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Synergistic Effects of Compost and Beneficial Bacillus Bacteria on Sudanese Grass Growth in Saline Soils

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ABSTRACT

This study investigated the physicochemical properties such as pH, EC, organic matter content, and nutrient concentrations of certain soils and assessed the quality of produced compost (produced from mixture of date palm wastes and animal manure) in comparison with various commercial compost types. The analyzed soils were found to be slightly alkaline, low in organic matter, and exhibited minor variations in NPK contents. However, significant differences were noted in total-P and available K levels, especially in sample S8. Texturally, the soils ranged from sandy to sandy clay loam. A comparative assessment of compost generated in this study with commercial compost variants revealed considerable disparities in physicochemical attributes such as pH, EC, organic matter content, nutrient concentrations, impurities, and carbon-to-nitrogen (C/N) ratio. The compost produced in our research exhibited remarkable properties, notably having a high nutrient content, low EC, and the least impurities. When juxtaposed with commercial versions, our compost demonstrated superior quality, attributed to optimal moisture, thorough mixing, and ideal temperatures during composting, resulting in the rapid production of premium-grade compost. This research emphasizes the importance of selecting composts based on specific attributes to maximize their benefits as soil amendments. The isolated *Bacillus* Spp. showed a substantial improvement of Sudanese grass growth and enhanced the chemical characteristics of soils. It's worth noting that combined application of compost and *Bacillus* inoculum was more effective compared to their sole applications.

Keywords: Soils; Compost; Bacillus; Salts; Nutrients

1. Introduction

The New Valley Governorate is one of the largest governorates in Egypt, accounting for more than 44% of the country's total land area. The majorities of soils in the New Valley have a coarse texture and are challenged by issues of salinity, as well as a lack of organic matter and essential nutrients. Compost's role as an influential soil amendment is well-documented. It not only enhances soil structure but also improves water-holding capacity, thereby reducing water stress in plants (Lazcano et al., 2013; Palm et al., 2014). These soil benefits translate into improved plant growth by providing better root penetration and aeration (Diacono and Montemurro, 2010). Various studies have delved into the nutrient composition of compost, highlighting its richness in essential macro and micronutrients such as nitrogen, phosphorus, potassium, calcium, and magnesium, which are made available to plants over time (Lazcano et al., 2013). Furthermore, compost has been shown to contain beneficial microorganisms that contribute to soil health, thereby offering another avenue through which it promotes plant growth (Diacono and Montemurro, 2010). Soil salinity is an escalating issue affecting arable lands across the globe, with adverse impacts on crop yield and quality (Munns and Tester, 2008). Salinity disrupts plant physiology at multiple levels, including ion homeostasis, osmotic balance, and nutrient uptake, leading to reduced growth and productivity (Zhang et al. 20023). Strategies to counter salt stress have included traditional breeding for salt-tolerant varieties, but this is a time-consuming process and often does not yield satisfactory results (Munns and Tester, 2008). Advanced techniques involving genetic engineering to induce salt tolerance have been explored, although they come with their set of challenges and ethical considerations (Shabala and Cuin, 2008). Salt-tolerant bacteria, often classified as plant growth-promoting rhizobacteria (PGPR), have shown promise in alleviating salt stress in various plant species (Egamberdieva et al., 2017). They employ multiple mechanisms, including ion sequestration, osmolyte production, and hormone secretion to improve plant tolerance to salinity (Yang et al., 2009; Upadhyay & Singh, 2015). Specific bacterial genera like *Halomonas*, *Bacillus*, and *Pseudomonas* have been studied in-depth for their salt-tolerance mechanisms and their ability to enhance plant growth under saline conditions (Egamberdieva et al., 2017). These bacteria have been shown to produce phytohormones like indoleacetic acid (IAA) and cytokinins, which encourage root elongation and better nutrient uptake, thereby helping the plant to better tolerate saline conditions (Upadhyay and Singh, 2015).

While both compost and salt-tolerant bacteria individually have well-documented benefits for plant growth, research exploring their synergistic effects is still in its infancy but shows significant promise (Mayak et al., 2004; Nadeem et al., 2014). Compost serves as an excellent medium for the proliferation of beneficial microbes, including salt-tolerant bacteria, which further enhances their potential to improve plant growth (Mayak et al., 2004). Moreover, compost's organic matter has been found to bind with salt ions, reducing their toxicity and bioavailability, which when combined with salt-tolerant bacteria, offers a dual mode of action against salt stress (Nadeem et al., 2014).

2. Materials and Methods

2.1 Site selection and soil Sampling:

Surface soil samples (0-15 cm) were collected from several sites in EL-Khargaa Oasis varying in their salinity levels (1.5 to 8.0 dS m^{-1}). Collected surface soil samples subsequently air-dried in a contamination-free environment, ensuring minimal exposure to external contaminants. Then, samples were air-dried and passed through a 2 mm sieve and subjected to chemical and physical analyses as presented below. Other soil portions were collected from the salt-affected areas using a sterile auger for microbial analyses. These samples were placed in sterile plastic bags, sealed, and then transported to the laboratory.

Date palm wastes (leaves, trunks, and fruit wastes) and animal manure (cow, sheep, goat, or poultry manure) were collected from the nearby areas for compost production. Sudanese grass seeds were obtained from the Agriculture Research Center (ARC), Egypt. Seeds of Sudanese grass were surface sterilized using 70% ethanol for 2 min., followed by a 10-minute soak in 10% bleach solution, and then rinsed five times in sterile distilled water (Nelson, 2017).

2.2 Methods of study

2.2.1. Compost Preparation

Composting date palm wastes and animal manure were done using the traditional composting technique with some modification of ensuring zero loss of compost material and maintaining high temperature during the composting process (Rynk, 1992; El Janati, et al., 2023).

- Shredding and mixing: Date palm wastes were chopped to accelerate their decomposition process. Thereafter, these wastes were mixed with animal manure prepared at a ratio of 3:1 browns (date palm wastes) to greens (animal manure). This mixture was moistened (40-60%), and piled into a heap of more than 3 feet high and regularly turned every 1-2 weeks to aerate it to speed up the decomposition process. During the composting process, temperatures were carefully monitored and maintained in the range of 60-71°C, a range that ensures the rapid decomposition of organic material while preserving beneficial microorganisms.

- Curing and screening: After the active composting phase was reached (3 months), we have left the compost cures for 2 weeks to allow the composting activity to slow, and the compost to fully stabilize and mature. The mature compost was screened for removing any large, un-decomposed materials, and becomes ready for analyses and application.

Finally, 5 commercial compost materials was collected and used for comparing the quality of the produced material and commercial one.

2.2.2. Isolation of bacteria

Salt tolerant bacteria were isolated from the investigates soils as follows: 10 g of each soil sample was added to 90 mL of sterile distilled water then shaken vigorously for 30 minutes (McPherson et al., 2018). Thereafter suspensions were serially diluted to attain 10-5 using sterile distilled water. A hundred µL of each dilution was spread onto Luria-Bertani (LB) agar plates that contained varying concentrations of NaCl (0.25%, 0.75%, 1%, 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, 22.5%, 25%, 27.5% and 30%), then incubated at 30°C under lab conditions for 48 h. We aimed to use different concentrations of salts in order to isolate bacteria that can thrive in varying degrees of salt stress. Specific colonies were picked up on bases of distinct morphological characteristics then streaked onto fresh nutrient agar plates with the corresponding NaCl concentration to obtain pure cultures (Sharma et al., 2021). Thereafter, the isolated bacterial strains were inoculated onto Luria broth (LB) containing different NaCl concentrations (0.25%, 0.75%, 1%, 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5%, 20%, 22.5%, 25%, 27.5% and 30%) then incubated at 30°C while being shaken at 150 rpm for 24 hours. Growth was assessed by measuring the optical density at 600 nm (OD600) using a spectrophotometer (Peñuelas-Urquides et al., 2013). Strains that exhibited growth at ≥5%NaCl were considered as salt-tolerant strains and were used in this investigation.

- Molecular Identification: DNA Extraction: Genomic DNA of the selected salt-tolerant strains was extracted using a commercial DNA extraction kit, following the manufacturer's instructions. The 16S rRNA gene was amplified using specific universal primers. PCR products were purified and sequenced (Tang et al., 2010). Thereafter, the obtained sequences were compared with sequences in the GenBank database using the BLAST algorithm. Phylogenetic trees were constructed using MEGA software (Kumar et al., 2016).
- Salt-tolerant bacterial strains were cultured in Luria-Bertani (LB) medium at 30°C until reaching the logarithmic phase. Before inoculation, Sundanese grass were coated with 20% gum Arabic as an adhesive material then immersed in a bacterial suspension of 10^8 CFU ml^{-1} 15 min before sowing (Egamberdieva et al., 2017). Control plants were immersed in sterile distilled water.

2.3. Greenhouse experiment

A complete randomized design experiment was conducted to attain the aim of the experiment comprising the following: no application (T1), *Bacillus* inoculum (T2), compost (applied at a rate of 19.2 cubic meters per acre) (T3) and the combined application of *Bacillus* inoculum +compost (T4). Plastic pots of 20 cm (diameter) and 15 cm height were therefore filled with 10 kg of the investigated salt-affected soils. These pots received the recommended doses of NPK as recommended by the Ministry Agricultural and Soil Reclamation as follows: NPK and compost as follows: 96 kg ordinary super phosphate (12.5%), 96 kg potassium sulphate (48%) and 345.0 kg ammonium nitrate (33.5%). Ten seeds of Sundanese grass were germinated in each pot. Later, plants were thinned to 3 per pot after germination.
Soil moisture was maintained gravimetrically at 70 % of the water holding capacity for one month (the duration of this experiment); thereafter plant materials were harvested to estimate the following parameters, shoots and roots lengths and chlorophyll contents. Also, soil samples were collected from the rhizosphere of grown plants in each pot.

Plant growth was assessed by measuring shoot length, root length, shoot and root fresh weights, and total chlorophyll content (Lichtenthaler and Wellburn, 1983). Measurements were taken at the harvest stage.

2.4. Soil and Plant analyses

2.4.1. Soil analyses

These samples were then subjected to various analyses procedures. Particle size distribution was measured by using the hydrometer method as described by (Gee and Or 2002). The soil acidic reaction (pH) and electrical conductivity (EC) was measured at room temperature in soil paste extract and 1:2.5 (soil/water) suspension, respectively via pH and EC meter meters. Total organic carbon content was determined using a Welkley-Black procedure (Nelson and Sommers, 1996). Soil samples for ammonium and nitrate determinations were extracted with 2M KCl. Ammonium-N was measured by indophenol method as described by Selmer-Olsen (Selmer-Olsen, 1971) and nitrate-N was measured by a spectrophotometer method according to (Doane and Horwath 2003). Following the extraction of soil samples with ammonium bicarbonate diethylene triamine pentaacetic acid (ABDTPA), chlorostannous acid method was adopted to determine available phosphorous (Kuo, 1996) using spectrophotometer instrument

(Spectrophotometer instrument) and Flame photometer was used for measuring K contents (Flame photometer instrument). To evaluate total contents of metals in soils, soil samples (1 g each) were digested in aqua regia, then totals content of P and K were measured using the instruments mentioned above. For N, it was determined by using the kjeldahl method according to (Chapman, 1961). The soluble cations and anaions in soil paste extract were determined according to standard methods described by (Jackson, 1967).

2.4.2 Plant and compost analyses

Plant samples were washed with tap water several times then deionized water, and the plant materials were oven-dried at 70°C for 48 h. A 0.2 g of oven dried plants was wet-digested by adding 10 mL nitric acid (60%) and 2 mL hydrogen peroxide, 30% (Chapman and Pratt, 1961). Carbon and total N contents of the produced compost and tested commercial types materials were measured by a CHNS elemental analyzer (Elementar Vario EL III, Germany). Plant and compost samples were dried at 70°C for 72 hours and ground to pass through a 2-mm sieve. Total nitrogen (N) content was determined using the Kjeldahl method (Bremner and Mulvaney, 1982). Phosphorus (P) content was determined by digesting the plant material in a mixture of nitric and perchloric acids and analyzing the extract colorimetrically (Murphy and Riley, 1962). In addition, K was measured by using Flame photometer instrument.

2.5 Statistical Analysis

Statistical analysis of data was performed using a post-hoc test, which was performed to measure specific differences between treatments using the Duncan's Multiple Range Test (DMRT). The significant diffidence between treatments means were determined using analysis of variance and means separation at a 5% significance level ($p \leq 0.05$).

3. Results and Discussions

3.1 Physiochemical properties of the investigated soil

The investigated soils were slightly alkaline (within the range of 7.1 to 8.5) of low organic matter contents (below 0.5%) and exhibited little variations in NPK contents, except for total-P and available K which were noticeably lower in S8. Soils were generally light textured ones, varying between sandy in S2 and S4 to sandy clay loam in S1.5, S6 and S8 (Table1).

3.2 Major characteristics of the produced compost compared to commercial types

The comparative assessment of the produced compost from the present study and various tested commercial compost types is presented in Table 2. The analyzed parameters include pH, electrical conductivity (EC), organic matter (OM) content, nitrogen (N), phosphorus (P), potassium (K), impurities (gravels and coarse sand), and the carbon-to-nitrogen (C/N) ratio.

The pH values of the compost samples were found to vary significantly among the different compost types. The optimal pH range for most compost is typically between 6 and 8. Within this range, the compost can effectively provide nutrients to plants and support microbial activity essential for soil health. We've incorporated this information into the discussion for clarity. The compost produced in the present study exhibited a pH of 7.37, which was statistically similar to compost type C2 (pH 7.38) and C5 (pH 7.59), but significantly lower than compost types C1 (pH 7.9), C3 (pH 7.31), and C4 (pH 7.21). The pH of compost is a crucial factor influencing nutrient availability and microbial activity in the soil (Smith, 2009).

Table 1. Physicochemical characteristics of the tested soils

Parameter	Soil salinity level				
	1.5	2.0	4.0	6.0	8.0
	dS m^{-1}				
pH	7.85^{a}	7.92^{a}	8.03± 0.3^{a}	7.74^{a}	7.71^{a}
EC, dS m^{-1}	1.5^{e}	2.00^{d}	4.00± 0.2^{c}	6.00^{b}	8.00^{a}
O.M. %	0.25^{b}	0.32^{a}	0.20± 0.0^{c}	0.19^{c}	0.16^{c}
Total N, %	0.11^{bc}	0.12^{ab}	0.10± 0.0^{c}	0.12^{a}	0.10^{c}
Total P, %	0.48^{a}	0.39^{b}	0.39± 0.0^{b}	0.43^{ab}	0.09^{c}
Total K, %	0.19^{b}	0.19^{b}	0.18± 0.0^{b}	0.20^{b}	0.23^{a}
Available N, mg^{-1}	26.33^{bc}	19.00^{d}	25± 2.7^{c}	30.33^{ab}	35.00^{a}
Available P, mg^{-1}	0.35^{b}	0.41^{ab}	0.46^{a}	0.48^{a}	0.38^{b}
Available K, mg^{-1}	151.37^{b}	134.95^{c}	103.88^{d}	166.83^{a}	82.79^{e}
Sand, %	65.44	57.44	45.44	61.44	49.23
Silt, %	4.00	4.00	4.00	4.00	17.77
Clay, %	30.56	38.56	50.56	34.56	33.00
Textural class	Sandy	Sandy	Sandy	Sandy clay	Sandy clay

Different letters show significant difference at $P < 0.05$ (values signify means ± SD).

Regarding electrical conductivity (EC), the compost from the present study exhibited an EC of 1.54dS m^{-1}. Compost types C3 (EC 10.35 dS m^{-1}) and C4 (EC 14.78 dS m^{-1}) showed significantly higher EC values, indicating a higher concentration of salts compared to the produced compost. High EC levels can impact plant growth and soil structure (Zucconi et al., 1981). The organic matter (OM) content in compost is a key indicator of its quality. The compost from the present study had an OM content of 55.92%, which was comparable to compost type C2 (OM 65.1%) and significantly higher than compost types C3 (OM 37.2%), C4 (OM 26.83%), and C5 (OM 33.38%). OM content influences soil structure, water holding capacity, and nutrient retention (Nardi et al., 2002).

In terms of nutrient content, the present study's compost exhibited nitrogen (N) content of 2.44%, phosphorus (P) content of 0.19%, and potassium (K) content of 0.71%. Compost types C1 and C3 had the highest N content (3.22% and 3.23%, respectively); while C2 showed the highest P content (0.49%) and C1 exhibited the highest K content (1.49%). Nutrient content is crucial for compost's potential as a soil amendment (Goldan et al., 2023). Impurities in compost, such as gravels and coarse sand, can affect its application and quality. The present study's compost contained 0.99% impurities, while compost types C1 and C2 had the lowest impurity levels (4.56% and 7.06%, respectively). Compost type C4 had the highest impurity content (22.28%). The carbon-to-nitrogen (C/N) ratio is an indicator of compost maturity and decomposition. The C/N ratio for the present study's compost was 13.3±0.40, similar to C1 (12.25) and significantly lower than C2 (33.84). Compost types C3, C4, and C5 exhibited C/N ratios of 17.59, 14.74, and 26.61, respectively. In conclusion, the comparative assessment revealed variations in pH, EC, OM, nutrient content, impurities, and C/N ratio among different compost types. The results highlight the importance of selecting compost types based on their specific properties to optimize their benefits when used as soil amendments. In this context, the compost generated in our study displayed exceptional characteristics, characterized by elevated nutrient content, a remarkably low EC value, and the lowest levels of impurities recorded among all samples. These results collectively underscore the superior quality of the compost produced in comparison to the commercially available variants. The achievement of such high-quality compost can be attributed to a combination of factors, including substantial moisture content, consistent and thorough mixing procedures, and the maintenance of optimal temperatures throughout the composting process. Notably, these factors collectively contribute to the rapid attainment of premium-grade compost within a notably abbreviated composting period.

Table 2. Comparative assessment of produced compost and tested commercial types

Compost type	pH	EC, dS m^{-1}	OM	N	P	K	Impurities	C/N ratio
					%			
Present study	7.37^{c}	1.54^{e}	55.92^{c}	2.44^{b}	0.19^{d}	0.71^{b}	0.99^{e}	13.30^{d}
C1	7.90^{a}	3.43^{d}	67.96^{a}	3.22^{a}	0.05^{e}	1.49^{a}	4.56^{d}	12.25^{d}
C2	7.31^{c}	2.68^{d}	65.10^{b}	1.12^{cd}	0.49^{a}	0.47^{b}	7.06^{c}	33.84^{a}
C3	7.31^{c}	10.35^{b}	37.20^{d}	1.23^{c}	0.34^{b}	0.51^{b}	15.37^{b}	17.59^{c}
C4	7.21^{c}	14.78^{a}	26.83^{f}	1.07^{d}	0.31^{c}	0.27^{b}	22.28^{a}	14.74^{d}
C5	7.59^{b}	4.59^{c}	33.38^{e}	0.73^{e}	0.20^{d}	0.12^{b}	3.68^{d}	26.61^{b}

Different letters show significant difference at $P < 0.05$ (values signify means ± SD).

3.3 Salt tolerant of isolated bacteria

The growth response of bacterial isolates was investigated across a range of NaCl concentrations, spanning from 0.25% to 30%. This investigation aimed to assess the isolates' tolerance to different salinity levels and identify their optimal growth conditions. The results obtained from this experiment revealed a significant ability of the bacterial isolate to thrive in saline environments, showcasing its adaptability to varying degrees of salinity. Remarkably, the bacterial isolate exhibited robust growth patterns even at elevated NaCl concentrations, which is a notable trait for organisms in environments characterized by high salinity. Notably, the bacterial isolate demonstrated particular resilience towards a salinity level of 27.5%, showcasing its capacity to flourish under conditions that mimic highly saline environments (Figure 1). The BLAST-N comparison of the searched sequences in the NCBI nucleotide database revealed > 88% similarity of the isolated bacteria with *Bacillus* Spp. (Figure 2). These findings provide valuable insights into the isolate's ecological niche and potential applications. The isolate's demonstrated tolerance to a salinity level of 27.5% suggests its suitability for environments where salt stress prevails, such as saline soils, brackish waters, or industrial processes involving high salt concentrations. This adaptability not only underscores the isolate's ecological versatility but also raises the prospect of utilizing it for biotechnological purposes, such as bioremediation or bioprocessing in saline-rich contexts. The growth experiment conducted across varying NaCl concentrations has shed light on the bacterial isolate's remarkable ability to tolerate high salinity levels, with a specific tolerance observed at 27.5%. This adaptability has implications for both environmental and industrial applications, warranting further exploration and study of the isolate's physiological mechanisms underlying its salt tolerance. The *Bacillus* spp. isolate is regarded as a promising salt-tolerant Plant Growth-Promoting Bacterium (PGPB). This particular strain exhibits notable capabilities in enhancing plant growth and development, especially in saline environments. Its salt tolerance makes it an appealing candidate for agricultural applications, where soil salinity can pose challenges to plant productivity.

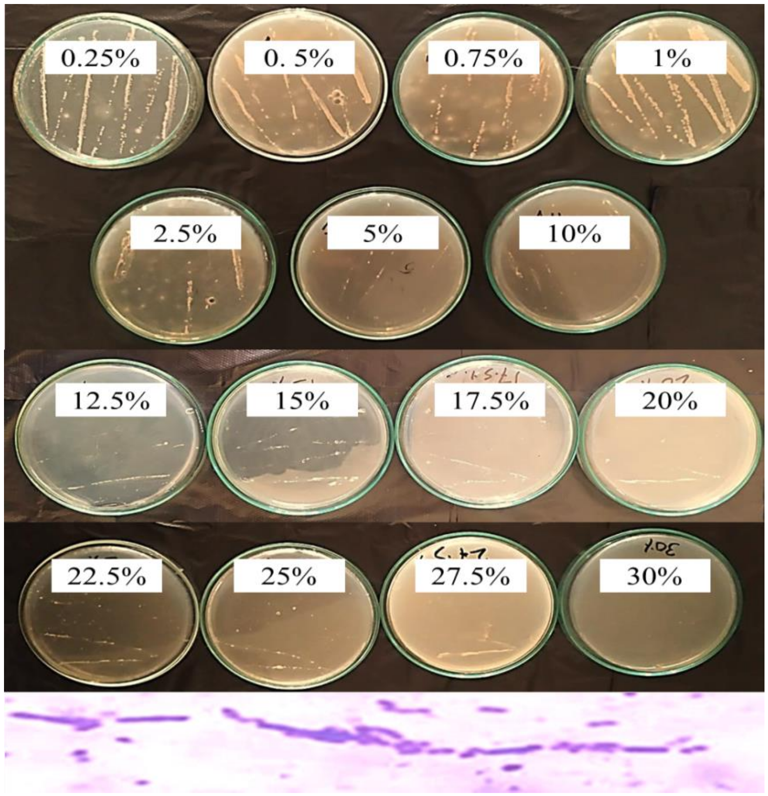

Figure 1. Growth of *Bacillus* Spp. isolates at different NaCl concentrations (0.25-30%)

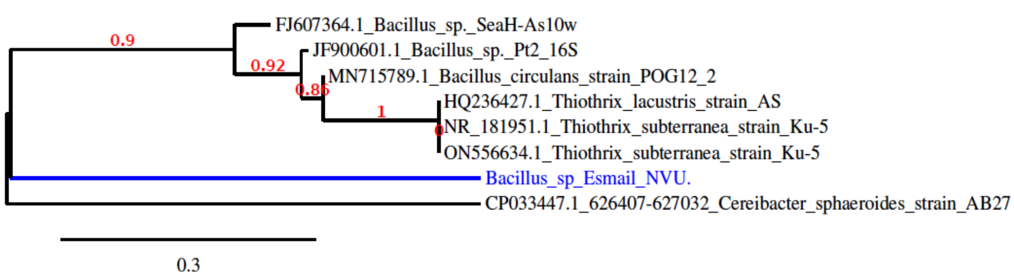

Figure 2. The phylogenetic of the bacterial isolate

3.4 Effect of Bacillus inoculum, compost and their combination on soil characteristics

In all soils, application of either *Bacillus* inoculum or compost alone raised significantly the bacterial counts in soil and also elevated soil organic matter content with superiority for the *Bacillus* inoculum treatment (Table 3). It is hypothesized that the compost could potentially be a source of PGPB, which will require further investigation for confirmation (Martínez-Cano et al., 2022). The combined application of *Bacillus* inoculum and compost recorded higher increases in both parameters than each treatment when applied solely. This is because of the nutritional content in compost that stimulated the activities of *Bacillus* inoculum (Hameeda et al., 2006). In soils of EC≥6 dS m^{-1}, these additives raised soil EC. This was probably the consequences of compost degradation in soil to set more nutrients in form of soluble salts (Abdelhafez et al., 2018). These salts increased salt stress in soil (Reddy and Crohn, 2013). In case of *Bacillus* inoculum, these bioagents synthesize low molecular weight compounds called siderophores to immobilize soil nutrients (Shilev, 2020; Javadzadeh et al., 2023; Kaur and Karnwal, 2023).

Table 3. Effect of applied *Bacillus* inoculum, compost and their combination on soil characteristics

Salinity level, dS m^{-1}	Soil amendment	pH	EC, dS m^{-1}	O.M., %	CFU × 10^3
1.5	Control	7.71^{b-e}	1.87^{no}	0.4^{d}	5.92^{j}
	Bacillus inoculum	7.65^{b-e}	1.8^{o}	0.52^{bc}	11.8^{c}
	Compost	7.92^{a}	1.9^{n}	0.54^{b}	9.75^{d}
	Bacillus inoculum +Compost	7.38^{hi}	1.8^{o}	0.654^{a}	15.94^{a}
2	Control	7.68^{b-e}	2.951^{l}	0.31^{e}	4.58^{k}
	Bacillus inoculum	7.58^{c-f}	2.25^{m}	0.52^{bc}	9.43^{de}
	Compost	7.46^{f-h}	3.27^{k}	0.38^{de}	10.00^{d}
	Bacillus inoculum +Compost	7.41^{g-i}	3.034^{l}	0.67^{a}	14.1^{b}
4	Control	7.79^{ab}	4.36^{i}	0.13^{f}	2.93^{lm}
	Bacillus inoculum	7.66^{b-e}	3.80^{j}	0.45^{cd}	8.23^{fg}
	Compost	7.52^{e-h}	4.44^{i}	0.13^{f}	8.73^{ef}
	Bacillus inoculum +Compost	7.24^{i}	4.75^{h}	0.65^{a}	11.3^{c}
6	Control	7.74^{a-d}	6.42^{g}	0.13^{f}	3.58^{l}
	Bacillus inoculum	7.57^{d-h}	6.58^{f}	0.32^{e}	6.87^{hi}
	Compost	7.81^{ab}	6.94^{e}	0.13	7.98^{fg}
	Bacillus inoculum +Compost	7.78^{a-c}	7.82^{d}	0.49^{bc}	9.53^{d}
8	Control	7.71^{b-e}	8.37^{c}	0.13^{f}	2.65^{m}
	Bacillus inoculum	7.74^{a-d}	8.94^{a}	0.49^{bc}	6.3^{ij}
	Compost	7.78^{a-c}	8.95^{a}	0.13^{f}	7.47^{gh}
	Bacillus inoculum +Compost	7.85^{ab}	8.83^{b}	0.54^{b}	8.43^{fg}

Different letters show significant difference at $P < 0.05$ (values signify means ± SD).

Excess siderophores probably increased soil salinity. A point to note is that the highest increases in soil EC was in S6 due to the combined application of *Bacillus* inoculum and compost, while this treatment exhibited the lowest EC value in S8. Neither of the application of *Bacillus* inoculum or compost solely affected soil pH yet the combined application of these two additives decreased significantly this parameter in low salinity soils (S1.5, S2 and S4). Thus occur because of the high buffering capacity of soil (Dvořáčková et al., 2022) while PGPB produced organic acids (Dhawi et al., 2015) while compost produced organic acids during its degradation that lowered soil pH (Farid et al., 2018 and 2023). In highly saline soils (S6 and S8), the combined application significantly raised soil pH versus the control. Maybe excess acidity raised the solubility of soil $CaCO_3$ that increased soil pH (Getahun et al., 2021).

3.5 Effects of applied Bacillus **inoculum***, compost and their combination on the growth of Sudanese grass*

Application of *Bacillus* inoculum enhanced significantly plant growth parameters versus the no application control treatment while the compost application recorded no significant effects on plant growth parameters. This might indicate the positive effects of the salt tolerant *Bacillus* inoculum on increasing plant tolerance to salinity stress via ameliorating osmotic-stress-producing osmolites (Shilev, 2020), formation of phytohormones and free radicals scavenging enzymes (Mishra et al., 2021). In case of compost, its application may lessen soil EC (Farid et al., 2020), nevertheless this mechanism could be temporarily. During compost degradation, many salts were again set free, in addition to the salts that were found in compost, hence this process increased salinity stress in plants (Reddy and Crohn, 2013; Goldan et al., 2020). The combined application of *Bacillus* inoculum and compost was more preferable than the single application of *Bacillus* inoculum for improving plant growth parameters in soils of EC ≤4 dS^{-1}. Similar results indicate the dual positive effects of compost and *Bacillus* inoculum on improving the growth of stevia plants (Alotaibi et al., 2022). In highly saline soil, no significant variations in plant growth parameters were detected between these two treatments. This probably occurred because of the low degradation rate of compost under such conditions (Al-Busaidi et al., 2014), and this explanation was confirmed by the results obtained herein of residual organic matter in soil by the end of the growing seasons (Table 4 and Figure 3).

Table 4. Effects of applied *Bacillus* inoculum, compost and on the growth of Sudanese grass grown on salt affected soils

Salinity	Soil amendment	Chlorophyll	Shoot	Root	Shoot	Root
1.5	Control	35.39^{b-d}	32.93^{ed}	8.67^{f}	5.37^{de}	1.49^{d}
	Bacillus inoculum	37.79^{a}	48.87^{b}	9.33^{ef}	10.52^{c}	2.34^{b}
	Compost	36.53^{ab}	60.07^{a}	11.87^{b-d}	5.02^{de}	1.21^{e}
	Bacillus inoculum +Compost	38.25^{a}	49.47^{b}	15.13^{a}	12.66^{b}	2.61^{ab}
2	Control	36.15^{ab}	32.0^{ef}	8.20^{g}	4.02^{e}	0.83^{f-h}
	Bacillus inoculum	34.69^{b-d}	41.60^{c}	13.13^{b}	12.46^{b}	2.32^{b}
	Compost	36.32^{ab}	34.70^{de}	11.0^{c-e}	5.33^{de}	1.14^{ef}
	Bacillus inoculum +Compost	38.18^{a}	48.07^{b}	12.67^{bc}	17.97^{a}	2.79^{a}
4	Control	0.00^{g}	0.00^{g}	0.00^{h}	0.00^{f}	0.00^{i}
	Bacillus inoculum	35.59^{bc}	31.67^{ef}	5.80^{g}	6.42^{d}	0.76^{gh}
	Compost	0.00^{g}	0.00^{g}	0.00^{h}	0.00^{f}	0.00^{i}
	Bacillus inoculum +Compost	33.39^{d}	34.60^{de}	9.13^{ef}	6.36^{d}	1.01^{e-g}
6	Control	0.00^{g}	0.00^{g}	0.00^{h}	0.00^{f}	0.00^{i}
	Bacillus inoculum	35.21^{b-d}	29.73^{f}	11.07^{c-e}	5.48^{de}	0.68^{gh}
	Compost	0.00^{g}	0.00^{g}	0.00^{h}	0.00^{f}	0.00^{i}
	Bacillus inoculum +Compost	33.81^{cd}	37.87^{de}	11.07^{c-e}	9.73^{c}	1.83^{c}
8	Control	0.00^{g}	0.00^{g}	0.00^{h}	0.00^{f}	0.00^{i}
	Bacillus inoculum	27.83^{f}	28.87^{f}	10.07^{d-f}	6.11^{d}	0.59^{h}
	Compost	0.00^{g}	0.00^{g}	0.00^{h}	0.00^{f}	0.00^{i}
	Bacillus inoculum +Compost	31.27^{e}	31.93^{ef}	12.07^{b-d}	5.0^{de}	0.89^{e-h}

Different letters show significant difference at $P < 0.05$ (values signify means ± SD).

Figure 3. Effect of applied *Bacillus* inoculum, compost and their combination on the vegetative growth parameters of Sudanese grass

3.6 Effects of applied Bacillus inoculum, compost and their combination on available N, P and K contents in soil

Available contents of nutrients increased significantly in soils owing to all additives versus the control (Figure 4). Such increases were more generally noticeable for the combined applications of *Bacillus* inoculum and compost rather than the single ones in all soils of EC≤4 dS m^{-1}. Probably, nutrients were released during degradation of compost (Farid et al., 2018 & 2021; Tolba et al., 2021; Farid et al., 2022; Hussein et al., 2022). Also, some N fixers are among PGPB in soil (Liu et al., 2011) and this explains the increases in N available contents in soils owing to

Bacillus inoculum application (either solely or with compost), with superior impacts for the combined treatment.

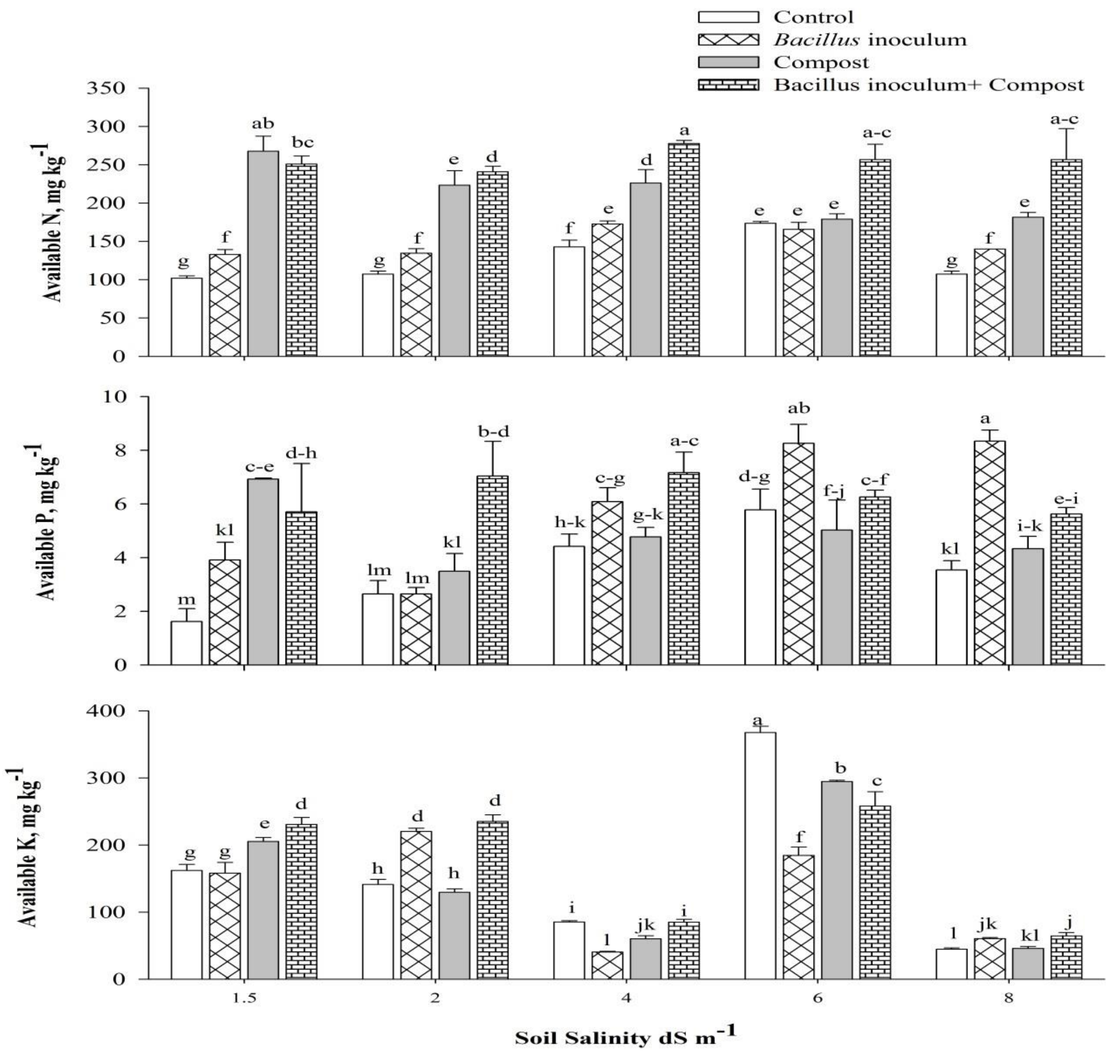

Figure 4. Effect of applied *Bacillus* inoculum, compost and their combination on available N, P and K contents in soil

Similar results indicate no significant variations among treatments

In soils of higher salinity, the highest increases in available content of N was recorded for the combined application of *Bacillus* inoculum and compost; yet for P and K, single additions noted comparable or probably superior impacts on this content versus combined applications. A point to note is that *Bacillus* inoculum probably raised the availability of soil nutrients (Esitken et al., 2010) while compost immobilized temporarily these nutrients (Elshony et al., 2019; Fan et al., 2019) in such highly saline soil. Accordingly, the corresponding increases in available N, P and K owing to the combined treatment were a bit higher than the ones obtained from *Bacillus* inoculum only.

3.7 Effects of applied Bacillus inoculum, compost and their combination on N, P and K contents in shoots and roots of Sudanese grass

N and P contents in shoots and roots of Sudanese grass increased significantly owing to all applications following the sequence of "*Bacillus* inoculum+compost application"> *Bacillus* inoculum >compost>control for N (Figures 5 and 6). A similar sequence was detected for P content in shoots, yet the control treatment recorded the highest increases in S1.5. This was probably the consequences of the dilution effect of this nutrient within plant tissues. In roots, the effects of single applications were superior to the combined one as if the combined application induced further translocation of P from roots to shoots. In highly saline soil (EC≥4dS m^{-1}), both *Bacillus* inoculum and "*Bacillus* inoculum+compost application" exhibited the highest increases in both N and P contents in roots and shoots with slight superiority for the combined treatment in shoot and for *Bacillus* inoculum in roots. Siderophores and organic acids were probably the main reasons beyond the significant increases in N and P contents in grass shoots and roots (Kartik et al., 2021). Also compost application could enrich soils with nutrients needed for plant growth (Kamel et al., 2016). In case of K, significant increases probably occurred in its content with plant tissues owing to these additives but because of the dilution effect resulted from increasing plant growth, no definite trend was noticed for K content, except that both *Bacillus* inoculum and "*Bacillus* inoculum+compost application" exhibited the highest increases in both roots and shoots.

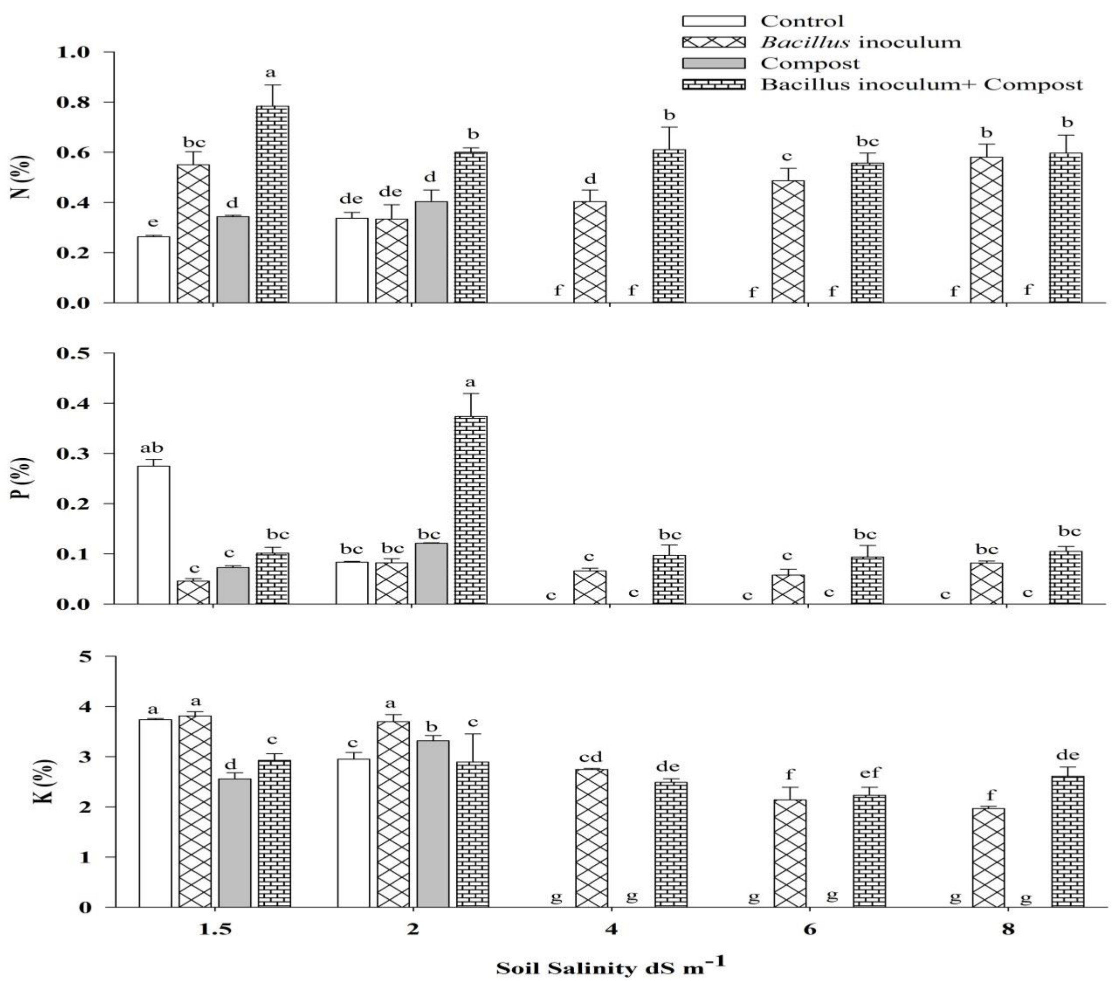

Figure 5. Effect of *Bacillus* inoculum, compost and their combination on N, P and K contents in shoots of Sudanese grass grown on salt affected soils

Similar results indicate no significant variations among treatments

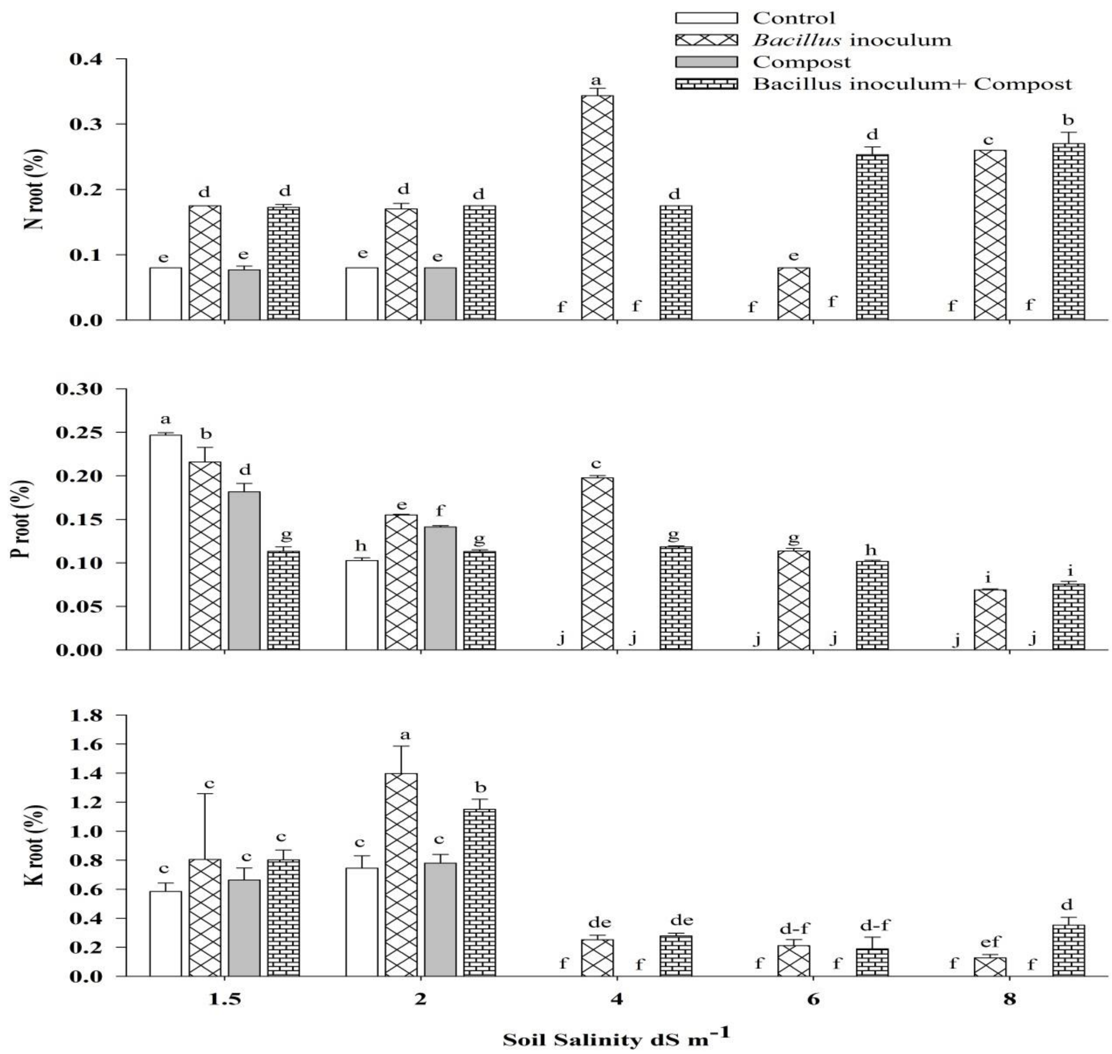

Figure 6. Effect of *Bacillus* inoculum, compost and their combination on N, P and K contents in roots of Sudanese grass grown on salt affected soils

Similar results indicate no significant variations among treatments

4. Conclusions

The study provides a comprehensive analysis of the physicochemical properties of selected soils and the quality of compost produced in comparison to commercial alternatives. Our findings indicate that the examined soils predominantly exhibit alkaline characteristics with varying textural classes. Importantly, the compost developed in this research stands out in terms of quality, boasting higher nutrient concentrations, a significantly low EC, and minimal impurities. This superior quality can be attributed to meticulous composting practices such as maintaining optimal moisture levels, thorough mixing, and consistent temperature regulation. The observed disparities among the compost variants underscore the necessity of judiciously choosing compost based on its inherent properties to harness its full potential as a soil amendment. In essence, the compost produced in this study offers an exemplary standard, potentially outpacing commercially available options in terms of its benefits to soil health and plant growth. Furthermore, the research highlighted the positive impact of the isolated *Bacillus* species on

Sudanese grass growth and soil chemical characteristics. The combined application of compost and bacillus inoculum proved to be more effective than their individual applications. This synergistic approach showcased enhanced benefits for soil fertility and plant growth.

Author Contributions

Conceptualization, S.M. and A.A; M.Y; validation, A.T.; formal analysis, M.Y.; investigation, M.Y.; resources, M.Y.; data curation, A.T.; writing -initial draft preparation, M.Y.; writing, reviewing and editing, M.A. and A.A. The final paper has been reviewed and approved by all authors.

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Conflicts of Interest

The authors declare no conflict of interest.

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