERCROP* OR (BREED* OR CULTIVAR* OR LANDRACE* OR GENOTYPE* OR ACCESSION* OR ECOTYPE*) OR (IRRIGATION OR {DEFICIT IRRIGATION} OR DROUGHT OR ((WATER OR SALT) AND STRESS) OR SALIN* OR (WATER AND (QUALITY OR MANAGEMENT))) OR (NITROGEN OR POTASSIUM OR PHOSPHORUS) OR (DISEASE OR FUNG* OR BIOTIC* OR BACTER*) OR (PEST* OR INSECT* OR ACARI*) OR (WEED* OR HERBICIDE) OR ROTATION) AND (CHICKPEA* OR GARBANZO OR {CICER ARIENTINUM}) AND ((PROTEIN AND (YIELD OR CONTENT)) OR YIELD)).

Upon conducting the initial search, a total of 1086 articles were obtained. Two selection criteria were evaluated for selecting papers for the bibliometric analysis: (I) studies conducted only under field conditions, but not under greenhouse conditions, pots, and in vitro culture, and (II) studies that focused on chickpea productivity. The time span indicated 1977–2022 was automatically selected from the number of articles identified from the search string. The year 2022 was selected as the last year because the year 2023 had not ended when the search string was produced. In addition, the 673 articles resulting from the screening were used to obtain numerical data within the methodologies used to draw up tables, graphs, and concept maps.

2.3 | Screening

Figure 1 describes the procedure for selecting articles. Eighty-six documents were excluded during the identification phase because they were neither journal articles nor written in a language other than English. Subsequently, in the screening of titles, abstract, and full-text studies, 327 articles were excluded because they were irrelevant to the topic, not accessible, no field experiment, or no effect size (yield or protein content) was available. As a result, only 673 documents satisfied the eligibility requirements and were included in the bibliometric analysis.

2.4 | Data Analysis

In this study, to examine the evolution of scientific production on chickpea cropping practices over a 45-year period (1977–2022), the bibliometric indicators such as the number of publications per scholar, number of citations, number of countries, number of institutions and journals, H-index, the journal impact factors as well as co-citation analysis, or keyword/terms co-occurrence analysis per periods are applied. The quantitative analysis was carried out utilizing the comprehensive science mapping analysis tools, bibliometrix package in R (Aria & Cuccurullo 2017). VOSviewer (van Eck & Waltman 2010), a conceptual network mapping software designed by scientists, was used to generate keywords and term maps. Citespace (Chen 2006) was also utilized to construct a co-cited reference network.

3 | Results and Discussion

3.1 | General Aspects of References

3.1.1 | Scientific Production and Authors

The screening revealed 673 papers that satisfied the inclusion and exclusion criteria selected in the first phase of the study. Figure 2 shows the development of scientific production over the last 45 years (1977–2022). Scientific research in the field of chickpea cultivation has grown at an average rate of 27, 25%, as confirmed by the trend line. At the end of the 1990s and in the last decade, significant increases compared to the average trend were recorded, in particular, 15 papers in 1999, 28 papers in 2009, and 35 papers in 2021. Figure 2 shows also two peaks in number of citations on 1991 and 2006.

The most important author who first published an article on chickpea cultivation was Sigh G. (Figure 3) in 1980. While the scientist who produced most articles in 1 year was Siddique Khm in 1999.

As shown in Figure 4, 57% of the scholars identified by the screening step published only one article in 45 years in this research area, followed by 30.5% of the scholars who published more than 3 articles, while the authors who published only 2 articles were 9.9%; 2.7% of scientists wrote 3 articles.

Table 1 illustrates the five most productive authors in field of chickpea cultivation practices. These five authors have written 50 articles and have been cited 1.485 times. An analysis of the

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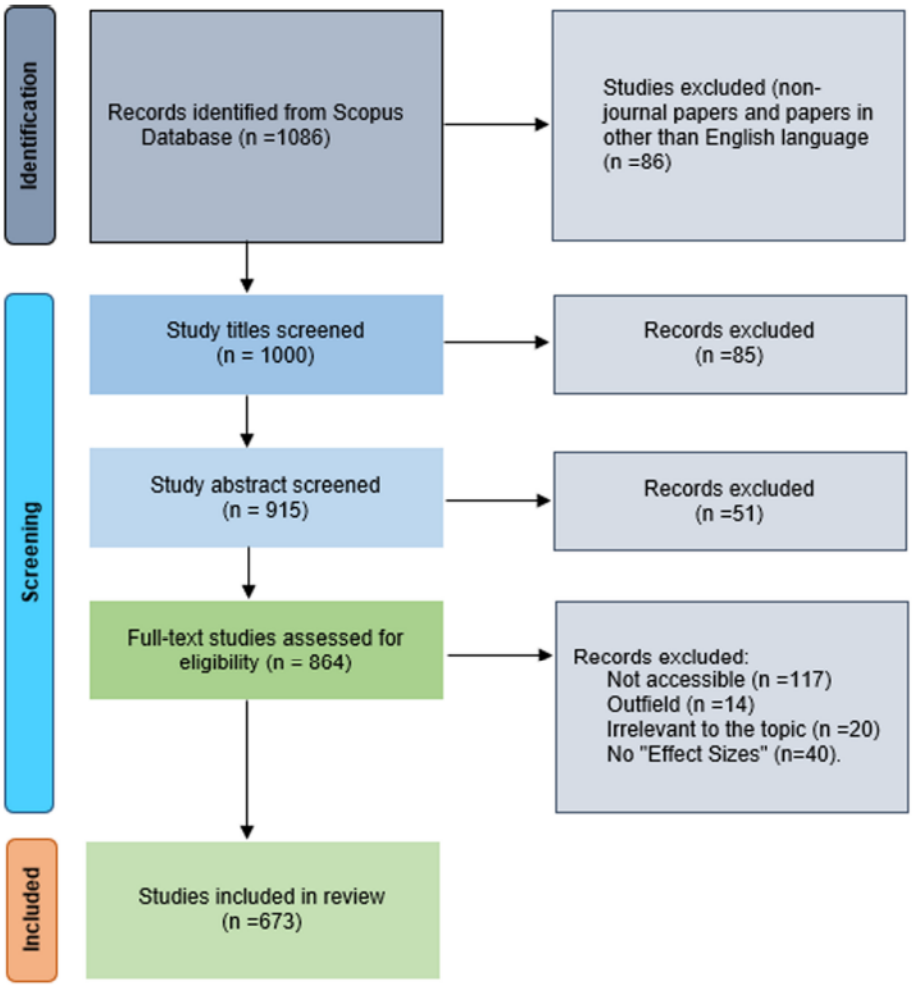

FIGURE 1 | PRISMA flowchart depicting the article selection procedure (n represent the number of studies).

bibliometric data shows that Sigh G. is the leading researcher, having published 13 articles and received 147 citations.

The 687 articles counted 1520 authors; 16 scholars contributed articles as single authors, and 17 articles were wrote by only two authors. The 22.22% of analyzed papers showed international co-authorship (Table 2).

3.1.2 | Scientific Production by Countries and Collaborations

Figure 5 shows the countries with the highest scientific production. It also shows which nations have collaborated with other countries. India is the most productive country in this area, followed by Pakistan. India is also the country with the highest number of collaborative articles with other international research institutions. Tunisia and Algeria despite having a low number of publications on chickpea show a high level of cooperation with international research organizations.

The 35 countries involved in international collaboration on chickpea cultivation research are grouped into four clusters (Figure 6), determined by the areas of cooperation. The closer two countries are on the map, the greater the collaboration between them. However, one of the most significant examples of international collaboration is the partnerships between India, Australia, and the United States.

3.1.3 | Trend and Analysis of Citation

The top 10 cited publications account for 18% of the total citations (8069 citations). Most of the cited articles (6/10) have as first author a researcher working for an Indian research institution. The article with the highest number of citations is entitled "Wheat and chickpea intercropping systems in an additives experiment: Advantages and weed smothering" and was cited 185 times (Figure 7). The following article is "Variability of root length density and its contributions to seed yield in chickpea (*Cicer arietinum* L.) under terminal drought stress," which received 168 citations (Figure 7).

Figure 8 represents the evolution of search terms focused on chickpeas. In recent years, the trend terms were protein, seed yield and herbicides, biofortification, and biofertilizers. Some terms like pulse, crop rotation, row spacing, and weed control were more frequent until 2010.

3.1.4 | Analysis of Sources Where Articles on Chickpea Have Been Published

Table 3 shows the 10 journals with the highest number of published articles and citations. The first journal is *Legume Research* with the highest number of published articles (24%). The journal *Field Crops Research* has the highest number of citations (31%). *Field Crops Research* is ranked in a higher quartile compared to *Legume Research*.

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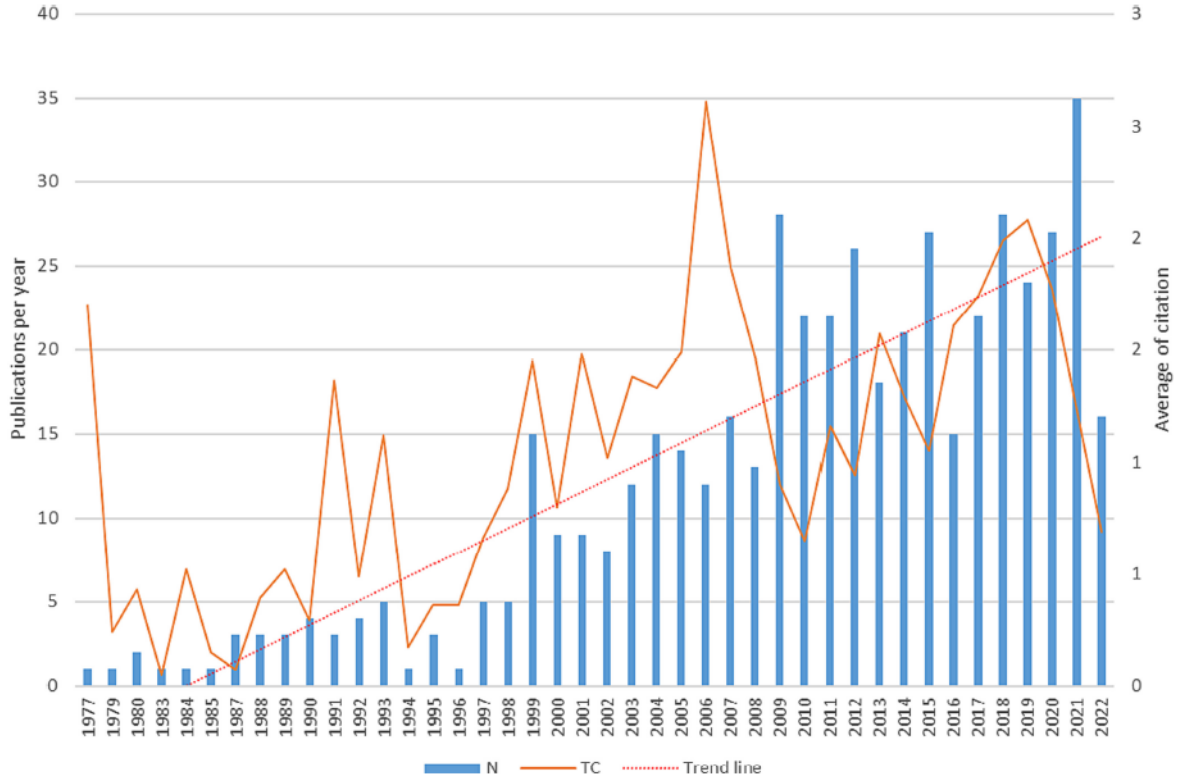

FIGURE 2 | This figure shows the number of total citations per year and the number of articles. The trend line is related to the number of articles. Number of publications (N) and total citation per year (TC).

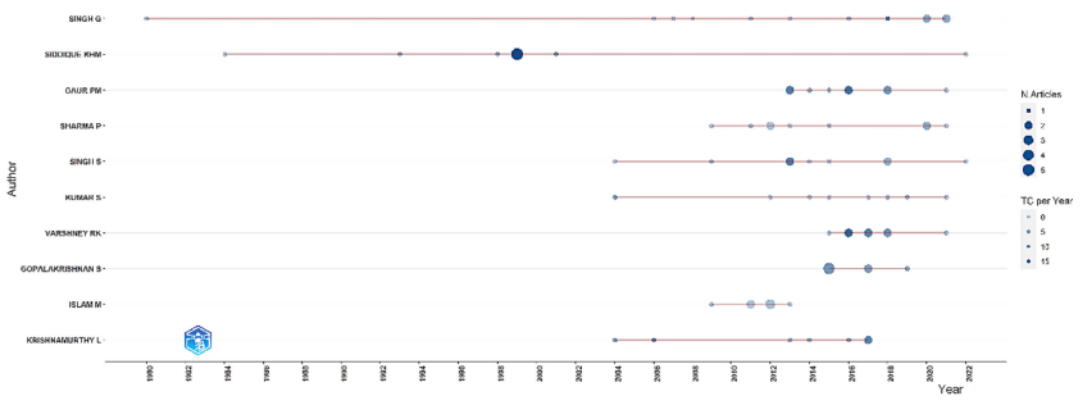

FIGURE 3 | The most productive authors by linking the period of production and the points at which the authors wrote the most papers. TC (Total Citation) and Number of articles are indicated.

Table 4 displays the top 10 institutions on chickpea research. In the first place is ICRISAT located in India with a total of 61 articles. Furthermore, 4 out of 10 institutes are Indian and account for 50.3% in terms of number of scientific articles.

Figure 9a shows the co-occurrence network analysis of the 132 most relevant terms that appeared at least 10 times in the title and abstract fields of 673 articles. It comprises 132 nodes, 4190 links, and 11,721 total links. Each node in the network represents a term and the size of the node reflects the number of times the term appeared. The connections between terms are determined by the frequency of their occurrence in the 673 publications. The higher the frequency with which two terms appear together, the closer they will be positioned on the map. There are three clusters of terms that share common topics. The co-occurrence network data show that the green term cluster is related to genetic improvement and selection of lines resistant to biotic and abiotic factors, for example, resistance to Ascochyta blight. The terms with the highest frequency in this cluster are "genotype" (frequency = 78), "pod" (frequency = 78), "cultivar" (frequency = 71), "trait" (frequency = 66), "environment" (frequency = 57), and "variation" (frequency = 50). The cluster of red terms is related to cropping systems and cultivation practices (fertilization, intercropping, and tillage) researched mainly in Indian territory. The terms in this cluster with the highest occurrences are "system" (frequency = 91), "fertilizer" (frequency = 60),

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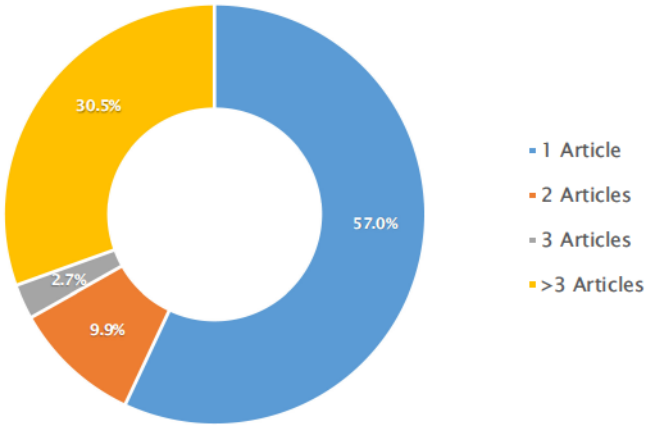

FIGURE 4 | Percentages of authors per published articles.

TABLE 1 | Top 5 authors in the period 1977–2022.

Authors	Articles	H_index	TC	PY_start
SINGH G	13	6	147	1980
SIDDIQUE KHM	10	9	786	1984
GAUR PM	9	8	311	2013
SINGH S	9	5	185	2004
SHARMA P	9	4	56	2009

Abbreviations: PY_start, publication year start; TC, total citation.

TABLE 2 | Number of authors and author collaboration.

Description	Result
Authors	
Authors	1520
Authors of single-authored docs	16
Authors collaboration	
Single-authored docs	17
Co-authors per doc	4.05
International co-authorships %	22.22
Document types	
Article	497
Article; early access	2
Article; proceedings paper	5

"wheat" (frequency = 45), "india" (frequency = 44), "dose" (frequency = 38), "tillage" (frequency = 30), and "nutrient uptake" (frequency = 26). The cluster of blue terms is related to agronomic techniques and protection of the chickpea crop (field, inoculation, and phosphorus). The terms in this cluster with the highest occurrences are 'field' (frequency = 85), 'inoculation' (frequency = 61), 'phosphorus' (frequency = 60), 'nodulation' (frequency = 45), 'species' (frequency = 41), 'disease' (frequency = 35), and 'rhizobium' (frequency = 29). The analysis revealed that the relative terms in the 1977–2022 timeframe, shown here in Figure 9b, focus on the evolution of the terms in the co-occurrence network between 2008 and 2014. The analysis revealed that the terms related to water use, tillage, tillage system, fixation, and grain legume appeared earlier than the other terms (in 2008, purple cluster). Field condition, trait, accession, drought stress, and drought tolerance appeared later than their predecessors (2014, yellow cluster). Between 2010 and 2012, the most frequently used terms were inoculation, cultivar, fertilizer, phosphorus, and environment.

Figure 10a depicts the co-occurrence network analysis of the terms of the 90 most relevant keywords that appeared at least 5 times in 673 articles, with 90 nodes, 507 links, and 1100 total links. Each node in the network denotes a keyword, and the node size reflects the amount of times the keyword appeared. The structure of the network is divided into 6 clusters of keywords that share related topics. The topic of the red cluster is nodulation and nitrogen fixation. The yellow cluster comprises all those keywords related to production characteristics. The green cluster represents and focuses on economic aspects and how these can be influenced by the agricultural practices adopted. The blue cluster focuses on biotic abiotic tolerance traits. The purple cluster is based on weeds and the pale blue cluster discusses elements used in chickpea cultivation such as zinc phosphorus and FYM (farmyard manure).

The evolution of the authors' keywords in the co-occurrence network is demonstrated in Figure 10b. This network map is based on the average number of times each keyword appears in our literature. In the beginning, the most used keywords were sowing date, nitrogen fixation, and tillage, and then around 2016, the research shifted towards aspects related to weed management, control of some diseases, biofertilizers, and the amount of nutrients absorbed. Economic aspects are compared to different farming systems between 2010 and 2014.

4 | Discussion

According to the results of the quantitative analysis, the number of scientific papers on the cultivation and quality characteristics of chickpeas is continuously increasing. This is probably because, in recent years, legumes have been at the center of the political debate for the achievement of the 2030 Agenda goals (UNRIC 2015). This is due to the capacity of legumes to fix 5–7 tons of atmospheric N per year (Kebede 2020; Vidigal, Manuel Romeiras, and Monteiro 2020). It is estimated that, on average, the environmental impact generated animal protein is 12–46 times greater than the legume proteins production (Arrigoni et al. 2023). All the positive aspect of pulses production have been highlighted by FAO in 2016 during the International Year of Pulses (FAO 2016); it is possible to see from the results how from 2016 onwards we have a growth in scientific research focused on the cultivation of pulses, all of which is surely favored by the increase in funds used for these crops.

Among the main active journals, it is essential to look at the relevance and reliability of the sources employed (Ahmad & Ayub Jan 2019; Sorenson 2016). The Agricultural research communication center has the highest number of published papers even though it is not in the first quartile. It is

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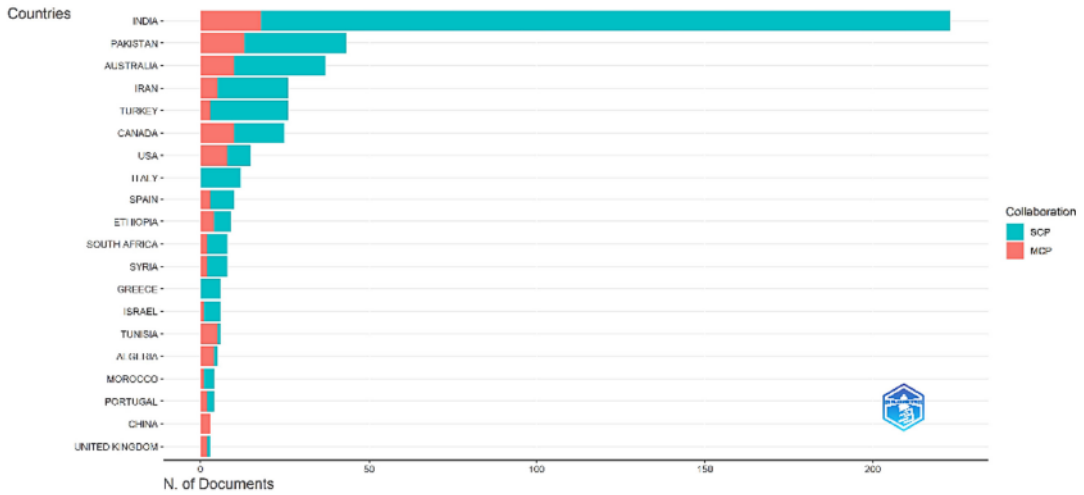

FIGURE 5 | The graph relates the number of papers to the country of production and it cooperation. MCP: multiple country publications; SCP: single country publications.

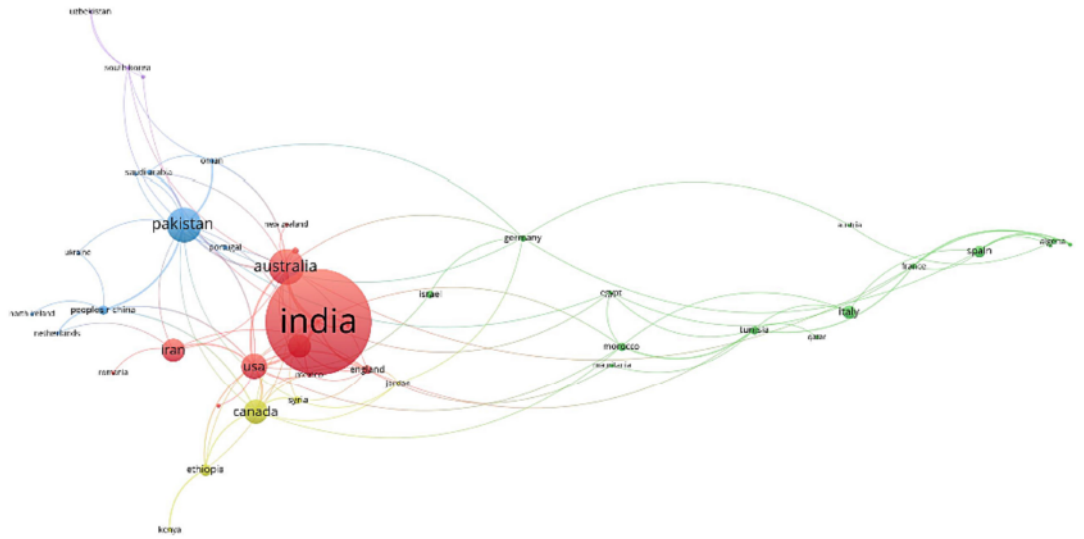

FIGURE 6 | Collaboration network of countries. The different colors represent the clusters of collaboration between countries in the field of chickpea research; nodes indicate countries (the size of nodes is based on several publications); links represent a collaboration between two countries. *Source:* VOSviewer (cluster resolution 0.5; minimum cluster size 1 and no merging of small clusters).

possible to remark that researchers prefer to cite articles in well-known and important journals, often falling in the first quartiles. Although some journals published few papers on the topic, these are distinguished by their high number of citations.

The analysis of terms and keywords helps understand the connections between the various issues in the research field (Molinillo et al. 2016). In recent years, the most common terms used focus on qualitative aspects (such as proteins) and agronomic traits such as herbicide use, biofertilizers, biocontrol, and so forth. In earlier years, research focused on the agronomic and genetic characteristics of chickpeas. Subsequently, the various studies focused on nitrogen fixation, Ascochyta control, and fertilization. From the keywords, it is further confirmed how research topics changed in a decade. In particular, the initial reference subjects were focused on traditional agricultural practices. Later, the focus was on economic analyses related to agricultural practices and in the last period on weed management and the use of sustainable practices that aim to reduce environmental impact.

The trend of last years in chickpea publications are probable due to the technological evolution of agricultural practices. The introduction of chickpea in cropping systems is important for (i) stabilizing food production (Renard & Tilman 2019), (ii) contributing to the reduction of synthetic fertilizers (Jensen et al. 2012) thanks to the organic N-fixation (Peoples et al. 2009; Steen Jensen, Carlsson, and Hauggaard-Nielsen, n.d.), (iii) support sustainable phytosanitary management (Voisin et al. 2014), and (iv) improve agricultural profitability (MacWilliam, Wismer, and Kulshreshtha 2014).

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FIGURE 7 | Top 10 cited articles in the chickpea literature.

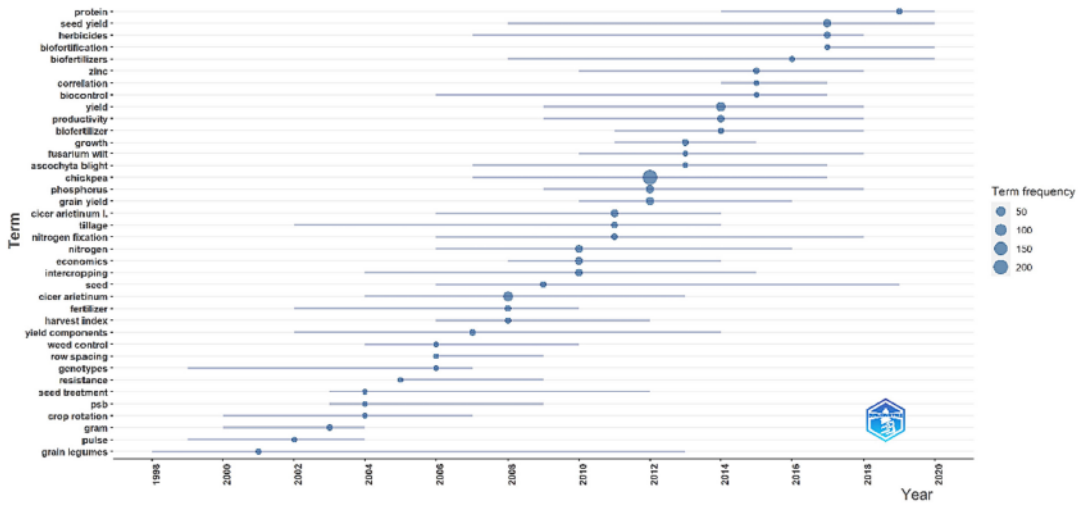

FIGURE 8 | Trend topics of the last 45 years.

This bibliometric analysis provides important information regarding chickpea cultivation practices. One of the most obvious gaps is the need for in-depth investigations into the quality characteristics of chickpeas needed to address the challenges of processing this product. In addition, there is a need for further investigation of cultivation-related issues in particular weed control, use of biofertilizers, and production characteristics, which are affected by both climate change (Phiri, Njira, and Chitedze 2023) and the low production yields of this crop (around 1 t/ha) far from the potential yields (Varshney et al. 2013; Zwart et al. 2019).

This bibliometric analysis shows the progress made in research towards the most common agricultural practices. The next research trends should focus on aspects related to biostimulants and agricultural practices that can significantly influence the qualitative aspects related to protein content, protein yield, reduction of antinutritional factors (Carbonaro 2011; Idate et al. 2021), bioactive properties, and polyphenolic compounds (Nartea et al. 2023). In addition, a further objective that research will have to achieve is to reduce inputs by ensuring greater environmental and economic sustainability aimed at circular agriculture (Reckling et al. 2016).

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TABLE 3 | Top 10 most productive journal.

No.	Element	NP	TC	H-index[a]	SJR[a]	Quartiles[a]	Publisher
1	*Legume Research*	53	168	18	0.39	Q2	Agricultural Research Communication Centre
2	*Indian Journal of Agricultural Sciences*	44	143	30	0.18	Q4	Indian Council of Agricultural Research
3	*Field Crops Research*	29	1153	174	1.4	Q1	Elsevier
4	*Indian Journal of Agronomy*	25	133	25	0.21	Q3	Indian Society of Agronomy
5	*Canadian Journal of Plant Science*	17	357	66	0.33	Q2	Agricultural Institute of Canada
6	*Journal of Plant Nutrition*	13	201	85	0.54	Q2	Taylor and Francis Ltd.
7	*Experimental Agriculture*	10	240	51	0.52	Q2	Cambridge University Press
8	*European Journal of Agronomy*	9	697	131	1.14	Q1	Elsevier
9	*Pakistan Journal of Botany*	9	66	67	0.3	Q3	Pakistan Botanical Society
10	*Australian Journal of Agricultural Research*	8	512	56	0.83	Q1	Wiley-Blackwell Publishing Ltd
	Subtotal	217	3670	70.3	0.584		

Abbreviation: SJR, SCImago Journal Rank.
[a]*Source:* SCImago Journal & Country Rank 2022 (www.scimagojr.com).

TABLE 4 | The 10 best institutes for the chickpea cultivation.

No.	Affiliation	Country	Articles
1	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)	India	61
2	The University of Western Australia	Australia	54
3	Punjab Agricultural University	India	53
4	Indian Agricultural Research Institute (ICAR)	India	50
5	University of Agriculture Faisalabad (UAF)	Pakistan	35
6	University of Saskatchewan	Canada	31
7	Islamic Azad University	United Arab Emirates	24
8	Akdeniz University	Turkey	19
9	Banaras Hindu University	India	19
10	University of Venda	South Africa	18
	Subtotal		364

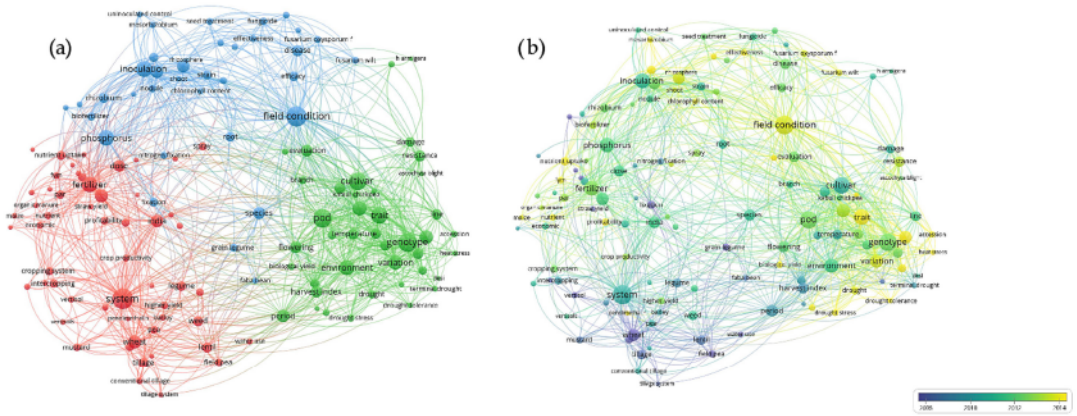

FIGURE 9 | (a) Co-occurrence network and (b) overlay visualization of 132 relevant terms which appeared at least 10 times in the title and abstract fields of 687 article.
Source: VOSviewer.

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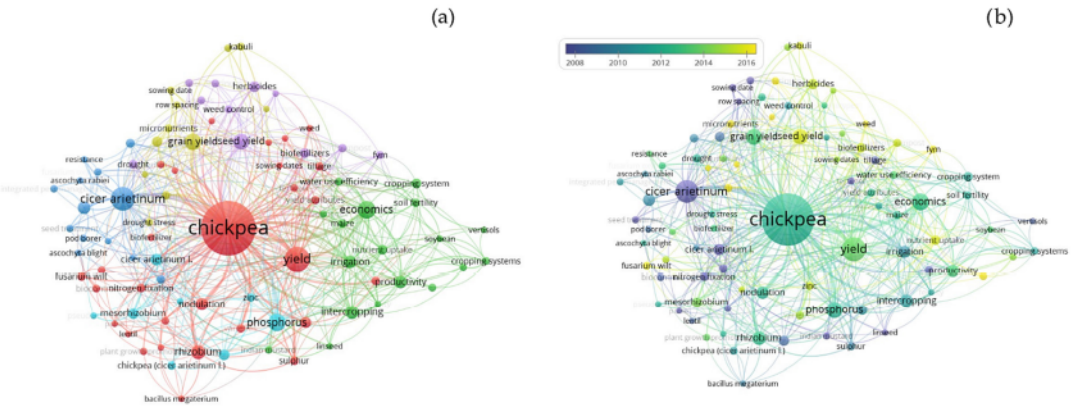

FIGURE 10 | (a) Co-occurrence network and (b) overlay visualization of 90 relevant keywords, which appeared at least 5 times in the title, and abstract fields of 687 articles.
Source: VOSviewer.

5 | Conclusions

The extensive bibliometric analysis conducted on chickpea cultivation practices spanning from 1977 to 2023 reveals several key insights and trends in the field of sustainable agriculture.

Research on chickpea cultivation has seen a significant increase over the years, and this growth can be attributed to the global recognition of pulses, as vital contributors to food security and sustainability goals.

India emerges as a central player in this research landscape, not only as the largest chickpea producer but also as a hub for scholarly contributions and international collaborations, emphasizing the field's global reach.

The choice of journals for publishing research findings plays a crucial role in determining the research's impact and reach, with high-impact journals being the preferred platforms for dissemination.

The evolving research focus, as reflected in the changing trends of research keywords, underscores the field's adaptability to emerging challenges. Recent research has shifted its emphasis towards qualitative aspects such as protein content and nutritional quality, alongside addressing environmental sustainability and circular agricultural practices.

The papers analysis also showed that cultivation practices such as biostimulants, fertilization, nodulation, genetic selection, weed control, and the management of some pathogens had a greater influence with a focus on improving yield, quality and sustainability. Furthermore, the literature shows that there is a growing interest in plant-derived proteins and extractable yield per hectare. Indeed, among the outputs of this research is the influence that agronomic practices have on protein yield.

Looking ahead, there are promising research avenues to explore, including a deeper investigation into chickpea quality characteristics, strategies to mitigate antinutritional factors, and improving yield. Climate change impacts and optimizing chickpea production will continue to be significant areas of concern.

This comprehensive bibliometric analysis not only highlights the evolution of chickpea cultivation practices but also underscores its growing importance in sustainable agriculture. It offers valuable insights for researchers, policymakers, and stakeholders, shaping the future of chickpea cultivation. This is vital in the quest for sustainable, protein-rich food production to meet global needs while minimizing the environmental footprint.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Chapter II:

The response of lentil (*Lens culinaris* Medik.) to different soil management systems and the application of biostimulants

agronomy

MDPI

(Article)

Combined effect of different soil management systems and biostimulants on qualitative and quantitative traits of two commercial varieties of lentil (*Lens culinaris* Medik.)

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Abstract: Over the past 50 years, increased agricultural production has reduced hunger but has also contributed to environmental and social challenges, leading to unsustainability. Climate change is causing desertification, salinization and compromising productivity. Biostimulants, derived from microorganisms and bioactive molecules, can promote plant growth, and enhance agricultural yields. Conservation agriculture offers solutions to improve soil health and reduce greenhouse gas emissions. The introduction of legumes into traditional cropping systems presents an opportunity for greater sustainability. An experimental trial was conducted in Poggiorsini (BA) over three consecutive years (2020-2023) in order to evaluate the effects of two different soil management systems (sod seeding and conventional tillage) and the use of biostimulants on qualitative and quantitative traits of two lentil varieties. The study revealed significant effects on lentil yield components and biometric parameters influenced by environmental conditions and applied treatments, including biostimulants and soil management systems. Water scarcity during the vegetative stages negatively affected yields, with significant reductions in the 2022 season. Sod seeding improved seed yields while maintaining soil moisture. The cultivar Dupuy was more sensitive to drought than the cultivar Crimson. Biostimulants increased yields by 8-13% by improving nutrient uptake and plant resistance.

Keywords: Lentil, biostimulants, soil management, *Trichoderma*, mycorrhizae

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1. Introduction

Over the past 50 years, efforts to boost crop yields and enhance production practices have played a key role in reducing hunger, increasing life expectancy, and addressing global food insecurity. However, these gains have come at a cost, as they have been offset by growing environmental and social unsustainability [1]. In recent years, Italian lentil production has increased by about 30% and the cultivation area by about 8% [2]. In Europe, lentil production demonstrated a growing trend between 2020 and 2022, with an increase of 32%. During the same period, the cultivated area expanded significantly, rising by 67%. [3].

Climate change and its consequences are now accelerating, time after time; the trend towards the adoption of conventional and intensive practices, making the agricultural sector one of the most impactful[1, 4, 5]. As global temperatures increase, there will be more land desertification and more soil salinization. This will hurt crop productivity and plant productivity[6]. Agricultural production, specifically livestock and agriculture, releases close to one-third of the global CO_2 output [4]. The successful transition to sustainable models by 2050 will require substantial changes, with a reduction of more than 50% in global consumption of animal-based and processed foods while increasing consumption of plant-based foods by more than 100% [1]. Climate change is also causing various biotic and/or abiotic stresses that may result in a strong variation in production. In addition to the reduction caused by climate change, a further challenge is the reduction of synthetic nitrogen fertilisers and the use of pesticides [7, 8].

One option to achieve high productivity in a sustainable way in cropping systems can be represented by biostimulants, originating from different matrices such as plant or organic extracts, microorganisms, algae etc. The use of biostimulants has been growing rapidly. These compounds can be based on different microorganisms (fungi and bacteria) and naturally bioactive molecules. Biostimulants can promote plant development and increase agricultural production by providing nutrients or making them more readily available to the crop [9]. Two types of soil fungi are particularly effective as natural plant stimulants: arbuscular mycorrhizal fungi and *Trichoderma* species. Mycorrhizae are fungi that can create a mutualistic symbiosis with plants, allowing them to multiply more easily while providing numerous benefits to plants. *Trichoderma* species have such positive feedback on the crop that they are included in the category of biofertilizers [10, 11]. *Trichoderma* was able to solubilize P resulting in growth promotion [12, 13].

Combinations of different biostimulants are significant on several biometric and productive parameters due to improved crop resistance to biotic and abiotic factors.

Another very important aspect concerns climate change's impact on agricultural soil health. Soil health is currently under threat worldwide due to factors such as erosion, compaction, salinization, nutrient depletion, pollution, and overgrazing [3]. Agricultural intensification has exacerbated soil degradation in recent decades, with the increased use of machinery and chemical inputs contributing to soil compaction, erosion, and salinization, while depleting organic matter and nutrients, thus undermining long-term soil productivity and sustainability [14].

Conservative agriculture (CA) offers a promising solution, promoting agroecosystem management practices that enhance agricultural sustainability while protecting the environment [15]. CA has been shown to reduce greenhouse gas emissions and improve soil carbon sequestration, while also enhancing soil resilience to drought and improving water and nutrient use efficiency—critical factors in maintaining sustainable agricultural production and mitigating the effects of climate change on global food systems [16]. Various agricultural practices, such as sod seeding, minimum tillage and intercropping, have been shown to contribute to the long-term conservation and increase of carbon stocks. This is independent of the type of crop or production season. In crops such as maize, wheat

and lentils, no-tillage has improved various quality and productive parameters. These benefits include increased water-retention capacity in the soil, more efficient root development and better distribution of soil aggregates. In addition, the transformation process of plant residues into stabilized organic matter is slower, which improves the soil's ability to absorb and retain nutrients [17, 18, 19].

The introduction of legumes, into traditional cropping systems holds great potential for making agricultural practices more sustainable [20].

Legumes are one of the world's most economically important crops, after cereals, and supply protein, carbohydrates, minerals and vitamins, as well as other phytochemicals, so they are recognized as an important source of nutrients [21]. Worldwide, about 6.10 million hectares are devoted to lentil cultivation, with annual production and yield of 6.33 million tons and just over one ton per hectare proposed [7]. In 2023, more than 5253 hectares of lentils were cultivated in Italy for a total production of about 4.56 thousand tons [2].

In this study, the authors aim to evaluate the combined effects of different biostimulants and two soil management systems (sod seeding and conventional tillage) on quality and quantity parameters of two lentil varieties, offering an innovative contribution in integrating sustainable agricultural practices with advanced biotechnological solutions. This approach represents a significant novelty, as it explores new treatment combinations that could improve not only productivity and quality, but also crop resilience in a context of climate change and global environmental challenges.

2. Materials and Methods

2.1. Experimental Site

A field experiment has been carried out at Poggiorsini (Bari, Italy) (40°53'14.16"N and 16°17'38.60"E; 720 m above the sea level) between 2020 and 2023. The soil texture of the experimental site was silty loam according to the USDA classification (Table 1). The area is characterized by a Mediterranean climate, with an average annual rainfall of 500 mm over the past 30 years. Most of the rainfall is concentrated in the autumn-winter period.

Table 1 Soil characteristics of the experimental field

Parameter	Unit	Value
Sand (2-0,05 mm)	g kg^{-1}	348
Silt (0,05-0,002 mm)	g kg^{-1}	426
Clay (< 0,002 mm)	g kg^{-1}	227
Textural Class (USDA)		Silty loam
pH		7.6
Electrical conductivity	dS m^{-1}	1.8
Organic Carbon	g kg^{-1}	11.8
Organic Matter	g kg^{-1}	20.4
Total Nitrogen	g kg^{-1}	1.2
C/N ratio		9.9
Available P_2O_5	mg kg^{-1}	14

Cation exchange capacity (CEC)	meq/100 g	23.6
Exchangeable K	mg kg^{-1}	363
Exchangeable Ca	mg kg^{-1}	5106
Exchangeable Mg	mg kg^{-1}	580
Exchangeable Na	mg kg^{-1}	532

2.2. Agronomic Management and Experimental Design

The experiment lasted for three crop seasons, and the sowing dates were respectively on January 31, 2021, February 6, 2022, and January 19, 2023. For all growing seasons, harvesting took place in July. Two lentil varieties (Cv) were cultivated: Crimson that is one of the most common commercial cultivars in the area and the Dupuy variety selected for industrial applications. For each variety, two soil managment systems (SM) were adopted: sod seeding (SS) and conventional tillage (CT).

In the SS system, glyphosate was used in the pre-sowing phase, sowing was carried out with the seed drill Laseminasodo disk 3960 (Valleverde - Gravina in Apulia) and post-emergence weed control was done using Piridate 45% and pure Cycloxydim.

For CT system subsoil tillage to a depth of 50 cm followed by a disc harrow to a depth of 20 cm was carried out; weed control was done in pre-emergence stage using Pendimetalin and in post-emergence using Piridate 45% and pure Cycloxydim.

Both soil systems were managed with low levels of chemical plant nutrition, applied according to the availability of nutrients in the soil and the expected effect of two different commercial biostimulants; at sowing one product with both fungi and bacteria (FB) (*Glomus* spp., *Azotobacter chrococcum*, *Bacillus subtilis*, *Streptomyces roseocinereus* and *Trichoderma longibrachiatum* - Tristart S, Agriges) and one containing only fungi (F) (*Trichoderma harzianum* T-22 - Trianum, Koppert) were applied.

The experimental design used was a split-split plot with four replicates. Each replicate consists of a main block, subdivided into two plots with the two different soil management(SS, CT). Subsequently, each main block was further divided into sub-plots, separated by variety(Crimson and Dupuy). Within each sub-plot, sub-sub-plots (133 m^2) with biostimulants, (F, FB, and Control). The meteorological data (Figure 1)(average, minimum and maximum air temperature, useful precipitation) were provided by an automatic weather station (model Davis) close to the experimental field.

2.3. Measurements

At maturity, 5 plants per sub-sub plot were harvested, and biometric parameters were measured. Additionally, to estimate the yield per hectare, 3 plots per treatment, each 10 m^2 in size, were harvested using a plot combine. The main biometric parameters assessed included plant height, plant weight, number of pods per plant, thousand-seed weight (TSW), harvest index (HI) — calculated as the ratio of seed weight to total plant weight — and seed yield.

The seed samples were analyzed for protein, fats, carbohydrates, dietary fiber, saturated fats, unsaturated fats, sterols, polyphenols, Oleic, Linoleic, α-Linolenic, ω3. Samples of legume seeds were ground in a laboratory grinder and ground to pass through a sieve size of 0.5 mm.

The proximate composition of lentils was assessed using the AOAC Official Methods. Moisture and ash content were determined gravimetrically by AOAC methods 930.04 and 930.05 [22], respectively. Total protein was determined by Dumas combustion method using a PRIMACS SNC-100 nitrogen analyzer (AOAC 992.23). The protein calculation is given by multiplying the nitrogen content by 5.75. Fat content was determined gravimetrically by extracting fat from lentils with petroleum ether, on a Soxtec extraction unit (Foss Soxtec 2050) (AOAC 2003.06). Total carbohydrate content was determined by calculating the difference 100 - (protein + ash + total fat + moisture). Quantification of fatty acids was carried out by cold methylation and subsequent gas chromatographic analysis with FID detector. A weighed aliquot of the lipid fraction obtained by Soxhlet extraction was subjected to cold methylation under agitation with a 2N potassium hydroxide solution in methanol and hexane. The ethereal fraction recovered is analyzed by capillary column gas chromatography (Shimadzu Nexis GC 2030). Quantification was obtained by a calibration curve with a FAMEs standard, with a calibration range of 50-500 μg/ml. Chromatographic conditions were as follows: DBfastFame 20 m column length 0.18 mm i.d and 0.20 μm film thickness; injection temperature: 250°C; split ratio: 1:20; thermal gradient: 190°C, for 3 min, then increased at 5°C/min, up to 210° at 240°C with an increment of 30°C/min and maintained at that temperature for 3 min; carrier hydrogen gas (H_2) at a linear rate of 30 cm per second. A weighed aliquot of the lipid fraction obtained by Soxhlet extraction, with added α-cholestanol as internal standards, is saponified with potassium hydroxide in ethanolic solution. The unsaponifiable matter is then extracted with ethyl ether. The different sterols compounds fractions are separated from the unsaponifiable matter by thin-layer chromatography on a basic silica gel plate. The fraction recovered from the silica gel separation is transformed into trimethylsilyl ethers and then analyzed by capillary column gas chromatography (ThermoScientific Trace GC Ultra). Chromatographic conditions were as follows: SPB-5 30m column length 0.25 mm i.d. and 0.25 μm film thickness; injection temperature: 300°C; split ratio: 1:20; oven 265°C for 55 min, carrier helium gas (He) at a constant flow 1ml/min.

A precisely weighed amount of homogenized sample (2.000g) was extracted with 20 mL of acetone/water/acetic acid (70:29.5:0.5, v/v/v) under sonication for 15 min at room temperature, The mixture was shaken for 3 h, centrifuged at 4000 g for 10 min and the supernatant was collected. The extraction was repeated with 20 mL of the same solution and the two supernatants were combined [25]. All legumes' extracts were adequately diluted in the appropriate solvent and immediately analyzed in triplicate for their total phenolic. Total phenolic contents were assayed according to Folin-Ciocalteu method [26]. For each food extract, an aliquot of 100 μl was added to 7 mL distilled water in volumetric flasck, afterward 0.5 ml of Folin-Ciocalteu reagent was added. The solution was mixed and allowed to equilibrate. After 5 min, 1.5 mL of sodium carbonate solution 20% (w/v) was

added. The mixture was shaken at 25 °C for 5 min. Then, the absorbance was measured at 740 nm, after 2 h, using UV-Visible spectrophotometer (PerkinElmer UV-Vis Lamda 365). Data were expressed as mg gallic acid equivalent per 100 g[23, 24].

2.4. Statistical Analysis

The collected data were subjected to a multivariate analysis of variance (MANOVA) test using R 4.3.2; the independent variables analyzed were four (years, soil management, cultivar and treatments). The normal distribution of each dependent variable was determined using the Shapiro-Wilk test and Bartlett's test was performed to confirm the homoscedasticity. A two-way ANOVA on the cultivar and treatment variable was performed for qualitative parameters. The average results were compared using the Student–Newman–Keuls (SNK) test at the P 0.05 probability level with the Rstudio agricultural package software.

3. Results

The main meteorological parameters collected during the three experimental years are shown in Figure 1. The last year had a higher concentration of precipitation from April to July, compared to the first and second year, and poliannual average values. In particular, the seasonal rainfall recorded was: 203 mm in 2021 with 84 mm of rain between March and April, 303 mm of rain in 2022 with 56 mm between March and April, and 459 mm in 2023 with 110 mm between March and April. May 2023 was the wettest month within the three years with 137.5 mm of rain, while the least rainy months were May 2021 (2.0 mm) and April 2022 (7.0 mm).

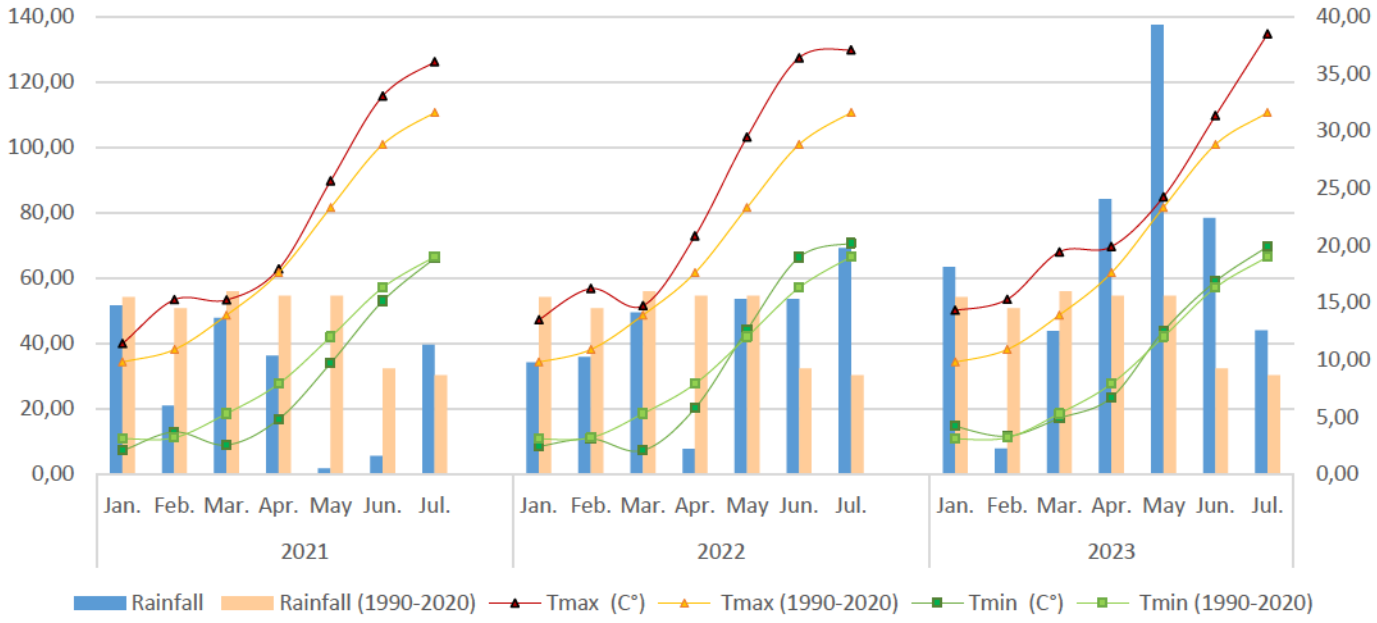

Figure 1 Climatic trends in the 3 years.

Biometric parameters analyzed are reported in Table 2. Results showed that the year effect was significant for all analyzed parameters. The highest values of plant weight (4.8 g) and

number of pods (47.8) were recorded in 2023 due to the favorable trend of the rainfall from the flowering until the seeds repening (March-June). Water stress conditions during the flowering phase lead to abscissions and abortions, resulting in fewer pods and a drop in production. This phenomenon was particularly evident in the second year, when rainfall in April was less than 10 mm, and in the first year, when rainfall in May and June was well below the seasonal average for the area. The years had a significant effect on plant height development, although with slight differences.

The cultivation system didn't have a significant effect on biometric parameters. This suggests that lentils have a good capacity to adapt to conservative cultivation practices, and in particular to no-tillage. The interaction between the cultivation system and the year was significant, confirming a positive effect of sod seeding in particularly dry years such as the first.

The cultivar effect was significant for plant height and number of pod values. Crimson recorded significant higher plant height compared to Dupuy. This was probably due to the year effect, which was also significant. On the other hand, Dupuy was more productive in terms of pods per plant, averaging 38.9 compared to 34.7 for Crimson.

The use of biostimulants did not lead to any improvement in the biometric parameters measured. In particular, F produced values for plant height and number of pods that were not significantly different from the control. The use of FB resulted in significantly lower values than the control and F.

The interaction between the Y and SM was significant for the number of pods produced per plant. The varying climatic conditions across the years and the soil's ability to retain moisture influenced this value. Additionally, the interaction between the four dependent variables was significant, with a greater impact on plant height, followed by plant weight.

Table 2 Biometric parameters of a single plant measured and collected in the field. Fungi and bacteria (FB); fungi (F); The independent variables are year (Y), soil management (SM), cultivar (Cv) and treatments (T). Differences at. *P≤0.05, **P≤0.01 and ***P≤0.001. ns indicates no significant difference. Means followed by different letters in each column are significantly different according to the *SNK* test (P=0.05).

	Plant height (cm)		Plant weight (g)		Pod number (n°)	
2021	34.7 ± 0.6	**a**	3.7 ± 0.7	**b**	35.7 ± 9.7	**b**
2022	34.3 ± 0.5	**b**	2.9 ± 0.6	**c**	26.9 ± 7.9	**c**
2023	33.3 ± 0.4	**c**	4.8 ± 0.7	**a**	47.8 ± 8.3	**a**
	***		***		***	
SS	34.1 ± 0.6		3.9 ± 1.0		37.2 ± 12.4	
CT	34.1 ± 0.8		3.7 ± 1.0		36.4 ± 11.9	
	ns		ns		ns	
Crimson	34.2 ± 0.8	**a**	3.8 ± 1.0		34.7 ± 11.0	**b**
Dupuy	34.0 ± 0.7	**b**	3.8 ± 1.0		38.9 ± 12.9	**a**
	**		ns		**	
FB	33.9 ± 0.8	**b**	3.7 ± 1.0		33.9 ± 10.8	**b**
F	34.2 ± 0.7	**a**	3.8 ± 1.1		38.7 ± 14.5	**a**
Control	34.2 ± 0.8	**a**	3.9 ± 0.9		37.8 ± 10.3	**a**
	**		ns		*	
Y x SM	ns		ns		***	
Y x Cv	*		ns		ns	
SM x Cv	ns		ns		ns	
Y x T	ns		ns		ns	
SM x T	ns		ns		ns	
Cv x T	ns		ns		ns	
Y x SM x Cv	ns		ns		ns	
Y x SMx T	ns		ns		ns	
Y x Cv x T	ns		ns		ns	
SM x Cv x T	ns		ns		ns	
Y x SM x Cv x T	**		*		ns	

The yield components values analyzed (harvest index, TSW and seed yield) are reported in table 3.

The year effect was significant, as the highest production was recorded in 2021 with 2.0 t ha^{-1}, followed by 1.8 t ha^{-1} in 2023 and 1.6 t ha^{-1} in 2022. This result is not supported by the higher number of pods detected in 2023 following the lower TSW (32.7 g) recorded in the same year. In this case, the TSW values reported again in 2021 (35.3 g) and 2022 (36.1 g) were significantly higher than in 2023. This parameter is justified by the close correlation between the number of pods and the TWS[25, 26]. As far as the

HI is concerned, it was the 2022 vintage that positively influenced this index (45.8) by determining a lower plant development, having recorded the lowest plant weight compared to the other vintages, a condition that, however, limited the productive expression. The 2021 (39.0) and 2023 (40.0) vintages had significantly lower values, but were not statistically different from each other, albeit with higher production levels than in 2022.

The positive effect of sod-seeding compared to conventional tillage in terms of seed yield was recorded, with an increase of the value of about 10%. On the other hand, the thousand-seed weight value is significantly higher in conventional sowing compared to sod seeding. 'Crimson' was the most productive variety in terms of seed yield value despite having a significant lower harvest index. Both biostimulants treatments increased significantly the seed yield value compared to the control treatment. Furthermore, the TR product also recorded a significant higher value in terms of TSW compared to the FB.

The interaction between the Y and SM was also significant for both yield and harvest index (HI). Furthermore, the interaction between Y and Cv proved significant, reflecting the influence of both climatic conditions and the thermal-pluviometric trends of the season. Additionally, the interaction between T and Y was significant, confirming the effectiveness of the biostimulant and highlighting how climatic conditions affect these variables.

Table 3 Productive parameters of lentils. Harvest index (H-index); Thousand seeds weight (TSW).); Fungi and bacteria (FB); fungi (F); The independent variables are year (Y), soil management (SM), cultivar (Cv) and treatments (T). Differences at *P≤0.05, **P≤0.01 and ***P≤0.001. ns indicates no significant difference. Means followed by different letters in each column are significantly different according to the SNK test (P=0.05).

	H-index (%)		TSW (g)		Seed yield (t ha^{-1})	
2021	39.0 ± 9.9	**b**	35.3 ± 3.1	**a**	2.0 ± 0.3	**a**
2022	45.8 ± 7.0	**a**	36.1 ± 2.4	**a**	1.6 ± 0.3	**c**
2023	40.0 ± 6.3	**b**	32.7 ± 1.2	**b**	1.8 ± 0.5	**b**
	*		***		***	
SS	42.0 ± 9.4		34.4 ± 2.9	**b**	1.9 ± 0.4	**a**
CT	41.2 ± 7.3		35.0 ± 2.7	**a**	1.7 ± 0.4	**b**
	ns		*		***	
Crimson	40.0 ± 9.5	**b**	35.0 ± 2.9		1.9 ± 0.4	**a**
Dupuy	43.2 ± 6.8	**a**	34.4 ± 2.6		1.7 ± 0.4	**b**
	**		ns		**	
FB	40.7 ± 9.9		34.1 ± 2.9	**b**	1.8 ± 0.4	**a**
F	43.3 ± 7.4		35.3 ± 2.9	**a**	1.9 ± 0.5	**a**
Control	40.8 ± 7.6		34.6 ± 2.5	**ab**	1.7 ± 0.4	**b**
	ns		*		***	

Y x SM	*	ns	***
Y x Cv	ns	ns	***
SM x Cv	ns	ns	ns
Y x T	ns	ns	**
SM x T	ns	ns	ns
Cv x T	ns	ns	ns
Y x SM x Cv	ns	ns	ns
Y x SMx T	ns	ns	ns
Y x Cv x T	ns	ns	*
SM x Cv x T	ns	*	ns
Y x SM x Cv x T	ns	*	ns

Fat content decreased significantly in the biostimulant treatment compared to the control. The treatments, on the other hand, did not show significance in macronutrients, except for fiber content(Table 4).

Table 4 macronutrient of lentils. Proteins, Carbohydrates, Dietary fiber and Fat; Fungi and bacteria (FB); fungi (F); The independent variables are treatments (T) and cultivar (Cv). Differences at *P≤0.05, **P≤0.01 and ***P≤0.001. ns indicates no significant difference. Means followed by different letters in each column are significantly different according to the SNK test (P=0.05).

	Proteins		**Carbohydrates**		**Dietary fiber**		**Fat**	
	g/100 g s.s.		**g/100 g s.s.**		**g/100 g s.s.**		**g/100 g s.s.**	
Crimson	28.1 ± 0.8	**b**	67.7 ± 0.8	**a**	15.4 ± 1.1	**a**	1.0 ± 0.1	**b**
Dupuy	28.9 ± 1.2	**a**	66.7 ± 1.4	**b**	14.5 ± 0.9	**b**	1.1 ± 0.1	**a**
	*		**		**		***	
FB	28.4 ± 1.5		67.4 ± 0.9		15.3 ± 1.1	**a**	1.1 ± 0.1	**a**
F	28.5 ± 0.7		67.3 ± 1.7		15.1 ± 1.2	**a**	1.0 ± 0.1	**b**
Control	28.5 ± 0.9		67.0 ± 0.9		14.5 ± 1.0	**b**	1.1 ± 0.1	**a**
	ns		ns		*		***	
Cv x T	ns		ns		*		*	

Table 5 shows fatty acids, sterols and polyphenols values. Crimson variety showed higher values of saturated fatty acids compared to Dupuy; Dupuy was significantly higher in unsaturated fatty acids, sterols and polyphenols compared to Crimson. The Dupuy variety recorded about 30% more polyphenols and sterols than the Crimson variety. In addition, the Dupuy variety has 17% less saturated fat than the Crimson variety. The FB treatment resulted in higher levels of both saturated (92.0) and unsaturated(514.0) fats compared to the F and the control. The Trichoderma treatment

showed significant lower levels for sterols and polyphenols compared to the control teratment and lower levels of sterols also compared to the FB tretament(Table 5).

Table 5 Qualitative parameters of lentils. Saturated fatty acids, unsaturated fatty, monounsaturated fatty acids, polyunsaturated fatty acids, sterols, and polyphenols; Fungi and bacteria (FB); fungi (F); The independent variables are treatments (T) and cultivar (Cv). Differences at *P≤0.05, **P≤ 0.01 and ***P≤0.001. ns indicates no significant difference. Means followed by different letters in each column are significantly different according to the SNK test (P=0.05).

	Saturated fatty acids		**Unsaturated fatty acids**		**Sterols**		**Polyphenols**	
	mg/100g s.s.		**mg/100g s.s.**		**mg/100g s.s.**		**mg/100g s.s.**	
Crimson	96 ± 5	**a**	466 ± 34	**b**	57 ± 10	**b**	819 ± 57	**b**
Dupuy	79 ± 12	**b**	507 ± 74	**a**	77 ± 5	**a**	1.050 ± 72	**a**
	***		**		***		***	
FB	92 ± 7	**a**	514 ± 88	**a**	70 ± 11	**a**	908 ± 162	**b**
F	84 ± 17	**b**	473 ± 34	**b**	63 ± 16	**b**	921 ± 113	**b**
Control	86 ± 11	**b**	471 ± 36	**b**	69 ± 9	**a**	975 ± 123	**a**
	**		*		*		*	
Cv x T	***		***		ns		*	

Table 6 shows the amount of mono- and polyunsaturated fatty acids in the samples analysed. All the parameters analysed are significant for the Cv. It is also evident that the biostimulant treatments yielded higher values for linoleic, α-Linolenic acid and ω-3 than the control, also influencing the distribution of the fatty acids present. The content of monounsaturated and polyunsaturated fatty acids was influenced by the interaction between Cv and T.

Table 6 qualitative parameters of lentils. Mono- and polyunsaturated fats; Fungi and bacteria (FB); fungi (F); The independent variables are treatments (T) and cultivar (Cv). Differences at *P≤0.05, **P≤0.01 and ***P≤0.001. ns indicates no significant difference. Means followed by different letters in each column are significantly different according to the SNK test (P=0.05).

	Oleic acid		**Linoleic acid**		**ω-3**		**α-Linolenic acid**	
	mg/100g s.s.		**mg/100g s.s.**		**mg/100g s.s.**		**mg/100g s.s.**	
Crimson	161,48 ± 14,07	**a**	231,14 ± 21,06	**b**	65,29 ± 6,30	**b**	65,29 ± 6,30	**b**
Dupuy	152,73 ± 24,72	**b**	274,22 ± 46,26	**a**	71,84 ± 11,41	**a**	71,84 ± 11,41	**a**
	*		***		*		*	
FB	165,37 ± 19,66	**a**	268,29 ± 60,41	**a**	72,44 ± 14,01	**a**	72,44 ± 14,01	**a**
F	147,53 ± 20,98	**b**	249,90 ± 15,09	**ab**	68,34 ± 5,20	**ab**	68,34 ± 5,20	**ab**
Control	158,42 ± 17,62	**a**	239,86 ± 27,68	**b**	64,91 ± 6,58	**b**	64,91 ± 6,58	**b**
	**		*		*		*	
Cv x T	***		**		**		**	

4. Discussion

The results highlighted that lentil productive and morphometric parameters were significantly influenced, in part, by both the year under consideration and the treatments applied, such as biostimulants and the soil management. Significant differences were also observed between the average values of the two varieties. Several scientific papers show that water plays an important role in the increase of lentil production; in fact, water stress in the vegetative stages can affect production[27, 28, 29]. In the recent study, which lasted three crop cycles, climatic trends proved to be decisive. In particular, the second year was the one with the lowest biometric characters and yield, except for the TSW, which was higher than in the other two years. It is plausible that the higher weight is due to a reduced harvest caused by the dry year. The 2022 crop season produced 13% and 25% less than the 2023 and 2021 crop years. Also, sod seeding showed to be a useful strategy to improve seed yield-reducing negative impacts on soil. Sod seeding performance can be explained by the higher soil moisture level that favors plant development and protects organic matter[30, 31, 32]. The rainfall favored the lengthening of the cultivation cycle and the development of the plants, generating an increase in production. The Dupuy cultivar was found to be much more sensitive than the crimson cultivar to drought years. The treatment with microorganisms increased production by a value between 8 and 13%. F proved to be the most effective in both sowing systems, sod seeding and in conventional sowing. Additionally, the weight of a thousand seeds in the experiment treated with F did not show a reduction despite producing more. The benefits of biostimulants and the use of microorganisms in agriculture are confirmed by several scientific articles, which highlight how treated plants have improved resistance to biotic and abiotic factors [33, 34]. As shown by Rukaitė et al. biostimulants in addition to having direct effects on cultivation can increase the amount of organic matter in the soil. It is known that the use of biostimulants combined with crop rotations with other species such as some vegetables or wheat increase production by increasing the amount of fertiliser[35, 36, 37, 38]. The effects of biostimulants were also verified on Fabacee, confirming an increase in production linked to two main factors, better absorption of nutrients and the production of hormone-like compounds and growth regulators [36, 39]. Laranjeira et al., highlighted another key aspect of chickpeas, where biostimulant and irrigation carried out in the flowering phase led to a production increase of between 5 and 17%. Furthermore, it can be stated that in all the theses tested, the yield was higher than in Italy by 0.89 t ha^{-1} [7]. The yield increases also occurred for cultivation on no-tillage, although the trial lasted only a few years. One possible reason could be the choice of commercial varieties with high seed purity and germinability. The growing environment and farming practices allowed for better nutrient utilization in the soil and better coping with biotic and abiotic stresses [33, 40]. Like many other legumes, lentils have gained increasing popularity due to their high nutritional value, with a composition comprising almost 60% carbohydrates and 25% to 30% protein [41, 42]. 'Crimson' showed higher macronutrient values than ‘Dupuy’, except for fat and dietary fibre. Treatments with biostimulants only showed an increase in fat and dietary fibre content. Among the

23 most studied legumes, lentils stand out as having the second highest starch content (47.1 %) and a higher amount of insoluble dietary fiber than other legumes. Legumes are also recognized as a good source of prebiotic carbohydrates that support the balance of the gut flora and help prevent gastrointestinal diseases[43]. In the varieties used in this study, the protein content ranged between 28% and 29%, confirming the role of lentils as an important protein source. Lentils contain a wide range of phenolic compounds, including phenolic acids, flavonoids and condensed tannins. The distribution of these compounds varies within the seed: flavonoids are the main constituents of the tegmen, while non-flavonoid compounds, such as hydroxycinnamic and hydroxybenzoic acids, are predominant in the cotyledons. In this study, the polyphenol content was significantly higher in the Dupuy cultivar than in the Crimson cultivar. However, the treatments did not have a significant positive impact on polyphenol levels nor macronutrients, except fat and dietary fiber. The fatty acid profile confirms the predominance of the following fatty acids linoleic acid, oleic acid and α-linolenic acid [44]. The use of FB had a significant effect on the distribution and concentration of fatty acids, with a significant increase in oleic acid, linoleic acid, and α-linolenic acid [45, 46]. F treatment also influenced the lipid composition, showing intermediate values between the FB and the control. However, the varietal variable remains the most influential element on quality parameters, followed by the treatment adopted. The results confirm that the use of FB and F can alter the content and distribution of fatty acids in lentils, significantly influencing their quality parameters.

5. Conclusions

The present study underscores the importance of selecting appropriate cultivars and cultivation practices to optimize lentil seed yield. The benefits of biostimulants and microorganisms in enhancing yield and quality parameters were evident. The findings provide valuable insights for sustainable agricultural practices, especially in the context of changing climatic conditions. The study highlights several key factors influencing the production performance of lentil, across different cultivation practices and environmental conditions. Climatic conditions played a decisive role in yield variability. Water stress during vegetative stages notably reduced grain yield, aligning with the other papers. Sod seeding emerged as a more productive method compared to conventional sowing due to higher soil moisture retention. The study confirmed that the cultivar plays a crucial role in determining qualitative traits. The Dupuy cultivar demonstrated higher protein and lipid contents, whereas the Crimson cultivar had higher fiber and carbohydrate levels. The Dupuy cultivar's sensitivity to drought highlights the need for selecting appropriate cultivars based on environmental conditions. Treatments with microorganisms, significantly increased production by 8-13%, regardless of the sowing method. Biostimulants and microorganisms' benefits in enhancing plant resistance to biotic and abiotic stress. Certain qualitative parameters can be influenced by the treatments and the cultivar used. Therefore, before cultivation, it is important to understand the production objectives and goals of the crop. This will help

in identifying the most suitable cultivars and agricultural practices for a specific environment.

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Chapter III:

Life cycle assessment of microbial plant biostimulant production for applications in sustainable agricultural system

Life cycle assessment of microbial plant biostimulant production for applications in sustainable agricultural system

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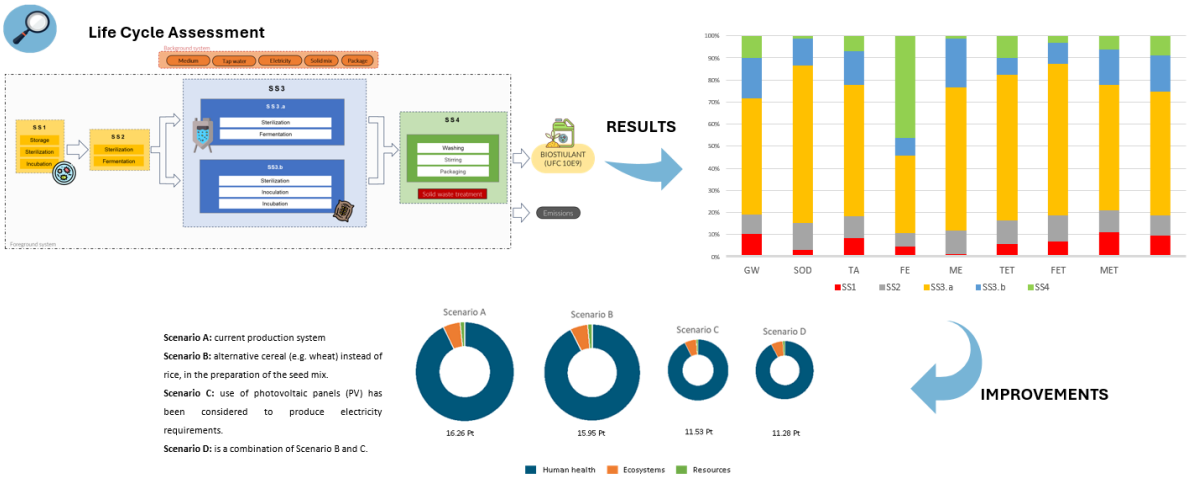
Graphical abstract:



Abstract:

By 2050, the global population will face increasing food demand due to population growth, prompting the need for sustainable agricultural practices. The European Union aims to address these challenges by reducing fertilizer and pesticide use, expanding organic farming, and limiting global warming to 1.5°C. Biostimulants have emerged as promising natural tools to boost agricultural productivity by 8-30% with minimal application per unit area. This study assesses the environmental impact of producing Trichoderma-based biostimulants using life cycle assessment (LCA) methodology to identify critical points and propose improvements to reduce their environmental footprint. The research reconstructed the biostimulant production cycle and inventoried inputs and outputs for each step. Environmental impacts were evaluated

using the ReCiPe 2016 Midpoint method for the current process and the Endpoint method for proposed improvements. The findings revealed that the liquid substrate production phase had the highest environmental impact, contributing 368 kg CO_2 eq emissions in the global warming category. Key contributors were Potato Dextrose Broth (40% of emissions) and electricity consumption (44%). To mitigate these impacts, two measures were implemented: replacing Potato Dextrose Broth with winter cereals and transitioning to self-generated electricity. These optimizations reduced the overall environmental footprint by approximately one-third, from 16.26 Pt to 11.28 Pt. This study highlights the potential of biostimulants as sustainable alternatives in green agriculture, offering insights into optimizing production processes to balance increased productivity with environmental sustainability. Biostimulants thus represent a key tool in addressing future agricultural challenges while minimizing ecological impacts.

Keywords: biostimulant, productivity, *Trichoderma*, sustainable agriculture

1. Introduction

In the foreseeable future, two major problems will be faced by the world: climate change and a growing global population. According to the UN DESA (2017), the world's population is projected to reach 10 billion people by 2050 (Rouphael & Colla, 2018) resulting how to feed the future world population in a sustainable way a major global societal challenge. This demographic growth leads to an increase in consumption, increasing greenhouse gas (GHG) emissions that modify the climate. In this sense, it is well known that rapid population growth worsens the impacts of climate change by depleting resources, increasing temperatures, and exposing more people to climate-related risks (Washington & Kopnina, 2022).

Global average surface temperatures are expected to increase by 0.3 to 4.8 °C by the end of this century. The imbalance caused by the carbon and nitrogen (N) cycles on the planet is a major driver of GHG fluxes and contributes to more than 90% of anthropogenic climate warming (Ma et al., 2025). As temperatures continue to rise, more soil desertification and salinization will be observed. This will have negative repercussions on agricultural productivity and plant growth (Hassani et al., 2021). As incomes rise, people will increasingly consume more resource-intensive, animal-based food (Nunes, 2023; Vladimir Kuzmanovski, 2019). In this sense, the transition to a more animal-centred diet is putting further pressure on crop productivity worldwide (Henchion et al., 2014). Global demand for animal-derived food products is expected to increase by more than 70% by 2050, which requires a significant increase in protein-rich forages. This demand arises not only from global population growth but also from rising animal welfare standards (Deolu-Ajayi et al., 2022). As animal welfare improves, the proportion of animal-based food products in daily diets also increases. For example, in Western diets, the proportion of protein derived from animal sources has increased from 40% in the 1960s to over 60% today (Deolu-Ajayi et al., 2022; Jalil et al., 2020). Consequently, the demand for animal feed is expected to increase again since to produce 1 kg of animal protein, up to 6 kg of plant protein is required (Deolu-Ajayi et al., 2022). At the same time, it is urgent to reduce GHG emissions and the agriculture sector plays a key role; in fact, one-quarter of the world's GHG emissions come from agriculture, forestry, and land-use change (Ahmed et al., 2020). Therefore, agricultural production is strongly affected by

and a major contributor to climate change. In this sense, agrochemicals have been at the centre of the agricultural sector in recent decades to improve growth and productivity, but their excessive use represents a threat to the environment and ecosystems (Hamedani et al., 2020; Zingale et al., 2024). The development of a sustainable agricultural sector, capable of addressing the key challenges of agricultural production in an environmentally friendly, cost-effective and food security-oriented manner for future generations, is essential. In the last years, various solutions have been proposed to improve the sustainability of production systems using innovative techniques that allow a reduction in the use of agrochemicals. In 2020, the European Commission (EC) introduced the 'Farm to Fork Strategy', setting ambitious targets for the agricultural sector: reduce the use of chemical pesticides by 50 per cent by 2030, reduce nutrient losses by at least 50 per cent, cut fertiliser use by 20 per cent and allocate 25 per cent of the agricultural area to organic cultivation (EC, 2020). Measures taken to promote greater sustainability in agriculture include the introduction of biostimulants in cultivation practices. These products represent an innovative and promising strategy to reduce the use of chemicals in agricultural production by improving the physiological and metabolic responses of plants to biotic and abiotic stresses (Magnabosco et al., 2023; Owen et al., 2015; Vishwakarma et al., 2022). Plant or agricultural biostimulants can be produced from different sources, even waste material. In this regard, biostimulants derived from algae, microbes, and other natural sources are gaining interest as a sustainable strategy that, when applied to plants or the rhizosphere, allows not only to improve the innate capacity of treated plants to cope with tolerance to the abiotic stress and increase productivity but also, to make more efficient use of available resources nutrients (Romagnoli et al., 2024; Shukla & Prithiviraj, 2021; Singh et al., 2018). However, the definition and concept of agricultural biostimulants are still developing, which is partly a reflection of the diversity of inputs that can be considered biostimulants. At this point it is important to highlight that biostimulants do not have direct action against pests, so they do not fall within the regulatory framework of pesticides (Calvo et al., 2014). The term biostimulant was first coined by Zhang and Schmidt (1997) to describe substances that, in small quantities, improve plant growth. The European Regulation EU 2019/1009(EC, 2009) defines biostimulants as a product that stimulates plant nutrition processes with the goal of improving efficiency in terms of nutrient use, tolerance to abiotic stress, quality characteristics and the availability of nutrients in the soil or rhizosphere. According to the literature (marketsandmarkets, 2024) the biostimulants market is estimated to be worth $4.3 billion by 2024 at a compound annual growth rate of 12% and will continue to grow to $7.6 billion by 2029(marketsandmarkets, 2023). Biostimulants could be considered a viable solution for the expansion of the organic food industry, reaching a value of $27.9 billion by 2028. The legume sector is the dominant sector in terms of biofertilizer use, accounting for 46% of the market in 2021. Nitrogen fixers have historically been used in this sector, especially for soybean cultivation (34% of the market in 2021 is represented using specific inoculum for this crop). In the herbaceous crops sector, the use of biofertilizers is predominant and represents 71% of the global market (DunhamTrimmer®, 2020). Biostimulants may contain microorganisms and/or hormone-like substances, whose function is to stimulate processes of nutrients uptake, tolerance to biotic and abiotic stress, and improve crop efficiency and quality(Franzoni et

al., 2022). It is almost impossible to isolate and study a solitary compound, which makes it highly improbable that the efficacy of a biostimulant can be attributed to a single component. Instead, it is believed that synergistic interactions between various bioactive molecules are responsible for its efficacy (Romagnoli et al., 2024; Rouphael & Colla, 2018; Vijay Anand et al., 2018). Within biostimulants, algae biostimulants offer great potential due to their unique qualities. The demand for these products thanks to their high content of bioactive metabolites (such as peptides and amino acids) and plant hormones (e.g., cytokinins) has increased significantly in recent years due to the need to replace commercial chemical fertilizers with biologically based molecules (Rojo et al., 2023; Singh et al., 2018). The cultivation of algae in the open sea eliminates the need to use land in conflict with food production and may help mitigate climate change(Deolu-Ajayi et al., 2022; Duarte et al., 2017; Singh et al., 2018). In addition, algae extracts have demonstrated the ability to increase resilience against abiotic stresses in numerous crops(Baum et al., 2015; Khan et al., 2009). Moreover, the application of seaweed biostimulants can effectively increase protein production in protein-rich crops(Deolu-Ajayi et al., 2022; Petropoulos, 2020). Open-field applications of biostimulants resulted in average yield increases ranging from 8.5% to 30.8%. Single foliar sprays proved effective in increasing yield, with a substantial increase of 14.9% (Li et al., 2022). While soil applications of biostimulants resulted in a yield increase of about 10% (Niu et al., 2021) Among the crops that responded best to the application of biostimulants were vegetables and legumes with an increase in yield per hectare (Hafeez et al., 2006; Li et al., 2022). Another type of biostimulant is produced from microalgae. Microalgae are single-celled, phototrophic organisms with a high capacity to adapt to the environment. They have high photosynthetic efficiency and the ability to grow in non-potable waters such as industrial waters. They have an enhancing impact on plant growth and soil health, probably due to the presence of N-fixing enzymes, soluble AA, bio-mineral conjugates, polysaccharides and phytohormones. Several publications confirm that microalgae metabolites improve soil fertility, provide plant resiliency against biotic and abiotic stresses and at the same time improve the uptake of soil nutrients such as phosphorus, potassium and nitrogen (Parmar et al., 2023; Rojo et al., 2024). In this context, the most relevant question that should be answered definitively is whether the use of biostimulants to improve agricultural productivity is environmentally sustainable? To determine and identify any possible improvement in the environmental impacts resulting from their application in agricultural systems, as a replacement or complement to mineral fertilizers, it is essential to estimate the various environmental impacts resulting from the production phase of biostimulants. The LCA is a powerful environmental knowledge tool, aimed both at communicating ecological information and analysing different scenarios that could improve environmental performance (Ingrao & Wojnarowska, 2023; Zingale et al., 2024). In this sense, the Life Cycle Assessment (LCA) methodology plays a fundamental role, being an efficient and acknowledged tool for quantifying the environmental impacts arising from many different production systems throughout their life cycle (ISO 14040 2006). LCA studies are based on collecting and quantifying the material and energy flows required in production processes, as well as the waste and emissions released into the environment during the entire life cycle (ISO 14040 2006). LCA studies are based on the collection and quantification of material and energy flows required in the production

processes, as well as waste and emissions released into the environment throughout the whole life cycle (ISO 14040 2006). In this study, the authors set out to model a microbial-based biostimulant derived from *Trichoderma s*pp. which can be considered efficient natural plants stimulant, as following a careful review of the literature, it emerged that LCA studies in the field of microorganisms are very limited. Therefore, this study investigates and seeks to better understand the impacts of the use of biostimulants in agriculture. This research encompasses two main goals: I) to conduct an environmental assessment of *Trichoderma spp*. biostimulant production from a LCA approach to anticipate environmental impacts, and II) to identify the environmental hotspots to propose improvement actions. In this regard, to the best of our knowledge, there have been no studies that have analysed the production (and use) of this type of biostimulant from an environmental perspective. The main target audience of this study are farmers, agricultural stakeholders and policy makers who promote biostimulants for sustainable development.

2. Materials and methods

2.1 Biostimulant production system under study

The research was focused on the production of microbial-based biostimulant from an attributional environmental perspective to identify the hotspots and potential environmental improvements. To this end, the LCA methodology was applied following the International Organization for Standardization ISO 14040 and 14044 guidelines (ISO, 2006a; 2006b). The study was restricted to the production at factory gate (cradle-to-gate approach). The processes were considered from the extraction of raw materials to obtaining the bio-product for its subsequent use. For this purpose, a production batch at pilot scale of one cubic meter of biostimulant was considered to study and model the production system. According to it, the functional unit was expressed in terms of volume (1 L) of biostimulant ready to be used in agricultural activities. The consideration of this unit is mainly functional to discuss the results obtained with those available in the literature, where the quantity of biostimulant produced in terms of volume is often taken as a reference (Egas et al., 2023; Rojo et al., 2024). Accordingly, the estimated environmental burdens will be those derived from the processes and the mass and energy flows involved throughout its life cycle. Energy and mass balances were applied to the factory in order to collect all necessary data for the life cycle inventory phase. The system boundaries included all phases from raw material extraction to biostimulant production and waste management. However, transport activities related to the distribution of inputs up to the factory gate and the final application of biostimulants in agricultural operations were excluded from the analysis. The reasons behind these exclusions are related to the authors' decision to focus only on the production cycle to be able to replicate the same modelling anywhere in the world.

Considering the wide range of microbial-based plant biostimulants on the market and the limited availability of data on the environmental impact of their production, it was decided to analyse, as a case study, the production process of a microbial biostimulant based on *Trichoderma* spp., with the technical support of EUROVIX, a leading company in the biostimulant production sector.

The production process has been analysed in detail, allowing the system to be modelled to have access to all inventory data. Figure 1 depicts the system boundaries as well as the flow chart of the microbial-based plant biostimulant production system.

The overall foreground production line has been divided into four main steps: **(1) Pre-culture (SS1),** it is the first step of the production process, in which a starter culture of microorganisms is added within a sterilized solution of water and potato dextrose broth (PDB). PDB is a growth medium that is widely used in the growth of microorganisms and fungi. It has been used because it is one of the most common and efficient substrates in microbiology (Karpagavalli et al., 2024; Obiedallah et al., 2024). The solution is set at 28°C for 5 days in an incubator; **(2) Pre-fermentation step (SS2),** it is the second step and takes place in a 150 L fermenter to which 7 L of pre-culture (two cycles) is added and subsequently brought up to volume with a previously sterilized solution of water and PDB. The culture inside the fermenter remains for 5 days at a temperature of 28°C. **(3) Microorganisms multiplication (SS3)**, it is characterized by the separation of the pre-fermented stream from SS2, in which a part of the product (5 L) is used as inoculum for the solid culture and the rest (145 L) is used as inoculum for the 1000 L fermenter. **Liquid fermentation (SS3.a),** this step is based on the addition and filling of the 145 L of pre-fermented stream with a previously sterilized solution of water and PDB. The growth times and temperatures are the same as in the previous step. **Solid substrate (SS3.b)** This stage aims to achieve the multiplication of those microorganisms that are characterized by the need for a normal level of oxygen. The substrate used is a mixture of grains chosen and used by the company, which is previously sterilized in an autoclave and then inoculated under a laminar flow hood. **(4) Biostimulant final preparation (SS4)**, this is the last step before obtaining the finished product and is structured as follows: the liquid substrate, colonized by microorganisms, ‘washes’ the solid substrate and cleans the seeds of the spores present in them. The resulting liquid is mixed to homogenize the microbial colonies present in the solution and is subsequently used to prepare the final product: 1 L biostimulant packages. The washed seeds are composted in a centre close to the farm. Mass balances have been applied to the production system and detailed information has been collected through specifically designed questionnaires and interviews among the factory workers. On the other hand, the energy consumption in the different steps was measured using meters specially installed for this study. Actual consumption is important to not overestimate electricity consumption and to be able to evaluate these impacts as accurately as possible (Ingrao et al. 2021).

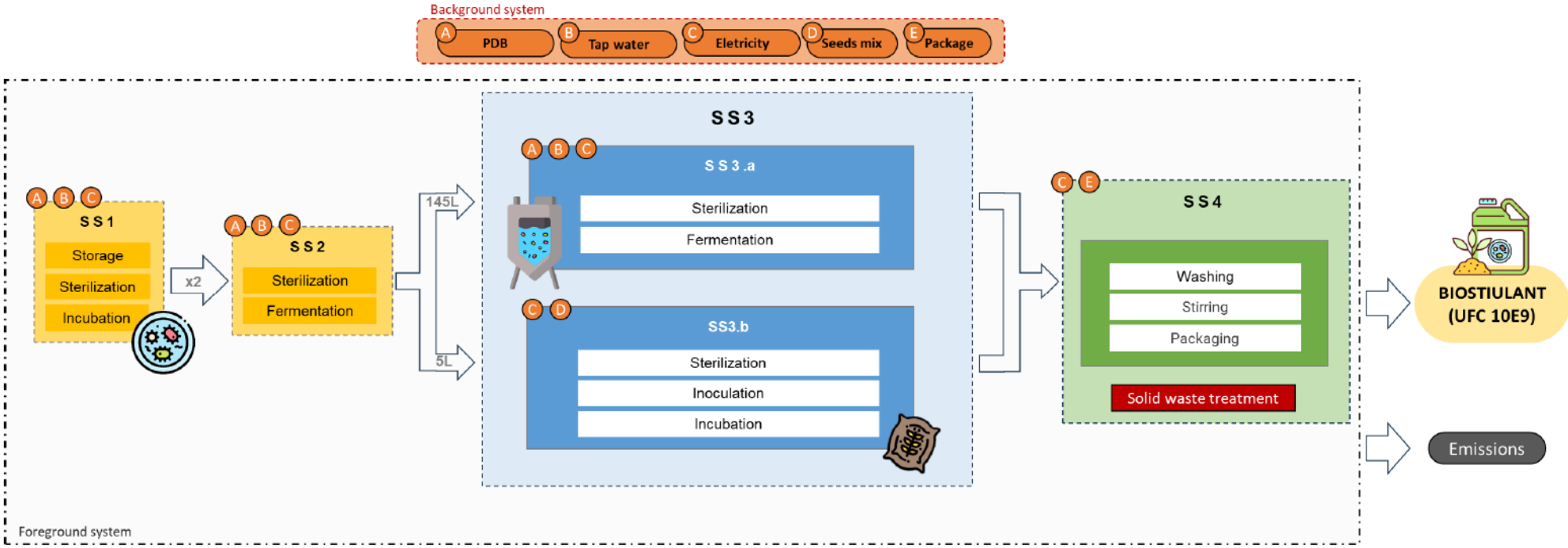


Figure 1 Flow chart of the microbial biostimulant production system under study.

2.2 Life Cycle Inventory

The Life Cycle Inventory (LCI) is the quantification of the most important process inputs and outputs within the system boundaries. LCA practitioners typically gather data from two data sources: primary data and secondary data. Primary data is raw data collected from primary sources (e.g., electricity consumption, water, PDB) and includes data specific to the process under study. Primary data should be used whenever possible, as it is representative of the system. In this study, primary data for the foreground system was used and was provided by EUROVIX operators (Tab. 1). While the secondary data used in this study were energy, water, grains used in the solid substrate, packaging and the input required to obtain PDB. Regarding the background processes associated to raw material production, the Ecoinvent® v3.1 database (Wernet et al., 2016) was used. On the other hand, the production process of PDB was modelled on the SuperPro designer® software, due to the lack of data in the Ecoinvent® database considering information that was gathered from the available literature (Arias et al., 2024; Rojo et al., 2024). The PDB process was modelled following the recipe indicated by the ATCC medium company (ATCC Medium 336, 2024). For each litre of PDB, the following processes were carried out: cutting 300 g of potatoes and subsequent boiling (100°C) in 0.5 L for one hour. This was filtered and brought to a volume of 1 L with the addition of 20 g glucose and finally sterilised in an autoclave at 121°C. Table 2 provides information on the processes considered in the Ecoinvent® v3.1 to produce the biostimulant (secondary data for background processes).

Table 1. Inventory of the production system related to the production of 1 m^3 of product.

Material	Amount	Unit
PDB (Potato Dextrose broth) production		
Water	1.00	kg
Energy	0.16	kWh
Glucose	0.02	kg
Potatoes	0.30	kg
SS1: Pre-culture production		
Energy	47.85	kWh
PDB	3.50	L
SS2: Pre-fermentation step		
Energy	30.35	kWh
Pre-culture (from SS1)	7.00	L
PDB	143.00	L
SS3.a: Liquid substrate		
Energy	173.12	kWh
Pre-fermented liquid (from SS2)	145.00	L
PDB	850	L
SS3.b: Solid substrate		

Energy	124.68	kWh
Pre-fermented liquid (from SS2)	5.00	L
Seeds company mix	14.25	kg
SS4: Biostimulant final preparation		
Liquid substrate (from SS3.a)	955	L
Solid substrate (from SS3.b)	5.00	L
Energy	2.28	kWh
Packaging	98.70	kg

209 **Table 2** Processes considered from the Ecoinvent® v3.1database for biostimulant production.

Process name
PDB (Potato Dextrose broth) production
Glucose {GLO}| market for glucose | Cut-off, S
Potatoes, cooled, market mix, at regional storage {IT} Economic, U
Tap water {RER}| market group for | Cut-off, U
SS1: Pre-culture production
Electricity, low voltage {IT}| market for | Cut-off, U
PDB
SS2: Pre-fermentation step
Electricity, low voltage {IT}| market for | Cut-off, U
PDB
SS3.a: Liquid substrate
Electricity, low voltage {IT}| market for | Cut-off, U
PDB
SS3.b: Solid substrate
Rice, non-basmati {GLO}| market for rice, non-basmati | Cut-off, U
Wheat bran {RoW}| market for wheat bran | Cut-off, U
Electricity, low voltage {IT}| market for | Cut-off, U
SS4: Biostimulant final preparation
Electricity, low voltage {IT}| market for | Cut-off, U
Packaging, for pesticides {GLO}| market for packaging, for pesticides | Cut-off, U
Transport, freight, lorry 7.5-16 metric ton, euro4 {RER}| market for transport, freight, lorry 7.5-16 metric ton, EURO4 | Cut-off, U
Compost {RoW}| treatment of biowaste, industrial composting | Cut-off,U

210 ***2.3 Life cycle impact assessment***

211 The Life Cycle Impact Assessment (LCIA) has been developed by aggregating material and energy
212 inventories into a limited set of intermediate impact categories which were quantified considering the

characterization factors of the ReCiPe 2016 V1.06 Hierarchist Midpoint World (2010) method (Huijbregts et al., 2017), which is included in the Simapro v9.4 software (Huijbregts et al., 2017). The inventory data were translated into environmental impacts and classified into nine impact categories according to their relevance in the agriculture sector (Costa et al., 2020; UNEP-SETAC Life Cycle Initiative, 2019) The impact categories selected are Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS). Following the objectives of this study, attention will not only be paid to identifying environmental burdens, but also to suggest alternatives in the production cycle to reduce the environmental impact of microbial biostimulant production by comparing different scenarios.

3. Results and Discussions

3.1 Environmental analysis

As detailed above, transportation activities related to product distribution as well as the delivery of inputs to the factory were excluded from the analysis First, because the location of the plant was not taken into account and, furthermore, because the analysis focused on the production chain and not on the entire value chain. The overall average environmental impacts of the evaluated production system are presented in Table 3. The relative contributions of the environmental impacts for the subsystems involved in the production of *Trichoderma*-based biostimulant are detailed in Figure 2, and the values of each midpoint can be found in Table 3. Firstly, the most impactful subsystem involves the production of the liquid substrate (SS3.a). According to the results, SS3.a has an impact of more than 50% in almost all categories. The category in which the value is highest is SOD (71%). With the exception of FE, this value decreases below the average to 35% because in this specific case, SS4 is the most important process with 46%. Eutrophication represents the absence of species in freshwater due to excessive phosphorus concentrations (Huijbregts et al., 2017). In the SS4 process, the impact is caused by plastic packaging, which significantly affects the FE. SS4 in the other impact categories is not a relevant cause. The second most impactful process in the production chain is SS3.b, particularly in impact categories ME (22%) and GW (18%). The other production processes appear less impactful, with a constant trend and percentage values generally not exceeding 10%. As demonstrated by Rojo et al. 2024, in the production of algae-based biostimulants, the cultivation process turns out to be the most impactful, the same applies to the analysed biostimulant, where the multiplication of microorganisms appears to be the most relevant process.

Table 3 Characterisation results for the different impact categories analysed. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

Impact category	Unit	Biostimulant
GW	kg CO_2 eq	368
SOD	g CFC11 eq	0.93
TA	kg SO_2 eq	1.57
FE	kg P eq	0.236
ME	kg N eq	0.158
TET	t 1,4-DCB	2.30
FET	kg 1,4-DCB	53.3
MET	kg 1,4-DCB	39.1
MRS	kg Cu eq	0.813

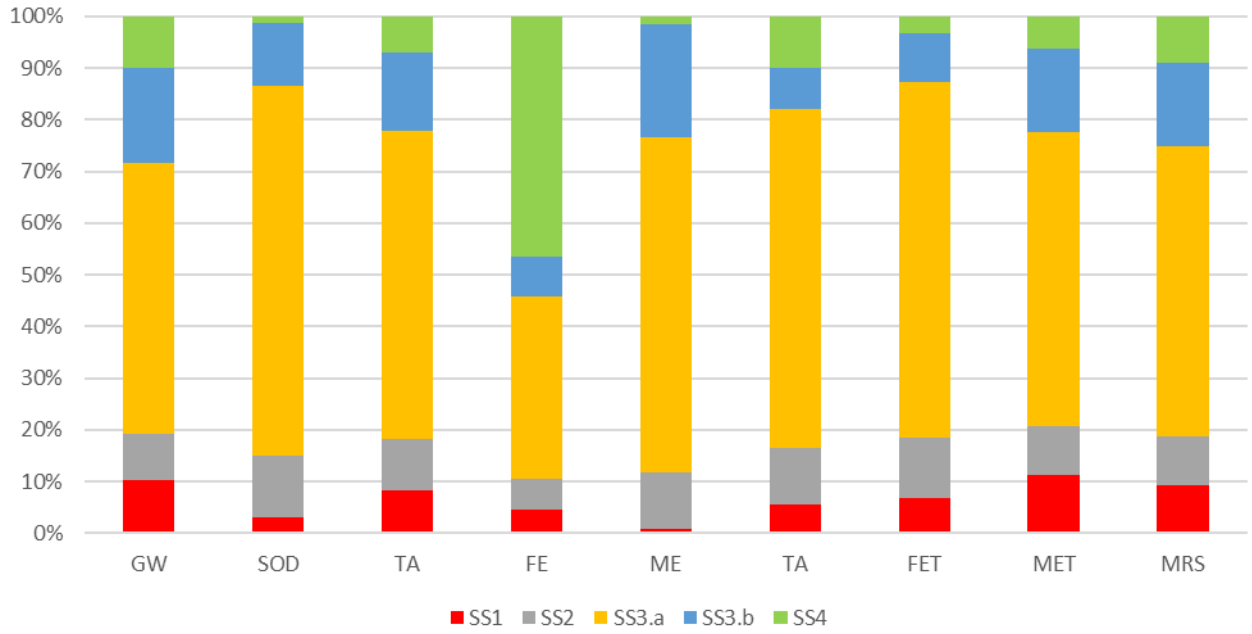


Figure 2 Environmental profile of the *Trichoderma*-based biostimulant production. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

To identify production alternatives and propose improvements, it was crucial to identify the processes or raw materials that have the greatest impact on the production system. PDB, electricity, and the seed mix were found to be among the main causes of impact (Figure 3). GW is mainly influenced by PDB (40%) and electricity (44%), mainly due to the amount of energy used and potato cultivation in PDB. The other group has an impact of 46% in the FE impact category. However, PDB is the most impactful element of the

production process, especially in the main SOD, ME, TET and FET categories. PDB is a necessary substrate for the multiplication of microorganisms (Karpagavalli et al., 2024; Obiedallah et al., 2024). In other studies, on algae-based biostimulants, electricity was found to be the component with the greatest environmental impact (Arias et al., 2024; Rojo et al., 2024).

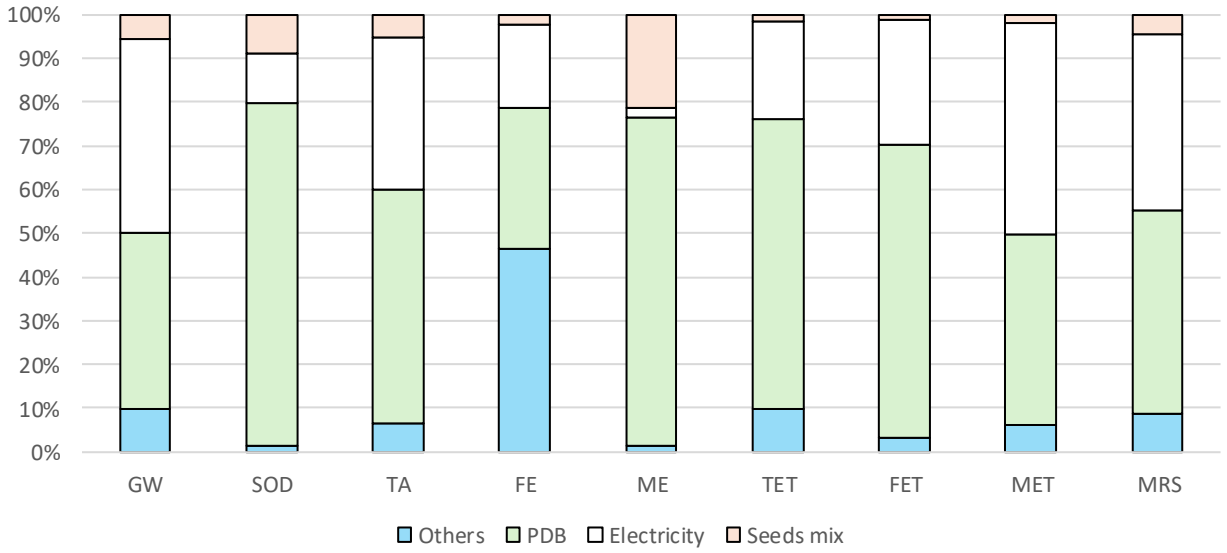


Figure 3 Production factors most impacting the environmental profile of the *Trichoderma*-based biostimulant production. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

Figure 4 shows the SS3.a production process, there are two elements used in this process, the PDB and electricity. PDB appears to have the greatest impact in all midpoints. For the ME and SOD categories, PDB is responsible for more than 90%, which is related to the use of fertilisers used in potato cultivation (Lago-Olveira et al., 2024).

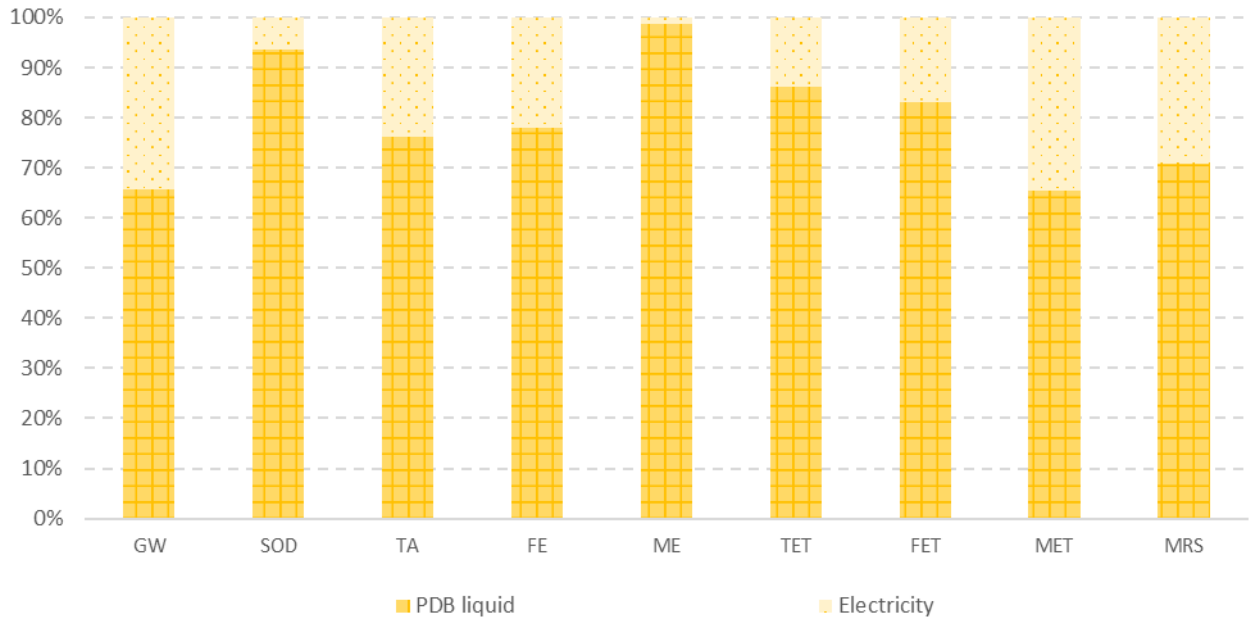


Figure 4 Distribution of the environmental impacts of SS3.a. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

The reason why the production of the PDB is so remarkable in terms of environmental impact is related to the production process of the PDB itself and specifically due to the main raw material needed to obtain it: the potato (Figure 5). Therefore, and because the limits of the system are cradle-to-gate, the production of the potato needed to obtain the PDB is the main responsible for the environmental impact. In this respect, it should be noted that potato production is a crop that requires many inputs, including the main fertilisers that are responsible for the high level of SOD and ME (González-García et al., 2021). Potato use is the main impactor, ranging from 38% to 91% of the impact. Electricity was found to be impacting 44% of the GW and glucose use in the MRS 40%.

The GW is split between the consumption of electricity and the PDB due to the consumption of fuel for agricultural processing or the use of fossil fuels for energy. The PDB was modelled with Superpro software (Arias et al., 2024; Rojo et al., 2024), , in this case potatoes were considered as the main source of carbohydrates, it would be useful to investigate this further and replace potatoes with another carbohydrate-rich plant matrix.

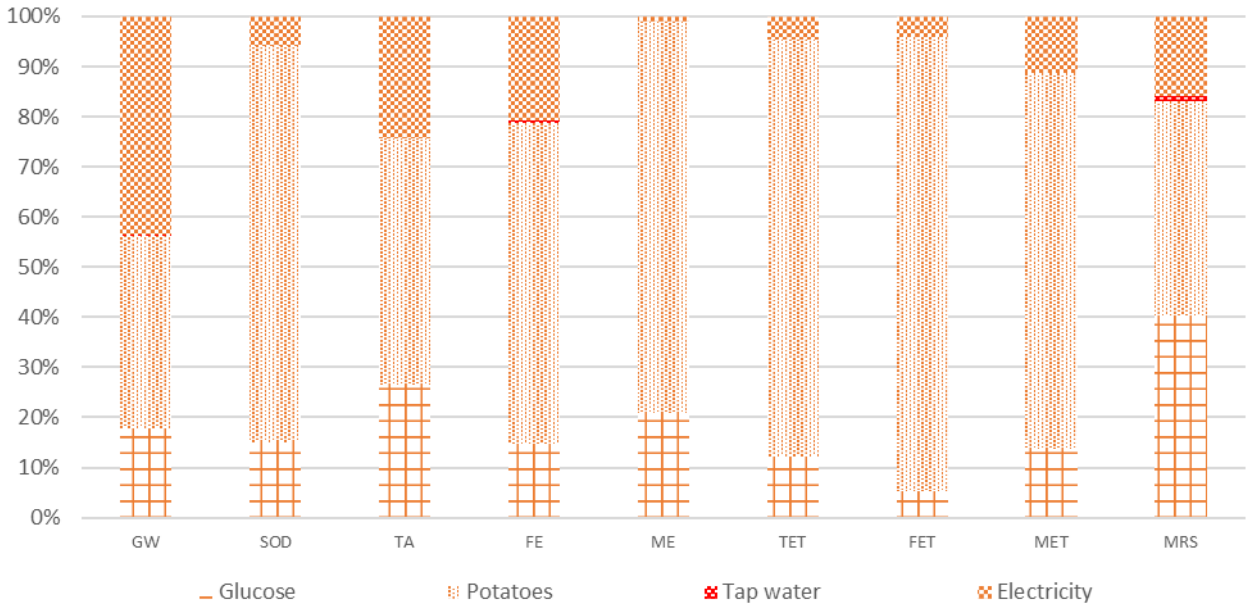
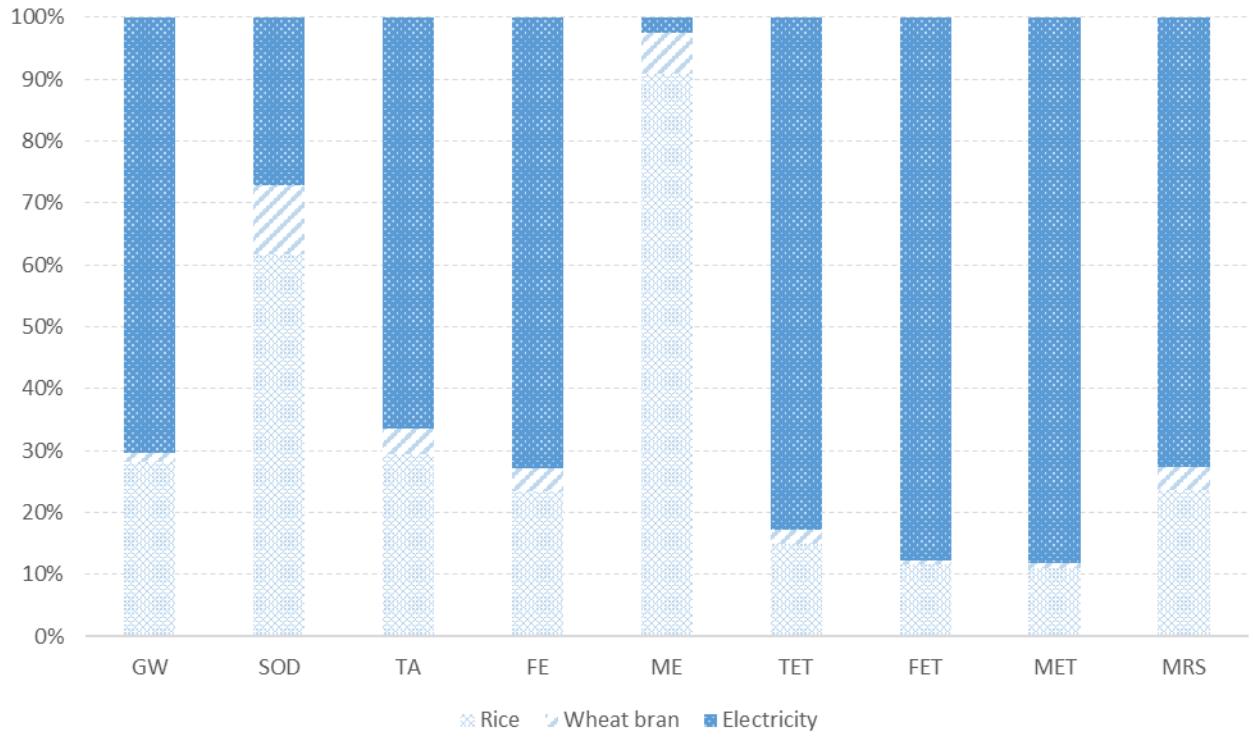


Figure 5 Distribution of the environmental impacts of PDB production. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

As shown in Table 1, the main components required to produce the solid substrate (SS3.b) are the seeds mixture used and energy. The production of the energy is the main contributor to the impacts in almost all impact categories with values usually above 70%. While the impact of the use of rice is highest in terms of SOD (62%) and ME (91%), where the production of the seeds mixture is the hot spot (Figure 6). As with potato cultivation in the case of PDB, the impacts of rice production are evident in the cultivation of this cereal.



297 **Figure 6** Distribution of the environmental impacts of SS3.b. Impact categories: Global Warming (GW),
298 Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE),
299 Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine
300 Ecotoxicity (MET) and mineral resource scarcity (MRS).

301 The last of the highlighted processes is SS4, which is among the least impactful, and in which the packaging
302 and transport stages for waste disposal are present. As shown in Figure 7, packaging is the most impactful
303 stage. Packaging is affecting more than 90% of all impact categories. Mainly for the following reasons, raw
304 material used, and quantity of plastic used.

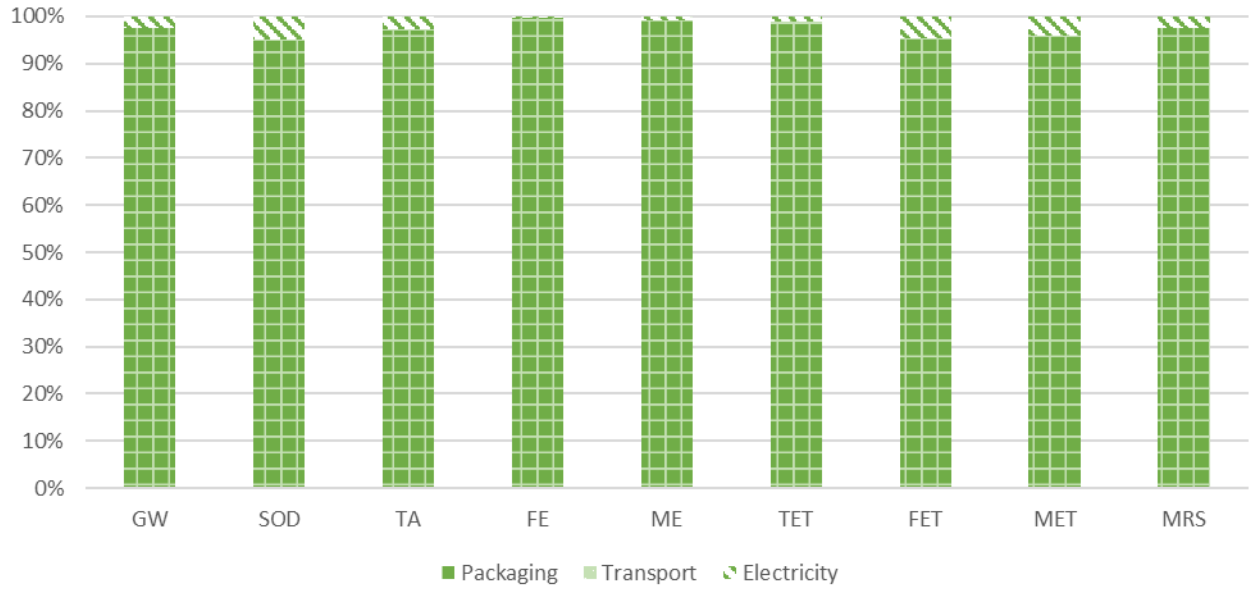


Figure 7 impact of the final process of production. Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS).

3.2 Production process improvement

There is great interest in the application of all those sustainable agricultural practices with a low environmental impact. Biostimulants can be an alternative or a valuable contribution to the reduction of chemically synthesised products (Egas et al., 2023; Magnabosco et al., 2023).

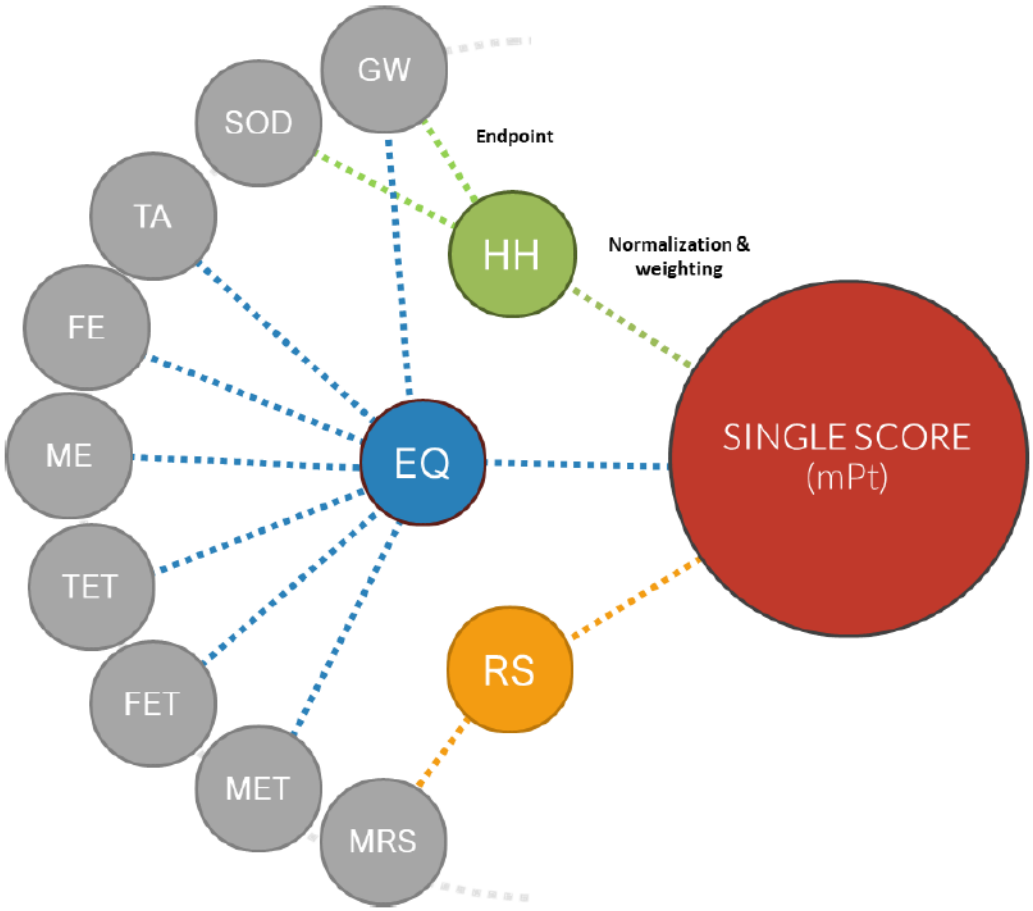
As specified by the company and scientific studies, PDB is essential for the growth of microorganisms, thus no alternatives could be proposed (Karpagavalli et al., 2024). Bearing in mind the environmental hotspots (seeds mix and electricity), improvements have been proposed to identify potential alternatives. There are three scenarios to compare with the current production system (henceforth Scenario A):

Scenario B: this alterative production system considers the use of an alternative cereal (e.g. wheat) instead of rice, in the preparation of the seed mix.

Scenario C: the production of electricity requirements has been identified as an environmental hotspot, which is directly taken from the Italian electricity mix and considerably depends on fossil sources. Therefore, the use of photovoltaic panels (PV) has been considered to produce electricity requirements.

Scenario D: is a combination of Scenario B and C.

To compare the different alternatives proposed in the study, an endpoint level methodology was used, which assigns a single environmental score for each production process (Pt). For this purpose, normalisation and weighting factors taken from the ReCiPe 2016 Endpoint method were considered. The analysis at endpoint level provides results through three main indicators representing the severity of damage in the following areas: Human Health Impact (HH), Ecosystem Quality (EQ) and Resource Scarcity (RS). Aggregating the midpoint categories and characterising them towards specific endpoints results in the three main endpoint (Figure 8).



330

331 **Figure 8** Schematization for estimating individual endpoints. Impact categories: Global Warming (GW),
332 Stratospheric Ozone Depletion (SOD), Terrestrial Acidification (TA), Freshwater Eutrophication (FE),
333 Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET), Freshwater Ecotoxicity (FET), Marine
334 Ecotoxicity (MET) and mineral resource scarcity (MRS).

335 The analysis focused on the influence of the change of two key variables identified and highlighted in Figure
336 3, representative of two of the system's environmental hotspots: the seeds mix and the self-production of
337 electricity through photovoltaic panels (instead of drawing electricity directly from the grid). The main
338 objective is to predict the possible environmental benefits of these potential changes. Comparing the
339 midpoints of the proposed alternatives, the best scenario appears to be the one where the seeds mix
340 alternative, and the self-production of photovoltaic panels are combined. The GW of biostimulant BS is
341 reduced by 52% (Table 4) compared to classical biostimulant (Table 3). The impact category TA is also
342 reduced by 30% compared to the classic biostimulant. However, the self-generation of electricity with
343 photovoltaic panels reduces several midpoints considerably.

344 **Table 4** Characterisation results for the different impact categories following the proposed improvement
345 Impact categories: Global Warming (GW), Stratospheric Ozone Depletion (SOD), Terrestrial Acidification
346 (TA), Freshwater Eutrophication (FE), Marine Eutrophication (ME), Terrestrial Ecotoxicity (TET),
347 Freshwater Ecotoxicity (FET), Marine Ecotoxicity (MET) and mineral resource scarcity (MRS)

Impact category	Unit	Scenario B	Scenario C	Scenario D
GW	kg CO_2 eq	358.12	184.51	175.22
SOD	g CFC11 eq	1.02	0.80	0.90
TA	kg SO_2 eq	1.62	1.06	1.12
FE	kg P eq	0.24	0.21	0.21
ME	kg N eq	0.28	0.16	0.28
TET	t 1,4-DCB	2.30	3.22	3.24
FET	kg 1,4-DCB	53.19	56.98	57.22
MET	kg 1,4-DCB	39.08	44.21	44.47
MRS	kg Cu eq	0.82	1.14	1.15

Vavrova et al. 2022 reported the GW 200 kg CO_2 eq to produce a biostimulant from chicken feathers; this value is superimposable to the production of the biostimulant PV. The percentages of the three endpoints are as follows in all 4 scenarios: 93% human health, 5% ecosystems, and 2% resources. Figure 9 shows the endpoints of the different alternatives proposed. With the use of photovoltaic systems and the use of winter cereal, the 'best case scenario' is reduced to 31% for human health, 18% for ecosystems and 52% for resources. It can also be seen that the application of photovoltaic panels alone drastically reduces the score compared to changing the seed mix. The authors proposed the use of autumn-winter cereals to produce the solid substrate, which requires less input than rice. However, the change compared to the original biostimulant is only 2%.

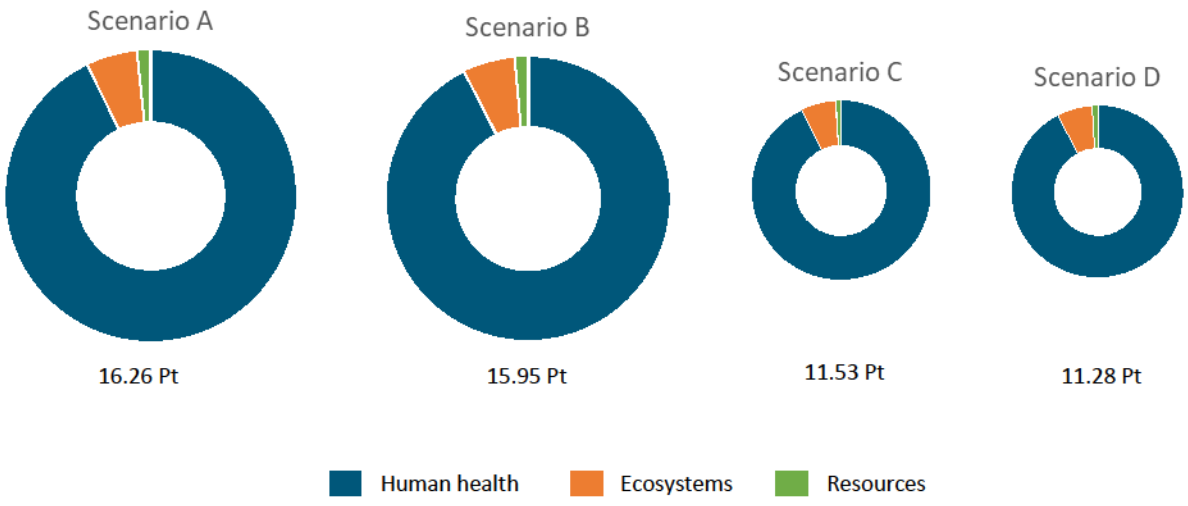


Figure 9 Impact of the *Trichoderma*-based biostimulant production process in alternative scenarios: autumn-winter cereals (Scenario B), photovoltaic panels (Scenario C) and a combination of Scenarios B and C (Scenario D).

4. Conclusions

In recent years, to ensure the food security of a growing world population, chemical fertilizers have been used intensively to increase agricultural productivity (Rose et al., 2014). However, this overuse poses a serious anthropogenic threat to global terrestrial and aquatic ecosystems. To solve this problem, it is imperative to develop sustainable strategies that increase agricultural production while reducing the use of chemicals (Shukla & Prithiviraj, 2021). The main causes of emissions in agriculture include nitrogen-based chemical fertilization (Hafeez et al., 2006; Rebolledo-Leiva et al., 2022). In this study, the modelling of a *Trichoderma*-based biostimulant production was investigated in depth. Biostimulants could contribute to reducing the use of chemical fertilizers by 2050, as requested by the European commission (Li et al., 2022). The research has achieved the proposed objective, namely, to model the entire production process of *Trichoderma*-based biostimulant production and to evaluate the environmental issues arising from the production of these products. Few articles in the literature focus on this topic. Most of the articles consulted focused on the environmental impact assessment of products based on algae but not on microorganisms. It was also difficult to find articles that modelled these processes. In recent years, the use of biostimulants and the attention to reducing environmental impacts has grown. It is important to understand the real advantage of these products compared to the traditional fertilizers used in agriculture. As can be seen from Figure 2, the production of liquid substrate is the most impactful process, mainly due to the amount of PDB used. Alternative or waste materials could be used to reduce the environmental impact of the *Trichoderma*-based biostimulant production process. In addition, autonomous energy production was effective in reducing much of the environmental impact. While the variation of cereal proposed as an alternative in the preparation of the solid substrate reduces the impact by 2%. In addition, a further topic that could be explored is the use of packaging capable of holding larger volumes or eco-friendly packaging that can be a viable alternative to reduce environmental impact. This article forms the basis for evaluating other aspects of cultivation and environmental impact assessment in the agricultural sector to make agriculture more sustainable and, above all, to have effective tools to show the real sustainability of agricultural practices and products used.

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Declarations

Ethics approval: Not applicable.

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Conclusions

In general, the research conducted during the PhD course focused on innovative and sustainable agricultural practices to improve crop productivity and quality while measuring and reducing environmental impact. The main objectives can be summarised as follows:

1. **Optimising crop productivity and quality through sustainable practices:** A central objective of the study is the adoption of sustainable agricultural practices, such as the use of biostimulants, microorganisms, and genetic selection, to improve crop yield and quality. Chapters I and II highlighted the importance of choosing varieties suited to specific climatic conditions and applying treatments that improve plant resistance to biotic and abiotic stresses. The use of biostimulants and microorganisms has shown a positive impact on plant productivity and nutritional value, demonstrating that the adoption of innovative practices can significantly improve crops in the context of climate change. The research emphasizes the importance of selecting varieties that can adapt to climate change, such as choosing drought-resistant cultivars for lentils or adopting agricultural practices that improve soil water retention, such as sod seeding (Chapter II).

2. **Modelling of biostimulants used in agriculture and experimental trials:** Assessing and quantifying the environmental impact of the *Trichoderma*-based biostimulant (Chapter III) was key to quantifying the effect in an agricultural production system. In addition, the proposed ecological solutions, such as variations in the substrates used in production and the self-production of energy, are some solutions proposed to further decrease the environmental impact. This article lays the foundation for subsequent work based on environmental impact assessment in agriculture combined with agricultural practices such as rotations and different soil management

Future research perspectives in legume science include the application of machine learning techniques. These tools can be used to analyse and predict agricultural trends, to optimize crop yields, and to improve the environmental sustainability of legume cultivation. The integration of artificial intelligence in agriculture allows the management of large datasets - including agronomic, climatic and soil variables - supporting more informed decision-making and customised cropping strategies based on specific growing areas. In this context, the modeling of microbial-based biostimulants, already initiated in this research, provides a solid basis for assessing environmental impacts not only to single crops but also in the context of crop rotations and different cropping scenarios. This approach will allow the exploration of systemic, long-term effects of biostimulant use on agroecosystems. In addition, the integration of machine learning with Life Cycle Assessment (LCA) provides a powerful approach to identifying environmentally optimal farming scenarios. This synergy makes it possible to simulate multiple growing conditions and identify the most sustainable strategies according to the specific characteristics of the growing region. The combined methodology can contribute to the development of a more efficient, resilient and green agricultural sector.
In conclusion, the main objectives of this research are to improve agricultural productivity, promote nutritional quality, reduce the environmental impact of agricultural practices, and make agriculture more resilient to climate change, all through the adoption of sustainable technologies and methods.

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