



RESEARCH ARTICLE

Mitigation of salinity stress on morpho-physiological and yield related parameters of rice using different organic amendments

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Abstract

Salinity is a pernicious abiotic element that hinders crop development. Utilizing organic amendments to remediate salt is crucial for enhancing soil function and promoting crop growth. Based on this, a pot experiment was carried out at the research field of Khulna Agricultural University, Khulna, to examine the physiology, growth and yield of transplanted aman rice in response to salinity stress using duckweed and dhaincha biomass supplement. Excess salinity with no organic amendments (OA) reduced plant growth and development, relative water content (26%) and membrane stability (28%), index compared to T₁S₁ (duckweed) and T₂S₁ (dhaincha) at 50 mM salinity. Salinity delayed the emergence of first flowering and the maturity of filled grains. The lowest grain yield was recorded in T₀S₃ (no treatment + 100 mM salinity). Application of dhaincha and duckweed biomass ameliorates salinity individually at all salinity levels. Rice grown in saline soil with the application of T₁ (duckweed biomass) and T₂ (*Sesbania* biomass) had an 87% spikelet fertility while rice grown in soil without T₁ and T₂ treatment had only 21.82%. The application of T₂ and T₁ @ 5 t ha⁻¹ increased grain yield by 33.29% and 4.70% compared to control. Furthermore, salinity stress @100 mM NaCl with duckweed decreased grain yield by 0.05% which was minimized to 12% by applying dhaincha green manure (T₂) @ 5 t ha⁻¹. The finding showed that the ameliorative impact of green manure at 5 t ha⁻¹ dose (T₂) was more effective compared to the duckweed (T₁) at the three salinity levels used in the study.

Keywords

salinity; organic amendments; mitigation; duckweed biomass; dhaincha biomass; yield

Introduction

Total cultivable land for food production has been declining over the last few decades owing to anthropogenic and natural calamities. Salinity is one of the threatening abiotic factors that is gradually engulfing the cultivable land and reducing the world's 30% rice production (1). Salinity stress results from salt build-up in the root zones of plants, which causes the plants to become physiologically floppy and unsuitable for production (2). It is assumed that salinity will worsen for cultivable land within 2050 and be alarming for rice cultivation too (3). Chemically formed sedimentary rock

created soil salinity as the soil was the disintegrating form of rock and minerals. Besides, human-induced inappropriate irrigation practices with salt-water irrigation, shrimp cultivation also jeopardize soil salinity. Salinity damages soil chemically, biologically, and physically by reducing soil aeration, water infiltration rate, soil structural stability and bulk density (4). Salinity lessens microbial community in soil and increased Na^+ accumulation with consequent decrease of K^+ (5), that hampered soil nutrient availability for crop production. In addition, soluble salts hold water more tightly to extract for plants and create osmotic stress, reducing plant growth. Salinity imposed oxidative stress and increased the production of H_2O_2 , MDA which reduced cell functioning with substantially reduced crop growth and production (6, 7). Salinity stress also damaged structure of chloroplast with consequent decrease of photosynthesis (8, 9). Some researchers revealed that rice crop is more sensitive to salinity at an early stage compared to the flowering and grain filling stage. Under salinity, osmotic stress-imposed restrictions on crop sources and sink assimilation, affect panicle length, flowering, grain filling, and finally fruit setting of rice. Rice is a halophytic crop and can tolerate a very negligible amount of salinity stress. To boost rice production and protect land from devastating effect of salinity, urged an eco-friendly, cost-effective approach. Organic amendments have the capacity to significantly improved the physicochemical properties of soil, which might make a substantial difference in converting the saline environment into a stable and healthy crop environment (2). Using different organic amendments like biochar from different sources, compost, farm yard manure and vermicompost have been used recently for the reclamation of soil salinity (4, 10, 11). Dhaincha also has very promising green manuring crops regarding those aspects (12). *Sesbania* is a nitrogen-fixing, modulating leguminous crop (13) which consists of calcium (Ca), nitrogen (N), phosphorus (P) and potassium (K). Furthermore, it also contributes to sustainable agriculture by reducing the use of chemical fertilizer and improving soil health (14). After decomposition, it increased soil organic matter, released various nutrients into the soil and made them available to the plants and succeeding crops too (15, 16). Green manure plants secrete certain organic acids from the roots into the rhizosphere, which increases the solubility of insoluble salts and reduces the total salinity of the soil. In addition, green manure plants penetrate hard and compact layers of saline soil with their roots, which improves soil aeration and increases the rate of water penetration, as well as improving ion exchange and binding. The organic matter returned to the soil with green manure acts as an excellent source of cation exchange, replacing sodium ions responsible for salinity with beneficial elements such as Ca^{2+} and Mg^{2+} . Mg^{2+} play an important role in photosynthesis. Duckweed (*Lemna minor*) is also an aquatic plant newly introduced for salinity mitigation that belongs to Lemnaceae family. Duckweed contains a high concentration of trace minerals like K, P and fat amino acids (17) that can tolerate a wide range of saline water

and significantly improve osmotic stress tolerance (18). Rice is considered salt sensitive and limited research is available about the effect of duckweed and *dhaincha* on their performances of rice growth and yield-related parameters under salinity. Thus, this study was performed to determine the comparative effect of duckweed and *dhaincha* on growth and physiological traits under salinity.

Materials and Methods

The experiment was carried out at Khulna Agricultural University, Khulna, during the period from June to October 2022. The soil was clay loam in texture with a soil pH of 6.43. The different treatments viz., control (T_0), duckweed biomass (*Lemna minor*) (T_1) and dhaincha biomass (*Sesbania rostrata*) (T_2) at the rate of 5 t h^{-1} as well as four levels of salinity viz. S_0 (Control), S_1 (50 mM), S_2 (75 mM) and S_3 (100 mM) were used and Transplant aman BRRi dhan66 which was collected from Bangladesh Rice Research Institute (BRRi) Gazipur, was considered as a test crop. The pot experiment was carried out using a completely randomized block design (RCBD) with three replications.

Plant materials and treatments

Two organic amendments, namely ex-situ dhaincha and duckweed biomass, were gathered, chopped and submerged in water for the next thirteen days to break down before being combined with pot soil. BRRi Dhan 66 seeds are drought tolerant high yielding rice varieties and were collected from Bangladesh Rice Research Institute (BRRi), Gazipur. Collected rice seeds of rice were soaked in water for 48 hours to germinate before sowing into a nursery. The recommended doses of fertilizer were applied per pot. After germination and raising seedlings in the seed bed, thirty days old seedlings were transplanted manually on 20 July (Fig 1) Three seedlings were on each hill

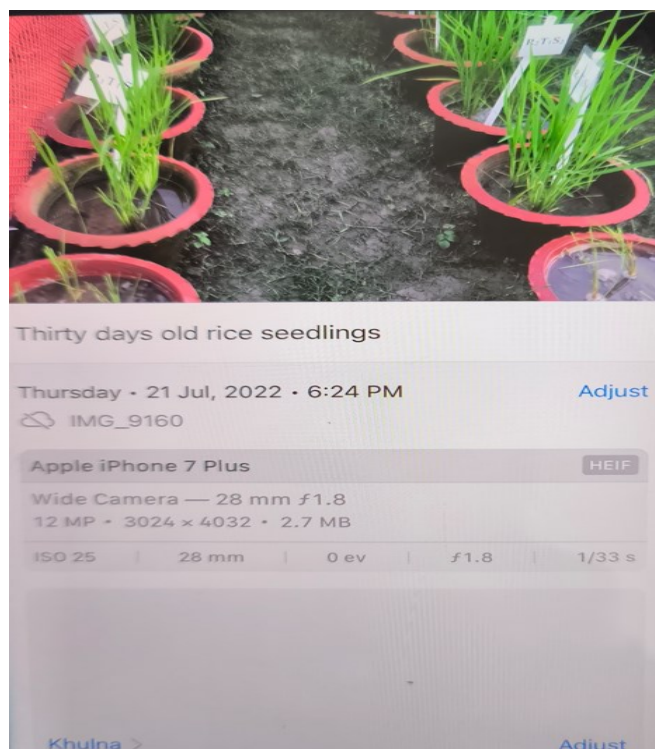


Fig. 1. Geotagged picture of 30 days old rice seedling

Pot soil Preparation

The pot contains 10 kg of loamy texture soil. All inert materials were removed once the soil had dried out in the sun and loosened. After that, the soil with dhaincha and duckweed biomass was filled in the pot. Three hills of rice were placed in each experimental pot, which represents a replication. The entire amount of triple super phosphate (TSP), muriate of potash (MoP), gypsum, zinc sulphate (ZnSO₄) and one-third of urea was applied during the last potting preparation. Two equal amounts of the remaining urea were top-dressed, one at 20 days after transplanting (DAT) and the other at 40 DAT.

Salinity imposition: Salinity stress at the levels of 50 mM, 75 mM and 100 mM NaCl were applied at three days intervals when the crop was at fifty days old. The experimental pot was covered with polyethene after saltwater application to avoid miscellaneous effects.

Data collection procedure

Two plants from each pot were harvested, and plant height, tiller plant⁻¹, root and shoot dry weight were recorded on different days after sowing (DAS). The physiological characteristics observed, including the relative water content (RWC) and the membrane stability index (MSI) were taken when the plant was at the maximum vegetative stage. The FW of the entire leaf lamina will be weighed, then floated on water in Petri dishes and kept in a dark place. Eight hours later, after removing the extra surface water, the leaf lamina was weighed again, which was then considered a turgid weight (TW). Then dry weight (DW) was weighed after drying at 80 °C for 48h. The calculation was done as, below following the procedure of Botânica *et al.* (19).

$$\text{RWC} = (\text{FW} - \text{DW}) / (\text{TW} - \text{FW}) \times 100$$

Leaf MSI was estimated according to Sairam *et al.* (20). Two sets of fresh leaf material were taken in test

tubes containing 10 ml of double distilled water. A conductivity bridge (C₁) was used to record the electrical conductivity of a solution after one set was heated to 40°C for thirty minutes in a water bath. After 10 minutes of boiling at 100°C in a boiling water bath, the conductivity of an additional sample was determined using a conductivity bridge (C₂) MSI was calculated as: $\text{MSI} = [1 - (C_1/C_2)] \times 100$. After full maturity, two plants of pots were harvested to determine paddy yield. The collected data were analysed statistically using the Statistix-10 computer package.

Results

Amelioration of salinity stress through T₁ followed by T₂ Morphological characters

The effect of different levels of salinity on plant height, effective tiller number, root weight hill⁻¹ and shoot weight hill⁻¹ was significant, as shown in Table 1. Results indicated that all the morphological parameters of rice decreased with increased salinity levels. The non-effective tiller number hill⁻¹ increased with increasing salinity levels. The results showed that, excess salinity (100 mM) decreased the plant height at 100 DAS (10%), tiller number (14.81%), root dry weight (28%) and shoot dry weight (2.35%), compared to control.

Application of organic amendments (T₁ and T₂) had a significant effect on all the studied morphological parameters (Table 3) and increased plant height by 58% compared to the control, which was 8% higher than duckweed biomass. With increasing salinity, both dhaincha and duckweed showed increased shoot and root biomass (34% and 14%) accumulation compared to control (Fig 3) at different salinity levels. The highest number of tillers, effective tillers hill⁻¹ were recorded at control with dhaincha biomass application (T₂S₀) which was statistically similar to T₂S₁.

Table 1. Effect of salinity stress on yield and yield attributes of transplant Aman rice var. BRRI Dhan66

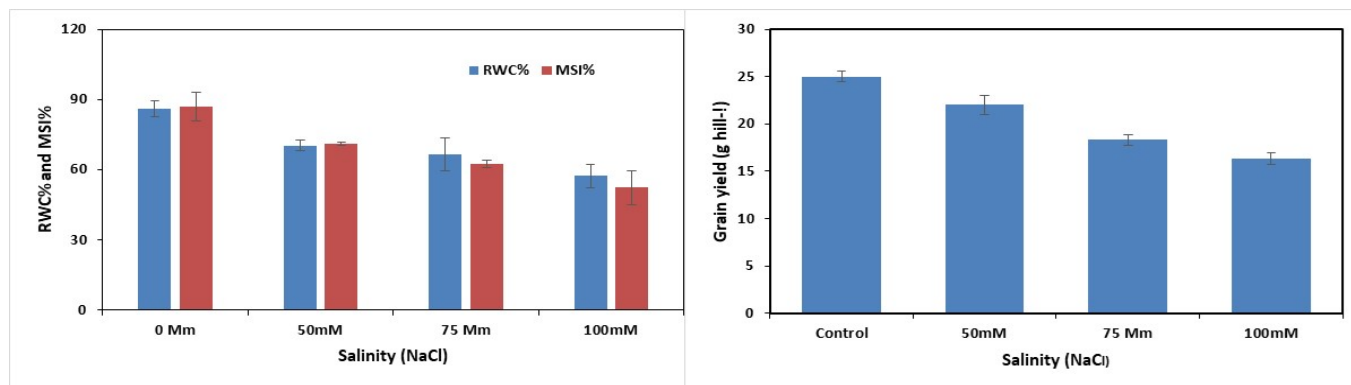
Salinity Stress	Plant height (cm)			Tiller plant ¹		Root DW (g)	Shoot DW (g)	Non-Effective tiller plant ¹
	60DAS	80DAS	100DAS	60DAS	90DAS			
T ₀ S ₀	55c	59.12c	94.11c	4.55cd	5.44b	3.07a	14.00a	2.35a
T ₀ S ₁	53.333d	58.00c	93.33c	4.33cd	5.33b	2.00ab	13.33a	2.00a
T ₀ S ₂	48.667e	59.33c	86.00cd	4.33cd	4.66b	2.00b	11.63ab	2.33ab
T ₀ S ₃	43.000f	60.00c	85.33d	3.66d	4.66b	1.48b	13.67b	3.33b

Here, T₀S₀ = no OA and no salt, T₀S₁ = no OA + 50 mM of salinity, T₀S₂ = no OA + 75 mM of salinity, T₀S₃ = no OA + 100 mM of salinity

Table 2. Effect of salinity stress on yield and yield attributes of transplant Aman rice var. BRRI Dhan66

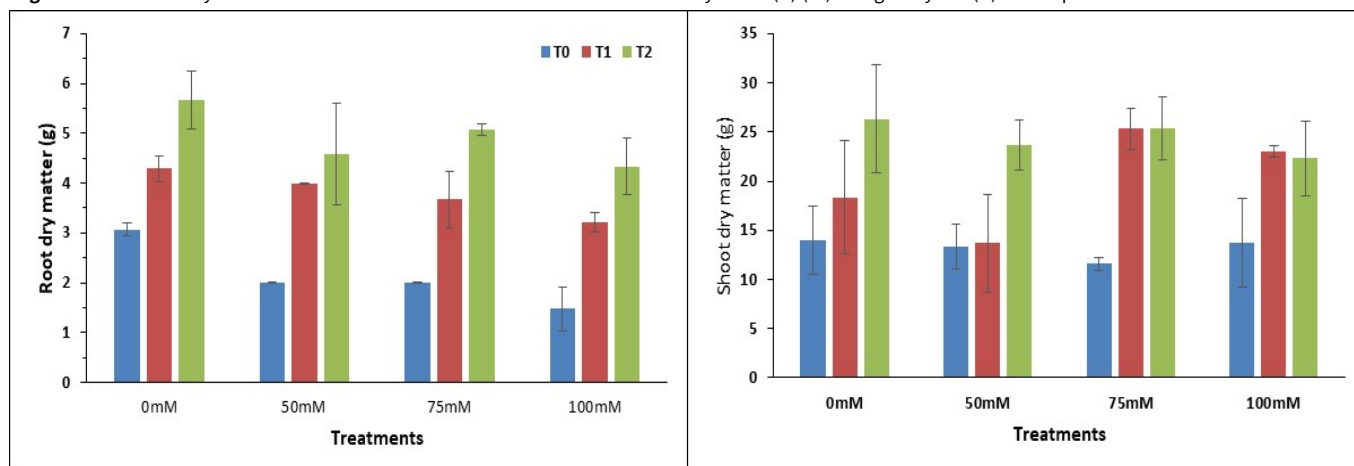
Salinity stress	Effective tiller	Spikelet panicle ⁻¹	Sterile grain panicle ⁻¹	1st day of emergence of panicle	Days to maturity	Panicle length (cm)
T ₀ S ₀	5.44b	115.11b	23.11a	85a	121.33a	24b
T ₀ S ₁	5.33bc	114.33b	26.00a	95a	123.67a	23.66b
T ₀ S ₂	3.66cd	107.67b	29.66a	95a	124.67a	23.0c
T ₀ S ₃	3.33d	98.77b	35.33b	97a	125a	22.66c

Here, T₀S₀ = no OA and no salt, T₀S₁ = no OA + 50 mM of salinity, T₀S₂ = no OA + 75 mM of salinity, T₀S₃ = no OA + 100 mM of salinity



Here, T_0S_0 = no OA and no salt, T_0S_1 = no OA + 50 mM of salinity, T_0S_2 = no OA + 75 mM of salinity, T_0S_3 = no OA + 100 mM of salinity. Bar shows mean values of three replicates and bar shows the standard error of means. (LSD value = 2.65, 3.05, 4.79)

Fig. 2. Effect of salinity stress on relative water content and membrane stability index (A) (%) and grain yield (B) of rice plant.



Here, S_0 = Control, S_1 = 50 mM of salinity, S_2 = 75 mM of salinity, S_3 = 100 mM of salinity, T_1 = Duckweed, T_2 = Sesbania, T_0 = Control. Bar shows mean values of three replicates and bar shows the standard error of means. (LSD value = 0.29, 3.09)

Fig. 3. Effect of organic amendments on root dry weight (A) and shoot dry matter (B) of rice plant under salinity.

Yield attributes and grain yield

Experimental results showed, that the highest effective spikelet panicle⁻¹, grain yield hill⁻¹ and panicle length were recorded in control and the above parameters decreased with increased salinity levels (Table 2). On the other hand, the number of sterile spikelets panicle⁻¹, 1st day of emergence of flower and days of maturity increased with increasing salinity levels. Results showed that, effective tiller (30%), spikelet panicle⁻¹ (14%) and panicle length (5.5) were reduced at the 100 mM salinity level compared to the control. The reduction in grain yield was 18%, 27%, and 37% for S_1 , S_2 and S_3 compared to the control (without salt), respectively. Application of organic amendments (T_1 and T_2) had a significant influence on grain yield (figure 5) and yield attributes of transplant Aman rice var. BRRI dhan 66 at different levels of salinity stress (Table 3). A maximum number of effective tillers hill⁻¹ (85% and 50%) spikelet's panicle⁻¹ (50%), filled grains panicle⁻¹ and grain yield (27% and 12%) were observed from T_2 and T_1 over control. With the increased salinity level, 12% and .05% grain yields were reduced by T_2S_3 and T_1S_3 compared to T_2S_0 and T_1S_0 whereas T_2 produced the highest grain yield (27%) over T_1 (12%).

Physiological parameters

Results showed that leaf water content and membrane stability index in leaves decreased with increasing salinity levels (Fig 2). Excess (100 mM) salinity reduced relative

water content and membrane stability index by 41% and 45% compared to control. These results indicate that salinity seriously hampered the physiological parameters of rice. Organic amendments had a significant influence on relative water content and membrane stability in BRRI dhan 66 at different levels of salinity stress (Figure 4). With the increased salinity, 24% and 30% reduction of RWC and 19% and 28% of MSI were noted by T_2S_3 and T_1S_3 compared to control but a minimum reduction was noted under control and 50 mM with T_2S_0 followed by T_1S_0 . On the other hand, the application of dhaincha amendments significantly increased RWC (8%) and MSI (16%) compared to duckweed.

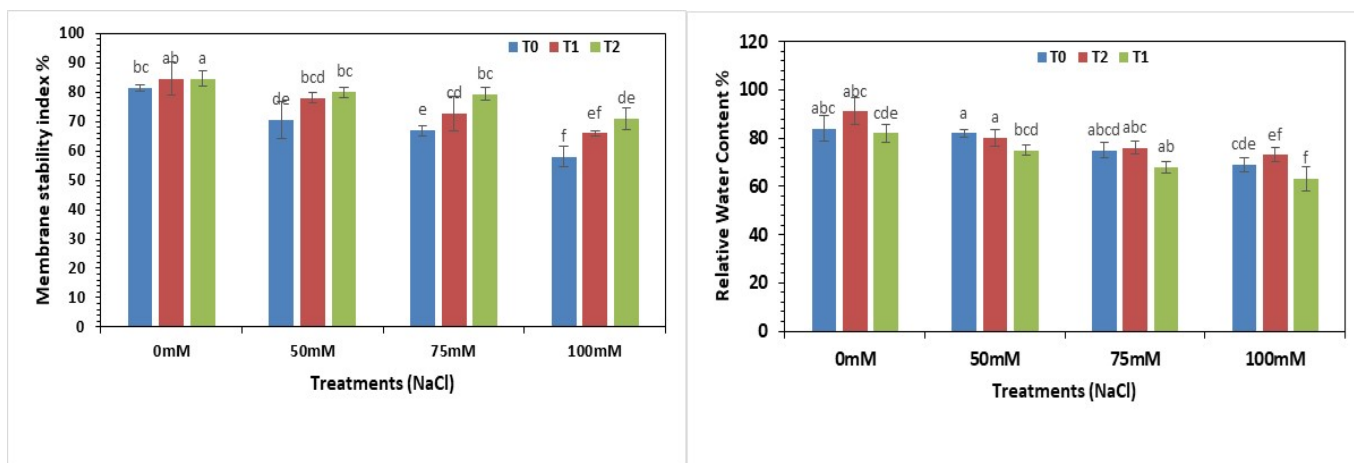
Comparison between two amendments regarding grain yield

The application of dhaincha green manure (T_2) @ 5 t ha⁻¹ and duckweed (T_1) @ 5 t ha⁻¹ increased grain yield by 37% and 24% under non-saline conditions and 12% and 31% under saline conditions (50 mM) over control (Table 4). On the other hand, imposition of salinity stress @ 100 mM NaCl with duckweed decreased grain yield by 0.05%, which was minimized to 12% by applying dhaincha green manure (T_2) @ 5 t ha⁻¹. The results revealed that the ameliorative effect of green manure at 5t ha⁻¹ dose (T_2) was more effective than that of duckweed (T_1) at both salinity levels used in the study.

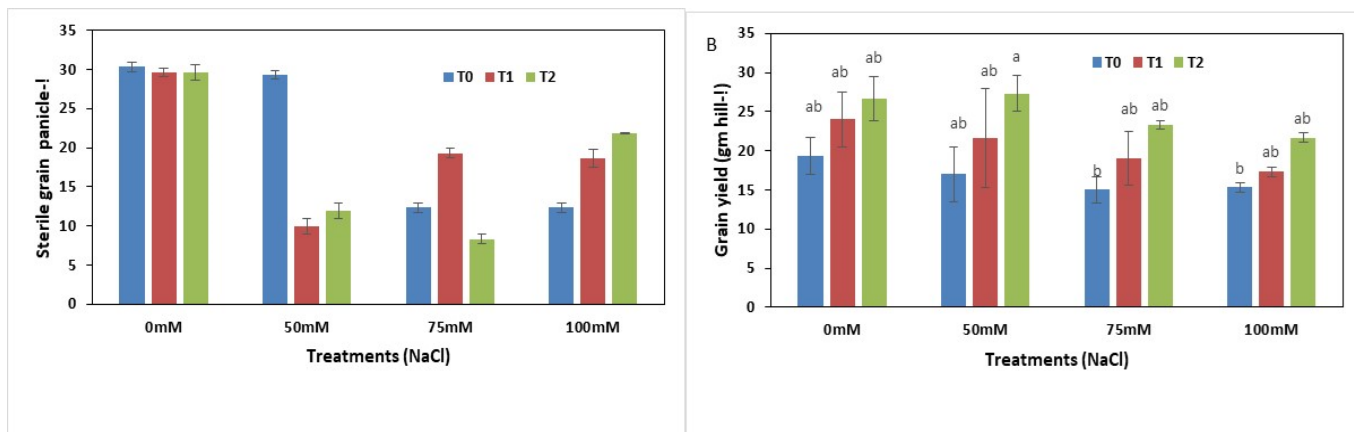
Table 3. Effect of Dhaincha and duckweed on crop growth and yield attributes of transplant Aman rice var. BRRI Dhan 66 under salinity.

Interaction	Plant height(cm)			Tiller plant ⁻¹		Effective tiller	Panicle length (cm)	Spikelet panicle ⁻¹	Filled grain panicle ⁻¹
	60DAS	80DAS	100DAS	60DAS	90DAS				
T ₀ S ₀	55c	59.12c	94.11c	4.55cd	5.44b	5.44b	24b	115.11b	85b
T ₀ S ₁	53.333d	58.00c	93.33c	4.33cd	5.33b	5.33bc	23.66b	114.33b	84.77b
T ₀ S ₂	48.667e	59.33c	86.00cd	4.33cd	4.66b	3.66cd	23.0c	107.67b	80.77b
T ₀ S ₃	43.000f	60.00c	85.33d	3.66d	4.66b	3.33d	22.66c	98.77b	69.77b
T ₁ S ₀	70a	84.10a	124a	7.11a	8.11a	8a	32a	158a	146a
T ₁ S ₁	69.00a	83.33a	122.00a	6.33ab	8.00a	7.66a	31.33a	157.33a	140.67a
T ₁ S ₂	65.33ab	83.33a	115.33a	7.33a	7.66a	7.66a	30.00a	143.00ab	136.00ab
T ₁ S ₃	56.00bc	74.33a	108.00b	5.00ab	6.66ab	6.66a	25.33b	134.67ab	124.00ab
T ₂ S ₀	72a	84.21a	135.55a	9a	10.11a	9.90a	37.65a	181.33a	159a
T ₂ S ₁	70.000a	83.33a	133.33a	8.33a	9.00a	9.00a	35.00a	177.33a	157.33a
T ₂ S ₂	65.333b	83.00a	127.33a	7.66ab	9.00a	9.00a	33.00b	176.77a	153.77a
T ₂ S ₃	56.660c	74.00b	112.67b	6.00bc	6.66b	6.66b	31.33b	148.67a	137.00b

Here, S₀= no salinity, S₁=50mM of salinity, S₂=75 mM of salinity, S₃=100 mM of salinity, T₀= no organic amendments, T₁=Duckweed, T₂=Dhaincha, and twelve treatments were, T₀S₀ = no OA and no salt, T₀S₁ = no OA + 50 mM of salinity, T₀S₂ = no OA + 75 mM of salinity, T₀S₃ = no OA + 100 mM of salinity, T₁S₀ = Duckweed OA and no salt, T₁S₁ = Duckweed OA + 50 mM of salinity, T₁S₂ = Duckweed OA + 75 mM of salinity, T₁S₃ = Duckweed OA + 100 mM of salinity, T₂S₀ = Dhaincha OA and no salt, T₂S₁ = Dhaincha OA + 50 mM of salinity, T₂S₂ = Dhaincha OA + 75 mM of salinity, T₂S₃ = Dhaincha OA + 100 mM of salinity



Here, S₀= Control, S₁=50mM of salinity, S₂=75 mM of salinity, S₃=100 mM of salinity, T₁=Duckweed, T₂=Dhaincha, T₀= Control. Bar shows mean values of three replicates and bar shows the standard error of means. Bar labeled with different alphabets show significant difference according to LSD test at 5% probability. (LSD value = 2.77, 2.13)

Fig. 4. Effect of organic amendments on membrane stability (A) (%) and Relative water content (B) (%) of rice plant under salinity.

Here, S₀= Control, S₁=50mM of salinity, S₂=75 mM of salinity, S₃=100 mM of salinity, T₁=Duckweed, T₂=Dhaincha, T₀= Control. Bar shows mean values of three replicates and bar shows the standard error of means. Bar labeled with different alphabets show significant difference according to LSD test at 5% probability. (LSD value = 2.01, 3.35)

Fig. 5. Effect of organic amendments on sterile grain panicle⁻¹ (A) and grain yield (B) (g hill⁻¹) of T. aman rice under salinity

Table 4. Interaction effect of organic amendments (T₁ and T₂) and salinity stress on yield and yield attributes of transplant Aman rice var. BRR1 dhan66

Treatments	Grain yield (g hill ⁻¹)	% increase or decrease over control
T ₀ S ₀	19.33	-
T ₁ S ₀	24	24.70%
T ₂ S ₀	26.66	37%
T ₁ S ₁	21.66	12%
T ₁ S ₂	20	3.46%
T ₁ S ₃	17	-0.05%
T ₂ S ₁	25.33	31.29%
T ₂ S ₂	23.33	21%
T ₂ S ₃	21.67	12%

T₀S₀ = no OA and no salt, T₂S₁= Dhaincha OA + 50 mM of salinity, T₂S₂= Dhaincha OA + 75 mM of salinity, T₂S₃= Dhaincha OA + 100 mM of salinity, T₁S₀ = Duckweed OA and no salt, T₁S₁= Duckweed OA + 50 mM of salinity, T₂S₂= Duckweed OA + 75 mM of salinity, T₂S₃= Duckweed OA + 100 mM of salinity.

Discussion

Salinity is most threatening for crop cultivation all over the world. Salinity significantly reduced plant growth and yield contributing parameters. The addition of leguminous green manuring crops such as dhaincha and duckweed biomass as organic amendments, reduces soil salinity (4) and increases crop growth. It was observed from the experiment that, excess salinity (100 mM) decreased the plant height, tiller number, root dry matter and shoot dry matter compared to the control. The reduction of the above parameter under salinity conditions might be due to an osmotic and ionic effect that caused nutrient imbalance along with hampered leaf water potential (21) resulting in poor morphological performance (22). Excess salinity reduces plant height by hampering nutrient uptake, cell division, and enzymatic activities (23, 24). The addition of dhaincha and duckweed amendments contains a substantial amount of nitrogen, phosphorus and potassium (4, 25) which reduced salinity stress, increased essential plant nutrients in rhizosphere areas and increased plant morphological performances in the present study.

The incorporation of dhaincha crops into soil produced a substantial amount of biomass, and different organic acids, amino acids, sugars and mucilage were also released (14) during crop growth. The organic matter returned into the soil through dhaincha green manuring crops acts as an excellent source for cation exchange, replacing the sodium ions responsible for salinity with beneficial elements like calcium and magnesium. These results indicated that under salinity stress, the use of organic amendments (T₁ and T₂) might decrease Na⁺ uptake, increasing the uptake of vital nutrients to boost root and shoot dry matter and crop growth (26). According to the study's findings, as salinity levels rose, panicle length, grain yield hill⁻¹ and the maximum effective spikelet panicle⁻¹ declined. Under saline stress, there may be a lesser assimilate supply to flowering that affects panicle formation and grain filling, resulting in more unfilled grains panicle⁻¹ with a consequent decrease in

yield and yield contributing parameters. Zhu et al. (27) and Zheng et al. (28) agreed that all the growth and yield parameters were reduced with increasing salinity levels.

The application of organic amendments had a significant effect on grain yield. Since dhaincha is a nitrogen-fixing leguminous green manuring crop, it improves soil salinity through ion exchange along with making available more nutrients needed for crop yield. Which notably increased the grain yield of dhaincha in comparison to the duckweed biomass. In addition, nitrogen is a vital nutrient for protein synthesis and cell structure that affects antioxidant activities, metabolic processes and enhanced salt tolerance. Antioxidants scavenge reactive oxygen species (ROS) and protect the plant from salt induced damaging effects (29, 30). Excess (100 mM) salinity reduced relative water content and membrane stability index by 41% and 45% compared to control, respectively, in the present study. These findings showed that rice's physiological characteristics were severely affected by salt. This might be due to osmotic effects that reduce water uptake by roots. Salt stress disrupted the turgidity of guard cells and decreased the membrane stability index (MRI), which had an impact on stomatal functioning (31). The addition of dhaincha and duckweed increased MSI leaching of NaCl below the root zone, with the consequent increase of essential Ca²⁺ and K⁺ ions. Further, decomposed green manuring replaces the sodium ions responsible for salinity with beneficial elements like Ca²⁺ and Mg⁺⁺ and CaCl₂ is very essential to maintaining membrane integrity in leaves (Talaat and Shawky, 32). Both duckweed and dhaincha were promising organic amendments that promoted soil juvenility and crop productivity by reclaiming soil salinity in the present study. Thus, the use of dhaincha green manure and duckweed can provide a robust, sustainable approach to tackling soil salinity, enhancing crop growth, and ensuring the sustainability of agriculture. By nature's design, the green manuring (dhaincha) technique appears to be an economically feasible, environmentally friendly, and methodically smart choice owing to its source of essential nutrient supply and salinity tolerance.

Conclusion

Green manure, dhaincha and duckweed are less costly, easily affordable, and eco-friendly organic amendments. The results showed that the application of *Sesbania rostrata* alleviated salinity stress in transplant Aman rice remarkably compared to duckweed. At 100 mM salinity, the addition of dhaincha increased the grain yield by 31%, 21%, and 12% over control. Whereas, grain yield decreased by 0.05% with duckweed addition, which was minimized to 12% by applying dhaincha green manure. The green manuring approach developed by dhaincha seems to be an environmentally sound and commercially viable solution for salinity reduction. Compared to duckweed, dhaincha showed to be a very efficient green leaf manure. Further field research is needed to investigate the potential use of duckweed and dhaincha in reclaiming saline soils and boosting crop yields.

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Authors' contributions

IJI performed the experiment and prepared the manuscript draft; IJI, KA, SR designed the experiment, analysed the data; IJA, TB, MMA revised the manuscript; all authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The Authors declare that there is no conflict of interest.

Ethical issues: None

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