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The role of biochar and some biostimulants in enhancing the ability of canola seedlings to tolerate salt stress

Heba Kalaf Razaq

Department of Soil Sciences and Water Resources/ College of Agriculture/ Wasit University

Corresponding author: hibaalquraishy@uowasit.edu.iq

Abstract:

The pot study was conducted in the research laboratory of the Department of Soil Sciences and Water Resources, College of Agriculture, University of Wasit to determine the effects of the combination of *Pseudomonas fluorescens*, Bacillus subtilis and biochar on the growth characteristics of Canola seedlings under salinity stress. The growthchamber experiment included three salinity levels of NaCl (0 mM NaCl, 50 mM NaCl and 100 mM NaCl), three biochar levels (0, 5 and 10 %) before planting, and add the biostimulants (*P. fluorescens* and *B. subtilis*) at a rate of 100 ml per pot separately after 3 weeks of planting. The results of the study showed a significant negative effect of salt levels on some traits and indicators of vegetative growth of canola seedlings, and on the amount of chlorophyll and the percentage of major nutrients (NPK). The bio-agents and Biochar applications mitigated the negative influence of salinity stress on plant growth characteristics of canola seedlings, enhancing chlorophyll content and plant nutrient element uptake. Therefore, it can be concluded that combining biochar and biostimulants could be used to minimize the detrimental impacts of salinity stress conditions in canola seedlings.

Keywords*: Pseudomonas fluorescens, Bacillus subtilis, biochar, Canola*

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دور الفحم الحيوي وبعض المحفزات الحيوية في تعزيز قدرة شتلات الكانولا على تحمل الجهد الملحي

هبه كلف رزاق

قسم علوم التربة والموارد المائية / كلية الزراعة / جامعة واسط

الخلاصه:

أجريت دراسة الأصص في مختبر أبحاث قسم علوم التربة والموارد المائية / كلية الزراعة / جامعة واسط لتحديد تأثير توليفة بكتيريا *Pseudomonas fluorescens* و *Bacillus subtilis* والفحم الحيوي على خصائص نمو شتلات الكانولا تحت ضغط الملوحة. وتضمنت تجربة غرفة النمو اضافة ثلاثة مستويات من ملوحة كلوريد الصوديوم (0 ملي مولاري، 50 ملي مولار، و100 ملي مولاري)، وثلاثة مستويات من الفحم الحيوي (0، 5، 10%) قبل الزراعة، واضافة المحفزات الحيوية (*P. fluorescens* و *B subtilis*) النامية على الوسط Nutrient Broth بمعدل 100 مل لكل اص بصورة منفردة بعد 3 اسابيع من الزراعة. أظهرت نتائج الدراسة التأثير السلبي مستويات الاملاح بشكل ملحوظ في بعض صفات ومؤشرات النمو الخضري لشتلات الكانولا وكمية الكلوروفيل ونسبة العناصر الغذائية الكبرى NPK، كما خففت العوامل الحيوية وتطبيقات

الفحم الحيوي من التأثير السلبي لإجهاد الملوحة على خصائص نمو شتلات الكانولا، مما عزز محتوى الكلوروفيل وامتصاص العناصر الغذائية. لذلك، يمكن الاستنتاج أن الجمع بين الفحم الحيوي والعوامل الحيوية يمكن أن يقلل من الآثار السلبية لإجهاد الملوحة على شتلات الكانولا.

الكلمات المفتاحية: *fluorescens*، *Bacillus subtilis* ، الفحم الحيوي ، الكانولا.

Introduction

Canola (*Brassica napus* L.) is an oilseed crop, commonly known as rapeseed (or colza). It is widely used as a source of oil and protein in food and industrial applications, as a medicinal plant, as a tourist attraction, or as an ornamental plant due to its diverse flower colors. All parts of rapeseed are useful, even its waste, which can be used for animal feed or recycled (Raboanatahiry et al., 2021). Global threats such as climate change, land degradation, environmental pollution, water shortages, and rapid population growth are causing economic, social, and environmental damage (Yuan et al., 2024). Ensuring food security has become an increasing challenge due to the growing global population and the ongoing impact of climate change. By 2030, the world population is expected to reach 8.5 billion, with a corresponding increase in food demand, totaling 11.6 billion tons (Ahmed et al., 2021). If current trends continue, the number of people suffering from hunger is projected to exceed 670 million by 2030 (FAO, 2022). Therefore, a 60–100% increase in crop productivity is needed to meet future food needs. However, achieving this goal presents significant challenges. The current agricultural sector and food production systems face multiple threats, including climate change, land degradation, water scarcity, the impact of the recent pandemic, and political conflicts (Lau et al., 2023). Soil nutrient deficiencies and invasions by plant pathogens or insects are also among the most significant destructive factors affecting global food production. Synthetic fertilizers and chemical pesticides are widely used to combat these problems. However, these pesticides have negative effects on microbial ecosystems and their functions (Thepbandit and Athinuwat, 2024). These stressors have significantly limited agricultural productivity worldwide. In the face of stressful conditions, traditional breeding methods, biotechnological approaches, the use of molecular markers, genetic modification techniques, and the development of resistant varieties, cultivars, or genotypes are among the most effective solutions for plant production. However, these methods can be time-consuming, expensive, and highly complex (Hamdan and Tan, 2025). Consequently, a 60–100% increase in crop yields is needed to meet future nutritional needs. Although the rhizosphere represents a closed nutrient reservoir containing all the macro- and micronutrients required by plants, it is the region where microbial activity is highest (Vejan et al., 2016).

Rhizosphere microorganisms have demonstrated their ability to enhance or manage plant nutrients to encourage plant growth, leading to increased yield and quality by converting organic and inorganic materials surrounding the rhizosphere into available plant nutrients (Thepbandit and Athinuwat, 2024). The use of biostimulants has been proven to be an effective tool and a suitable form of management, ensuring the efficient use of natural resources, food security, and positively impacting plant growth and productivity. Plant growth-promoting rhizobacteria (PGPR) are microbes associated with plant roots that can enhance plant growth in various ways, such as producing plant hormones and molecules to improve growth or increase mineral nutrition. They can colonize all root environments at all stages of crop growth and can directly influence plant growth and development by modifying plant hormone levels and enhancing the uptake of nutrients, such as potassium, phosphorus, nitrogen, and essential minerals (Sun et al., 2024). The bacterium Pseudomonas fluorescens is well known for its plant growth-promoting properties, including fixing atmospheric nitrogen, solubilizing phosphorus and potassium, and also producing plant hormones, degradative enzymes, volatile organic compounds, antibiotics, and secondary metabolites during stressful conditions. These compounds stimulate plant growth by inducing systemic resistance and inhibiting the growth of pathogens. Furthermore, Pseudomonas also protects plants during various stress conditions, such as heavy metal pollution, osmotic stress, temperature, oxidative stress, and others (Mehmood et al., 2023).

The genus Pseudomonas is often used as a crop inoculant due to its ubiquity and diverse metabolic potential, which enhances plant growth in numerous ways, including ACC deaminase activity, nutrient uptake, and antioxidant properties (Chandra et al., 2018). Selected strains of *P. fluorescens* have been used as seed inoculants in various crop plants to stimulate growth parameters and increase crop yield. These bacterial agents rapidly colonized the roots of potato, radish, and sugar beet, resulting in significant increases in plant productivity (Saranraj et al., 2022). *Pseudomonas fluorescens* may also help stimulate growth-related processes in cabbage, particularly by promoting rapid seedling growth and reducing transplant shock (Karungi et al., 2010). Bacillus bacteria can stimulate plant growth through a variety of strategies. Several studies have demonstrated the effectiveness of different strains of *Bacillus subtilis* in reducing the harmful effects of abiotic stresses.

Patel et al. (2023) reported that B. subtilis ER-08 exhibited significant growth-promoting properties in fenugreek plants in greenhouses, producing 83.7 g/ml of gibberellins (GA3) and 176.1 g/ml of indole-3 acetic acid. Furthermore, the BST strain also produced hydrogen cyanide, iron chelators, exopolysaccharides, ammonia, and various enzymes such as cellulase, protease, pectinase, and chitinase. The BST isolates showed good performance under several abiotic stress conditions, such as salinity (4 and 6 dS/M), pH (5, 7, and 9), drought (PEG6000 at 10%, 20%, and 30%), and temperature (25°C, 35°C, 37°C, and 55°C). Interestingly, previous studies have shown that if rhizobacteria possess appropriate solute transport systems or the ability to synthesize them, they can develop salt tolerance through the accumulation of appropriate solutes (Nagata et al., 2002). According to previous studies, Bacillus bacteria can achieve salt tolerance by accumulating glutamic acid and potassium ions in the cytoplasm as their primary solute and ion, respectively (Ikeuchi et al., 2003).

Biochar produced from organic waste using pyrolysis has also been shown to improve soil properties and quality by altering its structure, increasing nutrient content, and enhancing water-holding capacity, among other things (Hossain et al., 2020). Furthermore, biochar can enhance soil fertility by retaining nutrients and stimulating microbial activity (Minhas et al., 2020). Biochar is an emerging soil conditioner used to restore soil health. Biochar may be beneficial for soil reclamation due to its unique physical and chemical properties, including its high carbon and mineral fixation capacity. Furthermore, biochar has the potential to reduce the damage caused by environmental stress to plants (Agarwal et al., 2022). Given the current global situation of increasing human intervention and other natural processes that harm the ecosystem, soil researchers and stakeholders must find effective measures and reliable technologies to restore soil quality and fertility. This can enable the development or improvement of strategies to avoid or reduce crop losses, as well as to increase interest in propagating valuable traits in canola. Therefore, this study aims to find appropriate solutions, in line with sustainable development and environmental conservation, to enhance the ability of canola seedlings to withstand salt stress, the most important type of abiotic stress.

Materials and Methods

Study Site

The study on microbiological development was conducted in the Microbiology Laboratory of the Field Crops Department, College of Agriculture, University of Wasit. The laboratory experiment on pot culture was conducted in the Soil Science Research Laboratory of the Soil Science and Water Resources Department, College of Agriculture, University of Wasit.

Canola Seeds

Canola seeds of the AACC variety were obtained from the Field Crops Laboratory of the Field Crops Department, College of Agriculture, University of Wasit. The seeds were sterilized in a 50% sodium hypochlorite solution containing 0.2% Tween 20 for 10 minutes, rinsed five times with sterile distilled water, and stored at 4°C for 24 hours to promote germination and break the dormancy phase. The sterilized seeds were planted in soil to a depth of 1 cm under controlled conditions for 8 hours of light and 16 hours of darkness at a constant temperature of 22°C. 3-3- Adding Biochar Levels

The experiment used pre-made biochar produced by Miegos, packaged in 8 kg plastic bags, and manufactured from the remains of citrus trees used in the experiment. Prior to using the biochar, the biochar sample used for analysis was oven-dried at 68°C for 48 hours, then ground and screened to achieve particle sizes between 2.0 and 4.0 mm in diameter. The biochar was thoroughly mixed with the soil and added at rates of 0, 5, and 10% of the soil weight to 3 kg pots before planting (KUL, 2022).

Adding Sodium Chloride Levels

Salt levels were added approximately one week after the seedlings were planted in the pots. The saline treatments were applied with a nutrient solution containing zero (50) and (100) mM sodium chloride. At the end of the study, the electrical conductivity of the soil in all treatments was determined using a portable EC meter, in dS/m^{-1}.

Addition of Biostimulants

Biostimulants, Pseudomonas fluorescens and Bacillus subtilis, grown on nutrient broth medium, were obtained by Dr. Jawadin Talib Al-Kurani, Department of Plant Protection, College of Agriculture, University of Wasit. They were propagated on N.B. medium in 250 ml glass flasks. The flasks were sterilized in an autoclave at 121°C for 15 minutes. The medium was then inoculated with the bacteria individually, and 1 ml of the bacterial culture grown on N.B. The flasks were thoroughly mixed and incubated at 28°C for 48 hours (Bakker et al., 1986). Biostimulants were added at a rate of 100 ml per pot, individually, approximately one week after the seedlings were planted in the pots. 3-6: Pot Experiment

The experiment was conducted with 15 treatments, each with three replicates. Each replicate was housed in a 3 kg pot planted with ten AACC canola seeds. These seeds were thinned after germination to only five seedlings. The study was implemented as a factorial experiment according to a randomized complete block design (RCBD).1

1. A control treatment without any additions, designated "Control."

2. A salt stress level (50 mM), designated "S1."

3. A salt stress level (100 mM), designated "S2."

4. A 5% charcoal addition level, designated "B1."

5. A 10% charcoal addition level, designated "B2."

6- Treatment with the addition of *P. fluorescens* (PF)

7- Treatment with the addition of *B. subtilis* (BS)

8- Salt stress level (50 mM) + 5% charcoal (S1 B1).

9- Salt stress level (50 mM) + 10% charcoal (S1 B2).

10- Salt stress level (50 mM) + *P. fluorescens* (S1 PF).

11- Salt stress level (50 mM) + 10% charcoal (S1 BS).

12- Salt stress level (100 mM) + 5% charcoal (S2 B1).

13. Salt stress level (100 mM) + 10% charcoal addition, symbolized as S2 B2.

14. Salt stress level (100 mM) + *P. fluorescens* addition, symbolized as S2 PF.

15. Salt stress level (100 mM) + 10% charcoal addition, symbolized as S2 BS. Soil samples were taken and their chemical and physical properties were analyzed, as shown in Table (1).

Table 1. Physical and chemical properties of agricultural soil

Characteristics	Units	Values
pH	-	7.80
EC	d.s m^{-1}	1.78
Total N	g. kg^{-1}	0.11
Ready P	mg . kg^{-1}	0.06
K+	mmole . L^{-1}	0.10
Ca++	mmole . L^{-1}	3.21
Mg++	mmole . L^{-1}	1.20
Na+	mmole . L^{-1}	2.41
Cl-	mmole . L^{-1}	3.71
HCO-3	mmole . L^{-1}	4.00
Organic Matter	g. kg^{-1}	3.16
Lime	g. kg^{-1}	224
Gypsum	g. kg^{-1}	0.191
Soil Separators		
Sand	g. kg^{-1}	810
Silt	g. kg^{-1}	150
Clay	g. kg^{-1}	40
Texture		LS

After the experiment was completed, some plant growth parameters were calculated. Plant height was determined by measuring the distance from the root crown to the top of the plant using a ruler, as well as the number of branches and leaves. The green leaf area of the seedlings was measured in square centimeters using a leaf area meter (LI-3100, LI-COR). After measurements and analyses were completed, fresh plant and root samples were harvested after a 30-day growth period, and their fresh weight was determined. The dry weight of the seedlings and roots, which were dried at 70°C for 48 hours, was then determined using a precision balance (±0.001 g) and ground for mineral analysis. The relative leaf water content (LRWC) was determined using the method of Smart and Bingham (1974). The amount of chlorophyll was typically measured using a device called a chlorometer, such as the SPAD, and the plant macronutrient levels (NPK) were measured.

Statistical Analysis

The study was conducted as a factorial experiment according to a randomized complete block design (RCBD) with three replicates, with five plants per replicate. Averages were compared using the least significant difference (LSD) test to demonstrate statistical differences between treatments at a 5% probability level. Data were analyzed using the Genstat program.

Results and Discussion

Vegetative Growth Indicators of Canola Seedlings

The study results, shown in Table (2), revealed a negative effect of sodium chloride levels (50 mM and 100 mM) on some vegetative growth indicators of 30-day-old canola seedlings. Growth indicators decreased compared to the control treatment. The plant height, number of branches, number of leaves, fresh weight of the plant, dry weight of the plant, and leaf area in the control treatment were 30.61 cm, 8.02 branches per plant, 50.00 leaves/plant, 290.36 g, 85.05, and 80.6 cm2, respectively. While they were 24.78 cm, 6.81 branches per plant, 45.82 leaves/plant, 234.09 g, 71.88, and 73.8 cm2, respectively, in the 50 mM salt level treatment. Some indicators decreased significantly at the 100 mM salt level, reaching 23.06 cm, 6.13 branches per plant, 40.47 leaves/plant, 229.60 g, 60.15, and 61.8 cm2, respectively.

The results in Table (2) showed the role of biochar. Biostimulants in reducing the negative effect of salt stress on the growth of canola seedlings compared to treatments of adding salt levels only. The prominent role of microorganisms inhabiting the roots of canola plants was that they reduced the severity of the effect of the tested salt levels and even significantly increased growth indicators compared to the control treatment without addition, as the plant height, number of branches, number of leaves, fresh weight of the plant, dry weight of the plant and leaf area in the control treatment reached 33.78 cm and 11.11 branches per plant and 65.38 leaves/plant and 327.28 g and 88.08 and 83.8 cm2 respectively when adding bacteria Pseudomonas fluorescens at the 50 mM salt level. It also led to a significant increase in vegetative growth indicators at the 100 mM salt level, as it reached 37.33 cm and 10.56 branches per plant and 64.31 leaves/plant. 303.18 g, 84.81 g, and 76.0 cm², respectively. *Bacillus subtilis* also played a significant role in increasing growth indicators at a 50 mM salt concentration, reaching 33.06 cm², 11.83 branches per plant, and 64.72 leaves per plant, and 331.00 g, 88.66 g, and 91.4 cm², respectively. The same indicators reached 37.33 cm², 12.56 branches per plant, and 62.50 leaves per plant, and 310.20 g, 4266 g, and 77.2 cm², respectively.

These results are consistent with the results of previous studies that indicated the effect of salt stress on reducing growth indicators of eggplant seedlings (Kul, 2022, Yuan et al., 2023), as well as in radish plants, where high soil salinity led to a decrease in plant growth, photosynthetic pigment content, plant hormone content (indole-3-acetic acid (IAA) and gibberellic acid (GA3)), and mineral absorption, compared to non-saline soil (Mohamed and Gomaa, 2012). Other studies indicated the role of biochar in increasing and enhancing the ability of field crop seedlings to resist various abiotic and environmental stresses, including salt stress. Yuan et al. (2023) indicated that adding biochar significantly improved the physical and chemical properties of the soil by enhancing the stability of aggregates, porosity, and water-holding capacity, increasing the cation exchange capacity and organic carbon in the soil, and the availability of nutrients, in addition to reducing bulk density and alleviating salt stress. It can also improve biological health. For soil, particularly enhancing microbial biomass, facilitating enzyme activity, and ultimately increasing plant growth. However, biochar and biogrowth promoters applications improved rapeseed growth under salt stress.

The use of *Bacillus subtilis* Z-12 and *B. aryabhattai* Z-48 had a significant positive effect on canola growth parameters under salt stress. Z-48 significantly outperformed the other strains in increasing shoot length, root length, fresh biomass, and dry biomass under both normal and salt stress conditions (200 mM NaCl) (Khan et al., 2025). Mohamed and Gomaa (2012) reported that *P. floursecns* and *B. subtilis* were able to increase the growth of radish (Raphanus sativus) under salt stress, which could be attributed to phytohormones and iron chelators production. Furthermore, *B. subtilis* produced catalase, protease, cellulase, and amylase. On the other hand, *P. floursecns* produced both catalase and protease. Bacterial strains that exhibit catalase activity are known to be highly resistant to environmental, mechanical, and chemical stresses. The ability of these bacteria to increase plant growth under salt stress is likely due to the production of the enzyme ACC deaminase. This enzyme facilitates plant growth because it sequesters and decomposes plant-produced ACC (a direct precursor for ethylene synthesis in higher plants) into α-ketobutyrate and ammonia. The bacteria use the ammonia produced from ACC as a nitrogen source, which reduces ethylene levels (Mohamed and Gomaa, 2012; Joseph et al., 2007).

Table 2. Effect of salinity levels, biochar, and some biostimulants on some vegetative growth parameters of canola seedlings

Treatments	Plant height (cm)	Number branches/ plant	Number of leaves	Fresh weight (g)	Dry weight (g)	Leaf area (cm2)
Control	30.61*	8.02	50.00	290.36	85.05	80.6
S1	24.78	6.81	45.82	234.09	71.88	73.8
S2	23.06	6.13	40.47	229.60	60.15	61.8
B1	30.33	8.56	64.54	312.83	88.10	80.2
B2	31.61	9.02	69.81	323.59	90.28	86.6
PF	32.78	12.11	77.58	376.24	92.48	93.8
BS	35.06	12.83	81.36	355.93	90.56	98.3
S1 B1	37.33	8.56	60.52	295.03	83.21	66.2
S1 B2	31.61	9.02	57.27	291.36	84.28	69.6
S1 PF	33.78	11.11	65.38	327.28	88.08	83.8
S1 BS	33.06	11.83	64.72	331.00	88.66	91.4
S2 B1	33.33	7.56	57.52	271.17	81.11	66.8
S2 B2	35.06	7.83	55.81	261.20	82.21	66.1
S2 PF	37.33	10.56	64.31	303.18	84.81	76.0
S2 BS	37.33	12.56	62.50	310.20	86.42	77.2
L.S.D. 0.05	**2.39**	**0.504**	**10.32**	**9.11**	**1.345**	**3.61**

The effect of salinity levels, biochar, and some biostimulants on some chlorophyll content and macronutrients in canola seedlings. The results, shown in Table (3), showed a negative effect of sodium chloride levels (50 mM and 100 mM) on some chlorophyll content and the percentage of macronutrients (N, P, K) in 30-day-old canola seedlings. These decreased compared to the control treatment. The chlorophyll content and the percentage of nitrogen, phosphorus, and potassium in the control treatment were 1.585 mg/100 g, 1.225%, 0.559%, and 2.158%, respectively. They reached 1.752 mg/100 g, 1.201%, 0.356%, and 1.850%, respectively, when treated at a 50 mM salinity level. Some indicators decreased significantly at a 100 mM salinity level. It reached 1.590 mg/100 g, 1.214%, 0.378% and 1.637%, respectively. The results in Table (3) showed the role of biochar and biostimulants in reducing the negative impact of salt stress on the amount of chlorophyll and the percentage of nutrients nitrogen, phosphorus and potassium for canola seedlings compared to treatments that added only salt levels. They increased significantly over the comparison treatment without addition, reaching 1.752 mg/100 g, 2.305%, 0.856% and 2.835% respectively when adding *Pseudomonas fluorescens* bacteria at the 50 mM salt level. They also led to a significant increase in vegetative growth indicators at the 100 mM salt level, reaching 1.757 mg/100 g, 1.866%, 0.621% and 2.280% respectively. *Bacillus subtilis* also played a significant role in increasing growth parameters at a 50 mM salt concentration, reaching 1.590 mg/100 g, 1.814%, 0.778%, and 2.737%, respectively. The same parameters were 1.863 mg/100 g, 1.766%, 0.670%, and 2.280%, respectively.

Previous studies have indicated the negative impact of salt stress on the total chlorophyll content of plants. It has been reported that salt stress causes an enrichment of reactive oxygen species in plants, disrupting chlorophyll synthesis (Kul, 2022). Salt stress has also been found to cause a decrease in the amount of photosynthetic pigments (chlorophyll and carotenoids) in the light-harvesting complexes of photosystems (Parida and Das, 2005; Khan et al., 2025). It has been shown that excessive salt levels destroy chlorophyll by leading to the accumulation of sodium and chloride ions, which disrupts cellular metabolism and causes the decomposition of cell organelles in tissues (Ayaz Tilkat et al., 2019; Khan et al., 2025). Additionally, salt stress causes a decrease in chlorophyll content in plants by reducing chlorophyll synthesis, increasing chlorophyllase activity, and destroying pigment proteins (Anower et al., 2013). Khan et al. (2025) reported that inoculating rapeseed with the bioactive agents B. subtilis Z-12 and *B. aryabhattai* Z-48 under salt stress resulted in an effective increase in the content of total chlorophyll, soluble sugars, phenolics, flavonoids, and glucosinolates. Root-colonizing rhizobacteria increase iron availability to plants through their iron-chelating activity, increasing chlorophyll content, which in turn enhances photosynthetic activity and overall plant growth (Liu et al., 2024). This may be due to the effect of *P. fluorescens* and *B. subtilius* in biofertilizers, or to increased ACC-deaminase enzymes in plants treated with biostimulants, which slow chlorophyll degradation. It may also be due to increased photosynthetic rates or the role of nitrogen nutrition in producing growth-promoting substances, leading to more efficient nutrient uptake, which increases the main components of photosynthetic pigments and, consequently, chlorophyll content (Mohamed and Gomaa, 2012). Akhtar et al. (2015) also indicated that biochar mitigated the negative effects of soil salinity on potatoes (*Solanum tuberosum* sp.), attributing this to its ability to reduce soil bulk density and reduce the interaction between the root surface and sodium ions.

Table 3. The effect of salinity levels, biochar, and some biostimulants on some chlorophyll quantities and major nutrients of canola seedlings

treatments	Chlorophyll (mg/100g)	N%	P%	K%
Control	1.585*	1.255	0.559	2.158
S1	1.752	1.201	0.356	1.850
S2	1.590	1.214	0.378	1.637
B1	1.757	1.866	0.571	2.680
B2	1.585	1.991	0.587	2.658
PF	1.752	2.305	0.856	2.835
BS	1.590	2.114	0.878	2.737
S1 B1	1.757	1.466	0.571	2.680
S1 B2	1.585	1.491	0.659	2.658
S1 PF	1.752	1.305	0.756	2.835

S1 BS	1.590	1.814	0.778	2.737
S2 B1	1.757	1.766	0.371	2.180
S2 B2	1.590	1.314	0.378	2.137
S2 PF	1.757	1.866	0.621	2.280
S2 BS	1.863	1.766	0.670	2.280
L.S.D. 0.05	0.08	0.015	0.015	0.018

Conclusions

The study concludes that salinity is a widespread environmental constraint that negatively impacts agricultural productivity and food safety. The use of beneficial microorganisms and biochar is a sustainable and environmentally friendly approach to improving plant growth in organic farming practices. *P. fluorescens* and *B. subtilis* successfully rescued canola seedlings from salt stress during the study through multiple mechanisms. These bioactive agents enhanced plant growth and development under both saline and normal conditions and promoted increased chlorophyll and macronutrient (NPK) contents in the plants. These bacterial isolates and biochar can be used in biofertilizer formulations.

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