SUSTAINABLE IMPROVEMENT OF RICE GROWTH UNDER SALINITY STRESS USING AN ENDOPHYTIC FUNGUS-BASED BIOFERTILIZER

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ABSTRACT

Salinity stress adversely affects rice (*Oryza sativa L.)* growth, development and overall productivity. Multiple strategies have been implemented to enhance the resilience of rice plants, enabling them to grow better in saline environments. Use of biofertilizers, a sustainable alternative to chemical fertilizers, has gained significant attention in modern agriculture due to their potential to improve soil fertility, enhance crop productivity, and mitigate environmental concerns. Biofertilizers encompass a diverse group of microorganisms, including bacteria, fungi, and algae, which interact with plants through various mechanisms. These beneficial microbes have been reported to convert atmospheric nitrogen into plant-available forms and break down insoluble phosphates and zinc complexes in the soil. Additionally, they can also produce growth regulators, enhance nutrient availability, and protect plants against pathogens.

In our previous study, a salt-tolerant endophytic fungus isolated from the halophytic wild rice, *Oryza coarctata*, was identified as *Aspergillus welwitschiae Oc*streb1 by whole genome sequence analysis. During the in-vitro experiments, the endophyte showed several plant growth promoting activities such as, zinc and phosphate solubilization, siderophore, IAA, and ACC-deaminase production, nitrogen fixation, etc. in both normal and 900 mM salt stress. In this study, the endophyte was used to formulate a biofertilizer in combination with talcum powder to enhance the growth and yield of rice plants under salinity stress. This research investigated the effectiveness of the biofertilizer in four distinct salinity tanks under both non-saline and 6 dS/m saline conditions, each containing three different varieties of rice. Treatment of BRRI dhan28 (BD-28), BRRI dhan67 (BD-67) and BRRI dhan87 (BD-87) rice plants with the formulated biofertilizer significantly enhanced their yield in both non-saline and saline conditions. Among the three rice varieties, BD-28 showed the highest significant ($p<0.05$) yield increase, with 104.20% under normal conditions and 3080.56% under salt stress. BD-67 exhibited a 45.15% increase in normal conditions and 153.64% under salt stress ($P<0.05$). BD-87 showed a significant yield increase only under salt stress, at 293.63% ($p<0.05$).

From the results of the study, it can be proposed that the formulated biofertilizer is a potential eco-friendly and cost-effective solution to improve cultivation and yield of rice in the highly saline coastal regions of Bangladesh.

KEYWORDS: Salinity, Rice, endophytic fungus, Biofertilizer, Yield.

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Introduction

It is paramount to address the adverse impacts of climate change on agricultural productivity and crop susceptibility. This effort is critical for ensuring food security and sustaining progress in agriculture. Additionally, it is imperative to note that in order to meet the demands of a growing global population, food production must increase by 38% in 2025 and by 57% in 2050 (Wild, 2003). These figures underscore the urgency of proactive measures needed in the agricultural sector.

Along with the increasing food demand, the reduction in cultivable land is a matter of big concern. One of the significant challenges facing modern agriculture is soil nutrient depletion, which directly affects plant growth and yield. In order to combat this problem by conventional means, farmers rely on synthetic fertilizers to replenish the essential nutrients. However, the overuse of chemical fertilizers poses detrimental effects on the environment, such as soil degradation, water pollution, animal health risk, and the release of greenhouse gases (Savci, 2012).

Under these circumstances, biofertilizer comprising endophytic and rhizospheric micro-organisms can be economical, ecological and environment-friendly solution (Kumar *et al.*, 2022). These microbes are now recognized as an extension of the plant genome (Zaman *et al.*, 2023). They have been shown to exhibit several plant growth promoting activities and produce bioactive compounds which can ameliorate biotic and abiotic stressors (Kumar *et al.*, 2022, Mohamed and Abd-elsalam, 2021). It has been suggested that the endophytes isolated from the hosts endemic to highly saline regions can protect crop growth in salty conditions (Manjunatha *et al.*, 2022). In their study, Tisarum *et al.* (2020) reported that the arbuscular mycorrhizal fungi *Glomus etunicatum* inoculation can enhance rice salt tolerance. The fungal endophyte *Metarhizium anisopliae* has also been reported to enhance rice growth and yield by co-ordinating with the plant's salt tolerance mechanisms (Chowdhury *et al.*, 2024). The endophytic fungus *Piriformospora indica* improves the growth of *Arabidopsis thaliana* and regulates Na^+/K^+ balance under salt stress. (Abdelaziz *et al.*, 2017). These findings highlight the potential of fungal endophytes to improve crop resilience in saline environments.

In a previous study, we had isolated an endophytic fungus from wild halophytic rice *Oryza coarctata*, which demonstrated significant PGP (Plant growth promoting) activities and was identified as a novel strain of *Aspergillus welwitschiae* through whole genome sequencing. When inoculated into the salt-sensitive rice (*Oryza sativa*) in pots in a net house, it markedly improved the plant growth under normal and salt stress conditions. Yield increased by 203% and 167% in colonized plants in the absence and presence of salt, respectively. WGS data is available in NCBI GeneBank with BioProject ID: PRJNA985106 (Airin *et al.*, 2023).

In consideration of its excellent PGP activities, we used *Aspergillus welwitschiae Oc*streb1, as a potential candidate for formulation of a suitable biofertilizer to improve crop yield and establish a robust crop variety in regions where the soil is highly saline, such as, in the coastal areas. We formulated a biofertilizer with *Aspergillus welwitschiae Oc*streb1. The efficiency of the biofertilizer was assessed in salinity tanks at the Bangladesh Rice Research Institute (BRRI). The main purpose of this study was to do a preliminary investigation to determine whether the formulated biofertilizer can enhance rice growth and yield under both normal and salt stress in the presence of the recommended chemical fertilizer doses. For this purpose, the biofertilizer was applied to the three rice varieties, namely, BRRI dhan28, BRRI dhan67 and BRRI dhan87. We observed significant enhancement in the yield parameters of BD-28, BD-67, and BD-87 upon application of the biofertilizer both under non- stress and saline stress. Finally, the result of this study suggested that the biofertilizer comprising endophytic fungus *Aspergillus welwitschiae Oc*streb1 could be a holistic solution to combat climate change and promote a more sustainable future for agriculture and industry alike.

Methodology

Formulation of the biofertilizer

Biofertilizer was formulated by following the method of Zhu *et al.* (2022) and Din *et al.* (2019). At first, the fungus *Aspergillus welwitschiae Oc*streb1 was cultured in 250 mL Potato Dextrose Broth for 3 days. Then, 1 kg of wheat bran was moistened with 1 litre water and was autoclaved. The fungal broth was then mixed well with the sterilized wheat bran and kept at 28℃ for two weeks (until the wheat bran was fully utilized by the fungus) on a plastic tray covered with aluminium foil having small pores for aeration. After two weeks of incubation, the total wheat bran culture was mixed with 10 kg of sterilized talc powder and stored at 4℃ (Figure 1a).

Biofertilizer application and plant growth

For this experiment, three rice varieties were selected: BRRI dhan28, BRRI dhan67, and BRRI dhan87. Here, BD-28 is a salt sensitive rice variety whereas BD-67 is a moderately salt tolerant that can tolerate up to 8 dS/m salt stress with some loss in yield. Both of them are cultivated in the Boro season. On the other hand, BD-87 is cultivated in the Aman season. To inoculate the fungus into the roots of the rice plants, 80 gm of biofertilizer was thoroughly mixed with 8 kg of soil at first. The resulting mixture was then evenly spread on a plastic tray. Subsequently, 60 germinated rice seeds were sown onto this tray and allowed to grow for 20 days. Then they were transferred into the experimental tanks of BRRI.

BRRI experimental tank preparation

A total of two tanks were prepared where one tank served as the control tank and the other tank was designated as the saline tank with a salinity level of 6 dS/m. Before tank preparation, loamy soil was mixed with MOP (Muriate of Potash), DAP (Di-Ammonium Phosphate) and gypsum. Then the tanks were filled with the soil. For the biofertilizer treatment, each tank, measuring 9 square meters in total area, was divided into two equal sections of 4.5 square meters using a soil barrier. One section was treated with biofertilizer at a rate of 55.55 g/m², while the other section served as the untreated control. In each section, a total of seven rows were sown, with four plants in each row. There were two rows for BD-28, two rows for BD-67, and three rows for BD-87. The rows for each variety were arranged randomly. Urea and MOP were additionally provided to both of the tanks at 15, 30 and 45 days after the transplantation of the rice seedlings. Figure 1b illustrates the tank setup.

Data collection and statistical analysis

Rice seeds were harvested one month after flowering at maturity. After harvesting, several parameters such as plant height, tiller number, panicle number, filled grain number, filled grain weight, 1000 grain weight and yield were measured and analyzed. All the statistical analyses were performed using Graphpad Prism 9 and Microsoft Excel. t-test was performed for describing the variation between biofertilizer-treated and untreated plants in both saline stress and non-stressed conditions. Values are expressed as mean ± SD (standard deviation). Values of $p < 0.05$ and $p < 0.01$ were deemed significant and highly significant respectively.

Results

Effect of the biofertilizer on rice yield

Application of the biofertilizer generally led to comparable changes in various parameters across rice varieties. In some cases, the differences were statistically insignificant. However, under salt stress, the biofertilizer-treated plants consistently show significant increase in plant growth, several reproductive traits, and yield-related characteristics compared to control plants (Figure 2).

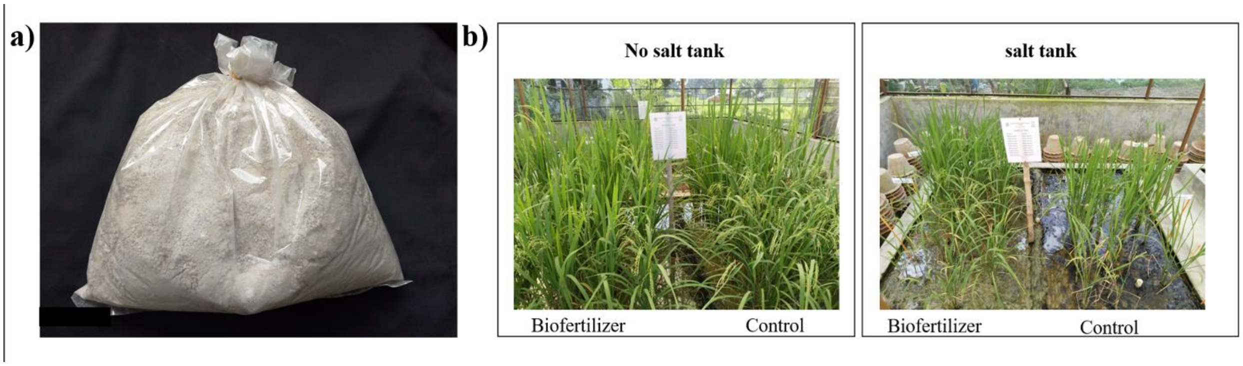

Figure 1. Experiment design. a) Physical appearance of the formulated biofertilizer, b) The soil tanks without salt and 6 dS/m salt tanks.

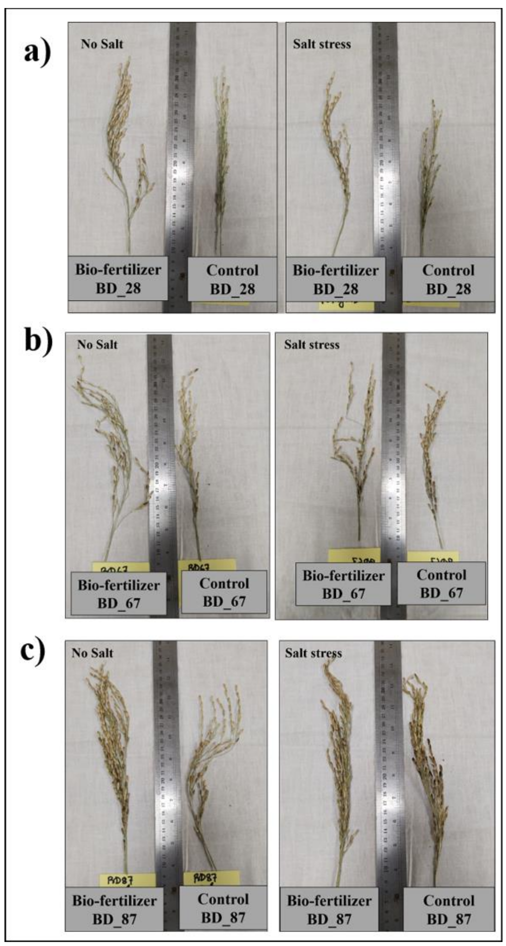

Figure 2. The effect of the biofertilizer on the panicles of control and biofertilizer inoculated rice; a) BD-28, b) BD-67 and c) BD-87 under no salt and 6dS/m saline conditions.

BD-28 exhibited significant changes in the biofertilizer treatment across various parameters under both normal and salt stress conditions (Figure 3 and Table. 1). Under normal conditions, fungal inoculation led to a statistically significant increase in plant height by 4.49%, along with significant improvements in tiller number (17.02%), panicle number (73.3%), filled grain number (80.98%), filled grain weight (104.2%), percent fertility (16.05%), 1000 grain weight

(6.33%), and yield per plant (104.2%). However, under 6 dS/m salt stress, a significant elevation was observed in total tiller number (85.1%), filled grain number (2352.6%), filled grain weight (3080.5%), percent fertility (245.1%) and yield (3080.5%) in the biofertilizer-inoculated plants.

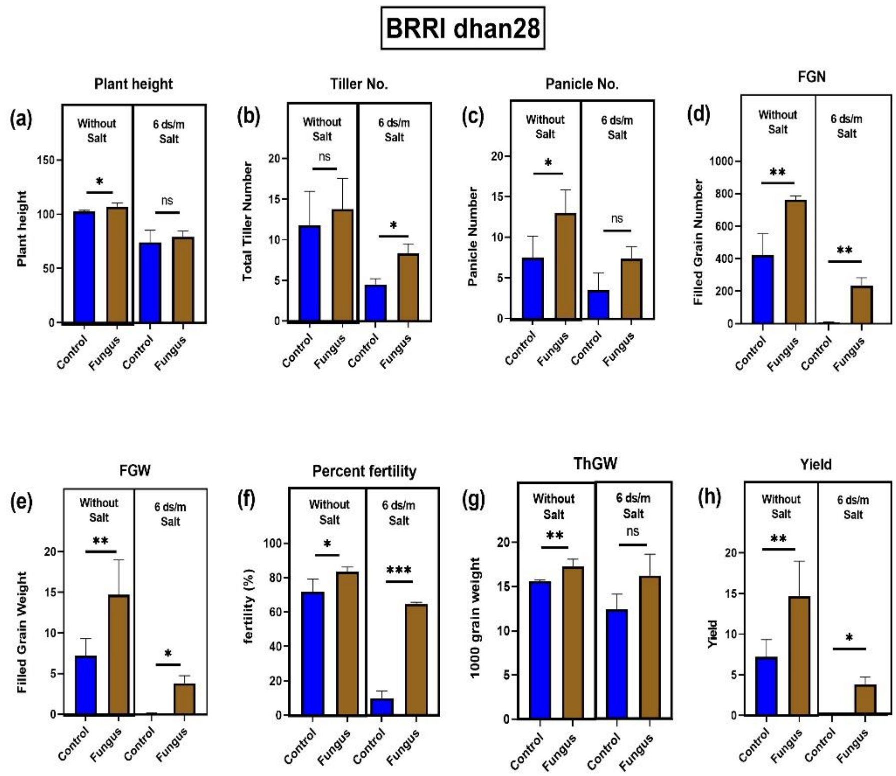

Figure 3. Effect of the biofertilizer on the yield parameters of BD-28 rice plant under normal and saline conditions. The bar plots show the changes in (a) plant height, (b) total tiller number, (c) panicle number, (d) filled grain number, (e) filled grain weight, (f) percent fertility, (g) 1000 grain weight, and (h) yield of control and *Aspergillus welwitschiae Oc*streb1-inoculated plants without and with salt. Bars represent mean ± SD. T-test was done for all parameters separately. Significant differences between control and treated plants are indicated by asterisks (*, **, ***) at $p \leq 0.05$, 0.01 and 0.001 respectively.

BD-67 showed similar trends to BD-28 in response to the biofertilizer treatment (Figure 4 and Supplementary Table 1). In non-saline conditions, plant height was increased by approximately 5.15%, tiller number by 33.06%, panicle number by 8.53%, and filled grain number by 41.08%. Additionally, filled grain weight and 1000 grain weight increased by 45.15% and 6.33%, respectively. Moreover, the percentage of the fertility of biofertilizer-treated plants showed a 4.43% increase compared to the control. Under salt stress conditions, the performance gap between control and biofertilizer-treated plants was widened. The biofertilizer-treated plants consistently outperformed the control group across all the parameters. Notably, tiller number, panicle number, filled grain number, filled grain weight, 1000 grain weight, and yield per plant increased by 117.5%, 73.33%, 157.97%, 153.64%, 6.39%, and 153.64%, respectively, compared to the control plants under salt stress.

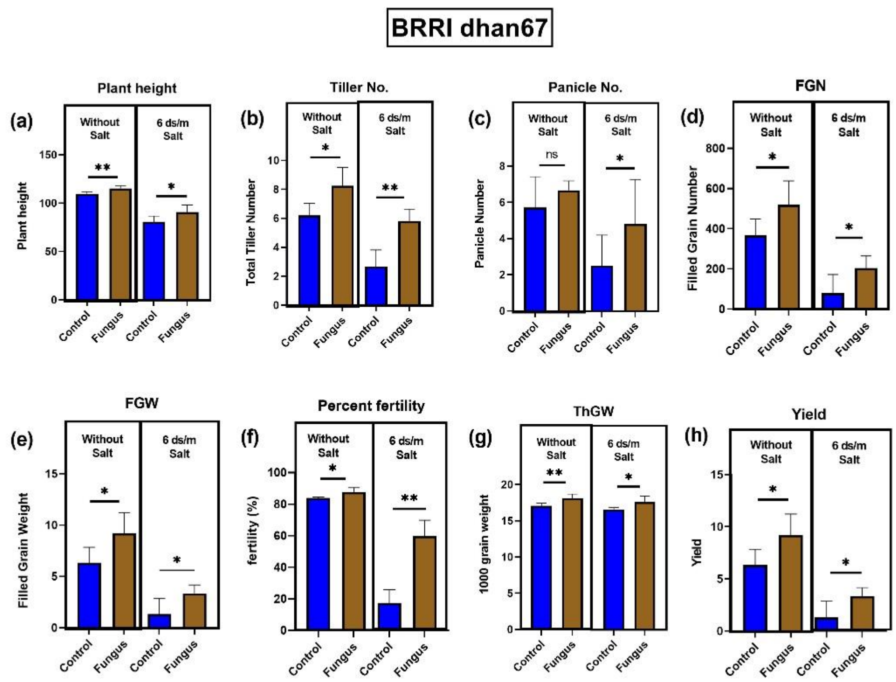

Figure 4. Effect of the biofertilizer on the yield parameters of BD-67 rice plant under normal and saline conditions. The bar plots show the changes in (a) plant height, (b) total tiller number, (c) panicle number, (d) filled grain number, (e) filled grain weight, (f) percent fertility, (g) 1000 grain weight, and (h) yield of control and *Aspergillus welwitschiae Oc*streb1-inoculated plants without and with salt. Bars represent mean ± SD. T-test was done for all parameters separately. Significant differences between control and treated plants were indicated by asterisks (*, **, ***) at $p \leq 0.05$, 0.01 and 0.001 respectively.

BD-87 also exhibited a significant increase in some yield parameters (Figure 5 and Supplementary Table 1). In the absence of salt stress, the biofertilizer-treated plants, demonstrated notable improvements across multiple metrics. Conversely, under salt stress conditions, the performance enhancement attributed to biofertilizer treatment was even more pronounced. Biofertilizer-treated plants showed substantial increases in plant height (37.37%), tiller number (141.98%), panicle number (92.73%), filled grain number (427.59%), filled grain weight (293.63%), percentage fertility (66.56%), 1000 grain weight (9.35%), and yield per plant (293.63%), as compared to their controls.

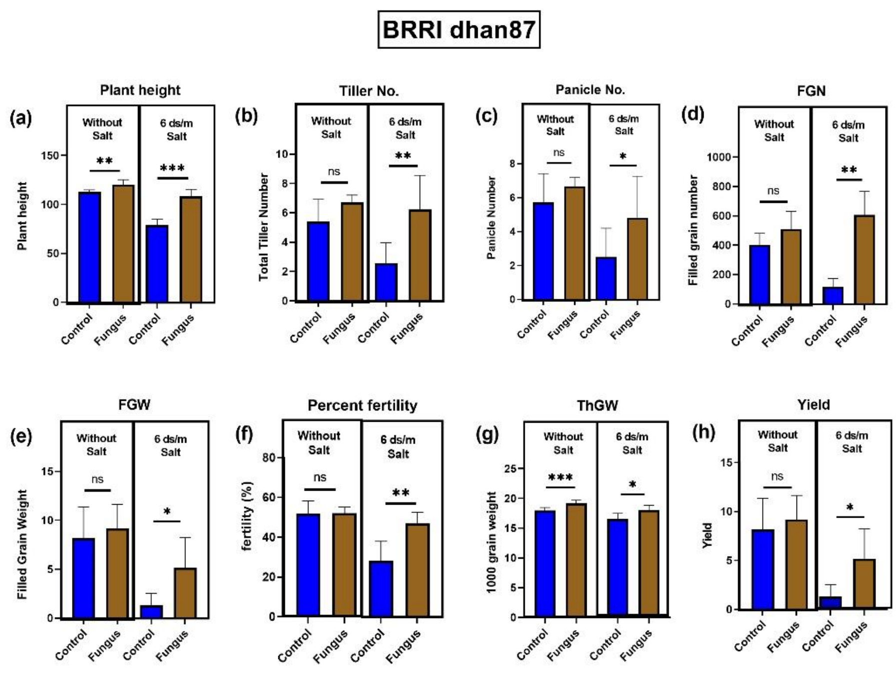

Figure 5. Effect of biofertilizer on the yield parameters of BD-87 rice plant under normal and salineconditions. The bar plots show the changes in (a) plant height, (b) total tiller number, (c) panicle number, (d) filled grain number, (e) filled grain weight, (f) percent fertility, (g) 1000 grain weight, and (h) yield of control and *Aspergillus welwitschiae Oc*streb1-inoculated plants without and with salt. Bars represent mean ± SD. T-test was done for all parameters separately. Significant differences between control and treated plants were indicated by asterisks (*, **, ***) at $p \leq 0.05$, 0.01 and 0.001 respectively.

Discussion

Mitigation of salt stress in plants by using plant growth-promoting microbes has become a promising technique for sustainable agriculture. Many microorganisms, specially, bacteria and fungi exhibit the capacity to induce crop resilience in extreme saline conditions. Plant growth-promoting endophytic fungi (PGPEF) inhabiting halophytic plants pose a symbiotic relationship with their hosts. They enhance the nutrient uptake of plants through their solubilization and produce compounds to reduce the toxic effects of soil contaminants on plants (Aizaz *et al.*, 2023). They help the plants to thrive in the highly saline condition by maintaining osmotic balance, delaying senescence, reducing ROS accumulation and oxidative damage and by maintaining Na^+/K^+ ratio. The fungus, *Aspergillus welwitschiae Oc*streb1, was isolated from the root interior of the wild halophytic rice, *Oryza coarctata*. This wild rice genotype can survive during submergence under extreme saline water such as sea water at 40-50 dS/m for a long period of time. It grows in abundance in the coastal regions (Garg *et al.*, 2014). However, *AwOc*sterb1 showed excellent PGP characteristics such as Nitrogen fixation, Zinc and Phosphate solubilization, Indole acetic acid production, etc. in both non-saline and extreme salt conditions (900mM salt) during in-vitro laboratory tests. The fungus showed its efficacy by significantly increasing the yield of BRRI dhan28 under 6 dS/m salinity during pot trials. In addition, plant metabolites such as chlorophyll content, protein, sugar, flavonoid, and phenolic contents were also elevated in fungus-inoculated plants with and without salt stress. In the current work, where a large-scale trial was done in saline tanks, the fungus enhanced the plant height, filled grain number, filled grain weight, 1000 grain weight, etc., leading to a remarkable elevation of the rice fertility and yield under both non-saline and 6 dS/m saline conditions.

Another strain of the fungus, *Aspergilus welwitschiae* BK, significantly promoted the growth of maize under the saline condition (Gul *et al.*, 2023). In addition, a novel PGPF named *Penicillium olsonii* A3, contributed to the better growth of tobacco plants under salt stress condition (Tarroum *et al.*, 2022). In our case, *Aspergillus welwitschiae Oc*streb1 strain significantly increased the filled grain number and filled grain weight, leading to a remarkable elevation of rice fertility and yield.

Biofertilizer is a bio-based fertilizer that can be prepared using plant or animal source or using beneficial microorganisms. Different carrier materials such as clay minerals, wheat bran, rice bran, saw dust support the growth of microorganisms and enhance their shelf life (Chaudhary *et al.*, 2022). Wheat bran is also suitable for the growth of *Aspergillus niger* (Fnca *et al.*, 2006). On the other hand, talc-based formulation of *Bacillus* species increased the growth of mung bean and rice (Pahari *et al.*, 2017). In addition, talc based formulation of *Streptomyces corchorusii UCR3-16* had longer shelf life while stored at both room temperature and at 4°C than the corn-starch based formulation (Tamreihao *et al.*, 2016). Application of biofertilizers is an eco-friendly and revolutionary method in sustainable agriculture, reducing the frequent use of chemical fertilizers and thereby lowering environmental pollution (Kumar *et al.*, 2022). As the cost of biofertilizer formulation is low, the production value will be minimal and may lead to adequate amount of cereal crops even under abiotic stresses such as salinity. This in turn will positively impact the country's economy, particularly for the benefit of resource-poor farmers in the southern coastal region of Bangladesh.

In this study, a formulation of biofertilizer was prepared using *Aspergillus welwitschiae Oc*streb1 and talc powder (as carrier). Upon formulation, the efficacy of the fungal biofertilizer was evaluated at Bangladesh Rice Research Institute. The biofertilizer was found to improve yield parameters of BD-28, BD-67, and BD-87 both without salt and under salt stress. Previously, several biofertilizers were reported to enhance plant growth and yield under several biotic and abiotic stress conditions. For example, inoculation of *P. oryzae* promoted rice growth and chlorophyll content (Zhu *et al.*, 2022). The phosphate solubilizing *Aspergillus niger* improved the growth and yield of *Lagenaria siceraria* (bottle gourd) and *Abelmoschus esculentus* (okra) (Din *et al.*, 2019). Application of biofertilizer containing *H. seropedicae* and *G. mosseae* significantly increased the yield of rice (kg ha^{-1}) by 20% and 35%, respectively (Hoseinzade *et al.*, 2016). Inoculums of *S. indica* including encapsulated spores can modulate the effect of drought stress on maize (Kaboosi *et al.*, 2023). Halotolerant microbial community-based biofertilizers enhance rice plant growth and mitigate saline stress effects (Shan *et al.*, 2023). The result of this study correlates with these previous reports. This indicates that *Aspergillus welwitschiae Oc*streb1 is suitable as a biofertilizer for improving rice growth, especially under salt stress. This means that this biofertilizer has the potential to promote rice yields in the salt-prone regions of the Southern coastal areas of Bangladesh.

Conclusion

Salt stress adversely affects the production of cereal crops in coastal regions that causes a negative impact on the economy of our country. By harnessing the beneficial effect of *Aspergillus welwitschiae Oc*streb1, the detrimental effect of soil salinity on crop yield can be reduced. *Aspergillus welwitschiae Oc*streb1 containing biofertilizer can decrease the excessive use of chemical fertilizer, resulting in lowering the production cost and environmental pollution as well as preservation of our eco-system. The efficiency of *Aspergillus welwitschiae Oc*streb1 based biofertilizer in increasing rice yield across different varieties underscores its potential to increase the yield of various cereal crops under both non-saline and extreme saline conditions. This study suggests that the eco-friendly biofertilizer based on *Aspergillus welwitschiae Oc*streb1 is suitable for further field trials to test the enhancement of crop yields in the saline coastal regions of our country. This work has also set the stage for determining whether this biofertilizer can serve as an alternative to chemical fertilizers.

Authors Contribution

AC and MIA formulated the biofertlizer, handled all the plants, analyzed data and wrote the manuscript, AAA helped in biofertilizer preparation, TA helped in planning the experimental set up, MSR designed and supervised the BRRI tank experiment, MRI, RAB, and ZIS designed the study, ZIS supervised the whole experiment, arranged funds for the study and edited the manuscript.

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