T.R.
ONDOKUZ MAYIS UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF SOIL SCIENCE AND PLANT NUTRITION

EKOFERTILE AND *MICROFERTILE* PLANT BIOSTIMULANTS POTENTIALS ON SOIL QUALITY AND WHEAT NUTRITION ENHANCEMENT

Master's Thesis

David TAVI AGBOR

Supervisor
Prof. Dr. Orhan Dengiz

II. Supervisor

Prof. Dr. Andon Vasilev

SAMSUN
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2023

.ACCEPTANCE AND APPROVAL OF THE THESIS

The study entitled **"*EKOFERTILE* AND *MICROFERTILE* PLANT BIOSTIMULANTS POTENTIALS ON SOIL QUALITY AND WHEAT NUTRITION ENHANCEMENT."** prepared by **David TAVI AGBOR**, and supervised by **Prof. Dr. Orhan Dengiz and Prof. Dr. Andon Vasilev,** was found successful and unanimously accepted by committee members as Master thesis, following the examination on the date …/…/2023 .

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Chairman	Prof. Dr. Orhan Dengiz Ondokuz Mayıs University Department of Soil Science and Plant Nutrition	☒ Accept ☐ Reject
Member	Prof. Dr. Violina Angelova Agricultural University of Plovdiv Department of General Chemistry	☒ Accept ☐ Reject
Member	Prof. Dr. Çoşkun Gülser Ondokuz Mayıs University Department of Soil Science and Plant Nutrition	☒ Accept ☐ Reject

This thesis has been approved by the committee members that already stated above and determined by the Institute Executive Board.

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Prof. Dr. Ahmet TABAK
Head of Institute of Graduate Studies

DECLARATION OF COMPLIANCE WITH SCIENTIFIC ETHIC

I hereby declare and undertake that I complied with scientific ethics and academic rules in all stages of my Master's Thesis , that I have referred to each quotation that I use directly or indirectly in the study and that the works I have used consist of those shown in the sources, that it was written in accordance with the institute writing guide and that the situations stated in the article 3, section 9 of the Regulation for TÜBİTAK Research and Publication Ethics Board were not violated.

Is Ethics Committee Necessary?

Yes ☐ (If it necessary, please add appendices.)

No x

23 /08 / 2023
David TAVI AGBOR

DECLARATION OF THE THESIS STUDY ORIGINALITY REPORT

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ÖZET

EKOFERTILE VE MICRO FERTILE BİTKİ BİYOSTİMÜLANLARI TOPRAK KALİTESİ VE BUĞDAYIN BESLENMESİNİ İYİLEŞTİRME POTANSİYELLERİ

David TAVI AGBOR
Ondokuz Mayıs Üniversitesi
Lisansüstü Eğitim Enstitüsü
Toprak Bilimi Ve Bitki Besleme Bölümü
Yüksek Lisans, Ağustos /2023
Danışman:Prof. Dr. Orhan DENGİZ
II Danışman: Prof. Dr. Andon Vasilev

Sürekli büyüyen nüfus ve kaliteli gıda ile besleme ihtiyacı göz önüne alındığında, tarımsal üretim sürecinde çevre dostu girdilerin kullanılması gerekmektedir. Sentetik gübrelerin gıda güvenliğini sağlamada önemi göz önüne alındığında, negatif etkileri aşırı derecede göz ardı edilmiş ve zararlı çevresel ve insan sağlığı tehlikelerine yol açmıştır. Bu nedenle, gıda üretimini nicelik ve nitelik olarak sürdürmek için bütünsel gübre kaynaklarına ihtiyaç vardır. Ekolive ekofertile® ve microfertile® bitki biyostimulanları, laboratuvar sonuçlarına dayanarak besin ve bitki büyüme teşvik eden mikroplar içeriğiyle birlikte Avrupa Komisyonu rehberine göre biyostimülan gereksinimlerini sergileyerek gıda ve beslenme güvencesini bütünsel olarak azaltmada harika bir uyum sağlıyor gibi görünmektedir.

Bu çalışma, ekofertile® ve microfertile® bitki biyostimulanlarının toprak kalitesini artırma ve buğday verimliliği üzerindeki potansiyelini araştırmak amacıyla yapılmıştır. Çalışma, sera ortamında Samsun, Türkiye'nin Karadeniz Bölgesi'nde kil ve killi-tınlı olmak üzere iki farklı toprak türünde üç kez tekrarlanan iki biyostimulan ve kontrol ile inorganik gübreleme dahil beş doz seviyesinden oluşan bir ayrık-parsel tasarımında gerçekleştirilmiştir. Toprak fiziko-kimyasal ve biyolojik özellikler ile buğday büyüme parametreleri ve verimi değerlendirilmiştir. Toplanan veriler R-programlama ve analitik hiyerarşi süreci kullanılarak ayrık-parsel modeli analizine tabi tutulmuştur. Sonuçlar, toprak fizikokimyasal ve biyolojik özelliklerin bir göstergesi olan toprak kalite indeksinin biyostimulan ile önemli ölçüde değiştiğini göstermiştir. Killi toprak için en iyi etkiyi %10 dozajda (0.66) elde edilirken, tınlı toprak için en yüksek etki %5 dozajında (0.65) görülmüştür. Bu doğrultuda, buğday biyolojik verimi tınlı toprakta en iyi sonuçları %10 dozajında (24.46 tha^{-1}) ve bitki başına tahıl ağırlığı (2.1 g) ile artırmıştır. Killi toprakta ise biyolojik verim (14.04 tha^{-1}) ve bitki başına tahıl ağırlığı (1.1 g) en iyi sonuçları %10 dozajında sağlamıştır.

Sonuçlar, ekofertile® ve microfertile®'ın inorganik gübreleme için harika sürdürülebilir alternatifler olduğunu ve harika toprak kalitesi ve ürün verimliliği yeteneklerine sahip olduklarını ortaya koydu.

Anahtar Sözcükler: dağ toprakları, eğim yönü, toprak sıcaklığı ve su tutma.

ABSTRACT

EKOFERTILE AND MICROFERTILE PLANT BIOSTIMULANTS POTENTIALS ON SOIL QUALITY AND WHEAT NUTRITION ENHANCEMENT

David TAVI AGBOR
Ondokuz Mayıs University
Institute of Graduate Studies
Department of Soil Science and Plant Nutrition
Master, August/2023
Supervisor: Prof. Dr. Orhan DENGİZ
II. Supervisor: Prof. Dr. Andon Vasilev

Considering the ever-growing population and the need to feed them with quality food requires eco-friendly inputs in agricultural production. Given that synthetic fertilizers have been gorgeous in ensuring food security, their pejorative effects have been overzealously ignored, leading to deleterious environmental and human health hazards. Therefore, there is a need for holistic fertilizer sources to sustain food production in quantity and quality. ekolive´s *ekofertile*® and *microfertile*® plant biostimulants appear as an awesome fit to abate food and nutrition insecurity holistically based on their laboratory results nutrient and plant growth-promoting microbes content with the exhibition of biostimulant requirements according to the European Commission guide on biostimulants.

Thus this work sought to investigate *ekofertile*® and *microfertile*® plant biostimulants' potential for soil quality enhancement and wheat productivity. The work was set out in a split-plot design consisting of two biostimulants with five dosage levels, including control and inorganic fertilization replicated three times across two soil types (clay and loam soil) in the greenhouse at the Black Sea Region of Samsun, Turkey, during the 2022 and 2023 growing seasons. Soil physicochemical and biological properties were assessed alongside wheat growth parameters and yield. The data collected was subjected to split-plot model analysis using R-programming and analytical hierarchy process.

The result revealed that the soil quality index, which is an embodiment of soil physicochemical and biological properties, was significantly modulated by the biostimulant with the highest at 5% dosage (0.65) for loam soil, while for clay soil, 10% dosage had the best effect (0.66). The biological wheat yield was significantly improved with the best result at 10% dosage (24.46 tha^{-1}) and the grains weight per plant (2.1 g) for loam soil, while for clay soil, biological yield (14.04tha^{-1}) and grains weight per plant (1.1 g) were best at 10% dosage. The result revealed that *ekofertile*® and *microfertile*® are great sustainable alternatives to inorganic fertilization with awesome soil quality and crop productivity abilities.

Keywords: Soil quality index, Biostimulants, Biological yield, *Ekofertile*® and *microfertile*®

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David TAVI AGBOR

CONTENTS

ABBREVIATION OF TERMS

MC	: Moisture content
SQI	: Soil quality index
LB	: Less is better
MB	: More is better
AHP	:Analytical Hierarchy Process
SSF	: Standard Scoring Function

FIGURES LEGENDS

TABLES LEGENDS

1. INTRODUCTION

The world population is expected to increase by 9 billion in 2050. This implies a massive increase in food demand, whereas there is a 30% increase in hunger prediction by this said year due to climate change (Tripathi et al., 2019; United Nations, 2019). Therefore, this warrants using sustainable alternatives to mitigate climate change while increasing food production to meet the global need (Kour et al., 2019; Agbor et al., 2022). The advent of synthetic fertilizers to tackle the problem of soil fertility to increase production has rather manifested in environmental and human health havoc like leaching and contaminating water bodies, reducing water quality, and at times causing eutrophication, killing aquatic lives (Koli et al., 2019; Pahalvi et al., 2021). They also introduce toxic chemicals into food that cause various human diseases, including cancer (Zhang et al., 2018; Rahman and Zhang, 2018).

The quest to search for sustainable alternatives to synthetic fertilizers for healthy food production emanated to biostimulants which are viewed as potential tools to limit induce climate change stress and lowering addiction to synthetic fertilizers (García-Fraile et al., 2017; Swift et al., 2018). With the definition of biostimulants still in contention, they primarily include natural plant and animal materials and have been classified into several categories by the European Commission (European Parliament, 2019). Biostimulants are sustainable alternatives for increasing agricultural production by enhancing nutrients uptake, nutrient use efficiency, tolerance to abiotic stress, increasing quality of crop yields and availability of confined nutrients in the soil or plant rhizosphere (Chiaiese et al., 2018; García-García et al., 2017). Improvement of agricultural crop yields has been the majority outcome from most of the biostimulants used so far, but crop yield is subject to the genotypic and phenotypic conditions of the crop (Schutz et al., 2018; Li et al., 2022). The biostimulant market is ever-increasing and was around 800 million euros in 2018, with an annual growth potential of more than 10% due to its safe environmental and human health potential (Traon et al., 2014). It is, therefore, necessary to test biostimulants through due process of scientific trials to ascertain their performance with results that will be vital to the farmers as different agronomic practices and environmental conditions may lead to different results for a given biostimulant (Ricci et al., 2019).

The biostimulants produced on the basis of bioleaching of sand and milled silicified rock residues after coal mining by EKOLIVE company have proven to improve nutrient use efficiency, tolerance to abiotic stress, crop quality traits or availability of confined nutrients in the soil and rhizosphere at random farmers' field usage. Lab analysis results for these products have revealed overwhelming macronutrients, micronutrients, beneficial microbes and organic acids presence with the potential to increase resistance to abiotic stress, increase nutrient use efficiency, crop quality traits or availability of confined nutrients in the soil and rhizosphere, all within the EU categorization of biostimulants. To ascertain these claims, it is therefore necessary to carry out scientific experimental trials of EKOLIVE biostimulants across different crops in different agroecological conditions in the lab, greenhouse and in the field to generate data that will warrant putting up the products in the EU fertilizer market as well as globally (Ricci et al., 2019). Thus this work was set out to test the performance of EKOLIVE biostimulant products based on bioleaching of sand and milled silicified rock residues after coal mining on soil quality and performance of wheat in the Black Sea region of Samsun, Turkey.

1.2. Problem statement

With the 30% increase in hunger projection by 2050 resulting from climate change and the problem exacerbated by the prediction of a 9 billion increase in human population, there is a need for eco-friendly alternative fertilizers to meet the global food demand (Del Buono, 2021). Over the years, chemical fertilizers have been used intensively to meet the food needs of the ever-growing world population. However, this has come with tremendous ecosystem and egregious human effects. The challenges of chemical fertilizers and climate change have made the world to evolved into an era of biostimulants that are ecosystem and human-health-friendly (Yakhin et al., 2017; Zulfigar et al., 2020). Demanding better biostimulants that can replace chemical fertilizers while ensuring an increase in food production prompted the production of biostimulants based on bioleaching of sand and milled silicified rock residues after coal mining by EKOLIVE company. Random results of these products from farmers have shown them to fall within the categorization of biostimulants by the European Commission. To ascertain the effectiveness of these products and to put them in the EU and global fertilizer markets, there is the need to test them through the due process of scientific trials and analysis (Krouk, 2015; Rouphael and Colla, 2018; Rouphael et al., 2018). Thus, this work was set to test biostimulants based on bioleaching of sand and milled silicified rock residues after coal mining produced by EKOLIVE company on soil quality and performance of wheat.

1.3. Significance of the study.

This study will test and deliver scientific results backing the effectiveness of biostimulants based on bioleaching of sand and milled silicified rock residues after coal mining produced by the EKOLIVE company. This work will prove claims of random farmers' results showing the biostimulants improving nutrient use efficiency, tolerance to abiotic stress, crop quality traits or availability of confined nutrients in the soil and rhizosphere. Soil quality and water use efficiency will be tested. This study will deliver eco-friendly biostimulant alternatives to synthetic fertilizers.

1.4. Objective of the study

This study seeks to generate scientific data that will back up the biostimulants based on bioleaching of sand and milled silicified rock residues after coal mining produced by EKOLIVE company to fall under the European Commission categorization of biostimulants into improving nutrient use efficiency, tolerance to abiotic stress, crop quality traits or availability of confined nutrients in the soil and rhizosphere.

Specific objective

Testing the role of the biostimulants in improving soil quality.

Testing the role of biostimulants in improving crop quality traits and increasing productivity.

Testing the potential of the biostimulants in increasing nutrients and water use efficiency.

1.5. Hypothesis

EKOLIVE´s biostimulants based on bioleaching of sand and milled silicified rock residues after coal mining will improve soil quality.

EKOLIVE´s biostimulants based on bioleaching of sand and milled silicified rock residues after coal mining will improve crop quality traits and increase productivity.

EKOLIVE´s biostimulants based on bioleaching of sand and milled silicified rock residues after coal mining will increase nutrients and water use efficiency.

2. LITERATURE REVIEW

2.1. Origin and distribution of wheat

It is thought that wheat originated in the Fertile Crescent, which included modern-day Turkey, Syria, Iraq, and Iran. There are six different species of wheat, emmer wheat, einkorn wheat, and spelt wheat being the earliest cultivated forms.

Durum wheat was domesticated in the Near East, and bread wheat, the most widely cultivated species, is a hybrid of three different species of wheat. Today, wheat is grown in almost every country in the world, with the top producers being China, India, and the United States (Hawkes, 1983, FAOSTAT, 2021)

Wheat belongs to the Poaceae family, also known as the grass family. Within the genus *Triticum*, there are six different species of wheat:

1. *Triticum aestivum L.* (bread wheat)
2. *Triticum turgidum L.* (durum wheat)
3. *Triticum dicoccum Schrank ex Schübl*. (emmer wheat)
4. *Triticum monococcum L.* (einkorn wheat)
5. *Triticum spelta L.* (spelt wheat)
6. *Triticum compactum Host* (club wheat)

Wheat is one of the most significant cereals farmed worldwide and a significant staple food crop (*Triticum aestivum*). It is rich in carbohydrates, protein, and dietary fibre and is used in various food products, including bread, pasta, and pastries (FAO, 2019). The most popular wheat is bread wheat, which accounts for over 95% of global wheat output (Kimber and Feldman, 1987).

2.1.1. Nutritional Properties of Wheat:

Wheat has been extensively studied for its nutritional properties. Products made from whole wheat have been demonstrated to have various health advantages, including a lower risk of heart disease, diabetes, and several cancers (Aune et al., 2016; Liu et al., 2017). Whole wheat contains important nutrients, including B vitamins, iron, and magnesium.

2.1.2. **Cultural and Historical Significance of Wheat:**

In addition to its nutritional and agricultural significance, wheat has also played a significant cultural and historical role. The crop has been farmed for thousands of years, and it is thought that domestication of the plant was a major factor in the rise of human civilization (Harlan, 1995). Wheat has also been used in religious and cultural ceremonies throughout history.

2.1.3. **Genetic Engineering of Wheat:**

Recent research has aimed to create new disease-resistant wheat types with environmental stresses, such as drought and heat. Advances in genetic engineering have allowed scientists to identify and modify genes responsible for these traits, leading to the development of more resilient wheat varieties (Mickelbart et al., 2015). This has the potential to increase wheat yields and improve food security.

2.1.4. **Cultivation of Wheat:**

Wheat is grown in various regions, from temperate to tropical climates. The crop needs soil with good drainage, a pH between 6.0 and 7.5, and a minimum annual rainfall of 250mm (Kumar et al., 2019). Wheat can be grown in winter, spring, or double crop in areas with mild winters.

2.1.5. **Seed Selection and Planting:**

Seed selection is a critical step in wheat cultivation. Farmers should choose seeds suited to their region's soil and climate, as well as seeds resistant to prevalent diseases and pests. Seed should be planted at 2-3 inches, depending on soil type and moisture conditions. Wheat requires a high level of nitrogen, phosphorus, and potassium, and farmers should apply these nutrients based on soil tests and crop requirements.

2.1.6. **Disease and Pest Management**:

Numerous diseases and pests can severely impair wheat yields because of their susceptibility to them. Rust, powdery mildew, and Fusarium head blight are prevalent diseases, and aphids, army-worms, and wire-worms are frequent pests (Roy et al., 2023). In addition to using chemical pesticides and fungicides, farmers can control these diseases and pests through cultural techniques like crop rotation and sanitation.

2.1.7. **Harvesting and Post-Harvest Handling:**

Wheat is typically harvested when the crop has reached physiological maturity, and the moisture content is between 13% and 18%. Farmers should use proper harvesting equipment and techniques to minimize losses and damage to the grain (Randby et al., 2019). After harvesting, wheat should be dried to a safe moisture level and stored in a cool, dry place to prevent spoilage and insect infestation.

2.2. **Biostimulants**

Various additives known as "biostimulants" are added to soil or plants to promote plant growth and health. Biostimulants are becoming increasingly popular in agriculture to improve plant performance in the light of climate change and the need for more sustainable agriculture.

2.2.1. **Definition of Biostimulants**:

Biostimulants are described by the European Biostimulant Industry Council (EBIC) (EBIC, 2021) as "materials and/or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality" . Biostimulants can be derived from various sources, including seaweed, plant extracts, and microbial extracts.

2.2.2. **Modes of Action:**

Biostimulants have a variety of modes of action that contribute to plant growth and health. These include:

1. Improving nutrient uptake and efficiency;
2. Enhancing root growth and development;
3. Stimulating plant growth and yield;
4. Increasing the ability of plants to withstand abiotic stresses like drought and severe temperatures.

2.2.3. **Types of Biostimulants:**

There are several types of biostimulants, including:

1. Seaweed extracts: Seaweed extracts are derived from various seaweed species and contain a range of nutrients, such as micronutrients and plant growth regulators, that can improve plant growth and health (Battacharyya et al., 2015).

2. Microbial extracts: Bacteria, fungi, and algae are a few microorganisms used to create microbial extracts. By promoting nutrient intake and plant health, these extracts can increase plant growth and enhance plant-microbe interactions (Elnahal et al., 2022).

3. Humic substances: A complex mixture of chemicals called humic substances derived from organic matter in soil and can enhance soil fertility and plant growth (Drobek et al., 2019).

4. Amino acids and peptides: Amino acids and peptides are organic compounds that can improve plant growth and health by enhancing nutrient uptake and stimulating plant metabolism (Colla et al., 2015).

2.2.4. **Benefits of Biostimulants**:

Biostimulants can benefit plants, including increased nutrient uptake, enhanced root growth and development, improved photosynthesis and carbon fixation, and increased resistance to abiotic and biotic stressors (Khan et al., 2020). In addition, biostimulants can improve crop yield, quality, and shelf life and reduce environmental impacts by decreasing the need and usage of chemical fertilizers and pesticides.

2.2.5. **Regulatory Framework:**

The regulatory framework for biostimulants varies by country and region and can be complex and inconsistent. In the European Union, biostimulants are regulated under the EU Fertilizing Products Regulation (Regulation (EU) 2019/1009), which sets out rules for placing biostimulants on the market and establishes their safety and efficacy criteria. Biostimulants are not subject to federal regulation in the United States, although some states have developed their own guidelines.

2.2.6. **Application of Biostimulants:**

Numerous methods, including foliar sprays, seed treatments, and soil applications, can apply biostimulants to plants or soil. The type of biostimulant, the crop being produced, and the effect intended can affect the timing and technique of application.

2.3. **Plant growth-promoting bacteria (PGPB)**

Microorganisms (like bacteria and fungi) that struggle for scarce resources like nutrients, water and space can be found in soil, a dynamic living source (Smith *et al.*, 2017). The rhizosphere is a microbial hot area where interactions between the host plant and different microorganisms can have a positive, neutral, or negative impact on the health and development of the plant (Berg *et al.*, 2016; Smith *et al.*, 2017). When it comes to endophytic bacteria, an additional stage is involved in colonizing interior plant tissues like roots (Berg *et al.*, 2016). The term "plant-growth-promoting bacteria" (PGPB) refers to bacteria that influence plant growth directly, indirectly (e.g., through biological control of plant diseases), or both ways (Naik et al., 2019). While many precise methods by which microorganisms encourage growth or suppress plant diseases are still unknown, several proven mechanisms have been identified. The four main direct plant growth promoters categories are biofertilizers, rhizoremediators, phytostimulators, and stress relievers. Through antibiosis, the generation of lytic enzymes, competition for nutrients, and induced systemic resistance (ISR) in the host plant, indirect plant growth promoters can lower the number of infections (Gopalakrishnan *et al.*, 2017; Tabassum *et al.*, 2017).

The plant heavily influences the rhizomicrobiome's makeup (Zhang *et al.*, 2017). According to Trabelsi & Mhamdi (2013), the plant produces root exudates of different compositions, some of which may be better suited than others to serve as sources of reduced C for bacteria. Additionally, the plant produces signalling molecules that attract particular species and control their genetic and metabolic activity (Massalha *et al.*, 2017; Smith *et al.*, 2017). The soil microbial population also engages in various self-regulation activities (Leach *et al.*, 2017). When conditions necessitate a general physiological shift, the microorganisms can create quorum-sensing chemicals to communicate (Chauhan *et al.*, 2015).

Finally, it is becoming clear that the phytomicrobiome has a hierarchy and that plants control some important members. These crucial members are called "hub species" (Agler et al., 2016) or "core species" (Toju et al., 2018). The term "endophytes" now refers to bacteria residing inside the root as opposed to those dwelling on the root surface, or "rhizoplane (Zhang *et al*., 2017). Free-living, specific symbiotic plant relationships (like those formed by Rhizobia species and Frankia species), bacterial endophytes that can colonize all or part of a plant's interior tissues, and cyanobacteria are examples of bacteria that can promote plant growth or PGPB.

2.3.1. **Biological Nitrogen Fixation** (BNF):

One of the eukaryotic microorganisms' most important ecological functions is their ability to reduce atmospheric nitrogen (N_2) to usable forms, a long-ago discovery only made by bacteria and archaea. Legumes are a major nitrogen source for farmed and natural ecosystems (Werner *et al*., 2014). A few lineages of angiosperms have developed intricate, extremely effective symbioses with bacteria that fix N_2 (Remigi *et al*., 2016). These symbioses are characterized by the development of specialized nodules in the roots (or rarely stems), which serve as tiny N_2-fixing factories for the plants and are heavily inhabited by bacterial partners. Seventy percent of legume species and many lineages of plants, primarily so-called actinorhizal plants, scattered over three Angiosperm orders, can nodulate thanks to the evolution of nodulation in plants some 100 million years ago (Van Valzen *et al.*, 2017). Parallel to this, only the Frankia in the *Actinobacteria phylum* can nodulate actinorhizal plants, but the ability to fix nitrogen with legumes has extended to hundreds of species in alpha- and beta-Proteobacteria, known as rhizobia (Remigi et al., 2016). The most popular legumes that can fix nitrogen are beans, chickpeas, cowpeas, lentils, pigeon peas, and peanuts. These legumes are frequently cultivated on rotation or intercropped with other crops. *Rhizobia*, a soil bacterium, and the soybean root system work together to fix nitrogen, which significantly aids growth, development, and maturity. Increased plant components, such as soybean pods, can be correlated with increased nitrogen fixation capacity (Tang *et al.*, 2016). On legume crops, symbiotic nitrogen-fixing bacteria are used to fix atmospheric nitrogen, such as *Rhizobium* species. To fix atmospheric nitrogen, *Rhizobium* spp. secretes nod factors in response to signals from root exudates in legumes, such as flavonoid compounds. The hairs of the legume plant subsequently sense these nod factors.

2.3.2. **Free-living N-fixation**:

Non-legume crop species that use rhizosphere-associated N_2-fixing bacteria include sugar beet, sugar cane, rice, jatropha, maize, and wheat (Olanrewaju *et al*., 2017). For example, *Bacillus* species research revealed greater cereal productivity (Tchakounté *et al*., 2020). Nitrogen can be fixed in either bulk or rhizospheric soil. The crop's nitrogen balance can be improved by adding fixed nitrogen acquired from root uptake (Olanrewaju *et al*., 2017; Tchakounté *et al*., 2018). Bacteria such as *Enterobacter*, *Klebsiella*, *Burkholderia*, and *Stenotrophomonas* have recently received attention due to their ties to important crops and their potential to enhance plant development (Geddes., 2015). *Pseudomonas putida* RC06, *Paenibacillus polymyxa* RC05 and RC14, and *Bacillus* OSU-142, which fix N_2, have excellent potential and are used as biofertilizers in formulations to promote wheat, sugar beet, and spinach development (Tahir *et al*., 2017; Tchakounté *et al*., 2018). The N_2-fixing *Bacillus* strains and A. brasilense sp246 can influence plant development activities in organic and low-N input agriculture (Geddes *et al*., 2015).

2.3.3. **Siderophores production**:

Iron is the fourth most prevalent element in this sphere. Iron is a micronutrient essential for practically all organisms' existence and survival. Still, absorption by bacteria and plants is difficult. Fe^{3+} is the most common form on Earth; nevertheless, it is very mildly soluble. Therefore the amount of Fe available for absorption by living organisms is quite limited. Plants and microorganisms require adequate iron, which is especially important in the root zone, where plants and microbes compete for iron. To deal with such a limited supply and to make iron available to plants in an iron-deficient environment, PGPR secretes low molecular weight siderophores (~400-1500 Da) and molecules with an increased affinity for Fe^{3+} (*KKaa* between 1023 and 1052). Siderophores are classified into three types based on their functional groups: hydroxamates, catecholates, and carboxylates. Several studies have demonstrated the significant benefits of PGPR-produced siderophores on plant growth (Duca *et al*., 2014; Mandal and Kotasthane, 2014).

The bacterium that first generated the siderophores picks up the iron siderophore complex by using a complex-specific receptor in the bacterium's outer cell membrane. The iron is released once within the cell and is then available to assist microbial development. PGPR can inhibit the growth of fungi and other diseases by creating siderophores that bind most of the Fe around the plant root. The consequent shortage of iron inhibits germs from growing in this area (Kesaulya *et al*., 2018). In general, iron-sensitive fur proteins, global regulators (GasS and GasA), sigma factors (RpoS, PvdS, and Fpv1), quorum-sensitive autoinducers (N-acyl homoserine lactone), and several site-specific recombinases govern siderophores synthesis in bacteria. *Pseudomonas* strains that produce siderophores colonized the roots of numerous crops quickly, resulting in higher output.

2.3.4. **Phosphorus solubilization:**

Phosphorus is more important than any other element after nitrogen. Phosphorus levels in the soil are often high (between 400 and 1,200 $mgkg^{-1}$ of soil), but it is insoluble and unavailable to promote plant growth. Unavailable phosphorus can be found in inorganic minerals such as apatite or organics such as inositol phosphate, phosphomonoester, and phosphodiester (Rizvi *et al*., 2014). Bacteria release organic acids, which lowers the pH in the root zone, allowing trapped forms of phosphate such as $Ca_3(PO_4)_2$ in calcareous soils to be freed (Oteino *et al*., 2015). Aside from delivering available cumulated phosphate (through solubilization), phosphorus biofertilizers also contribute to increasing organismal N-fixation and making Zn, Fe, and other trace elements available through the creation of some plant growth-promoting chemicals (Meena *et al*., 2015). Phosphate-solubilizing bacteria play a biotechnological role in sustainable agriculture, particularly in phosphorus-deficient soils. Phosphate-solubilizing bacteria use many processes (s) to convert insoluble phosphate forms to soluble forms (Meena *et al*., 2015; Naik *et al*., 2019). Organic acids produced by microorganisms operate as effective chelators of divalent Ca^{2+} cations, accompanied by releasing phosphates from insoluble phosphatic compounds. Organic acids can also form soluble complexes with metal ions coupled with insoluble 'P,' releasing the phosphate (Kumar *et al*., 2017). Microorganisms were discovered to be involved in the solubilization of inorganic phosphates as early as 1903 (Kucey *et al*., 1989). P-solubilizing microorganisms are believed to comprise

20 to 40% of the culturable population of soil microorganisms, with a considerable fraction of them isolated from rhizosphere soil.

2.3.5. **PGPR in potassium solubilization**:

The third most important macronutrient is potassium. Soluble potassium concentrations are generally low in the soil, and approximately 90% of total potassium is found in insoluble rocks or silicate minerals (Han *et al*., 2006). Potassium depletion is quickly becoming one of the most serious issues in agricultural productivity. Plant roots develop poorly when potassium levels are low. The plant grows slowly, producing little seeds, and yields fall short of expectations. This underlined the need to identify additional potassium sources for plant uptake and to manage soil potassium levels to sustain agricultural productivity (Kumar *et al*., 2017). Through organic acid production, PGPR can solubilize potassium (Bhardwaj *et al*., 2014). *Acidithiobacillus ferrooxidans*, *Burkholderia* spp., *Bacillus mucilaginous*, *Bacillus edaphic*, *Paenibacillus* spp., and *Pseudomonas* have all been characterized as potassium solubilizing PGPR (Liu *et al*., 2016). As a result, using potassium-solubilizing PGPR as a biological fertilizer for crop enhancement can reduce the use of agrochemicals while encouraging environmentally friendly crop production practices.

2.4. Biostimulants from ekolive

2.4.1. Manufacturing process

The company EKOLIVE from Slovakia claims to be the first and leading provider of a certified novel eco-bioleaching technology (called *InnoBioTech*®) that can be used to clean or upgrade soils/minerals and to produce inexpensive but unique biostimulants for plants and soil – consisting of liquid natural minerals, trained healthy probiotics and plant growth-promoting bacteria, dozens of proteins produced by them, organic acids, alcohols and microalgae.

The process itself is essentially a replication of the natural microbiological weathering process of minerals occurring in nature in healthy soils using heterotrophs – in which nutrients are oxidized, released and made available to plants. ekolive has brought this process under control to such an extent that it can be used on an industrial scale for the purposes of the above applications. The resulting products are then the following biostimulants, which are brought back into the natural process in the soil through their application.

EKOLIVE has received many awards from the world's most recognized agricultural University in Wageningen, the largest chemical company BASF, as well as it is sponsored by the European Commission, EIT Food, EIT RawMaterials, VUB Foundation, BMW Foundation, and others.

All of this made it obvious to deal with the biostimulants produced by ekolive in the present work.

2.4.2. *ekofertile® plant*

ekofertile® plant is a liquid biostimulant containing natural minerals dissolved in the bioleaching process of silica sand – mainly liquid iron, manganese, growth-promoting probiotic bacteria as well as the metabolites produced bythem: a wide range of proteins and organic acids such as butyric, lactic, acetic, propionic, formic, diketopiperazines, hydroxy derivatives, fatty acids, 3-phenyl lactate, antibacterial bacteriocins and bacteriocin-like inhibitors, hydrogen peroxide, pyrrolidone-5-carboxylic acid, diacetylene and reuterin, which shall directly stimulate the production of plant hormones and siderophores, and methanol and ethanol for drought resistance.

2.4.3. *microfertile® plant*

microfertile® plant is a liquid biostimulant containing all the essential micronutrients dissolved in the bioleaching process of milled silicified rock residues after coal mining, as well as chlorella microalgae, *thiobacillus* bacteria and their metabolites: protein rusticyanin, oxaloacetic, isovaleric and pyruvic acid.

2.4.4. Classification and evaluation

Following the nomenclature set out above (under point 2.2.3.), both biostimulants from EKOLIVE cannot be clearly assigned to one of the four given categories. Rather, the two preparations are somehow at odds with all categories, as they contain both, algae and their nutrients, other microorganisms that stimulate directly, but above all indirectly, namely through the metabolites produced by them, namely, among other things, humic substances, amino acids and peptides, which in turn improve soil fertility, plant growth and nutrient uptake and stimulate plant metabolism.

This is also a reason to deal explicitly with the two biostimulants in the present work, since they are complex, naturally produced preparations and not – as in many other cases – just one or the other auxiliary substance or a mixture of such.

3. MATERIALS AND METHODS

3.1. Description of the site.

The experiment was conducted at the greenhouse of Ondokuz Mayis University's Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Samsun, Turkey (Fig. 3.1). The site location is coordinated with 264201 E and 4582754 N (WGS-84, Zone37 and UTM m). The mean annual maximum and minimum temperatures are 5°C to 27.7°C, and the relative humidity is 73%. The average annual precipitation is 937.26 mm per year.

Figure 3.1. Location of the study area

3.2. Candidate Biostimulants.

In this study, two candidate products produced by EKOLIVE company from Slovakia were tested for biostimulant activity as a measure to ascertain the biostimulant potential of the products as shown by the laboratory result of their content according to Yahkin et al. (2017). Table 1 displays the organic acid content of *ekofertile*® and *microfertile*® plant biostimulants, while Tables 2 and 3 show the chemical and biological constituents and functions of the biological properties respectively for *ekofertile*®. Similarly, Tables 4 and 5 show the chemical and biological constituent and functions of the biological properties, respectively, for *microfertile*®.

Table 3.1. Organic acid constituent of *ekofertile*® and *microfertile*® plant biostimulants

Sample	Formic acid (mg/l)	Lactic acid (mg/l)	Acetic acid (mg/l)	Propionic acid (mg/l)	Butyric acid (mg/l)	Methanol (mg/l)	Ethanol (mg/l)
ekofertile® plant	<5	9320	1550	19*	900*	8.6**	610
microfertile® plant	<5	<5	<5	<5	<5	<5	<20

*= HS-GC-MS measurement with internal standard calibration (4-methyl valeric acid)
**=HS-GC-MS measurement with external standard calibration

Table 3.2. Chemical and microbial constituents of *ekofertile*® plant (sand based) biostimulant

Chemical content			Microbial content	
Constituent	Unit	Quantity	Genus	Species
Dry matter	%	0.91	Lactobacillus	*Lactobacillus satsumensis*
Organic matter	%	0.27		*Lactobacillus diolivorans*
Ash	%	0.53		*Anaeromassilibacillus senegalensis*
Total Nitrogen	%	0.040		*Lactobacillus bifermentans*
NH_4^+	%	0.01		*Lactobacillus perolens*
NO_3^-	%	< 0.01		*Lactobacillus nagelii*
Available Nitrogen	%	0.01	Clostridium_IV	*Clostridium tyrobutyricum*
Carbamide N	%	< 0.05		*Clostridium ljungdahlii*
P_2O_5 mineral acid soluble	%	< 0.01	Clostridium_sensu_stricto	
K_2O	%	0.0840		
Total MgO	%	0.0275	Bifidobacterium	*Bifidobacterium mongoliense*
Total CaO	%	0.0855		
Total Sulphur	%	0.025	Leuconostoc	*Leuconostoc fallax*
Sodium	%	0.0895		
Silicon	%	< 0.0100	Acetobacter	*Acetobacter indonesiensis*
Alkaline active components	%	0.44	Macellibacteroides	*Macellibacteroides fermentans*
Boron	mg/kg	< 2.00		
Cobalt	mg/kg	0.117	Bacteroides	*Bacteroides luti*
Iron	mg/kg	142		
Copper	mg/kg	< 2.00		
Manganese	mg/kg	6.58		
Molybdenum	mg/kg	< 0.100		
Zinc	mg/kg	< 2.00		
pH		4.5		
Salt content	% KCl	0.782		

Table 3.3. Role of beneficial microbes found in *ekofertile*® plant biostimulant

		Coal	
	Genus	Species	Function
1	Lactobacillus	*Lactobacillus satsumensis*	catalyzes the hydrolytic depolymerization of polysaccharides in soil. Breakdown of complex polysaccharides, including starch, to a readily available form of glucose, extracellular polymeric substances secretion & fermentation [30]
		Lactobacillus diolivorans	Solubilize insoluble inorganic phosphate [31]
		Anaeromassilibacillus Senegalensis	
		Lactobacillus bifermentans	
		Lactobacillus perolens	
		Lactobacillus nagelii	
2	Clostridium_IV	*Clostridium tyrobutyricum*	Free Nitrogen fixation release polysaccharides and carboxylic acids like tartaric acid and citric acid to solubilize K, breakdown organic matter releasing citric acid, formic acid, malic acid, and oxalic acid, making K available, fermentation [32]
		Clostridium ljungdahlii	obligatory anaerobic heterotrophs only capable of fixing N2 in the complete absence of oxygen, isolated from rice fields [32]
3	Clostridium_sensu_stricto		Fermentation [32]
4	Bifidobacterium	*Bifidobacterium mongoliense*	degradation of non-digestible carbohydrates, protection against pathogens, production of vitamin B, antioxidants, and conjugated linoleic acids, and immune system stimulation [33].
5	Leuconostoc	*Leuconostoc fallax*	catalyzes the hydrolytic depolymerization of polysaccharides in soil. Breakdown of complex polysaccharides, including starch, to a readily available form of glucose, fermentation
7	Macellibacteroides	*Macellibacteroides fermentans*	Fermentation [34]
8	Bacteroides	*Bacteroides luti*	Pathogen-suppressing contributes prominently to rhizosphere phosphorus mobilization, express constitutive phosphatase activity, and organic matter degradation [35]

Table 3.4. Chemical and microbial constituents of *microfertile®* plant (milled silicified rock residues after coal mining based) biostimulant

Chemical content			Microbial content	
Constituent	Unit	Quantity	Genus	Species
Dry matter	%	< 0.32	Thiobacillus	
Organic matter	%	< 0.01	Shinella	
Ash	%	0.4	Comamonas	
Total Nitrogen	%	0.020	Bosea	
NH_4^+	%	< 0.01	Thermomonas	*Thermomonas koreensis*
NO_3^-	%	< 0.01	Clostridium_sensu_stricto	*Clostridium saccharobutylicum*
Available Nitrogen	%	< 0.01	Pseudomonas	*Pseudomonas* sp.
Carbamide N	%	< 0.05	Unclassified at the Genus level	
P_2O_5 mineral acid soluble	%	< 0.01	Castellaniella	*Castellaniella daejeonensis*
K_2O	%	< 0.0285	Petrimonas	*Petrimonas sulfuriphila*
Total MgO	%	0.0155	Tepidibacillus	*Tepidibacillus fermentans*
Total CaO	%	0.023		*Sedimentibacter saalensis*
Total Sulphur	%	0.0465		
Sodium	%	0.102		
Silicon	%	< 0.0100		
Alkaline active components	%	0.555		
Boron	mg/kg	< 2.00		
Cobalt	mg/kg	0.361		
Iron	mg/kg	12.2		
Copper	mg/kg	< 2.00		
Manganese	mg/kg	< 2.00		
Molybdenum	mg/kg	< 0.100		
Zinc	mg/kg	4.30		
pH		7.8		
Salt content	% KCl	0.574		

Table 3.5. Role of beneficial microbes found in *microfertile®* plant biostimulant

		Coal	
	Genus	Species	Function
1	Thiobacillus		release polysaccharides and carboxylic acids like tartaric acid and citric acid to solubilize K, breakdown organic matter releasing citric acid, formic acid, malic acid, and oxalic acid, making K available
2	Shinella		Biosurfactant producers capable of degrading crude oil components within 14 days, bioremediations.
3	Comamonas		Alleviate salinity stress, and degrade phenol and 4-chlorophenol mixtures completely through a meta-cleavage pathway, beneficial for enhanced cell growth and the biotreatment of both compounds, bioremediation, biofertilizer
4	Bosea		Bioavailability of nutrients, N-fixation, denitrifier.
5	Thermomonas	*Thermomonas koreensis*	nutrient cyclings, such as nitrogen respiration, nitrate reduction, nitrate respiration, fermentation, and cellulolysis
7	Clostridium_sensu_stricto	*Clostridium saccharobutylicum*	Fermentation [34]
8	Pseudomonas	*Pseudomonas* sp.	Free Nitrogen fixation, solubilize insoluble inorganic phosphate and K Indole-3-acetic acid, wheat, A combined bio-inoculation of diacetyl-phloroglucinol producing PGPR and AMF and improved the nutritional quality of the wheat grain, organic compounds degradation, auxins
9	Castellaniella	*Castellaniella daejeonensis*	acid phosphatase and invertase activities, available potassium and iron, and organic matter content
10	Petrimonas	*Petrimonas sulfuriphila*	Anaerobic and fermentative, Degradation of high insulable organic molecules, plant residues decomposition
11	Tepidibacillus	*Tepidibacillus fermentans* *Sedimentibacter saalensis*	ferment yeast extract and mono-, oligo-, and polysaccharides, including starch and xanthan gum

3.3. Experimental design

The experimental greenhouse design is a split-plot design consisting of two factors (Table 3.6). The factors are dosage and biostimulant type tested on two soil types (loam soil gotten from Samsun Turkey Bafra plain and clay soil gotten from the Faculty of Agriculture practicing field). Factor 1, dosage, had 5 levels (control, inorganic fertilization, 2.5%, 5% and 10% biostimulant), and biostimulant type had 2 levels (*ekofertile*® and *microfertile*® plant biostimulants). This gave 10 treatments replicated three times in the greenhouse per soil type (Fig. 3.3a). About 300 kg of soil was collected from the field, 150 kg from the Faculty of Agriculture practicing field Ondokuz Mayis University and 150kg from Samsun Turkey Bafra plain. The soils were placed in the shade to air dry for two weeks. The large clumped soils were crushed and sieved through a 4 mm sieve to obtain fine particle soil for growing crops in the greenhouse (Fig. 3.4a). Three-kilogram soil was placed in a 5 l bucket of 0.031 m^2 surface area without perforations to avoid leaching (Fig. 3.2a). The moisture content of the soil was calculated to estimate the field capacity of the soil. The wheat seeds were sown following the treatments (Table 3.6) according to the layout below (Table 3.7). Given that 500 wheat seeds are sown per m^2, 15 were sown per pot (Fig. 3.2b) and watered after seeding (Fig. 3.2c).

Table 3.6. Treatments combination

Loam Bafra soil	Biostimulant	*ekofertile*®					*microfertile*®				
	Dosage	control	Inorganic fertilization	2.5%	5%	10 %	control	Inorganic fertilization	2.5%	5%	10%

Clay school soil	Biostimulant	*ekofertile*®					*microfertile*®				
	Dosage	control	Inorganic fertilization	2.5%	5%	10 %	control	Inorganic fertilization	2.5%	5%	10%

Table 3.7. Greenhouse layout

Replicate 1		Replicate 2		Replicate 3	
ekofertile®	*microfertile*®	*ekofertile*®	*microfertile*®	*ekofertile*®	*microfertile*®
Control	Control	Control	Control	Control	Control
Inorganic fertilizer	Inorganic fertilizer	Inorganic fertilizer	Inorganic fertilizer	Inorganic fertilizer	Inorganic fertilizer
2.5%	2.5%	2.5%	2.5%	2.5%	2.5%
5%	5%	5%	5%	5%	5%
10%	10%	10%	10%	10%	10%

Figure 3.2. Greenhouse design. **3.2a** experimental design, **3.2b** wheat planting, **3.2c** watering.

3.4. Greenhouse Management.

The wheat plants were irrigated to field capacity in the evening periods of the day at 2 days intervals to prevent drought stress. Weeding was done manually. Figure 3 displays the greenhouse progress and activities. The pots were weighed at regular intervals for field capacity (Fig. 3.3a), pots were weeded to reduce weed competition with wheat (Fig. 3.3b), and growth parameters were collected (Fig.3.3c). Figures 3.3d and 3.3e shows plants at head formation while figure 3.3f, 3.3g and 3.3h shows plant at head drying

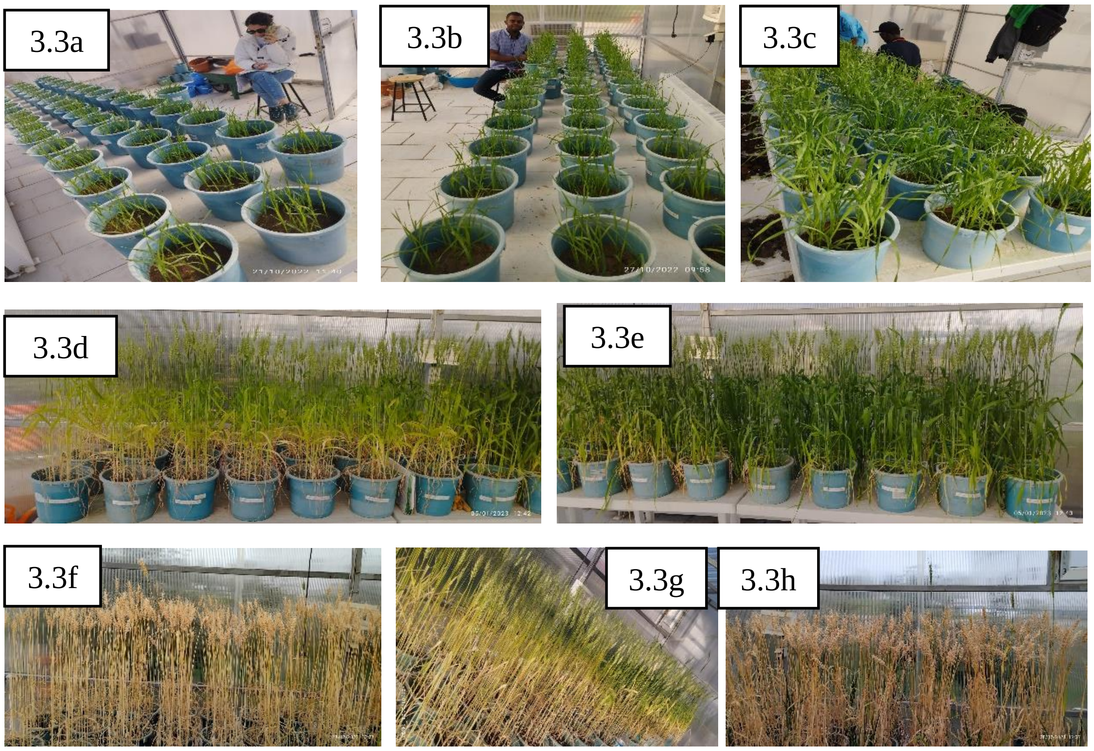

Figure 3.3. Wheat growing. **3.3a** pot weighing for field capacity, **3.3b** Weeding in pots, **3.3c** growth data collection, **3.3d** wheat growth in clay soil, **3.3e** wheat growth in loam soil, **3.3f** dry heads in clay soil, **3.3g** general view after heading, **3.3h** dry heads in loam soil.

3.5. Data collection

3.5.1. Soil physicochemical analysis

The soil quality status and dynamics were determined by collecting pre-planting baseline soil samples from the two field sites using the X format at 15 cm depth, and each bulked to one composite sample per field site (Fig. 3.4a). Two different soil types were classified as Lithic Haplusert (Verisol) taken from Ondokuz Mayis University greenhouse area and Typic Ustipomment (Entisol) collected from Ondokuz Mayis University Bafra experimental station area were used in this study. The soil physicochemical properties from the two field sites are given in Table 3.8 below.

Table 3.8. Pre-soil physicochemical properties

	Texture			pH	EC	$CaCO_3$	OC	OM	N	MC	BD
Soil Type (Soil Taxonomy, 2014)	Clay (%)	Silt (%)	Sand (%)		(µscm^{-1})	(%)					(gcm^{-1})
Lithic haplustert	48	16	36	6.95	520	1.79	0.85	1.46	0.12	6.75	1.17
Typic ustipomment	23	40	37	7.66	972	12.67	2.20	3.78	0.23	2.17	1.12

	K	Na	Mg	Ca	P	Mn	Fe	Zn	Cu
	(meq/100g)				(ppm)				
Lithic haplustert	1.09	0.28	13.49	46.85	42.44	41.83	27.05	1.43	5.0
Typic ustipomment	2.79	0.49	14.33	29.94	178.98	10.42	34.13	5.83	3.10

While post-planting soil samples were collected from each experimental unit by mixing the soil and collecting one sample per replicate per treatment (Fig. 3.4b and c). Pre and post-soil samples were analysed at the Department of Soil Science and Plant Nutrition laboratory of the Faculty of Agriculture, Ondokuz Mayis University, Turkey.

The particle size distribution of the soil was determined using the hydrometer method with sodium hexametaphosphate as dispersing agent (Kalra *et al.*, 1991). Aggregate stability was analysed, and moisture content was determined. $CaCO_3$ content by the volumetric method (Martin and Reeve, 1955), pH in 1:1 (w/v) in soil: water suspension by pH-meter (Rowell, 2014), electrical conductivity (EC) in the same soil suspension by EC-meter (Rowell, 2014). The soil's organic carbon content was derived by the Walkey-Black method (Walkey and Black, 1934). Exchangeable bases extraction was done with neutral Ammonium acetate solution (Fig. 3.4d).

Calcium (Ca) and Magnesium (Mg) were derived by titration with 0.01N EDTA, while Potassium (K) and Sodium (Na) by flame photometry (Benton et al., 2001). The total Nitrogen (N) was gotten by the macrokjeldahl digestion method (Bremner and Mulvaney, 1982) (Fig.3.4f), while the available Phosphorus (P) was by the Bray II method (Benton *et al.*, 2001).

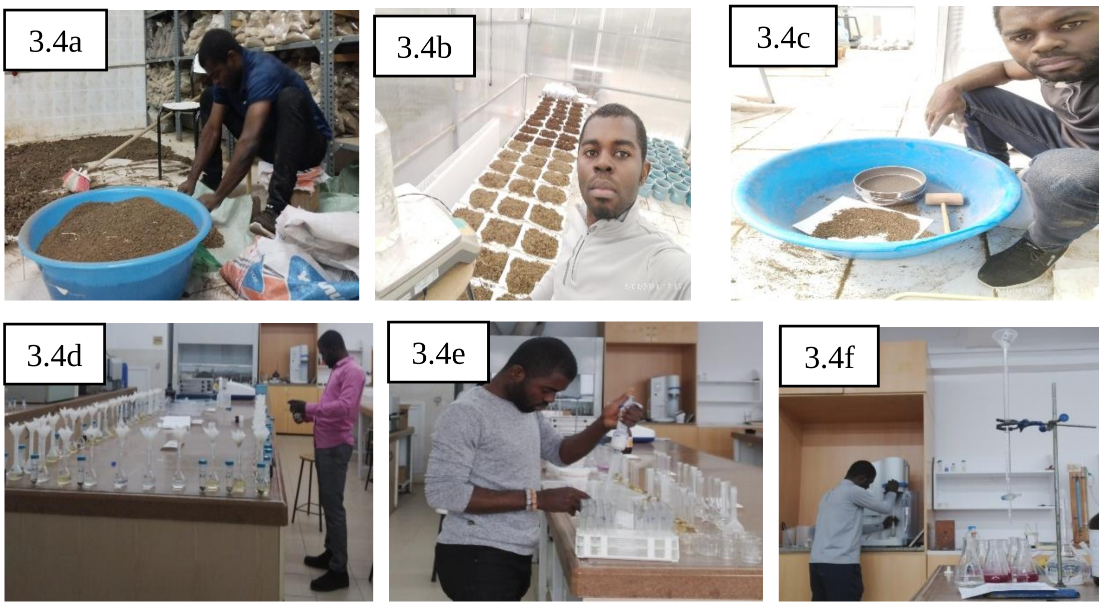

Figure 3.4. Soil preparation. **3.4a** pre-soil preparation for the greenhouse, **3.4b** post-soil air drying for analysis, 3.**4c** post soil collection, 3.**4d** exchangeable cation analysis, 3.**4e** micronutrients analysis, 3.**4f** nitrogen analysis.

3.5.2. Soil microbial analysis

Figure 3.5a and b represents soil sample preparation for biological analysis. Basal soil respiration: The measurement of basal soil respiration (BSR) was conducted at field capacity without the addition of glucose for CO_2 production at 22°C according to Anderson (1982) during a 24-hour incubation period was determined using alkali ($Ba(OH)_2.8H_2O$ + $BaCI_2$) for the absorption of produced CO_2 (Fig. 3.5d). Following the addition of phenolphthalein as an indicator, the residual OH ions were titrated with standardized hydrochloric acid. Each soil sample was evaluated from the three replicates, and the data were expressed as g CO2-Cg^{-1} of the dry soil sample.

Microbial biomass carbon: Anderson and Domsch (1978) described the substrate-induced respiration method for determining microbial biomass carbon (Cmic). A moist sample comparable to 100 g of oven-dry soil was modified with a powder mixture containing 400 mg of glucose. The rate of CO_2 production was measured hourly per Anderson's (1982) approach. The respiratory response was recorded for 4 hours, and the maximum initial respiratory response was used to calculate microbial biomass carbon (Cmic) in terms of $mgCg^{-1}$ soil, using the equation 40.04 $mgCO_2g^{-1}$+3.75. The soil sample was examined from the three replicates, and the results were represented as $mgCO_2$-$C100g^{-1}$ of dry soil per hour.

Dehydrogenase activity: Dehydrogenase activity (DHA) was measured using the method described by Pepper et al. (1995). Six grams of soil (Fig. 3.5c), 30 mg of glucose, 1 ml of 3% 2,3,5-triphenyltetrazoliumchloride (TTC) solution, and 2.5 ml of pure water were added to the samples using this procedure. After that, the samples were incubated at 37°C for 24 hours. TPF (1, 3, 5 triphenylformazan) production was quantified spectrophotometrically at 485 nm and reported as g $TPFg^{-1}$ dry sample

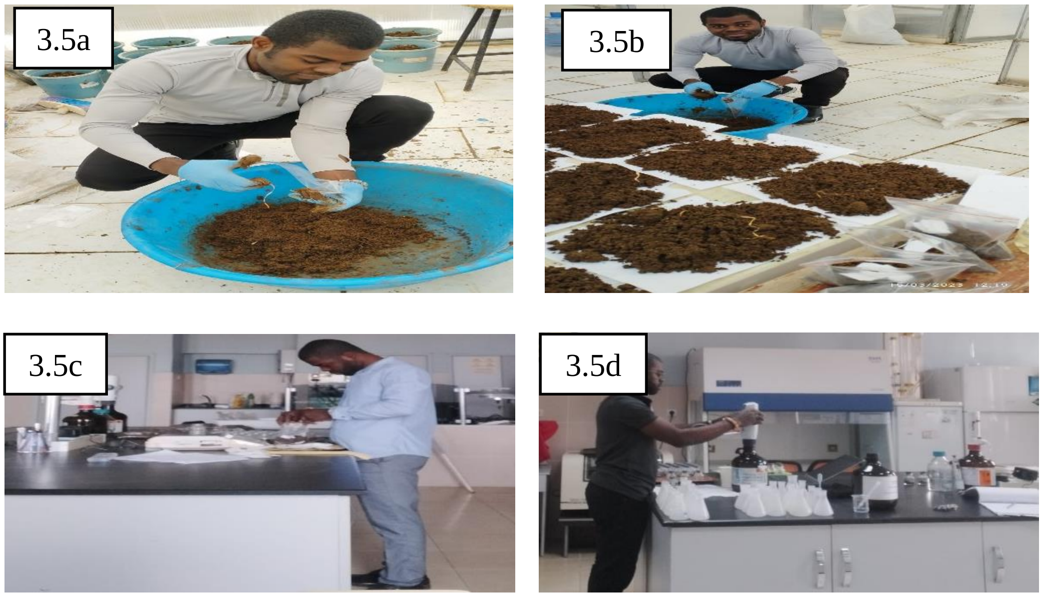

Figure 3.5. Biological analysis. 3.**5a** and 3.**5b** biological soil collection, 3.**5c** post soil weighing, 3.**5d** basal respiration analysis.

3.5.3. analysis of soil quality utilizing the analytical hierarchy process (AHP) method and the standard score function.

The experiment used 21 soil indicators to determine the soil quality index. Since each soil indicator uses a distinct unit, they were first transformed into a unit-less form between 0.1 and 1 using the standard scoring function described by Mukherjee and Lal (2014). After that, the soil indicators were subjected to analytic hierarchy process (AHP) to generate weighted values in determining the effective levels of the soil indicators (Saaty, 2001). In this experiment, two standard scoring functions were used, less is better (LB) and more is better (MB) (Tongsiri et al., 2020). Soil indicators under more is better included MC, AS, OC, OM, N, P, K, Mg, Zn, Cu, dehydrogenase and microbial biomass carbon (Table 8). Those under less is better were pH, EC, Na, Ca, Mn, $CaCO_3$, Fe, C/N ratio and basal soil respiration (Table 3.9).

Table 3.9. Soil parameter, functional types and standard scoring functional equation

Parameter	Functional types	Standard Scoring function (SSF) equations
pH	LB	$$f(x)=\begin{cases}0.1 & x\geq L\\ 1-0.9\times\frac{x-L}{U-L} & L\leq x\leq U\\ 1 & x\leq U\end{cases} \quad (1)$$
EC	LB	
Na	LB	
Mn	LB	
$CaCO_3$	LB	
Fe	LB	
C/N	LB	
basal soil respiration	LB	
MC	MB	$$f(x)=\begin{cases}0.1 & x\geq L\\ 0.9\times\frac{x-L}{U-L}+0.1 & L\leq x\leq U\\ 1 & x\leq U\end{cases} \quad (2)$$
AS	MB	
OC	MB	
OM	MB	
NPK	MB	
Mg	MB	
Zn	MB	
Cu	MB	
Dehydrogenase (DHA)	MB	
microbial biomass carbon (Cmic)	MB	

LB-less is better, MB – more is better, U and L are the upper and lower threshold values, respectively.

The AHP approach (Saaty 1980) was chosen and applied in this study for weighting the criteria and sub-criteria for assessing the land's suitability for rice growing. To solve difficulties, AHP employs the notion of building hierarchies. The hierarchy provides for the evaluation of each criterion at a lower level's contribution to a criterion at a higher level of the hierarchy.

The weightings of parameters were derived using the Pair Wise Comparison Matrix (PWCM) by comparing two parameters. The PWCM approach is used with a scale of 9 to 1/9 or 0.111 (Saaty 1980). If available, a nine-point scale or real-world data can be used to compare (Saaty and Vargas 2001). The nine-point scale includes a scale where 9 means extreme preference, 7 means very strong preference, 5 means strong preference, and so on, down to 1, which means no preference (Table 3.10).

This pair-wise comparison allows for an independent evaluation of each parameter's contribution, simplifying decision-making (Rezaei-Moghaddam and Karami 2008; Şener et al. 2010; Dengiz et al. 2015).

Table 3.10. The comparison scale in AHP (Saaty, 1980).

Intensity of importance	Definition	Explanation
1	Equal importance	Two activities contribute equally to the objective
3	Weak importance of one over another	Experience and judgment slightly favour one activity over another
5	Essential or strong importance	Experience and judgment strongly favour one activityover another
7	Demonstrated importance	Activity is strongly favoured, and its dominance is demonstrated in practice
9	Absolute importance	The evidence favouring one activity over another is of the highest possible order of affirmation
2, 4, 6, 8	Intermediate values between the two adjacent judgments	When compromise is needed
Reciprocals of the above nonzero	If activity i has one of the above nonzero numbers assigned to it when compared with activity j, then j has the reciprocal value when compared with i	

The pair-wise comparisons of the various criteria were grouped into a square matrix in the current study. The diagonal elements of the matrix were assigned a score of one. The comparison matrix's major eigenvalue and related normalized right eigenvector gave the relative importance of the compared criterion. The normalized eigenvector elements were weighted about the criterion or sub-criterion and scored about the alternatives (Bhushan and Rai 2004). The consistency of the order n matrix was then assessed. If the consistency index did not reach a certain level, the results of the comparisons were re-examined. The consistency index, CI, was calculated as follows:

$$CI = (\lambda_{max} - n)/(n-1) \qquad (3)$$

Where: CI is the consistency index, λ_{max} is the largest or principal eigenvalue of the matrix, and n is the order of the matrix. This CI can be compared to that of a random matrix, RI (Table 3.11), and the resulting ratio, CI/RI, is the consistency ratio, CR. In general, a value of CR 0.1 should be maintained to keep the matrix consistent.

Table 3.11. Values of Random Index (RI)

n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
RI	0.00	0.00	0.58	0.90	1.12	1.24	1.32	1.41	1.45	1.49	1.51	1.48	1.56	1.57	1.59

In other words, for all RIs in the single and general hierarchies, the findings were less than 0.1. The consistency index is improved by indicator homogeneity within each group, fewer elements in the group, and a better knowledge of the decision problem (Saaty 1993).

3.5.4. Plant data collection

Five plants were tagged randomly for vegetative and yield data collection from each unit.

3.6. Statistical Data Analysis

All data sets were keyed into Excel version 16 and later analysed using the R-programming package. A split plot model at $P \leq 0.05$ was used to test the effect of treatments as categorical predictors. Significantly different data means were separated using post-hoc LSD ($P \leq 0.05$). Correlation ($P = 0.05$) analyses were performed to determine the degree of association between dependent variables.

4. RESULTS AND DISCUSSION

4.1. Treatment effect on wheat parameters under typic ustipomment (loam) soil

4.1.1. Sole factors modulated wheat parameters under typic ustipomment soil

Table 4.1 shows the effect of different biostimulants (*ekofertile*® and *microfertile*®) and dosages, including control and inorganic fertilization, on wheat growth parameters.

Plant height was highest in *ekofertile*® (74.6 cm), significantly different from *microfertile*®. Similar trends were seen in grains number (29), grains weight (2.1 g), head weight (2.5 g) and plant biomass (72 g), while leaf area and head length did not experience significant differences between the biostimulants with *ekofertile*® having larger leaf area (45.3 cm^2) and longer head length (14.6 cm). Several studies have investigated the effects of biostimulants on plant growth parameters. For instance, Kaushal and Wani (2016) found that applying biostimulants increased crop yield, plant growth, and nutrient uptake. Caruso et al. (2019) and De Pascale et al. (2018) also reported that biostimulant application improved plant growth by enhancing nutrient uptake and stress tolerance. Mosa et. (2023) showed that different biostimulants increase shoot length and diameter, leaf area and chlorophyll differently in apple trees when they used moringa leaf extract, seaweed extract and fulvic acid in apple plantation under loam soil which is in line with this study.

Also, the results showed that the biostimulant effect is dosage dependent, with 10% biostimulant dosage significantly increasing height weight by 23% compared to the control. This result is supported by Mosa et al. (2023), who utilised different dosages of moringa leaf extract, seaweed extract and fulvic acid in apple plantations under loam soil, with the highest result coming from the highest dosage. The significant dominant effect of the 10% biostimulant dosage continued for head length (14.8 cm), plant biomass (78.4 g), grains number (31) and grains weight (2.1) except for leaf area (45.8 cm^2) where inorganic fertilization had best result (Table 4.1). Other previous studies have shown that the effectiveness of biostimulants can be dose-dependent. For example, Bulgari et al. (2015) found that increasing the concentration of a biostimulant improved plant growth parameters up to a certain threshold, beyond which no further benefits were observed. The results in this study support this finding, as the highest values for wheat growth parameters were generally observed at the highest biostimulant dosage (10%).

Table 4.1. Effect of sole factors of biostimulants and dosages on wheat parameters for typic ustipomment

	Plant height (cm)	Leaf area (cm^2)	Head length (cm)	Head weight (g)	Plant biomass (g)	Grains number	Grains weight (g)
			Biostimulant type				
ekofertile®	74.6^a	45.3^a	14.6^a	2.5^a	72.0^a	29^a	2.1^a
microfertile®	73.1^b	43.2^a	13.8^a	2.2^b	65.8^b	25^b	1.8^b
Significance level	0.05	1	0.1	0.05	0.05	0.01	0.05
			Dosage				
Control	69.6^c	41.1^d	13.3^b	1.9^d	60.3^c	23^d	1.5^d
Inorganic	74.0^a	45.8^a	13.6^b	2.1^c	64.5^{bc}	26^b	1.8^b
2.5% biostimulant	72.8^b	42.8^c	13.6^b	2.2^c	62.0^{bc}	25^c	1.7^c
5% biostimulant	74.0^a	44.3^b	14.2^{ab}	2.3^b	66.4^b	26^b	1.9^b
10% biostimulant	74.8^a	45.5^a	14.8^a	2.6^a	78.4^a	31^a	2.1^a
Significance level	0.001	0.001	0.05	0.001	0.001	0.001	0.001

4.1.2. Interaction of factors modulated on wheat parameters under typic ustipomment soil

Table 4.2 shows that both biostimulant products at different dosages significantly affect wheat growth parameters compared to the control. The 10% dosage of both *ekofertile®* and *microfertile®* produced the highest values for most of the growth parameters observed. The head weight of wheat was highest for both biostimulant products at the 10% dosage (*ekofertile®*: 2.8 g; *microfertile®*: 2.3 g) compared to the control (1.90 g) and the lowest dosage groups. The grains number (*ekofertile®*: 35; *microfertile®*: 26) and grains weight (*ekofertile®*: 2.3 g; *microfertile®*: 1.9 g) also followed a similar trend with the highest values observed at the 10% dosage of both biostimulant products. Plant height showed no significant differences among the different dosages of the biostimulant products, with the best result from *ekofertile®* 10% dosage (75.4 cm) than the control. A similar result was seen with head length, 15.3 cm length for *ekofertile®* 10% dosage. The highest leaf area was observed at the 10% dosage of *ekofertile®* (46.5 cm^2), and the highest plant biomass was observed at the 10% dosage of *ekofertile®* (85.0 g). These findings are consistent with previous studies that have reported the positive effects of biostimulants on plant growth. For instance, the study by Mosa et al. (2023) showed that using biostimulants with the right dosage could improve plant growth and yield, as seen in the apple plantation they experimented on.

Table 4.2. Interaction effect of biostimulants and dosage on wheat growth parameters for typic ustipomment soil

Biostimulant and dosage interaction		Plant height (cm)	Leaf area (cm^2)	Head length (cm)	Head weight (g)	Plant biomass (g)	Grains number	Grains weight (g)
ekofertile®	Control	69.6^{a}	41.1^{e}	13.3^{a}	1.9^{b}	60.3^{d}	23^{d}	1.5^{d}
	Inorganic	74.0^{a}	45.8^{ab}	13.6^{a}	2.1^{ab}	64.5^{cd}	26^{c}	1.8^{c}
	2.5%	73.7^{a}	43.4^{cd}	14.1^{a}	2.3^{ab}	62.9^{cd}	26^{c}	1.9^{bc}
	5%	74.8^{a}	46.1^{ab}	14.6^{a}	2.4^{ab}	68.2^{bc}	27^{b}	2.1^{b}
	10%	75.4^{a}	46.5^{a}	15.3^{a}	2.8^{a}	85.1^{a}	35^{a}	2.3^{a}
microfertile®	Control	69.6^{a}	41.1^{e}	13.3^{a}	1.9^{b}	60.3^{d}	23^{d}	1.5^{d}
	Inorganic	74.0^{a}	45.8^{ab}	13.6^{a}	2.1^{ab}	64.5^{cd}	26^{c}	1.8^{c}
	2.5%	71.9^{a}	42.3^{de}	13.2^{a}	1.9^{ab}	61.1^{d}	23^{d}	1.6^{d}
	5%	73.3^{a}	42.7^{d}	13.8^{a}	2.3^{ab}	64.7^{cd}	26^{c}	1.8^{c}
	10%	74.3^{a}	44.6^{bc}	14.3^{a}	2.3^{ab}	71.8^{b}	26^{bc}	1.9^{bc}
Significance level		1	0.05	1	0.01	0.05	0.001	0.01

4.2. Treatment effect on typic ustipomment soil physicochemical properties

4.2.1. Sole factors effect on typic ustipomment soil physicochemical properties

Table 4.3a shows the effect of biostimulant types and dosage levels on post-soil physicochemical properties.

pH and Na were significantly affected by biostimulant types, with *ekofertile®* raising the pH (7.83) more than *microfertile®* (7.77), whereas *microfertile®* affected Na content (1.43 meq/100g) more than *ekofertile®* (1.37 meq/100g). This result contradicts Yousfi et al. (2021), who reported decreased soil pH after rhizospheric biostimulant application under sandy and sandy loam soil. The effect of biostimulant types did not differ between each other for EC, Ca, Mg, K, $CaCO_3$, OC, OM and N, with *ekofertile®* exerting more effect on EC (666), Ca (68 meq/100g), Mg (18.9 meq/100g), K (1.91 meq/100g), $CaCO_3$ (13.48%), OC (3.02%), OM (5.21%) and N (0.28%) as shown in Table 4.3a due to its high nutrient content and microbial diversity (Table 3.2 and 3.3).

Dosage levels of biostimulants, including control and inorganic fertilization, demonstrated significant effects on post-soil physicochemical properties, as displayed in Table 4.3a. Biostimulant, 10% dosage, raised the pH highest (7.84), whereas EC was highest at the control (714). Biostimulant, 10% dosage, had highest Ca (70.6 meq/100g), Mg (21.1 meq/100g), Na (1.42 meq/100g), K (1.91 meq/100g), $CaCO_3$ (14.15%), OC (3.03%), OM (5.22%) amounts except for N (0.29%) which was highest in inorganic fertilization (Table 4.3a). Yousfi et al. (2021) showed that biostimulants increased organic matter and soil nutrient content under sandy and sandy loam soil, supporting this study's result. The high mineral content in biostimulant treatments shows the ability of microbial content in biostimulants to decompose the organic matter by immobilization and mineralization (Meena *et al*., 2015; Oteino *et al*., 2015; Naik *et al*., 2019). The high soil potassium content was a result of potassium-solubilizing bacteria such as *Lactobacillus,* which can solubilize K into assimilated forms from K minerals to soluble K in the soil for plants to be easily absorbed (Meena *et al.*, 2015; Tchakounté *et al.*, 2018) through the synthesis of organic acids (Figueiredo et al., 2020; Lidbury et al., 2021).

Table 4.3a. Sole factors effect of biostimulants and dosage on loam soil chemical properties

	pH	EC	Ca	Mg	Na	K	$CaCO_3$	OC	OM	N
		(μscm^{-1})	(meq/100g)				%			
Biostimulant type										
ekofertile®	7.83^{a}	666^{a}	68.0^{a}	18.9^{a}	1.37^{b}	1.91^{a}	13.48^{a}	3.02^{a}	5.21^{a}	0.28^{a}
microfertile®	7.77^{b}	647^{a}	67.3^{a}	18.4^{a}	1.43^{a}	1.82^{a}	13.45^{a}	2.92^{a}	5.04^{a}	0.27^{a}
Significance level	0.05	1	1	1	0.05	0.1	1	1	1	0.1
Dosage										
Control	7.78^{bc}	714^{a}	64.7^{c}	13.6^{d}	1.20^{d}	1.75^{c}	12.12^{d}	2.54^{d}	4.38^{d}	0.25^{c}
Inorganic	7.71^{d}	678^{ab}	68.0^{b}	13.9^{cd}	1.26^{c}	1.87^{ab}	13.61^{b}	2.75^{c}	4.74^{c}	0.29^{a}
2.5% biostimulant	7.76^{c}	641^{c}	65.4^{c}	16.1^{c}	1.36^{b}	1.81^{bc}	12.86^{c}	2.91^{b}	5.01^{b}	0.27^{bc}
5% biostimulant	7.81^{ab}	680^{ab}	67.0^{b}	18.8^{b}	1.43^{a}	1.87^{ab}	13.23^{bc}	2.98^{a}	5.13^{a}	0.28^{ab}
10% biostimulant	7.84^{a}	649^{bc}	70.6^{a}	21.1^{a}	1.42^{a}	1.91^{a}	14.15^{a}	3.03^{a}	5.22^{a}	0.28^{ab}
Significance level	0.001	0.01	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01

Table 4.3b showed that there existed no significant differences across the two biostimulant types in P, Fe, Zn, C/N ratio, moisture content (MC) and aggregate stability (AS) except for Mn (27.4 ppm) and Cu (4.7 ppm) where *ekofertile*® was significantly different from *microfertile*®. On the contrary, dosage levels significantly affected P, Fe, Zn, C/N ratio, and MC, including Mn and Cu, except AS (Table 4.3b). Biostimulant, 10% dosage, had higher P (71.9 ppm), Fe (79.8 ppm), Mn (26 ppm), Zn (1.2 ppm), and MC (1.84%) content, while 5% biostimulant dosage had highest Cu (4.7 ppm) with best C/N ratio (9.5) coming from inorganic fertilization. A similar result was reported by Tchakounté *et al.* (2018), who demonstrated the nutrient mobilization potential of beneficial microbes.

Table 4.3b. Sole factors effect of biostimulants and dosage on loam soil chemical properties

	P	Fe	Mn	Zn	Cu	C/N	MC	AS
	(ppm)					ratio	%	
Biostimulant type								
ekofertile®	70.7^{a}	77.5^{a}	27.4^{a}	1.2^{a}	4.7^{a}	10.80^{a}	1.79^{a}	15.41^{a}
microfertile®	67.9^{a}	71.2^{a}	26.3^{b}	1.0^{a}	4.5^{b}	10.79^{a}	1.84^{a}	14.64^{a}
Significance level	1	1	0.05	1	0.05	1	1	1
Dosage								
Control	62.5^{c}	44.9^{c}	20.7^{c}	0.5^{c}	4.0^{b}	10.0^{b}	1.75^{ab}	16.33^{a}
Inorganic	64.4^{c}	48.6^{c}	24.3^{bc}	0.7^{bc}	4.1^{c}	9.5^{b}	1.57^{b}	18.38^{a}
2.5% biostimulant	66.4^{bc}	60.8^{b}	25.5^{ab}	0.9^{b}	4.5^{b}	10.8^{a}	1.82^{a}	15.03^{a}
5% biostimulant	69.7^{ab}	82.4^{a}	29.2^{a}	1.2^{a}	4.7^{a}	10.8^{a}	1.77^{a}	18.18^{a}
10% biostimulant	71.9^{a}	79.8^{a}	26.0^{ab}	1.2^{a}	4.6^{b}	10.8^{a}	1.84^{a}	11.88^{a}
Significance level	0.01	0.001	0.01	0.001	0.001	0.01	0.05	1

4.2.2. Interaction of factors effect on typic ustipomment soil physicochemical properties

Table 4.4a shows no significant effect of biostimulants and dosage interaction on soil's Ca, Mg, Na, $CaCO_3$, OC, OM and N content after harvest. Nonetheless, *ekofertile®* at 10% dosage had the highest Ca (71.6 meq/100g), Mg (21.3 meq/100g), $CaCO_3$ (14.53%), OC (3.07%) and OM (5.29%) compared to control and the other dosages while *microfertile®* at 5% dosage had the highest Na content (1.46 meq/100g) than the other dosages with control the least (1.20 meq/100g) and N content (0.29%) was highest at both biostimulants inorganic fertilization treatment. On the contrary, pH, EC and K were significantly affected by biostimulants and dosage interaction with *ekofertile®* at 10% dosage, raising pH more (7.91) and had high K (1.99 meq/100g) content while EC (714) was highest at control treatment in both biostimulants (Table 4.4a). Treatment application increases the soil nutrient status compared to the control. This is attributed to the rich nutrient content of the biostimulants, as stated by Agbor et al. (2022). Also, the biostimulant contains a variety of beneficial microorganisms involved in nutrient fixation and solubilization.

Table 4.4a. Biostimulant and dosage interaction effects on loam soil chemical properties

Biostimulant and dosage interaction		pH	EC	Ca	Mg	Na	K	$CaCO_3$	OC	OM	N
			(µscm^{-1})	(meq/100g)				%			
ekofertile®	Control	7.78[bc]	714[a]	64.7[a]	13.6[a]	1.20[a]	1.75[d]	12.12[a]	2.54[a]	4.38[a]	0.25[a]
	Inorganic	7.71[d]	678[abc]	68.4[a]	14.9[a]	1.26[a]	1.87[bc]	13.61[a]	2.75[a]	4.74[a]	0.29[a]
	2.50%	7.72[d]	630[cd]	65.7[a]	16.3[a]	1.30[a]	1.82[cd]	12.88[a]	2.96[a]	5.10[a]	0.27[a]
	5%	7.87[a]	667[abc]	67.0[a]	19.0[a]	1.40[a]	1.93[ab]	13.02[a]	3.04[a]	5.23[a]	0.28[a]
	10%	7.91[a]	702[ab]	71.6[a]	21.3[a]	1.42[a]	1.99[a]	14.53[a]	3.07[a]	5.29[a]	0.29[a]
microfertile®	Control	7.78[bc]	714[a]	64.7[a]	13.6[a]	1.20[a]	1.75[d]	12.12[a]	2.54[a]	4.38[a]	0.25[a]
	Inorganic	7.71[d]	678[abc]	68.4[a]	14.9[a]	1.26[a]	1.87[ab]	13.61[a]	2.75[a]	4.74[a]	0.29[a]
	2.50%	7.80[a]	652[bc]	65.2[a]	15.9[a]	1.42[a]	1.81[cd]	12.84[a]	2.86[a]	4.92[a]	0.27[a]
	5%	7.74[cd]	694[ab]	66.9[a]	18.6[a]	1.46[a]	1.82[cd]	13.43[a]	2.91[a]	5.02[a]	0.27[a]
	10%	7.77[bc]	595[d]	69.7[a]	20.8[a]	1.43[a]	1.82[cd]	13.77[a]	2.99[a]	5.16[a]	0.28[a]
Significance level		0.001	0.01	1	1	0.1	0.05	1	1	1	1

Similarly, no significant effects of biostimulants and dosage interaction were observed for P, Mn, Zn, C/N ratio, MC and AS, as shown in Table 4.4b. Nevertheless, P content (73.3 ppm) was highest in 10% *ekofertile*® dosage, while Mn (30.2 ppm) was highest in 5% *ekofertile*® dosage, same with Zn (1.2 ppm) and AS (18.38%), inorganic fertilization had the best C/N ratio (9.5). Biostimulants and dosage interactions significantly affected Fe and Cu, with Fe (82.8 ppm) content highest in 5% *ekofertile*® dosage, same with Cu (4.8 ppm). The high available P and micronutrient content in biostimulant treatments demonstrates the ability of single and synergistic microbes to solubilize P through the displacement of sorption equilibria, which results in an increased net transfer of phosphate ions into soil solution or an increase in the mobility of organic forms of P, as well as through the stimulation of metabolic processes that are effective in directly reducing P.

Solubilizing and mineralizing P from inorganic and organic P in poorly accessible forms (Etesami et al., 2021). These mechanisms include hydrogen ion excretion, organic acid release, siderophores generation, and the development of phosphate enzymes capable of hydrolyzing soil organic P. (Etesami et al., 2020). Organic acids and associated protons, in particular, are effective at dissolving precipitated forms of soil P (e.g., Fe- and Al-P in acidic soils, Ca-P in alkaline soils), chelating metal ions that may be associated with complexed forms of P, or facilitating the release of adsorbed P via ligand exchange reactions (Rawat et al., 2021).

Table 4.4b: Biostimulant and dosage interaction effects on soil chemical properties

Biostimulant and dosage interaction		P	Fe	Mn	Zn	Cu	C/N	MC	AS
		(ppm)					ratio	(%)	
ekofertile®	Control	62.5^{a}	44.9^{d}	20.7^{a}	0.5^{a}	4.0^{d}	10.0^{a}	1.75^{a}	16.33^{a}
	Inorganic	64.4^{a}	48.6^{cd}	24.3^{a}	0.7^{a}	4.1^{d}	9.5^{a}	1.57^{a}	18.38^{a}
	2.5%	67.7^{a}	67.8^{b}	25.8^{a}	1.0^{a}	4.8^{a}	10.8^{a}	1.71^{a}	14.85^{a}
	5%	71.1^{a}	82.8^{a}	30.2^{a}	1.2^{a}	4.8^{a}	10.9^{a}	1.76^{a}	18.38^{a}
	10%	73.3^{a}	82.0^{a}	26.2^{a}	1.2^{a}	4.6^{b}	10.7^{a}	1.90^{a}	13.02^{a}
microfertile®	Control	62.5^{a}	44.9^{d}	20.7^{a}	0.5^{a}	4.0^{d}	10.0^{a}	1.75^{a}	16.33^{a}
	Inorganic	64.4^{a}	48.6^{cd}	24.3^{a}	0.7^{a}	4.1^{d}	9.5^{a}	1.57^{a}	18.38^{a}
	2.5%	65.1^{a}	53.9^{c}	25.1^{a}	0.8^{a}	4.3^{c}	10.8^{a}	1.94^{a}	15.21^{a}
	5%	68.2^{a}	81.9^{a}	28.2^{a}	1.2^{a}	4.6^{b}	10.7^{a}	1.78^{a}	17.98^{a}
	10%	70.5^{a}	77.7^{a}	25.8^{a}	1.1^{a}	4.5^{b}	10.8^{a}	1.81^{a}	10.74^{a}
Significance level		1	0.05	1	1	0.001	1	1	1

4.3. Utilising analytic hierarchy process (AHP) for the assessment of the indicator weighted

Table 4.5 displays the weight of soil properties evaluated using the AHP. Here the major soil properties, physicochemical, fertility and biological, are weighted generally against each other. Subsequently, the soil indicators were weighted per major category according to their significance using the AHP scale. The combined weight of the soil quality indicators was gotten by multiplying the general weight of the major soil categories with the weight of the soil indicators per category. This gave the weight of each soil quality parameter per category it belongs to.

Considering the major categories (Table 4.5), the highest values were obtained in the physicochemical (0.5396) category, while fertility yielded the least value (0.1634). The best contribution for soil indicators per category came from OM (0.2843), N (0.2153), and MBC (0.4330), respectively. Soil physicochemical and fertility properties come to mind when discussing soil fertility and quality (Tian et al., 2022). However, soil biological properties are pivotal in soil fertility enhancement (Sofo et al., 2022). Under loam soil conditions, microbial biomass carbon (0.4330) contributed the highest to the soil quality index using the AHP, followed by dehydrogenase enzyme activity(0.3085). OM's dominant contribution in the physicochemical category is consistent with other studies which suggested a similar trend (Olorunfemi et al., 2018; Alaboz and Hasan, 2020). This is because the decomposition of organic materials significantly affects the soil's fertility and biological properties. Macronutrients contributed better than micronutrients under the fertility category, with N having the highest value (0.21530). This is because plants generally require macronutrients in higher amounts than micronutrients, with severe deficiencies in plants if their amounts are inadequate in the soil.

Table 4.5. Contribution weight of soil indicators to soil quality calculated by the AHP

	Physico-chemical	Fertility	Biology	Wi
	0,5396	0,1634	0,297	Birleştirilmiş Ağırlık Toplam AixCi
MC	0,1234			0,0666
AS	0,1943			0,1048
pH	0,0677			0,0365
EC	0,0427			0,0230
$CaCO_3$	0,0604			0,0326
OC	0,2272			0,1226
OM	0,2843			0,1534
N		0,2153		0,0352
P		0,1889		0,0309
K		0,1482		0,0242
Ca		0,1185		0,0194
Mg		0,096		0,0157
Na		0,0218		0,0036
Fe		0,0719		0,0117
Cu		0,0369		0,0060
Zn		0,0567		0,0093
Mn		0,0430		0,0070
MBC			0,4330	0,1286
CO_2			0,3085	0,0916
C/N			0,1645	0,0489
qCO_2			0,0940	0,0279
	1.0000	1.0000	1.0000	1.0000

4.4 Treatment effect on typic ustipomment soil biological properties, soil quality and wheat yield

4.4.1. Sole effects of factors on typic ustipomment soil biological properties, soil quality and wheat yield

Table 4.6 shows the effect of different biostimulants (*ekofertile*® and *microfertile*®) and dosages, including control and inorganic fertilization, on soil biological properties dynamics.

Dehydrogenase (DHA) enzyme activity was highest in *ekofertile*® (55.88 µgTPFg^{-1}), which was not significantly different from *microfertile*®. Similar trends were seen in microbial biomass carbon (Cmic) (25.34 mgCO_2-C100g^{-1}), microbial basal soil respiration (BSR) (0.107 µgCO_2-Cg^{-1}), and biological wheat yield (22.70 tha^{-1}), while soil quality index (SQI) was not significant modulated with highest effect from *ekofertile*® (0.64).

Also, the results showed that the biostimulant effect is dosage dependent, with 10% biostimulant dosage significantly increasing Dehydrogenase (DHA) enzyme activity (59 µgTPFg^{-1}) compared to the control (36.57 µgTPFg^{-1}). The significant dominant effect of the 5% biostimulant dosage continued for microbial biomass carbon (Cmic) (26.22 mgCO_2-C100g^{-1}) and soil quality index (SQI) (0.65). Biostimulant 10% significantly modulated microbial basal soil respiration (BSR) (0.109 µgCO_2-Cg^{-1}) and biological wheat yield (24.46 tha^{-1}) with the highest values compared to the control (Table 4.6), while soil quality indexed (SQI) was highest at 5% biostimulant dosage (Fig. 4.1).

The increase in dehydrogenase activity and microbial biomass carbon with the application of biostimulants is consistent with previous studies. For instance, Nanda et al. (2022) reported that applying a biostimulant based on amino acids and seaweed extracts increased soil microbial biomass and enzyme activities. Similarly, Canellas et al. (2002) and Holatko et al. (2020) found that applying a humic acid-based biostimulant enhanced the soil's dehydrogenase activity and microbial biomass.

The higher basal soil respiration observed with the application of biostimulants suggests an increase in soil microbial activity and organic matter decomposition. This is supported by the findings of Piotrowska et al. (2012) and

Silva et al. (2023), who reported that applying a biostimulant increased soil respiration rates due to the stimulation of microbial activity.

The dosage dependency of biostimulants is evident in the results, with higher dosages generally leading to greater improvements in soil biological properties and biological wheat yield. The 10% biostimulant dosage consistently outperformed other dosages and the control or inorganic fertilizer treatments regarding DHA activity, Cmic, BSR, SQI, and wheat yield. These findings align with previous studies that have reported the dosage-dependent effects of biostimulants on crop growth and soil health (De Pascale et al., 2018).

Table 4.6. Sole factors effect of biostimulants and dosage on loam soil biological properties

	DHA $\mu gTPFg^{-1}$	Cmic, mg CO_2-C $100g^{-1}$	BSR, µg CO_2-Cg^{-1}	SQI	Biological yield (th^{e-1})
		Biostimulant type			
ekofertile®	55.88^{a}	25.34^{a}	0.107^{a}	0.64^{a}	22.70^{a}
microfertile®	55.70^{a}	22.31^{a}	0.097^{a}	0.58^{a}	21.94^{a}
Significance level	1	0.1	1	0.1	0.1
		Dosage			
Control	36.57^{b}	18.63^{c}	0.093^{bc}	0.38^{d}	19.66^{c}
Inorganic	50.75^{a}	22.10^{b}	0.089^{c}	0.54^{c}	22.38^{b}
2.5% biostimulant	52.34^{a}	19.85^{c}	0.096^{bc}	0.56^{bc}	20.66^{c}
5% biostimulant	59.39^{a}	26.22^{a}	0.102^{ab}	0.65^{a}	21.85^{b}
10% biostimulant	55.66^{a}	25.41^{a}	0.109^{a}	0.62^{ab}	24.46^{a}
Significance level	0.01	0.001	0.01	0.001	0.001

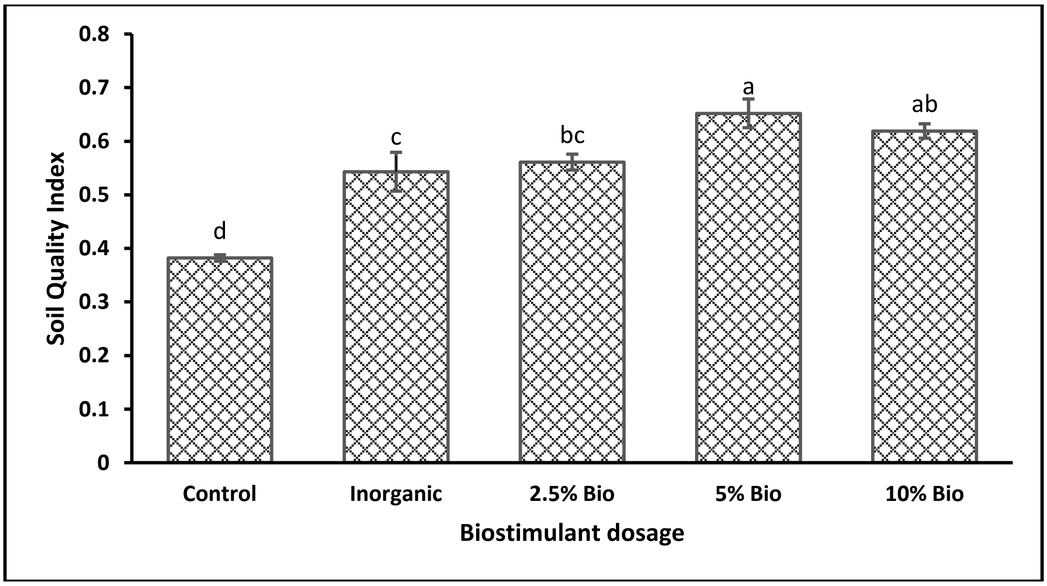

Figure 4.1. Dosage modulated loam soil quality index

4.4.2. Interaction effects of factors on typic ustipomment soil biological properties, soil quality and wheat yield

The results in Table 4.7 showed that both biostimulant products at different dosages did not significantly modulate Dehydrogenase (DHA) enzyme activity, microbial biomass carbon (Cmic), soil quality index and wheat biological yield, while microbial basal soil respiration (BSR) was significantly modulated. The 5% dosage of *microfertile®* produced the highest Dehydrogenase (DHA) enzyme activity (60.42 $\mu gTPFg^{-1}$). The microbial biomass carbon (Cmic) was highest at *ekofertile®* 5% dosage (27.96 $mgCO_2$-$C100g^{-1}$) and lowest in control (18.63 $mgCO_2$-$C100g^{-1}$). The microbial basal soil respiration (BSR) was highest (0.121 μgCO_2-Cg^{-1}) at *ekofertile®* 10% dosage, with significant differences across the other treatment interactions. Despite the Soil Quality index showing no significant differences among the different dosages of the biostimulant products, the best result was recorded in *ekofertile®* 5% dosage (0.69), while the best wheat biological yield, 25.01 tha^{-1} was recorded in *ekofertile®* 10% dosage with no significant differences among the different dosages of the biostimulant products (Table 4.7).

Table 4.7. Biostimulant and dosage interaction effects on loam soil biological properties

Biostimulant and dosage interraction		DHA $\mu gTPFg^{-1}$	Cmic, mg CO_2-C $100g^{-1}$	BSR, μg CO_2-Cg^{-1}	SQI	Biological yield (tha^{-1})
ekofertile®	Control	36.57^a	18.63^a	0.093^{bc}	0.38^a	19.66^a
	Inorganic	50.75^a	22.10^a	0.089^c	0.54^a	22.38^a
	2.5%	53.79^a	20.52^a	0.098^b	0.59^a	20.98^a
	5%	55.35^a	27.96^a	0.103^a	0.69^a	22.13^a
	10%	55.51^a	27.54^a	0.121^a	0.64^a	25.01^a
microfertile®	Control	36.57^a	18.63^a	0.093^{bc}	0.38^a	19.66^a
	Inorganic	50.75^a	22.10^a	0.089^c	0.54^a	22.38^a
	2.5%	50.88^a	19.18^a	0.094^a	0.54^a	20.33^a
	5%	60.42^a	24.48^a	0.100^a	0.61^a	21.56^a
	10%	55.81^a	23.28^a	0.096^a	0.60^a	23.92^a
Significance level		1	0.1	0.05	1	1

4.5. Treatment effect on wheat parameters under lithic haplustert (clay) soil

4.5.1. Sole factors modulated wheat parameters under Lithic haplustert soil

Biostimulant types significantly ($P \leq .05$) affected the growth of wheat (Table 4.8). *ekofertile®* plant biostimulant had the best growth performance of the two biostimulants used in the lithic haplustert soil of Samsun, Turkey. Significant differences were recorded between the biostimulants for the head weight (1.3 g), leaf area (31.6 cm^2) and biomass (38.4 g). However, despite *ekofertile*® superior results in grains number (19), grains weight (1 g), and plant height (71.4 cm), no statistically significant differences were observed between the two biostimulants (Table 4.8).

Despite the biostimulant performance, the results revealed that the application of biostimulants is dosage dependent. This can be seen in leaf area (36.6 cm^2) and biomass (42.1 g), with 10% biostimulant dosage exhibiting clear statistical superiority among other dosages and inorganic fertilization applied compared to the control. No significant differences were observed for head length even though *ekofertile*® (11.7 cm) had a longer head length than *microfertile*® (11.1 cm). Also, 10% biostimulant dosage showed equal strength with inorganic fertilization applied for grains number (21), grains weight (1.1 g) and plant height (73.8 cm) with no statistical deviations between the pair and was only overshadowed statistically at the head weight (1.6 g) by inorganic fertilization (Table 4.8). This study's results align with Grichar et al. (2023), who found increased maize vigor when treated with biostimulants under clay conditions. Similar results were reported by Kumar et al. (2018), who witnessed improved potato growth when treated with biostimulants.

Table 4.8 Effect of sole factors of biostimulants and dosages on wheat parameters for lithic haplustert soil

	Plant height (cm)	Leaf area (cm^2)	Head length (cm)	Head weight (g)	Plant biomass (g)	Grains number	Grains weight (g)
Biostimulant type							
ekofertile®	71.4^a	31.6^a	11.7^a	1.3^a	38.4^a	19^a	1.0^a
microfertile®	71.1^a	29.1^b	11.2^a	1.1^b	36.3^b	17^a	0.8^a
Significance level	1	0.05	1	0.01	0.05	0.1	0.1
Dosage							
Control	67.9^c	20.6^d	9.1^d	0.9^e	30.1^c	14^d	0.7^c
Inorganic	71.2^{ab}	23.3^c	12.2^a	1.6^a	37.5^b	21^a	1.1^a
2.5% biostimulant	68.7^{bc}	25.9^c	10.8^c	1.0^d	31.7^c	16^c	0.8^{bc}
5% biostimulant	71.2^{ab}	28.6^b	11.5^b	1.2^c	38.2^b	17^b	0.9^b
10% biostimulant	73.8^a	36.6^a	11.9^{ab}	1.4^b	42.1^a	21^a	1.1^a
Significance level	0.001	0.001	0.001	0.001	0.001	0.001	0.001

4.5.2. Interaction of factors modulated wheat parameters under Lithic haplustert soil

Factors interaction showed a significant ($P \leq .05$) effect for head weight (1.5 g) and grains number (22). Despite inorganic fertilization (1.6 g) having better results for head weight, there existed no statistical difference with *ekofertile*® 10%

biostimulant dosage (1.5 g) while *microfertile*® 10% biostimulant dosage (1.3g) was second best. *ekofertile*® 10% biostimulant dosage gave the best result with statistical significance for grains number (22) compared to control (14), inorganic fertilization (21) and the other biostimulant dosages. The Interaction of factors revealed no significant differences in grains weight, plant height, leaf area, head length and biomass. Nonetheless, *ekofertile*® 10% biostimulant dosage had taller plants (74.5 cm), larger leaf area (37.2 cm^2), longer head length (12.2 cm) and more plant biomass (43.7 g) compared to the other treatment combinations while inorganic fertilization had more grains weight (1.17 g) as shown in Table 4.9. This study showed that biostimulant with the right dosage increases plant growth which has been supported by other works (De Pascale et al., 2018; Grichar et al., 2023).

Table 4.9 Interaction effect of biostimulants and dosage on wheat growth parameters for lithic haplustert soil

Biostimulant and dosage interaction		Plant height (cm)	Leaf area (cm^2)	Head length (cm)	Head weight (g)	Plant biomass (g)	Grains number	Grains weight (g)
ekofertile®	Control	67.9^a	20.6^a	9.1^a	0.9^e	30.1^a	14^e	0.66^a
	Inorganic	71.2^a	23.3^a	12.2^a	1.6^a	37.5^a	21^b	1.17^a
	2.5%	69.3^a	26.8^a	11.0^a	1.1^d	31.7^a	17^d	0.80^a
	5%	70.4^a	30.9^a	11.8^a	1.2^c	39.7^a	17^d	0.89^a
	10%	74.5^a	37.2^a	12.2^a	1.5^a	43.7^a	22^a	1.15^a
microfertile®	Control	67.9^a	20.6^a	9.1^a	0.9^e	30.1^a	14^e	0.66^a
	Inorganic	71.2^a	23.3^a	12.2^a	1.6^a	37.5^a	21^b	1.17^a
	2.5%	68.1^a	25.0^a	10.6^a	1.0^e	31.6^a	15^e	0.70^a
	5%	72.0^a	26.3^a	11.3^a	1.1^{cd}	36.7^a	17^d	0.81^a
	10%	73.1^a	36.1^a	11.6^a	1.3^b	40.6^a	19^c	0.99^a
Significance level		1	1	1	0.001	1	0.01	0.1

4.6. Treatment effect on lithic haplustert soil physicochemical properties

4.6.1. Sole factors modulated lithic haplustert soil physicochemical properties

Table 4.10a shows the effect of biostimulant types and dosage levels on post-lithic haplustert soil physicochemical properties.

EC, OC and OM were significantly affected by biostimulant types, with *ekofertile*® raising the EC (620.84) more than *microfertile*® (545.19), whereas *microfertile*® affected OC (1.90%) and OM (3.27%) contents more than *ekofertile*®. The effect of biostimulant types did not differ between each other for pH, Ca, Mg, Na, K, $CaCO_3$, and N, with *microfertile*® exerting more effect on pH (7.04 (H_2O)). Meanwhile, *ekofertile*® had a higher effect on Ca (50.33 meq/100g), Mg (15.91 meq/100g), K (0.86 meq/100g), $CaCO_3$ (3.27%) and N (0.148%) as shown in Table 4.10a.

Dosage levels of biostimulants, including control and inorganic fertilization, demonstrated significant effects on post-soil physicochemical properties, as displayed in Table 4.10a. Control treatment had a higher pH (7.08), whereas EC was highest at a 10% dosage level (613). Biostimulant, 10% dosage, had the highest Ca (52.30 meq/100g), Mg (15.95 meq/100g), Na (0.80 meq/100g), K (0.87 meq/100g), $CaCO_3$ (3.41%), OC (1.96%), OM (3.38%) amounts except for N (0.162%) which was highest in inorganic fertilization (Table 4.10a).

Table 4.10a. Sole factors effect of biostimulants and dosage on clay soil physicochemical properties

	pH	EC	Ca	Mg	Na	K	$CaCO_3$	OC	OM	N
		(µscm^{-1})	(meq/100g)				%			
Biostimulant type										
ekofertile®	6.99^{a}	620.84^{a}	50.33^{a}	15.91^{a}	0.72^{a}	0.86^{a}	3.27^{a}	1.83^{b}	3.15^{b}	0.148^{a}
microfertile®	7.04^{a}	545.19^{b}	49.02^{a}	15.38^{a}	0.69^{a}	0.84^{a}	3.07^{a}	1.90^{a}	3.27^{a}	0.147^{a}
Significance level	1	0.01	1	0.1	0.1	1	1	0.05	0.05	1
Dosage										
Control	7.08^{a}	537.53^{c}	44.85^{d}	12.78^{b}	0.48^{d}	0.77^{c}	2.81^{c}	1.55^{c}	2.67^{c}	0.125^{d}
Inorganic	6.99^{c}	592.63^{b}	49.04^{bc}	14.94^{a}	0.57^{c}	0.78^{c}	3.01^{b}	1.60^{c}	2.75^{c}	0.162^{a}
2.5% biostimulant	7.00^{bc}	548.83^{c}	46.82^{cd}	15.26^{a}	0.59^{c}	0.83^{b}	3.03^{b}	1.71^{b}	2.94^{b}	0.139^{c}
5% biostimulant	6.99^{c}	587.12^{b}	49.90^{ab}	15.72^{a}	0.73^{b}	0.85^{ab}	3.08^{b}	1.93^{a}	3.32^{a}	0.148^{bc}
10% biostimulant	7.06^{ab}	613.10^{a}	52.30^{a}	15.95^{a}	0.80^{a}	0.87^{a}	3.41^{a}	1.96^{a}	3.38^{a}	0.156^{ab}
Significance level	0.05	0.001	0.001	0.05	0.001	0.001	0.001	0.001	0.001	0.001

Table 4.10b showed that there existed no significant differences across the two biostimulant types in P, Fe, Mn, Zn, Cu and C/N ratio, except for moisture content (MC) (5.92%) and aggregate stability (AS) (56.83%) where *microfertile*® was significantly different from *ekofertile*®. On the contrary, dosage levels significantly affected P, Fe, Mn, Zn, Cu, C/N ratio, MC and AS (Table 4.10b). Biostimulant, 10% dosage, had higher P (7.79 ppm), Fe (60.06 ppm), Mn (59.81 ppm), Zn (2.47 ppm) and Cu (2.52 ppm), while MC (6.47%) was best in control. Biostimulant, 2.5% dosage, had the best aggregate stability (57.67%), with the best C/N ratio (9.87) coming from inorganic fertilization (Table 4.10b).

Table 4.10b. Sole factors effect of biostimulants and dosage on lithic haplustert soil physicochemical properties

	P (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	C/N Ratio	%MC	%AS
Biostimulant type								
ekofertile®	7.06[a]	49.55[a]	46.56[a]	2.35[a]	2.47[a]	12.37[a]	5.27[b]	53.25[b]
microfertile®	5.71[a]	50.46[a]	46.46[a]	2.25[a]	2.46[a]	12.99[a]	5.92[a]	56.83[a]
Significance level	1	1	1	0.1	1	1	0.01	1
Dosage								
Control	4.12[c]	28.75[d]	26.93[d]	1.98[b]	2.30[c]	12.43[a]	6.47[a]	52.45[bc]
Inorganic	4.55[c]	47.21[bc]	40.87[bc]	2.40[a]	2.51[a]	9.87[b]	5.01[c]	48.31[c]
2.5% biostimulant	5.47[b]	41.81[bc]	36.01[c]	2.05[b]	2.40[b]	12.34[a]	5.66[b]	57.67[a]
5% biostimulant	5.88[b]	48.15[b]	43.70[b]	2.37[a]	2.47[ab]	12.62[a]	5.58[b]	53.86[ab]
10% biostimulant	7.79[a]	60.06[a]	59.81[a]	2.47[a]	2.52[a]	13.08[a]	5.55[b]	53.59[ab]
Significance level	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.05

4.6.2. Interaction of factors modulated lithic haplustert soil physicochemical properties

Table 11a shows no significant effects of biostimulants and dosage interaction on the soil's pH, Ca, Mg, K, $CaCO_3$, and N content after harvest. Nonetheless, the control treatment had the highest pH (7.08) while *ekofertile*® at 10% dosage had the highest Ca (53.32 meq/100g), Mg (16.37 meq/100g), K (0.88 meq/100g), $CaCO_3$ (3.44%), and N content (0.162%) was highest at both biostimulants inorganic fertilization treatment. On the contrary, EC, Na, OC and OM were significantly affected by biostimulants and dosage interaction with *ekofertile*® at 10% dosage, raising EC more (697.60), had high Na (0.82 meq/100g), OC (1.97%) and OM (3.39%) compared to the other factor interactions (Table 11a). The potential of these biostimulants to increase soil nutrient content was discussed by Agbor et al. (2022), which is evident in this study. Also, given that microbes are involved in nutrient solubilization, the increase in soil nutrients is a reflection of the rich nature of microbes contained in these biostimulants as reported by Divjot et al. (2021) and Figueiredo et al. (2020).

Table 4.11a. Biostimulant and dosage interaction effects on clay soil chemical properties

Biostimulant and dosage interaction		pH	EC	Ca	Mg	Na	K	$CaCO_3$	OC	OM	N
			(μscm^{-1})	(meq/100g)				%			
ekofertile®	Control	7.08^{a}	537.53^{de}	44.85^{a}	12.78^{a}	0.48^{f}	0.77^{a}	2.81^{a}	1.55^{c}	2.67^{c}	0.125^{a}
	Inorganic	6.99^{a}	592.63^{b}	49.04^{a}	14.94^{a}	0.57^{e}	0.78^{a}	3.01^{a}	1.60^{c}	2.75^{c}	0.162^{a}
	2.50%	6.96^{a}	555.63^{cd}	47.68^{a}	15.43^{a}	0.64^{d}	0.82^{a}	3.15^{a}	1.58^{c}	2.73^{c}	0.142^{a}
	5%	6.93^{a}	609.30^{b}	49.99^{a}	15.93^{a}	0.70^{c}	0.85^{a}	3.23^{a}	1.94^{a}	3.34^{a}	0.148^{a}
	10%	7.07^{a}	697.60^{a}	53.32^{a}	16.37^{a}	0.82^{a}	0.88^{a}	3.44^{a}	1.97^{a}	3.39^{a}	0.160^{a}
microfertile®	Control	7.08^{a}	537.53^{de}	44.85^{a}	12.78^{a}	0.48^{f}	0.77^{a}	2.81^{a}	1.55^{c}	2.67^{c}	0.125^{a}
	Inorganic	6.99^{a}	592.63^{b}	49.04^{a}	14.94^{a}	0.57^{e}	0.78^{a}	3.01^{a}	1.60^{c}	2.75^{c}	0.162^{a}
	2.50%	7.04^{a}	542.03^{de}	45.95^{a}	15.09^{a}	0.55^{e}	0.83^{a}	2.90^{a}	1.83^{b}	3.15^{b}	0.136^{a}
	5%	7.03^{a}	564.93^{c}	49.82^{a}	15.51^{a}	0.76^{b}	0.84^{a}	2.93^{a}	1.92^{a}	3.31^{a}	0.147^{a}
	10%	7.04^{a}	528.60^{e}	51.28^{a}	15.53^{a}	0.77^{b}	0.85^{a}	3.39^{a}	1.95^{a}	3.36^{a}	0.152^{a}
Significance level		1	0.001	1	1	0.001	1	1	0.001	0.001	1

No significant effects of biostimulants and dosage interaction were observed for Fe, Mn, Zn, Cu, C/N ratio and AS, as shown in Table 4.11b. Nevertheless, Fe (60.79 ppm) and Zn (2.56 ppm) contents were highest in 10% *ekofertile*® dosage, while Mn (61.17 ppm) was highest in 10% *microfertile*® dosage, same with Cu (2.53 ppm). Inorganic fertilization had the best C/N ratio (9.87) as 2.5% *microfertile*® dosage had the best stable aggregates (57.88%). P and MC were significantly affected by biostimulants and dosage interactions, with P (9.36 ppm) content highest at 10% *ekofertile*® dosage and control treatment having more MC (6.47%) as shown in Table 4.11b. The high nutrient content is also associated with organic acids, which effectively solubilize precipitated soil forms and chelate metal ions (Agbor et al., 2022). Again, they decompose the organic residue by immobilization and mineralization (Oteino *et al*., 2015; Naik *et al*., 2019).

Table 4.11b. Biostimulant and dosage interaction effects on soil chemical properties

Biostimulant and dosage interaction		P (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	C/N ratio	%MC	%AS
ekofertile®	Control	4.12^{d}	28.75^{a}	26.93^{a}	1.98^{a}	2.30^{a}	12.43^{a}	6.47^{a}	52.45^{a}
	Inorganic	4.55^{cd}	47.21^{a}	40.87^{a}	2.40^{a}	2.51^{a}	9.87^{a}	5.01^{e}	48.31^{a}
	2.5%	5.69^{b}	39.25^{a}	37.08^{a}	2.07^{a}	2.41^{a}	11.70^{a}	5.19^{de}	57.45^{a}
	5%	6.11^{b}	48.59^{a}	43.28^{a}	2.41^{a}	2.48^{a}	13.09^{a}	5.40^{d}	52.37^{a}
	10%	9.36^{a}	60.79^{a}	58.44^{a}	2.56^{a}	2.51^{a}	12.31^{a}	5.23^{de}	49.93^{a}
microfertile®	Control	4.12^{d}	28.75^{a}	26.93^{a}	1.98^{a}	2.30^{a}	12.43^{a}	6.47^{a}	52.45^{a}
	Inorganic	4.55^{cd}	47.21^{a}	40.87^{a}	2.40^{a}	2.51^{a}	9.87^{a}	5.01^{e}	48.31^{a}
	2.5%	5.25^{bc}	44.36^{a}	34.95^{a}	2.03^{a}	2.40^{a}	12.98^{a}	6.14^{b}	57.88^{a}
	5%	5.64^{b}	47.71^{a}	44.12^{a}	2.34^{a}	2.46^{a}	13.07^{a}	5.75^{c}	57.26^{a}
	10%	6.22^{b}	59.32^{a}	61.17^{a}	2.38^{a}	2.53^{a}	12.93^{a}	5.86^{bc}	55.34^{a}
Significance level		0.01	1	1	1	1	1	0.001	1

4.7. Treatment effect on lithic haplustert soil biological properties, soil quality and wheat yield

4.7.1. Sole factors modulated lithic haplustert soil biological properties, soil quality and wheat yield

Table 4.12 shows the effect of different biostimulants (*ekofertile*® and *microfertile*®) and dosages, including control and inorganic fertilization, on soil biological properties dynamics, soil quality index and biological wheat yield.

Dehydrogenase (DHA) enzyme activity was highest in *ekofertile*® (49.60 $\mu gTPFg^{-1}$) and significantly differed from *microfertile*®. Similar trends were seen in microbial biomass carbon (Cmic) (24.41 $mgCO_2$-$C100g^{-1}$), while microbial basal soil respiration (BSR) was highest in *microfertile*® (0.081 μgCO_2-Cg^{-1}) with significant differences between the two biostimulants (Table 4.12). Soil quality index and biological wheat yield did not demonstrate significant differences between the biostimulants. *Microfertile*® had a better SQI (0.64) compared to *ekofertile*® (0.60), while *ekofertile*® modulated wheat biological yield (12.78 tha^{-1}) better than *microfertile*® (12.09 tha^{-1}) as displayed on Table 4.12.

Also, the results showed that the biostimulant effect is dosage dependent, with 10% biostimulant dosage significantly increasing Dehydrogenase (DHA) enzyme activity (51.00 $\mu gTPFg^{-1}$) compared to the control (41.67 $\mu gTPFg^{-1}$) and inorganic fertilization (36.92 $\mu gTPFg^{-1}$). The significant dominant effect of the 10% biostimulant dosage continued for microbial biomass carbon (Cmic) (24.86 $mgCO_2$-$C100g^{-1}$), microbial basal soil respiration (BSR) (0.082 μgCO_2-Cg^{-1}), soil quality index (SQI) (0.66) (Fig. 4.2) and wheat biological yield (14.04 tha^{-1}) as can be seen in Table 4.12.

The increase in microbial activities seen in this study aligns with other studies. For instance, Nanda et al. (2022) reported that applying a biostimulant based on amino acids and seaweed extracts increased soil microbial biomass and enzyme activities. Similarly, Canellas et al. (2002) and Holatko et al. (2020) found that applying a humic acid-based biostimulant enhanced the soil's dehydrogenase activity and microbial biomass.

The dosage dependency of biostimulants is evident in the results, with higher dosages generally leading to greater improvements in soil biological properties and biological wheat yield. The 10% biostimulant dosage consistently outperformed other dosages and the control or inorganic fertilizer treatments regarding DHA activity, Cmic, BSR, SQI, and wheat yield. These findings align with previous studies that have reported the dosage-dependent effects of biostimulants on crop growth and soil health (De Pascale et al., 2018).

Table 4.12. Sole factors effect of biostimulants and dosage on lithic haplustert soil biological properties, soil quality index and wheat biological yield

	DHA $\mu gTPFg^{-1}$	Cmic, mg CO_2-C $100g^{-1}$	BSR, µg CO_2-Cg^{-1}	SQI	Biological yield (tha^{-1})
		Biostimulant type			
ekofertile®	49.60^{a}	24.41^{a}	0.072^{b}	0.60^{a}	12.78^{a}
microfertile®	45.40^{b}	22.70^{b}	0.081^{a}	0.64^{a}	12.09^{a}
Significance level	0.01	0.05	0.05	1	0.1
		Dosage			
Control	41.67^{cd}	17.49^{c}	0.068^{b}	0.42^{d}	10.02^{c}
Inorganic	36.92^{d}	21.09^{b}	0.054^{c}	0.47^{c}	12.72^{b}
2.5% biostimulant	43.29^{bc}	22.85^{b}	0.072^{ab}	0.55^{b}	10.55^{c}
5% biostimulant	48.21^{ab}	22.97^{ab}	0.077^{ab}	0.64^{a}	12.50^{b}
10% biostimulant	51.00^{a}	24.86^{a}	0.082^{a}	0.66^{a}	14.04^{a}
Significance level	0.01	0.001	0.01	0.001	0.001

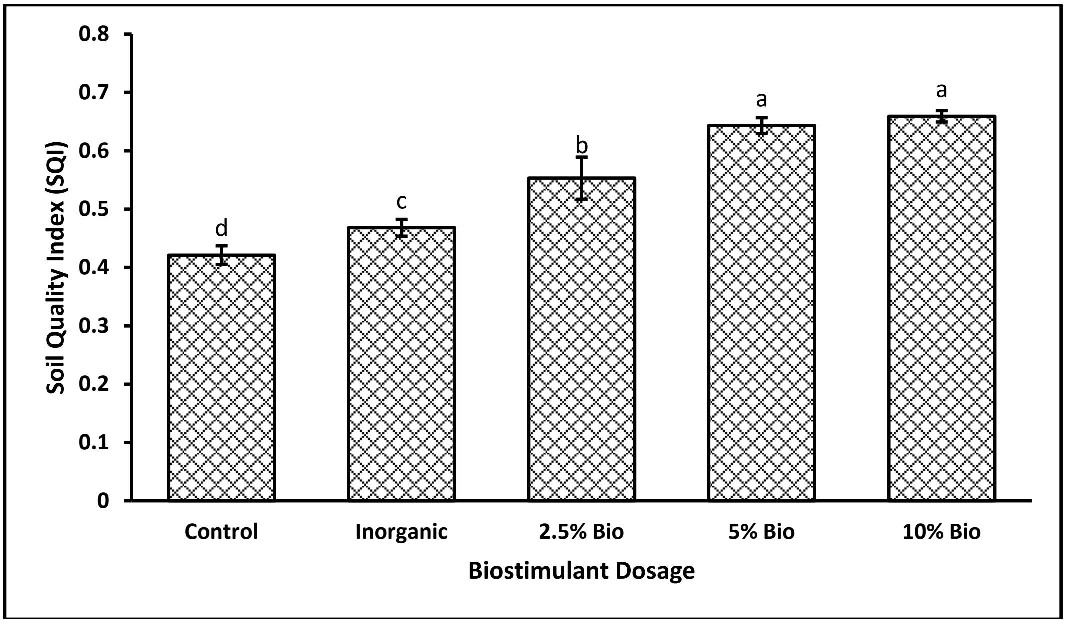

Figure 4.2. Dosage modulated lithic haplustert soil quality index

4.7.2. Interaction of factors modulated lithic haplustert soil biological properties, soil quality and wheat yield

The results in Table 4.13 showed that both biostimulant products at different dosages did not significantly modulate Dehydrogenase (DHA) enzyme activity, microbial basal soil respiration (BSR) and biological wheat yield, while microbial biomass carbon (Cmic) and soil quality index were significantly modulated. The 10% dosage of *ekofertile*® produced the highest Dehydrogenase (DHA) enzyme activity (54.15 $\mu gTPFg^{-1}$). The microbial biomass carbon (Cmic) was highest at *ekofertile*® 10% dosage (27.68 $mgCO_2$-$C100g^{-1}$) and lowest in control (17.49 $mgCO_2$-$C100g^{-1}$) with significant differences across the treatment interactions. The microbial basal soil respiration (BSR) was highest (0.088 μgCO_2-Cg^{-1}) at *microfertile*® 10% dosage with no significant differences across the other treatment interactions. Soil Quality index showed significant differences among the different dosages of the biostimulant products, with the best result recorded *in ekofertile*® 10% dosage (0.68) (Fig. 4.3), while the best wheat biological yield, 14.56 tha^{-1} was recorded in *ekofertile*® 10% dosage with no significant differences among the different dosages of the biostimulant products (Table 4.13).

Table 4.13. Biostimulant and dosage interaction effects on lithic haplustert soil biological properties, soil quality index and wheat biological yield

Biostimulant and dosage interaction		DHA $\mu gTPFg^{-1}$	$mgCO_2$-C $100g^{-1}$	BSR, µg CO_2-Cg^{-1}	SQI	Biological yield (tha^{-1})
ekofertile®	Control	41.67[a]	17.49[c]	0.068[b]	0.4[b]	10.02[a]
	Inorganic	36.92[a]	21.09[b]	0.054[c]	0.47[b]	12.72[a]
	2.5%	43.73[a]	22.78[b]	0.073[a]	0.48[b]	10.57[a]
	5%	50.92[a]	22.79[b]	0.069[a]	0.65[a]	13.22[a]
	10%	54.15[a]	27.68[a]	0.076[a]	0.68[a]	14.56[a]
microfertile®	Control	41.67[a]	17.49[c]	0.068[b]	0.42[b]	10.02[a]
	Inorganic	36.92[a]	21.09[b]	0.054[c]	0.47[b]	12.72[a]
	2.5%	42.85[a]	22.91[b]	0.071[a]	0.63[a]	10.53[a]
	5%	45.51[a]	23.16[b]	0.084[a]	0.64[a]	12.22[a]
	10%	47.84[a]	22.04[b]	0.088[a]	0.64[a]	13.52[a]
Significance level		1	0.05	1	0.01	1

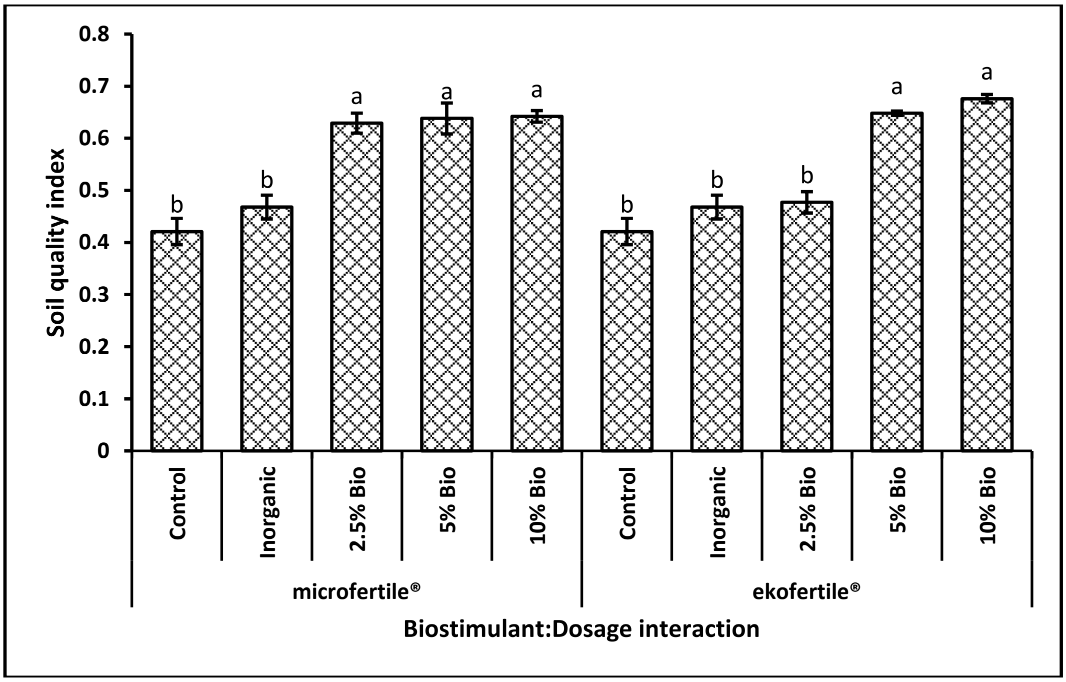

Figure 4.3. Biostimulant and dosage interaction modulated lithic haplustert soil quality index

4.8. Comparing soil quality index across loam and clay soil as modulated by biostimulants

Figure 4.4 shows that *ekofertile*® enhanced soil quality more in loam soil compared to clay soil. This is likely because loam soil is alkaline while *ekofertile*® is acidic, thus buffering the soil to limits where conditions become favourable for nutrient availability and high biological activity, supported by Agbor et al. (2022). Whereas *microfertile*® meliorated soil quality more in clay soil than loam soil, which also may result from pH buffering as clay soil has acidic pH and *microfertile*® has alkaline pH, thus increasing soil biological activity and nutrient content. While the contrary, *ekofertile*® acidic pH in clay soil may be associated with the lower soil quality observed in clay soil with a similar reason for *microfertile*®.

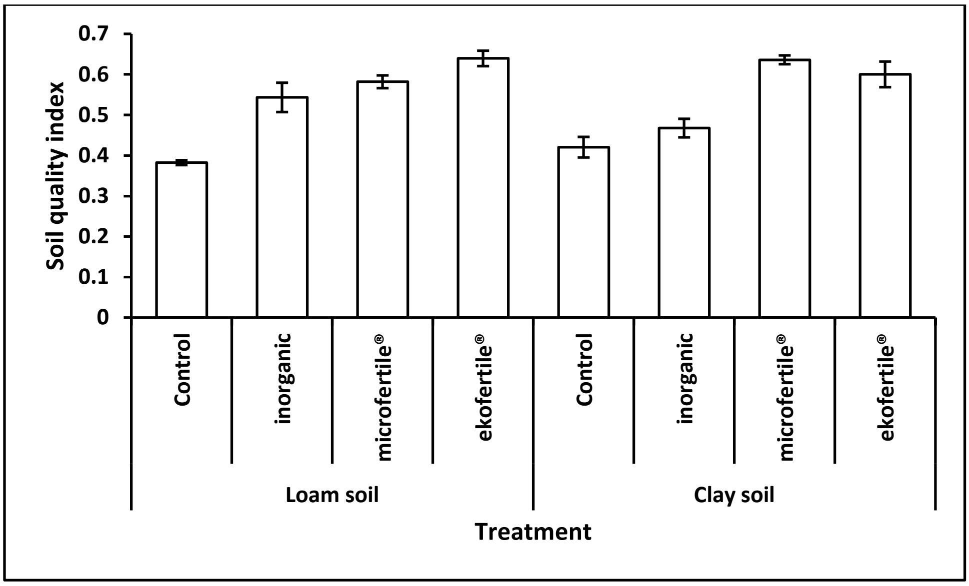

Figure 4.4. *ekofertile*® and *microfertile*® biostimulants affect soil quality index across loam and clay soil

Figure 4.5 shows that control has the least effect on soil quality across the two soils but clay soil quality in control was better than loam soil quality which can be a result of clay soil particles fixed to each other with a higher propensity to hold more nutrients and stimulate better biological activity compared to loose loam soil particles with a contrary view. Despite the loose nature of loam soil particles, they easily respond to additives as can be seen in inorganic fertilization, *ekofertile*® 2.5%, and 5% dosage levels with contrast at *ekofertile*® 10% level, which, probably due to higher dosage levels, was able to modulate clay soil better thus producing better soil quality outcomes. Whereas *microfertile*® 2.5%, 5% and 10% modulated clay soil is better, probably due to buffering effect of the alkaline pH.

Figure 4.5 shows biostimulant and dosage interaction modulation on soil quality. As explained in Figure 4.4, control interaction with both biostimulants had a similar trend with inorganic fertilization. 2.5% dosage interaction with *ekofertile*® saw loam soil quality improve better than 2.5% dosage interaction with *microfertile*®, which may be due to the reason explained in Figure 8, supported by Agbor et al. (2022). A similar pattern was seen at 5% dosage interaction with both biostimulants.

Nonetheless, the 10% dosage had a similar interaction with both biostimulants in modulating soil quality.

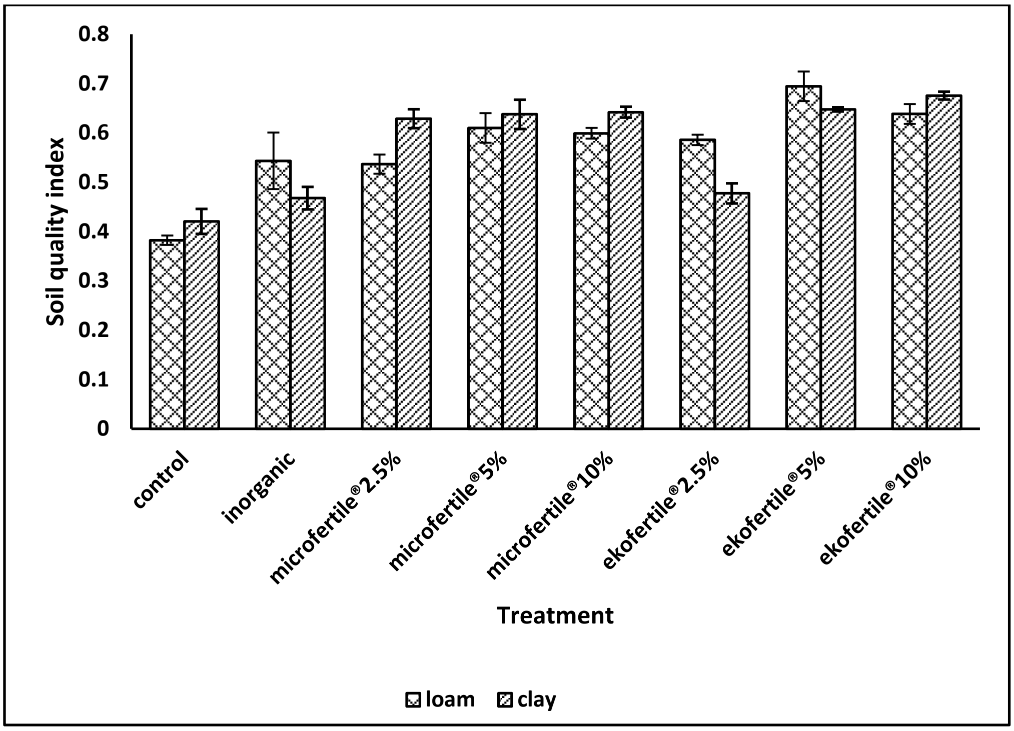

Figure 4.5. Dosage affects soil quality index across loam and clay soil

5. CONCLUSION

This study has shown that *ekofertile®* and *microfertile®* plant biostimulants significantly affected wheat growth and yield parameters, soil physicochemical and biological properties and greatly enhanced soil quality compared to inorganic fertilization. Thus, these biostimulants' uniqueness in consisting of beneficial microbes and mineral nutrients with their eco-friendly nature is a tremendous fertilization product for agriculture. Our results also showed that while 10% biostimulant has largely increased soil physicochemical and biological properties, 5% dosage had a better propensity to enhance soil quality index.

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CURRICULUM VITEA

About me

I am interested broadly in research and pro-poor community development projects. Soil Science, Plant Nutrition and data analysis.

Education

a) Erasmus Mundus + European Union Joint Master's Degree in soil science (On going). Turkey, Poland, Bulgaria and Jordan (GPA- 3.81/4.00).

1) Ondokuz Mayis University, Samsun-Turkey. 2) Agricultural University-Plovdiv, Bulgaria. https://emissmaster.omu.edu.tr

b) M.Sc. Agronomy, Faculty of Agriculture and Veterinary Medicine (FAVM), University of Buea, Cameroon (GPA- 3.55/4.00)

c) B.Sc. Agronomy, Faculty of Agriculture and Veterinary Medicine (FAVM), University of Buea, Cameroon (GPA- 3.54/4.00)

Contact Information :

ORCID ID : https://orcid.org/0000-0002-7070-1000

Publications:

1. Nkongho, R. N., Efouba-Mbong, J. T., Ndam, L. M., Ketchem, G. A., Etchu-Takang, E. G., & **Agbor, D. T.** (2022). Seed production system and adaptability of okra (Abelmoschus esculentus L.) cultivars in Buea, Cameroon. Plos one, 17(12), e0278771.

2. Nkongho, R. N., Ndam, L. M., Akoneh, N. N., Tongwa, Q. M., Njilar, R. M., **Agbor, D. T.,** ... & Ngone, A. M. (2023). Vegetative propagation of F1 tomato hybrid (Solanum lycopersicum L.) using different rooting media and stem-nodal cuttings. Journal of Agriculture and Food Research, 11, 100470.

3. **AGBOR, D. T.**, ŠTYRIAKOVÁ, D., & DENGİZ, O. (2022). Agroecological significance of ekofertile™ plant biostimulant on tropical soils and crop improvement. Conference: INTERNATIONAL SOIL SCIENCE SYMPOSIUM on SOIL SCIENCE & PLANT NUTRITION (7th International Scientific Meeting) 2–3 December 2022 Samsun, Türkiye ISBN: 978-605-63090-8-3 At: Samsun, Türkiye.

4. SAMA, D. K., **AGBOR, D. T.**, DOHNJI, J. D., & KIZILKAYA, R. (2022). Exploring the soil fertility and plant nutrition potential of LAB isolated from palm wine and sha'. Conference: INTERNATIONAL SOIL SCIENCE SYMPOSIUM on SOIL SCIENCE & PLANT NUTRITION (7th International Scientific Meeting) 2–3 December 2022 Samsun, Türkiye ISBN: 978-605-63090-8-3 At: Samsun, Türkiye.

Won Awards, Incentives and Scholarships

1. Won Erasmus Mundus Joint Master's Degree in Soil Science, European Union Scholarship Awarding institution: Erasmus Mundus Master Program (emiSS; https:// emissmaster.omu.edu.tr) of the European Union

2. Best Faculty president, the University of Buea Confederation of Faculties Students' Association, University of Buea, Cameroon. Awarding institution: University of Buea