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**Maestría en Ciencias en Recursos Naturales y Medio
Ambiente en Zonas Áridas**

**USO DE ZEOLITA Y ALGAENZIMAS PARA MITIGAR EL
ESTRÉS POR DÉFICIT HÍDRICO EN TOMATE (*Solanum
lycopersicum* L.) EN LA REGIÓN NORTE DE MÉXICO**

**USE OF ZEOLITE AND ALGAENZYMES TO MITIGATE
WATER DEFICIT STRESS IN TOMATO (*Solanum
lycopersicum* L.) IN THE NORTHERN REGION OF MEXICO**

T H E S I S

Submitted as a partial requirement to obtain the degree of:

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MEDIO AMBIENTE EN ZONAS ÁRIDAS**

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**USE OF ZEOLITE AND ALGAENZYMES TO MITIGATE WATER
DEFICIT STRESS IN TOMATO (*Solanum lycopersicum* L.) IN THE
NORTHERN REGION OF MEXICO**

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**MAESTRO EN CIENCIAS EN RECURSOS NATURALES Y MEDIO
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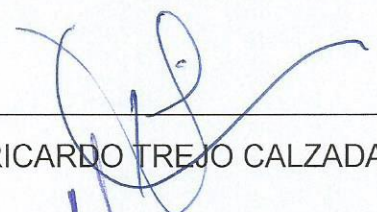
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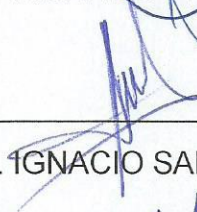
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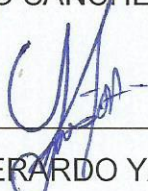
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1. José Antonio Miranda Rojas. (2023) **TESIS** “Agricultura de temporal en el estado de durango, México: pronóstico de rendimiento mediante uso de modelo hidrológico calibrado con datos observados”
2. Ignacio Sánchez Cohen, Sergio Iván Jiménez Jiménez, Ricardo Trejo Calzada, Aurelio Pedroza Sandoval, José Antonio Miranda Rojas, Marco Antonio Inzunza Ibarra, Isaac Gramillo Ávila. (2023) **MANUAL TECNICO PARA PRODUCTORES** “Manejo de siembras en la agricultura de temporal deficiente para el estado de durango, México.
3. Pedroza-Sandoval, Aurelio, Luis Ángel González-Espíndola, Isaac Gramillo-Ávila and José Antonio Miranda-Rojas. 2025. **ARTICULO** "Certain Physiological and Chemical Indicators Drive the Yield and Quality of Cladode Mucilage in

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GENERAL ABSTRACT

Use of zeolite and Algaenzymes to mitigate water deficit stress in tomato (*Solanum lycopersicum* L.) in the northern region of Mexico

This study evaluated the effect of zeolite and seaweed extracts on the physiological, biochemical, and productive responses of tomato (*Solanum lycopersicum* L. var. Saladette) grown under limited irrigation in an arid environment in northern Mexico. An open-field experiment was established using a randomized complete block design with split plots and three replications. Two soil moisture levels were applied—suboptimal ($25 \pm 2\%$) and deficient ($20 \pm 2\%$)—along with four treatments: seaweed extract (SE), zeolite (Z), the combination of both (SE+Z), and a control without application.

The results showed that water deficiency reduced photosynthesis, stomatal conductance, and transpiration, while the application of zeolite under suboptimal moisture significantly improved the photosynthetic rate and CO₂ fixation efficiency. Photosynthetic pigments showed no significant variations, indicating stability of the photosynthetic apparatus. The combined application of SE+Z promoted greater antioxidant activity and accumulation of phenols and tannins, suggesting an adaptive metabolic response to water stress. In contrast, the exclusive use of zeolite reduced the accumulation of secondary metabolites, likely due to a lower induction of oxidative stress.

At the agronomic level, the SE+Z combination favored balanced growth, greater leaf biomass, and a significant increase in both fruit number and weight, particularly under suboptimal moisture. This synergy between the slow nutrient release from zeolite and the bioactive compounds of the seaweed extract optimized water use and improved crop productivity. Overall, the joint application of zeolite and seaweed extract represents a sustainable strategy to strengthen tomato resilience under water scarcity, enhancing both physiological performance and yield in arid regions.

Keywords: Biostimulants; Antioxidant activity; Secondary metabolites; Photosynthesis; Plant resilience.

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RESUMEN GENERAL

Uso de zeolita y Algaenzimas para mitigar el estrés por déficit hídrico en tomate (*Solanum lycopersicum* L.) en la región norte de México

El presente estudio evaluó el efecto de la zeolita y los extractos de algas marinas sobre la respuesta fisiológica, bioquímica y productiva del tomate (*Solanum lycopersicum* L. var. Saladette) bajo condiciones de riego limitado en un ambiente árido del norte de México. Se estableció un experimento en campo abierto con un diseño en bloques completamente al azar con parcelas divididas y tres repeticiones. Se aplicaron dos niveles de humedad del suelo, subóptimo ($25 \pm 2\%$) y deficiente ($20 \pm 2\%$), y cuatro tratamientos: extracto de algas (SE), zeolita (Z), la combinación de ambos (SE+Z) y un testigo sin aplicación.

Los resultados demostraron que la deficiencia hídrica redujo la fotosíntesis, la conductancia estomática y la transpiración, mientras que la aplicación de zeolita bajo humedad subóptima mejoró significativamente la tasa fotosintética y la eficiencia de fijación de CO_2 . Los pigmentos fotosintéticos no mostraron variaciones relevantes, indicando estabilidad del aparato fotosintético. La aplicación combinada de SE+Z promovió una mayor actividad antioxidante y acumulación de fenoles y taninos, lo que sugiere una respuesta metabólica adaptativa frente al estrés hídrico. En contraste, el uso exclusivo de zeolita redujo la acumulación de metabolitos secundarios, probablemente por una menor inducción de estrés oxidativo.

A nivel agronómico, la combinación SE+Z favoreció el crecimiento equilibrado, mayor biomasa foliar, y un incremento significativo en el número y peso de frutos, especialmente bajo humedad subóptima. Esta sinergia entre la liberación lenta de nutrientes de la zeolita y los compuestos bioactivos del extracto de algas optimizó el uso del agua y mejoró la productividad del cultivo. En conjunto, la aplicación conjunta de zeolita y extracto de algas representa una estrategia sostenible para fortalecer la resiliencia del tomate ante la escasez de agua, mejorando tanto su desempeño fisiológico como su rendimiento en regiones áridas.

Palabras Clave: Bioestimulantes; Actividad antioxidante; Metabolitos secundarios; Fotosíntesis; Resiliencia vegetal

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CHAPTER 1

GENERAL INTRODUCTION

In recent decades, agriculture in arid and semi-arid regions has been increasingly affected by climate change and the growing pressure on water resources. Rising global temperatures, irregular rainfall patterns, and more frequent extreme events — such as prolonged droughts — have created difficult conditions for agricultural production (IPCC, 2022; FAO, 2021). Those areas often deal with little water and high evaporation, so horticultural activity doesn't always perform well. This problem ends up affecting food availability and the economy of small rural communities.

Tomato (*Solanum lycopersicum* L.) is a key crop in arid and semi-arid zones. It is widely consumed and plays an important role in local farming and rural income (FAOSTAT, 2023; Heuvelink & Dorais, 2019). However, tomato plants are very sensitive to water shortage, especially during key growth stages such as flowering and fruit development. For this reason, they are often used as indicators of how vulnerable horticultural systems can be under limited water conditions (Patane & Cosentino, 2010; Sánchez-Rodríguez et al., 2010).

When water is limited, tomato plants react through different physiological and biochemical changes that influence their growth and performance. Under drought stress, they tend to accumulate more reactive oxygen species, which can harm cell structures, promote lipid peroxidation, and reduce the amount of photosynthetic pigments (Farooq et al., 2009; Hasanuzzaman et al., 2013). These reactions weaken the plants, making them shorter and less vigorous. They also affect the fruits, reducing both their number and quality — their weight, firmness, sugar level, and nutritional value (Sánchez-Rodríguez et al., 2012). In arid regions, the challenge is not only the lack of water but also the absence of

affordable and sustainable ways to lessen drought effects without raising production costs too much.

To face these challenges, farmers and researchers have tried diverse ways to use water more efficiently and lessen the impact of drought on crops. Among these efforts are modern irrigation methods, like drip systems and soil moisture sensors, along with the selection of varieties that can better handle dry conditions. The use of organic or mineral materials that help soils hold water has also proven helpful (Chartzoulakis & Bertaki, 2015; Fereres & Soriano, 2007).

In the last few years, natural biostimulants — such as seaweed extracts — have gained attention. These products provide phytohormones, polysaccharides, amino acids, and antioxidants that support plant metabolism and strengthen tolerance to environmental stress (Khan et al., 2009; Craigie, 2011; Rouphael & Colla, 2020).

Zeolites are another option. They are natural minerals that can hold water and exchange nutrients with the soil. When used in agriculture, they improve soil structure, increase nutrient availability, and reduce water loss from evaporation (Leggo, 2017; Ramesh & Reddy, 2011).

Although both materials have shown reliable results when used separately, only a few studies have evaluated the combination of seaweed extracts and zeolites in tomato plants under drought in open fields. This topic still needs more research and could bring useful results for farming in dry areas.

This research comes from the need to look for simple and sustainable ways to keep agriculture working in dry areas. Farmers in arid and semi-arid regions often deal with water shortages, which reduce yield and can affect the quality of their crops. One possible solution is to use seaweed extracts as biostimulants. They are natural products that help plants stay healthy under stress by improving

photosynthesis and helping them form protective substances (Battacharyya et al., 2015; Sharma et al., 2014). Zeolites can also be useful because they help the soil keep both water and nutrients, which is important where water is limited (Polat et al., 2004; Ramesh & Reddy, 2011). Using seaweed extracts and zeolites together could help tomato plants produce more and better fruits in dry conditions. This approach might also work for other crops and support farming that can adapt better to a changing climate.

1 Objectives

1.1. General Objective

To help develop ways to use water more efficiently in tomato crops (*Solanum lycopersicum* L.) grown in open fields in a dry region of northern Mexico

1.1.2 Specific Objectives

Evaluate algae extract and zeolite as biostimulants to reduce water stress and its impact on plant growth, chlorophyll index, photosynthesis, and tomato yield.

Analyze antioxidant activity and photochemical pigments in response to hydration stress and biostimulants.

1.2 Hypothesis

Applying seaweed extracts and zeolite, alone or together, will help tomato plants cope with water shortage, improving growth and yield, and making them more tolerant to drought compared to plants that do not receive any treatment.

CHAPTER 2

Literature Review

2.1 Climate change, drought, and agriculture in dry regions

Dry regions already face limited water, and climate change is making farming there even harder. Higher temperatures, irregular rainfall, and longer dry periods create tough conditions for crops, especially in arid and semiarid areas. According to various reports from the Intergovernmental Panel on Climate Change, rising global temperatures and changing precipitation patterns are increasing the frequency and severity of storm events (IPCC, 2022). These changes have a direct impact on agricultural productivity by reducing water availability, increasing evapotranspiration, and worsening soil degradation. According to the FAO (2021), approximately 45% of the world's agricultural surface is in areas with water scarcity. Food security in these areas relies heavily on innovative practices that optimize water usage.

Mexico's arid regions, like the Comarca Lagunera, face serious water problems. Farmers struggle with overused aquifers, salty soil, and inefficient water use, and climate change makes things even harder (CONAGUA, 2020). Long periods without rain have reduced both surface and underground water. Because of this, farmers often have to rely on expensive solutions that are not always good for the environment (Magaña et al., 2012). Tomatoes need water regularly to grow well. This makes farming in these dry areas even more challenging (Patane & Cosentino, 2010).

Water stress affects both farming and the daily lives of people in rural areas. When crops produce less, farmers earn less, and communities face bigger challenges (Altieri & Nicholls, 2017). At the same time, the need for food keeps

growing. Water is also becoming harder to get for farms, cities, and industries (Foley et al., 2011). Finding ways to help crops survive these conditions is key for keeping farming sustainable.

When plants do not get enough water, the tiny pores on their leaves, called stomata, close. This slows down photosynthesis and makes the plants grow more slowly, which lowers the harvest (Chaves et al., 2003; Flexas et al., 2014). Dry soil also makes it harder for plants to take up nutrients such as nitrogen, sulfur, and potassium (Farooq et al., 2009). When water stress is high, harmful molecules build up in plant cells. These molecules can damage membranes and proteins, which lowers crop quality (Hasanuzzaman et al., 2013).

In dry areas, farmers need to manage water carefully. They can try methods like planting crops in ways that save water, using less water at certain times, or checking soil moisture to avoid wasting water. Many developing countries still find these methods hard to use because they cost a lot, the infrastructure is missing, or farmers lack training. Natural products like seaweed extracts or minerals such as zeolites can help. They are easier to use and can improve water use and help crops survive and produce under dry conditions (Rouphael & Colla, 2020).

2.2. Importance of tomato cultivation

Tomatoes are grown in many countries around the world. People eat them in diverse ways. Some eat them fresh, while others are made into sauces, juices, or pastes. Because of this, tomatoes are an important crop and provide income for many farmers (FAOSTAT, 2023; Heuvelink & Dorais, 2019). Tomatoes also contain compounds that are good for our health. One of them is lycopene, which gives tomatoes their red color. Another is beta-carotene, which helps the body in different ways. Tomatoes also have vitamin C, which supports the immune

system. In addition, they contain polyphenols. These substances help protect our cells and may reduce the risk of some health problems like heart disease or certain cancers (Canene-Adams et al., 2005; Dorais et al., 2008; Raiola et al., 2014).

The plant itself, not just the fruit, also makes protective substances and enzymes. These include superoxide dismutase, catalase, and peroxidase. They help the plant stay healthy when it faces stress from harmful molecules that appear under dry conditions (Hasanuzzaman et al., 2013; Farooq et al., 2009). This study focuses on these natural defenses. It looks at how the plant reacts to water scarcity, rather than the nutritional content of the fruits.

In 2024, global tomato production was estimated at 47.2 million tons, with substantial contributions from countries like China, India, and Mexico (World Processing Tomato Council, 2024). In Mexico, tomato production is a critical crop for both domestic consumption and export. In 2023, national production reached 3.64 million tons, with approximately 49,461 hectares harvested (Secretaría de Agricultura y Desarrollo Rural (SADER), 2024; Servicio de Información Agroalimentaria y Pesquera (SIAP), 2023). Tomatoes are especially important in northern states like Sonora, Baja California, Sinaloa, and the Comarca Lagunera. These areas are dry, and water is limited. Because of this, growing tomatoes costs more. The plants are also more affected by changes in climate (CONAGUA, 2020; Magaña et al., 2012). If drought reduces production, it can affect both local and national economies. (Ramírez-Pérez et al., 2019).

Tomatoes need a lot of water. During the growing season, they may need 4,000 to 6,000 cubic meters per hectare, depending on the irrigation system and climate (Patane & Cosentino, 2010). If they do not get enough water, the plants grow less and produce smaller fruits. They also change the way they use energy. The plants make protective substances called antioxidants. These substances may

help the plant deal with stress. Tomato plants also adjust how they manage water inside their cells (Chaves et al., 2003; Flexas et al., 2014). These natural responses help the plant survive drought and keep producing as much as possible. Understanding these antioxidants in the plant is important, not just the ones found in the fruits.

Tomatoes often face dry conditions. When this happens, the plants respond in different ways. One way is by producing substances called compatible solutes such as proline. These substances help keep proteins and cell membranes safe. The plant makes more enzymes. These enzymes help protect it from harmful molecules, called free radicals (Farooq et al., 2009; Hasanuzzaman et al., 2013). These changes help the plant survive. They also allow it to keep producing fruits when water is low. Tomato is an appropriate plant to study how water shortage affects growth and how biostimulants, like zeolite and algae extracts, can help to deal with it. (Mohkami et al., 2020; Rouphael & Colla, 2020).

Tomatoes are also important for research and improving farming. Farmers use different methods to grow better crops, such as protected agriculture, fertilization, biological control, and biostimulants (Heuvelink & Dorais, 2019; Battacharyya et al., 2015). In open fields in dry areas of Mexico, these methods are not always easy to use. This shows the need for simple and affordable ways to produce vegetables, fruits, and crops under water-limited conditions, like using algae extracts and minerals such as zeolite.

2.3. Effects of water deficit on tomato physiology and productivity

Tomatoes need water to grow and make fruit. Water helps them to do photosynthesis, move nutrients, keep cells firm, and control temperature. If there is not enough water, the plant reacts quickly to protect itself. This helps the plant survive, but it can also decrease fruit yield and quality.

When water is scarce, tomatoes close their stomata on their leaves. This slows down water loss and helps the plant save water (Chaves et al., 2003). Consequently, less carbon dioxide gets into the leaves, and photosynthesis slows down. The plant grows more slowly and produces less biomass (Flexas et al., 2014). If the tiny pores stay closed for a long time, reactive oxygen species can form inside the cells. These molecules may damage proteins, cell membranes, and pigments used in photosynthesis (Farooq et al., 2009; Hasanuzzaman et al., 2013). Water shortage also changes pigments used in photosynthesis, such as chlorophyll a, chlorophyll b, and carotenoids. Research shows that drought lowers chlorophyll levels and reduces the efficiency of photosystem II in tomato plants (Sánchez-Rodríguez et al., 2010; Mafakheri et al., 2010). This makes it harder for the plant to capture sunlight, fix carbon, and grow leaves and fruits.

When tomatoes do not get enough water, they respond in different ways. One main effect is that the water inside their cells becomes unbalanced. To deal with this, the plants produce small molecules called osmolytes, including proline, glycine betaine, and soluble sugars. These molecules help cells stay firm and protect proteins and membranes (Ashraf & Foolad, 2007; Farooq et al., 2009). Plants also make antioxidant enzymes, including superoxide dismutase, catalase, and peroxidase. These enzymes remove harmful molecules called reactive oxygen species, which appear when the plant is under stress (Hasanuzzaman et al., 2013). Drought slows growth and reduces the number of fruits. It also makes the plant use more energy to maintain these defenses, which lowers yield.

Flowers and fruits are also affected by water shortage. Fewer flowers grow, fewer fruits form, and the fruits are smaller. Severe drought can cut total yield by half (Patane & Cosentino, 2010). Water stress also changes fruit quality, including

sugar content, acidity, and firmness (Sánchez-Rodríguez et al., 2012). This affects both production and market value.

The timing, severity, and length of drought are important. When it shows at early growth phases, it slows vegetative development. Later, during flowering and fruiting, it directly reduces fruit production and ripening (Jones, 2004). Studying how tomatoes respond to drought can show whether biostimulants or minerals like zeolite improve physiological and morphometrical traits. Any improvement in photosynthesis, antioxidant activity, or water balance may increase yield and fruit quality (Rouphael & Colla, 2020; Battacharyya et al., 2015).

2.4. Biostimulants in sustainable agriculture

Biostimulants are widely used in farming because they help plants grow and cope with stress. They are not like fertilizers, which supply nutrients directly. Instead, biostimulants help plants take up nutrients, grow roots and leaves, and protect themselves from problems like drought or salty soil (du Jardin, 2015; Rouphael & Colla, 2020). They include seaweed extracts, humic and fulvic acids, amino acids, protein hydrolysates, helpful bacteria, and mycorrhizal fungi (Calvo et al., 2014).

These products support sustainable farming. They help plants use water and nutrients more efficiently and reduce the need for chemicals. Natural extracts can help crops deal with water shortages. Research shows that biostimulants raise levels of molecules like proline and sugars, activate antioxidant enzymes, and improve photosynthesis, helping plants survive drought (Ertani et al., 2013; Colla et al., 2015).

Biostimulants work in several ways at once. In tomatoes, they can increase chlorophyll, photosynthesis, and leaf water content. They also increase the

number of fruits and their size (Battacharyya et al., 2015; Bulgari et al., 2019). These effects are especially useful in dry regions, where water is scarce.

Biostimulants are an important tool for making agriculture more resilient and sustainable. The European Commission, through Regulation 2019/1009, officially recognized biostimulants as a separate category of agricultural inputs, reflecting their growing relevance in production systems (European Commission, 2019). This recognition highlights the need for farming practices that reduce environmental impact without lowering productivity, a goal that is especially important in areas vulnerable to climate change.

Many studies have tested biostimulants under water stress. Colla et al. (2014) showed that applying hydrolyzed proteins to tomatoes with limited water increased the flowers' water content and antioxidant activity. Ertani et al. (2013) found that plant extracts help tomatoes grow and carry out photosynthesis during drought. These results show that biostimulants not only reduce the immediate effects of water shortage but also help plants prepare for future stress (Rouphael & Colla, 2020).

Biostimulants help crops grow and deal with stress. They also bring benefits for the environment and local communities. They decrease the need for chemical fertilizers and help plants use water better. This can reduce pollution in soil and aquifers and make farming easier for small producers (Sharma et al., 2014; du Jardin, 2015).

Products from natural sources, like seaweed or crop residues, can make farming more sustainable. Biostimulants help plants deal with drought. They make plants grow better and protect them from harmful molecules. Using them combined with zeolites may help crops produce more and better fruits.

2.5. Seaweed Extracts as Biostimulants

Seaweed extracts are now used as biostimulants because they help plants deal with stress from low availability of water. They have natural compounds, including plant hormones, amino acids, vitamins, sugars, and phenols. These compounds work together to improve plant growth and help plants survive when water is limited (Khan et al., 2009; Craigie, 2011; Chojnacka et al., 2015).

Various studies have shown that the application of seaweed extracts improves physiological parameters such as relative water content in leaves, photosynthetic rate, and photosystem II efficiency, which contribute to better growth maintenance under water stress (Ertani et al., 2013; Colla et al., 2015; Rouphael & Colla, 2020). In particular, the activation of antioxidant systems is one of the most documented effects. Drought causes an increase in reactive oxygen species, which damage membranes, proteins, and pigments; in response, seaweed extracts induce the activity of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase, as well as promote the accumulation of non-enzymatic compounds such as phenols and flavonoids, which help neutralize oxidative damage (Hasanuzzaman et al., 2013; Mohkami et al., 2020). It is important to emphasize that these antioxidant responses are mainly manifested in the vegetative tissues of the plant, such as leaves and stems, which are the organs that first perceive water stress and play a crucial role in the protection and survival of the plant.

In tomato cultivation, the application of seaweed extracts has shown positive results under both optimal and drought conditions. Battacharyya et al. (2015) reported that plants treated with seaweed extracts exhibited greater biomass accumulation and improved photosynthetic performance under water deficit, while other studies have noted increases in yield parameters such as fruit number and weight in different growing environments (Sharma et al., 2014; Bulgari et al., 2019). These effects come from the extracts' capacity to improve water use,

which is important in dry regions where water is limited and expensive (Rouphael & Colla, 2020).

Another important aspect is that the effects of seaweed extracts depend on factors such as the dose, the method of application, and the specific composition of the product. Extracts obtained from brown algae such as *Ascophyllum nodosum* usually have a high concentration of polysaccharides, cytokinins, and betaines, compounds that have been associated with the induction of tolerance to water stress (Chojnacka et al., 2015). Foliar applications allow rapid responses in the photosynthetic apparatus, while soil applications can improve the rhizosphere and the absorption of water and nutrients (Colla et al., 2015). This versatility makes seaweed extracts practical and adaptable tools for different production systems.

Under moderate drought, seaweed extracts generally provide benefits, but when water shortage becomes extreme, and plants are near permanent wilting, their effectiveness is limited (Rouphael & Colla, 2020). Nevertheless, studies indicate that these extracts can help plants handle water stress by supporting key physiological processes and boosting antioxidant defenses in leaves and stems.

In dry areas, seaweed extracts help crops grow better and cope with water stress. They improve plant water status, aid photosynthesis, and enhance antioxidant activity in vegetative tissues. Combining them with other methods, like adding mineral amendments to the soil, can further strengthen plant resilience and increase crop stability.

2.6. Zeolite as a mineral amendment in agriculture

Zeolite is a mineral from volcanic rocks and contains aluminosilicates that form from volcanic rocks. Their tiny pores can trap water and release it slowly, which makes them particularly useful in dry regions where water is limited (Mumpton,

1999; Leggo, 2017). Because of this property, farmers have increasingly used zeolites to help crops endure and grow in challenging, arid conditions.

When zeolite is added to soil, it can improve its characteristics in multiple ways. For example, they help retain humus and allow air to circulate more easily. Zeolites help prevent nutrients from being lost in the soil. At the same time, they create a more stable environment that allows roots to develop properly (Polat et al., 2004; Ramesh & Reddy, 2011). These effects together create better conditions for plant development.

Zeolites can hold important nutrients such as potassium, calcium, magnesium, and ammonium. Over time, these elements are gradually released, giving plants what they need in smaller amounts. This process helps prevent nutrient loss and encourages stronger plant growth (Polat et al., 2004). In areas with heavy irrigation, where nutrients might easily wash away, zeolites act like tiny reservoirs. By slowly supplying nutrients as plants require them, they improve fertilization efficiency and support more sustainable crop production (Mumpton, 1999; Margeta et al., 2013).

Zeolite also helps with nutrients. It can store potassium, calcium, magnesium, and ammonium. Plants take these nutrients slowly over time. This reduces waste and helps them grow healthier (Polat et al., 2004). In areas with heavy irrigation, where nutrients can wash away, zeolite acts like a small reservoir. It slowly gives nutrients to plants and helps fertilization work better (Mumpton, 1999; Margeta et al., 2013). Research shows that zeolite does more than hold water. Ramesh and Reddy (2011) found that tomatoes with zeolite kept more water and grew better than untreated plants. Similar results were seen in cucumber, maize, and pepper. Zeolite helped plants do photosynthesis and survive during the season (Polat et al., 2004; Margeta et al., 2013).

Zeolite can be combined with biostimulants like seaweed extracts. Zeolite improves water and mineral supply. Seaweed extracts help plants respond to stress with antioxidants. Together, they make plants stronger and improve yields under moderate stress (Rouphael & Colla, 2020; Miranda-Rojas et al., 2025). If drought is severe, zeolite cannot replace missing water (Margeta et al., 2013).

Zeolite should be used as part of a larger soil and water plan. It helps farmers use resources efficiently. It lasts in the soil for several growing cycles and is low-cost (Leggo, 2017). This is very useful in dry regions, where water and nutrients must be used carefully. In short, zeolite has many benefits. It improves soil, saves water, provides nutrients gradually, and helps plants survive drought. Using it with biostimulants, like algae extracts, can further improve tomato growth and sustainability.

2.7. Combination of algae and zeolite extracts in the management of water deficit

Water is often scarce in dry regions. This makes farming very difficult. One way to help plants is to use biostimulants together with minerals. Seaweed extracts help plants grow by increasing antioxidants. They also support hormone activity and improve photosynthesis (Khan et al., 2009; Battacharyya et al., 2015; Rouphael & Colla, 2020). Zeolite works differently. It keeps water and nutrients in the soil. Plants can access them steadily during the whole growing season (Mumpton, 1999; Leggo, 2017). Using both together helps plants grow better in dry conditions.

The logic behind this combination is based on the fact that each input's action mechanisms operate at different levels of the soil-plant system. While algae extracts promote internal physiological changes—such as an increase in antioxidant enzymes and the accumulation of osmolytes in leaves and stems—

zeolite contributes to maintaining edaphic humidity and reducing nutrient leaching, ensuring that plants have access to water and minerals. Together, these actions promote better hydration and nutrient balance, resulting in more energy, higher water content in leaves, and improved photochemical stability under stress.

Recent studies on tomato, broccoli, strawberry, and lettuce support this complementarity. In field experiments, it has been observed that the application of algae extracts, and zeolite not only increases vegetative growth but also improves performance parameters such as fruit number, average weight, and post-harvest quality when compared to individual treatments (Rouphael & Colla, 2020; Bulgari et al., 2019; Bernardi et al., 2010). The results indicate that plants benefit from both internal changes and improved environmental conditions.

Tomatoes in northern Mexico often grow in dry conditions. According to Miranda-Rojas et al. (2025), using algae extracts together with zeolite can help the plants grow stronger when water is scarce. Their leaves retain more water, and chlorophyll levels rise, which improves photosynthesis. Plants also produce more antioxidant compounds. Algae extracts help plants protect themselves internally. Zeolite keeps water available in the soil. Using both together usually gives higher yields than using either one alone when water is moderately scarce.

The success of this combination depends on factors such as the amount applied, the timing, and how severe the drought is. If soil moisture becomes too low, algae extracts and zeolite cannot fully make up for the lack of water (Margeta et al., 2013; Rouphael & Colla, 2020). This shows that these treatments should be part of a broader soil and water management plan, not used on their own.

Evidence suggests that combining algae extracts with zeolite is a practical way to grow tomatoes in dry areas. This approach reduces the need for synthetic

fertilizers. It also improves water use. At the same time, it helps soil and plants stay healthy. It supports immediate growth under water stress. It also helps make production more stable over time.

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CHAPTER 3.

Effect of seaweed extract and zeolite on tomato: photosynthesis and antioxidants under water stress

Efecto de extracto de algas y zeolita en tomate: fotosíntesis y antioxidantes bajo estrés hídrico

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ABSTRACT. Water scarcity in arid regions such as northern Mexico negatively affect the physiological performance and productivity of tomatoes grown in open fields. The aim of this study was to evaluate the effect of seaweed extract and zeolite on photosynthesis, photosynthetic pigments, and antioxidant activity in tomato under open-field water stress conditions. A randomized complete block design with split plots and three replicates was used, under suboptimal soil moisture content (SSMC: 23–27%) and deficient soil moisture content (DSMC: 18–22%). Treatments included seaweed extract (50 L ha⁻¹), zeolite (20 t ha⁻¹), their combination, and control (no product applied). Under suboptimal moisture, zeolite registered the high photosynthetic rate in both soil moisture content (20.99 CO₂ m⁻²s⁻¹ in SSMC and 15.01 CO₂ m⁻² s⁻¹ in DSMC), while the combined application showed the highest antioxidant activity (9.7 μMg-1 FW). In contrast, under deficient moisture, gas exchange was strongly reduced and did not differ among treatments. Photosynthetic pigments were not affected by treatments. However, phenolic and tannin contents increased under water deficit, particularly with seaweed extract application. Overall, seaweed extract and zeolite improved physiological performance under moderate water stress, mainly by enhancing antioxidant metabolism, whereas their effects were limited under

severe stress. These findings highlight their potential as management strategies to mitigate water stress in tomatoes under arid conditions.

Keywords: photosynthetic gas exchange, plant antioxidant response, phenolic compounds, soil water management, arid agroecosystems.

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RESUMEN. La escasez de agua en regiones áridas, como el norte de México, afecta negativamente el desempeño fisiológico y la productividad del tomate cultivado en campo abierto. El objetivo de este estudio fue evaluar el efecto del extracto de algas marinas y la zeolita sobre la fotosíntesis, los pigmentos fotosintéticos y la actividad antioxidante en plantas de tomate sometidas a estrés hídrico. Se empleó un diseño de bloques completos al azar con parcelas divididas y tres repeticiones, bajo dos niveles de humedad del suelo: subóptima (SSMC: 23–27%) y deficiente (DSMC: 18–22%). Los tratamientos consistieron en extracto de algas marinas (50 L ha⁻¹), zeolita (20 t ha⁻¹), su combinación y un control sin aplicación. Bajo condiciones de humedad subóptima, la zeolita presentó la mayor tasa fotosintética (20.99 CO₂ m⁻² s⁻¹ en SSMC y 15.01 CO₂ m⁻² s⁻¹ en DSMC), mientras que la combinación de ambos productos mostró la mayor actividad antioxidante (9.7 µM g⁻¹ de peso fresco). En condiciones de humedad deficiente, el intercambio gaseoso disminuyó notablemente sin diferencias entre tratamientos. Los pigmentos fotosintéticos no se vieron afectados. Sin embargo, los compuestos fenólicos y taninos aumentaron bajo déficit hídrico, especialmente con la aplicación de extracto de algas marinas. En conjunto, el extracto de algas marinas y la zeolita mejoraron el desempeño fisiológico del tomate bajo estrés hídrico moderado, principalmente mediante el fortalecimiento del sistema antioxidante. No obstante, sus efectos fueron limitados bajo condiciones de estrés hídrico severo, lo que resalta su potencial como estrategia de manejo en zonas áridas.

Palabras clave: intercambio gaseoso fotosintético, respuesta antioxidante vegetal, compuestos fenólicos, manejo del agua en el suelo, agroecosistemas áridos.

INTRODUCTION

Water scarcity and extreme temperatures in northern Mexico are the major abiotic stress factors that significantly reduces crop productivity under open field. This environmental condition limits metabolic activity, reduces chlorophyll content and photosynthetic efficiency, and restricts leaf expansion (Chandra *et al.* 2018). Nevertheless, plants possess adaptive mechanisms that allow them to tolerate environmental stressors like water

scarcity, maintaining physiological function and plant development (Seleiman *et al.* 2021).

Plant biostimulants, applied to plants or soil, promote plant growth and improve crop health through various biochemical and physiological mechanisms. These products are a sustainable and ecological alternative to conventional agrochemicals in modern agriculture (Saldivar and Cruz 2024). As global demand for food production intensifies, particularly due to population increase and the negative impacts of climate change, the use of biostimulants offers a viable strategy to mitigate plant stress and thereby enhance agrifood production. These substances have shown a great capacity to alleviate abiotic stressors such as drought and nutrient deficiencies (Rai *et al.* 2021). Biostimulant formulations enrich the soil with nutrients, enhance microbial biodiversity, and improve water retention, which are key factors in maintaining resilient agricultural systems (Karami *et al.* 2020).

Among the diverse biostimulant strategies, mineral-based amendments such as zeolite have gained attention for their potential to improve soil structure and plant performance under environmental stress. Zeolite is a naturally occurring porous aluminosilicate mineral with high cation-exchange capacity and strong water-retention properties. It also contains beneficial mineral elements, including silicon (Si), which can enhance nutrient availability in the rhizosphere and promote plant vigor and growth (Cataldo *et al.* 2021; Kaur *et al.* 2023). In addition, its porous structure may stimulate microbial activity and improve soil water dynamics, thereby contributing to higher crop productivity under limiting conditions (Mondal *et al.* 2021). The primary physical and chemical properties of the zeolite used in this study were previously described by Miranda *et al.* (2025)

Organic biostimulants, such as seaweed extracts, have also shown significant benefits for plant growth under stress conditions. Seaweed extracts are high in bioactive compounds, including phytohormones, amino acids, and micronutrients, that enhance photosynthetic efficiency, improve nutrient uptake, and increase resilience to both biotic and abiotic stressors such as drought and salinity (Hernández *et al.* 2022). These extracts promote physiological responses and crop performance, particularly in intensive agricultural systems where conventional fertilizers may be less effective (Ali *et al.* 2021).

Tomato is a globally important horticultural crop that is especially sensitive to water deficiency, which adversely affects its growth, yield, and fruit quality (Chakma *et al.* 2021). Global tomato production was estimated at 47.2 million Mg in 2024, with substantial contributions from China, India, and Mexico (World Processing Tomato

Council 2024). In Mexico, tomato production is vital for both domestic consumption and export markets; production reached 3.64 million tons from approximately 49,461 harvested hectares in 2023 (SADER 2024 and SIAP 2023). In the Comarca Lagunera region of Coahuila and Durango states, Mexico, which is the area of this study, tomatoes are grown under protected agriculture systems, such as shade netting or greenhouses to mitigate extreme temperatures and dry weather. These systems yield 145 769 tons with an average yield of 135 t ha⁻¹ (SIAP 2021). However, the high technology costs associated with protected agriculture remain a limiting factor for local producers.

Several studies have addressed strategies to mitigate water deficit in tomato and other crops, including the use of zeolite as a soil amendment to improve water retention and nutrient availability. For example, Méndez-Argüello *et al.* (2018) reported enhanced growth and chlorophyll content in tomato cultivated using zeolite as a substrate, while Elseedy *et al.* (2023) highlighted zeolite's potential for agricultural water management, but emphasized the need for validation under diverse crop and environmental conditions. However, most available evidence derives from controlled or substrate-based systems, and the combined use of zeolite and seaweed extracts as soil water retainers and/or biostimulants in tomato crops under water stress conditions remains poorly explored.

From a physiological perspective, zeolite and seaweed extract are expected to act through complementary mechanisms as zeolite modifies the root-zone environment by stabilizing soil moisture and ion exchange capacity, whereas seaweed extract stimulates plant metabolic activity through hormone-like compounds and signaling molecules that regulate antioxidant and stress response pathways (Shahad *et al.* 2025). Their combined application, therefore, represents a targeted strategy to simultaneously modulate soil water dynamics and plant biochemical responses, rather than a simple additive treatment. Tomato was selected as a model plant due to its high sensitivity to water availability and its well-characterized physiological responses. Based on recent studies on abiotic stress and tomato physiology, various exogenous agents and internal mechanisms such as photosynthetic gas exchange, photosynthetic pigment stability as well as antioxidant activity make the integration of these physiological indicators feasible, improving plant growth and development under adverse conditions (Miranda *et al.* 2025).

Based on this framework, we hypothesized that the application of a mineral soil amendment (zeolite) and an organic biostimulant (seaweed extract) would improve

tomato physiological responses under limited water availability. Therefore, this study aimed to evaluate the single or together effects of zeolite and seaweed extract on photosynthesis, photosynthetic pigments, and antioxidant activity in tomato plants grown under suboptimal and deficient soil moisture conditions under open-field in northern Mexico.

MATERIALS AND METHODS

Geographic location

The study was conducted at the experimental area of the Unidad Regional Universitaria de Zonas Aridas (URUZA) of the Universidad Autonoma Chapingo in Bermejillo, Durango, Mexico. The experimental area is located at 25° 53' 39'' N and 103° 36' 14'' W, at an elevation of 1 130 m (Figure 1). According to the Köppen climate classification, the region is categorized as BWhw(e), signifying a very dry, semi-warm climate. The average annual temperature is 21 °C, with maximum and minimum temperatures of 33.7 °C and 7.5 °C, respectively. Rainfall occurs primarily during the summer and winter, with an average annual rainfall of 199.60 mm (Medina *et al.* 2005).

Climatic conditions

Monthly rainfall in the area was 23.6 mm, 0 mm, 0.4 mm, 2 mm, and 0.6 mm in February, March, April, May, and June, respectively; these levels did not affect the experimental area. Maximum temperatures reached 35 °C in June (Figure 2). This thermal stress, especially when combined with water deficits, can trigger floral abscission in tomato plants (Rai *et al.* 2024).

Experimental design

A randomized block design in a split-plot arrangement with three replicates was used. The main plots consisted of two soil moisture levels: suboptimal soil moisture content (SSMC) in a range of 23–27%, and deficient soil moisture content (DSMC) in a range of 18–22 %. These ranges were based on soil moisture properties, corresponding to a field capacity (FC) of 32% and a permanent wilting point (PWP) of 18%. Moisture contents were determined using a pressure membrane pot (Model 1500F1®, Santa Barbara, CA, USA) (Richards 1948). The sub-plots comprised four treatments: seaweed extract (SE) applied at 50 L ha⁻¹; zeolite (Z) applied at 20 t ha⁻¹; a combination of both at 25 L ha⁻¹ (SE) and 10 t ha⁻¹ (Z); and a control (no biostimulant application). These doses were selected to evaluate the physiological and biochemical effectiveness of commonly recommended field applications of each treatment, including the organic biostimulant (seaweed extract) and the mineral soil amendment (zeolite), under contrasting soil moisture conditions.

Seaweed extracts were applied twice: first at 14 days after transplanting (DAT) the tomato plants into the open field and again at 56 DAT, corresponding to the seedling and flowering stages. In contrast, granulated zeolite was applied once during soil preparation. The experimental unit consisted of nine tomato plants arranged in four 4.5-m-long rows, with 0.80 m between rows and 0.2 m between plants. Four plants were randomly selected from the two median rows during the flowering stage (56 DAT) to measure plant variables.

Seaweed and zeolite features and application system

Algae extract was obtained from the brown alga *Ascophyllum nodosum* (L) following the methodology described by Drygas *et al.* (2024) and as reported by Miranda *et al.* 2025. In addition to hormones, seaweed extracts contain high concentrations of nutrients, including nitrogen derivatives, vitamins, macro- and micronutrients, and trace microelements such as silicon (Si), which provide several beneficial effects for plants. The aqueous extract was applied using a foliar sprayer (Lola Safe 289 020, SWISSMEX, Mexico City, Mexico) at rates of 0.4 mL L⁻¹ and 0.8 mL L⁻¹, corresponding to doses of 25 L ha⁻¹ and 50 L ha⁻¹, respectively.

The zeolite used in this study was extracted from the subsoil and donated by Grupo AG Zeolitas (Chihuahua, Chih. Mexico). Application was performed 20 days before transplanting tomato into the open field. Soil preparation included harrowing and leveling using agricultural machinery. Subsequently, the granulated product was applied manually using a multi-perforated dosing tube (1 kg capacity) at doses of 10 and 20 t ha⁻¹. Product volume was adjusted for a surface area of 14.4 m² per treatment (4.5 × 3.2 m). Finally, 20-cm-high furrows were formed using a plow to ensure that the product was covered with soil.

Irrigation system setup

Irrigation was carried out using a polyvinyl chloride (PVC) piping system, consisting of a 2" main line connected to 1/2" perpendicular lateral lines supplying each experimental main plot. Each lateral line fed four crop rows through one drip tape per row. Self-compensating drippers (Chapin® drip tape, Jain Irrigation Systems Ltd., Watertown, NY, USA) were used, spaced 0.20 m apart between emitters and 0.80 m between lines, with a discharge rate of 2 L h⁻¹ per emitter. Based on this irrigation system, the hourly application rate was calculated as 12.5 mm h⁻¹. Soil moisture was monitored at a depth of 30 cm using a digital meter (model PMS-714, Lutron Electronic Enterprise Co., Taipei, Taiwan). After an initial period of uniform irrigation to reach field capacity (32%), two soil moisture regimes were established: suboptimal soil moisture content (SSMC, 25% ± 2) and deficit soil moisture content (DSMC, 20% ± 2). Once the treatments were differentiated, irrigation was applied for 4 h per event up to reach an approximate gross

water depth of 50 mm (equivalent to a net depth of 40 mm) and was repeated each time soil moisture dropped to the lower limit of each water regime.

Antioxidant activity and antioxidant metabolites

Total antioxidant activity (TAA) was determined using the DPPH method (Parejo *et al.* 2000). Plant extracts (200 µg of sample in 10 ml of 7:3 acetone-water) were shaken for 30 min and centrifuged at 5000 rpm at 4°C for 15 min. The resulting supernatant was incubated in the dark for 60 min, and the absorbance was measured spectrophotometrically at 515 nm. Results were expressed as µM Trolox (6-hydroxy-2,5,7,8-tetramethylchromium-2-carboxylic acid 97%, Sigma Aldrich, Co., UK) equivalent (TE) per gram of fresh matter (TE µM g⁻¹ FW). To determine total phenolic content (TPC), total flavonoid content (TFLC), and total tannin content (TTC), a separate extract was prepared by mixing 100 mg of sample in 1 mL of methanol and agitating on ice for 24 h prior to use.

Total phenolic content (TPC) was determined using a modified Folin-Ciocalteu method (Lamuela 2018). A reaction mixture containing 50 µl of the plant extract, 3 ml of distilled H₂O, and 250 µl of Folin-Ciocalteu reagent was incubated for 5 minutes. Then, 750 µl of 20% Na₂CO₃ was added and vortexed for 2 minutes. After 30 minutes of incubation at room temperature, absorbance was measured at 760 nm. Gallic acid (10 mg ml⁻¹ in a methanol solution) was used as the standard. TPC was expressed as mg of gallic acid (GA) eq g⁻¹ FW.

Total flavonoid content (TFLC) was determined using the aluminum chloride method (Barnum 1977). The reaction mixture was prepared with 100 µl of extract and 1.4 ml of distilled H₂O, followed by 500 µl of flavonoid reagent. After incubation in the dark, it was left undisturbed for 30 min at room temperature, after which the samples were read at 415 nm in a spectrometer. The TFL was determined using a calibration curve with quercetin (QE, Sigma Aldrich, Co. UK.) at a concentration of 1 mg ml⁻¹. The TFLC was expressed as QE eq mg 100 g⁻¹ FW.

Total tannin content (TTC) was assessed using the method described by Harborne (1998). The reaction mixture included 100 µl of extract, 5 ml of distilled water, 500 µl of Folin-Ciocalteu reagent, and 1 ml of 35% Na₂CO₃. Following homogenization and 30 minutes of incubation at room temperature, absorbance was read at 724 nm. Quantification was performed using a gallic acid standard (1 mg ml⁻¹ GA), and results were expressed as mg GA eq mg⁻¹ FW.

Photosynthesis and photosynthetic pigments

Leaf gas exchange was assessed on fully expanded leaves to determine photosynthetic rate (Pn), stomatal conductance (gs), intercellular CO₂ concentration (Ci), and

transpiration rate (E). Measurements were carried out under clear-sky conditions between 10:00 and 11:00 h using a portable infrared gas analyzer (LI-COR 6400, Lincoln, NE, USA). During data acquisition, chamber CO₂ concentration was maintained at 400 ppm, photosynthetically active radiation (PAR) was fixed at 750 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and cuvette temperature was maintained at 25 °C. Leaf samples were allowed to equilibrate in the cuvette for approximately one minute prior to recording measurements, ensuring stabilization of physiological parameters, as confirmed by a low coefficient of variation.

Photosynthetic pigments were quantified following the method described by Wellburn (1994). The spectral determination of chlorophylls *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. According to this author the original formulas are as follows:

$$\text{Chl } a = [(15.65)(A_{666}) - (7.34)(A_{653})]$$

$$\text{Chl } b = [(27.05)(A_{653}) - (11.21)(A_{666})]$$

$$\text{Car} = [[1000(A_{470}) - 2.86 \cdot (\text{Chl } a) - 129.2 (\text{Chl } b)]/221]$$

Ten fresh leaves were collected, from which 5-mm diameter discs were cut and placed in test tubes. Each disc was mixed with 10 mL of methanol (CH₃OH) and incubated in darkness at room temperature for 24 hours. Absorbance was measured with a spectrophotometer at 470 nm for carotenoids (Car), 653 nm for chlorophyll *b* (Chl *b*), and 666 nm for chlorophyll *a* (Chl *a*), using the following formulas:

$$\text{Chl } a = [(15.65)(A_{666}) - (7.34)(A_{653})] [V/1000 \times W]$$

$$\text{Chl } b = [(27.05)(A_{653}) - (11.21)(A_{666})] [V/1000 \times W]$$

$$\text{Car} = [[1000(A_{470}) - 2.86 \cdot (\text{Chl } a) - 129.2 (\text{Chl } b)]/221] [V/1000 \times W]$$

$$\text{Chl } t = \text{Chl } a + \text{Chl } b$$

Statistical Analysis

Data was tested for normality (Shapiro Wilk). A one-way analysis of variance (ANOVA), followed by Tukey's test ($p \leq 0.05$), was used to compare treatments. Pearson correlation and regression analyses were also performed using the statistical programs SPSS version 18 (SPSS Inc., Chicago, IL, USA) and SAS version 9.0.

RESULTS

The ANOVA indicated significant treatment effects on key biochemical and physiological traits of tomatoes plants. Significant differences were detected for total phenolic content (TPC), total tannin content (TTC), antioxidant activity (TAA) net photosynthesis (Pn) stomal conductance (gs), intercellular CO₂ concentration (Ci), and transpiration rate (E) ($P < 0.05$). In contrast, total flavonoid content and photosynthetic pigments were not significantly affected ($P > 0.05$) (Table 1).

Table 1. Analysis of variance of different physiological and biochemical variables measured in tomato plants

Variables	Source of variation	Sum of squares	df	Mean square	F-value	P-value
TPC	Inter-groups	20.464	7	2.923	1.488	0.018
	Total	146.192	71			
TFLC	Inter-groups	23.527	7	3.361	1.483	0.189
	Total	168.550	71			
TTC	Inter-groups	814.065	7	116.295	2.874	0.011
	Total	3403.639	71			
TAA	Inter-groups	3.242	7	0.463	1.057	0.042
	Total	31.298	71			
Car	Inter-groups	.191	7	0.027	1.343	0.245
	Total	1.488	71			
Chl b	Inter-groups	.091	7	0.013	1.831	0.096
	Total	.544	71			
Chla	Inter-groups	.295	7	0.042	2.212	0.065
	Total	1.515	71			
Chl t	Inter-groups	290578.986	7	41511.284	12.173	0.100
	Total	508833.653	71			
Pn	Inter-groups	2638.842	7	376.977	25.878	0.000
	Total	3571.163	71			
gs	Inter-groups	0.218	7	0.031	6.957	0.000
	Total	0.505	71			
Ci	Inter-groups	3880427.351	7	554346.764	5.047	0.000
	Total	1.091E7	71			
E	Inter-grupos	306.419	7	43.774	7.898	0.000
	Total	661.143	71			

Antioxidant activity and secondary metabolite concentrations

Antioxidant activity was significantly higher ($P < 0.05$) when SE + Z was applied under suboptimal soil moisture content (SSMC), with a value of $9.7 \mu\text{Mg}^{-1} \text{FW}$. Conversely, the lowest values were obtained in the Z treatments under the same moisture conditions (Figure 3A).

TPC was significantly ($P < 0.05$) lower in SSMC with the application of zeolite, with a value of $6.33 \text{ mg GA g}^{-1} \text{ FW}$; a contrasting trend was observed in the SE + Z (SSMC) and SE (DSMC) treatments. The remaining treatments showed intermediate values, regardless of soil moisture content (Figure 3B). TFLC variation was not statistically significant across biostimulant treatments at both soil moisture contents (Figure 3C). The TTC evaluations showed a similar trend to TPC; zeolite showed the lowest value with $8.34 \text{ mg g}^{-1} \text{ FW}$ in SSMC, while SE (DSMC) and SE + Z (SSMC) presented the highest values at $16.78 \text{ mg g}^{-1} \text{ FW}$ and $16.54 \text{ mg g}^{-1} \text{ FW}$, respectively (Figure 3D).

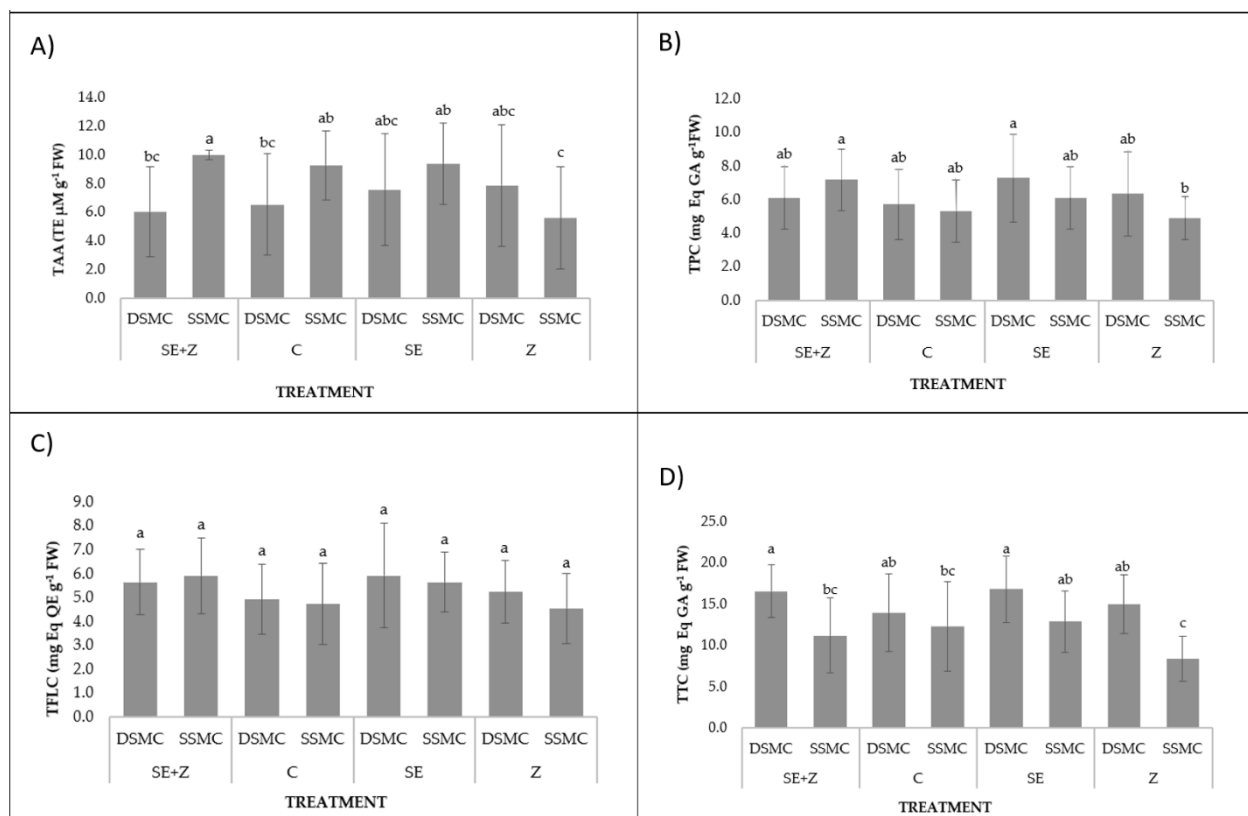


Figure 1. Effects of seaweed extracts (SE) and zeolite (Z) applied separately or together (SE + Z) in tomato on total antioxidant activity (TAA) (3A), total phenolic content (TPC) (3B), total flavonoid content (TFLC) (3C), and total tannin content (TTC)

Photosynthetic pigments

Photosynthetic pigments (Chl a, Chl b, and carotenoids) did not vary significantly ($P < 0.05$) with the use of seaweed and zeolite, whether applied separately or combined. All treatments performed similarly to the control under both soil moisture contents (SSMC and DSMC) (Figures 4A, 4B, and 4C). Chl a levels ranged from 0.75-0.95 mg g⁻¹ FW across all treatments (Figures 4A and 4B), whereas Chl b concentrations ranged from 0.45 to 0.60 mg g⁻¹ FW (Figure 4B). Carotenoid concentrations remained between 0.70 and 0.90 mg g⁻¹ FW (Figure 4C).

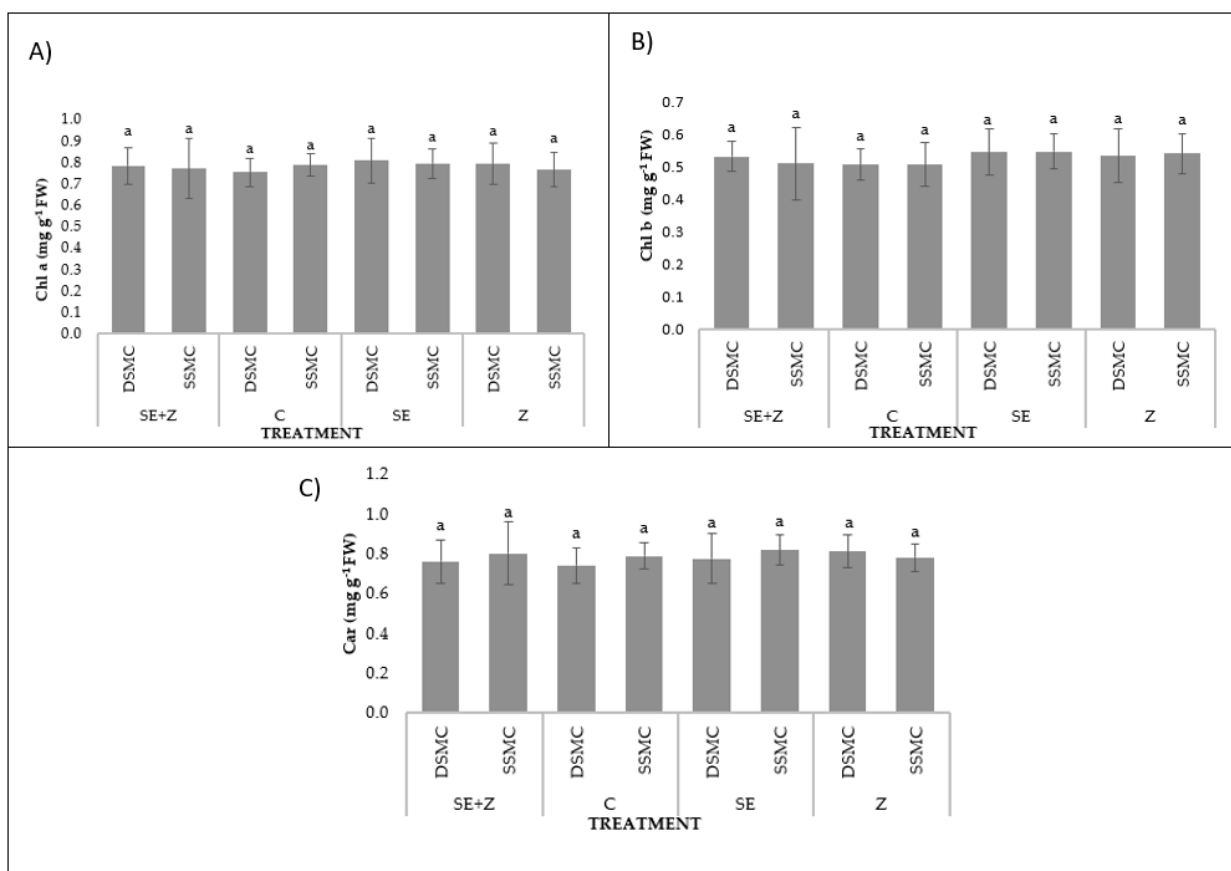


Figure 2. Effects of seaweed extracts (SE) and zeolite (Z) applied separately or together (SE + Z) in tomato on photosynthetic pigments [Chlorophyll a (Chl a) (4A), Chlorophyll b (Chl b) (4B), and Carotenoids (Car) (4C)]. Measurements were taken at suboptimal soil moisture content (SSMC) and deficient soil moisture content (DSMC). Bars labeled with the same letter are not statistically different according to Tukey's test ($P < 0.05$).

Photosynthesis and photosynthetic pigments

Net photosynthesis (PN), stomatal conductance (gs), and transpiration rate (E) showed a notable decrease in tomato plants under water deficiency compared to suboptimal treatments. The highest PN was observed under SSMC with the Z treatment. All treatments under SSMC, including the control, exhibited higher stomatal conductance and a similar trend for transpiration. Conversely, intercellular CO₂ and VPD_L reached their highest values under DSMC, with concentrations of 255.77 and 4.19 mol m⁻² s⁻¹, respectively (Table 2).

Table 2. Effects of applied biostimulants — zeolite (Z), seaweed extracts (SE), their combination (SE + Z), and the control (C) on leaf gas exchange under different soil moisture contents in tomato: photosynthesis (PN); stomatal conductance (gs); intercellular CO₂ (Ci); transpiration (E); and leaf-level vapor pressure deficit (VPDL).

Soil Moisture Content (%)	Biostimulants	P _N (μmol CO ₂ m ⁻² s ⁻¹)	g _s (mmol H ₂ O m ⁻² s ⁻¹)	E (mol H ₂ O m ⁻² s ⁻¹)	Intercellular CO ₂ (μmol CO ₂ mol air ⁻¹)	VPDL (kPa)
25% ± 2	Control	17.79 ^b ± 3.04	0.199 ^a ± 0.063	8.39 ^a ± 2.35	205.26 ^{ab} ± 19.58	3.91 ^{ab} ± 0.25
	SE	15.05 ^c ± 1.86	0.208 ^a ± 0.075	8.07 ^a ± 0.04	207.76 ^{ab} ± 34.45	3.73 ^b ± 0.56
	Z	20.99 ^a ± 3.86	0.225 ^a ± 0.070	9.05 ^a ± 2.19	192.82 ^b ± 50.73	3.80 ^{ab} ± 0.31
	SE + Z	18.28 ^b ± 3.12	0.150 ^b ± 0.053	6.61 ^b ± 2.13	186.78 ^b ± 70.10	4.05 ^{ab} ± 0.36
20% ± 2	Control	8.16 ^{de} ± 3.79	0.077 ^c ± 0.033	3.40 ^c ± 0.99	191.78 ^b ± 72.65	4.08 ^{ab} ± 0.61
	SE	6.97 ^e ± 2.55	0.056 ^c ± 0.024	2.68 ^c ± 1.12	255.77 ^a ± 99.96	4.19 ^a ± 0.42
	Z	15.01 ^c ± 3.31	0.089 ^c ± 0.078	3.54 ^c ± 2.31	197.01 ^b ± 35.36	4.04 ^{ab} ± 0.63
	SE + Z	8.36 ^d ± 3.54	0.071 ^c ± 0.027	3.28 ^c ± 1.25	178.37 ^b ± 45.64	4.10 ^{ab} ± 0.59

Correlation analysis

The Pearson correlation matrix provides insight into the relationships among physiological, biochemical, and phytochemical variables. These variables exhibited modulated regulations in the studied tomato plants. As expected, gas exchange variables displayed strong, significant associations with one another, underscoring their functional integration. However, the vapor pressure deficit at the leaf level (VPDL) did not correlate significantly with any physiological variables. Additionally, while photochemical pigments were positively correlated with one another, they showed no significant correlations with gas exchange parameters. Secondary metabolites exhibited strong internal correlations, consistent with their shared biosynthetic origin via the phenylpropanoid pathway (Table 3).

Table 3. Pearson correlation matrix among physiological (gas exchange), photochemical (pigments), and biochemical (phenolic) traits.

	TPC VPDL	TFLC	TTC	TAA	Car	Chl b	Chl a	Pn	gs	Ci	E
TPC	P	1									
	Sig.										
TFLC	P	.636	1								
	Sig.	.000									
TTC	P	.344	.306	1							
	Sig.	.003	.009								
TAA	P	.035	.193	.026	1						
	Sig.	.771	.104	.829							
Car	P	.114	.251	-.106	.303	1					
	Sig.	.342	.034	.375	.010						
Chl b	P	.088	.217	.062	.244	.723	1				
	Sig.	.463	.067	.605	.039	.000					
Chl a	P	.180	.384	.081	.249	.803	.851	1			
	Sig.	.131	.001	.497	.035	.000	.000				
PN	P	-.084	.142	-.178	.570	.326	.159	.257	1		
	Sig.	.484	.234	.136	.000	.005	.181	.029			
gs	P	-.246	-.146	-.356	.223	.096	.040	-.020	.559	1	
	Sig.	.038	.223	.002	.059	.423	.742	.865	.000		
Ci	P	.089	.014	.037	-.149	-.216	-.153	-.253	-.411	-.022	1
	Sig.	.456	.910	.755	.210	.068	.200	.032	.000	.855	
E	P	-.221	-.089	-.363	.247	.130	.034	.024	.628	.972	-.117
	1 Sig.	.062	.457	.002	.037	.277	.778	.844	.000	.000	.326

VPDL	P	.214	.279	.024	-.108	.141	-.050	.184	-.029	-.631	-.184
	-.483	1									
	Sig.	.072	.017	.843	.367	.239	.679	.123	.807	.000	.121
	.000										

TPC is total phenolic content; TFLC is total flavonoid content; TTC is total tannin content; TAA is total antioxidant activity; Car is carotenoids; Chl a is chlorophyll a; Chl b is chlorophyll b; P_N is photosynthesis; gs is stomatal conductance; C_i is intercellular CO_2 ; E is transpiration; VPDL is leaf-level vapor pressure deficit.

Regression analysis

Regression analyses revealed a set of statistical linear relationships among biochemical and physiological variables, although with different treatments (Table 4). TFLC was positively and significantly correlated with TPC, indicating that increases in TPC explain about 30% of the variability in TFLC; this moderate association suggests that additional biochemical factors likely influence these levels. Photosynthetic pigments exhibited a strong relationship with carotenoids. *Chl b* and *Chl a* were both strongly and positively associated with carotenoid content, with carotenoids explaining over 80% of the variation in chlorophyll levels, highlighting tight coordination among photosynthetic pigments. Furthermore, photosynthetic rate (P_N) was positively related to total antioxidant activity (TAA), suggesting that antioxidant capacity contributes moderately to photosynthetic performance, which is also linked to increased stomatal opening. Additionally, E rate showed a moderate positive relationship with photosynthesis and a very strong dependence on stomatal conductance. The latter accounted for more than 94% of the variability in E, identifying stomatal regulation as the primary driver of transpiration. Collectively, these results demonstrate a coherent physiological performance in tomato plants, where pigment composition strongly influences photosynthetic capacity, stomatal conductance tightly regulates transpiration, and gas exchange processes are moderately modulated by biochemical status and treatment.

Table 4. Significant linear regression between evaluated variables in tomato plants

Equation	R ²	p
TFLC = 0.3969 TPC + 2.9675	0.304	0.001
Chl b = 0.683 Car - 0.033	0.806	0.011
Chl a = 0.956 Car - 0.013	0.856	0.001
P_N = 5.028 TAA + 1.193	0.325	0.005
gs = 0.04 P_N + 0.007	0.302	0.037
E = 1.790 P_N + 0.270	0.386	0.006

E = 0.917 gs + 34.95	0.944	0.001
VPDL = 4.493 gs + 3.631	0.390	0.002
VPDL = 4.429 E + 0.077	0.223	0.001

TPC is total phenolic content; TFLC is total flavonoid content; TTC is total tannin content; TAA is total antioxidant activity; Car is carotenoids; Chl a is chlorophyll a; Chl b is chlorophyll b; P_N is photosynthetic rate; E is transpiration; VPDL is leaf-level vapor pressure deficit, R^2 is the coefficient of determination; p is the significance level of the statistical test.

DISCUSSION

Cumulative rainfall during the experimental period was 30.4 mm, with the highest level recorded in February (23.6 mm). Precipitation during April, May, and June totaled only 3 mm; these minimal levels were continuously monitored using a digital sensor to maintain precise soil moisture conditions for each treatment. This rainfall did not interfere with the evaluated soil moisture treatments, as seedlings were initially grown in trays under shade mesh prior to field transplanting. Furthermore, water deficit studies are commonly conducted in low-rainfall regions to minimize precipitation as a confounding factor affecting soil moisture levels (Chen *et al.* 2025, Pedroza *et al.* 2024)

Despite contrasting soil moisture regimes, photosynthetic pigment concentrations did not show significant differences among treatments ($P > 0.05$), as chlorophyll a ranged from 0.75 to 0.95 mg g⁻¹ FW, chlorophyll b from 0.45 to 0.60 mg g⁻¹ FW, and carotenoids from 0.70 to 0.90 mg g⁻¹ FW (Figs. 4A–C). This stability contrasts with the significant variation observed in antioxidant activity and secondary metabolite accumulation (Fig. 3), suggesting that the applied amendments primarily modulated oxidative metabolism rather than directly affecting chloroplast structure or pigment biosynthesis (Abd-Elrahman *et al.* 2022; Hernández *et al.* 2022). Similar pigment stability under moderate abiotic stress has been reported in crops such as *Olea europaea* cv. Dezful (Khaleghi *et al.* 2012). Although abiotic stress can negatively impact photosynthesis by degrading pigments such as chlorophylls and carotenoids (Liu *et al.* 2011), the absence of significant changes in this study indicates that stress intensity remained within a physiological tolerance range.

Antioxidant activity showed a marked response under suboptimal soil moisture conditions (SSMC), particularly in the combined SE + Z treatment, which reached 9.7 μM g⁻¹ FW (Fig. 3A). This value was slightly higher than the control (9.2 μM g⁻¹ FW), representing an increase of approximately 5.4%, and higher than SE alone (9.3 μM g⁻¹ FW; 4.3% increase). In contrast, the Z treatment showed substantially lower antioxidant activity (5.5 μM g⁻¹ FW), indicating a reduction of approximately 43% compared to the

control. These results suggest that zeolite alone does not stimulate antioxidant metabolism to the same extent as seaweed extract, while its combination with SE enhances the overall biochemical response. Seaweed extracts have been reported to stimulate antioxidant metabolism through hormone-like compounds, osmoprotectants, and signaling molecules, whereas zeolite improves soil water retention and nutrient availability (Gneedy *et al.* 2022; Kavvadias *et al.* 2023). The combined application likely promoted a more balanced physiological response under moderate water stress conditions.

Under deficient soil moisture conditions (DSMC, $20\% \pm 2$), antioxidant activity varied among treatments, with the highest values observed in Z ($7.8 \mu\text{M g}^{-1}$ FW) and SE ($7.5 \mu\text{M g}^{-1}$ FW), followed by the control ($6.5 \mu\text{M g}^{-1}$ FW), whereas the combined SE + Z treatment showed the lowest value ($6.0 \mu\text{M g}^{-1}$ FW) (Fig. 3A). Compared with suboptimal soil moisture conditions (SSMC, $25\% \pm 2$), zeolite increased antioxidant activity from 5.5 to $7.8 \mu\text{M g}^{-1}$ FW, representing an increase of approximately 41.8%. This response suggests that under severe water limitation, zeolite may contribute to maintaining metabolic activity by improving soil water availability. In contrast, the lower antioxidant response in the combined treatment may indicate reduced metabolic efficiency or a differential allocation of stress-response resources under stronger water deficit.

TPC was also affected by soil water content and amendment type. Higher phenolic accumulation in the SE and SE + Z treatments under water deficit supports their role as stress-induced metabolites involved in reactive oxidative species (ROS) scavenging and cellular protection (Atencio 2023). Conversely, lower phenolic concentrations in zeolite treatments reflect reduced metabolic stress due to improved hydration, emphasizing that phenolic synthesis is more closely linked to stress intensity than to the presence of the amendment itself.

Flavonoids, the major subclass of polyphenol compounds, contribute significantly to antioxidant capacity and stress tolerance (Shen *et al.* 2022). Although the total flavonoid content (TFLC) did not differ statistically among treatments, consistently higher values in SE + Z treatments under both moisture conditions indicate a trend toward enhanced flavonoid biosynthesis. However, the strong correlation observed between flavonoids and total phenolics supports the concept of coordinated regulation within the phenylpropanoid pathway (Del Giudice *et al.* 2017), reinforcing their synergistic contribution to antioxidant activity in tomato plants.

Total tannin content showed a more pronounced response to specific treatment combinations. The highest tannin accumulation under SE + Z application in suboptimal

moisture conditions highlights the effectiveness of combined biostimulants in activating defense-related secondary metabolism. Conversely, tomato plants treated with Z alone exhibited a lower tannin concentration, confirming its limited role in directly stimulating antioxidant metabolism. These results underscore the primary role of seaweed extracts in inducing biochemical defenses, particularly under moderate water stress where plants remain metabolically active (Kumar *et al.* 2025). Such responses are agronomically relevant for tomato production in arid regions (Miranda *et al.* 2025), where improving stress resilience without compromising productivity is a major challenge.

The lack of impact on photosynthetic pigments further indicates that neither water deficit nor amendment application imposed severe physiological stress. This aligns with findings by Méndez-Argüello *et al.* (2018), who observed stable pigment levels in tomato plants grown on various substrates such as peat moss, perlite, zeolite, and mixtures thereof. Although drought-induced pigment degradation has been documented in different tomato cultivars (Zhou *et al.* 2017), the maintained chlorophyll and carotenoid levels observed in this study suggest that stress intensity remained within a tolerable range, preserving the photosynthetic apparatus (Li and Mattson 2015, Hurtado *et al.* 2017).

Previous studies have reported mixed responses of chlorophyll and carotenoid content to biostimulant applications, depending on plant species, growth stage, and environmental conditions (Calvo *et al.* 2014, Xu and Leskovar 2015). Preserved pigment levels suggest that treatments did not impair pigment production in tomato plants, aligning with their overall healthy appearance and productivity reported in parallel measurements (Miranda *et al.* 2025).

Carotenoids play a central role in photoprotection, excess energy dissipation, and membrane stability under stress conditions (Son *et al.* 2020, Sun *et al.* 2022). The consistent carotenoid concentration across treatments further supports the conclusion that plants maintained effective photoprotective mechanisms. The values obtained are comparable to those reported for tomato cultivars (*S. lycopersicum* var. Saladette, *S. lycopersicum* var. Moneymaker and *S. chilense*) exposed to moderate water stress (Loyola *et al.* 2012), emphasizing the physiological stability of plants in this study.

The lack of correlation between leaf-level vapor pressure deficit (VPDL) and gas exchange parameters indicates that atmospheric demand — typically driven by temperature and humidity rather than soil water — was not the primary constraint on photosynthesis (Middleby *et al.* 2024), explaining its limited influence under these experimental conditions. Furthermore, the absence of a strong correlation between

photosynthetic pigments and gas exchange parameters suggests that pigment abundance was not the limiting factor for carbon assimilation. Instead, photosynthetic regulation likely occurred at the stomatal or biochemical level, consistent with observations in well-adapted tomato plants (Jiang *et al.*, 2017).

Secondary metabolites exhibited a clear decoupling from gas exchange parameters, suggesting their role as stress response compounds rather than a direct contribution to primary carbon assimilation (Erb and Kliebenstein 2020). This functional separation highlights the importance of antioxidant metabolism as a protective strategy operating independently of photosynthetic activity in plant physiology (Maury *et al.* 2020).

Regression analyses provided further mechanistic insight into the integrated biochemical and physiological responses underlying plant performance under the evaluated treatments. The results showed a structured and biologically coherent system in which secondary metabolites, photosynthetic pigments, and gas exchange parameters are tightly interconnected, albeit with varying magnitudes of response. This suggests that the production of these metabolites was likely part of a coordinated biological response that helped the plant manage environmental stressors while maintaining essential functions like photosynthesis (Pandey *et al.* 2022).

The positive and significant relationship between total flavonoid content and total phenolic content indicates that phenolic metabolism is a major contributor to flavonoid accumulation (Chandra *et al.* 2024). Gas-exchange relationships revealed a clear hierarchy of control: stomatal conductance (g_s) was positively related to photosynthetic rate, indicating that increases in carbon assimilation were associated with greater stomatal conductance (Aryal *et al.* 2019).

CONCLUSIONS

Seaweed extracts and zeolite modified tomato responses to water stress mainly through biochemical adjustments rather than through changes in photosynthetic pigments, which remained stable across all treatments. Under deficient soil moisture conditions, seaweed extract induced the greatest antioxidant response, reaching 7.20 mg GA g⁻¹ FW of total phenolics and 16.78 mg g⁻¹ FW of tannins, indicating that activation of secondary metabolism was a key mechanism under severe stress. Under suboptimal soil moisture, the combined application of seaweed extract and zeolite produced the highest phenolic accumulation (7.15 mg GA g⁻¹ FW), suggesting that improved soil water availability together with biostimulant action enhances protective metabolism when stress is moderate. In contrast, zeolite applied alone showed the lowest phenolic and tannin values under SSMC, consistent with a lower oxidative stress demand. Collectively, these findings demonstrate that tomato tolerance to limited water availability depends more on antioxidant metabolic plasticity than on pigment adjustments, and that seaweed extracts, alone under severe stress or combined with zeolite under moderate stress, constitute an effective management strategy for tomato production in arid and semi-arid environments.

CONFLICT OF INTEREST

The authors have no competing interests to declare that are relevant to the content of this article.

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CHAPTER 4.

Article

Algae Extracts and Zeolite Modulate Plant Growth and Enhance the Yield of Tomato *Solanum lycopersicum* L. Under Suboptimum and Deficient Soil Water Content

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Abstract

Drought and water scarcity are some of the most important challenges facing agricultural producers in dry environments. This study aimed to evaluate the effect of algae extract and zeolite in terms of their biostimulant action on water stress tolerance to obtain better growth and production of tomato *Lycopersicum esculentum* L. grown in an open field under suboptimum and deficient soil moisture content. Large plots had a suboptimum soil moisture content (SSMC) of 25% $\pm$ 2 [28% below field capacity (FC)] and deficient soil moisture content (DSMC) of 20% $\pm$ 2 [11% above permanent wilting point (PWP)]; both soil moisture ranges were based on field capacity FC (32%) and PWP (18%). Small plots had four treatments: algae extract (AE) 50 L ha⁻¹ and zeolite (Z) 20 t ha⁻¹, a combination of both products (AE + Z) 25 L ha⁻¹ and 10 t ha⁻¹, and

a control (without application of either product). By applying AE, Z, and AE + Z, plant height, plant vigor, and chlorophyll index were significantly higher compared to the control by 20.3%, 10.5%, and 22.3%, respectively. The effect on relative water content was moderate—only 2.6% higher than the control applying AE, while the best treatment for the photosynthesis variable was applying Z, with a value of $20.9 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, which was 18% higher than the control. Consequently, tomato yield was also higher compared to the control by 333% and 425% when applying AE and Z, respectively, with suboptimum soil moisture content. The application of the biostimulants did not show any mitigating effect on water stress under soil water deficit conditions close to permanent wilting. These findings are relevant to water-scarce agricultural areas, where more efficient irrigation water use is imperative. Plant biostimulation through organic and inorganic extracts plays an important role in mitigating environmental stresses such as those caused by water shortages, leading to improved production in vulnerable agricultural areas with extreme climates.

Keywords: water stress; plant growth; organic and inorganic amendments; drylands

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1. Introduction

About 85% of the planet's agricultural surface is negatively impacted by drought, which has increased in intensity and frequency due to climate change [1]. Drought and the resulting water shortage impact agricultural processes and increase the risk of food insecurity [2]. The negative impact depends on the stage of plant development and the level of stress induced by water deficiency [3], causing leaf senescence, flower abscission, chlorophyll destruction, and wilting [4]. Deficient soil moisture content during the reproductive phase of most plants reduces yield [5].

The use of water stress-tolerant plant species [6] complemented with different crop management practices to mitigate water deficit in plants is a real strategy to mitigate the impact of water deficit in agricultural areas with low water availability. Several agronomic practices have been reported to alleviate plant water stress, such as the use of soil moisture retainers based on mulch, or the use of hydrogel or compost [7,8], as well as the use of organic and inorganic biostimulants that improve plant growth, plant vigor, and yield [9,10]. Also, organic product groups such as symbiotic mycorrhizae [11] and some types of algae extracts, as well as inorganic products like zeolite, are playing an important role as bio-stimulants to enhance plant growth and productivity under constraint conditions [12].

Algae extracts contain a wide variety of biocompounds, such as polysaccharides, phytohormones, and amino acids, that play an important role in plant physiology and are vital to crop growth and yield [13]. Biostimulant application is a promising innovation for the sustainability of modern agricultural systems. These products may improve plant nutrition processes by increasing nutrient use efficiency, tolerance to abiotic stress, quality of traits, and availability of nutrients [14]. Almaroai et al. [15] highlight that the application of 9% algal extract on onion grown under water stress increased the bulb yield by 67 and 102% in 2018 and 2019, respectively. Hassan et al. [16] found that the application of algae *Pediastrum boryanum* at a rate of 2.0 mL L⁻¹ significantly increased plant height, number of branches, number of umbels, and fruit yield in fennel (*Foeniculum vulgare* Mill.) plants. In addition, algae and zeolite applied together have been used for the treatment of

wastewater for agriculture, improving soil fertility and the protein and carbohydrate contents in maize crops [17].

Inorganic products like zeolite contain different compounds, such as selenium (Se) [18,19], acetylsalicylic acid [9,20], abscisic acid [21], and aluminum polyciliate. Zeolite is being used as a biostimulant to enhance the growth and production of agriculture under water stress conditions. Zeolite is rich in aluminosilicate minerals and other important microelements with beneficial properties for human, animal, and plant health. Zeolite is proving to be very useful for plants, mainly as a mitigator of environmental stress and as a soil moisture-retaining agent when water availability is limited, as well as for detoxification processes, antioxidant action, and assimilation of different nutrients in plants [22]. In addition, zeolite has been evaluated as a soil moisture retainer [23] and as a soil fertility enhancer [24], as well as for improving the physical and chemical properties of soil water [10]. It is a compound whose structure has an oxygen/metal ratio of 2, tetrahedra of SiO_2 and AlO_2 composition, and a negative charge equal to the number of aluminums in the structural position [25]. This inorganic product has properties that improve soil moisture retaining, and facilitate the gradual and continuous uptake of nutrients by the plant, acting as a fertilizer [26].

Silicon (Si) is the second most abundant element on Earth. This element has been classified by the International Plant Nutrition Institute as a beneficial nutrient in plants [27]. Si has been associated with plant responses to several biotic and abiotic stresses. The role of Si in response to heavy metals, salinity, water stress, and extreme temperatures has been associated with its effects on secondary metabolism and particularly the production of phenolic and polyamine compounds [28,29]. The other important microelement contained in zeolite is aluminum (Al), which is the third most abundant metal in the Earth's crust. Aluminum is not an essential element for plants. However, there are many scientific articles evidencing beneficial effects on plants [30,31], such as increasing plant growth and mitigating biotic and abiotic stress when applied at a low concentration [32,33]. These microelements (Si and Al) are subjects of important studies in agriculture. Hazrati et al. [34] reported that zeolite application alleviates water stress and improves plant growth and yield of *Aloe vera* by increasing water use efficiency, while Selahvarzi et al. [35] showed that treatment with 20% zeolite resulted in the highest leaf area, total dry weight,

and carotenoid contents in *Festuca arundinacea* grass grown under water stress conditions.

For all the above, it is important to develop innovative strategies to aid marginal agriculture in drylands, such as those in northern Mexico. This region has an intensive agricultural production system, with crops such as fodders, fruit trees, and vegetables being the most common farming activity in the valley. All these crops have a high water demand and are irrigated with groundwater, which has caused a gradual depletion of the aquifers, with lower volumes of water pumped that also has a high salt content and heavy metals [36]. Integrated water management in arid-zone agriculture is crucial to improve the use efficiency of this vital natural resource, mostly where water scarcity is the main limiting factor [37,38].

Moreover, tomato is one of the most important vegetables in the agri-food industry worldwide, and it is widely cultivated in northern Mexico. However, tomato plants are highly sensitive to various environmental constraints, especially water stress [39]. In addition, in the survey area, tomato are grown year-round under greenhouse conditions due to the extreme climate and, in very small areas, this vegetable is cultivated under open-field conditions where the high temperatures and water deficit in summer are the cause of a high rate of floral senescence and consequently drastically decrease tomato yield [40]. The assumption of this study was to develop a tomato production strategy under open-field conditions in extreme environments such as arid zones and explore the possibility of making a more efficient use of water by applying low water volumes and using native materials native to the region to mitigate plant water stress. The study aimed to evaluate algae extract and zeolite as biostimulants to mitigate water stress and its impact on the water status of plants, growth, chlorophyll index, photosynthesis, and yield of tomato (*Solanum lycopersum* L. Cv. Saladette) under suboptimum and deficient soil water contents in open fields in northern Mexico.

2. Materials and Methods

2.1. Geographic Location

The study was conducted in the Unidad Regional Universitaria de Zonas Áridas of the Universidad Autónoma Chapingo, Bermejillo, Durango, Mexico, located at 25.8° NL and 103.6° WL at

1130 m. The climate is BWhw(e) semi-warm with summer rainfall and extreme temperatures, with an average temperature of 21 °C, a maximum of 33.7 °C, and a minimum of 7.5 °C. The average annual precipitation is 199.60 mm [41].

2.2. Chemical Features of Organic and Inorganic Products

2.2.1. Algae Extract

Algae extract was obtained according to Drygas et al. [42] from the brown alga *Ascophyllum nodosum* (L). Twenty-five grams of crushed plant and algal material were weighed using an analytic balance (SHIMADZUM, AW320, Kyoto Japan) and subsequently extracted with a 1:1 (v/v) mixture of H₂O (0.05 µS deionized water, and EtOH (analytical grade PA-01-0300-W, PAE001, 110 Pol-Aura) in an ultrasonic bath for two 30 min intervals at 40 °C. The resulting extract was filtered through a 1 mm mesh and combined, and the final volume was adjusted to 500 mL in a volumetric flask.

Algae extract analysis shows a relatively high content of organic compounds, including nitrogen derivates, hormones, and vitamins. Also, this extract contained macronutrients, micronutrients, and trace elements. It is noteworthy that there was a high content of silicon, a beneficial element (Table 1).

Table 1. Physical–chemical–biological characteristics of the algae extract.

Physical Characteristics	Chemical and Biological Characteristics		
	Macronutrients (ppm)	Micronutrients (ppm)	Phytohormones and Vitamins (ppm)
Humidity: 97.02	N: 5500	Zn: 2005	Cytokinin: 2900
Proteins: (Nx6,25) 2.27	P: 67,300	Mg: 1204.5	Ascorbic acid: 129
Total solids: 2.98	K < 43,700	Fe: 932.6	Carotenes: 60
Biochar: 0.406	Ca: 1660	Si: 3800	Niacin: 30
Organic matter: 0.700	Mg: 882.5	Bo 1:00	Riboflavin: 10
Carbohydrates >40.00	S: 1200		Thiamin: 5

2.2.2. Zeolite

Zeolite is a granulate inorganic compound and contains four macronutrients and two micronutrients. It also contained high concentrations of aluminum and silicon, both recognized as beneficial elements [43] (Table 2).

Table 2. Physical and chemical characteristics of zeolite.

Physical Characteristics	Chemical Characteristics
Color: Greenish pink	Al ₂ O ₃ (11.99%)
Odor: Odorless–earthy	SiO ₂ (53.45%)
pH of 5% aqueous solution: 7.9	CaO (8.06%)
Element adsorption: Rapid	Na ₂ O (1.16%)
Selectivity: N > P > K > Ca	Fe ₂ O (33.96%)
Element release: Slow	K ₂ O (6.11%)
	MgO (4.76%)
	MnO (0.09%)
	P ₂ O (50.23%)

2.3. Experimental Design

A randomized block experimental design in a split-plot arrangement with three replicates was used. The large plots were the soil moisture contents: suboptimum soil moisture content (SSMC) of $25\% \pm 2$ (28% below of FC) and deficient soil moisture content (DSMC) of $20\% \pm 2$ (11% above WP). The soil moisture content at FC and PWP were 32% and 18%, respectively. A soil water potential curve was determined using a Soil Moisture Equipment® model 1500F1 membrane pot (St. Barbara, CA, USA) according to the methodology described by Richards [44] (Figure 1). The small plots had four treatments: algae extract (AE) 50 L ha⁻¹, zeolite (Z) 20 t ha⁻¹, a combination of both products (AE + Z) at doses of 25 L ha⁻¹ and 10 t ha⁻¹, respectively, and the control (without application of either product). This experimental design was selected to facilitate the application of irrigation levels, establishing the two-soil moisture contents into each large plot to turn on/off irrigation control valves according to the irrigation timing for each soil moisture content evaluated in this study (Figure 2).

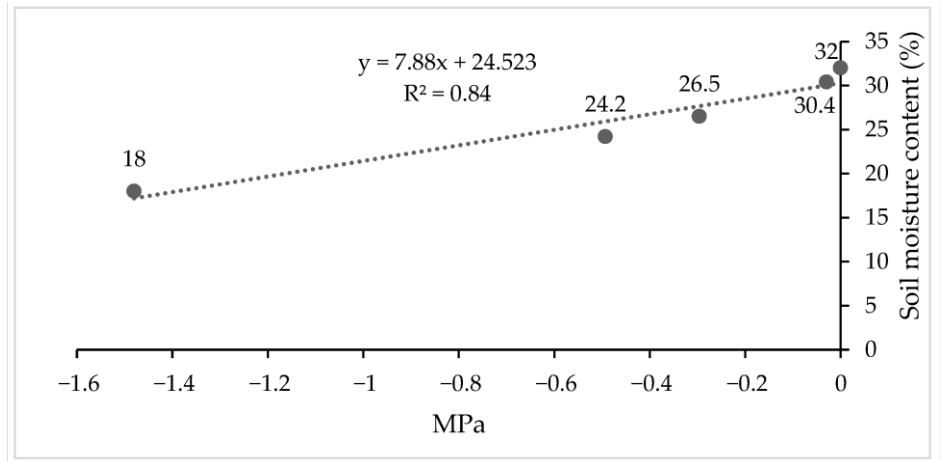


Figure 1. Soil moisture depletion from field capacity (FC) to permanent wilting point (PWP) in terms of MPa vs. moisture percentage in a clay-loam soil.

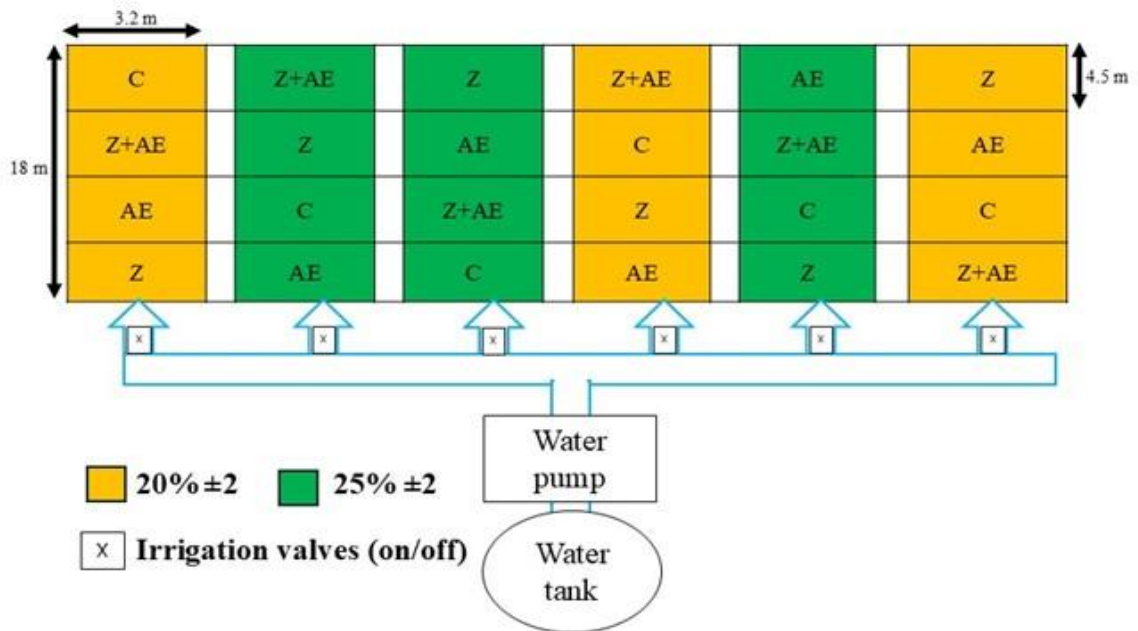


Figure 2. Randomized block experimental design in a split-plot arrangement for the evaluation of algae extract and zeolite applied separately and combined, plus the control, on each soil moisture content (20% ± 2 and 25% ± 2). AE is algae extract (50 L ha⁻¹); Z is zeolite (20 t ha⁻¹); AE + Z is both products combined (25 L ha⁻¹ of AE + 10 t ha⁻¹ of Z, respectively); C is the control (no application of either product).

2.4. Application of Algae Extract and Zeolite

Two field applications of AE and Z were made at the indicated rates for each treatment. The aqueous algae extract was applied using a foliar sprayer (Lola Safe 289020, SWISSMEX, State of Mexico, Mexico) at rates 0.4 mL L^{-1} and 0.8 mL L^{-1} according to doses of 25 L ha^{-1} and 50 L ha^{-1} . Two applications were made: the first at 14 days after transplanting (DAT) and the second at 56 DAT.

As for zeolite, which is a granulated powder, it was applied once to the soil during soil preparation, 20 days before tomato transplanting in the field. Soil preparation included harrowing and leveling with agricultural machinery. The zeolite was applied manually using a 1 kg-capacity multi-perforated cap test tube, spreading the granulated powder evenly in each treatment, according to doses of 10 and 20 t ha^{-1} , and adjusting the product volume to a surface area of 14.4 m^2 per treatment ($4.5 \times 3.2 \text{ m}$). After applying zeolite, furrows were formed with a plow so that the product was covered by the soil. The furrows were 20 cm high.

2.5. Irrigation System

Irrigation treatments were applied through a polyvinyl chloride (PVC) main irrigation line (2"), which was connected to PVC perpendicular irrigation lines (1/2") for each large plot (soil moisture content). The irrigation water supply was controlled by on/off valves, which irrigated four rows through one line tape per row (see Figure 2). Self-compensating drippers (Chapin® drip tape, Jain Irrigation Systems Ltd., Watertown, NY, USA) were used 20 cm apart, with an emission of 2 L h^{-1} for each one. Each large plot corresponded to the moisture content in each replicate; the water flow was controlled according to the irrigation program using a stopcock. Pre-transplant irrigation was standardized to FC with an irrigation time of 15.4 h, with a flow rate per dripper of 30.8 L h^{-1} at a pressure of 2.7 kg cm^{-2} , corresponding to an irrigation depth of 30 cm. For 7 days, the watering was uniform to FC in the whole experimental area, and after this date, the two soil moisture regimes were programmed and maintained in their corresponding ranges: SSMC to $25\% \pm 2$ and DSMC to $20\% \pm 2$. To maintain the soil moisture regimes, after reaching an SSMC of 27%, the soil moisture content was allowed to drop to 22% and then it was increased to 27%; for DSMC, after reaching the upper soil moisture limit (22%), irrigation was stopped until it dropped to 18%, after which irrigation was reinitiated until it rose to the upper limit of 22%. The above means

that once the moisture contents were differentiated, the subsequent irrigation time was 4 h every third day to recover the moisture content to the maximum limits from the lower levels of each soil moisture range. Soil moisture content was measured periodically using a digital moisture meter reading in real time (Lutron model PMS-714, Lutron Electronic Enterprise Co., Taipei, Taiwan) to a depth of 30 cm.

2.6. Variables

2.6.1. Climate

The environmental temperature and pluvial precipitation were recorded from February to June 2024, which is the tomato cycle time. A Davis Instruments model 6162 microclimatic station (Hayward, CA, USA) located 600 m from the experimental area was used.

2.6.2. Plant Growth (Plant Height, Stem Thickness, and Plant Vigor)

Plant height was measured using a tape graduated in cm, and stem thickness was measured using a vernier caliper in mm; plant vigor, rated on a scale of 0 to 10, where 0 is no vigor (wilted plant) and 10 is a completely vigorous plant, was determined according to the scale proposed by Horsfall & Barrat [45]. Plant growth variables were measured at 42 DAT, corresponding to the vegetative stage.

2.6.3. Physiological Indicators

Three physiological indicators were measured. The first was the chlorophyll content index (CCI), obtained using a CM 1000 chlorophyll meter (model 29950, Brand Spectrum Technologies Inc., Aurora, IL 60504, USA) according to the methodology reported by De Lima et al. [46]. The second physiological indicator was the relative water content (RWC), measured using the formula proposed by Browne et al. [47]. Saturated weight was stirred after immersing the tissue in distilled water for 24 h. Dry mass was obtained by drying the plant in an oven with air circulation at 65 °C until constant weight was reached. The results were expressed as a percentage (%) using the following formula:

$$RWC = \frac{(fm - dm)}{(sm - dm)} \times 100$$

where fm = fresh mass, dm = dry mass, and sm = saturated mass.

In addition, the photosynthetic activity ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was measured using the Infrared Gas Analyzer Brand LI-6400XT (LI-COR®, Inc. Lincoln, NE, USA).

2.6.4. Yield Components and Tomato Yield

The components of yield such as number of fruits per plant and fruit weight per plant (kg) were recorded, as was tomato yield (kg m^{-2}). These variables were measured in two harvest stages from 56 to 70 DAT, corresponding to early and late maturity stages.

2.7. Data Analysis

An ANOVA–Tukey statistical analysis was performed to contrast treatments, along with a simple Pearson correlation analysis using the Minitab 16 and SAS version 9.0 statistical programs. Excel program v. 6.0 was used for figure design.

3. Results

3.1. Climatic Behavior in the Study

From January to June 2024, the highest rainfall was in February, with 23.6 mm; after this time, the pluvial precipitation was low, with values of 0, 0.4, 2, and 0.6 mm in March, April, May, and June, respectively. Maximum and minimum temperatures showed an upward trend during this period, reaching up to about 35 °C and 20 °C in June, respectively (Figure 3). These high temperatures are harmful to tomato plants, especially in conditions of water deficit, resulting in floral abscission during the fruiting step [9].

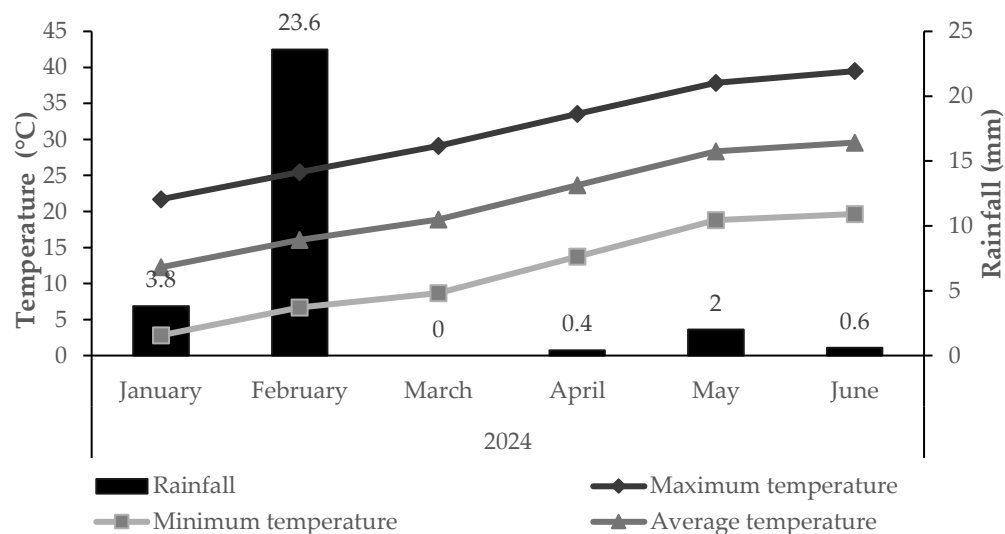


Figure 3. Temporal dynamic of maximum and minimum temperatures and rainfall in the experimental area during the crop cycle from February to June 2024.

3.2. Analysis of Variance

According to the analysis of variance, the significance for the source of variation in “blocks” was significant ($p < 0.05$) and highly significant ($p < 0.01$) for all the variables measured, which means that the randomized block design used was correct, based on the assumption that there was variation in uncontrolled factors in the experimental area and that they were part of the experimental error. Meanwhile, in the variation factor “treatments,” a highly significant response was recorded ($p < 0.01$), so it was correct to perform the statistical analysis with all possible interactions of soil moisture content and biostimulation treatments plus the control (Table 3).

Table 3. Analysis of variance of different morphometric variables, chlorophyll index, tomato yield, and productivity indicators.

VS	DF	Variable	Prob. > F	Significance
Blocks	2	Height plant	<0.0173	*
Treatments	7		<0.0001	**
Blocks	2	Stem thickness	<0.0142	*
Treatments	7		<0.0001	**
Blocks	2	Plant vigor	<0.0195	*
Treatments	7		<0.0001	**
Blocks	2	Relative water content	<0.0001	**
Treatments	7		<0.0001	**
Blocks	2	Chlorophyll index	<0.0001	**
Treatments	7		<0.0001	**
Blocks	2	Photosynthesis	<0.0001	**
Treatments	7		<0.0001	**
Blocks	2	Number of fruits harvested per plant ⁻¹	<0.2907	*
Treatments	7		<0.0001	**
Blocks	2	Fruit weight per plant ⁻¹	<0.0001	**
Treatments	7		<0.0001	**
Blocks	2	Yield m ⁻²	<0.0001	**
Treatments	7		<0.0001	**

VS is variation source; DF is degrees of freedom; * means significant probability ($P < 0.05$), and ** means highly significant probability ($P < 0.001$).

3.3. Plant Growth (Plant Height, Stem Thickness, and Plant Vigor)

At 42 DAT, the height and vigor of the tomato plants had values of 51.5 cm and 9.4, respectively, when algae extract was applied (Figure 4A), and 47.3 cm and 9.0 when zeolite was applied (Figure 4C), respectively. These values were statistically higher than those of the control under suboptimum soil moisture content ($25\% \pm 2$). Also, they were equivalent to increases of 20.3% and 10.5% in plant growth, and of 14.6% and 9.7% in plant vigor when applying AE and Z, respectively. Stem thickness did not vary among treatments involving the application of the biostimulant products, which were statistically equal to the control under suboptimum soil moisture content, but slightly higher than the treatments under deficient soil moisture content, mainly when Z was applied, with 7.5 mm, compared to the control, which was 8.4 mm. There was no effect on plant height or chlorophyll index content, since differences were not significant under suboptimum soil moisture conditions ($25\% \pm 2$) (Figure 4A). Stem thickness and plant vigor did not vary significantly under suboptimal soil moisture conditions ($25\% \pm 2$)

and only slight variations under deficient soil moisture content ($20\% \pm 2$) according to Figures 4B and 4C, respectively.

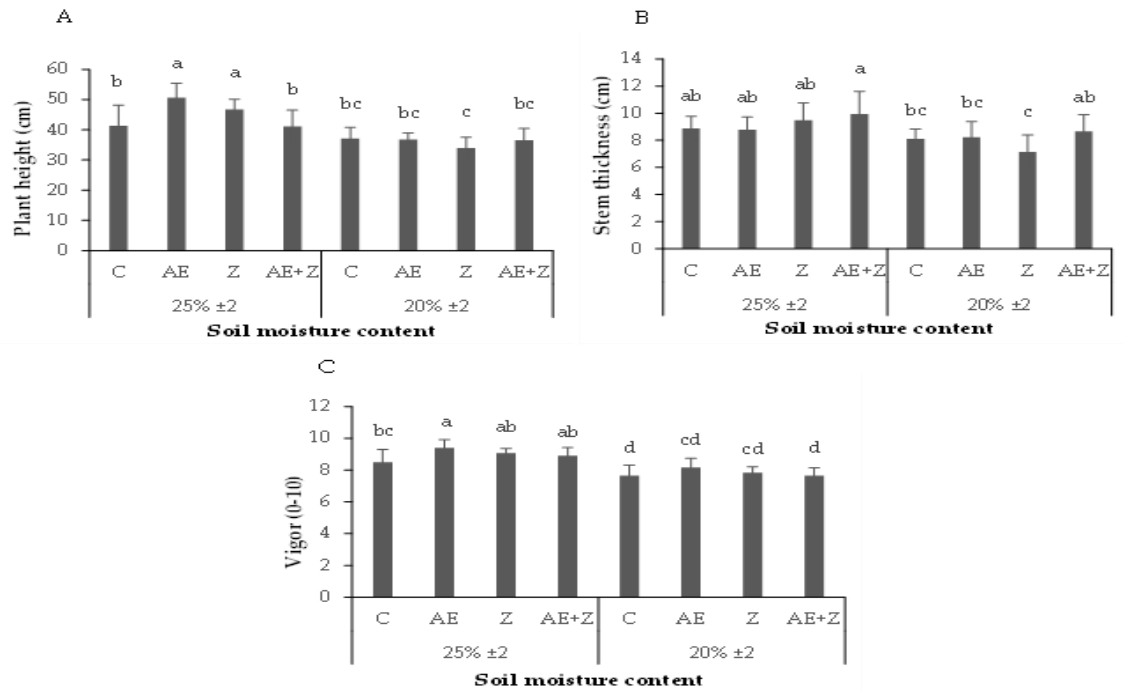


Figure 4. Effect of algae extract and zeolite on plant height (A), stem thickness (B), and plant vigor (0–10) (C) on tomato plant *Solanum lycopersicum* L. var. Saladette under suboptimum ($25\% \pm 2$) and deficient ($20\% \pm 2$) soil moisture contents. Tukey test ($p < 0.05$). In treatments C is control, AE is algae extract; Z is zeolite; and AE + Z is algae extract plus zeolite. Figures with the same letters on the bars are statistically equal. The vertical line on each bar is the standard deviation.

3.4. Physiological Indicators

Overall, relative water content (CRA) had low treatment effect, with a slightly better biostimulant response from AE, Z, and AE + Z under suboptimum soil moisture conditions compared to deficient soil water in the soil. Algae extract (AE) showed the greatest effect, with 4% higher CRA when applied at 50 L ha^{-1} under suboptimal soil moisture conditions ($25\% \pm 2$) compared to deficient soil water conditions ($20\% \pm 2$), and 2.6% more compared to the control (Figure 5A).

Chlorophyll index content registered a similar behavior, with results statistically higher than those of the control, with a value of

295.2 for the AE + Z treatment, representing an increase of 22.34% compared to the control under suboptimum moisture content. Second in importance were the treatments AE and Z, with values of 281.4 and 270.7, respectively, without a statistical difference between them. These results are equivalent to increases of 16.6% and 12.1% compared to the control. The lowest treatment values were obtained under deficient soil moisture content, which were not statistically different compared to the control (Figure 5B).

Photosynthesis showed a similar pattern to the behavior of the other plant growth and physiological variables, with a biostimulating effect at suboptimum soil moisture content ($25\% \pm 2$), where the best treatment was applying Z, which had an 18% higher photosynthesis rate compared to the control, and the lowest effect was applying AE, which was even lower than the control, with values of 20.9, 15.5, and 17.8 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. The AE + Z treatment was statistically equal to the control. No effect was found for these products when applied under deficient soil moisture contents ($20\% \pm 2$) (Figure 5C).

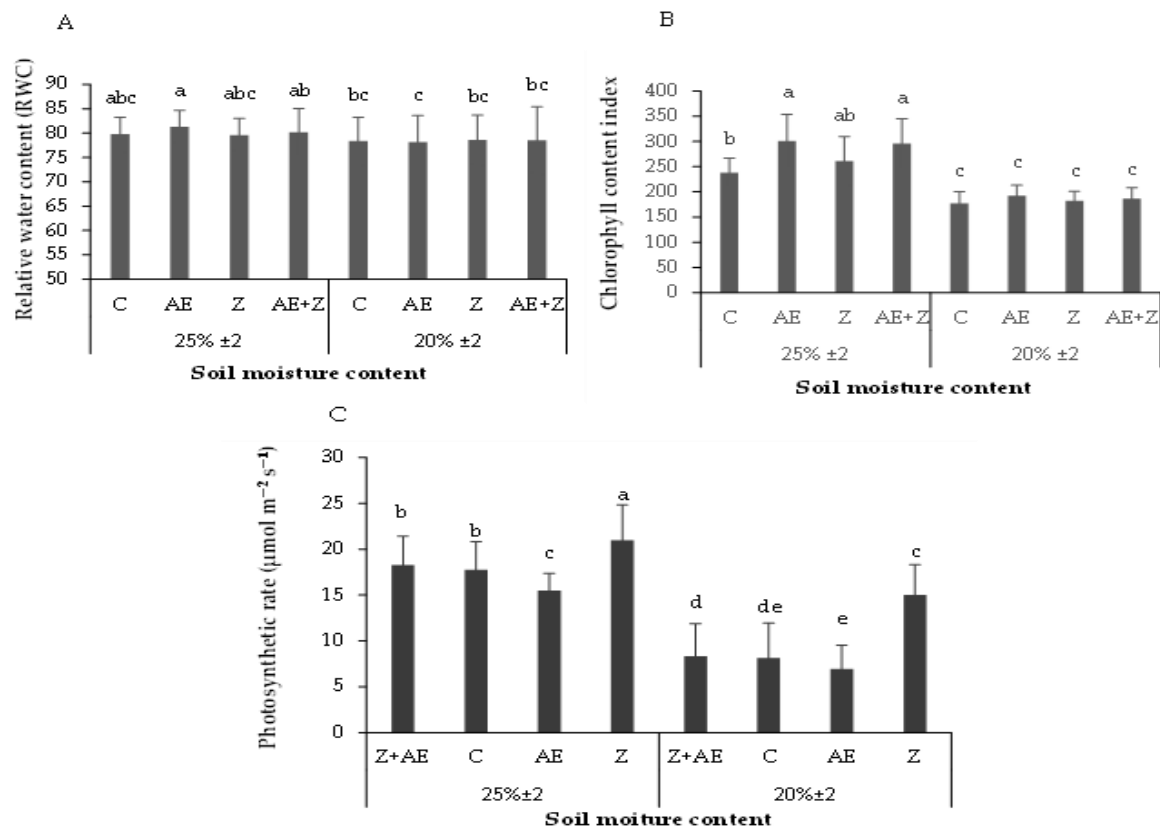


Figure 5. Effect of algae extract and zeolite on relative water content (A), chlorophyll index (B), and photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) (C) on tomato plant *Solanum lycopersicum* L. var. Saladette under suboptimum ($25\% \pm 2$) and deficient ($20\% \pm 2$) soil moisture contents at 42 DAT. In the treatments C is control, AE is algae extract, Z is zeolite, and AE + Z is algae extract plus Zeolite. Tukey test ($p < 0.05$). Figures with the same letters on the bars are statistically equal. The vertical line on each bar is the standard deviation.

3.5. Productivity Indicators and Tomato Yield

The number of tomato fruits per plant was significantly higher when AE, Z, and AE + Z were applied compared to the control, with values of 6.62, 5.62, and 6.41, respectively. The differences were not significant, which means an average increase of 248.4% compared to the control. In terms of fruit weight per plant and yield m^{-2} , application of Z and AE recorded $0.17 \text{ kg plant}^{-1}$ and 0.9 kg m^{-2} . These results mean increases of 425% and 333.3%, respectively, with both results under suboptimum soil moisture conditions. On the other hand, under deficient soil moisture conditions there was no

biostimulant effect of these products applied as AE, Z, or AE + Z, with values statistically equal to those of the control, with only a slight variation in the case of the number of fruits per plant, with better action when AE + Z was applied and a statistically lower value than the control when applying Z (Table 4).

Table 4. Tomato yield of *Solanum lycopersicum* L. var. Saladette as a result of the application of algae extract and zeolite under soil water deficit. The values are an accumulation of two harvest dates, 56 and 70 DAT.

Soil Moisture Content (%)	Organic and Inorganic Products	NFHPP	Fruit Weight (kg plant ⁻¹)	Yield (kg m ⁻²)
25% ± 2	Control	2.5 bc	0.04 ab	0.27 ab
		± 0.35	± 0.009	± 0.01
	Algae extract (AE)	6.62 a	0.16 ab	0.90 a
		± 0.17	± 0.02	± 0.04
	Zeolite (Z)	5.62 a	0.17 a	0.3 ab
		± 1.23	± 0.1	± 0.2
	AE + Z	6.41 a	0.15 ab	0.63 ab
		± 0.38	± 0.09	± 0.31
20% ± 2	Control	1.85 bc	0.03 b	0.18 b
		± 0.17	± 0.01	± 0.09
	Algae extract (AE)	2.00 bc	0.03 b	0.16 b
		± 0.35	± 0.01	± 0.1
	Zeolite (Z)	1.75 c	0.03 b	0.1 b
		± 1.41	± 0.02	± 0.06
	AE + Z	4.5 ab	0.04 ab	0.20 b
		± 0.7	± 0.02	± 0.12

Tukey test ($p \leq 0.05$). Figures with the same letters within the same column are statistically equal. NFHPP = number of fruits harvested per plant. ± The value of standard deviation.

Finally, a simple Pearson correlation analysis showed a highly significant correlation ($p < 0.01$) among all variables, which means that plant height, stem thickness, plant vigor, and chlorophyll content index contributed to a greater number of fruits harvested per plant (NFHPP), weight of fruits harvested per plant (WFHPP), and the consequent yield of tomato per unit of surface area (kg m⁻²) (Table 5).

Table 5. Correlation between growth, development, and physiological variables with yield and the components of tomato *Solanum lycopersicum* L. var. Saladette.

	CCI	PH	ST	VIG	NFHPP	WFHPP	YIELD
CCI	1.00000	0.54398	0.36819	0.52466	0.50271	0.53735	0.53735
		<.0001	0.0002	<.0001	<.0001	<.0001	<.0001
PH		1.00000	0.42651	0.53038	0.3890	0.50671	0.50671
			<.0001	<.0001	<.0001	<.0001	<.0001
ST			1.00000	0.42944	0.31766	0.29390	0.29390
				<.0001	0.0016	0.0037	0.0037
VIG				1.00000	0.57788	0.71558	0.71558
					<.0001	<.0001	<.0001
NFHPP					1.00000	0.73931	0.73931
						<.0001	<.0001
WFHPP						1.00000	1.00000
							<.0001
YIELD							1.00000

Simple Pearson correlation ($p < 0.01$) is highly significant. CCI = chlorophyll content index; PH = plant height; ST = stem thickness; VIG = vigor; NFHPP = number of fruits harvested per plant; WFHPP = weight of fruits harvested per plant.

4. Discussion

The accumulated rainfall in the study region from January to June 2024 was 30.4 mm, with maximum rainfall in February of 23.6 mm, which shows the non-interference of this environmental phenomenon in the moisture content of the soil in the open field since, at this time, the plants were growing in trays under controlled shade mesh conditions.

4.1. Plant Growth

The potential benefit of the organic and inorganic products on the plant was evident under suboptimum soil moisture content ($25\% \pm 2$), possibly by a biostimulant action influenced by better plant nutrition, as reported by Murnane et al. [24], and by improving the quantity of irrigation water [10]. Arminjon & Lefort [48] reported that applying plant growth-promoting microorganisms (PGPMs) at the seedling stage increased tomato productivity under salinity stress conditions, since the irrigation water in our experiment is based on using saline water pumped from a deep well [49].

Algae extract contains a great diversity of biomolecules such as phytohormones, amino acids, vitamins, and macro- and microelements to promote better plant vigor and growth [50]. Additionally, it contains active compounds such as polysaccharides, phenols, sterols, and betaines, which are biostimulants in the growth of plants [51], particularly in the early stages of tomato development, since the tomato seedling stage is sensitive to adverse environmental factors linked to the stomatal regulatory function [52]. Moreover, Ntanasi et al. [53] reported that tomato plants can respond and be resilient to adverse factors such as salinity through biostimulant activity induced by applying nutrient solutions based on algae extracts.

In addition, Sfechis et al. [54] conducted a review to highlight the potential of zeolite-based amendments in agriculture, reporting several benefits to plants. Chávez et al. [55] found that applying zeolite at the pre-harvest phase improves the growth of the tomato plant (*Solanum lycopersicum* L.). Zhang et al. [56] reported an improvement in growth parameters such as plant height, stem thickness, root length, and photosynthesis rate in tomatoes by applying zeolite. Additionally, applying macronutrients and zeolite together improves the physical-chemical properties of the soil and makes efficient use of water and nutrients for better plant growth [57,58]. According to Méndez et al. [59], tomato seedlings in a substrate mixture with 30% zeolite had improved leaf area, root length, and stem diameter, but there was no significant effect on the chlorophyll index. Deshmuck et al. [60] reported that Si, which is contained in zeolite, could improve stomatal function and root hydraulic conductance by regulating aquaporins, while aluminum, also contained in zeolite, can stimulate plant growth, modulate the color of flowers, and increase the vase life of some plant species [61].

Thus, positive plant growth response shown by indicators such as plant height, stem thickness, and vigor, with treatment average values of 47.2 cm, 9.84 mm, and 9.1 when AE, Z, or AE + Z were applied, respectively, under suboptimum soil water content 28% below that of FC may be due to an increase in plant nutrient availability [16,17,24,35]. In particular, the application of Z showed, additional availability of nutrients due to better soil moisture retention [34]. However, in deficient soil moisture content (11% upper of PWP), the tomato plants were unable to respond to the biostimulant benefits applied either as AE, Z, or AE + Z, where the growth and yield indicators were negatively affected.

4.2. Physiological Indicators

Relative water content (RWC) is directly related to the plant's water potential [62], which is an indicator of tissue water content and is essential for continued metabolic activity and plant growth [63]. This variable was only biostimulated under suboptimal soil moisture content (25% $\pm$ 2), not under deficient conditions (20% $\pm$ 2), where biostimulants apparently no longer achieved their beneficial action and the response was equal to that of the control. The beneficial action at medium soil moisture content was achieved by applying AE, Z, or AE + Z at doses of 50 L h⁻¹, 10 f ha⁻¹, or 25 L ha⁻¹ + 5 t ha⁻¹, respectively, mainly when AE was applied, suggesting that this organic product may be a viable alternative for mitigating water stress by maintaining better cell turgor and plant vigor [64,65] (Figure 5A).

Chlorophyll index is an indicator of photosynthesis, and this variable showed the lowest values of plant growth and chlorophyll index in the deficient soil moisture content (20% $\pm$ 2), with no statistical difference among treatments under deficient soil moisture content in comparison to the control (Figure 5B). These results suggest that the tomato plant is highly sensitive to water deficit, in accordance with the findings reported by Florido et al. [66]. AE and AE + Z were the best biostimulating treatments for the chlorophyll index under moderate soil moisture conditions (25% $\pm$ 2), compared to the control and Z. These results may be indicative that AE and AE + Z have an effect on chlorophyll stability in addition to the effect on maintaining the turgor of the cells through a better RWC, which allows better growth and development [64]. The effects of the applied biostimulants may be achieved through different routes, such as the availability of macronutrients and vitamins, and growth promotion [67].

Photosynthesis is directly linked to the water and chlorophyll content of the plant, due to the relationship between the stability of the cellular organelles responsible for the metabolic activity for the growth and development of the plant [56,68]. In this case, the best treatment was the application of zeolite, which suggests that it is related to the dual function of this inorganic compound, as a water retainer in the soil, and through the contribution of some microelements such as Si [28,29] and Al [30,31], among other minerals that act as nutrients. Also, Z could influence a greater availability of macro- and micronutrients for the plant by maintaining better moisture content in the soil [23,24,]. Algal extract

did not seem to have a biostimulating effect on photosynthetic activity under suboptimal soil moisture conditions ($25\% \pm 2$) and even less under water deficient conditions for the plant ($20\% \pm 2$).

4.3. Productivity Indicators and Tomato Yield

The application of either of the two products evaluated in this study failed to mitigate water stress in tomato plants under deficient soil moisture content. This suggests that the plant was unable to respond to the biostimulant action of algae extract and zeolite under extreme water stress conditions due to a general weakening of vigor and a high rate of flower absorption, with a significantly lower fruit production per plant and consequently a lower yield [69]. The worst and best response of chlorophyll content under deficient and suboptimum conditions of water availability in the soil is a result of the genotype–environment interaction effect, as reported by Majid et al. [70] in their study on chlorophyll content and yield of different maize cultivars under water stress conditions.

This means that if the objective is to make efficient use of water in drylands, medium–high-level irrigation can be sufficient for growing tomatoes and obtaining a moderate yield under open-field conditions in drylands. However, when the necessary water is available, irrigation can be applied to FC, where the response can be improved in terms of growth and tomato yield using either algae extract or zeolite or both together.

4.4. Tomato Yield

In terms of tomato yield, a very similar dynamic was recorded in the treatments under deficient soil moisture content ($20\% \pm 2$) but with less difference between them over time, where the control practically behaved the same as or slightly better than the treatments where the algae extracts and zeolite products were applied, mainly at the end of the growth stage, confirming the null compensatory or mitigation effect of these products under deficient soil moisture content. Contrary to these results, but under optimum irrigation conditions, Soca & Lorente [71], in a study with a mixture of zeolite and compost, found a positive effect on tomato yield. This suggests that the benefit of some biostimulants, such as algae extracts or zeolite, occurs under suboptimum (28% low FC) or deficient soil water (11% upper PWP) conditions, but the effect of mitigating environmental stress not occurs when the water stress is induced by soil moisture content close to the wilting point.

Therefore, in agricultural areas with sufficient and high-quality water availability, biostimulant products can be useful for productivity purposes, and in agricultural areas with scarce and poor-quality water, these products can be useful for mitigating water stress and improving crop growth and yield.

The above confirms the beneficial action of the two organic and inorganic products, applied either together or separately, but only when there is a suboptimum soil moisture content close to FC. The productivity increases shown in tomato yield and its components, such as the number of fruits per plant and the weight of fruits per plant, are sufficiently encouraging to justify promoting tomato productivity in areas where water is not a limiting resource. The average tomato yield under suboptimum soil moisture ($25\% \pm 2$) in the open field is 9 t ha^{-1} when applying algae extract, for instance, which can be considered relatively low. However, the very unfavorable environmental conditions of the region must be considered, particularly the extremely high temperatures of greater than 35°C in summer and water with a high salt content with an electrical conductivity of approximately 2.5 dS m^{-1} , very close to the maximum value of 3 dS m^{-1} allowed for irrigation water based on the Official Mexican Standard NOM-127-SSA1-2021 [72], although its effect is cumulative, and the value can reach and exceed this limit over time. Mesa et al. [73] reported that tomato is widely affected by temperatures above 32°C , resulting in decreased fruit production even when the plant produces antioxidant activity to mitigate heat stress, while Biglia et al. [74] showed that tomato plants are sensitive to water and salt stress, similar to the conditions under which the tomato plants were studied in the open field in this study.

Zeolite and algae extracts contain beneficial elements such as silicon and aluminum. There is evidence that silicon modulates the secondary metabolism of plants, thereby increasing antioxidant responses to biotic and abiotic stresses [29]. Silicon may alleviate the effects of several environmental stress factors, such as drought, salinity, pests, and diseases [61]. In tomato grown under suboptimum soil moisture conditions and high temperatures, it is likely that the silicon contained in the zeolite and algae extract contributed to a better response by reducing deleterious effects and promoting a greater number of fruits, with a higher weight and final yield than control plants.

Also, extreme temperatures recorded from March onwards could have influenced the response of the tomato yield. This is because the plant is very susceptible to high temperatures, causing flower abortion, which reduces the number of fruits per plant and, therefore, decreases the tomato yield per hectare [74]. This extreme environment is the main reason why tomato cultivation in the region is carried out under greenhouses, which increases production costs and reduces the harvested area [75], hence the interest in evaluating some innovative management practices that can mitigate plant stress under open-field conditions.

Overall, the lack of response to algae extracts and zeolite under deficient soil moisture content close to PWP suggests that the plant is entering a severe stress condition that inhibits the response to the biostimulating effects of these organic and inorganic products. Thus, a medium-high and possibly optimum water content to trigger the physiological processes for good plant growth and yield is required [76].

Finally, according to the Pearson Simple Correlation, the results suggest that with a higher chlorophyll content, there is greater photosynthetic activity that produces a greater quantity of photo-assimilates, which results in greater plant growth, better vigor, and, therefore, a higher tomato yield per unit of surface area. Murshed et al. [77] reported antioxidant activity as a mitigation mechanism for water stress during the fruiting phase of tomato (*Solanum lycopersicum* L.). Xiao et al. [78] reported that the intensity and quality of light influence plant growth and leaf size in tomatoes. Studies in tomato found that chlorophyll content, photochemical efficiency of PSII, and the activities of superoxide dismutase (SOD) and catalase (CAT) increased due to silicon application, reducing the negative effects of salt stress. One of the possible effects of algae extracts and zeolite may be related to their silicon content [79]. It is recognized that a series of adaptation strategies are involved in the tomato plant, mainly the redox activities of antioxidants that activate cellular processes to mitigate oxidative stress and induce acclimatization to drought [80]. In this way, plants have developed complex physiological and biochemical adaptations to adjust and adapt to a variety of environments that produce stress [81].

5. Conclusions

These results provide some highlights into the use of algae extract applied at a rate of 0.8 mL L⁻¹, or algae extract and zeolite

(AE + Z) at a rate 0.4 mL L⁻¹ and 10 t ha⁻¹, respectively, since they were the best treatments, with tomato yield up to 333%, and 442% higher than the control, respectively, under suboptimum soil moisture content (25% ± 2). This increase in the tomato yield was obtained due to a favorable response of biostimulation in growth and physiological indicators such as plant height and plant vigor, as well as chlorophyll index, relative water content, and photosynthesis. Tomato plants were unable to respond to the biostimulant action of these products to mitigate plant stress induced by deficient soil moisture content (20% ± 2). This is relevant to water-scarce agricultural areas, where more efficient water use is imperative. The findings of this study show that tomato cultivation with moderate irrigation is possible by supplementing the application of biostimulants such as algae extract and zeolite, which mitigate water stress without the need for field capacity irrigation (30%), thereby saving water in dry areas.

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