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Effect of digestate application replacement to chemical fertilizer on the growth, quality and salt stress resistance of vegetable

(消化液の化学肥料の代替が野菜の成長、品質及び塩ストレス耐性に及ぼす影響に関する研究)

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Abstract

Anaerobic digestion technology, which is extensively used for commercial processing of agricultural and other wastes worldwide, only a small part of digestate reused directly or indirectly. If this excess digestate cannot be properly managed, new environmental issues will arise. From a circular economic perspective, the application of digestate as organic fertilization is an interesting scenario. With the goal of chemical fertilizer reduction and organic fertilizer replacement of chemical fertilizer in sustainable agriculture, it is important and urgent to explore the reasonable application of organic fertilizer and chemical fertilizer in the production of vegetables in facilities. Therefore, this work mainly focuses on digestate as a fertilizer. Based on this, the following studies were conducted using tomato and lettuce as test crops and digestate as representative organic fertilizer.

In this work, firstly, the digestate as an alternative or partial replacement to inorganic fertilization for tomato production was tested. Digestate was added to agricultural soil, either individually or along with inorganic fertilizer, and tomato plants were cultivated under open field and plastic greenhouse conditions. Inorganic fertilizer alone was also applied, with unfertilized soil used as a control. The impact of three fertilization strategies at same nitrogen dose and a control on growth trait and yield was investigated under both cultivation environments. The results showed that the application of digestate significantly increased growth traits of tomato including height, stem diameter, leaf chlorophyll content index and net photosynthetic rate of tomato plant and sugar-acid ratio, protein, and ascorbic acid of tomato fruit as well as decreased nitrate concentration in fruit compared to inorganic fertilizer and untreate plants. Combined digestate to inorganic fertilization had the greatest increase in tomato yield, which is up to 174.28% and 67.37% under open field and plastic greenhouse, respectively, compared to untreated control.

In order to further elucidate the effect of digestate replacement of chemical fertilizers on tomato quality, bioactive compounds and antioxidant capacity in tomato fruits were analyzed. This study investigated the sugar, organic acid and phenolic compound levels, total phenolic (TP) and antioxidant capacity of four different fertilizer applications. Analysis of sugars, organic acid, phenolic compound, TP and antioxidant capacity levels in tomato fruit showed statistically significant differences under fertilizer applications. Chlorogenic acid was the predominant phenolic compounds found in tomato fruit of both cultivation environments. The highest chlorogenic acid value (41.04 mg kg^{-1} FW) was found in the application of digestate and the lowest (18.35 mg kg^{-1} FW) in control under greenhouse condition. Fructose and glucose were the predominant saccharides found in all treatments. Citric acid content was the dominant organic acid in tomato fruits, with the lower citric acid value found in the application of digestate. The bioactive compounds were significantly higher in the application of digestate as compared to other fertilization strategies.

This next part of work investigated the effect of digestate replacement of chemical fertilizers on soil soil chemical properties and enzymatic activities during tomato growth. The results showed that the application of digestate significantly increased the activities of urease, sucrase, protease and nitrate reductase in the soil. Also, the application of digestate neutralized the soil pH and increased the soil organic carbon content. In addition, the application of digestate also increased the soil nitrogen and ammonium nitrogen content. Fertilization with digestate increased soil fertility, including nitrogen and carbon levels, and enhanced soil enzyme activities. In short, the combined use of digestate along with inorganic fertilizer allows for reduced inorganic fertilization while maintaining tomato fruit yield, enhancing tomato fruit quality, and improving soil characteristics.

Finally, a pot experiment was used to examine the changes in physiological and biochemical parameters of lettuce under salt stress adversity. Experimental treatments comprised application of two types of fertilizer (mineral fertilizer and digestate) and three NaCl concentrations (0, 3, and 7.5 dS m^{-1}). High NaCl concentrations resulted in significantly lower photosynthesis, growth, and physiological indices compared with those under no NaCl addition. However, under the 7.5 dS m^{-1} NaCl condition, digestate application increased the fresh weight (42%), dry weight (27%), photosynthesis (20%) of lettuce compared with that under chemical fertilizer application. Accumulation of reactive oxygen species was markedly lower, and the membrane stability index was therefore higher, under digestate compared with under application of chemical fertilizer within the same salinity level. Lipid peroxidation was lower under digetstae compared with under chemical fertilizer in all salinity treatments. In addition, the total water use was lower and water-related indices, such as water use efficiency of fresh weight, water use efficiency of dry weight and relative water content, were higher under digestate treatment compared with under chemical fertilizer treatment.

In this work, application of digestate obtained the highest yield of tomatoes and achieved higher fruit quality while promoting the growth traits in the tomato plant. The synthesis of bioactive compounds (including sugar, phenolic components and some acids) and antioxidant capacities in tomato fruits were also enhanced under application of digestate. Moreover, digestate was found to neutralize soil pH significantly increase soil C, N, and enzyme activities. In addition, application of digestate instead of mineral fertilizer offers potential for growth of lettuce in saline soils.

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Chapter 1. General introduction

1.1 Excessive use of chemical fertilizer and the risks

Chemical fertilizers play a crucial role in modern agriculture by providing essential nutrients to crops for their optimal growth and increased yield. However, excessive and improper use of these fertilizers can have detrimental effects on the environment, human health, and long-term sustainability of agricultural systems. In the environmental impacts, excessive use of chemical fertilizers can result in several environmental problems (Savci et al. 2012). When chemical fertilization was over-applied, they can leach into water bodies, causing eutrophication, algal blooms, and the subsequent depletion of oxygen levels, which harm aquatic life (Da Costa et al. 2013; Simpson et al. 2011). Moreover, runoff from agricultural fields can carry nitrogen and phosphorus from fertilizers into rivers and lakes, further exacerbating water pollution issues. In soil degradation, continuous and excessive use of chemical fertilizers can lead to soil degradation (Wu et al. 2020). Fertilizers high in nitrogen and phosphorus can disrupt the soil's natural balance, negatively affecting its fertility and nutrient-holding capacity (Lv et al. 2020). This can result in nutrient imbalances, reduced soil biodiversity, and increased vulnerability to erosion. Over time, the soil becomes less productive, requiring even higher fertilizer application rates to achieve desired crop yields (Geng et al. 2019). In health Risks, chemical fertilizers, if not used judiciously, can pose health risks to humans. Nitrogen-based fertilizers, when applied excessively, can contaminate drinking water sources with nitrates (Ye et al. 2020). Elevated nitrate levels in drinking water have been linked to various health issues, particularly affecting infants, such as methemoglobinemia (Richard el al. 2014). Furthermore, the excessive use of pesticides and herbicides, often associated with chemical fertilizers, can have adverse effects on human health and contribute to the development of pesticide resistance (Baweja et al. 2020; Warra et al. 2020). In loss of diodiversity, the overuse of chemical fertilizers can lead to a decline in biodiversity in agricultural landscapes. Excessive nutrient inputs favor the growth of fast-growing, competitive species, which can outcompete and suppress native plant species (Byun et al. 2023). This loss of plant diversity can disrupt ecological balances, reduce habitat availability for wildlife, and impact beneficial insect populations, including pollinators (Vanbergen et al. 2013). In the sustainable Alternatives, to mitigate the negative impacts of excessive chemical fertilizer use, sustainable agricultural practices should be promoted. These include integrated nutrient management, organic farming methods, crop rotation, and precision agriculture techniques (Da Costa et al. 2013; Lv et al. 2020; Lambert et al. 2015; Sumner et al. 2018). Integrated approaches that incorporate organic matter, cover crops, and biofertilizers can enhance soil fertility, reduce nutrient losses, and improve long-term sustainability (Panhwar et al. 2019). While chemical fertilizers have contributed significantly to increased agricultural productivity, their excessive and improper use poses significant risks to the environment, human health, and biodiversity (Ye et al. 2020). It is essential to promote sustainable agricultural practices that minimize the reliance on chemical fertilizers, improve nutrient management, and prioritize the long-term health of agricultural systems. By adopting these practices, a more sustainable and balanced approach can be striven to crop production while minimizing the hazards associated with excessive fertilizer use.

1.2 Digestate: Composition and properties

1.2.1 Sources of digestate

The origin of digestate can be attributed to the process of anaerobic digestion, which converts organic waste into a nutrient-rich residue (Levén et al. 2012). Digestate is a by-product of this biogas production method and has diverse sources (Nkoa et al. 2014). Digestate primarily originates from the decomposition of organic materials through anaerobic digestion (Fig. 1.1). Anaerobic digestion is a natural process that occurs in oxygen-deprived environments, such as biogas plants or anaerobic digesters (Zappi et al. 2021). Various organic waste materials are fed into these systems, including agricultural residues, food waste, sewage sludge, and energy crops (Duku et al. 2011). The organic waste is subjected to anaerobic conditions, where a consortium of microorganisms breaks down the complex organic compounds present in the feedstock (Saravanan et al. 2021). This microbial activity results in the production of biogas, which mainly consists of methane and carbon dioxide. However, not all of the organic matter is converted into biogas (Antoniou et al. 2019; Bolzonella et al. 2018).

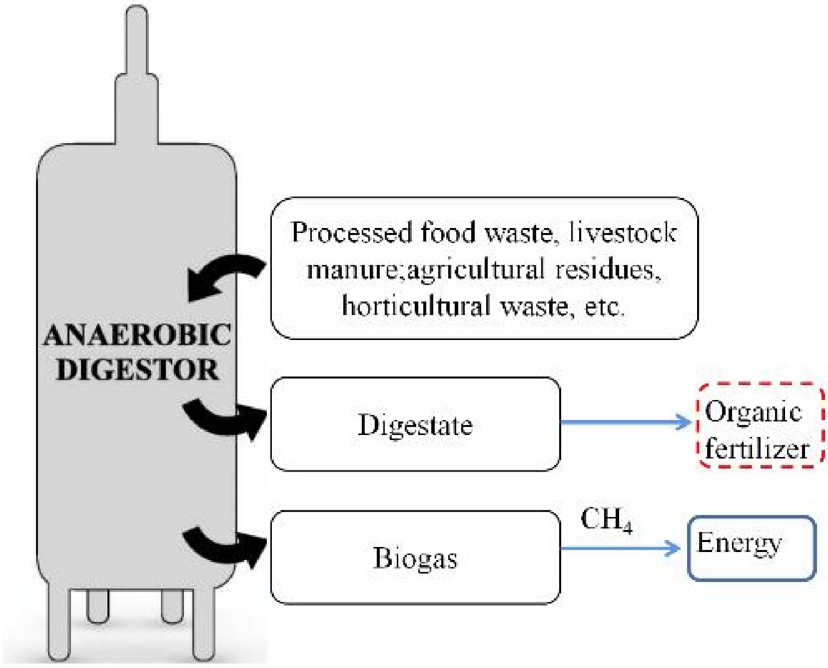


Fig. 1.1 Basic anaerobic digestion process

A portion of the material undergoes partial decomposition, forming a nutrient-rich residue known as digestate. Digestate composition can vary depending on the type of organic waste used as feedstock. It typically contains water, organic matter, nutrients (such as nitrogen, phosphorus, and potassium), and trace elements (Bolzonella et al. 2018; Antoniou et al. 2019). The nutrient content in digestate makes it a valuable agricultural fertilizer and soil amendment. In addition to organic waste, co-substrates may be added during anaerobic digestion to enhance the process and the quality of the digestate (Sambusiti et al. 2013; Yao et al. 2020). Co-substrates can include energy crops like maize, beet, or grass, as well as byproducts from industries such as breweries or food processing plants (Braun et al. 2003). These co-substrates contribute additional organic matter and nutrients to the digestate. The quality and characteristics of digestate can be influenced by various factors, including the composition of the feedstock, the operational parameters of the anaerobic digester, and the duration of the digestion process (Mata-Alvarez et al. 2014). Digestate can undergo further treatment or processing to optimize its properties for specific agricultural applications, such as solid-liquid separation or nutrient concentration (Nkoa 2014). It is a valuable resource that provides essential nutrients for agricultural purposes. By utilizing digestate as a fertilizer and soil amendment, it can effectively close the loop in organic waste management, promoting sustainable practices and reducing environmental impacts.

1.2.2 Main components of digestate

Table 1.1 Biochemical properties of anaerobic digestate

Parameters	Value range	Reference
N (%, dm)	3.0-14.0	Möller 2012; Scaglia 2015; Ronga 2019; Giulio 2019; Giulio 2020.
P (%, dm)	0.2-3.5	Teglia 2011a,b; Scaglia 2015; Giulio 2020
K (%, dm)	1.9-4.3	Möller 2012; Nkoa 2014; Aihemaiti 2019; Giulio, 2020.
Micro element (Mn, Zn, Cu, Fe, Mo, Pb, Cd)	non-phytotoxic; lower than the limit values in soil	Makádi 2012; Li 2016; Monlau 2016; Bolzonella 2018; Giulio 2019; Giulio, 2020; Cheong, 2020; Lamolinara 2022.
pH	7.3-9.0	Makádi 2012; Chantigny 2008; Fouda 2011; Aihemaiti 2019; Baryga 2020.
EC (dS m^{-1})	7.1-19.8	Yu 2010; Scaglia,2015; Aihemaiti 2019; Baryga 2020.

dm: Dry matter.

Organic matter is a significant component of digestate, consisting of partially decomposed organic materials. These can include agricultural residues, food waste, sewage sludge, and energy crops (Teglia et al. 2011; Nkoa 2014). The organic matter provides the foundation for nutrient-rich content in digestate. Nutrients, such as nitrogen, phosphorus, and potassium, are essential elements for plant growth and development (Wang et al. 2017). Digestate serves as a valuable source of these nutrients, which are released during the anaerobic digestion process (Bolzonella et al. 2018; Antoniou et al. 2019). The nutrient composition of digestate depends on the original organic materials used as feedstock. In addition to the major nutrients, digestate can contain a range of trace elements (Nkoa 2014). These elements include micronutrients like iron, zinc, copper, and manganese, which are required in small quantities for optimal plant growth (Li et al. 2023; Antoniou et al. 2019). The presence of trace elements in digestate contributes to its overall nutritional value. Furthermore, digestate may contain other organic compounds, such as beneficial bacteria and enzymes that aid in nutrient availability and soil health (Nkoa 2014).

Overall, digestate serves as a valuable resource in agriculture due to its organic matter, nutrient content, trace elements, and water (Table 1.1). By applying digestate to fields, farmers can enhance soil fertility, improve nutrient cycling, and contribute to sustainable waste management practices.

1.2.3 Advantages of replacing chemical fertilizers with digestate

Digestate, derived from anaerobic digestion, offers several advantages over chemical fertilizers. Firstly, digestate is rich in organic matter, which helps improve soil structure and enhances its water-holding capacity (Leno et al. 2021). This organic matter serves as a long-term source of nutrients and promotes beneficial microbial activity in the soil, leading to improved soil health and fertility (Tan et al. 2021). In contrast, chemical fertilizers provide a concentrated dose of specific nutrients but lack organic matter and do not contribute to long-term soil health (Monlau et al. 2016). Another benefit of digestate is its balanced nutrient content. It contains not only the primary nutrients nitrogen, phosphorus, and potassium but also essential micronutrients (Nkoa 2014; Yao et al. 2020). This balanced nutrient profile helps prevent nutrient imbalances in the soil and reduces the risk of excessive nutrient runoff, which can lead to water pollution. Digestate also promotes sustainable waste management by recycling organic materials (Lamolinara et al. 2022). It allows for the repurposing of agricultural residues, food waste, and other organic byproducts into a valuable resource. In contrast, chemical fertilizers rely on the extraction and production of finite resources, such as fossil fuels and mineral deposits, leading to environmental concerns and contributing to carbon emissions during manufacturing and transportation (Cristina et al. 2020). Furthermore, digestate can enhance soil fertility and productivity over the long term. By improving soil health, digestate helps build resilient agricultural systems that can better withstand environmental stresses, such as drought or disease (Schröder et al. 2018). Chemical fertilizers, while providing an immediate nutrient boost, do not address long-term soil health concerns and can contribute to soil degradation and reduced ecosystem resilience (Simpson et al. 2011). However, it is essential to consider some challenges associated with digestate. Its nutrient content can vary, requiring careful analysis and management to ensure appropriate application rates. While there are considerations and challenges, the use of digestate represents a valuable approach towards sustainable and environmentally friendly agriculture.

1.3 Effect of digestate on the growth of vegetables

1.3.1 Nutritional effects of Digestate

Digestate, as a nutrient-rich residue from anaerobic digestion, can have significant positive effects on crop growth and nutrition. When used as a fertilizer, digestate provides a wide range of essential nutrients for crops (Makádi et al. 2012). It contains nitrogen, phosphorus, potassium, and micronutrients necessary for plant growth and development (Bolzonella et al. 2018; Wang et al. 2019; Ivanchenko et al. 2021). These nutrients are released slowly and steadily, ensuring a continuous supply throughout the crop's growth cycle. Nitrogen is essential for promoting vegetative growth and enhancing leaf and stem development (Leghari et al. 2016). Phosphorus plays a vital role in root development, energy transfer, and flowering (Malhotra et al. 2018). Potassium contributes to overall plant health, water regulation, and disease resistance (Rawat et al. 2016). Micronutrients, such as iron, zinc, copper, and manganese, are crucial for various physiological processes and enzyme activities in plants (Hänsch et al. 2009). The organic matter content in digestate improves soil structure, moisture retention, and nutrient-holding capacity (Tan et al. 2021). It enhances microbial activity and the availability of nutrients to plants (Valentinuzzi et al. 2020). The organic matter also acts as a slow-release reservoir of nutrients, providing a sustained supply over time (Wu et al. 2022). By using digestate as a fertilizer, farmers can promote soil health and fertility. It helps improve soil biodiversity, microbial activity, and nutrient cycling. The organic matter in digestate increases the soil's ability to retain moisture and enhances its overall nutrient-holding capacity (Weldon et al. 2022). These soil improvements contribute to better nutrient availability for crops, leading to improved growth, higher yields, and increased resistance to environmental stresses (Yu et al. 2010; Nkoa 2014; Panuccio et al. 2019). Digestate offers a comprehensive nutrient package and soil-building properties that positively influence crop growth and nutrition (Hills et al. 2021). Its use can contribute to sustainable agriculture practices, improved soil health, and enhanced crop productivity while minimizing environmental impacts.

1.3.2 Hormonal regulatory effects of digestate

Digestate, as a residue of anaerobic digestion, can potentially exhibit hormone-regulating effects on crop growth. It contains organic compounds, such as humic acids, fulvic acids, and other bioactive substances, which have the potential to influence plant hormone regulation (Nkoa 2014; Panuccio et al. 2019). These compounds can interact with the hormonal pathways of crops, leading to various physiological responses and growth stimulation. Studies have suggested that digestate application may promote plant growth by influencing hormone levels and activities. For instance, digestate has been reported to enhance the production of auxins, a class of plant hormones that play a crucial role in cell elongation, root development, and overall growth. Increased auxin levels can lead to enhanced root growth, nutrient uptake, and improved plant vigor (Yu et al. 2010; Jabeen et al. 2017; Cristina et al. 2020). Additionally, digestate has been associated with the modulation of cytokinin levels, another group of plant hormones involved in cell division, leaf expansion, and shoot development (Nardi et al. 2021). The application of digestate has been shown to increase cytokinin levels, potentially leading to increased shoot growth and branching. Moreover, digestate may affect the balance between plant hormones, such as auxins and gibberellins, which are involved in regulating plant growth and development (Scaglia et al. 2015; Rékási et al. 2019; Ronga et al. 2019). By influencing the hormone ratios, digestate can potentially promote desirable growth responses in crops. However, it is important to note that the hormone-regulating effects of digestate can be influenced by various factors, including the specific composition of the digestate, crop species, and environmental conditions. Further research is needed to fully understand the specific mechanisms and effects of digestate on hormone regulation in different crops.

1.4 Characteristics of digestate as fertilizer

1.4.1 Digestate regulation of vegetable quality

The application of digestate as a fertilizer can positively impact the quality of vegetables. Digestate contains a diverse range of nutrients, organic matter, and bioactive compounds that contribute to the improvement of various quality attributes (Möller et al. 2012; Nkoa 2014). Firstly, the organic matter present in digestate enhances soil structure, water-holding capacity, and nutrient availability (Komilis and Ham 2003). This can result in improved plant growth, leading to higher yields of vegetables with better texture, color, and overall appearance (Tei et al. 2020; Weimers et al. 2022). The increased organic matter content also enhances soil fertility, which can positively influence the nutritional value of the vegetables ((Jenkinson et al. 1990; Diacono et al. 2011; Cesarano et al. 2017). Digestate is rich in essential nutrients, including nitrogen, phosphorus, potassium, and micronutrients (Möller et al. 2012; Wang et al. 2019; Ivanchenko et al. 2021). These nutrients play a vital role in the synthesis of plant compounds such as proteins, carbohydrates, and vitamins (Tariq et al. 2023). As a result, digestate application can enhance the nutritional content of vegetables, increasing their vitamin and mineral levels. Moreover, the bioactive compounds present in digestate, such as humic and fulvic acids, can have positive effects on vegetable quality (Campitelli and Ceppi 2008; De Corato 2020; Tan et al. 2021). These compounds have been reported to improve nutrient uptake, enhance antioxidant activity, and stimulate plant metabolism ((Nkoa 2014; Canellas et al. 2015). Consequently, digestate-treated vegetables may exhibit increased antioxidant capacity, prolonged shelf life, and improved flavor profiles. Furthermore, the use of digestate as a fertilizer promotes sustainable agricultural practices by recycling organic waste. By diverting waste from landfills and utilizing it as a nutrient source, digestate application reduces environmental pollution and contributes to a circular economy (Jabeen et al. 2017; Saqib et al. 2017; Cheong et al. 2020). However, it is important to note that the specific effects of digestate on vegetable quality can vary depending on factors such as crop type, application rate, timing, and environmental conditions. Careful management practices and appropriate dosage of digestate are necessary to maximize the desired effects on vegetable quality while avoiding any potential negative impacts.

1.4.2 Effect of digestate on soil properties

Digestate application can improve soil properties and contribute to enhanced soil health and fertility. The organic matter content in digestate serves as a valuable source of carbon, providing energy for soil microorganisms and promoting their activity (Provenzano et al. 2011). Increased microbial activity leads to improved nutrient cycling and organic matter decomposition, resulting in enhanced soil structure (Teglia et al. 2011b). The addition of digestate to the soil improves soil aggregation and porosity, leading to better water infiltration and drainage (Cristina et al. 2020). The organic matter acts as a binding agent, binding soil particles together and creating stable aggregates. This improves soil structure, reduces compaction, and enhances root penetration and nutrient uptake by plants (Nkoa 2014; Panuccio et al. 2019). Moreover, digestate contains a range of essential nutrients, including nitrogen, phosphorus, potassium, and micronutrients (Wang et al. 2019; Ivanchenko et al. 2021; Bolzonella et al. 2018). These nutrients become available to plants as they are slowly released from the digestate. The application of digestate helps replenish nutrient levels in the soil, contributing to improved nutrient availability for plants and supporting healthy growth (Li et al. 2023). Digestate also enhances the water-holding capacity of the soil. The organic matter in digestate increases the soil's ability to retain moisture, reducing water stress for plants and improving their resilience during dry periods (Głowacka et al. 2020). This can be particularly beneficial in arid or salt stress regions. In addition, the use of digestate as a fertilizer promotes the recycling of organic waste materials, reducing the need for chemical fertilizers (Jabeen et al. 2017; Cristina et al. 2020). This sustainable approach helps minimize environmental pollution and supports a circular economy by reusing valuable nutrients. However, it is crucial to consider the appropriate application rates and timing of digestate to avoid potential nutrient imbalances or excessive nutrient loading in the soil (Michalzik et al. 2001; Thornton and McManus 1994). In addition, digestate application as a fertilizer can have a significant impact on soil enzyme activity. The organic matter and bioactive compounds present in digestate can stimulate the activity of soil enzymes, such as cellulases, proteases, and amylases (Nkoa 2014). These enzymes play a crucial role in breaking down complex organic matter into simpler forms that plants can readily absorb. The addition of digestate to the soil provides a rich source of organic carbon, which serves as an energy substrate for soil microorganisms (Teglia et al. 2011a, b; Campitelli and Ceppi 2008;). As microorganisms break down the organic matter, they release enzymes that facilitate the decomposition process. This enzymatic activity promotes nutrient cycling, releasing essential elements like nitrogen, phosphorus, and potassium for plant uptake (Larsen et al. 2007; Canali et al. 2011). The stimulation of soil enzyme activity through digestate application also contributes to the overall health and fertility of the soil (Siebert 2008). It enhances the decomposition of organic residues, leading to the release of valuable nutrients and the improvement of soil structure. The increased enzymatic activity aids in the breakdown of complex organic compounds, making them more accessible to plants and promoting nutrient cycling within the soil ecosystem (Weiland 2010; Scaglia et l. 2015). Proper management practices, including soil testing and nutrient management plans, should be followed to optimize the benefits of digestate while minimizing any potential risks. Digestate application as a fertilizer can improve soil properties by enhancing soil structure, nutrient availability, water-holding capacity, and microbial activity (Jabeen et al. 2017; Cristina et al. 2020). These positive effects contribute to overall soil health and fertility, supporting sustainable agriculture practices and promoting the long-term productivity of agricultural lands.

1.5 Application of digestate in vegetable production

Digestate has a variety of applications in vegetable production, some of which are commonly used as follows: 1） Soil conditioner: Digestate can be used as a soil conditioner to improve soil structure, increase organic matter content and improve soil water retention. By mixing digestate with soil, it can improve the fertility and water retention capacity of the soil and enhance the environment for vegetable roots to grow (Möller and Müller 2012). 2) Organic fertilizer: Digestate is rich in organic matter and nutrients and can be used as organic fertilizer in vegetable cultivation. By applying digestate to the growing bed or soil of vegetables (Cristina et al. 2019), it can provide balanced nutrition to the plants and promote the growth and development of vegetables. 3）Hydroponic systems: Digestate can be used as a fertilizer component in the nutrient solution of hydroponic systems (Ronga et al. 2019). By dissolving the nutrients in digestate in water, it provides plants with the nutrients they need and supports the growth and development of vegetables in a hydroponic system. Greenhouse Growing: Digestate is widely used in greenhouse growing. By applying digestate to greenhouse growing media, such as soil or hydroponic systems, it can provide rich nutrients to vegetables, improve soil quality, and promote vegetable yield and quality (Chantigny et al. 2008). When using digestate as a fertilizer, attention needs to be paid to the proper application rate and method to ensure a balanced nutrient supply and optimal results.

1.6 Problem statement of research, hypothesis, and objectives

Based on the above research background, the aim of this work on the replacement of chemical fertilizers with digestate is to explore the potential of using digestate as a sustainable alternative in agricultural practices. The objective is to investigate the efficacy of digestate in providing nutrients to crops and improving soil health compared to traditional chemical fertilizers. The focus of the study was to assess the nutrient content of digestate in different cultivation environments and crop systems. In addition, the impact of digestate application on crop growth, yield and quality was assessed. This study also examined the effects of digestate on soil fertility, enzymatic activity and nutrient. Also, the effect of digestate replacement to chemical fertilizers on the resistance of vegetables to salt stress was explored. The aim is to provide scientific evidence and guidelines for farmers, policy makers and agricultural stakeholders on the safe and effective use of digestate as a sustainable fertilizer. The results of the study contribute to sustainable agricultural practices by promoting resource cycling, reducing dependence on chemical fertilizers, and minimizing nutrient loss and environmental pollution. Therefore, the hypothesis of this research is that digestate replacement chemical fertilizeron on crop producation could enhance crop yield and quality as well as reduction of abiotic stresses by altering soil chemical properties and enzymatic activities. The technological roadmap for this study showed Fig 1.2.

Hence, the main objectives of the research are:

1) To investigate the response to singular and combination of organic and inorganic fertilization on tomato by analyzing tomato growth and fruit yield/quality.

2) To reveal the synthesis of bioactive compounds and antioxidant activity of tomato fruits as affected by application of digestate.

3) To explore the interaction effect between soil indicators and yield/quality of tomato fruits.

4) To compare the physiological and antioxidant responses of lettuce on chemical fertilizer and digestate under salt stress.

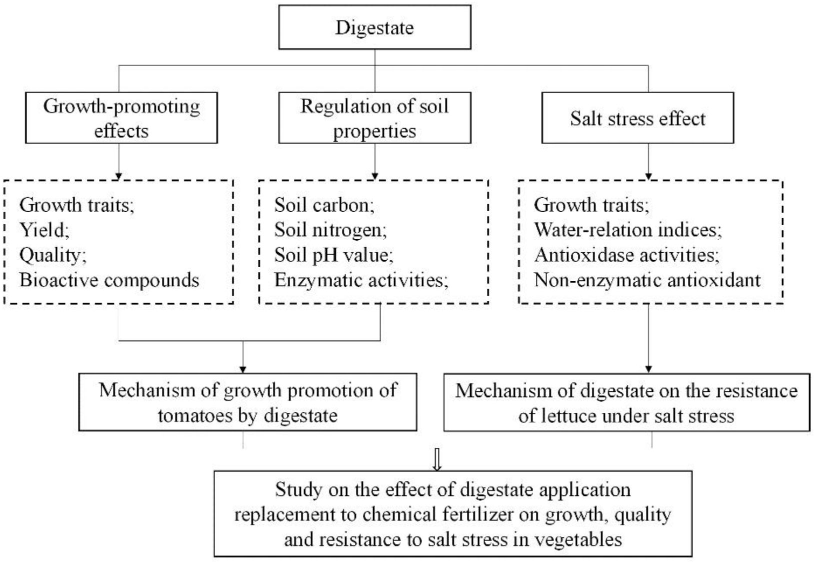


Fig. 1.2. Technological roadmap for this study

Chapter 2. Effect of digestate on the growth, yield and quality of tomato

2.1 Introduction

The Chemical fertilization is one of the most widely used regimes in intensified agriculture (Hernández et al. 2014). However, the long-term use of chemical fertilizers has many harmful effects, is a huge drain on mineral resources, and is not beneficial for sustainable agricultural development. Chemical fertilizers are the source of most of the nitrogen lost from farmlands, which is then released into the water or the atmosphere, leading to greenhouse gas emissions and soil salinization (Da Costa et al. 2013; Hutton et al. 2017). In addition, excessive chemical fertilization results in decreases in food quality, such as increased nitrate accumulation and lower synthesis of ascorbic acid (AsA), and phenols in cultivated plants (Ye et al. 2020; Kerckhoffs et al. 2021). Several studies have shown that organic fertilization could serve as a good substitute for chemical fertilizers and potentially minimize their adverse impacts (Asadollahi et al. 2022; Balík et al. 2022). Organic fertilizers generally promote crop growth and increase the nutritional properties of plants (Wang et al. 2019; Zhang et al. 2020). The integrated nutrient management method of fertilization management has been proposed as a solution to agro-environmental problems (Mahmoud et al. 2009; Kugedera et al. 2022). The aim is not to remove chemical fertilizers completely, but to use a combination of organic and inorganic fertilizers, thus reducing the amount of chemical fertilizer applied in agricultural production. Many studies have confirmed that the use of organic fertilizer as an alternative or partial replacement for chemical fertilizers provides a reliable supply of nutrients during crop growth, increases crop yields and reduces farmland pollution (Mousa et al. 2009; De Corato 2016; Brunetti et al. 2019; Qaswar et al. 2019;).

Anaerobic digestion technology is one of the most important waste management strategies, and is extensively used for the commercial processing of agricultural and other wastes worldwide (Yao et al. 2020). It not only helps to reduce greenhouse gas emissions in the agricultural production but also generates biogas, which is a biofuel that could be used for heat and electricity generation (Sambusiti et al. 2012). However, anaerobic digestion only partially addresses the issue of material and energy recovery, because a significant portion of organic matter and mineral elements still remain in the digestate (Antoniou et al. 2018; Bolzonella et al. 2018). Thus, the ture value of this waste product is not fully captured. In addition, due to the accumulation of biogas plants in certain regions with intensive livestock farming, an oversupply of digestate is expected to be generated (Antoniou et al. 2018). If this excess digestate cannot be properly managed, new environmental issues will arise. Therefore, it is essential to devise some methods for the value-added utilization of digestate. From a circular economy, the utilization of digestate as organic fertilization is an interesting scenario. After anaerobic digestion, many nutrients from the feedstock, such as macroelements (N, P, K), and microelements (B, Cu, Mn, Zn) remain in the digestate (Bolzonella et al. 2018; Li et al. 2023). The utilization of digestate as fertilizer could therefore further enhance the sustainable development of agriculture. However, the application of digestate is not widely acknowledged to increase crop yield and quality.

Tomato (Solanum Lycopersicon) is among the most economically significant and nutritional vegetable crops cultivated worldwide (Brunetti et al. 2019). Over 5 million hectares of land were used for tomato cultivation in 2019, with outputs of more than 180 million tons (FAOSTAT. 2020). In Japan, where tomato plays an important role in vegetable production, and the output value of tomatoes accounts for 10% of all vegetables (MAFF. 2022). In addition, tomato fruits contain various bioactive substances, such as organic acid, AsA, and phenols (Ilahy et al. 2011). Regular consumption of tomatoes is thus very beneficial for human health. The yield and fruit quality of tomato are affected by the type of fertilization. For example, Bilalis et al. found that organic fertilization regime resulted in tomato fruits with a higher sugar-acid ratio (SAR) than that of those grown with conventional inorganic fertilizer (Bilalis et al. 2018). Hernández et al. (2014) reported that the amount of mineral nitrogen could be reduced by approximately 40% by using combined organic and inorganic fertilization while achieving similar tomato fruit yields. In some studies, tomatoes grown in a nutrient system of organic fertilizer have shown improved quality characteristics. For example, Wang et al. found that, compared with chemical fertilization, organic fertilization decreased the nitrate content and enhanced the SAR of tomato fruit (Wang et al. 2017). In another study, the levels of AsA and phenolic compounds in tomato fruits were enhanced by organic fertilization as compared with conventional fertilization (Anton et al. 2014). This study is the first experimental comparison of the effects of different fertilization treatments (digestate, chemical fertilizer, and a combination of the two) on the growth, yield, and fruit quality of tomato.

The objective of this study was to evaluate the influence of applying digestate for tomato production as a replacement or partial substitution to NPK fertilizers. It was assumed that the application of digestate or digestate combined with chemical fertilizer could enhance the growth traits and yield/quality of tomato. Two experiments were therefore conducted under field and greenhouse conditions. The effects on growth traits and fruit yield/quality of tomato was investigated by comparing NPK fertilizer, digestate and digestate combined with chemical fertilizer.

2.2 Materials and methods

2.2.1 Fertilizer sources

Fig. 2.1 Digestate from a pilot-scale anaerobic digester used in this study.

The chemical fertilizer used in the current work was purchased from the Hokuren Fertilizer Co. (Sapporo, Japan). Digestate was collected from a pilot-scale cattle farm waste recycling system located on the campus of Hokkaido University, Hokkaido, Japan (Fig. 2.1). This farm produces livestock manure (approximately 98% cattle manure). The livestock manure is digested into 80–120 m^3 biogas containing 60%–65% methane, thereby producing digestate as a by-product of anaerobic digestion.

2.2.2 Experimental set-up

Four experimental treatments were used in this work: (1) CK, no fertilizer; (2) NPK, fertilization with 180 kg N ha^{-1} of NPK fertilizer (N–P–K: 14–14–14); (3) D, fertilization with 180 kg N ha^{-1} of digestate; (4) NPK-D, fertilization with 90 kg N ha^{-1} of NPK fertilizer and 90 kg N ha^{-1} of digestate. At the same time, two cultivation experiments were conducted under field and greenhouse conditions. The experiment was performed using a completely randomized factorial design, with three plots per treatment, for a total of 12 plots for each cultivation experiment. The chemical properties of different treatments are shown in Table 2.1.

Table 2.1 Characteristics of fertilizers used in different fertilization treatments.

Treatment	C	N	P	K	Ca	Mg
	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
CK	---	---	---	---	---	---
NPK	---	180	180	180	---	---
D	3413.64	180	123.48	377.16	194.64	81.24
NPK–D	1706.82	180	151.74	278.58	97.32	40.62

2.2.3 Experimental site and crop management

Experiments were conducted from June 20, 2021, to October 24, 2021, in an open field and from June 29, 2021, to November 23, 2021, in a greenhouse on the campus of Hokkaido University (43°4′ N, 141°20′ E; 20 m above sea level), Hokkaido, Japan (Fig. 2.2). Meteorological conditions from transplanting to harvest are listed in Table 2.2. The indeterminate tomato variety cultivated in this experiment, Medium Matina, is popular with local growers and was purchased from Greenfield Project Corporation (Nagano, Japan). Prior to experiments, soil samples were characterized from a depth of 0 to 20 cm in the field and greenhouse as shown in

Table 2.3. At the four-leaf stage, uniform healthy tomato seedlings were transplanted into the experimental plots. Each plot was 2.2 m long and 1.3 m wide, comprising a total area of 2.86 m^2, and consisted of two rows. Plants were spaced 35 cm apart, with 50 cm between rows, for an average planting density of 3.5 plants m^2. The shortest spacing between tomato plants in each plot and neighboring plots was 80 cm.

Fig. 2.2 Experimental sites at First Farm of Hokkaido University

Table 2.2 Climatic conditions of experimental sites during production season

	Air temperature (°C)				Precipitation (mm)
	Field		Greenhouse		
	Max	Min	Max	Min	Sum
June	30.6	8.8	38.4	12.1	50.5
July	34.3	16.4	41.1	17.1	7.5
August	34.4	13.9	42.8	15.6	108.5
September	26.6	11.1	37.6	12.8	73
October	27.3	7.3	36.6	9.5	150
November			29.5	8.6	

Agronomic management was the same for all treatments, including fertilization time, de-worming and de-leafing. The only difference was that the greenhouse was irrigated regularly every 3 days, whereas the field was irrigated less frequently during the rainy season, for a total of 24 times during the growing season. In addition, tomato plants were trellised using vertical strings in the greenhouse but were staked with canes and covered with bird-proof nets in the field (Fig. 2.3).

Table 2.3 Characteristics of soil used on the two cultivation conditions.

Parameter	Unit	Field	Greenhouse
Attributes		Sandy soil	Loamy soil
P-absorption coefficient		480	1099
CEC	me $100g^{-1}$	9.7	29.3
N	g kg^{-1}	0.89	1.982
Olsen-P	mg $100g^{-1}$	6.4	38.1
K exchangeable	mg $100g^{-1}$	22	61.0
Ca exchangeable	mg $100g^{-1}$	157.8	401.1
Mg exchangeable	mg $100g^{-1}$	15.8	39.4
Cu	ppm	3.93	2.86
Zn	ppm	3.17	25.75
Mn	ppm	19.18	156.11
B	ppm	0.30	0.73

CEC, Cation exchange capacity.

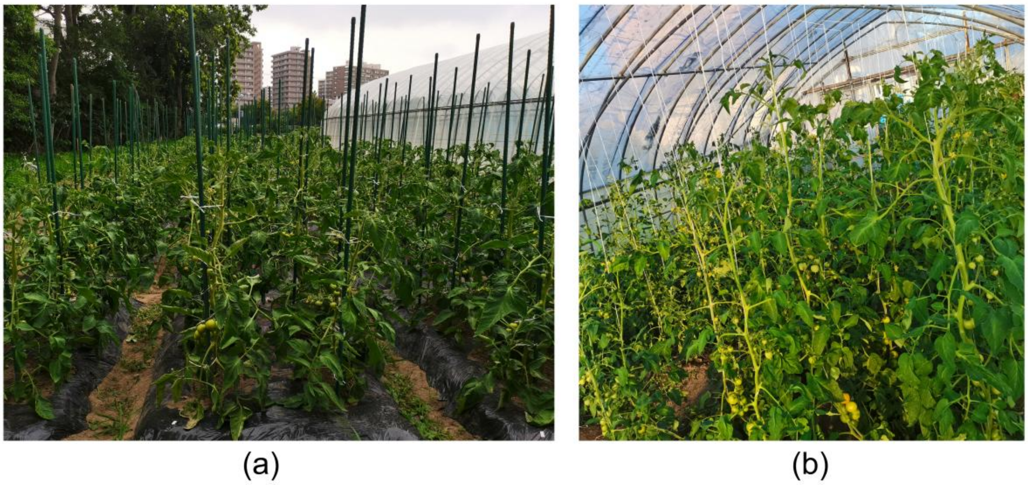


Fig.2.3 Experimental sites used in the study. (a)Field; (b) Greenhouse.

2.2.4 Sampling and analytical methods

Plant growth was measured on September 10th, 2021, for plants in the field and on October 4th, 2021, for plants in the greenhouse. The height and stem diameter were measured and then the chlorophyll content indices were recorded by SPAD values which were measured by method described previously in 2020 (Cristina et al. 2020) with a portable chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd., Japan). The photosynthetic rate was measured as described in our previous study (Li et al. 2023) with a plant photosynthesis meter (miniPPM-300, EARS, Delft, Netherlands) in the tomato plant.

Red-ripened fruits were harvested until the end of the crop production. Tomato fruit yield was measured as the total weight of tomato fruits per m^2 of plants. During the tomato fruiting period, at least 30 fruits were collected from 10 plants per plot to generate a representative pooled fruit sample. Before further analysis, the tomato fruits were washed and sterilized. Tomato fruits were sliced and then homogenized in a blender for analysis of quality parameters. The soluble protein content, soluble sugar content, organic acid content and nitrate content of tomato fruits were determined using the described method of Wang et al. (2017) and SAR was defined as the ratio of soluble sugar to organic acid. The AsA content in tomato fruits was measured using the molybdenum blue colorimetric method (Li et al. 2023).

2.2.5 Statistical analysis

All values were represented as the mean ± SE of three replicates (n = 3). The data was subjected to oneway ANOVA followed by Duncan's post-hoc test at $P < 0.05$ to assess the significance of differences among means. Means (± SE, n = 3) with the same letter in the same cultivation condition are not significantly different from each other ($P < 0.05$) in all figures and tables.

2.3 Results

2.3.1 Growth traits of tomato plants

The height, stem diameter, SPAD, and photosynthetic rate of tomato plants were affected by the fertilization treatments (Fig. 2.4). Plant height was significantly greater in all fertilization treatments than in CK ($P < 0.05$), by 62.36% in NPK-D, 60.82% in D and 41.67% in NPK under field conditions. Similar trends in plant height were observed under greenhouse conditions. Plant height was significantly higher in the NPK-D and D treatments ($P < 0.05$) than in the NPK treatment under both cultivation conditions, except in the D treatment under greenhouse condition. The stem diameters of the tomato plants in the NPK-D and D treatments was not significantly different but was significantly higher in both of these treatments than in NPK and CK under both cultivation conditions ($P < 0.05$). The SPAD of the plants was not comparable between the D and NPK-D treatments and was higher in both of those treatments than in NPK. The photosynthetic rate of tomato plants was significantly higher in the D and NPK-D treatments than in CK and NPK, with that in the D treatment being a 4.28 and 1.34-folds higher than CK and NPK, respectively, under field conditions. Under greenhouse condition, plants in the D and NPK-D treatments showed a statistically higher photosynthetic rate than that of plants in the NPK treatment.

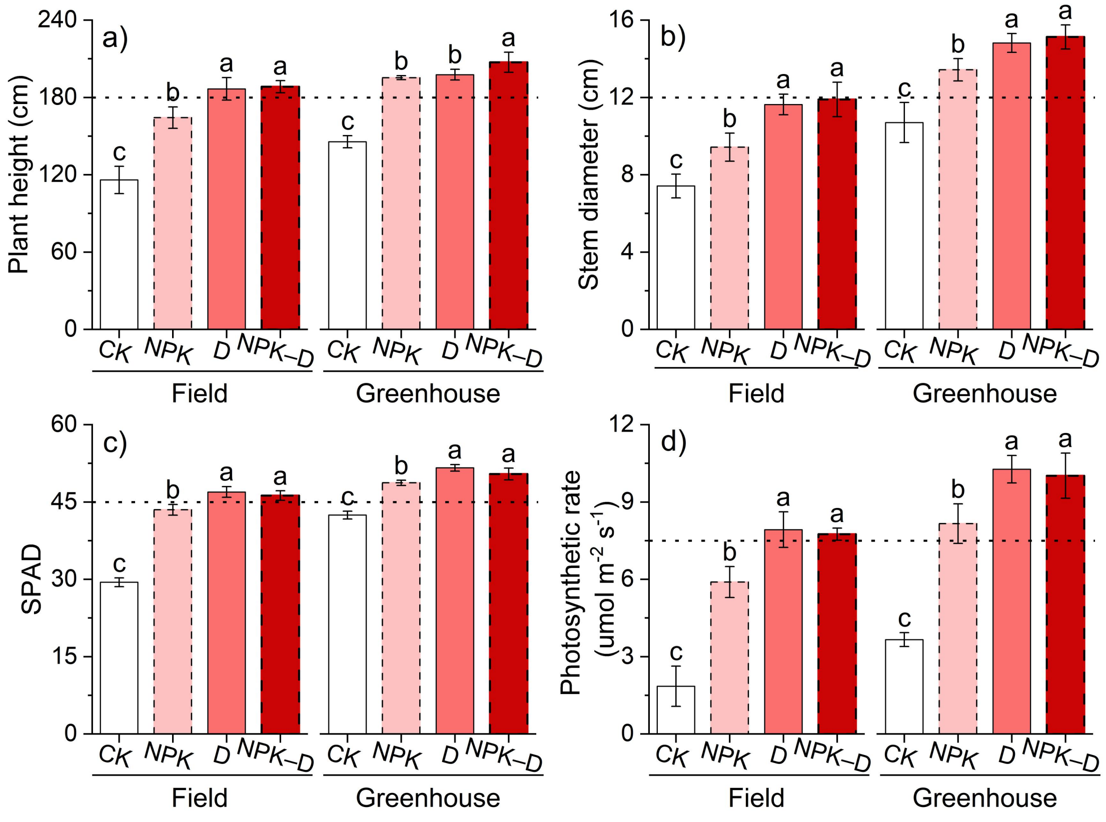


Fig. 2.4 Effects of fertilization strategies on growth traits of tomato plants in field and greenhouse conditions.

2.3.2 Yield of tomato fruits

The fruit yields of tomato plants were markedly higher ($P < 0.05$) in the D and NPK-D treatments than in CK and NPK under both cultivation environments (Fig.

2.5). Under field and greenhouse conditions, the highest fruit yield was in the NPK-D treatment. The fruit yield in NPK-D treatment was 174.28% and 26.29% higher than that in CK and NPK in the field, respectively, under field conditions, and 67.37% and 10.78% higher than that in CK and NPK, respectively, under greenhouse conditions. However, no significant difference in tomato fruit yield was observed between the D and NPK-D treatments in the field or the greenhouse.

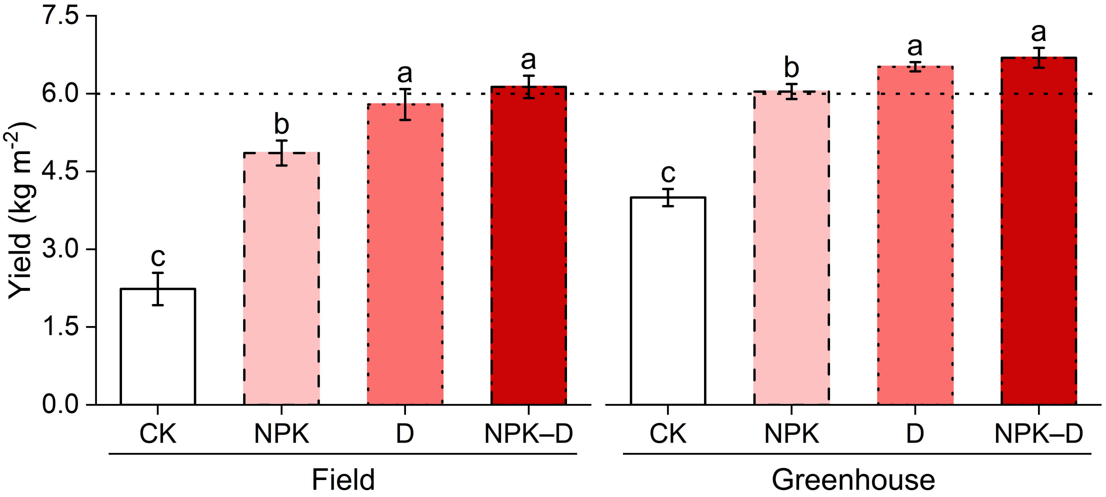


Fig. 2.5 Effects of fertilization strategies on tomato yield in field and greenhouse conditions.

2.3.3 Quality of tomato fruits

Growth The quality parameters of SAR, soluble protein content, AsA content, and nitrate content of tomato fruits are shown in Fig. 2.6. In both field and greenhouse conditions, the fruit SAR, soluble protein content, and AsA content were significantly higher in the D treatment than in the other treatments. The SAR of NPK-D was 18.30% and 63.93% higher than those of NPK and CK, respectively, under field conditions, and 13.29% and 26.68% higher than those of NPK and CK, respectively, under greenhouse conditions. The soluble protein content of NPK-D was 19.81% higher than that of NPK under field conditions, but was not significantly different ($P > 0.05$) from that of NPK under greenhouse conditions (Fig. 2.6b). The AsA content in tomato fruits was higher in NPK-D than in NPK under both cultivation conditions. Th nitrate contents treatment in tomato fruit were higher in the NPK treatment than in the other treatments under both cultivation conditions (Fig. 2.6d). The nitrate contents in tomato fruit were significantly lower in D treatment than in NPK-D, by 17.84% under field conditions and by 18.51% under greenhouse conditions.

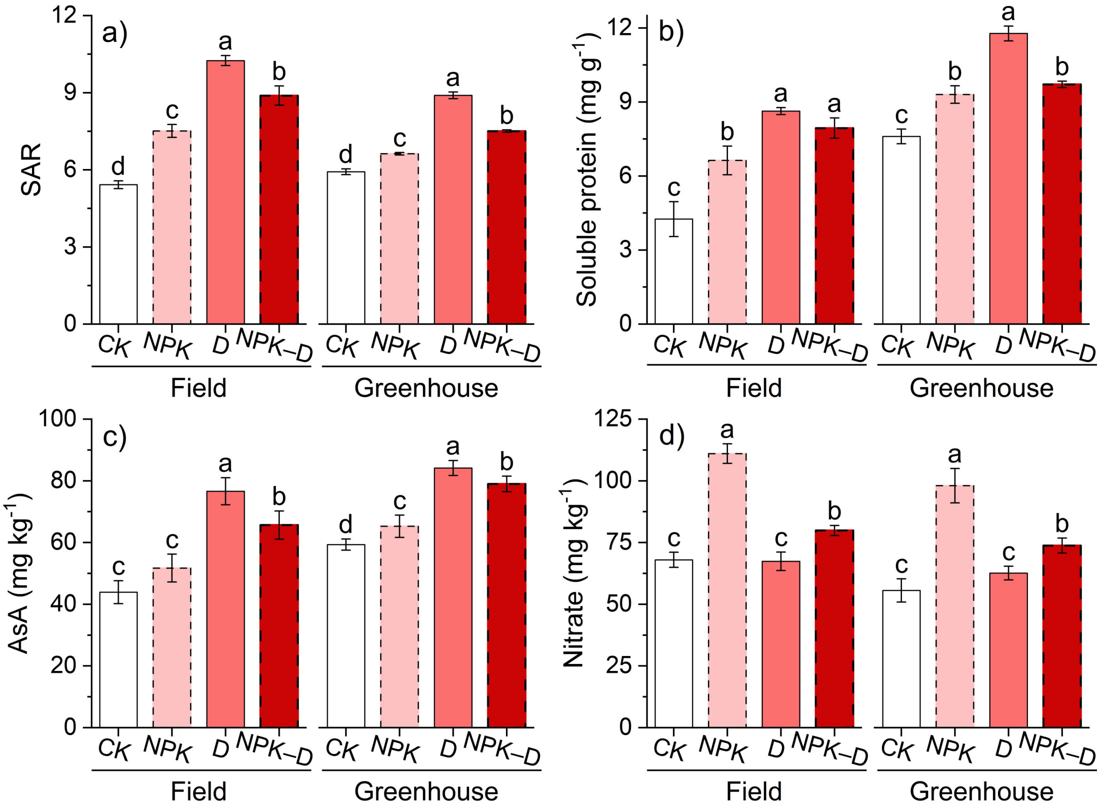


Fig. 2.6 Effects of fertilization strategies on tomato qualities in field and greenhouse conditions.

2.4 Discussion

Organic fertilization as a full or partial replacement for inorganic fertilization is increasingly recommended for crop production (Mahmoud et al. 2009; Hernández et al. 2014). Previous studies have reported inconsistent results in terms of impacts of organic fertilizer on crop yield. Some researchers reported lower yields under organic fertilization than under chemical fertilization (Yu et al. 2010; Wang et al. 2017; Bilalis et al. 2018). It was proposed that this was due to the slow mineralization of organic nitrogen in organic fertilizers, leading to slower crop growth because of the relatively lower levels of available nitrogen in the early growth period (Hartz et al. 2010). In contrast, other studies reported that the application of organic fertilizer as a full or partial replacement for chemical fertilizer achieved similar or higher yields in agricultural production (Mousa et al. 2009; Nkoa 2014; Wu et al. 2022). Consistent with those findings, in this study, the yields of tomato were comparable or even higher in the digestate treatments than in the chemical fertilizer treatment. Cristina et al. (2020) found that digestate had a potentially positive influence on tomato growth, and Wu et al. reported that the combined organic-inorganic fertilization improved tomato yield (Wu et al. 2022). Further elucidation of the mechanisms underlying the effect of digestate as a full or partial replacement for NPK fertilization is therefore essential for improved tomato production.

Digestate is a good fertilizer as it is a source of macro and micro mineral elements and abundant organic matter (Cristina et al. 2020). Compared with chemical fertilizer, it has a stronger effect to improve soil fertility, thereby increasing crop production and promoting crop growth (Cristina et al. 2019; Brtnicky et al. 2022). The results of this study are in agreement with those of a previous work (Cristina et al. 2020), in which the addition of digestate increased plant height and stem diameter. Interestingly, compared with chemical fertilizer, the digestate combined with chemical fertilizer had stronger growth-promoting effects on tomato plants in this study. Similar results were obtained in other studies (Hernández et al. 2014; Zhang et al. 2020), where the combination of organic-inorganic fertilization led to a higher nitrogen level in the soil, thus promoting higher crop production. Brtnicky et al. suggested that the enhancements in crop growth may be partially due to the large increase in soil microbial biomass after use of digestate, resulting in more bioactive soil components in the digestate treatment (Brtnicky et al. 2022).

Fertilization can increase the photosynthesis in plants, thereby promoting the accumulation of organic matter. Higher photosynthetic rate has also been reported when organic fertilization was applied simultaneously with chemical fertilization (Efthimiadou et al. 2010). Results of the present work confirmed the positive effects of digestate application on photosynthetic rate (Mohamed et al. 2018; Cristina et al. 2019). Moreover, compared with CK and the chemical fertilizer treatment, the digestate fertilizer treatments resulted in increased SPAD values in tomato plants. Similar beneficial effects of organic fertilizer have been reported for in cucumber, kale, and lettuce (Wang et al. 2019 Panuccio et al. 2018; Lee et al. 2021). Leaf chlorophyll content is directly related to indirect chlorophyll measurements, such as SPAD values (Yuan et al. 2016). Chlorophyll is the key pigment for photosynthesis, hence an increase in chlorophyll content will enhance photosynthesis, thereby increasing yield. Organic fertilization was shown to improve the status of soil TN and promote nitrogen uptake by plants, thus promoting chlorophyll synthesis (Wang et al. 2017). Consistent with those results, this work found that the application of digestate increased the soil TN, accompanied by an increase in plant leaf SPAD values. In contrast, another study found that application of digestate led to a decrease in leaf chlorophyll content in tomato plants (Elloumi et al. 2016). This was probably because of excessive levels of heavy metals in the digestate used in that study (Singh et al. 2006). The digestate used in this study was from a pilot-scale cattle farm and almost all of the input was cow manure, which has a lower heavy metal content than other digestate inputs (Table 2.4) (Nkoa 2014). Although the SPAD value was not decreased after the application of digestate in the current study, it is still possible that heavy metals could accumulate over long-term cultivation and suppress tomato growth and yield. Further research is required to explore the heavy metal contents in this and other digestates, and to monitor their fate in soil after the application of digestates as fertilizers.

Table 2.4 Heavy metals in the dry matter of digestate used in this study and literatures.

Reference	Mn ($\mu g\ g^{-1}$)	Zn ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	Ni ($\mu g\ g^{-1}$)	Co ($\mu g\ g^{-1}$)
This study	183.56	337.02	55.36	1950	14.82	9.63
Cristina [2020]	268	849	406	39900	155	
Singh [2007]	186.2	785.3	317.7		47.17	
Perez-Espinosa[1999]	221	660	425	46402		13

The results of this study showed that fruit quality in tomato was significantly influenced by fertilizer treatments. The nitrate content in tomato fruits was lower in the digestate treatments than in the chemical fertilizer treatment, consistent with the results of a previous study (Wang et al. 2017). Including mixed microelements such as magnesium, zinc, manganese, and boron in fertilization strategies can decrease the nitrate content of tomato fruits by 20% (Qin et al. 2008). The lower nitrate contents in tomato fruit in the digestate treatments than in the chemical fertilizer treatment may be related to the supply of available micronutrients from digestate. The digestate used in this study was rich in potassium under the same amount of N dose, an element previously reported to enhance tomato fruit yield and quality (Çolpan et al. 2013). Furthermore, the application of organic fertilizer increased the SOC. In another study, fruit quality parameters such as AsA and soluble sugar contents were significantly positively correlated with SOC (Jindo et al. 2016), indicating that tomato fruit quality could be improved by adding digestate. In this study, addition of digestate increased the AsA content and SAR of tomato fruit, similar to the results reported by Wu et al (2022). Tomato fruit quality is a complex character with multiple interactivities among the contributing factors. The results confirmed the positive influence of digestate application on tomato fruit quality. In addition, Digestates contain some phytohormones (e.g., gibberellins, indoleacetic acid, and vitamins) (Nkoa 2014; Panuccio et al. 2019), and these bioactive compounds can significantly enhance crop quality. Another study found that the application of digestate increased SOC and soil fertility, resulting in a larger yield than that obtained using a balanced chemical fertilizer (Wu et al. 2022). The correlation analysis in this study also revealed a significant positive correlation between fruit quality and soil parameters (SOC and TN). These results may explain why tomato plants treated with digestate produced higher-quality fruit. It was also found that growth and fruit quality of tomato were clearly lower in field cultivation than in greenhouse cultivation under the same fertilization treatment. There are several possible explanations for this. First, frequent rain during the harvest period in the field resulted in a higher soil moisture content (Table 2.5). The excess water decreased plant growth and metabolism, thereby reducing the synthesis of compounds related to fruit quality, such as AsA and proteins. Ultimately, such changes in metabolism resulted in a low yield, consistent with the results of other studies (Yang et al. 2015; Zhang et al. 2018). Second, the soil in the field was sandy soil, whereas that in the greenhouse was loamy soil. Sandy soils lack nutrients, and this nutrient depletion limits plant growth and decreases the synthesis of proteins, AsA, and carbohydrates (Cristina et al. 2020).

Table 2.5 Soil moisture content at harvest under different fertilization treatments

Site	CK	NPK	D	NPK–D
Humidity (%)				
Field	18.52±0.59d	20.28±0.65c	24.80±1.15a	22.20±0.67b
Greenhouse	11.14±0.79c	12.35±0.62b	13.77±0.80a	13.49±0.55a

Overall, these findings suggest that the combined fertilization, namely, the addition of digestate to NPK fertilizer, can significantly enhance tomato growth, yield and fruit quality and resulted in improved height, stem diameter, SPAD and photosynthesis rate. The highest yield of tomatoes from a combined digestate and NPK fertilization was obtained and higher fruit quality while promoting the growth traits in the tomato plant were achieved.

Chapter 3. Bioactive compounds and antioxidant activity of tomato fruits as affected by fertilization

3.1 Introduction

In modern agricultural production, livestock manure recycling systems have emerged as a sustainable approach to agricultural waste management. Livestock manure recycling systems convert livestock manure into organic fertilizer by effectively utilizing waste resources while reducing environmental pollution and chemical fertilizer use (Vijay 2011). Compost is known to provide abundant available nutrients to plants as organic fertilizer (Boldrin et al. 2009), improve soil physicochemical properties (Zhang et al. 2006), and promote the proliferation of beneficial microorganisms (Hagreaves et al. 2008), thereby promoting increased crop yields and reducing the application of chemical fertilizers. However, there is a large semi-solid or liquid fraction of the livestock manure recycling system, including urine, water cleanup collection, etc. (Sorathiya et al. 2014), which is difficult to compost. Therefore, anaerobic biomass digestion for gas production is used as another important waste management strategy in livestock manure recovery systems (Qi et al. 2018). It not only helps to reduce greenhouse gas emissions from the agricultural sector, but also produces biogas, a biofuel that can be used to generate heat and electricity (Sambusiti et al. 2012). The very important environmental aspect of biogas production is the fact that anaerobic digestion installations generate large amounts of by-product called digestate (Baryga et al. 2020). In addition, the digestate produced after anaerobic digestion still retains most of its organic matter and mineral elements (Bolzonella et al. 2018; Li et al. 2023). In recent years, excess digestate is expected to be generated due to the accumulation of biogas plants in some areas of intensive livestock farming (Antoniou et al . 2019). If this excess digestate is not properly managed, new environmental problems will arise. Therefore, it is necessary to provide some directions for the utilization of digestate for value addition.

Tomatoes are rich in several bioactive substances such as antioxidants, flavonoids and organic acids that are important for human health (Ilahy et al. 2011).

Several studies have shown that organic fertilization could serve as a good substitute to chemical fertilizers and potentially minimize the adverse impacts of chemical fertilization (Luthria et al. 2010; Asadollahi et al. 2022). As nutrient systems, organic fertilizers generally increase the nutritional properties of plants (Wang et al. 2019; Zhang et al. 2020). Previous studies have shown that tomato yield and fruit quality can be influenced by fertilization practices. For example, Hernández et al. (2014) reported that combined organic-inorganic fertilization could reduce mineral N by approximately 40% and while obtaining similar tomato fruit yields. Bilalis et al. (2018) found higher levels of sugar-to-acid ratio (SAR) in organic fertilizers compared to tomato fruit grown in conventional inorganic fertilizers. In some studies, tomatoes grown in organic fertilizer nutrient systems had better quality characteristics: for example, organic fertilization increased the levels of AsA and phenolic compounds in tomato fruit compared to conventional fertilization (Anton et al. 2014).

The objective of this study was to evaluate the effect of application of digestate or combined application of digestate as fertilizer in tomato production on bioactive substances and antioxidant activity of tomato fruits compared with NPK fertilizer under field and greenhouse conditions. In addition, the changes in tomato yields and major sugars and acids were explained under different fertilization practices. A systematic analysis of the effect of fertilization with digestate of bioactive substances in tomato fruits provides a scientific basis for optimizing the use of agriculture waste and fertilization strategies for tomato production.

3.2 Materials and methods

3.2.1 Fertilizer sources

The fertilizer sources were as same as 2.2.1.

3.2.2 Experimental set-up

The fertilizer sources were as same as 2.2.2. The chemical properties of different treatments are shown in Table 2.1.

3.2.3 Experimental site and crop management

The experimental site and crop management were as same as 2.2.3.

3.2.4 Sampling and analytical methods

During the tomato fruiting period, at least 30 fruits were collected from 10 plants per plot to generate a representative pooled fruit sample. Prior to further analysis, the tomato fruits were washed and sterilized. Tomato fruits were sliced and then homogenized in a blender for analysis of physicochemical parameters, sugars, acids, and phenolic compounds.

Sugars in tomato fruits were extracted according using the described method of Xi et al. (2014). Approximately 5 g of homogenate was accurately weighed, diluted to 20.0 mL with ultrapure water (Millipore, Bedford, MA, USA), and incubated for 20 min in a 35 °C water bath. After centrifugation of samples at 10,000 ×g for 10 min (MX-305, TOMY, Tokyo Rikakikai, Tokyo, Japan), the supernatant was removed and filtered through a 0.22-μm, 25-mm-diameter syringe filter (PTFE, Merck Millipore, Ireland). The filtered solution was then used for sugar and organic acid analyses. Monosaccharide (fructose and glucose) contents in tomato fruits were detected by high performance liquid chromatography (HPLC) with an RI detector on an Agilent 1260 series instrument fitted with a Sugar SH-1821 column and a SH-G guard column (Shodex, Tokyo, Japan). The separation of sugars was conducted using 2 mM H_2SO_4 as the mobile phase with an injection volume of 50 μL, and a flow rate of 0.6 mL min^{-1}.

Acids in tomato fruits were extracted using the described method of Xi et al. (2014). Contents of citric and malic acids in tomato fruits were detected by HPLC with a UV detector at a wavelength of 210 nm on an Agilent 1260 series instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with an RSpak KC-811 column and a KC-G guard-column (Shodex, Tokyo, Japan). The separation of acids was conducted using 1 M $HClO_4$ as the mobile phase with a flow rate of 0.7 mL min1 and an injection volume of 50 μL.

Phenolic compounds were extracted in tomato fruits as described by Anton et al. (2014). Approximately 5 g of homogenate was accurately weighed and then diluted to 20.0 mL with 80% methanol solution. The samples were shaken for 5 h in a rotator (BioSan, Riga, Latvia), left at 4 °C overnight, and then subjected to ultrasonication for 10 min. After centrifugation of samples at 10,000 ×g for 10 min at 20 °C, the supernatant was filtered through a 0.22-μm, 25-mm-diameter syringe filter. The filtered solution was then used for analysis of phenolic compounds. Concentrations of phenols (caffeic acid, chlorogenic acid, ρ-coumaric acid, ferulic acid, gallic acid, syringic acid, kaempferol, naringenin, catechin, quercetin,and rutin) were determined by HPLC with a UV detector at a wavelength of 290 nm with a C18M 4E column (Shodex, Tokyo, Japan). The separation of phenolic compounds was detected by gradient elution using methanol (solvent A) and 0.5% acetic acid (solvent B) as the mobile phase at a flow rate of 0.8 mL min1 and an injection volume of 20 μL. The protocol was determined following from 30% solvent A up to 90% and from 70% solvent B down to 10% over 25 min. The percentage of solvent A was then reduced to initial conditions, and the column was re-equilibrated for 10 min. The run time for one sample was 35 min.

Total phenolic content (TPC) in tomato fruits was measured by the Folin–Ciocalteu method (Rajapaksha et al., 2020). Total flavonoid content (TFC) in tomato fruits was detected using the colorimetric method described by Yuan et al. (2023). A modified method was used to detect the DPPH radical scavenging activity (Zhang et al. 2004) and the antioxidant capacity of tomato fruits was expressed as μg trolox equivalent per kg fresh weight (μg g^{-1}).

3.2.5 Statistical analysis

All values were represented as the mean ± standard error (SE) of three replicates (n = 3). Data was subjected to analysis of variance (ANOVA) and differences between the means were compared by Duncan's post-hoc test at a probability of 95%. Means (± SE, n = 3) with the same letter in the same cultivation condition are not significantly different from each other ($P < 0.05$) in all figures and tables. Statistical analyses were conducted using SPSS 24.0 (SPSS. Inc., Chicago, IL, USA). All figures were Origin 2022.

3.3 Results

3.3.1 Sugars were affected by fertilization

To investigate how the fertilizing to affect fruit sweetness, it was explored the contents of glucose, fructose and sucrose in tomato fruit (Fig. 3.1). Digestate and

NPK-D fertilized plants had higher amounts of saccharides, including monosaccharides (fructose and glucose) and sucrose compared to those of CK under both field and greenhouse conditions. These were even significantly higher than the NPK treatments. Fructose sugars were 35.98% and 13.23% higher in digestate treatment than in NPK under field and greenhouse conditions, respectively; and 26.04% and 6.34% higher in NPK-D treatment than in NPK under field and greenhouse conditions, respectively. The glucose had a same trend as fructose under field and greenhouse conditions.

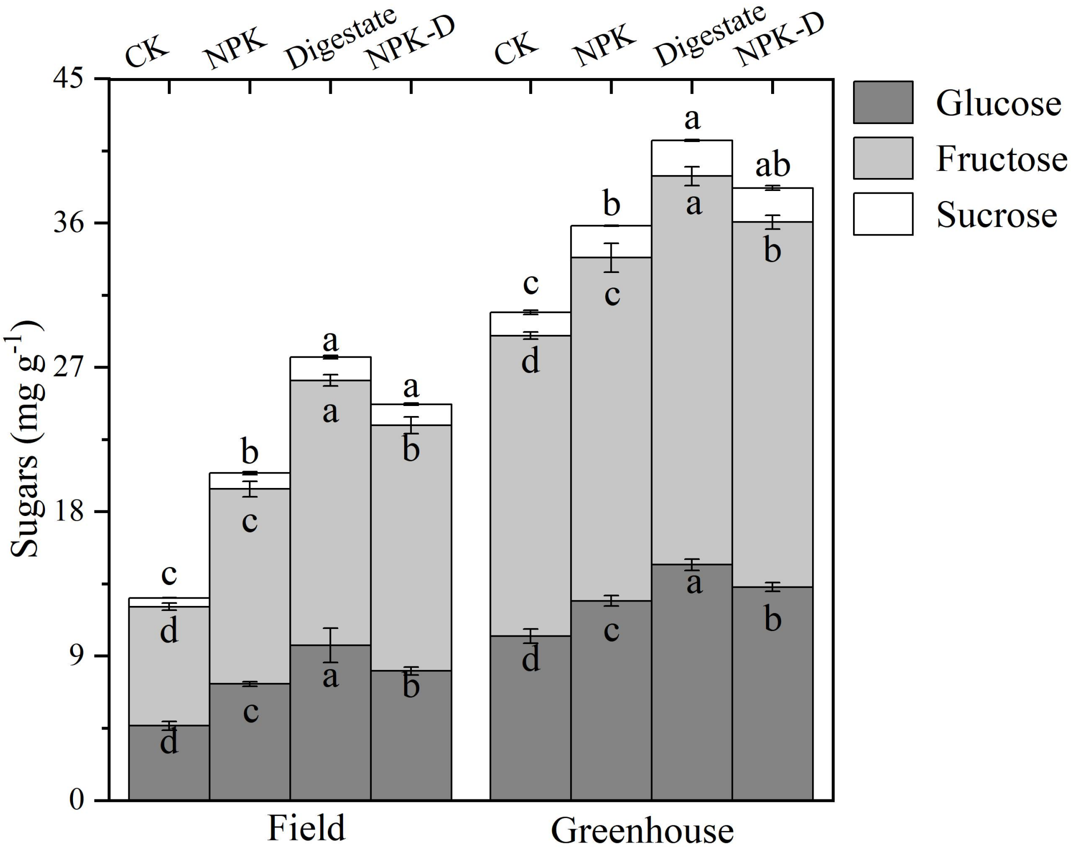


Fig. 3.1 Effects of fertilization treatments on fruit sugars (glucose, fructose and sucrose) in field and greenhouse conditions.

3.3.2 Organic acids were affected by fertilization

Acid contents of tomato fruits were affected by fertilization treatment, including in five kind of acid (Table 3.1). Citric acid was higher than malic acid, and did not differ significantly between the digestate and NPK-D treated fruits, but they were significantly higher than the CK treated fruits. Citric acid contents of tomato fruits were visibly higher in NPK than in the other three treatments under field and greenhouse conditions. Citric acid contents following the NPK treatment were significantly enhanced in tomato fruits under field and greenhouse conditions, respectively, compared to those of digestate. Malic acid contents were substantially lower than those of citric acid in both cultivation environments. Malic acid fruit contents of digestate-treated tomato plants significantly improved compared to those of NPC and CK. Malic acid contents did not differ greatly between digestate and NPK-D treatments in the field. The other acids were affected under field and greenhouse conditions in the different fertilization strategies.

Table 3.1 Effects of fertilization treatments on organic acid in open field and plastic greenhouse environments.

Treatment	Citric acid	Fumaric acid	Malic acid	Oxlic acid	Succinic acid
Field	(mg 100g^{-1})	(mg 100g^{-1})	(mg 100g^{-1})	(mg 100g^{-1})	(mg 100g^{-1})
CK	404.87±17.51d	0.64±0.07c	25.94±1.27c	0.36±0.05d	22.73±3.34b
NPK	621.73±31.67a	2.33±0.39b	53.56±3.05b	0.85±0.04c	50.90±5.75a
Digestate	489.29±20.60c	4.10±0.47a	72.52±2.45a	1.08±0.05b	54.14±4.98a
NPK-D	533.85±21.38b	3.56±0.42a	69.47±2.94a	1.24±0.06a	51.86±6.74a
Greenhouse					
CK	626.48±7.81d	1.29±0.17c	41.10±1.40d	0.65±0.00d	43.66±4.04b
NPK	747.30±17.16a	3.17±0.41b	63.98±1.42c	1.36±0.01c	66.45±7.05a
Digestate	652.42±9.34bc	4.52±0.28a	75.76±2.80a	1.48±0.02b	66.09±5.21a
NPK-D	699.81±14.02b	4.22±0.32a	70.86±1.51b	1.78±0.01a	62.81±5.15a

3.3.3 Phenol compounds was affected by fertilization

In this study, contents of 11 phenolic compounds was analyzed including caffeic acid, catechin, chlorogenic acid, ρ-coumaric acid, ferulic acid, gallic acid, kaempferol, naringenin, quercetin, rutin, and syringic acid (Table 3.2). Tomato fruits from fertilization treatments that included digestate or NPK-D had higher phenolic components compared with CK and NPK under both cultivation conditions. Chlorogenic acid was the predominant phenolic compound in tomato fruits in both cultivation environments. The highest chlorogenic acid content of tomato fruits was in digestate (34.90 mg kg^{-1}) under field and digestate (41.04 mg kg^{-1}) under greenhouse, respectively. Correspondingly, chlorogenic acid contents of digestate treatments in tomato fruits were notably higher than that of other treatments under field and greenhouse conditions. Gallic acid and naringenin contents of tomato fruits showed similar trends to that of chlorogenic acid. In addition, differences in the remaining phenolic compounds in this study varied according to fertilization treatments; however, digestate resulted in higher phenolic compound contents compared with the other treatments. In the two cultivation environments, ferulic acid was the only phenolic compound that did not differ significantly in all treatments except for CK.

Table 3.2. Content of phenolic compounds in tomato fruits of different fertilization treatments (ug g^{-1}) under two cultivation environments.

Site	CK	NPK	Digestate	NPK-D
Caffeic acid				
Field	0.13±0.01d	0.20±0.01c	0.29±0.00a	0.26±0.01b
Greenhouse	0.45±0.01c	1.35±0.04b	2.22±0.03a	2.17±0.06a
Catechin				
Field	8.15±0.85d	9.76±0.31c	12.82±0.26a	11.52±0.36b
Greenhouse	9.84±0.37c	12.33±0.54b	14.73±0.54a	14.19±0.57a
Chlorogenic acid				
Field	9.36±1.35d	14.57±1.63c	34.90±1.72a	21.61±3.01b
Greenhouse	18.36±1.30d	29.83±1.54c	41.04±2.92a	37.55±1.23b
ρ-Coumaric acid				
Field	0.22±0.02d	0.32±0.02c	0.66±0.03a	0.38±0.01b
Greenhouse	1.22±0.10d	1.74±0.10c	2.45±0.12a	1.69±0.18b
Ferulic acid				
Field	1.04±0.04b	1.47±0.04a	1.45±0.06a	1.35±0.12a
Greenhouse	2.61±0.09b	2.88±0.07a	2.94±0.08a	2.99±0.76a
Gallic acid				
Field	4.18±0.25d	6.33±0.36c	9.88±1.00a	7.70±0.51b

Greenhouse	6.87±0.35d	10.84±0.38c	14.06±0.45a	12.40±0.41b
Keampferol				
Field	0.19±0.01c	0.29±0.00b	0.35±0.01a	0.34±0.01a
Greenhouse	0.36±0.01c	0.43±0.01b	0.55±0.01a	0.54±0.02a
Naringenin				
Field	0.05±0.01d	0.15±0.02c	0.24±0.01a	0.18±0.01b
Greenhouse	0.15±0.01d	0.17±0.01c	0.26±0.00a	0.24±0.01b
Quercetin				
Field	0.92±0.08d	1.46±0.06c	1.83±0.07a	1.61±0.07b
Greenhouse	2.30±0.04d	2.73±0.10c	3.16±0.11a	2.76±0.09b
Rutin				
Field	2.62±0.14d	4.15±0.13c	5.40±0.11a	4.78±0.16b
Greenhouse	9.28±0.61d	11.18±0.75c	14.76±0.94a	12.87±0.52b
Syringic acid				
Field	4.18±0.21d	5.55±0.27c	8.61±0.33a	6.09±0.23b
Greenhouse	9.15±0.17b	9.63±0.15ab	9.92±0.17a	9.76±0.25a

3.3.4 Total phenolic and flavonoid contents was affected by fertilization

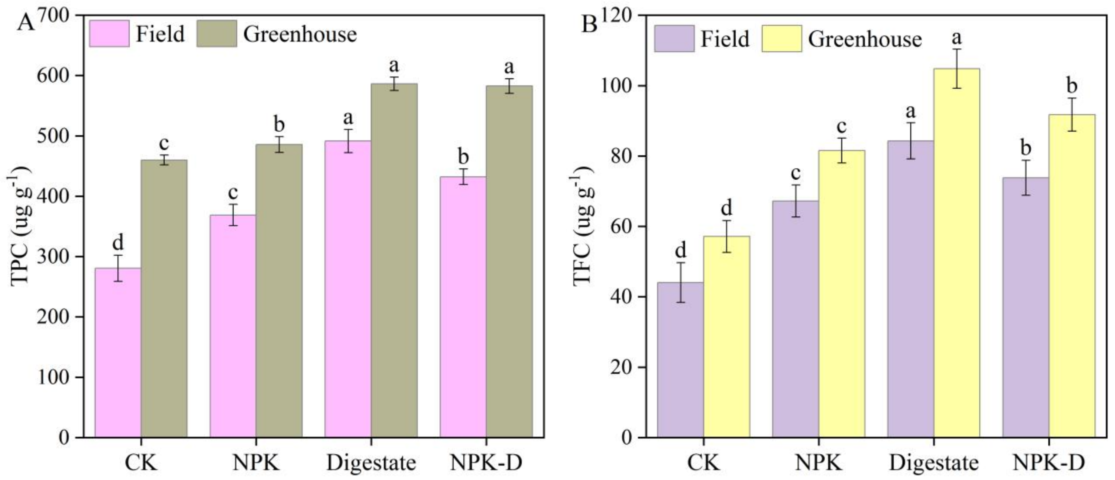


Fig. 3.2 Effects of fertilization treatments on total phenolic contents and total flavonoid contents in field and greenhouse conditions.

TPC and TFC of tomato fruits are shown in Fig. 3.2. In both field and greenhouse conditions, fruits from digestate had significantly higher amounts of TPC and TFC than those in other treatments (Fig. 3.2). Under field conditions, TPC of digestate was 25.38% and 15.76% higher than those of NPK, whereas no significant difference was observed between digestate and NPK-D under greenhouse. Tomato fruits of both digestate and NPK-D had obviously higher TFC contents than those of NPK in the two cultivation experiments (field and greenhouse conditions). In addition, TFC of digestte were significantly higher than those of other three treatments under both cultivation environment conditions.

3.3.5 Antioxidant capacity was affected by fertilization

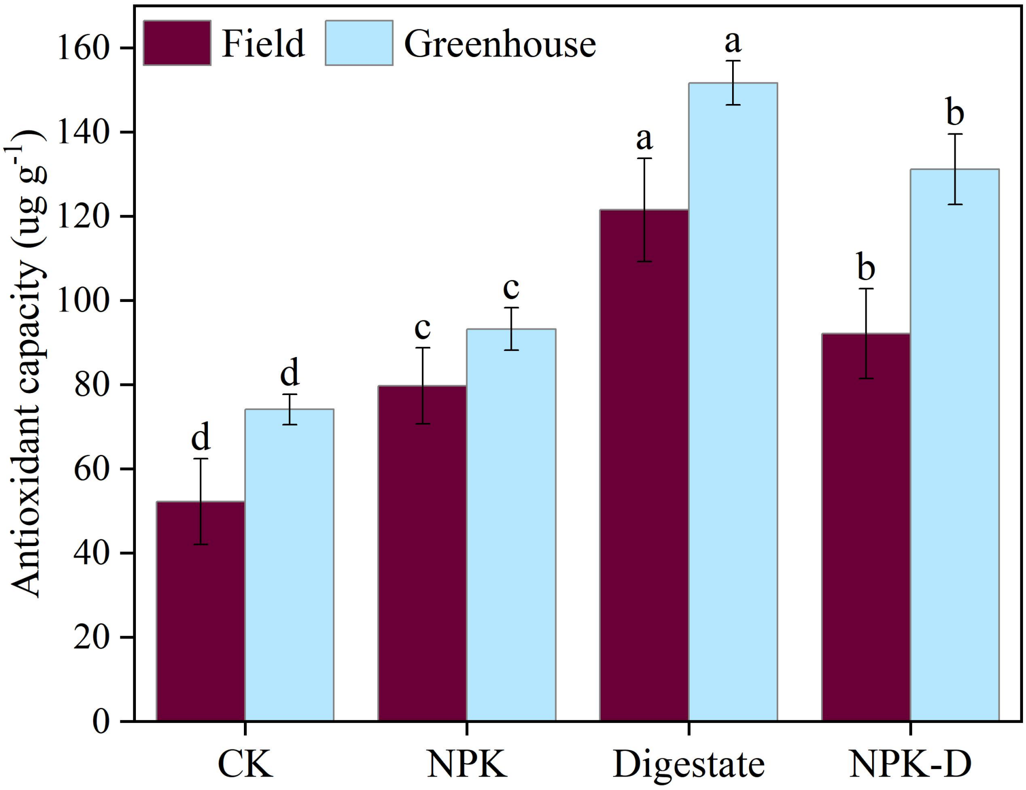


Fig. 3.3 Effects of fertilization treatments on antioxidant capacity in field and greenhouse conditions.

The antioxidant capacity of tomato fruits are shown in Fig. 3.3. The highest antioxidant capacity was in digestate under field and the same result occurred under greenhouse condition in all treatments. In both field and greenhouse conditions, fruits from digestate and NPK-D had significantly higher amounts of antioxidant capacity than those in CK and NPK treatments. The antioxidant capacity of digestate and NPK-D was 52.44% and 15.54% higher than those of NPK treatment, respectively, under field condition, and 62.69% and 40.71% higher than those of NPK treatment, respectively, under greenhouse condition. The antioxidant capacity tomato fruits in between digestate and NPK-D treatments had significant difference under both conditions, and antioxidant capacity of digestate was 31.94% and 15.61% higher than this of NPK-D treatment under field and greenhouse, respectively.

3.4 Discussion

Livestock waste could be recycled in many ways to address rising energy prices, sustainable agriculture and reduce the environmental threat of traditional livestock waste management practices (Sorathiya et al. 2014). Proper use of livestock manure for biogas, composting and vermicompost manufacturing is very useful for improving crop yield and sustainability (Chew et al. 2019). Recovery of digestate as organic fertilizer after biogas fermentation is considered a suitable use because it recovers plant nutrients and reduces the consumption of chemical fertilizers (Qi et al. 2018). Our study also confirms these ideas that the application of digestate and NPK-D can reduce the application of chemical fertilizers while maintaining good tomato yields. Organic fertilizers are increasingly recommended for crop production as an alternative to inorganic fertilizers (Hernández et al. 2014; Wu et al. 2022). Many studies have been interested mainly in the improvement of crop quality or yield by organic fertilizers (Asadollahi et al. 2022; Zhang et al. 2020), without systematically focusing on the effects of bioactive substances and antioxidant activity of the fruit, and even less on the effects of digestate application on these parameters. In the present work, the application of digestate or NPK-D as an alternative to chemical fertilizers significantly increased tomato yields. The results are consistent with the findings of Zhao et al. (2009), where different organic fertilizers were found to increase crop yield. Cristina et al. (2020) found a potential positive effect of digestate on tomato growth and Wu et al. (2022) reported that application of organic fertilizer could increase tomato fruit yield. The results show that organic fertilizer applications, i.e., digestate from the same cattle farm waste recycling system, both maintain tomato yields and increase the and synthesis of bioactive substances in tomato fruits.

Organic fertilizers play an important role in the organic acids and sugars in tomato fruits (Terada et al. 2023). These results showed that organic fertilizers significantly promoted the accumulation of sugars in tomato fruit, whether digestate or NPK-D treatments. Hu et al. presented similar evidence (2022). Riahi et al. (2009) also reported that organic farming systems regulated the substantial accumulation of sugars in organic tomato fruit and that these differences were statistically significant. Another study also reported similar results to this work, where different organic fertilizers increased the soluble sugar content of tomato fruits (Ma et al. 2021). Organic fertilizer application may also affect the acidity of tomato fruit, with chemical fertilizer-applied crops containing more organic acids compared to organic fertilizer application (Hallmann 2012). Different results were given by Pieper et al. (2009), who showed that organic fertilizer application resulted in higher organic acid content in tomato fruit, but no statistical difference compared to inorganic fertilizer. In this study, the application of digestate promoted an increase in some organic acids, such as malic, fumaric and oxalic acids, however, citric acid was significantly lower in the fruits of digestate applications than in the chemical fertilizer treatments. This could be due to the difference caused by the difference in the amount of nitrogen applied. The most pronounced effect on fruit citric acid levels was observed with a reasonable amount of N application (Taghavi et al. 2019). Much of the acidity in tomato fruit is attributed to citric acid, which is the predominant organic acid in tomato fruit and is responsible for the sour taste of tomatoes (Dong et al. 2023). In many studies, it has been shown that the application of organic fertilizers reduces the level of titratable acidity in tomato fruits compared to chemical fertilizer applications (Hernández 2014; Bilalis et al. 2018). Almost all of these reports measured titratable acidity content with citric acid as a conversion factor, which is consistent with the results of the present study. Li et al (2022) also reported similar results, where organic fertilizer application reduced the citric acid content but increased the malic acid content of tomato fruits and promoted the glucose and fructose content. In this study, tomato fruits treated with organic fertilizer had lower citric acid content and higher malic acid content compared to chemical fertilizer treatment, but significantly higher sugar content, which could be due to loss of citric acid due to sugar synthesis. The sugar to acid ratio is one of the main indicators affecting the taste of tomato fruits. In this study, tomato fruits treated with digestate and NPK-D had a higher sugar-acid ratio than the chemical fertilizer treatment (Fig. 2.6). This observation is consistent with other studies (Wang et al. 2017; Bilalis et al. 2018; Wu et al. 2022;). The lack of flavor in fruits treated with inorganic fertilizers may be due to low sugar to acid ratio (Li et al. 2022). Many factors, such as cultivation conditions, temperature, light and humidity, could affect the flavor of tomato fruits. However, studies have shown that fertilizer availability is the most important factor affecting fruit flavor (Frías-Moreno et al. 2021).

Organic fertilization as a substitute or partial replacement to inorganic fertilization is increasingly recommended for crop bioactive components (Hernández et al. 2014; Wu et al. 2022). The fruit bioactives of tomato are a complex system with multiple interactions. According to previous studies, tomato fruits are rich in bioactive compounds, including secondary metabolites (e.g. phenolics and flavonoids) (Anton et al. 2014). The current study confirms that the application of digestate and NPK-D has a positive impact on the synthesis of phenolic compounds. The application of digestate improved most phenolic compounds as well as total phenol and total flavonoid content in tomato fruits under field and greenhouse cultivation. The beneficial effects of organic fertilizer were also observed in cucumber (Panuccio et al. 2019), kale (Lee et al. 2021) and lettuce (Wang et al. 2019). Many studies have found that organic fertilizers increase the synthesis of flavonoids in plants (Ilahy et al. 2011; Anton et al. 2014). These results also confirm this finding, as the treatment with digestate or NPK-D had higher levels of flavonoids, such as rutin, quercetin, and naringenin, compared to tomato fruits from chemical fertilizer and CK treatments. In addition, digestate contains high amounts of organic matter and phytohormones (Nkoa 2014), and these compounds could significantly enhance the synthesis of bioactive substances in crops. Meanwhile, recent studies have reported antioxidant and free radical scavenging properties of polyphenolic compounds in several plant extracts, suggesting a possible protective role in reducing the risk of cardiovascular diseases in humans (Kaushal 2022). he findings in this study regarding the major phenolic compounds are similar to previous findings (Erdinc et al. 2018), where the application of organic fertilizers increased the synthesis of phenolic compounds such as chlorogenic acid and rutin in tomato fruits. This can be explained by the fact that some organic substances provided in organic fertilizers are able to promote the synthesis of phenolic compounds in plant growth (Naguib et al. 2012). Tomatoes are considered a nutritional indicator of good dietary habits and a healthy lifestyle (Salehi 2019) because their fruit is a food with high levels of antioxidants. Oxidation plays an important role in the emergence of certain diseases and in human aging, and antioxidant capacity that helps to limit the oxidative process is a highly desirable property in foods (Lenucci et al. 2006). In the present study, high antioxidant capacity was found in tomato fruits treated with digestate. The antioxidant capacity was significantly different in different fertilization treatments. Organic treatment has a stimulating effect on the biosynthesis of phenolics which possessed high potential activity as antioxidant when compared with control and bio-organic treatments (Naguib et al. 2012). A strong correlation between antioxidant activity and lycopene content was reported by Zanfini et al. (2010). Ilahy et al. (2011) stated that tomatoes containing high amounts of lycopene have high antioxidant activity. Although the fruit lycopene content was not examined in this study, increased antioxidant capacity of tomato fruits in treated digestate did be observed.

Tomatoes occupy an important position among vegetables in terms of both production and consumption. Traditionally a warm-weather crop, they can also be produced in controlled environments with optimum conditions. The application of digestate enhanced significantly levels of antioxidants under two cultivation environments. The present study found some significant differences ($p < 0.05$) in the phenolic compound, saccharides and organic acid levels, TP and TF contents and antioxidant activity of two different cultivation conditions grown using four different fertilizer strategies.

Chapter 4. Variation in soil chemical properties and enzymatic activities under different fertilization strategies

4.1 Introduction

Many studies have confirmed that organic fertilization or combined organic-inorganic fertilization provides a reliable supply of nutrients during crop growth, increases crop yields, promotes soil health, and reduces farmland pollution (Wu et al. 2022). According to the analysis, in addition to rich nitrogen, phosphorus, potassium and trace elements and other essential nutrients for plant growth and development, the digestate also contains organic matter, humic acid, hemicellulose, cellulose, lignin, which is a high-quality soil conditioner (Nkoa 2014). Digestate can significantly increase soil organic matter content, improve soil total nitrogen, total phosphorus, total potassium and alkaline ammonia, fast-acting phosphorus content, increase soil nitrogen, phosphorus, potassium and other nutrients (Tambone et al. 2010). Among them, organic matter can adsorb more cations, which makes the soil fertility-holding and buffering. The acids such as huminic acid and fulvic acid in digestate eliminate sodium carbonate, the main salt substance that causes soil alkalinization, and reduce soil alkalinity. In addition, humus is a weak acid containing multi-acidic functional groups and its salts have amphiphilic colloidal action with a strong ability to buffer acid-base changes (Doyeni M. 2021). It also loosens and forms structures in the soil, thus improving the physical properties of the soil, increasing soil porosity, and promoting the formation of soil colloids and soil agglomerate structures. Bian et al. found that the content of organic matter, total N, fast-acting N, fast-acting P, and fast-acting K in agricultural soils with continuous application of digestate was higher than those with chemical fertilizers, while the porosity increased (Kizito et al. 2019).

Digestate can also provide essential carbon and energy sources for soil microorganisms and promote the balanced growth of soil microorganisms. Holatko et al. (2021) found that the digestate is beneficial to the balanced growth of multiple microorganisms in the soil and to the uniform distribution of soil microbial populations, and the chasing of biogas is more beneficial than the chasing of chemical fertilizers to improve soil enzyme activity and soil respiration intensity, thus improving the soil. Soil enzymes are the products of plant, animal and microbial activities in the soil and play an important role in increasing soil labile nutrients by catalyzing biochemical reactions in the soil (Brunetti et al. 2019; De Corato et al 2016). Their activity is one of the important indicators of soil biological activity and soil fertility. The application of digestate could significantly improve the enzymatic activity of soil urease, sucrase, protein, polyphenol oxidase, nitrate reductase, inorganic pyrophosphatase and phospholipase, and improve soil fertility (Ren et al. 2021). Supplementary application of methane can increase the activities of soil urease and convertase to varying degrees, with the greatest increase in soil pulse enzyme activity (Holatko et al. 2021). In addition, the application of digestate could increase the number of soil bacteria, yeast, fungi and actinomycetes, resulting in an increase in the soil microbial (Manici L. et al. 2021). Lee et al. (2018) reported that the number of soil auto-nitrogen-fixing bacteria increased gradually with the advancement of tomato fertility in each fertilization treatment, and basal application of methane was more beneficial than basal application of pig manure in promoting the growth of soil auto-nitrogen-fixing bacteria. Therefore, the present study was conducted to assess the soil chemical characteristics and soil enzymatic activities in two cultivation conditions under four different fertilizer strategies. In addition, the correlations between growth traits and yield/quality of tomato and soil properties indicators was explained in different fertilization strategies.

4.2 Materials and methods

4.2.1. Fertilizer sources

The fertilizer sources were as same as 2.2.1.

4.2.2. Experimental set-up and site

The fertilizer sources were as same as 2.2.2. The chemical properties of different treatments are shown in Table 2.1.

4.2.3 Experimental site and crop management

The experimental site and crop management were as same as 2.2.3.

4.2.4. Sampling and analytical methods

Soil samples (0–20 cm) were collected at 20 points around tomato roots in each plot and then mixed to obtain a composite sample. Soil samples were immediately stored at 80 °C until analyses.

The soil pH was determined in a 1:2.5 (w/v) soil/water slurry. Soil organic carbon (SOC) was measured by the potassium dichromate–sulfate colorimetric method (Qaswar et al. 2020). The total nitrogen (TN) and ammonium nitrogen (AN) contents in soil were determined in accordance with method of Lu (2000).

Urease, sucrase, protease, and nitrate reductase (NR) activities in soil were measured according to Schinner et al. (2012). Control tests without soil or substrate were conducted to evaluate the abiotic transformation or spontaneity of all analyzed enzymes.

4.2.5 Statistical analysis

All values were represented as the mean ± SE of three replicates (n = 3). The data was subjected to oneway ANOVA followed by Duncan's post-hoc test at $P < 0.05$ to assess the significance of differences among means. Means (± SE, n = 3) with the same letter in the same cultivation condition are not significantly different from each other ($P < 0.05$) in all figures and tables. Red and blue circles represent positive and negative correlations, respectively. *, $p < 0.05$ and **, $p < 0.01$.

4.3 Results

4.3.1 Soil chemical characteristics

The properties of soil from the field and greenhouse after harvest following the different fertilization treatments are summarized in Fig. 5. The soil pH values ranged from 5.13 to 6.03 under field conditions and from 5.55 to 6.08 under greenhouse conditions. The SOC was significantly higher in digestate ($P < 0.05$) than in CK, by 80.69% under field conditions. Similar trends in SOC were observed under greenhouse conditions. Under both cultivation conditions, the trends in soil AN were similar to those of SOC under field conditions. The lowest TN contents in soil were in CK. The TN content soil was lower in NPK than in digestate traetment, by 37.41% under field conditions and by 20.03% under greenhouse conditions. There was no significant difference in TN content between the digestate and NPK–D treatments under both cultivation conditions.

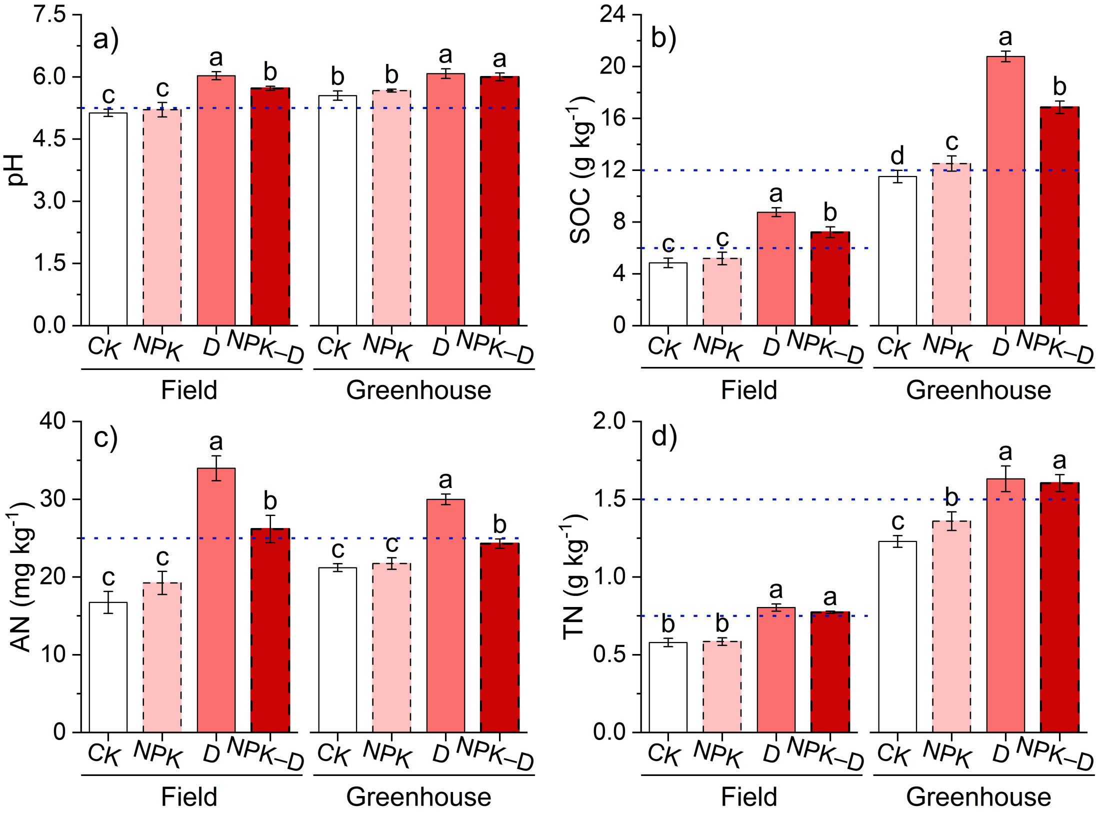


Fig. 4.1 Effects of fertilization types on soil chemical properties in field and greenhouse conditions.

4.3.2 Soil enzyme activities

Fertilization enhanced soil enzymatic activities in all treatments compared with CK under both cultivation conditions (Fig. 6), except for soil urease activity in NPK under greenhouse conditions. The lowest soil urease activities were in CK followed by NPK and NPK–D under the two cultivation conditions, and the highest soil urease activity was in D treatment (Fig. 6a). However, sucrase activity showed a different trend, being highest in the NPK–D treatment under both cultivation conditions. No statistical difference in soil sucrase activity was found between the NPK–D and D treatments under field conditions. Under greenhouse conditions, however, the sucrase activity was significantly higher ($P < 0.05$) in NPK–D than that in D. The soil protease activities differed significantly among all treatments under both field and greenhouse conditions, and soil NR activity showed a similar to that of protease activity under both cultivation conditions.

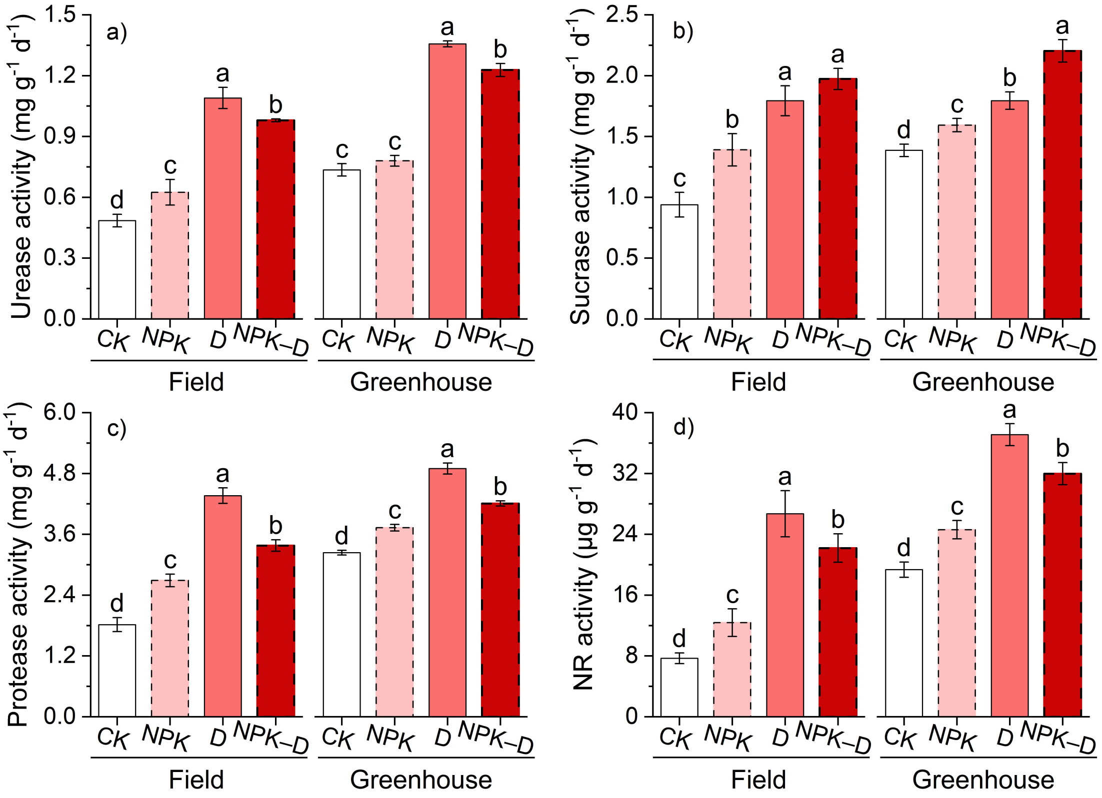
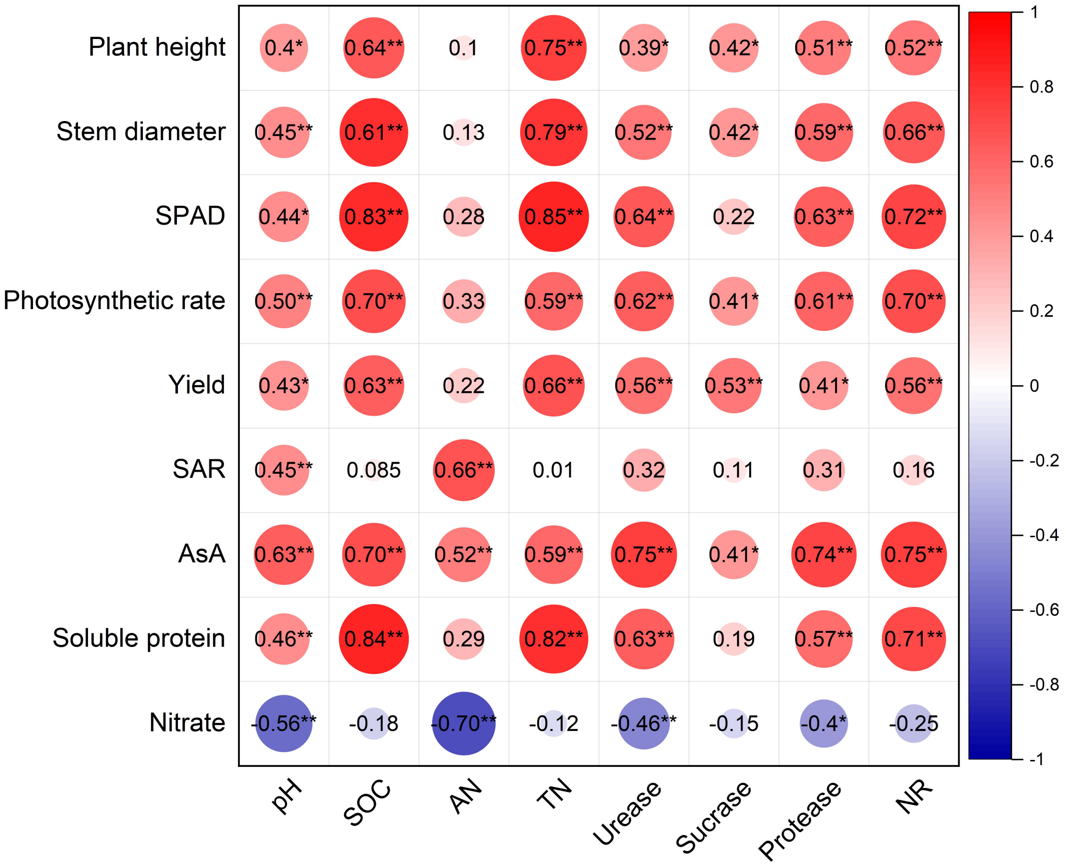


Fig. 4.2 Effects of fertilization types on soil enzyme activities in field and greenhouse conditions.

4.3.3 Correlations of growth and yield/quality of tomato with soil properties

To further characterize the effect of fertilization treatments, it was conducted a Spearman's correlation analysis to explore the relationships between the growth, yield, and fruit quality of tomato and soil chemical properties and enzyme activities (Fig. 7). A positive correlation was found between soil properties and growth, yield, and fruit quality indicators of tomato. Only the nitrate content of tomato fruit was negatively correlated with soil properties. Most growth indicators were significantly and positively correlated with soil properties. The maximum correlation coefficient (0.85) was between SPAD and TN ($P < 0.01$). According to this analysis, tomato fruit yield was remarkably and positively related to soil properties except for AN in soil (SOC: $r = 0.63$, $P < 0.01$; TN: $r = 0.66$, $P < 0.01$). The SAR was significantly and positively correlated with soil pH and AN. Correspondingly, the AsA and soluble protein contents in tomato fruit were positively correlated with most soil properties. The nitrate content of tomato fruits was negatively correlated with pH, AN, and urease and protease activities in soil. Therefore, the growth, yield, and fruit quality of tomato were related to most soil properties affected by the fertilization treatments.



	pH	SOC	AN	TN	Urease	Sucrase	Protease	NR
Plant height	0.4*	0.64**	0.1	0.75**	0.39*	0.42*	0.51**	0.52**
Stem diameter	0.45**	0.61**	0.13	0.79**	0.52**	0.42*	0.59**	0.66**
SPAD	0.44*	0.83**	0.28	0.85**	0.64**	0.22	0.63**	0.72**
Photosynthetic rate	0.50**	0.70**	0.33	0.59**	0.62**	0.41*	0.61**	0.70**
Yield	0.43*	0.63**	0.22	0.66**	0.56**	0.53**	0.41*	0.56**
SAR	0.45**	0.085	0.66**	0.01	0.32	0.11	0.31	0.16
AsA	0.63**	0.70**	0.52**	0.59**	0.75**	0.41*	0.74**	0.75**
Soluble protein	0.46**	0.84**	0.29	0.82**	0.63**	0.19	0.57**	0.71**
Nitrate	-0.56**	-0.18	-0.70**	-0.12	-0.46**	-0.15	-0.4*	-0.25

Fig. 4.3 Spearman correlations between soil indicators and tomato growth and yield/quality under different fertilization types. Red and blue circles represent positive and negative correlations, respectively. *, $p < 0.05$ and **, $p < 0.01$.

4.4 Discussion

Application of digestate could therefore further enhance the sustainable development of agriculture. Fertilizers are reported to increase crop yields by changing soil chemical properties, such as soil pH, and the contents of TN and other nutrients (Mahmoud et al. 2009; Qaswar et al. 2020). Organic fertilizers could neutralize the pH in soil, as found in this study and another study (Bolzonella et al. 2018). Organic fertilizers normally contain large amounts of organic matter, and their application provides many carbon sources for SOC improvement, thereby promoting soil humification (Tsiknia et al. 2014). SOC is one of the most relevant parameters to reflect soil quality and fertility (Wu et al. 2022). In the current study, SOC was enhanced by the addition of digestate, consistent with previous findings (Qaswar et al. 2020; Ye et al. 2020). A higher SOC content could increase nutrient retention capacity of soil and facilitate nutrient availability to plant. Nitrogen is a key nutrient for plant growth in agricultural production. The application of digestate resulted in a significant increase in soil carbon and nitrogen contents, and these are key indicators of soil quality (Rice et al. 1996). Another study reported that, compared with unfertilized, all fertilizer treatments increased the nitrogen content in soil (Yang et al. 2015). Similar results were obtained under greenhouse conditions, where the highest soil TN was in the digestate treatments. Thus, the addition of digestate resulted in satisfactory soil pH and soil carbon and TN for tomato plant growth, resulting in better yield and fruit quality. In addition, our study showed that the soil TN was not significantly different between the chemical fertilizer treatment and CK under field conditions, similar to the results of another study (Wu et al. 2022). In this study, it was detected differences between sandy soil and loamy soil with respect to the trends in soil TN among the various treatments, consistent with the findings of another study (Cristina et al. 2020). These differences are likely related to differences in the properties of sandy soil (field) and loamy soil (greenhouse).

Enzymes are important participants in soil nutrient cycling, and plant growth is closely related to soil enzyme activity (Ren et al. 2021). It was observed that digestate application resulted in increased values for soil chemical properties and soil enzyme activities. Chemical fertilizers have been reported to reduce the activity of hydrolases associated with carbon and nitrogen (Zhu et al. 2019). However, the opposite trend was observed in this study, namely, increased activities of urease, sucrase, protease, and nitrate reductase in all treatments. The fact that the highest soil urease activities were in the digestate treatments is consistent with the findings of other studies in which microbial urease production increased under high soil nitrogen status (Dai et al. 2021; Tsiknia et al. 2014; Zhu et al. 2013). Similar to the results of another study (Zhu J. et al. 2019), the highest sucrase activity and the second-highest protease activity were observed in plants in the organic fertilizer treatments. These results indicate that application of digestate could promote carbohydrate transformation and sucrose hydrolyzation, thus increasing crop yield (Zhang et al. 2018). An increase in the NR activity is correlated with improvement in the potential for nitrate reduction, which provides a stronger capacity for protein synthesis or nitrogen assimilation (Piotrowska et al. 2021), leading to better nitrogen utilization by the plant.

Many studies have focused on the ability of organic fertilizers to improve crop quality or yield without linking these changes to soil chemical properties and enzymatic activities (Asadollahi et al. 2022; Li et al. 2022; Zhang et al. 2020). Even less attention has been paid to the the impact of digestate application on these parameters. However, soil properties reflect soil fertility and are essential indicators of crop growth and yield formation (Du et al. 2020). The growth, yield, and fruit quality of tomato were significantly correlated with most soil properties in the present study. Treatments consisting solely of digestate boosted fruit quality and resulted in the highest SOC but not the highest tomato yield, in agreement with previous studies (Brunetti et al. 2019; Wang et al. 2017). It has been reported that an increased carbon content in soil can improve tomato fruit quality (Jindo et al. 2016). In this study, parameters such as soluble protein and AsA contents in tomato fruits were significantly and positively correlated with SOC, indicating that fruit quality could be improved by the application of digestate. Compared with chemical fertilizers, organic fertilizers have been shown to enhance SOC and soil fertility, resulting in increased plant height, stem diameter, SPAD value, and yield (Wang et al. 2017). In this study, it was also detected significant positive relationship between most growth and fruit quality of tomato and soil nitrogen status. These findings, which agree with those of Barzee et al. (2019), confirm the superior performance of digestate compared with chemical fertilizer, possibly because of the higher soil TN after application of digestate. Other studies have shown that the massive increase in soil microbial biomass after the application of organic fertilizers can lead to improvements in crop growth, yield, and quality (Brunetti et al. 2019; Yang et al. 2015; Zhu et al. 2019). It was did not be detected a significant relationship between soil microbial biomass and tomato growth, yield, and fruit quality. However, it did be observed that the growth, yield and fruit quality of tomato were positively associated with most of the soil enzyme activities determined in the current study. Therefore, the results could conclude that digestate enhanced tomato growth, yield, and fruit quality by altering soil properties.

Although these findings suggest that digestate combined with chemical fertilizer enhances tomato growth and yield and improves the soil nutrient status. However, it was not significantly different from the digestate treatment, even the similar or lower fruit quality than in digestate treatment. Therefore, the optimum ratio of digestate to chemical fertilizer for tomato production remains to be determined. Future research should include a more in-depth analysis of the aspects addressed in the current study and determine the optimum ratio of organic to inorganic fertilizer.

Chapter 5. Comparative effects of chemical fertilizer and digestate on growth, antioxidant system, and physiology of lettuce under salt stress

5.1 Introduction

Lettuce (Lactuca sativa L.) is among the most economically significant leafy vegetable crops cultivated worldwide. Lettuce is rich in several nutritive and healthful compounds, such as minerals, protein, and carbohydrates (Pérez-López et al. 2015), and provides a pleasant flavor to salads and other dishes. In addition, lettuce contains various antioxidants, such as ascorbic acid (AsA), phenols, and carotenoids, and thus its regular consumption can improve antioxidant status and prevent cardiovascular disease (Nicolle et al. 2004). However, lettuce plants are sensitive to moderate salinity (Andriolo et al. 2005; Neocleous et al. 2014).

Anaerobic digestate is proposed to be a cleaner and more efficient organic fertilizer for agricultural use. Digestate can be sustainably used as a substitute to mineral fertilizer (Plaimart et al. 2021). After anaerobic digestion, some nutrients from the feedstock remain in the digestate, such as potassium, nitrogen and phosphorus (Bolzonella et al. 2018). In addition, the digestate is enriched in microelements (such as boron, copper, manganese and zinc), which are vital for plant growth but are not usually incorporated into the majority mineral fertilizers (Wang et al. 2019; Ivanchenko et al. 2021). Digestate is also considered to be an environmentally friendly soil amendment for management of salinization (Hamid et al. 2021). Bioactive substances, such as saccharides, vitamins, organic acids, and phytohormones (e.g., indoleacetic acid and gibberellins), in digestate can promote plant growth and mitigate plant exposure to salt stress (Yu et al. 2010; Nkoa 2014; Panuccio et al. 2019). Digestate can not only provide substances of mineral nutrients but also hinders soil degradation attributed to salinity and confers several benefits simultaneously on the physical and chemical properties of the soil, such as enhanced carbon isolation and soil water-holding capacity (Jabeen et al. 2017; Cristina et al. 2020).

Salt stress severely limits horticultural productivity and plant growth. Salinity is a severe impediment to agricultural production worldwide (Haddadi et al. 2016). Currently, salinization affects approximately 74% of agricultural land worldwide and over-salinization affects more than 397 million ha of soil worldwide (Gong et al. 2013; Orosco-Alcalá et al. 2021). Salt stress adversely affects many metabolic processes in plants and leads to a reduction in biomass accumulation. Salt stress also induces hyperionic and hyperosmotic effects, resulting in elevated accumulation of reactive oxygen species (ROS) (Elsawy et al. 2018). Plants possess a complex defensive system of antioxidants, including antioxidative defense enzymes and non-enzymatic antioxidant substances, to alleviate the impacts of ROS. Antioxidative defense enzymes (e.g., catalase [CAT], ascorbate peroxidase [APX], peroxidase [POD], and superoxide dismutase [SOD]) and non-enzymatic antioxidant substances (e.g., AsA, glutathione [GSH], carotenoids, and phenolics including flavonoids and tocopherols) in plants are efficient scavengers of ROS and suppressants of lipid peroxidation, and hence are extremely relevant in relieving salt stress (Arora et al. 2020).

A variety of methods in plants are effective to reduce salt stress, such as application of organic and mineral amendments, phytohormones, nano-based products, etc. Application of organic matter in digestate can improve the cation exchange capacity and fertility of saline soils (Saqib et al. 2017). Most studies involving digestate application (DA) have been conducted on non-salinity-affected soils (Lee et al. 2018; Cheong et al. 2020). However, the few studies of DA to saline soils suggested that digestate may have a favorable impact on plant growth and the physicochemical characteristics of these problematic soils (Jabeen et al. 2017; Saqib et al. 2017; Hamid et al. 2021). The interaction between DA and the antioxidative defense system is not well understood. However, DA may alleviate the detrimental impacts of salt stress on plants on account of the high organic matter content in digestate and promotion of salt uptake capacity (Nkoa 2014; Panuccio et al. 2019; Hamid et al. 2021).

The usage of digestate as a fertilizer has received increased attention recently (Cheong et al. 2020). Little literature is available regarding the effect of digestate amendment on the antioxidant system of plants under salt stress. In previous studies, the influence of digestate as a soil fertilizer was studied. However, the comparative salt stress response of lettuce to mineral fertilizer and digestate remains unknown. In the present experiment, the effect of DA was investigated under different NaCl concentrations in edible parts of lettuce. The objective was to investigate the response to application of two fertilizer types (mineral fertilizer and digestate) on lettuce grown in non-saline and saline soil by analyzing the growth and antioxidative defense system. In addition, it was explored the differences in water-relation indices, photosynthesis, oxidative stress, and lipid peroxidation among the treatments.

5.2 Materials and Methods

5.2.1 Digestate acquisition

Digestate was collected as showing 2.2.1.

5.2.2 Plant materials and experimental design

Fig. 5.1 Experimental environment used in the study. (a) Anaerobic digester; (b) transplanted lettuce in pots in the summer of 2021

Lettuce ‘Grand Rapids’ seeds were purchased from the Sakata Seed Corporation Ltd. (Yokohama, Japan). The experiment was conducted in the summer of 2021 (22 May to 13 July; Fig. 1b) under natural conditions in a plastic-film greenhouse on the campus of Hokkaido University (43°4′ N, 141°20′ E; 20 m above sea level). The seeds were washed and soaked in warm water at 55°C for 5 min and then wrapped in wet gauze to accelerate germination for 48 h at 25°C. After germination, the seeds were sown in a seeding tray with 60 cells and two seeds per cell on 24 May. Commercial nutrition soils were used as seedling medium and purchased from the Iris Ohyama Corporation Ltd. (Sendai, Japan). Uniform healthy seedlings were selected at 20 days after sowing (DAS) and randomly transplanted into 3-L commercial plastic pots (diameter 18 cm, height 16 cm).

Table 5.1 Partial elemental profile of mineral fertilizer and digestate in the quantity applied during this study

Treatment	N (mM)	P (mM)	K (mM)	Ca (mM)
CFA	143	116	62	15
DA	143	13	100	20

CFA: application of chemical fertilizer; DA: application of digestate.

A pot experiment was performed using a completely randomized factorial design with four replications (four pots per replication). Ninety-six 3-L pots were each filled with soil with a field water-holding capacity of 22.94%. The field capacity of the soil was measured using the descriptive method of Mehdizadeh et al. (2020). The experimental treatments comprised two types of fertilizer (mineral fertilizer and digestate) and three salinities (0, 3.0, and 7.5 dS m^{-1}). Mineral fertilizer (NPK 10-18-12) was purchased from the Hokuren Fertilizer Co., Ltd. (Sapporo, Japan). Characterization of the digestate is summarized in Table 5.2. For each fertilizer application as a soil drench, either 100 mL digestate or 2 g of mineral fertilizer dissolved in 100 mL pure water was applied to the respective pots with a same nitrogen dosage (Table 5.1). After the final irrigation session, the fertilizer treatments were applied in the late afternoon at 20 and 36 DAS. An application of 100 mL was less than the volume pre-determined gravimetrically to be the soil saturation volume of a 3-L pot. Three salinities were applied by supplying NaCl in the irrigation water. Over the entire experimental period, irrigation was provided to maintain the soil water content at field capacity.

All plants were irrigated with tap water at 17:00–19:00 daily before 25 DAS. The salinity treatments were applied from 26 to 50 DAS. Each pot was weighed daily to confirm the plant water status and to guide the irrigation volume required following the method of Liu et al. (2021). The difference in weight between the day before around 18:00 and the day around 18:00 was recorded as the irrigation volume per day during the salinity treatment period. In addition, throughout the experimental period, on each day a known volume of water was placed in a small evaporator comprising a round metal basin (diameter 20 m, height 10 cm) and, after 24 h, the remaining water volume was measured with a measuring cup. The reduction in water volume was recorded as the daily evaporation. The daily evaporation and temperature during the growth period are shown in Fig. 5.2. All plants were harvested at 50 DAS.5.2.3 Sampling and analytical methods

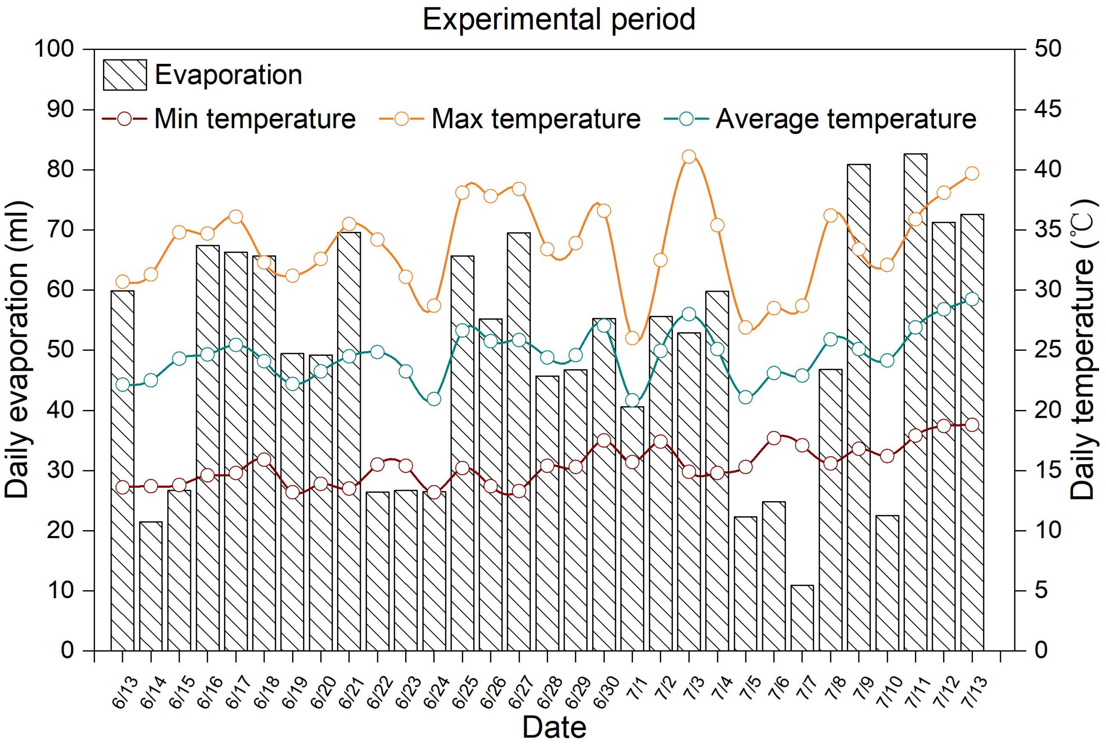


Fig. 5.2 Temperature and daily evaporation during the experimental period

5.2.3 Sampling and analytical methods

5.2.3.1 Growth parameters

At 50 DAS, the photosynthetic rate (P_n) was measured from 10:00 to 11:00 using a mini plant photosynthesis meter (miniPPM-300, EARS, Delft, The Netherlands) before harvest. The second, third, and fourth fully expanded leaves were selected for the photosynthesis measurements, and measurements were taken three times under about 1000 μmol m^{-2} s^{-1} PPFD illumination and 30°C average temperature on each leaf sheet. Leaves were assessed in position near the major veins, and the mean value was calculated as the P_n of each plant. Three plants were selected randomly per treatment for measurement.

After harvest, the edible parts of six plants per treatment were excised. The edible parts of three plants were immediately weighed to record the fresh weight (FW) using an electronic scale (XS204V, Mettler Toledo, Greifensee, Switzerland). To measure the mean dry weight (DW), these three plant parts were dried at 75°C to a constant weight in a drying oven (WFO-420W, EYELA, Tokyo Rikakikai Co., Ltd., Tokyo, Japan). The other three plants were used to measure physiological indices.

5.2.3.2 Water use efficiency and relative water content

The water use efficiency (WUE; g L^{-1}) was determined following Barbieri et al. (2012). The relative water content (RWC; %) was determined using the method of Galmés et al. (2007).

5.2.3.3 Photosynthetic pigment contents

Chlorophyll (Chl), comprising Chl *a* and Chl *b*, and carotenoid contents were measured using the methods of Lichtenthaler (1987) and Popescu et al. (2017). These were extracted from fresh leaf tissues with 95% ethanol and magnesium oxide. The absorbance was calculated with a spectrophotometer (UV/VIS-560, Jasco Co., Tokyo, Japan) at 665, 646, and 470 nm.

5.2.3.4 Superoxide anion radical, lipid peroxidation, and membrane stability index

Superoxide anion radical ($O^{2\bullet-}$) concentration was measured using the described method of Rauckman et al. (1979). Malondialdehyde (MDA) content was determined in accordance with the method of Li (2000). The membrane stability index (MSI; %) was measured in accordance with the procedure of Sairam et al. (1997).

5.2.3.5 Antioxidant enzyme activity

For enzyme extraction, frozen samples in edible parts of lettuce were homogenized with 100 mM phosphate buffer (pH 7.0) containing 1% PVPP (w/v) at 4°C and centrifuged for 10 min at 15,000 × g. The extract solution was used to measure enzyme activities.

The SOD activity was measured at 560 nm sustained to prevent photoreduction of nitro blue tetrazolium (NBT) by about 50% following the method of Li (2000). The CAT activity was determined as the decrease in absorbance at 240 nm following the method of Aebi (1984) due to the decline of extraction of H2O2. The POD activity was measured as the increase in absorbance at 470 nm following the method of Urbanek et al. (1991) due to the guaiacol oxidation. The APX activity was measured following the method of Nakano et al. (1981), which depends on the decrease in absorbance at 290 nm as ascorbate was oxidized.

5.2.3.6 Non-enzymatic antioxidant components

The AsA content was determined using the molybdenum blue colorimetric method (Bajaj and Kaur 1981). The GSH content was determined following the method of Li (2000). The total phenolic content (TPC) was determined using the Folin–Ciocalteu method in accordance with the procedure described by Rajapaksha and Shimizu (2020).

5.2.4 Statistical analysis

The values are presented as the mean ± SE of three biological replicates (n = 3). The data were subjected to two-way ANOVAs using SPSS 25.0. In addition, one-way ANOVAs were performed followed by Duncan's post-hoc tests at $P < 0.05$ to assess the significance of differences among the means. All figures were generated with OriginPro 2021. Means (± SE, n = 3) with the same letter in the same cultivation condition are not significantly different from each other ($P < 0.05$) in all figures and tables.

5.3 Results

5.3.1 Plant growth

Plant FW and DW, under both CFA and DA treatments, decreased with increasing NaCl concentration (Fig. 5.3). Under the non-saline condition, the FW and DW of edible parts of lettuces were higher under CFA than under DA. In contrast, DA markedly increased FW (42%) and DW (27%) compared with CFA under the 7.5 dS m^{-1} NaCl treatment. Fertilizer treatment did not significantly affect ($P > 0.05$) the FW or DW of the edible parts of lettuce plants under the 3 dS m^{-1} NaCl treatment.

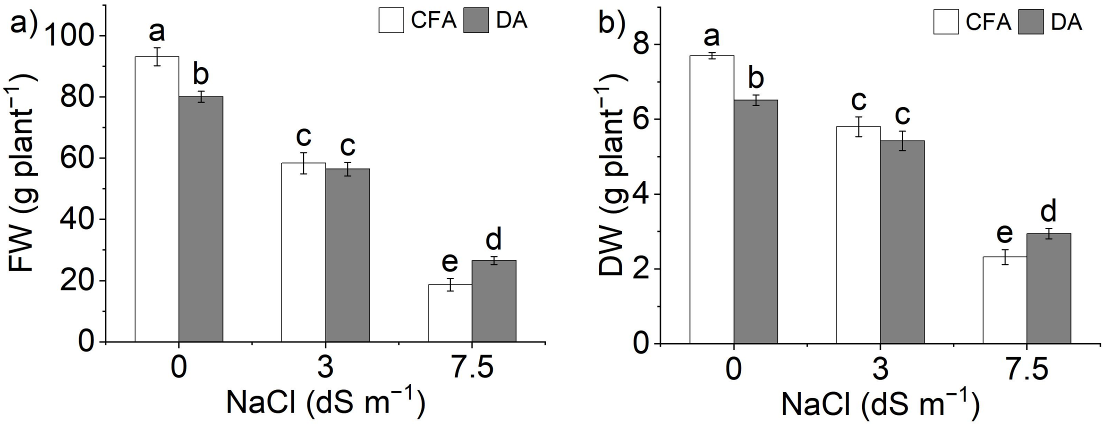


Fig. 5.3 Fresh weight and dry weight in response to application of two fertilizers as affected by salt stress in lettuce plants.

5.3.2 Water-relation indices

Water relations were affected by salt stress during plant development (Fig. 5.4). The WUEFW, WUEDW, and RWC in edible parts of lettuce plants decreased with increasing salinity; however, DA-treated plants were less severely affected than CFA-treated plants by salt stress ($P < 0.05$; Fig. 5.4a–c). Specifically, the WUEFW, WUEDW, and RWC in edible parts of the plants were reduced by 29%, 15%, 13% and 17%, 2%, 9% ($p < 0.05$) under 3 dS m−1 NaCl treatment compared with levels under the non-saline condition under CFA and DA, respectively. Under the 7.5 dS m^{-1} NaCl treatment, these indices declined more substantially. Inconsistent with the reduction in WUE, the total water use was significantly higher ($P < 0.05$) throughout the growth period in CFA-treated plants compared with in DA-treated plants under the same salinity (Fig. 5.4d). Furthermore, with increasing duration of salt exposure, DA markedly reduced the volume of water consumption compared with CFA, and the water consumption declined with increasing salinity under both CFA and DA treatments (Fig. 5.4e).

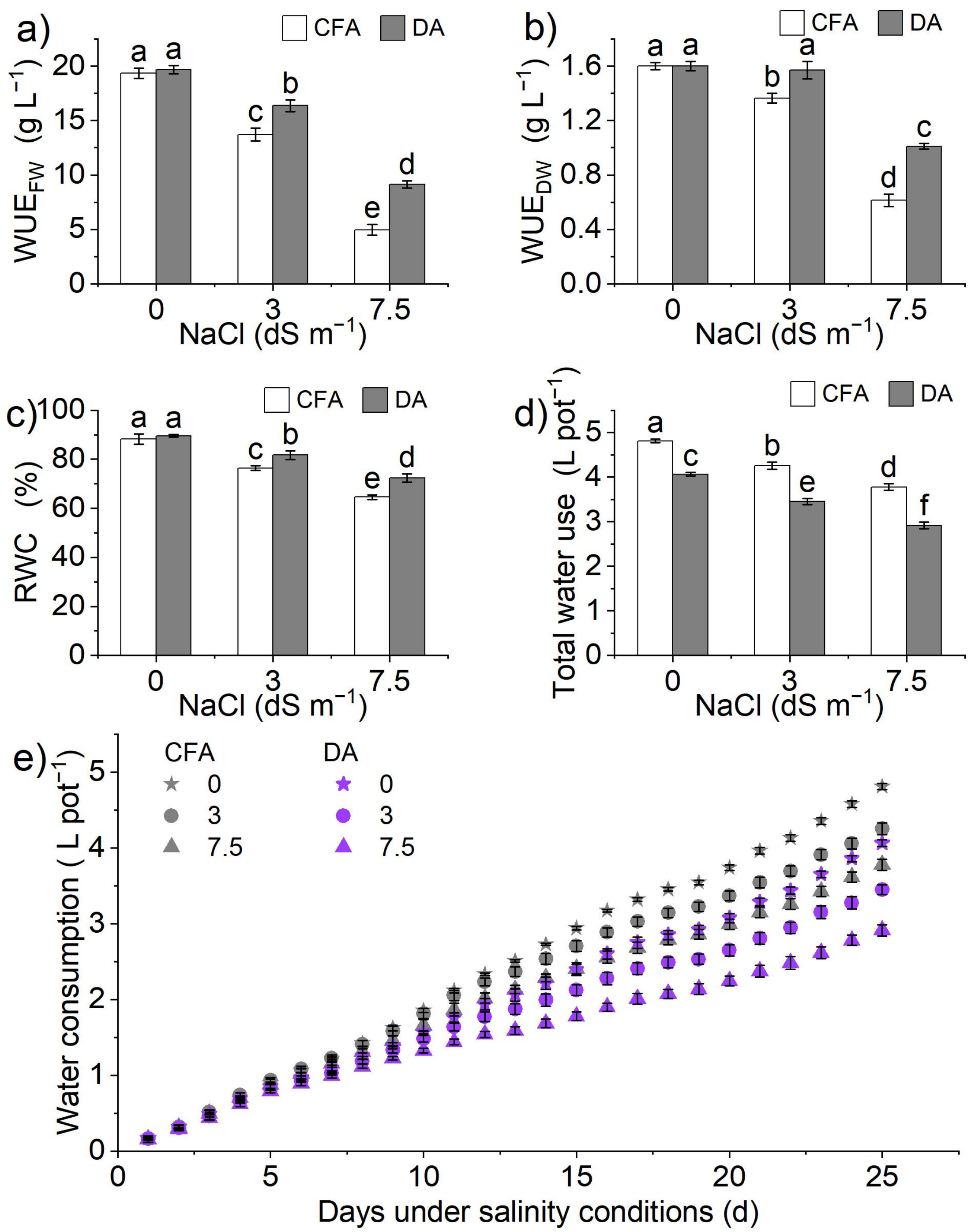


Fig. 5.4 Fresh-weight water use efficiency, dry-weight water use efficiency , relative water content, total water use, and water consumption in response to two fertilizers as affected by salt stress in lettuce during the salinity treatment period.

5.3.3 Photosynthetic pigment contents and Pn

Salt stress reduced the concentrations of photosynthetic pigments and Pn (Fig. 5.5). Compared with CFA treatment, lower contents of Chl a and Chl b were observed under DA treatment in the non-saline condition (Fig. 5.5a,b). However, these contents were 10% and 11% higher ($P < 0.05$), respectively, in the DA treatment compared with in the CFA treatment under 7.5 dS m^{-1} NaCl. The Chl a content in DA-treated plants was elevated visibly ($P < 0.05$), whereas the Chl b content showed no significant difference, under the 3 dS m^{-1} NaCl treatment compared with that of CFA-treated plants. The carotenoids content in DA-treated plants was considerably higher compared with that of CFA-treated plants (Fig. 5.5c), increasing by 1%, 7%, and 20% under 0, 3, and 7.5 dS m^{-1} NaCl, respectively. The Pn decreased with increasing NaCl concentration (Fig. 5.5d). In addition, Pn differed significantly ($P < 0.05$) between fertilizer treatments only under the 7.5 dS m^{-1} NaCl concentration; at this concentration, Pn was higher (20%) under DA than under CFA.

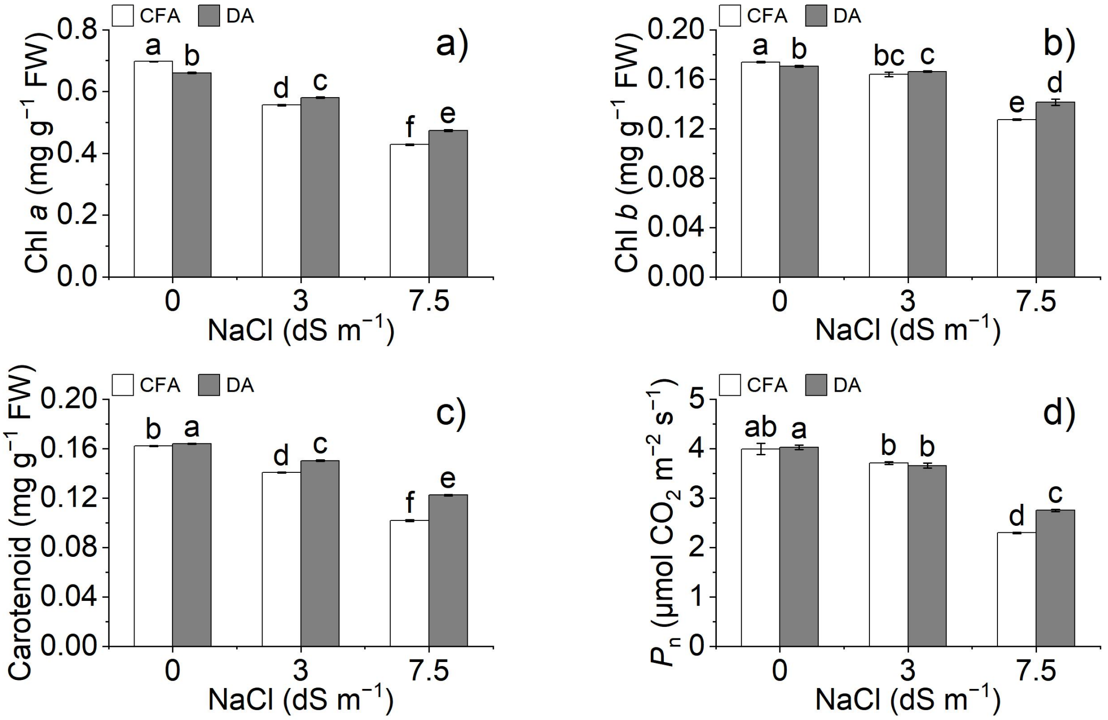


Fig. 5.5 Contents of chlorophyll a, chlorophyll b, and carotenoids, and net photosynthetic rate in response to two fertilizers as affected by salt stress in lettuce.

5.3.4 $O_2^{\bullet-}$ concentration, MDA content, and MSI

Salt stress conditions increased $O_2^{\bullet-}$ and MDA concentrations compared with the corresponding non-saline condition. In this study, under the 3 dS m^{-1} NaCl concentration, the increase in concentrations of $O_2^{\bullet-}$ and MDA was 57.2% and 27.6% in CFA- and DA-treated plants, respectively, resulting in a 6% decrease in the MSI, compared with the corresponding non-saline condition (Fig. 5.6). Under exposure to 7.5 dS m^{-1} NaCl, $O_2^{\bullet-}$ and MDA increased further and MSI decreased further under both CFA and DA treatments. However, the $O_2^{\bullet-}$ and MDA concentrations were higher and the MSI was lower in CFA-treated plants compared with in DA-treated plants at the same salinity.

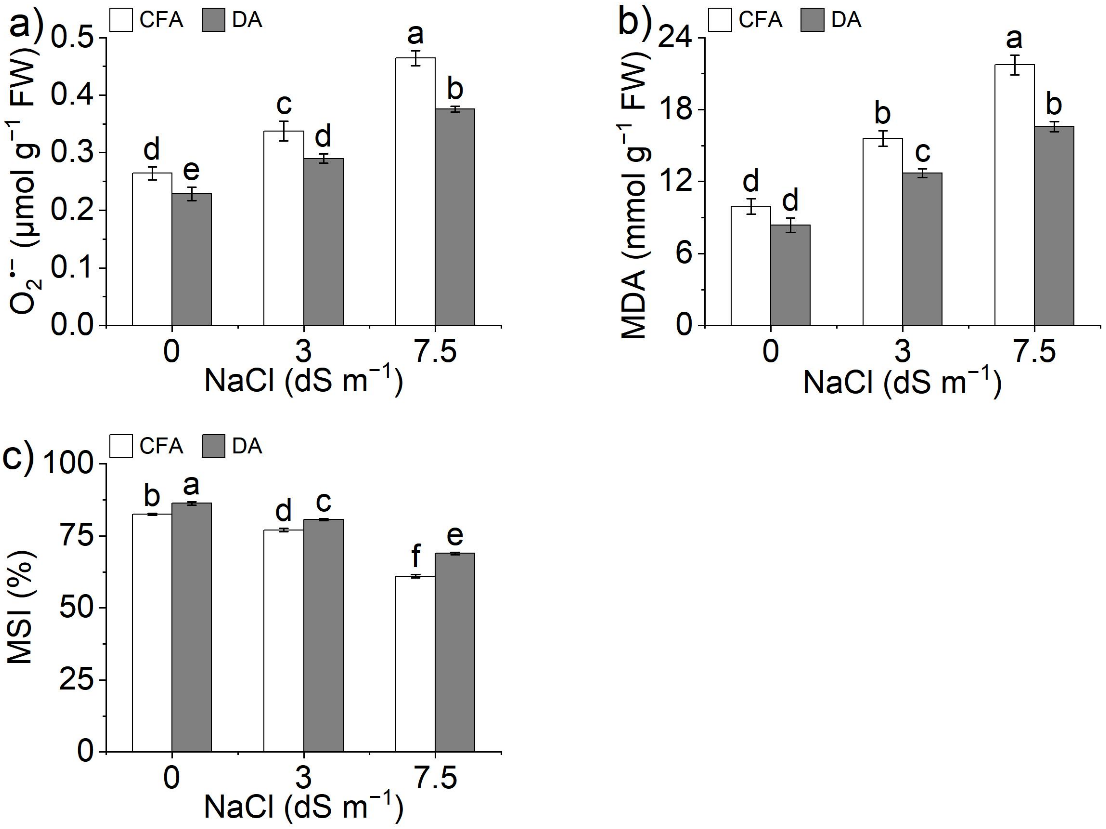


Fig. 5.6 Contents of superoxide anion radical, malondialdehyde, and membrane stability index in response to two fertilizers as affected by salt stress in lettuce.

5.3.5 Activities of antioxidant enzymes

The activities of SOD, CAT, POD, and APX in the edible parts of lettuces were markedly elevated under both fertilizer treatments under salt stress (Fig. 5.7). In addition, DA treatment increased the activities of antioxidant enzymes compared with the CFA treatment under the same salinity. The activities of SOD and CAT in the non-saline condition were 36% and 74% ($P < 0.05$) higher in DA-treated plants than in CFA-treated plants (Fig. 5.7a,b). In contrast, the activities of POD and APX were not significantly different ($P > 0.05$) between the two fertilizer sources under the non-saline condition (Fig. 5.7c,d).

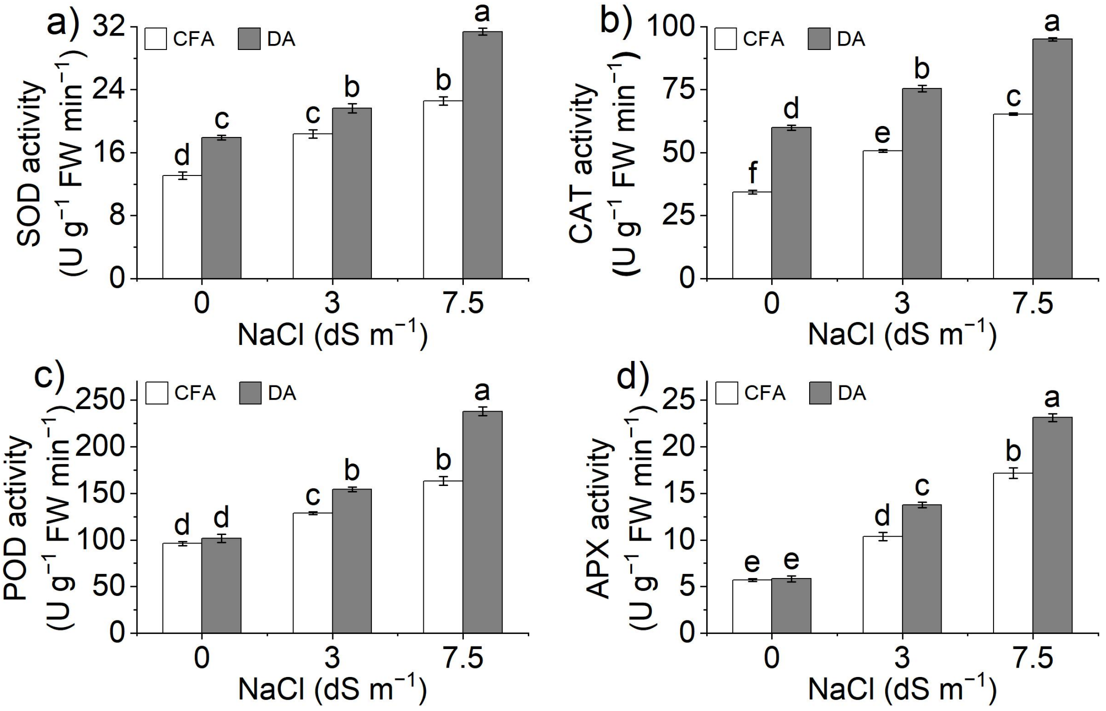


Fig. 5.7 Activity of superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase in response to two fertilizers as affected by salt stress in lettuce.

5.3.6 Non-enzymatic antioxidants

Treatment with DA significantly increased ($P < 0.05$) the AsA, GSH, and TPC contents compared with those of CFA-treated plants under the same salinity (Fig. 5.8). Compared with the CFA treatment, DA treatment increased accumulation of these antioxidants by approximately 11%, 10%, and 27% for AsA, 24%, 38%, and 11% for GSH, and 20%, 10%, and 16% for TPC under 0, 3, and 7.5 dS m^{-1} NaCl, respectively. The GSH and TPC contents increased with increasing salinity (Fig. 5.8b,c). In contrast, AsA content increased from the 0 to 3 dS m^{-1} NaCl treatment but decreased from the 3 to 7.5 dS m^{-1} NaCl treatment under both CFA and DA (Fig. 5.8c).

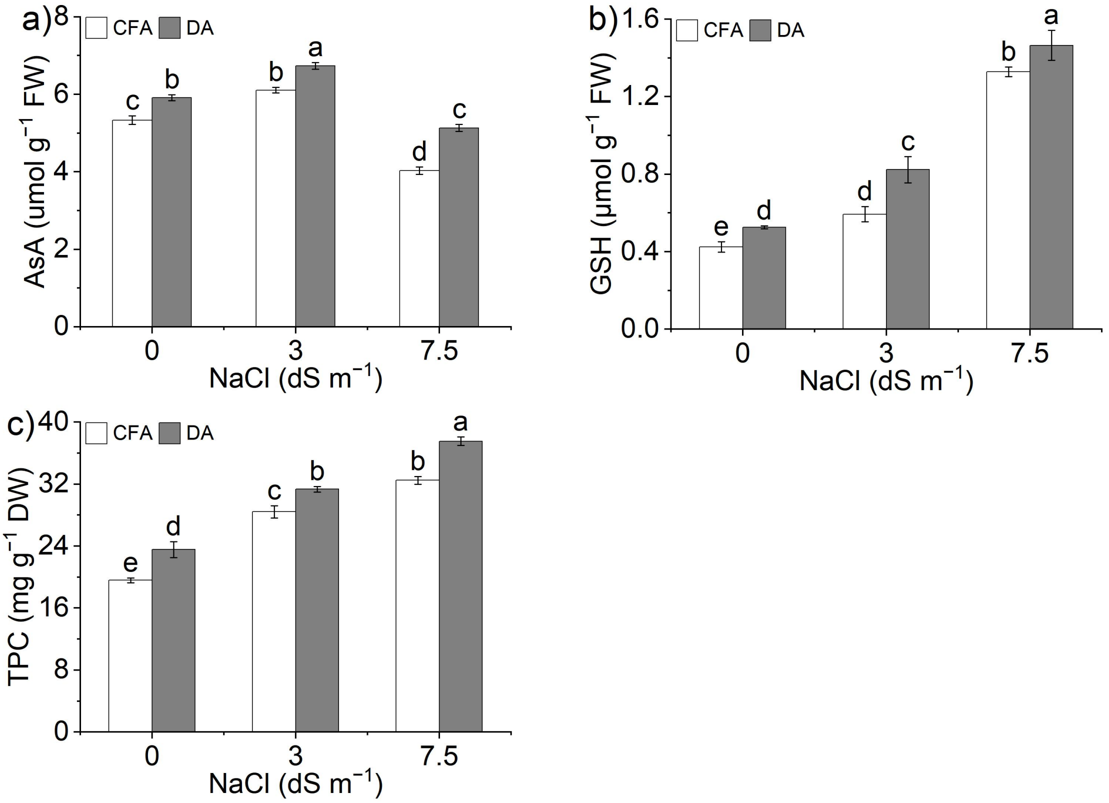


Fig. 5.8 Ascorbic acid, glutathione, and total phenolic content in response to two fertilizers as affected by salt stress in lettuce.

5.4 Discussion

Soil salinity represents a major challenge for agricultural production in all climates worldwide (Evelin et al. 2019). The current study evaluated differences in the edible parts of lettuce in response to salt stress between CFA and DA treatments. The results revealed that salt stress had a detrimental effect on accumulation of biomass in lettuce. The studies of Pérez-López et al. (2015) and Shams et al. (2016) demonstrated similar results. Biomass is an optimal plant index for evaluation of various stresses, and its accumulation indicates the life-sustaining activities of plants. In the present study, biomass accumulation under DA was obviously lower than that observed under CFA in the non-saline condition. Similar results have been reported using digestate originating from poultry manure and food waste as a replacement for mineral fertilizer in leafy vegetables (Wang et al. 2019; Cheong et al. 2020). Digestate may be an unbalanced fertilizer because the lack of phosphorus in the digestate limited the growth of lettuce. Li et al. (2016) also reported a deficiency of phosphorus in digestate applied as fertilizer. In addition, comparisons of DA and CFA based on an equivalent nitrogen dose have shown that DA provides lower fertilizer nitrogen amounts than mineral fertilizers (Quakernack et al. 2012). It was notable that, especially under the 7.5 dS m^{-1} NaCl concentration, the FW and DW of DA-treated plants were markedly greater than those of CFA-treated plants. The reduced biomass under the 3 dS m^{-1} NaCl concentration was ameliorated by DA because the element imbalance in the digestate limited plant growth. The FW and DW biomasses under the 3 dS m^{-1} NaCl concentration were inconsistent with those under the non-saline condition. The DA treatment ameliorated the negative consequences of salt stress by promoting biomass accumulation under the different salinities; DA under 7.5 dS m^{-1} NaCl achieved superior performance compared with CFA in this regard. Hamid et al. (2021) reported that DA enhanced the K^+/Na^+ ratio in sunflower, improved plant growth, and declined the harmful effects of salinity in saline soil (EC $\leq$ 8 dS m^{-1}).

Water uptake by plants is lowered due to high soil salinity concentration (Phogat et al. 2018). Consistent with this phenomenon, the total water use was lower during the growth period under saline conditions. Saline irrigation water negatively affects plant growth due to lower soil osmotic potential, leading to lower RWC and plant dehydration (Maggio et al. 2004; Larbi et al. 2020). The current results demonstrated that DA improved the WUE of FW and DW biomass (WUEFW and WUEDW) under salt stress compared with those under CFA, including at 3 and 7.5 dS m^{-1} NaCl concentrations. WUE decreased with increasing salt concentration. However, different crops have different salinity thresholds. The WUE of bell pepper under saline conditions (EC $\leq$ 8 dS m^{-1}) (Orosco-Alcalá et al. 2021) is similar to that of lettuce recorded in the current work. In contrast, wheat WUE was higher under a saline condition (EC $\leq$ 8 dS m^{-1}) than under a non-saline condition (Khataar et al. 2018), which contrasted with the present results for lettuce under salt stress (EC $\leq$ 8 dS m^{-1}), because the salinity threshold of wheat is about 8 dS m^{-1} and wheat WUE declined with increasing salt concentration (EC $\geq$ 8 dS m^{-1}). A reduction in RWC denotes loss of turgor with a limited supply of water for cell expansion. In the current study, salinity decreased the RWC in edible parts of lettuce plants under both CFA and DA.

Kaya et al. (2007) reported a reduction in RWC in salt-stressed melon plants compared with in unstressed melon plants. The decrease in RWC under DA was significantly greater than that under CFA at the 3 and 7.5 dS m^{-1} NaCl concentrations. Therefore, DA was associated with a superior water retention in soil. Similar to most organic fertilizers, digestate has a high organic matter content and improves plant water relations during growth (Jabeen et al. 2017). In addition, DA resulted in relatively lower integrated total water use than CFA in the growth period when the field capacity was used to determine the irrigation requirement. This may be attributed to the improvement in soil properties resulting from the micronutrients and high organic matter content in digestate (Cristina et al. 2019). In short, the results of the current study support the notion that DA improves the water relations of lettuce under salt stress. Hamid et al. (2021) reported that water use and salt stress tolerance of plants might be increased by DA. The present results corroborate this suggestion.

Reduction in photosynthetic pigment contents of salt-stressed plants inhibits chlorophyll synthesis and activity of enzymes implicated in carbohydrate metabolism (Castañares and Bouzo 2019). Chlorophyll is the most pivotal pigment for photosynthesis, hence a decrease in chlorophyll content will decrease photosynthesis. The current study indicated that DA suppressed chlorophyll degradation under 7.5 dS m^{-1} NaCl. Decreased plant biomass under salt stress is often a direct result of photosynthesis inhibition (Gong et al. 2013). The leaf Pn was lowest in CFA-treated lettuce under the 7.5 dS m^{-1} NaCl concentration, consistent with the lowest biomass. Under 3 dS m^{-1} NaCl, some aspects of chlorophyll synthesis were promoted by DA compared with CFA, such as Chl a accumulation. Application of certain mineral nutrients, such as calcium, magnesium, zinc, and manganese, can promote chlorophyll synthesis and alleviate salt stress (Bohn et al. 2004; Li et al. 2017; Nadeem et al. 2020). In the current study, calcium and microelement concentrations in lettuce were not measured under DA and CFA, but DA provided higher calcium and potassium concentrations than CFA at the same nitrogen dose. A variety of microelements are present in digestate (Nkoa 2014; Wang et al. 2019; Ivanchenko et al. 2021). In addition, the results of Wang et al. (2019) indicated that the calcium content in lettuce leaves under DA treatments were significantly higher than those under CFA. Larbi et al. (2020) reported similar findings in which supplementary potassium and calcium mitigated salt stress. Therefore, DA improved chlorophyll synthesis in lettuce compared with CFA, which may reflect that digestate provides certain amounts of calcium and micronutrients.

Cell membrane damage is among the first subcellular impacts of salt stress. The loss of cellular membrane integrity and stability is caused by lipid peroxidation (Castañares and Bouzo 2019). In the current study, the MSI was higher under DA compared with under CFA in the same salinity. This response likely strengthened the cellular membranes by reducing water deficiency and decreasing ion leakage (Tabaei et al. 2000). Presumably, this reinforcement of cellular membrane stability aids in absorption of essential minerals and hence improved plant growth. Salt stress can cause enhanced lipid peroxidation (Meloni et al. 2003), and MDA is a result of oxidative stress as the final product of lipid peroxidation. Inhibition of oxidative stress can be prevented by reducing MDA levels (Koca et al. 2007). In the current results, MDA contents in the edible parts of lettuce plants were lower under DA compared with under CFA in the non-saline condition and salt stress conditions, indicating that DA inhibited membrane damage. These findings are concordant with the result of Ali et al. (2019). Damage to membrane lipids is usually provoked by oxidants, including $O_2^{\bullet-}$ (Elsawy et al. 2018), which explains why $O_2^{\bullet-}$ was observed in all lettuce plants . $O_2^{\bullet-}$ accumulation was substantially higher in leaves of CFA-treated lettuce than in those of DA-treated plants under the same salinity. The high $O_2^{\bullet-}$ concentration in lettuce tissue might explain the higher MDA content and lower MSI observed in the CFA treatment compared with in the DA treatment.

To relieve oxidative stress, the ROS-scavenging system in plants include enzymes and non-enzymatic antioxidant components. Antioxidant enzymes (e.g., SOD, CAT, POD, and APX) have an efficient scavenging effect on ROS. Enhanced activities of these enzymes are commonly associated with decreased oxidative damage; thus, these enzymes could mitigate salt stress (Barbieri et al. 2012). Antioxidant enzymes in plants frequently operate in concert to achieve ROS detoxification. The action of SOD is enabled to scavenge $O_2^{\bullet-}$, which is the most abundant component of ROS (Elsawy et al. 2018). Hence, SOD represents the main line of defense to remove ROS. Subsequently, the H_2O_2 generated by dismutation of $O_2^{\bullet-}$ must be eliminated because its accumulation readily causes oxidative damage. At this point, other antioxidant enzymes (e.g., CAT, POD, and APX) are vital as defense against oxidative stress. These enzymes play an influential role in scavenging H_2O_2, which is produced under salt stress (Gong et al. 2013; Elsawy et al. 2018). In the current research, activities of antioxidant enzymes increased with increasing salinity under both fertilizer applications. It should be noted that SOD and CAT activities under the non-saline condition and salt stress conditions were higher with DA than with CFA, whereas fertilizer treatment did not significantly affect activities of GPX or APX under the non-saline condition. Antioxidant enzymatic activities of plants exhibit varying sensitivities and responses to digestate treatments (Aihemaiti et al. 2019). The current results confirmed that SOD and CAT are more responsive to DA than other antioxidant enzymes in lettuce. Therefore, DA can alleviate the harmful effect of biomass accumulation and lipid peroxidation in lettuce induced by salt stress, which is presumably mediated by the increase in antioxidant enzymatic activities to various degrees.

The non-enzymatic components under salt stress are also important elements of antioxidant defense systems. In the current research, the concentrations of AsA, GSH, and TPC in edible parts of lettuce were relatively higher under DA compared with under CFA. These findings are similar to the results of Panuccio et al. (2019), who reported that digestate enhances phenol and flavonoid contents in cucumber. Under salt stress, plants produce non-enzymatic antioxidant substances to scavenge ROS. A previous study (Ashraf et al. 2008) showed that the antioxidant capability of plants is directly associated with salt tolerance. In addition, a previous study of lettuce observed markedly higher radical-scavenging capability in plants containing high contents of antioxidant substances (GSH and TPC) (Shams et al. 2016). As an essential non-enzymatic scavenger of ROS, AsA effectively plays an influential role to repair the damage induced by salt stress. A previous study indicated that DA enhances the AsA content in lettuce leaves (Wang et al. 2019). In the present work, the AsA content increased initially and thereafter reduced with increasing salt concentration, but the AsA content under DA was always higher than that under CFA. This is consistent with the findings of Gong et al. (2013), who observed that GSH content rose consistently with increasing NaCl concentration, whereas AsA content increased under a low NaCl concentration and decreased under higher NaCl concentrations. The present results indicate that DA may contribute to the mitigation of oxidative damage induced by ROS by promoting the actions of antioxidant enzymes and non-enzymatic components in edible parts of lettuce.

In the present study, lettuce plants did not grow optimally under irrigation to field capacity following application of digestate instead of mineral fertilizer because biomass in the edible parts of the plants were reduced. However, DA led to superior salt tolerance compared with CFA under the salinity treatments. This study revealed that DA ameliorated most of the variables studied under NaCl stress. In particular, the amelioration was more pronounced in plants exposed to 7.5 dS m^{-1} NaCl stress. Therefore, the present results corroborated the presence of a window within which DA is potentially beneficial to lettuce for overcoming salt stress. In conclusion, we found that DA alleviated, at least during the short term, the salt stress in lettuce. This could be explained by the reduced oxidative stress and increased photosynthetic pigment contents and water relations, which were conferred by the increased activities of antioxidant enzymes and non-enzymatic substances in edible parts of lettuce plants. These results indicate that application of digestate instead of mineral fertilizer offers potential for growth of lettuce in saline soils.

Chapter 6. Conclusion and prospects

6.1 General discussion and conclusion

Organic fertilizer application is a sustainable approach to achieve high crop quality and maintain yield and soil fertility. In contrast to compost, the agronomic properties of digestate from by-products of anaerobic digestion are not well characterized. The impact of three fertilization strategies and a control on tomato yield/quality and soil properties was investigated under field and greenhouse. The results showed that the application of digestate significantly increased the growth and fruit quality of tomato including height, stem diameter, leaf chlorophyll content index, and photosynthetic rate of tomato plant and sugar-acid ratio, protein content, and ascorbic acid content of the fruit. The nitrate contents in tomato fruit were lower in the digestate treatment and digestate combined with chemical fertilizer treatment than in the chemical fertilizer. The digestate combined with chemical fertilization resulted in the greatest increase in tomato yield, up to 26.29% and 10.78% higher than that in the chemical fertilizer treatment under field and greenhouse conditions, respectively. In both cultivation environments, digestate and combined chemical fertilizer with digestate had strong effects on tomato fruit bioactive compounds and secondary metabolites, especially phenols and flavonoids.

Furthermore, the application of digestate and combined chemical fertilizer with digestate increased the levels of sugar compounds, phenolic compounds, and a small amount of organic acids in tomato fruits. It was noted that the application of digestate and combined chemical fertilizer with digestate reduced the citric acid content in fruits compared to the chemical fertilizer treatment. Moreover, the application of both organic fertilizers improved the total phenol and total flavonoid contents in tomato fruits, and their antioxidant capacity was significantly higher than that of the chemical fertilizer treatment. The application of digestate as a full or partial replacement for chemical fertilizer resulted in fruit with considerably superior bioactive compounds.

Both fertilization with digestate treatment and treatment with digestate combined with chemical fertilizers under field and greenhouse conditions can improve soil fertility. For example, digestate and digestate combined with fertilizer treatments significantly increased soil carbon content and total nitrogen content. Also, digestate substitution or partial substitution of fertilizer enhanced soil enzyme activities such as urease, sucrase, protease and nitrate reductase activities. In addition, correlation analysis revealed that growth, yield and fruit quality of tomatoes were significantly correlated with soil chemical properties and soil enzyme activities.

Salt stress in plants presents a major challenge to future agricultural production. Digestate has various effects on plant growth, but little information is available on its effects on the antioxidant system and physiological characteristics of lettuce under salt stress. In this study, the impacts of chemical fertilizer and digestate application on edible parts of lettuce were compared under three salinities. Under the 7.5 dS m^{-1} NaCl condition, digestate application increased the fresh weight (42%), dry weight (27%), photosynthesis of lettuce compared with that under chemical fertilizer application. Salt stress up-regulated the antioxidant system and digestate application further increased the enzymatic and non-enzymatic antioxidant capability compared with that under chemical fertilizer application. In addition, the total water use was lower and water-related indices were higher under digestate application compared with under chemical fertilizer application.

In short, the effects of digestate treatments to maintain a stable tomato yield and improve fruit quality may be due to the enhanced soil enzymatic activities and chemical properties. These results suggest that the use of digestate as a full or partial replacement for chemical fertilizer could improve the growth and fruit quality of tomato, maintain the yield, and reduce the use of inorganic fertilizers in tomato production. In addition, the application of digestate instead of chemical fertilizer could be a promising practice to alleviate the negative impact of salt stress on the productivity and physiological characteristics of lettuce plants.

6.2 Future Recommendations

This research trial was conducted for only one year under field and greenhouse conditions and obtained some benefits of digestate replacement or partial replacement of chemical fertilizers. However, further research is needed to see if it can be applied in continuous practical production in fields and the crop types need to be diversified.

This study used HPLC to perform a detailed biochemical analysis of different cultivation environments grown using different fertilization strategies. Future detailed studies should also be conducted using other fertilizer applications.

In this work, the mechanism of action of digestate replacement fertilizer in inducing resistance to salt stress was investigated, and the results showed that digestate has good resistance under salt stress for a short period of time. However, further studies were conducted to investigate the response of digestate application over a longer period of time and whether it has the characteristics to reduce the negative effects of stress under other adversity conditions.

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