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ORIGINAL RESEARCH

Effects of bio-fertilizers on the seedling of rice (*Oryza sativa* L.) in in-vitro and in-vivo conditions under salt stress

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Abstract

Purpose: Rice is the most important staple crop around the globe which has been prone to low productivity due to various abiotic factors. To address this challenge, we researched to describe the effect of biofertilizers on the morpho-physiology of rice seedlings *in-vitro* and *in-vivo* conditions under salt stress.

Method: The effects of biofertilizers on the growth characteristics of Rice were assessed in *in-vitro* and under salt stress in *in-vivo* on the open-pollinated, non-aromatic rice variety Pk-386. Different treatments of the individual biofertilizer and the biofertilizer combination were used to assess their effects on germination, plant length, fresh weight, dry weight, moisture percentage, vigor, and chlorophyll content of the leaves.

Results: In *in-vitro*, the best root length was obtained from Biozote + *Trichoderma* (BT) of (7.00) cm. A substantial increase in plant fresh weight was obtained from Reclaimer (R) with a weight of (47.33) milligrams. In *in-vivo*, the longest shoot length was observed in Reclaimer's 0 mM (15.23), and the highest root length was obtained from the Reclaimer (R) 75 mM of (12.33). Vigor (2517.66) in Reclaimer's 75 Mm. A fresh weight of (900.33) milligrams was obtained from Reclaimer's 75 mM. The Reclaimer (R) 25 mM treatment with (78.80 %) moisture was found to have the highest moisture percentage.

Conclusion: Based on the findings of this study, biofertilizer, and biofertilizer mixture in *in-vitro* conditions have resulted in a considerable increase in rice seedling growth. In contrast, *in-vivo* conditions, Reclaimer treatment shows remarkable results.

Keywords: Abiotic Stress, Bio-fertilizers, *Oryza sativa L.,* Rhizobacteria (PGPR), Salinity, *Trichoderma*

Introduction

Rice (*Oryza sativa L.*) is among the three leading food crops in the world and the most important food crop in the developing world. However, the per-acre production of rice remains low in many countries. While low in fiber and fat, rice is a good source of calories, magnesium, phosphorus, manganese, selenium, iron, folic acid, thiamin, and niacin below. Rice is a staple food for more than half of the world's population 14. 10.9% of the cultivated land is taken up by rice, or 2.5 million hectares, which yields 5.1 million tonnes of milled rice. Rice has a variety of roles in Pakistan's rural economy. For starters, it is the second most common basic food, accounting for about 2 million tonnes of our national dietary requirement; however, the average yield remained at 1.4 tonnes per acre.

Global food output can be increased by agricultural intensification; however, this will increase dependency on chemical agro-inputs such as fertilizers, which have several negative environmental consequences 14. For example, chemical fertilizers are closely linked to greenhouse gas emissions that drive climatic change and global warming 14. Ironically, long-term artificial fertilization may also contribute to the general decline of soil quality and production through acidification 13.

Biofertilizer is defined as a substance that contains effective living microorganisms (EM) that can be applied to seeds, plant surfaces, and soil 13, which colonize the rhizosphere or the inside of the plant to increase the supply or availability of primary nutrients and/or growth stimulants to the target crop 14and hence, stimulate the growth. The plant growth-promoting rhizobacteria (PGPR), with several beneficial tasks in plant rhizospheres, including the solubilization of nutrients, are among the most fascinating plant microbiomes 14, prevention of plant diseases **Error! Bookmark not defined.**, fixing nitrogen (N2) 14, as well as enhanced phytochemical content 14, as well as others. To promote plant development and soil fertility, biofertilizers are PGPR strains that can be either immobilized or trapped on inert carrier materials **Error! Bookmark not defined.**. Significant progress has been achieved over the years in the understanding, investigation, and formulation of diverse PGPR as alternative crop fertilization methods 13.

The primary rice-growing regions in Asia that produce more than 85% of the world's rice are frequently threatened by severe abiotic pressures, especially drought, and salinity 15. Since rice is mostly farmed with irrigation, intermittent water stress throughout key stages may significantly reduce productivity and cause crop failure 14. Drought and salinity have caused the rice plant to develop a few osmotic stress adaptations 14. Under abiotic stress conditions, salinity is especially detrimental to the growth and productivity of significant crops 15. Osmotic stress conditions brought on by salinity stress disturb cell homeostasis, cause redox imbalance, hamper photosynthesis, and deplete cellular energy 14, but the level of tolerance can be increased by using helpful microorganisms associated with roots that have positive effects on growth, physiology, and yield indices under stress 14.

Azotobacters are free-living bacteria that fix atmospheric nitrogen in cereal crops without the aid of symbiosis and without the necessity for a particular host plant 14, while *Trichoderma* has developed the ability to connect with the plant and provide a host with a wide range of advantageous effects 15. Through the use of biofertilizers, the yield of different crops can be raised by around 25%, and the consumption of inorganic N (nitrogen) and P (phosphorus) fertilizers can be decreased by about 25–50 and 25%, respectively 14. Microbial inoculants are widely acknowledged as being a crucial part of integrated nutrient management, which promotes sustainable agriculture 13.

Biofertilizers can play a vital role in plant growth and have been proven as an eco-friendly way compared to synthetic fertilizers. The study was conducted to describe the effects of biofertilizer and biofertilizer mixtures on the physiological and morphological aspects of rice seedlings in a controlled environment and under salt stress in *in-vivo* conditions.

Materials and Methods

Individual and interactive effects of Reclaimer (R), Biozote (B) and *Trichoderma* (T) on productivity of Rice seedlings in *in-vitro* and in *in-vivo* conditions were studied through a seedling tray and Petri dish experiment executed on May 19th and 23rd and harvested on June 6th (*in-vitro*) and 12th (*in-vivo*), 2023 on soil and compost media at the Department of Agriculture and Agribusiness Management, University of Karachi, Karachi, Pakistan.

The experiments were designed to study the effect of biofertilizers plant growth-promoting rhizobacteria PGPR, and beneficial fungi *Trichoderma* on the open-pollinated, non-aromatic variety PK-386 of Rice (*Oryza sativa L.*) cultivar for growth characters. Where Reclaimer (R) is a liquid biofertilizer containing *Azotobacter* and *Azospirillum* and Biozote (B) is produced by Pakistan Agricultural Research Council (PARC), Islamabad, and contains living bacteria TAL169 in the carrier material 14. The liquid bio-fertilizer Reclaimer (R) solution of 20 ml Reclaimer and 1-liter Distilled water, and the solid biofertilizer Biotoze (B) and *Trichoderma* (T) mixture were added as a seed coating after seeds had been mixed with a sucrose solution, then seeds were dried, sown and irrigated immediately. Ten seeds were placed into each hole in seedling trays, and fifteen seeds were sown into each autoclaved Petri dish manually.

The following treatments were evaluated: Biofertilizer individual Reclaimer (R), Biozote (B), *Trichoderma* (T), and Biofertilizer mixture Reclaimer + Biozote (RB), Reclaimer + *Trichoderma* (RT), Biozote + *Trichoderma* (BT), Reclaimer + Biozote + *Terichoderma* (RBT)) and control, for salinity stress, 0mM, 25 mM, 50 mM, 75 mM, and 100 mM of NaCl replicates set, all in triplicate, While the first irrigation and every seven days were when the salt stress was administered, whereas in *in-vitro*, only 2 ml of water or solution were administered.

Germination Percentage (GP) was calculated three, seven, and fourteen days after sowing. After harvesting Shoot length (SL) and Root length (RL) were calculated with the help of scale in cm. Fresh weight (milligram) was calculated by using an Analytical Balance Scale Weighing Machine, while the Dry weight (milligram) was calculated 24 days after harvesting. Moisture percentage (MP) and Vigor index (VI) were calculated by using a standard formula in (*in-vitro*), (*in-vivo*) Germination Percentage (GP), Shoot length (cm), Root length (cm), Fresh weight (milligram), Dry weight (milligram), Moisture percentage, Vigor index (VI), and Chlorophyll content by using spectrometer were among the features that were measured. Using SPSS software, the acquired data were statistically examined individually using the mean, standard deviation, and the analysis of variance (ANOVA) **Error! Bookmark not defined.**, and Tukey b Test (5 %) to explain the difference among the treatments.

Results and discussion

In **Error! Reference source not found.** Reclaimer (R), and biofertilizer mixture Reclaimer + Biozote (RB), and Biozote + *Trichoderma* (BT) fertilizers treatment have generally improved growth parameters of Rice (*Oryza sativa L.*) Seedlings, However, as shown in **Error! Reference source not found.**, 14 observed that some plants' plant height was significantly lower under the biofertilization treatment than under the control treatment. The greatest plant shoot was obtained from Control (C) can be seen in with values of (11.33) cm for the first, the second treatment was Reclaimer + Biozote (RB) with a plant shoot of (11.13) cm, and the lowest plant shoot was obtained from Biozote (B) of (7.43) cm.

Table 1. Effects of biofertilizers and biofertilizer mixtures on the seedlings of rice in *in-vitro* conditions

Treatments	GP	RL(cm)	SL(cm)	FW (mg)	DW (mg)	VI	MP

	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation
C	100.00±000a	6.67±1.49a	11.33±1.47a	26.67±7.28b	8.33±1.10a	1760.00±272.25a	67.70±6.74a
R	100.00±0.00a	5.47±0.39a	10.23±0.85a	47.33±10.19a	10.33±2.51a	1570.00±75.44a	76.6±9.07a
B	100.00±0.00a	5.70±1.83a	7.43±1.57ab	26.67±8.50b	10.00±2.65a	1313.33±215.45a	61.81±4.22a
T	97.78±3.80a	4.77±0.76a	10.90±0.76a	32.00±8.50ab	10.67±2.51a	1535.78±206.57a	66.18±5.96a
R + B	100.00±0.00a	5.10±1.67a	11.13±1.04a	36.00±3.65ab	12.00±1.00a	1623.33±198.58a	66.60±0.82a
R + T	100.00±0.00a	6.17±2.46a	10.80±1.18a	36.33±7.75ab	11.33±2.07a	1696.67±260.80a	67.81±9.60a
B + T	95.56±3.80a	7.00±0.64a	11.00±0.52a	39.33±6.86ab	11.33±1.53a	1719.33±90.61a	71.63±1.11a
R + B + T	100.00±0.00a	4.93±0.15a	10.53±0.26a	40.67±2.07ab	12.00±1.00a	1546.67±20.86a	70.46±2.5a
ANOVA DF **F Sig.**	7 2.21 0.08	7 0.93 0.51	7 4.24 0.01	7 2.83 0.04	7 1.20 0.01	7 1.68 0.18	7 1.65 0.19
HSD (5 %)	5.4	3.8	2.9	20.6	5.4	533.5	16.8

Remark: Germination (GP), Shoot Length (SL), Fresh Weight (FW), Dry Weight (DW), Vigor Index (VI) and Moisture Percentage (MP). Values are means of three replicates ± standard error. Means with different letters are significantly different from each other compared to the Tukey b test at $p \leq 0.05$.

The greatest root length was obtained from Biozote + *Trichoderma* (BT) treatment of (7.00) cm can be seen in, the second treatment was Control (C) with a root length of (6.67) cm, and the lowest root length was obtained from *Trichoderma* (T) (4.77) cm. A substantial increase in plant fresh weight was obtained from the Reclaimer (R) treatment with a weight of (47.33) milligrams, and the second one was the Reclaimer + Biozote + *Trichoderma* (RBT) treatment with a weight of (40.67) milligrams and the lowest weight was obtained from Control and *Trichoderma* with the same reading of (26.67). 14 reported that, Because *Azotobacter* can create growth hormones like auxins and gibberellins, which promote root growth, more root areas may become available for rhizobia to infect. Increased nodulation, nitrogen fixation, and eventually higher crop yields would follow from this.

Control (C) treatment had the lowest biomass, with only (8.33) milligrams present. The maximum biomass was seen in the different biofertilizer mixtures in which Reclaimer + Biozote had (12.00) milligrams and Reclaimer + Biozote + *Trichoderma* (R + B + T) also had (12.00) milligrams dry weight. The Reclaimer (R) treatment of (1313.33) had the lowest calculated vigor, whereas the Control (C) treatment of (1760.00) had the highest and the Biozote + *Trichoderma* (B + T) treatment of (1719.33) was close to control. The Reclaimer (R) treatment with (76.6 %) moisture was found to have the highest moisture percentage, followed by the Biozote + *Trichoderma* (B + T) treatment with (71.63 %) moisture, and the Biozote (B) (61.81 %) treatment with the lowest moisture percentage.

The diazotrophic bacteria that make IAA with or without tryptophan precursors include *Rhizobium*, *Azotobacter*, and *Azospirillum*, all of which are categorized as H-PGPR **Error! Bookmark not defined.**. The ability of *Azotobacter* and *Azospirillum* to enhance root development, water and mineral uptake rate, and biological nitrogen fixation are the primary factors that determine the benefits of co-inoculating a crop 14. 15 found that the germination percentage and plant growth characteristics of hopbush shrub (*Dodonaea viscosa* L.) seeds infected with *Azospirillum* + *Azotobacter* were improved. The biofertilizer application stimulated nutrient accumulation and plant growth compared to the non-treated plants 13.

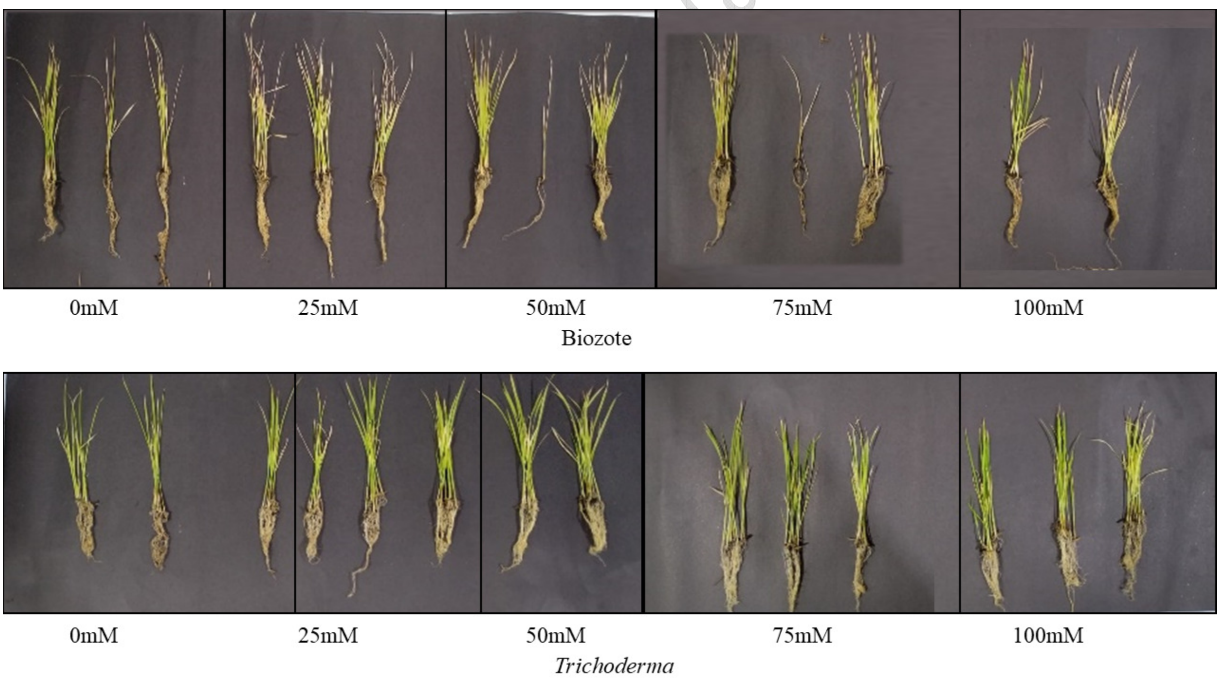
In the *in-vivo* condition under salt stress as shown in Table 2**,** the results of the effects of different treatments on plant shoot, root, fresh weight, dry weights, moisture percentage, and vigor fluctuating with Reclaimer (R) biofertilizer giving the longest shoot length in both the first 0 mM (15.23) cm can be seen in Fig. 1 and second 25 mM (15.13) cm treatments and the lowest shoot length under salt stress were obtained from Biozote (B) fertilizer's 100 Mm treatment of (8.43) cm.

The highest root length was obtained from the Reclaimer (R) 75 mM treatment of (12.33) cm and the second highest was obtained from Biozote (B) treatment 0 mM of (11.23) cm can be seen in Fig. 2 and the lowest root length was obtained from the Reclaimer + *Trichoderma's* (RT) 100 mM treatment of (4.46) is visible in Fig. 3. In both control and salt stress situations, *Azotobacter* and *Azospirillum* enhanced the growth metrics and antioxidant activities 13. 14 reported that Under salinity stress, the plant's growth, dry weight, and root dry weight were all positively significantly impacted ($p < 0.01$) by the combined application of *Azotobacter* and *Azospirillum* bacteria.

The process of biological nitrogen fixation, which occurs either symbiotically or freely between microorganisms and plants, is responsible for over two-thirds of nitrogen fixed globally 14. Legumes and symbiotic microorganisms like *Rhizobium* fix nitrogen symbiotically. *Rhizobium* is a nitrogen-fixing PGPR that has been shown to have a strong ability to increase plant development and yield 14. Conversely, it has been demonstrated that free-living nitrogen-fixing PGPR, such as *Azotobacter* and *Azospirillum*, can adhere to roots and effectively colonize root surfaces 13.

As the *in-vitro* result shows, the same results can be seen in the *in-vivo* condition regarding weight; the highest fresh weight was obtained from Reclaimer's 75 mM treatment of (900.33) milligram, and second highest from the Reclaimer + Biozote + *Trichoderma's* (RBT) 25 mM of (819.66) milligram treatment is visible in Fig. 4, and the lowest weight was in Biozote (B) 100 mM treatment with only (143.6667) milligram. The Biozote (B) 100 mM treatment had the lowest biomass, with only (53.6667) milligrams present. The maximum biomass was seen at the different salt stress levels of the control (C); the highest concentrations were found at 0 mM and 25 mM with (218.66) and (218.33) milligrams of control (C). The Biozote (B) 100 mM treatment of (834.66) had the lowest calculated vigor, whereas the Reclaimer 75 mM treatment of (2517.66) and the Reclaimer treatment of (2192.66) were close to 25 mM each. The Reclaimer (R) 25 mM treatment with (78.80 %) moisture was found to have the highest moisture percentage, followed by the Reclaimer (R) 75 mM treatment with (78.02 %) moisture, and the Reclaimer (R) 50 mM (41.85 %) treatment with the lowest moisture percentage.

Fig 1. Effects of different salt stress levels on control and Reclaimer treatment



7. Effects of different salt stress levels on Biozote and *Trichoderma* treatment

Fig 3. Effects of different salt stress levels on Reclaimer + *Trichoderma* and Reclaimer + Biozote treatment

Fig 4. Effects of different salt stress levels on Biozote and Reclaimer treatment

Table 2. Effects of biofertilizer treatments on the seedlings of rice under salt stress in *in-vivo* conditions

Treatment	GP	RL (cm)	SL(cm)	FW (mg)	DW (mg)	VI	MP
	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Devia-tion	Mean ± Std. Deviation
C 0_{mM}	100.00±0.00a	6.96±1.30abc	13.53±1.01a	736.33±47.01abc	218.66±8.62ab	2050.00±222.71ab	70.17±3.04a
C 25_{mM}	66.66±15.27a	5.90±1.21bc	14.00±0.26a	680.33±8.14abc	218.33±4.61ab	1312.00±217.77ab	67.90±.90a
C 50_{mM}	93.33±5.77a	7.60±1.93abc	13.53±2.13a	701.33±183.84abc	211.66±25.42ab	1958.66±266.30ab	68.38±8.74a
C 75_{mM}	93.33±5.77a	6.50±1.12abc	12.70±1.27a	631.33±148.13abcde	185.00±35.59abcd	1794.33±208.50ab	70.44±1.84a
C 100_{mM}	56.66±49.32a	4.43±3.86c	8.60±7.54a	330.33±295.61bcde	87.00±75.38bcd	1113.33±993.11ab	48.75±42.37a
R 0_{mM}	83.33±20.81a	10.60±2.94abc	15.23±2.00a	744.33±321.31abc	178.00±46.18abcd	2131.66±424.27ab	74.12±7.03a
R 25_{mM}	90.00±10.00a	9.20±1.56abc	15.13±1.55a	721.00±221.96abc	146.66±15.63abcd	2192.66±279.08ab	78.80±4.16a
R 50_{mM}	36.66±35.11a	5.30±4.84bc	7.76±6.72a	322.00±286.33bcde	118.33±102.75abcd	701.66±639.88b	41.85±36.41a
R 75_{mM}	96.66±5.77a	12.33±1.87a	13.60±1.85a	900.33±56.04a	199.33±58.07abcd	2517.66±429.65a	78.02±0.60a
R 100_{mM}	83.33±20.81a	9.20±0.50abc	13.60±1.01a	599.66±168.63abcde	150.66±47.43abcd	1893.33±464.17ab	75.04±1.49a
B 0_{mM}	46.66±37.85a	11.23±4.33ab	13.66±0.95a	231.66±93.27cde	83.66±30.55bcd	1064.66±677.80ab	62.49±7.77a
B 25_{mM}	80.00±20.00a	9.36±0.56abc	13.86±1.00a	316.66±54.63bcde	133.33±8.02abcd	1860.00±467.72ab	57.28±5.34a
B 50_{mM}	70.00±51.96a	9.86±1.58abc	12.83±1.13a	260.33±202.60cde	89.00±83.64abcd	1562.00±1150.79ab	68.60±7.57a
B 75_{mM}	66.66±49.32a	9.76±2.40abc	13.13±2.11a	344.33±23.43bcde	180.00±6.08abcd	1651.00±1283.85ab	47.53±4.43a
B 100_{mM}	40.00±40.00a	5.63±4.88bc	8.43±7.32a	143.66±159.93e	53.66±66.45d	834.66±816.64b	43.59±38.21a

T 0$_{mM}$	50.00±45.82a	5.20±4.57bc	9.16±7.97a	251.33±217.68cde	86.33±75.14bcd	1062.00±942.72ab	65.66±2.42a
T 25$_{mM}$	83.33±11.54a	9.76±1.36abc	13.20±0.51a	553.66±150.99abcde	178.66±37.80abcd	1921.66±342.68ab	66.75±9.01a
T 50$_{mM}$	93.33±11.54a	8.16±1.25abc	12.63±0.77a	683.00±101.53abc	204.00±40.73abc	1936.66±237.13ab	70.27±2.04a
T 75$_{mM}$	73.33±5.77a	8.66±1.60abc	12.86±0.72a	500.33±76.87abcde	142.00±28.00abcd	1579.66±169.21ab	71.71±1.57a
T 100$_{mM}$	100.00±0.00a	9.73±1.77abc	11.96±0.15a	493.33±175.28abcde	135.33±52.63abcd	2170.00±165.22ab	72.28±4.93a
R + B 0$_{mM}$	76.66±32.14a	6.80±0.65abc	14.53±0.45a	738.33±158.59abc	191.00±42.72abcd	1656.33±742.79ab	74.17±0.87a
R + B 25$_{mM}$	86.66±5.77a	6.93±0.37abc	14.40±2.16a	539.33±67.88abcde	142.00±15.00abcd	1854.33±298.16ab	73.51±3.03a
R + B 50$_{mM}$	63.33±25.16a	6.70±0.65abc	13.36±0.70a	743.00±174.15abc	184.66±29.50abcd	1287.33±582.90ab	74.81±2.14a
R + B 75$_{mM}$	43.33±20.81a	6.06±0.56abc	14.23±0.81a	707.00±354.20abc	177.33±82.61abcd	894.00±460.12ab	74.37±1.66a
R + B 100$_{mM}$	100.00±0.00a	6.10±0.62abc	13.03±1.05a	684.66±82.03abcd	194.00±19.28abcd	1913.33±141.53ab	71.61±0.98a
R + T 0$_{mM}$	90.00±10.00a	5.20±0.80bc	13.50±0.70a	385.00±98.53bcde	165.00±55.38abcd	1682.00±206.13ab	57.84±4.15a
R + T 25$_{mM}$	80.00±17.32a	4.83±0.28bc	13.90±0.79a	520.33±12.74abcde	182.33±8.73abcd	1506.00±370.67ab	64.97±0.85a
R + T 50$_{mM}$	96.66±5.77a	5.73±0.68bc	12.66±0.57a	251.00±102.80cde	88.66±50.20bcd	1779.33±122.39ab	66.25±6.23a
R + T 75$_{mM}$	73.33±37.85a	4.66±0.57c	11.46±1.55a	175.66±77.66de	64.00±37.24cd	1228.66±720.62ab	66.01±8.28a
R + T 100$_{mM}$	93.33±5.77a	4.46±0.46c	12.16±0.28a	659.00±110.81abc	242.66±43.59a	1555.33±168.58ab	63.21±0.85a
B + T 0$_{mM}$	86.66±5.77a	5.76±0.57bc	12.63±0.83a	747.66±91.35abc	208.33±65.37abc	1590.33±51.03ab	72.51±5.50a
B + T25$_{mM}$	80.00±17.32a	6.86±0.95abc	14.66±0.72a	710.00±154.79abc	186.33±28.91abcd	1727.00±422.90ab	73.53±1.55a
B + T 50$_{mM}$	86.66±15.27a	8.33±2.27abc	12.40±0.20a	690.33±207.23abc	193.66±49.08abcd	1784.33±281.37ab	71.48±3.05a
B + T 75$_{mM}$	96.66±5.77a	7.66±1.91abc	14.20±2.16a	727.66±119.20abc	212.00±29.10ab	2098.66±240.38ab	70.78±0.90a
B + T 100$_{mM}$	83.33±15.27a	6.03±0.64abc	12.66±1.20a	693.66±70.88abc	192.00±20.66	1565.33±385.38ab	72.18±3.60a

R + B + T 0_{mM}	76.66±11.54a	5.80±0.60bc	13.30±0.26a	545.00±37.64abcde	175.33±9.01abcd	1470.33±286.03ab	67.80±0.714a
R + B + T 25_{mM}	100.00±0.00a	5.46±0.40bc	13.46±0.55a	819.66±158.10ab	216.00±30.04ab	1893.33±66.58ab	73.44±1.91a
R + B + T 50_{mM}	93.33±11.54a	6.46±0.40abc	14.56±1.60a	642.33±148.50abcd	175.66±32.47abcd	1962.66±266.42ab	72.46±1.25a
R + B + T 75_{mM}	96.66±5.77a	6.70±0.45abc	12.96±0.55a	724.66±83.26abc	188.33±17.61abcd	1901.66±158.61ab	73.96±0.55a
R + B + T100_{mM}	93.33±11.54a	5.90±0.65bc	12.20±0.36a	649.66±74.84abcd	188.00±9.84abcd	1695.33±276.95ab	70.89±2.31a
ANOVA df F Sig.	39 1.794 0.014	39 3.072 0.000	39 1.314 0.152	39 4.862 0.000	39 3.356 0.000	39 1.861 0.010	39 1.66 0.029
HSD (5 %)	76.04	6.56	8.55	520.25	148.78	1687.62	42.72

Remark: Germination Percentage (GP), Root Length (RL), Fresh Weight (FW), Dry Weight (DW), Vigor Index (VI), and Moisture Percentage (MP). Values are means of three replicates ± standard error. Means with different letters are significantly different from each other compared to the Tukey b test at p ≤ 0.05.

In Table 3 The Reclaimer + Biozote + *Trichoderma* (RBT) treatment had the highest total chlorophyll content (7.04), the biozote (B) 100 mM treatment had the second-highest total chlorophyll content (5.40), and the biozote (B) 0 mM treatment had the lowest total chlorophyll content (2.31).

Thus, under salt stress *in-vivo* circumstances, the Reclaimer (R) treatment exhibits exceptional outcomes in almost all metrics, including shoot length, root length, fresh weight, moisture percentage, and vigor. 13 also reported that bio-fertilizers (*Azotobacter* and *Azospirillum*) increased nutrient concentration and uptake by cereal crops, which led to luxurious growth and better crop development.

Table 3. Effects of biofertilizer treatments on the chlorophyll content of rice under salt stress in in-vivo conditions

Treatment	Chlorophyll a	Chlorophyll b	Carotenoid	Total Chlorophyll
	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation	Mean ± Std. Deviation
C 0_{mM}	2.19±0.08efghij	1.13±0.013ijklm	103.59±1.80ghij	3.33±0.09ghijklmn
C 25_{mM}	2.45±0.04defgh	1.28±0.09hijkl	132.46±7.36efgh	3.74±0.13fghijklm
C 50_{mM}	2.63±0.06de	1.88±0.18gh	197.84±.18ab	4.52±0.24bcdef
C 75_{mM}	1.76±0.05jklmno	0.91±0.16ijklm	94.16±11.31hijk	2.68±0.21mn
C 100_{mM}	2.12±0.06fghijkl	0.66±0.10klm	68.43±7.31jk	2.78±0.17lmn
R 0_{mM}	1.22±0.20pq	2.12±0.30defg	143.96±22.92cdefg	3.34±0.51ghijklmn

R 25_{mM}		1.02±0.15q	1.94±0.27efgh	124.46±21.04fghi	2.97±0.42jklmn
R 50_{mM}		1.40±0.13opq	2.59±0.23abcde	183.37±17.11abcd	4.00±0.36cdefghij
R 75_{mM}		1.67±0.09lmnop	2.54±0.08bcdef	184.28±9.7abcd	4.22±0.17cdefghi
R 100_{mM}		1.56±0.42mnop	2.98±0.86ab	192.38±55.72abc	4.54±1.28bcdef
B 0_{mM}		1.58±0.15mnop	2.25±0.25cdefg	164.08±21.04bcdef	3.84±0.41efghijkl
B 25_{mM}		1.61±0.26mnop	2.41±0.54bcdefg	178.97±41.70abcde	4.03±0.81cdefghij
B 50_{mM}		1.70±0.16klmno	2.90±0.16abc	203.08±13.55ab	4.61±0.33bcdef
B 75_{mM}		2.00±0.22hijklm	2.90±0.40abc	220.39±28.34a	4.91±0.63bcde
B100_{mM}		1.82±0.05ijklmno	3.24±0.06a	227.69±4.84a	5.07±0.12bc
T 0_{mM}		1.88±0.11ijklmno	2.75±0.23abcd	190.24±19.16abc	4.63±0.34bcdef
T 25_{mM}		1.48±0.07nop	2.71±0.14abcd	190.15±14.27abc	4.20±0.22cdefghi
T 50_{mM}		1.6±0.12mnop	2.71±0.22abcd	181.32±15.63abcd	4.34±0.35bcdefgh
T 75_{mM}		1.58±0.25mnop	2.74±0.45abcd	179.41±34.26abcde	4.33±0.70bcdefgh
T 100_{mM}		1.58±0.25ijklmno	2.74±0.45abcde	179.41±34.26ab	4.33±0.70bcdefg
R + B 0_{mM}		3.39±0.00c	1.33±0.00hijkl	107.82±0.00ghij	4.72±0.00bcdef
R + B 25_{mM}		1.79±0.01jklmno	0.52±0.05m	63.73±3.93jk	2.31±0.07n
R + B 50_{mM}		1.85±0.03ijklmno	0.52±0.05m	74.60±1.89jk	2.37±0.08n
R + B 75_{mM}		2.03±0.07ghijklm	0.63±0.07lm	71.14±2.80jk	2.67±0.14mn
R + B 100_{mM}		2.61±0.02de	1.29±0.02hijkl	137.60±21.96defgh	3.90±0.00defghijk
R + T 0_{mM}		2.04±0.05ghijklm	0.65±0.07klm	61.61±4.35jk	2.70±0.13mn
R + T 25_{mM}		3.60±0.05c	1.40±0.05hij	130.32±1.85fgh	5.00±0.11bcd
R + T 50_{mM}		2.23±0.08efghij	0.65±0.08klm	73.69±3.77jk	2.88±0.17klmn
R + T 75_{mM}		2.48±0.04defg	0.75±0.07ijklm	90.08±2.07hijk	3.24±0.02hijklmn
R + T 100_{mM}		2.52±0.01def	0.84±0.02ijklm	89.46±2.52hijk	3.36±0.01ghijklmn
B + T 0_{mM}		1.79±0.00jklmno	0.52±0.02m	51.24±1.48k	2.31±0.02n
B + T 25_{mM}		2.17±0.02efghijk	0.69±0.06klm	66.30±1.41jk	2.87±0.09klmn
B + T 50_{mM}		4.04±0.05b	1.35±0.06hijk	131.83±5.60efgh	5.40±0.00ab
B + T 75_{mM}		2.30±0.05defghi	0.72±0.00jklm	76.51±0.87ijk	3.03±0.05jklmn
B + T 100_{mM}		1.91±0.01ijklmn	0.54±0.01m	73.45±0.02jk	2.45±0.02n
R + B + T 0_{mM}		3.25±0.61c	1.44±0.02hi	125.60±0.63fghi	4.69±0.63bcdef
R + B + T 25_{mM}		2.74±0.03d	1.31±0.05hijkl	112.30±6.06ghij	4.05±0.02cdefghij
R + B + T50_{mM}		2.54±0.02def	0.80±0.02ijklm	77.94±0.94ijk	3.34±0.04ghijklmn
R + B + T 75_{mM}		2.29±0.04defghi	0.84±0.02ijklm	87.98±1.38hijk	3.13±0.07ijklmn
R + B + T 100_{mM}		5.11±0.06a	1.92±0.17fgh	144.20±1.58cdefg	7.04±0.11a
ANOVA	Df	39	39	39	39
	F	76.313	46.304	29.647	23.845
	Sig.	0.000	0.000	0.000	0.000
HSD (5%)		0.52	0.751	54.55	1.19

Remark: Chlorophyll a, Chlorophyll b, Carotenoid, and Total Chlorophyll. Values are means of three replicates ± standard error. Means with different letters are significantly different from each other compared to the Tukey b test at $p \leq 0.05$.

Conclusion

Based on the findings of this study, it is concluded that the use of a biofertilizer and biofertilizer mixture in *in-vitro* conditions resulted in a considerable increase in growth. Where second highest shoot was recorded in the treatment of Reclaimer + Biozote (RB) with plant shoot of (11.13) cm, the greatest root length was recorded in the treatment of Biozote + *Trichoderma* (BT) of (7.00) cm, and the highest fresh weight was recorded in the treatment of Reclaimer (R) weight of (47.33) milligram and the highest moisture percentage was recorded in the treatment of Reclaimer (R) of 76.97 %.

While *in-vivo* conditions Reclaimer treatment shows remarkable results in almost all the growth parameters of seedlings, starting from the shoot length of (15.23) cm, root length (12.33) cm, fresh weight of (900.33) milligram, highest vigor of (2517.66), and the highest moisture percentage 78.80 %, followed by the Reclaimer's another treatment with (78.02 %). In *in-vivo* conditions, biofertilizers cope with the adverse effects of salt stress and show remarkable growth in many growth parameters. Therefore, further development is needed to increase the awareness of using biofertilizers among the farming communities of developing countries.

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