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The Influence of Biofertilizers on Growth and Yield of Rice (*Oryza sativa* L.)

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ABSTRACT: Over half of the world's population consumes rice as a staple food. Sustainable rice (*Oryza sativa* L.) production is required to feed the growing population. Therefore, farmers are force to apply chemical fertilizers to increase rice sustainable rice production. It is not affordable for poor farmers due to the high market costs of chemical fertilizer. Alternative crop and resource management strategies, in addition to chemical fertilizer, are required to sustain crop productivity and profitability. A high output from agricultural systems is required for sustainable agriculture and food security. These systems must be economically viable, environmentally responsible, and socially acceptable. In this case, biofertilizers could be a novel approach for sustainable rice production, low cost, and pollution reduction. Crop productivity dramatically benefits from the use of biofertilizers. This study was done to find out how biofertilizers will help maximize rice yield. The treatments were T_1: POP, KAU + Azolla, T_2: POP, KAU + AMF (Arbuscular Mycorrhizal Fungi), T_3: POP, KAU + PGPR Mix I (Plant Growth Promoting Rhizobacteria Mix 1), T_4: POP, KAU + PSB (Phosphorus solubilizing bacteria), T_5: POP, KAU + KSB (Potassium solubilizing bacteria), T_6: T_1 + AMF, T_7: T_1 + PGPR Mix I, T_8: T_1 + PSB, T_9: T_1 + KSB, T_{10}: POP, KAU (Control) and T_{11}: $ZnSO_4$ spray. Applying different bio-fertilizers increased plant height, number of tillers per plant, root length, number of grains per panicle, 1000 grain weight, and grain yield. When compared to the control, the incorporation of Azolla and AMF, along with POP and KAU recommendations (T_6), recorded the highest values in terms of plant height (90.72 cm), number of tillers (23.56), and root length (35.62 cm). T_6 (POP, KAU + Azolla + AMF) had the highest number of grains per panicle (155.37), 1000 grain weight (24.16 g), and grain yield (3718.52Kg per ha (32.84%) when compared to the control. Results revealed the greater scope of biofertilizers application for achieving greater yield in rice variety Uma. We hope that this finding is beneficial for farmers to improve rice production.

Keywords: Rice, Bio-fertilizers, Growth parameters, Yield parameters.

INTRODUCTION

Rice (*Oryza sativa* L.) is a major cereal crop and staples food in most countries, especially in Asia (Debnath *et al.*, 2020). It provide a goodsource of energy (21%) and protein (15%) for more than 50% of the world's population (Maclean *et al*. 2002; Depar *et al*., 2011). The fast-growing human population increases the global demand for food (Maja and Ayano, 2021). Hence the insignificant increase in the total global land under cultivation in the last 25 years (O'Mara, 2012) with the fast-growing human population and global climate change raise serious concerns about food security. Godfray *et al*. (2010) reported that the human population will reach 9.6 billion by 2050, which marks a 33% increaseinthe world population. However, the yield of rice has been declining over the past years. In recent years, the importance of sustainable agriculture has been widely recognized, and it has emerged as the most pressing issue in agriculture. Sustainable agriculture is the management and utilization of agricultural systems to maintain their productivity, regeneration capacity, biological diversity, vitality, and ability to function without harming other ecosystems. Biofertilizers could be a novel approach to sustainable rice production and pollution reduction (Iniesta *et al.*, 2021). Crop productivity dramatically benefits from the use of biofertilizers (Nosheen *et al.*, 2021).

Biofertilizer is one of the sources that support chemical fertilizers. Biofertilizersare potential environmentally friendly supplemental inputs for healthy plant growth (Suhag, 2016). It is a component made up of living, helpful bacteria that, when added to soil, surfaces, or plant seeds, colonize the plant's rhizosphere and promote development by giving the host plant more essential nutrients or by giving it phytohormones. With their host plants, biofertilizers engage in symbiotic and associative microbial interactions. These are organic mini-fertilizer factories that increase agricultural production while enhancing soil fertility. They are less expensive and safer sources of plant nutrition. Different biofertilizers are used to boost agricultural productivity because they have been shown to fix nitrogen (N), phosphate (P), and produce phytohormones. One theory for how biofertilizers increased crop yield was suggested involved increased uptake of nutrients like N, P, and K. (Lucy *et al*., 2004; Solaiman and Hirata (1997) reported that AMF application at the nursery stage of rice increased the rice yield by 14-21%. Similarly, Oyange *et al*., (2019) reported that Azolla incorporation in rice fields increased the grain weight, panicle length, percent grain filling, and grain yield. This result revealed that the incorporation of Azolla increases the rice yield by 5-42 % compared to the control. Furthermore, the application of potassium solubilizing bacteria (KSBs) enhanced the grain yield of riceby 20–38% in the pot and 20–52% in the field conditions, mainly when half of the prescribed potassium fertilizer (K_2SO_4, 44% K_2O) was applied, as compared to the control. Application of plant growth-promoting bacteria (PGPB) strains considerably improved fruit weight (13.9–25.5%), cumulative yield (26.0–88.0%), shoot diameter (15.9–18.4%), and shoot length (16.4–29.6%) in apple (Karlidag *et al*., 2007). Other research has found that using PGPRs alone or in combination with organic amendments can boostplant development and yields of maize (Shahzad *et al*., 2017). The experiment showed that P application at greater levels combined with phosphorus solubilizing bacteria (PSB) seed treatment might improve maize's growth, yield, and yield qualities, as well as its nutritional content and uptake.

Therefore, applying biofertilizers is a promising approach for improving crop yield and as well as improving crop quality. Also, it helps to reduce the usage of chemical fertilizers. It is expected that the outcomes of this research may help to develop climate-smart agricultural practices and, in turn, solutions to the severe food scarcity of the global population in the present scenario.

MATERIALS AND METHODS

A field experiment was carried out in the year 2021 august at the College of Agriculture, Vellayani, Kerala, India, which is located at 8°5' N latitude, 76°9' E longitude, and 29 m above mean sea level. The investigation included 11 treatment combinations tested in the field with the rice variety Uma. One of the most popular rice varieties of Kerala is Uma (Mo.16) developed by the Rice Research Station, Moncompu of Kerala Agricultural University and it occupies more than 60 percent of the paddy cultivation. The experiment was designed with three replications using Randomized Block Design (RBD). Two weeks before sowing, FYM at 10 tonnes ha^{-1} was applied. Bio-fertilizers (PGPR Mix I, AMF, PSB and KSB) were collected from Department of Microbiology, College of Agriculture, Vellayani.

The treatments were T_1: POP, KAU + Azolla, T_2: POP, KAU + AMF (Arbuscular Mycorrhizal Fungi), T_3: POP, KAU + PGPR Mix I (Plant Growth Promoting Rhizobacteria Mix 1), T_4: POP, KAU + PSB (Phosphorus solubilizing bacteria), T_5: POP, KAU + KSB (Potassium solubilizing bacteria), T_6: T_1 + AMF, T_7: T_1 + PGPR Mix I, T_8: T_1 + PSB, T_9: T_1 + KSB, T_{10}: POP, KAU (Control) and T_{11}: $ZnSO_4$ spray.

Phosphorus Solubilising Bacteria (PSB) Carrier based phosphobacteria can be applied as seed treatment and field application. 2 kg PSB is mixed with 50 kg of dried powdered farm yard manure and then broadcast in one ha of main field just before transplanting. In case of Potassium mobilizing bacteria Mix 500 ml of liquid formulation (Fraturia aurentia) with 50 kg of FYM for field application in one ha. PGPR mix 1 apply as Field application @ 2.5 kg/ one litre in 100kg organic manure for one hectare. Azolla can be used as green manure before transplanting rice. Azolla is grown 15-20 days before rice transplanting in a well-prepared field with 1-2 t fresh inoculum per ha. Rock phosphate is applied in three equal splits at seven-day intervals at a rate of 62.5 kg/ha. After the thick mat has formed, the water is drained and the field is ploughed in preparation for the incorporation of Azolla. Treatment with Arbuscular Mycorrhizal Fungi (AMF), 200 g of AMF inoculum was applied per square meter of nursery area at the tie of nursery preparation.

Plant height measurement was taken from the plant base to the tip of the panicle at the maturity stage and expressed in centimetres. The total number of tillers present in each plant was counted at the time of the maximum tillering stage. Plant samples from each treatment were uprooted, washed, sun-dried and oven dried at 70±5°C to constant weight. Dry matter production was recorded and expressed in g $plant^{-1}$. Root length was measured from the root-shoot junction to the tip of the longest rootlet and expressed in centimeters.

The number of panicle-bearing tillers present at the time of harvest in each replication is termed a productive tiller number. Panicles of rice crops were hand threshed and the total grains per plant were counted and recorded as the Number of Grains per Panicle. Filled, bold one thousand grains were collected from the harvested grains, weighed and expressed in 1000 grain weight in grams. The grains were harvested from each plant separately and dried in sun to a moisture content of 14 per cent and its weight was recorded as grain yield and expressed in g $plant^{-1}$.

The data analysis was done using the statistical package, GRAPES. Multiple comparisons among treatment means, where the F test was significant (at a 5 % level) were done with Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Growth parameters. It is well known that application of biofertilizer desirably affects plant growth and development. In this present study, analysis of plant height (Table 1) exhibited significant differences among plants under all the biofertilizer treatments when compared to the control. Treatment 6 with Azolla + AMF and Treatment 7 with Azolla + PGPR Mix I inoculated plant along with POP recommendations showed the highest mean in plant height (90.72 cm and 88.74 cm) which is followed by T_9 (POP, KAU + Azolla + PSB) (85.24 cm), T_8 (POP, KAU + Azolla + KSB) (84.99 cm) and T_1 (POP, KAU + Azolla) (82.14 cm) respectively. Diedhiou *et al.* (2016) investigated the response of rice plants to inoculation with AMF and PGPB under field and green house conditions. In this study revealed that incorporation of AMF and PGPB improve the rice plants growth and development. In the case of rice this was due to N-fixing activity of azolla and the secretion of growth promoting substances such as indole-3-acetic acid (IAA), gibberellins, cytokinin (ElShanshoury, 1995), and nutrient mineralization in AMF inoculated plants (El-Demerdash *et al.*, 1992). Allah *et al*. (2015) reported that the AMF inoculation in rice increased the growth hormone, including IAA, IBA and GA by 9.4%, 1.9% and 153.1%, respectively. These phytohormones are essential for plant growth and development. Also AMF helps to improve the nutrient uptake efficiency in plants. Bhuvaneshwari and Singh (2015) found that Azolla incorporation with rice and dual cropped system also increased the growth parameters like plant height, the number of effective tillers and dry matter production compared to control. Also, the results are in agreement with Bheemareddy and Lakshman (2011) reported that AMF inoculation in the soil increased the growth parameters like plant height, biomass, leaf area, and the number of leaves per plant.

The effect of the bio-fertilizer application on the number of tillers per plant was studied and the data are presented in Table 1. Azolla + AMF (23.56) and Azolla + PGPR Mix I (21.86) inoculated plants along with POP recommendations showed the highest mean value and were significantly on par followed by T_8 (20.13) and T_9 (20.89). The activities of Azolla and AMF result in increased nitrogen uptake, phosphorus solubilization, siderophores production, and phytohormone secretion required to chelate iron and make it available to the plant. Various biologically significant metabolites and enzymes are promoted by the increased nutrient absorption caused by AMF (Yuan *et al*., 2010). These will result in improved water and nutrient status of the plants, which will result in improved photosynthetic efficiency and higher assimilate accumulation. This will be reflected in the production of tillers (Gyaneshwar *et al*., 1998). This observation is in parallel with the report by According to Razavipour *et al*. (2018), the Azolla rice ecosystem positively increased the rice tiller number and yield. This suggests improved photosynthetic apparatus performance, which is undoubtedly responsible for the enhanced growth shown in rice plants colonized with AMF (Ruz-Sánchez *et al*., 2011). Similarly, Nayak *et al.* (2019) reported that combining the application of microalgal-based organic fertilizer with chemical fertilizer helps to improve the plant height, tiller number, biomass, and grain yield of rice.

The highest mean root length was recorded from T_6 (POP, KAU + Azolla + AMF) (35.62 cm) followed by T_7 (POP, KAU + PGPR mix 1) with 33.52 cm. The least value was recorded from the control (POP, KAU) (23.11cm). Indoleacetic acid (IAA) is a common phytohormone produced by bacteria that promotes plant root growth. The extensive root architecture assists plants in absorbing nutrients and water from their surroundings. Because of AMF inoculation, increased root length and root dry weight, via N fixation, growth-promoting hormone secretion, and mineral nutrient uptake via root elongation. The primary methods of biofertilizers for enhancing plant growth include increasing nitrogen availability by fixing atmospheric nitrogen; solubilizing insoluble organic and inorganic P by producing organic acids and enzymes; and boosting root growth via IAA.

Table 1: Effects of application of bio-fertilizers on growth parameters of rice plant at maximum tillering stage.

Treatments	Plant height (cm)	Number of tillers per hill	Root length (cm)
T_1- POP, KAU + Azolla	82.14^{c}	19.75^{cd}	29.22^{d}
T_2- POP, KAU + AMF	81.33^{c}	19.01^{cde}	28.00^{de}
T_3- POP, KAU + PGPR 1	80.58^{cd}	18.74^{de}	27.13^{e}
T_4- POP, KAU + K solubilizing bacteria	78.96^{d}	17.67^{ef}	27.45^{de}
T_5- POP, KAU + P solubilizing bacteria	80.08^{cd}	17.23^{ef}	28.56^{de}
T_6- T_1+ AMF	90.72^{a}	23.56^{a}	35.62^{a}
T_7- T_1+ PGPR 1	88.74^{a}	21.86^{ab}	33.52^{b}
T_8- T_1+ K solubilizing bacteria	84.99^{b}	20.13^{bcd}	31.41^{c}
T_9- T_1 + P solubilizing bacteria	85.24^{b}	20.89^{bc}	31.26^{c}
T_{10}- POP, KAU	73.21^{e}	15.69^{f}	23.11^{f}
T_{11}-Zinc sulphate (0.5%)	74.56^{e}	15.94^{f}	24.13^{f}
SEm (±)	0.71	0.679	0.68
CD (0.05)	2.10	2.004	2.02

Yield parameters. Yield is the product of the yield parameters including number of grains per panicle and the weight of 1000 grains. Analysis of yield parameters (table 2) exhibited significant differences among plants under all the treatments when compared to the control. The highest mean value of 1000 grain weight was recorded in Azolla + AMF (24.91 g) inoculated plants along with POP recommendations followed by T_7 (23.66 g) which is on par with T_8 (23.65 g), T_9 (23.56 g), T_1 (23.47g), T_2 (23.40 g) and T_4 (23.41 g). All the treatments resulted in significant variations in the number of grains per panicle. Control plants recorded a mean value of 111.23 grains per panicle. The highest mean value of grain yield (Fig. 1) was recorded in T_6 with 155.37 grains per panicle, followed by T_7 (142.64). The highest mean value for grain yield was recorded in Azolla + AMF (3718.52kg/h) inoculated plants along with POP recommendations (T_6) followed by T_7 (3624.35 kg/ha), whereas least mean value was recorded in control (2799.18 Kg/ha). Similarly, Diedhiou *et al*. (2016) showed that the application of AMF and PGPB improves the number of tillers, grain yield, and 1000-grain weight of rice plants.

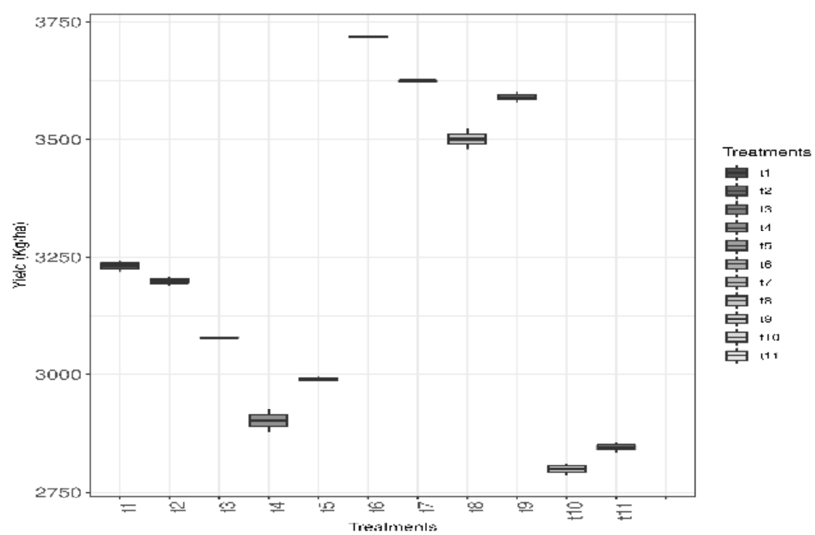

Fig. 1. Effect of biofertilizers on Grain yield in kg/ha.

A variety of morphological and physiological mechanisms interact to produce grain yield. According to the current program, AMF inoculation improved rice water and mineral uptake, IAA, and GA status. All of this would have resulted in improved photosynthetic efficiency and dry matter accumulation in the treated plants (Kucey *et al.*, 1989; Tiwari *et al*., 1989). Biofertilizers stimulate growth parameters as well. AMF-inoculated plants allocate more N, P, and water to rice panicles, resulting in more grains per panicle and an increase in 1000 grain weight and grain yield (Manske, 1990; Manske *et al*., 1995; George *et al*., 1995). Similar findings were reported earlier by Oyange *et al*. (2020). He noticed that Azolla incorporation at the transplanting stage of rice seedlings positively influenced the spikelet number per panicle and 1000 grain weight and also Oyang *et al*. (2019) conducted a study to know the effect of Azolla and inorganic N fertilizer on the yield and growth parameters of rice. He reported that Azolla incorporation in rice fields increased the grain weight, grain yield, percentage grain filling, and grain yield compared to inorganic nitrogen fertilizer application. Azolla compost boosted the formation and translocation of assimilates from source to sink, resulting in high spike weight and high grain yield, it is possible that the increased grain yield was caused by the effective absorption of N and possibly other nutrients (Razavipour *et al.,* 2018).

The present study agrees with Zhang *et al*. (2017), during the grain filling stage, AMF inoculated plants allocate more nitrogen and P and water to rice panicles helping to produce more number of grains per panicle and also improve 1000 grain weight and grain yield. Although AMF colonization in rice fields is typically modest, it might be able to increase by introducing AMF inoculum. A higher harvest index results from improved rice plant performance due to increased RLC (root length colonization), which also increases grain yields. These benefits are probably mediated by both nutrients and the control of plant hormones, particularly IAA. Diedhiou *et al*. (2016) discovered that AMF inoculated plants, grew taller, matured earlier, and produced more grain than AMF non-inoculated plants and he discovered that upland varieties responded best to inoculation, particularly in terms of grain yield, harvest index, and spikelet fertility. Under conditions of elevated CO_2, the combination of mycorrhized rice seedlings and basal application of AMF improved the microbiological and enzymatic characteristics of the soil, increasing rice yield (Panneerselvam *et al.,* 2019). Therefore, biofertilizer is a promising ingredient for improving plant growth and development.

Table 2: Effects of bio-fertilizers on yield parameters of rice plant at harvest stage.

Treatments	Number of grain per panicle	1000 grain weight (g)	Grain yield (Kg/ha)
T_1- POP, KAU + Azolla	130.12^{e}	23.47^{bc}	3231.67^{e}
T_2- POP, KAU + AMF	126.62^{f}	23.40^{bc}	3198.56^{f}
T_3- POP, KAU + PGPR 1	122.23^{h}	23.24^{c}	3078.17^{g}
T_4- POP, KAU + K solubilizing bacteria	123.97^{g}	23.41^{bc}	2901.96^{i}
T_5- POP, KAU + P solubilizing bacteria	121.65^{h}	22.36^{d}	2990.45^{h}
T_6- T_1+ AMF	155.37^{a}	24.91^{a}	3718.52^{a}
T_7- T_1+ PGPR 1	142.64^{b}	23.66^{b}	3624.35^{b}
T_8- T_1+ K solubilizing bacteria	138.29^{c}	23.65^{b}	3501.56^{d}
T_9- T_1 + P solubilizing bacteria	134.75^{d}	23.56^{bc}	3589.83^{c}
T_{10}- POP, KAU	111.23^{j}	21.41^{e}	2799.18^{k}
T_{11}-Zinc sulphate (0.5%)	116.48^{i}	22.33^{d}	2845.90^{j}
SEm (±)	0.46	0.11	7.64
CD (0.05)	1.35	0.34	22.56

CONCLUSION

These results highlights the importance of incorporation of AMF in rice- production systems. The field performances of rice variety Uma was improved to a great extent under the application of biofertilizer in addition to chemical fertilizers. This was reflected in the significant enhancement in growth and yield parameters. Growth parameters and yield parameters were raised by using biofertilizers in addition to chemical fertilizers. Azolla and AMF incorporation with POP recommendation (T_6) revealed maximum values for growth and yield parameters. Azolla + AMF + KAU POP greatly enhanced the plant's growth characteristics, including height (90.72 cm), tiller count (23.56), and root length (35.62 cm). T_6: T_1 + AMF + KAU POP biofertilizer combination enhanced the number of grains per panicle (155.37), 1000 grain weight (24.91 g), and grain production (3718.52Kg/ha). When Azolla and AMF with POP recommendations (T_6), there was a 32.84% increase in yield compared to control. With its multifaceted potential, AMF and azolla can be used as one of the tools for sustainable rice production. This study will help to draw attention to natural AMF colonization and application of azolla in rice-growing areas. This will also facilitate designing future rice production technologies with the incorporation of biofertilizers and exploitation of symbiosis in the field of agriculture.

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Conflict of Interest. None.

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