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Assessment of rhizobacteria-derived biofertilizers associated with *Celosia argentea* L. under sodic-salinized soil conditions

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Abstract

This study evaluated the phytoassessment potential of biofertilizers produced from rhizobacteria associated with *Celosia argentea* L. cultivated in sodic–salinized soil. Soil samples were collected from the Botanic Garden of the Department of Plant Biology and Biotechnology, University of Benin, and analyzed for physicochemical properties prior to use. Microbial inoculants were incorporated into various organic substrates to enhance microbial diversity and biofertilizer efficacy. A potted field experiment was conducted using 44 soil-filled bowls assigned to 7 treatment groups, with 2 bowls serving as untreated controls. The treatment groups received biofertilizers formulated from pap waste, rice hulls, groundnut husk, plantain peel, banana peel, sorghum chaff, and charcoal, respectively. Seeds of *C. argentea* (0.5 g per plot) were sown, and growth parameters were assessed during weeks 6 and 7. Biofertilizer application significantly improved plant growth and rhizobacterial activity compared to the control. Plant height ranged from 15.1–35.2 cm, number of leaves from 8.7–23.1, leaf area from 2.10–15.18 cm^2, root length from 6.5–12.0 cm, and rhizobacterial counts from $5.65 \times 10^3$to 8.09×10^3CFU/g. These findings demonstrate that biofertilizers enhance plant performance under saline conditions and support the role of beneficial rhizobacteria in improving salinity tolerance in vegetable crops. The study underscores the importance of further research into biofertilizer–plant–microbe interactions to advance sustainable agricultural practices in salt-affected soils.

Keywords: Rhizobacteria; Biofertilizers; Salinity stress; Sodic–salinized soil; Plant growth performance; Sustainable agriculture

1. Introduction

Biofertilizers are products containing live microorganisms, such as fungi, algae, and bacteria. They jump-start the activity of beneficial soil microbes and make nutrients more readily available to plants (Atieno et al., 2020). Sure, inorganic fertilizers boosted farm productivity for a while, but they pushed the limits of renewable resource use. Farmers still use a lot of chemical fertilizers, hoping for bigger harvests, but this overuse comes with a cost: polluted soil, toxic buildup, water contamination, and environmental damage. It's also linked to more cases of cancer and heart disease in people (Bharti & Suryavanshi, 2021). Quick results and low costs made these fertilizers popular, especially with farmers trying to get by on smaller plots. When it comes to crops, vegetables stand out. They grow fast, adapt easily, and deliver big yields, so they matter a lot for both health and trade. Vegetables give us essential nutrients—fiber, vitamins, minerals, and phytochemicals that aren't just "nice to have." They're loaded with vitamins like C, A, B1, B6, B9, and E, plus antioxidants that help our bodies repair and grow, and even cut down the risk of serious diseases like cancer (Fasusi et al., 2021).

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Organic farming really makes a difference in the quality of vegetables, and honestly, if you want modern agriculture to keep up sustainably, you need to use microbial biofertilizers. They're a big part of organic farming because they help soil stay fertile much longer (Joshi & Gauraha, 2022). The microbes in these fertilizers do a lot. They feed plants nutrients in different ways, boost plant immunity, and protect them from diseases, pests, and even tough weather. Biofertilizers work by adding nutrients naturally—think nitrogen fixation, phosphorus solubilization, and the production of growth-promoting substances that help plants grow strong. You'll find a few types out there: symbiotic nitrogen-fixing biofertilizers, free-living ones, and associative symbiotic nitrogen fixers (Kremsa, 2021).

You can make biofertilizer in solid or liquid form, depending on how you plan to use it. Most of the time, people mix biofertilizers with carrier materials to boost their effectiveness and help them hold onto water. Adding microorganisms to these carriers makes the biofertilizer easier to handle, store, and use. Sawdust, talcum dust, manure, and earthworm castings all work well for this (Kopittke et al., 2019). Biological fertilizers really help improve vegetable yields and push agriculture in a more sustainable direction. When it comes to managing the rhizosphere—the area around plant roots—the main goal is to extract the most from soil nutrients, so plants grow healthier and produce more. A variety of soil microbes, such as mycorrhizal fungi and plant growth-promoting rhizobacteria (PGPR), play key roles in this process (Kumawat et al., 2021). Take Rhizobium, for example. You'll find it in the root nodules of legumes, where it pulls nitrogen straight from the air and makes it available for plants (Lehmann et al., 2020). The aim of this study is to determine the phytoassessment of biofertilizer produced from rhizobacteria associated with *Celosia argentea* L. exposed to sodic-salinized soil.

1.1. Description of the Study Area

This study will be conducted in the Botanic Garden of the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria. The garden provides a controlled environment suitable for experimental cultivation and soil-based research. Materials required for the study include a weighing balance, a pH meter, measuring cylinders, beakers, conical flasks, safety goggles, nose masks, spatulas, vernier calipers, a meter rule, a stirrer, an autoclave, and a mortar and pestle.

2. Methodology

2.1. Biofertilizer Preparation

Organic wastes, including plantain peels, banana peels, rice hulls, charcoal, sorghum residue, pap residue, and groundnut pods, will be used to formulate bio-organic fertilizers. These substrates will serve as carriers for microbial inoculants.

2.2. Soil Collection and Preparation

The experimental site will be identified and cleared prior to setup. Approximately 50 kg of soil will be collected from the Botanic Garden and evenly distributed into 44 plastic bowls. To simulate saline conditions, the soil will be pre-treated with 500 mL of sodium nitrate ($NaNO_3$) solution daily for one week to ensure adequate sodification. A representative soil sample will then be taken to the laboratory for microbial analysis.

2.3. Media Preparation and Microbial Isolation

All culture media will be sourced from Oxoid and prepared according to the manufacturer's specifications. The media to be used include Nutrient Agar, Eosin Methylene Blue (EMB) Agar, MacConkey Agar, Triple Sugar Iron (TSI) Agar, Simmons Citrate Agar (SCA), Bile Esculin Agar, Pikovskaya's Agar, and Nitrogen Free Medium (Willey et al., 2008). Soil samples will be diluted in series using a 10^{-2} dilution factor. A stock solution will be prepared by homogenizing 10 g of soil in 90 mL of sterile distilled water. Serial dilutions will be performed across five test tubes. From the first dilution tube, 0.1 mL of inoculum will be aseptically transferred into sterile Petri dishes, followed by the addition of molten nutrient agar supplemented with 1% fluconazole to inhibit fungal growth. Bacterial cultures will be prepared in replicates using the pour plate method.

2.4. Planting and Experimental Setup

A total of 0.5 g of *Celosia argentea* seeds will be evenly sown into each bowl containing the treated soil. The bowls will be watered immediately after sowing, and subsequent irrigation will be carried out daily for six weeks. Growth parameters will be monitored throughout the experimental period. Replicates of samples will be prepared for both bacterial cultures using the pour plate method. The formula for the dilution factor is given below in equation (1)

$$Dilution\ factor\ = \frac{final\ volume}{aliquot\ volume} (1)$$

$$where: final\ volume = aliquot\ volume\ (sample\ volume)\ + diluent\ volume$$

Enumeration of the bacteria will be carried out using the formula delineated by Willey et al. (2008), and it is shown in equation (2) below.

$$\frac{cfu}{g} = \frac{number\ of\ colonies\ x\ dilution\ factor}{volume\ of\ inoculum}$$

3. Results

The result of the following experiment describes the assessment of biofertilizer produced using a rhizobacterial isolate of *Celosia argentea* exposed to sodic soil. Table 4.1 provides a description of the experimental treatment and the various codes used to represent it. Fz1 Klebsiella inoculated in Pap, Fz6 Bacillus inoculated in Rice, and Fz9 Bacillus inoculated in dried blended groundnut.

Table 4.2 presents the viability test results for the bioferilizer produced after 2 months. The result showed that after 2 months. Results showed that the bacterial isolates were still viable even after 2 months upon innovation in the various sold carrier molecules; for example, during the first month, the bacterial count ranged from 5.00 – 8.00 log10, whereas in the 2 months, the bacterial count ranged from 5.37-8.09 log10. This indicated a constant viability for 2 months.

Table 1 Treatment designations

Code	Description
Fz1	PAP + *Klebsiella*
Fz2	PAP + *Serratia*
Fz3	PAP + *Bacillus*
Fz4	RICE + *Klebsiella*
Fz5	RICE + *Serrtia*
Fz6	RICE + *Bacillus*
Fz7	Gnut + *E. coli*
Fz8	Gnut + *Klebsiella*
Fz9	Gnut + *Bacillus*
Fz10	BAN + *Serratia*
Fz11	BAN+ *Klebsiella*
Fz12	BAN+ *Bacillus*
Fz13	CHAR+ *Klebsiella*
Fz14	CHAR+ *Serratia*
Fz15	CHAR+ *Bacillus*
Fz16	PLAN+ *E. coli*
Fz17	PLAN+ *Klebsiella*
Fz18	PLAN+ *Bacillus*
Fz19	SORG + *Klebsiella*

Fz20	SORG + *E. coli*
Fz21	SORG + *Bacillus*

Table 2 Test of viability of the biofertilizers produced after the first two months

Fertilizers	**1 month**	**2 month**	**Dilution**	**vol. inoculum**	**log_{10} (cfu/g)**	
					1st month	**2nd month**
PAP + *Klebsiella*	524	608	100	0.1	5.72	5.78
PAP + *Serratia*	450	504	100	0.1	5.65	5.70
PAP + *Bacillus*	604	802	100	0.1	5.78	5.90
RICE + *Klebsiella*	54	87	10000	0.1	6.73	6.94
RICE + *Serrtia*	87	200	10000	0.1	6.94	7.30
RICE + *Bacillus*	1002	1222	10000	0.1	8.00	8.09
Gnut + *E. coli*	11	17	10000	0.1	6.04	6.23
Gnut + *Klebsiella*	780	840	100	0.1	5.89	5.92
Gnut + *Bacillus*	112	269	10000	0.1	7.05	7.43
BAN + *Serratia*	328	288	100	0.1	5.52	5.46
BAN+ *Klebsiella*	321	288	100	0.1	5.51	5.46
BAN+ *Bacillus*	200	234	100	0.1	5.30	5.37
CHAR+ *Klebsiella*	35	31	10000	0.1	6.54	6.49
CHAR+ *Serratia*	129	137	10000	0.1	7.11	7.14
CHAR+ *Bacillus*	153	122	10000	0.1	7.18	7.09
PLAN+ *E. coli*	336	300	100	0.1	5.53	5.48
PLAN+ *Klebsiella*	288	305	100	0.1	5.46	5.48
PLAN+ *Bacillus*	323	300	100	0.1	5.51	5.48
SORG + *Klebsiella*	137	114	10000	0.1	7.14	7.06
SORG + *E. coli*	159	437	10000	0.1	7.20	7.64
SORG + *Bacillus*	30	45	10000	0.1	6.48	6.65

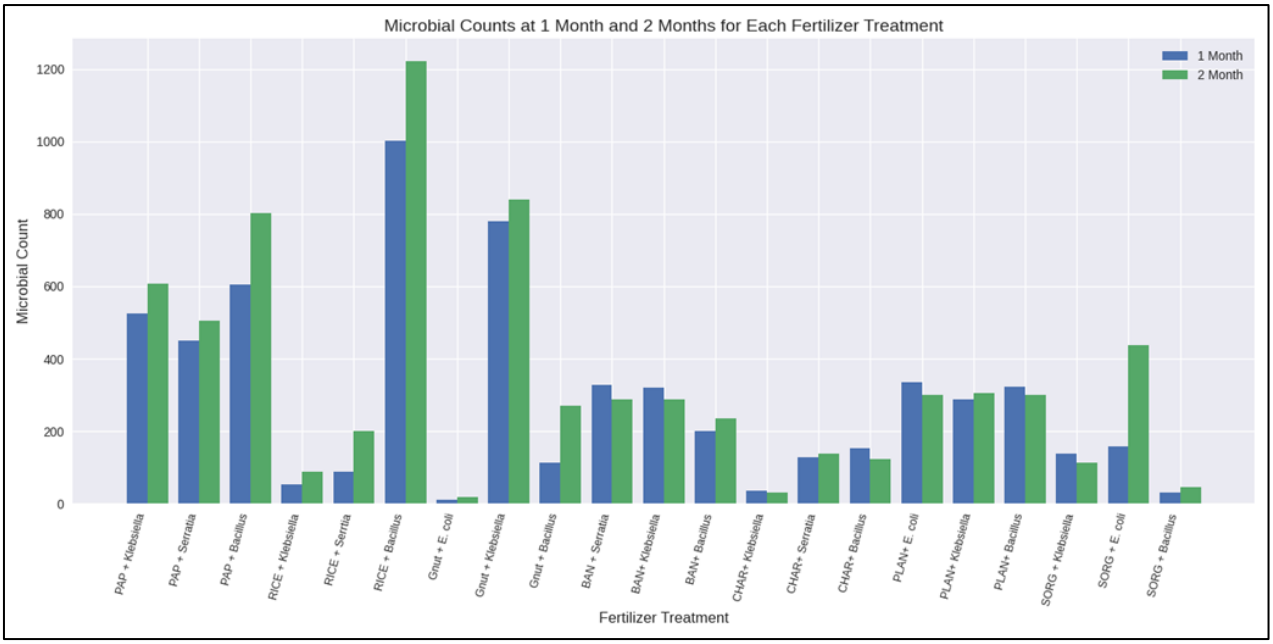

Figure 1 Growth response of *Celosia argentea* under different rhizobacteria-derived biofertilizer treatments in sodic–salinized soil conditions, showing variations in plant height, leaf development, and overall vigor compared to control treatments

Table 3 shows the effect of biofertilizer application on sodic salinized soil compared to that in the control soils. Generally, the number of emergents at 33 days after seeding in biofertilizer sodic-saline soil was higher than in control soils. Emergent time was not affected by biofertilizer application, with a range of 8 to 11 days. Plant height at 48 days ranged from 15.1cm in a few sodic saline soils that received biofertilizer to 35.2cm. Generally, plant height was absent; in the sodized soil, it was 30.2cm, compared to 19.12 cm in the control soil. Although it was expected that plant height in sodic soil would be lower, it was relatively higher with the addition of biofertilizer. There were generally more leaves in the sodized soil than in the unsodized soil; one explanation is the presence of nitrate in the oil-nitrate compound used to sodize the soil.

Table 4.4 presents the biogram parameters of test plants after 48 days of exposure to biofertilizers in sodic-salinized soil. Results showed that Fz1, Fz2, and Fz7 had significantly more primary root branches than Fz8 and Fz9, which each had 3 primary root branches. Generally, in non-sodic soils, the number of root branches is significantly reduced. The length of the longest root ranged from 6.5 to 12.0cm in the biofertilized sodized soil, whereas the biofertilized controlled soil ranged from 6.0 to 12.0cm.

Table 3 Morphological characteristics of Celosia after exposure to biofertilizers in a sodic soil

Bowls	**No of emergents at 33 days**	**Emergence time (days)**	**Height of plants(cm) (at 48days)**	**no of leaves per plant (at 48days)**	**Leaf area at 48 days (cm^2)**
sds+Fz1	84	8	28.3	14.1	9.97
sds+Fz2	115	9	27.2	13.1	5.56
sds+Fz3	99	11	30.2	13.1	5.72
sds+Fz4	95	8	32.2	17.9	9.47
sds+Fz5	124	9	26.2	16.9	6.28
sds+Fz6	94	9	17.1	14.1	5.15
sds+Fz7	120	9	23.2	16.1	5.87
sds+Fz8	207	9	27.2	15.1	6.43

sds+Fz9	132	10	30.2	17.1	8.26
sds+Fz10	233	8	32.2	19.1	7.62
sds+Fz11	85	10	25.2	13.1	6.00
sds+Fz12	237	7	33.2	21.1	6.43
sds+Fz13	208	7	35.2	19.1	7.91
sds+Fz14	120	8	15.1	13.6	8.12
sds+Fz15	85	9	30.2	19.1	7.15
sds+Fz16	206	6	35.2	13.1	15.18
sds+Fz17	222	7	26.2	17.7	4.86
sds+Fz18	100	9	34.2	23.1	6.27
sds+Fz19	166	9	29.2	13.9	7.50
sds+Fz20	104	9	24.2	14.8	7.96
sds+Fz21	100	9	25.2	14.1	8.37
Sds	105	11	30.2	18.1	8.58
ws+Fz1	44	11	30.2	17.1	2.10
ws+Fz2	85	10	25.2	13.8	4.33
ws+Fz3	117	9	19.1	13.7	3.19
ws+Fz4	62	10	22.1	17.9	4.56
ws+Fz5	101	9	17.1	9.5	2.48
ws+Fz6	139	8	16.1	10.8	1.64
ws+Fz7	126	8	24.2	14.9	3.55
ws+Fz8	199	8	19.2	12.1	4.56
ws+Fz9	99	9	16.1	10.9	5.31
ws+Fz10	89	10	22.2	12.8	3.60
ws+Fz11	48	9	20.1	12.9	4.46
ws+Fz12	102	9	25.2	14.8	4.91
ws+Fz13	85	9	12.1	8.7	1.56
ws+Fz14	88	9	21.2	9.7	2.47
ws+Fz15	78	8	38.2	11.1	6.37
ws+Fz16	72	9	19.1	9.8	2.96
ws+Fz17	102	10	26.2	19.1	4.42
ws+Fz18	109	9	16.1	9.9	3.21
ws+Fz19	75	9	22.2	13.9	4.19
ws+Fz20	86	9	19.2	10.8	3.64
ws+Fz21	125	10	19.2	9.9	3.86
Ctr	146	8	28.2	11.7	3.27

Table 4 Below-ground parameters of test plant after 48 days of exposure to biofertilizers in a sodic-salinized soil

Bowls	No of primary roots	Length of longest root(cm)
sds+Fz1	18	9.5
sds+Fz2	13	6.5
sds+Fz3	11	6.6
sds+Fz4	10	6.0
sds+Fz5	8	7.6
sds+Fz6	9	7.8
sds+Fz7	10	9.7
sds+Fz8	3	7.0
sds+Fz9	3	12.0
sds+Fz10	8	12.0
sds+Fz11	3	8.0
sds+Fz12	2	5.0
sds+Fz13	3	10.0
sds+Fz14	6	12.0
sds+Fz15	9	8.5
sds+Fz16	6	5.0
sds+Fz17	11	13.0
sds+Fz18	4	7.1
sds+Fz19	7	7.5
sds+Fz20	8	10.3
sds+Fz21	7	7.5
Sds	3	10.5
ws+Fz1	8	7.0
ws+Fz2	9	6.7
ws+Fz3	6	16.5
ws+Fz4	10	10.3
ws+Fz5	13	6.6
ws+Fz6	6	6.1
ws+Fz7	12	8.8
ws+Fz8	3	11.0
ws+Fz9	1	12.0
ws+Fz10	3	8.0
ws+Fz11	5	13.0
ws+Fz12	3	7.0
ws+Fz13	2	6.0

ws+Fz14	4	6.0
ws+Fz15	5	7.0
ws+Fz16	4	6.6
ws+Fz17	3	6.0
ws+Fz18	4	7.9
ws+Fz19	5	7.2
ws+Fz20	1	6.5
ws+Fz21	1	11.0
Ctr	1	4.9

Table 4 shows the yield parameters of the test plants after 48 days of exposure to biofertilizer in sodic-salinized soils. Results generally showed differences in plant weight. In SDS + Fz1, the single-plant weight was 1.13g at 1 day. However, this was reduced to 0.51 g dry weight when F27 was used as a biofertilizer in the sodic-salinized soil. Without fertilization, the single plant weight was 0.80g in the sodic soil but 0.3g in the control. Although it was ordinarily expected that the control soils would be the better performers in terms of plant biomass, the worst performer may be due to onion nitrate, which is known to enhance plant reproductive and vegetative development.

Table 5 Yield parameters of test plant after 48 days of exposure to biofertilizers in a sodic-salinized soil

Bowls	**No of plants**	**Weight of harvested plants(g)**	**Single plant weight (g DW)**
sds+Fz1	78	88.4	1.13
sds+Fz2	64	48.0	0.75
sds+Fz3	67	77.0	1.15
sds+Fz4	49	47.0	0.96
sds+Fz5	92	38.1	0.41
sds+Fz6	19	11.0	0.58
sds+Fz7	54	27.7	0.51
sds+Fz8	83	59.8	0.72
sds+Fz9	58	51.3	0.88
sds+Fz10	47	27.5	0.59
sds+Fz11	71	32.9	0.46
sds+Fz12	132	83.1	0.63
sds+Fz13	47	39.7	0.84
sds+Fz14	136	52.8	0.39
sds+Fz15	36	42.4	1.18
sds+Fz16	54	43.0	0.8
sds+Fz17	101	76.1	0.75
sds+Fz18	68	53.4	0.79
sds+Fz19	140	92.9	0.66
sds+Fz20	104	56.7	0.55
sds+Fz21	94	48.2	0.51

Sds	24	19.2	0.8
ws+Fz1	21	21.3	1.01
ws+Fz2	77	49.2	0.64
ws+Fz3	36	24.5	0.68
ws+Fz4	35	21.5	0.61
ws+Fz5	31	25.7	0.83
ws+Fz6	130	70.0	0.54
ws+Fz7	120	48.4	0.4
ws+Fz8	26	17.4	0.67
ws+Fz9	40	22.5	0.56
ws+Fz10	42	27.1	0.65
ws+Fz11	40	16.9	0.42
ws+Fz12	70	29.1	0.42
ws+Fz13	75	40.4	0.54
ws+Fz14	60	28.0	0.47
ws+Fz15	97	61.3	0.63
ws+Fz16	84	44.6	0.53
ws+Fz17	61	54.9	0.9
ws+Fz18	98	49.0	0.5
ws+Fz19	97	47.3	0.49
ws+Fz20	77	49.7	0.65
ws+Fz21	104	58.6	0.56
-+Ctr	103	37.0	0.36

4. Discussion

Salinity basically, when too much salt builds up in the soil, really holds back crop production around the world. Most crops just don't handle salt well. It messes with how they grow, throwing off everything from photosynthesis to protein synthesis and energy metabolism. People make it worse with things like drilling, dumping waste where it shouldn't go, or letting salty water spill, but sometimes it happens naturally, too (Pretty & Bharucha, 2015). When salt levels climb, plants struggle. They can't pull in water the way they need to, and their roots struggle to cope with the stress. Over time, this results in weaker plants, smaller harvests, and lower-quality crops.

The biggest problem? Osmotic stress. Plants can't take up enough water, so they end up thirsty even if the soil looks damp. Soil fertility drops, and over time, things get even worse: salt starts poisoning the plant tissues, nutrients get out of balance, and growth just stalls out. Some plants even die off, which can change the entire species mix in a field.

Vegetables have it especially tough. Research shows that tomatoes, chilies, cucumbers, okra—you name it struggle to even germinate or grow properly when there's too much salt. Because of this, scientists have been working hard to find ways to help crops handle salt (and drought, too), and some strategies are really starting to pay off in the field.

One approach that's catching on is using biofertilizers. These are fertilizers packed with helpful microbes. They do more than just feed the plants; they help them withstand salt stress by boosting nutrient uptake, balancing ion levels, and keeping key processes running smoothly. Take lettuce grown in semi-hydroponic systems with salty water, for example. Adding biofertilizers can improve water use, nutrient uptake, and how plants handle salt.

Even under stress from salty soils, plants treated with biofertilizers often grow better and stronger. The research supports this: Biofertilizers help crops perform, even under tough conditions (Mahanty et al., 2017). They're turning out to be a promising, eco-friendly way to help vegetables thrive in salty soils.

5. Conclusion

In conclusion, this study provides clear evidence that biofertilizers substantially enhance the growth and physiological performance of *Celosia argentea* cultivated under saline soil conditions. The improved plant height, leaf development, root architecture, and rhizobacterial activity observed across treatments demonstrate the capacity of biofertilizers to mitigate the detrimental effects of salinity stress. These findings reinforce the growing body of research indicating that biofertilizer-mediated microbial interactions play a pivotal role in promoting nutrient availability, maintaining ionic balance, and strengthening plant tolerance mechanisms in salt-affected environments. Overall, the study highlights the practical value of biofertilizers as a sustainable, low-cost, and environmentally friendly strategy for improving vegetable crop productivity in saline soils. By supporting both plant growth and beneficial soil microbial communities, biofertilizers offer a promising pathway toward resilient agricultural systems that can withstand increasing soil salinization pressures.

Recommendation

Based on the findings of this study, several recommendations are proposed to advance the application and scientific understanding of biofertilizers in saline soil management:

Field-Scale Validation and Multi-Season Trials. Although the present study demonstrates the effectiveness of biofertilizers in enhancing the growth of *Celosia argentea* under controlled conditions, future research should extend these evaluations to field-scale and multi-season trials. Such studies will help determine the consistency, scalability, and long-term benefits of biofertilizer application across diverse agro-ecological zones and varying levels of soil salinity. This will provide more robust evidence to support their adoption in real-world farming systems.

Mechanistic Studies on Plant–Microbe–Soil Interactions. Further investigation is recommended to elucidate the physiological, biochemical, and molecular mechanisms through which biofertilizers enhance salinity tolerance. Understanding how microbial inoculants influence ion homeostasis, osmotic adjustment, antioxidant activity, and rhizosphere microbial dynamics will enable the development of more targeted and efficient biofertilizer formulations. Such insights will also help optimize application rates, timing, and substrate combinations for maximum effectiveness.

Evaluation Across Multiple Vegetable Crop Species Since salinity stress affects a wide range of vegetable crops, future studies should assess the performance of these biofertilizers on other economically important species. Comparative evaluations will help identify crop-specific responses and broaden the applicability of biofertilizer technology in saline environments.

Integration into Sustainable Soil Management Practices. It is recommended that biofertilizers be incorporated into broader soil restoration and salinity management strategies, including organic amendments, improved irrigation practices, and salt-tolerant crop varieties. Integrated approaches will enhance soil health, promote microbial diversity, and improve long-term agricultural resilience in salt-affected regions.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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