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Research article

Evaluation of Organic Matter for Enhancing the Agro-physiological Traits of Rice cv. Banyuasin Planted under Saline Conditions

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Abstract

Salinity stress can inhibit the metabolic processes in plants via ionic stress, osmotic stress, and ion imbalance, affecting their agro-physiology. The application of organic matter (OM) in the planting media can improve soil quality so the plants can grow and develop optimally. Therefore, the use of superior rice variety as well as good land management, can be a solution for rice cultivation under saline conditions. This study evaluated the application of various sources of OM under saline conditions and their influence on the agro-physiological traits of rice cv. Banyuasin. This study was conducted from March to August 2021 using a pot experiment in the screen house of Universitas Perjuangan Tasikmalaya. Treatment was performed using one factor, types of OM, and four types (cow manure, rice husk, rice straw, and *Azolla pinnata*) were investigated. The four treatments were arranged in a completely randomized design with five replications. The study results showed that the addition of cow manure produced the highest shoot biomass, plant biomass, and chlorophyll content. *Azolla pinnata* produced the highest proline content, whereas rice husk and rice straw increased the nitrate reductase activity. Among the type of OM tested under saline conditions, the best agro-physiological variables were cow manure, followed by rice husk, rice straw, and *Azolla pinnata*. Hence, the use of rice cv. Banyuasin combined with four types of OM under saline conditions using agro-physiological traits was a novel approach in this study.

Keywords: biotic stress; metabolic process; organic ameliorant; paddy; superior rice

1. Introduction

Salinity is a wide agricultural problem as it causes a reduction in plant productivity by up to 65% (Shafi et al., 2013). A high salt concentration in a rice field caused by climate change decreases the water and soil quality (Feng et al., 2021). Ibarra-Villarreal et al. (2021) stated

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that salinity occurs due to interactions between environmental factors such as climate, topography, soil properties, water, and vegetation around agricultural land. Salinity causes changes in soil properties such as high salt content, decreased hydraulic systems, soil productivity, and organic matter content (Xiaoqin et al., 2021). In plants, salinity disrupts plant metabolic processes through three mechanisms: osmotic stress, ion imbalance, and ionic stress (Anshori et al., 2019). A high concentration of Na^+ ion makes it difficult for plants to absorb water and minerals (Munns & Tester, 2008), while the limited water uptake by plants causes ion toxicity and decrease in plant cell turgor in the leaves (Acosta-Motos et al., 2017). Muflikhah et al. (2018) reported that irrigation water with salt concentration up to 2.14 dS m^{-1} decreased the leaf area index, photosynthesis rate, and root length and increased the proline content of rice. Arif et al. (2020) stated that salinity decreased the photosynthesis rate, chlorophyll content, and carotenoid, and increased proline content as an osmoregulatory accumulation. Saline conditions with electrical conductivity (EC) value 5 dS m^{-1} had a negative impact on rice plants, decreasing plant height, leaf area, and plant biomass (Adlian et al., 2020), and decreasing growth and physiological variables in tomato plants (Ali et al., 2021). A salt concentration with EC 8 dS m^{-1} was shown to decrease plant height, chlorophyll-a, and chlorophyll-b content in rice plants (Nasrudin et al., 2022a).

The impact of high salinity on plant metabolism is an obstacle to rice growth and production; it is necessary to use technology to improve rice plant growth and production optimally. Cheng et al. (2023) stated that salinity causes changes in soil structure and texture, inhibiting plant root growth, which ultimately reduces plant productivity. The use of organic matter (OM) under saline conditions can improve soil properties (Matosic et al., 2018) by triggering several activities such as aggregation, mineralization, and C and N cycling (Zhou et al., 2023). Liu et al. (2023) stated that the use of OM was able to stimulate microbial activity in the soil, thereby minimizing C-organic loss. Furthermore, microbial activity in the soil is important for the maintenance of soil health and support of nutrient cycling (Unnikhrishnan et al., 2022). In a previous study, Shaaban et al. (2023) found that OM in the form of manure and rice straw was able to increase dissolved C-organic, dissolved N-organic, and minerals (NO_3^- and NH_4^+), and increase enzyme activities and available P. Soil improvement under saline conditions is a key to optimizing the agro-physiological traits of plants. To increase plant tolerance to salinity, OM can be used to improve soil properties. Wibowo & Harahap (2018) observed that OM as an ameliorant played a role in improving soil microbe populations and increasing the chemical content in the soil. The improvement of soil properties helps rice plants to grow and develop optimally. The physical soil properties help to improve the decomposition process, increase the number of soil microbes that can reduce salinity stress, and increase soil nutrients that can be absorbed by plants (Subardja et al., 2016). In soil containing a high salt concentration, the addition of OM was shown to improve soil biological traits (Elhabet, 2018).

Furthermore, selecting a rice genotype that has a tolerance to salinity is one of the efforts explored by researchers and farmers. Knowledge of rice variant agro-physiology and biochemical traits are needed when attempting to improve rice genotype tolerance of salinity. Several genotypes are susceptible to salinity and cause a decrease in biochemical and physiological variables. Rice cv. Banyuasin is a superior rice variety that is tolerant to salinity stress and can be planted in tidal swamp areas (Oelviani et al., 2024). In a previous study, this rice variety with more optimal morpho-physiological traits was shown to tolerate salinity conditions of up to 150 mM NaCl (Hariadi et al., 2015). Other studies stated that salinity inhibited rice growth when the plants entered the vegetative stage, the flowering stage, and at grain filling (Rad et al., 2012). A number of previous studies that were carried out such as the use of organic and inorganic matter in saline soil to improve soil properties, the use of OM to increase microbial activity, as well as the use of several tolerance rice varieties cultivated under saline conditions. To the best of our knowledge, limited information is available on the soil changes that occur under saline conditions with the use of OM as a planting media to cultivate the rice cv. Banyuasin. In the present study, we evaluated changes in soil properties that occur due to the application of OM under saline conditions and their influence on the agro-physiological traits of rice cv. Banyuasin.

2. Materials and Methods

2.1 Pot experiment

A pot experiment was conducted in the screen house of Universitas Perjuangan Tasikmalaya (7º21'10.0"S 108º13'22.1"E) from March to August 2021. Treatments were arranged in a non-factorial completely randomized design (CRD) with five replications. The treatment tested involved one factor, namely type of OM, and the types were cow manure, rice husk, rice straw, and *Azolla pinnata*. Each treatment used three plants in different polybags. The rice tested was cv. Banyuasin which has a tolerance gene to salinity. The saline condition was NaCl 8 dS m^{-1} (based on the technical instruction for the soil chemical content of Indonesian Soil Research Institute, constitutes a very high EC value). The observation of salinity was conducted using an Hanna instruments DiST 3 waterproof EC tester HI98303. The analysis of soil properties was conducted in the Assessment Institutes for Agricultural Technology of Yogyakarta and the analysis of plant physiology was conducted in Plant Science Laboratory Universitas Gadjah Mada, Yogyakarta.

2.2 Procedures and planting media analyzed

The planting media used was latosol soil taken from rice fields in Tasikmalaya Regency. The soil was mixed with OM according to the treatments (each treatment used 20 tonnes $hectare^{-1}$ or 600 g pot^{-1}) as planting media, and was then analyzed for chemical properties, as presented in Table 1. The OM types used in this study were first composted for 30 days under anaerobic conditions. Each OM was fermented using additional molasses and microorganisms in a container and tightly closed. Ripening of OM was characterized by a change in color to dark, a stable temperature (ranging between 30ºC-35ºC), and slightly clumpiness when held. A total of 8 kg of planting media was put into a pot measuring 50 cm x 60 cm. Meanwhile, saline conditions were provided using NaCl (8 dS m^{-1}). NaCl was dissolved in water in a bucket and the salinity was measured using a portable EC & TDS meter until it reached EC 8 dS m^{-1}. The saline conditions were provided when the rice plants entered 3 WAP and 7 WAP by pouring the NaCl solution with EC 8 dS m^{-1} through the planting media. Rice seeds cv. Banyuasin were sowed into trays for 21 days after sowing (DAS). The planting media used in the sowing process was latosol soil mixed with cow manure in a ratio of 1:1 (w/w). After 21 DAS, the seeds were transplanted into pots (2 rice seeds were planted in each hole) in the morning. The microclimate conditions in the study areas at mid-day were temperature ranging between 25ºC-33.1ºC and relative humidity ranging between 47-93%. The average temperature at mid-day was 27.73ºC and the average relative humidity was 67.67%.

Table 1. Soil properties on planting media

Parameters	Unit	Types of OM							
		CM	C	RH	C	RS	C	*AP*	C
pH		5.49	A	6.60	N	4.71	SA	6.01	A
C-organic	%	5.45	VH	0.97	VL	5.96	VH	3.69	H
Total N	%	0.29	M	0.09	VL	0.30	M	0.26	M
Available N	ppm	48.92	VH	22.01	VH	.135.67	VH	40.09	VH
Exchangeable K	ppm	268	VH	248	VH	346	VH	258	VH
P_2O_5	ppm	1	VL	5	H	11	VH	3	M
Total Na	ppm	0.20	L	0.14	L	0.77	M	0.21	L

Note: CM (cow manure); RH (rice husk); RS (rice straw); *AP* (*Azolla pinnata*); C (criteria); A (Acid); N (neutral); SA (strongly acid); VH (very high); VL (very low); H (high); M (medium); L (low); chemical analysis was analyzed in Assessment Institute for Agricultural Technology of Yogyakarta

2.3 The growth and agro-physiological variables

Leaf area (cm^2) was observed using a gravimetry method when the plants were 8 weeks after planting (WAP). The leaves were drawn on millimeter block sheets, and then each part was calculated manually. Root length (cm), root biomass (g), shoot biomass (g), and plant biomass (g) were observed using destructive methods. Roots were separated from the shoots, and then the root lengths were measured using a ruler. Plants were removed from the planting medium, and then dried at 80ºC for 48 h using a Memmert oven type UN 260. After drying, the roots and shoots were weighed using a digital scale with 0.01 x 500 g accuracy. The total plant dry weight was used as a measure of plant biomass. Root length, root biomass, shoot biomass, and plant biomass were observed when the plants entered 8 WAP.

Total chlorophyll content (mg g^{-1}) was observed using extraction methods. Approximately 1 g of leaves was separated from the bones, mashed with a mortar, and then added with 20 mL of acetone pro analysis. The solution was filtered using a Whatman No. 42 to get the filtrate, and then two cuvettes were filled with the filtrate and acetone, respectively. Acetone pro analysis was used as the standard in the chlorophyll analysis. The total chlorophyll was measured using a UV VIS spectrophotometer, model 752AP, at wavelengths of 663 nm and 645 nm. The total chlorophyll was calculated using equation 1.

$$\text{TCC (mg g}^{-1}\text{)} = (17.3 \times \text{A645}) + (7.18 \times \text{A663}) \quad (1)$$

The proline content (PC) (µmol g^{-1}) was determined as follows. Leaves (0.5 g) were mashed using a mortar, and added with 10 mL sulfosalicylic acid 3%. Then, the mixture was filtered and mixed with 2 mL of ninhydrin and glacial acetic acid, respectively. The extract was kept at 100ºC for 1 h. After the extract had cooled, it was mixed with 4 mL toluene. The change to a red color indicated that proline was present in the filtrate. Two cuvettes were filled with filtrate and toluene, respectively. Toluene was used as a standard in the proline analysis. The absorbance was monitored at 520 nm using a UV VIS spectrophotometer model 752AP, and proline was calculated using equation 2.

$$\text{PC } (\mu\text{mol g}^{-1}) = (64.3649 \times \text{A520}) + (-5.2987 \times 0.347) \quad (2)$$

Nitrate reductase activity (µmol NO_2^- g^{-1} h^{-1}) was determined by extraction methods adopted from the procedure of Maghfiroh et al. (2020). One hundred grams of the leaves were cut, then mixed with 5 mL phosphate buffer, $Na_2HPO_{4.2}H_2O$ (pH 7.5). The mixed solution was put into a dark tube for 24 h. The buffer solution was removed and replaced with a new solution of 5 mL and added with 0.1 mL 5M $NaNO_3$, which was then put into the dark tube for 2 h. After that, the solution was filled with 0.2 mL sulfanilamide 1% and napthylethylendiamide 0.02%. The solution was then shaken for 15 min until it changed to a pink color. The filtrate was monitored at 540 nm using a UV VIS spectrophotometer model 752AP.

2.4 Statistical analysis

The quantitative data were subjected to an analysis of variance. The least significant difference test α 5% was performed to evaluate the agro-physiological responses of rice cv. Banyuasin planted under salinity conditions. The data were processed using Statistical Tools for Agricultural Research version 2.0.1. Graphing and determination of standard deviation values were done with Microsoft Excel.

3 Results and Discussion

A planting medium is a location where plants grow optimally which is influenced by the soil properties. The soil's chemical properties are one of the things that affect plant growth under abiotic stress conditions. Based on Table 1, the planting medium with cow manure had several chemicals that made it suitable for rice cultivation; however, it had low pH, which caused acidity in the planting medium. The presence of rice straw had the strongest acidity among all of OM. Even though the three OM differed in pH levels, the other chemicals contained in the three made them suitable for rice cultivation. then the fixation by Glutamine Oxoglutarate Aminotransferase (GS/GOGAT) pathway into amino acids. Moreover, the addition of rice husk produced a neutral planting medium; however, c-organic and n-total were very low. Based on the research of Staszel et al. (2022), a high concentration of organic C supported root growth.

Saline conditions can inhibit the metabolic processes in rice plants. Healthy roots and well-biomass produced indicate one thing: plant resistance to abiotic stress. It is well known that roots absorb water and minerals, and then distribute them to other plant organs. A high Na^+ concentration inhibits root growth through ionic stress. The use of OM can improve the soil properties and make the soil into a planting medium suitable for rice cultivation (Sudaryanto et al., 2015). As we know, the accumulation of Na^+ ion can reduce the ability of other ions such as K^+, Ca^{2+}, and Mg^{2+} (Ijaz et al., 2023). Application of OM can increase the exchangeable K around plant roots as per the results in this study (Table 1) and thus reduce Na^+ and Cl^- (Irin & Hasanuzzaman, 2024). This condition causes an increase in the K^+/Na^+ ratio, and OM can accumulate Ca^{2+}, which plays a role in increasing cell membrane integrity (there is no Ca^{2+} measurement in this study) (da Silva et al., 2023). Hence, the application of OM can reduce ionic stress for plants, thereby influencing the increase in the agro-physiological activity of rice.

Roots are key functional plant organs and are factors that can control environmental conditions for acquiring water and nutrients (Fromm, 2019). Figure 1a shows that the type of OM had no statistically significant differences in root length. High salt concentration causes osmotic stress, water may flow from the plant's roots back into

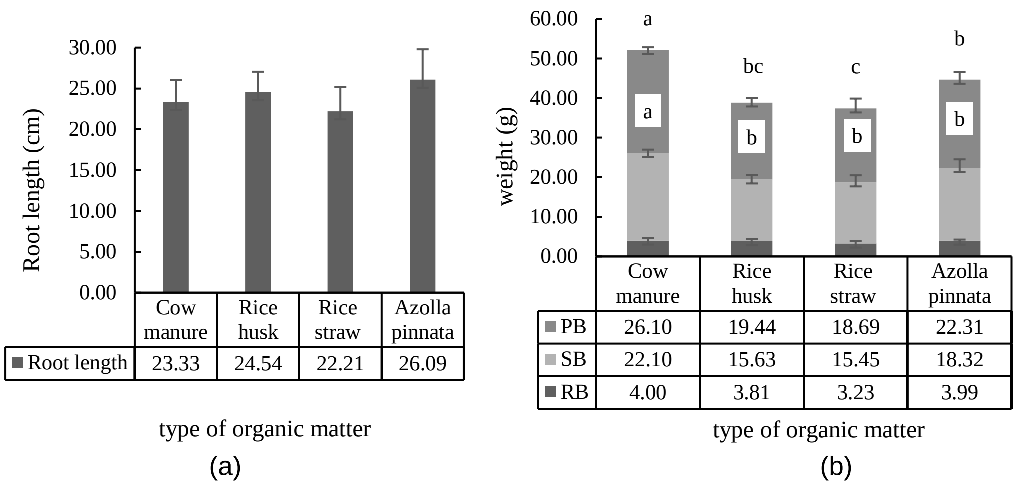


Figure 1. (a) Root length of rice cv. Banyuasin in various types of OM. (b) Root biomass (RB), shoot biomass (SB), and plant biomass (PB) of rice cv. Banyuasin in various types of organic OM. The data showed for each treatment uses four samples, with five repetitions.

the soil. Osmotic stress causes *reactive oxygen species* (ROS) accumulation and cell membrane damage (Seeda et al., 2022). ROS causes plants to experience water imbalance resulting in low soil water potential and plasmolysis (Naseem et al., 2023). Low water potential also has an impact on various reductions in the physio-biochemical activities of rice plants. This activity causes osmotic stress for plants which affects the decrease in the assimilates in plants, so no significant differences were seen in root lengths. Diacono & Montemurro (2015) stated that salinity affects changes in soil properties, i.e., pH, C-organic, and alters the osmotic potential.

From the study, there was no statistically significant difference in root biomass (RB) across the OM types (Figure 1b). Salinity caused a decrease in root growth due to osmotic and ion toxicity. Under the high salt conditions (8 dS m^{-1}), OM was not able to improve the physical properties of the soil in the planting media. The high salt level inhibited plant root development under various OM additions. Salinity inhibited plant growth and development via three mechanisms: ion imbalance, osmotic stress, and ion toxicity (Anshori et al., 2018), and OM was unable to improve physical properties caused by high salt concentrations (Voltr et al., 2021). Furthermore, salinity causes assimilate partitioning in plants, and inhibits root and shoot elongation (Lokeshkumar et al., 2023). On the other hand, shoot biomass (SB) was affected by the addition of OM (Figure 1b). Cow manure was the highest SB compared to the other OM due to available nitrogen being the essential nutrient. Kusmiyati et al. (2018) showed that the application of cow manure increased the plant biomass of S. *grandiflora* although it was in a slow-released character. Wang *et al.* (2020) stated that the OM improved nutrients (nitrogen, phosphor, and potassium) in soil and reduced the salt content of the soil. Nevertheless, nitrogen in high salt stress can leach and migrate, becoming unavailable for plants (Chen et al., 2023). Hence, in the present investigation, it was concluded that minerals contained in cow manure improved the metabolic process in plants.

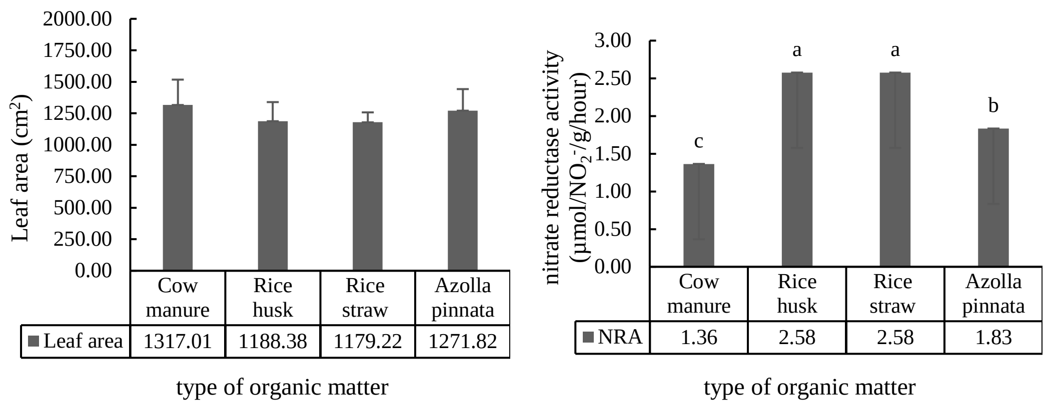
A similar trend was observed in plant biomass (PB) which was affected by OM. Cow manure had the highest PB, *Azolla pinnata* produced a higher PB that was not significant different to rice husk, and rice straw produced the lowest PB without any significant difference to rice husk (Figure 1b). The PB in cow manure produces assimilates from photosynthesis which is also supported by the high nitrogen availability in the soil. The PB being high likely caused by the translocation of assimilates needed for plant growth. According to the study of Sudarsono et al. (2014), the application of cow manure at 10 t ha^{-1} increased plant dry weight due to nitrogen availability and also supported the photosynthesis rate. Furthermore, *Azolla pinnata* provides a high nitrogen availability through the roots and is absorbed by the plant and affects the synthesis of protein and chlorophyll. The high chlorophyll content plays a role in capturing proton to produce assimilates. *Azolla pinnata* can also be used for phytoremediation, increasing plant growth, and plant biomass to promote better rice growth under saline conditions (Da-Silva et al., 2022).

One of the important organs in plants that has the main function of creating a carbohydrate as a source is the leaf (Sun et al., 2023). Wide leaves of green color affect optimal metabolic activities such as photosynthesis. Green leaves are also affected by the uptake of nutrients from the soil, especially nitrogen. Nitrogen is essential in the formation of protein and chlorophyll (Zhou et al., 2023).

Leaf area is influenced by various factors such as nutrients, environment, and management (Winck et al., 2023). The present study showed that the OM did not affect the leaf area (Figure 2a). The effect of the four OM on nitrogen availability in the soil is relatively very high. High nitrogen available affects cell division and elongation, increases the number of cells during initiation of shoot apical meristem, and the duration of meristemoid division (Moreno et al., 2020). In addition, the environment used in this study was relatively homogenous. Under saline conditions, plants tend to experience decreased metabolic activity due to nutrient imbalance, osmotic stress, and ionic stress (Ran et al., 2023). Under these conditions, nutrients and nitrogen cannot be absorbed by plants. This causes nitrogen to have no significant impact on the leaf area.

Furthermore, the availability of nitrogen absorbed from the soil by plants is usually in the form of nitrate. The catalytic reduction of nitrate (NO_3^-) to nitrite (NO_2^-) by enzyme nitrate reductase, and further reduction to ammonia by enzyme nitrite reductase, and then fixation by Glutamine Oxoglutarate Aminotransferase (GS/GOGAT) pathway into amino acids (Berger et al., 2020). The formation of amino acids occurs in plants (Yang et al., 2023), and it affects plant growth. In this study, it was shown that OM affected nitrate reductase activity (NRA). Rice husk and rice straw had the highest NRA, cow manure had the lowest NRA, while *Azolla pinnata* produced lower NRA than rice husk and rice straw but higher than cow manure (Figure 2b). High NRA indicates the ability of OM to help plants reduce nitrate to nitrite by enzyme which is then converted into ammonia to produce amino acid and nitrogen compounds of the cell. Table 1 shows that the rice straw had the highest available N compared to other types of OM. Nevertheless, fertilization activity also affects the nutrient uptake and their availability (Ma et al., 2023). This affects the activity of the enzyme nitrate reductase, which is illustrated by the NRA content.

Plant metabolic activities, especially the formation of amino acids and other activities that are influenced by nitrogen availability, also have an impact on plant chlorophyll content. Nasrudin et al. (2022b) stated that chlorophyll is an important component in plants that plays a role in energy metabolism and production of biomass from photosynthesis activity.



(a) (b)

Figure 2. (a) Leaf area, and (b) Nitrate reductase activity of rice cv. Banyuasin in various types of OM. The data showed for each treatment uses four samples which were repeated five times

The presence of chlorophyll in plants is influenced by nutrients such as nitrogen and magnesium, which are involved in chlorophyll synthesis (Li et al., 2021). Rice cv. Banyuasin treated with cow manure under saline conditions produced the highest chlorophyll content, followed by rice straw and *Azolla pinnata*, while the rice husk produced the lowest chlorophyll content in rice plants (Figure 3a). This makes the nitrogen contained in the cow manure more available for the rice plants, so it affects the chlorophyll content in the leaves. As we know, nitrogen is one of the nutrients that affect the greenness of the leaf (Zhang et al., 2019). Under saline conditions, cow manure was also indicated to be able to maintain nitrogen content in the soil and be easily absorbed by plant roots (Zurhalena et al., 2023). On the other hand, the use of rice husk had the lowest nitrogen content among other OMs (Table 1). A low availability of nitrogen affects the absorption process in rice plants, and even under saline conditions. This causes a low chlorophyll content in rice which is indicated by a yellowish-green color in the leaves due to salt stress. According to Wang et al. (2022), salinity inhibits nitrogen absorption and thus decreases the chlorophyll content.

Furthermore, under saline conditions, nitrogen also plays a role in the formation of amino acids, which affects the accumulation of proline, which is a secondary metabolite. Proline acts as an osmolyte in a defense mechanism against salt stress (Mona et al., 2017). Figure 3b shows that the OM of *Azolla pinnata* produced the highest proline content in the tested rice followed by rice straw and cow manure, while rice husk produced the lowest proline. The high proline accumulation indicates and mediates in a plant under salinity stress (Zheng et al., 2023). Proline is a form of signal or indicator of the resistance of rice plants under salinity and drought stress (Yamika et al., 2018). This study showed that the rice husk had a low nitrogen content, but was able to improve the physical soil properties so that the saline conditions around the growing environment did not affect the absorption of water and nutrients in the rice plants. Demir & Gülser (2021) stated that rice husk can improve water use efficiency and inhibit salt absorption by the roots. Its use lowers salinity stress in rice plants and this is reflected in the low accumulation of proline. In contrast, *Azolla pinnata* contains a higher N-total but lower water-holding ability to inhibit salt absorption by roots (Setiawati et al., 208). Its use increases salt stress conditions and may inhibit the plant growth.

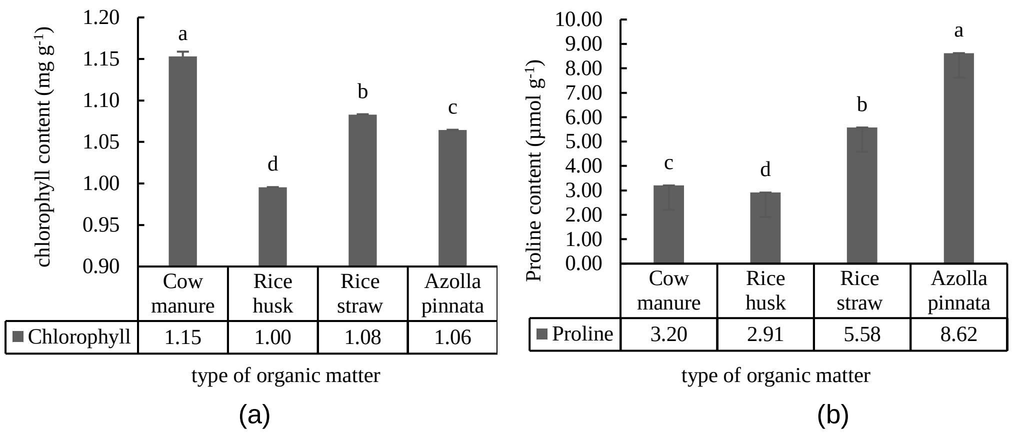


Figure 3. (a) Chlorophyll content, and (b) Proline content of rice cv. Banyuasin in various types of OM. The data showed for each treatment uses four samples which were repeated five times.

In the present study, we evaluated various agro-physiological parameters and processes of the rice cv. Banyuasin through given OM sources under saline conditions. This study showed that the rice cv. Banyuasin had a tolerance to salinity. Furthermore, the use of OM also affected the ability of plants to handle salinity stress. Based on the study results, we suggest that the use of cow manure can improve the agro-physiological traits of rice cv. Banyuasin under saline conditions.

4 Conclusions

The results of this study indicated that the various sources of OM used in this study produced different chemical constituents present in soil media. These varying conditions affected the adaptability of the plants by increasing plant defense mechanisms. Therefore, this study found the influence of the OM used on the agro-physiological traits of rice cv. Banyuasin. The study results showed that the addition of cow manure had the highest shoot biomass, plant biomass, and chlorophyll content. *Azolla pinnata* had the highest proline content, whereas the rice husk and straw increased the nitrate reductase activity. Among the addition of OM tested under salinity conditions, the best agro-physiological variables were shown by cow manure, followed by rice husk, rice straw, and *Azolla pinnata*. Overall, the OM can improve soil traits under saline conditions. However, the OM dose needs to be studied. In a further study, we hypothesize that the appropriate dose and the application of a combination of OM and ameliorants can reduce salt levels in the soil. The lower salt in the soil is thought to increase rice growth and production.

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6 Conflicts of Interest

The authors declare that no conflicts of interest exist.

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