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Effect of Microbial Consortium Application on Growth And Yield of *Oryza sativa* L.

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Abstract: A group of microorganisms (bacteria, viruses, fungi, and other microbes) working together to perform a specific task is called a consortium. This study aims to study and determine the growth and yield response of rice plants to the application of a consortium of phyllosphere microbes Fm48 and rhizosphere microbes R15. This research has been conducted since from August to November 2021 in the rice fields of Boya Baliase Village, Marawola District, Sigi Regency, Central Sulawesi Province. This study used a one-factor randomized block design method with grouping based on plot height. The treatment that was tried consisted of four levels, that is: Control = Without applied microbes, Fm48 = Applied a consortium of phyllosphere microbes Fm48, R15 = Applied a consortium of rhizosphere microbes R15 and Fm48R15= Applied a consortium of phyllosphere microbes Fm48 and rhizosphere microbes R15. To determine the effect of the treatment being tested, a variance analysis was carried out. Analysis of variance which showed a significant effect, further test was carried out for LSD α = 0.5. The results showed that the treatment of various consortiums of phyllosphere microbes Fm48 and R15 rhizosphere microbes had no significant effect on growth parameters but had a significant effect on the number of hollow grains and grain weight per 1000 rice grains. The consortium of phyllosphere microbes Fm48 and rhizosphere microbes R15 gave the best results for the lowest number of hollow grains and the highest grain weight per 1000 rice grains.

Keywords: Consortium; Hollow Grains; Microbes; *Oryza sativa;* Weight 1000 Seeds.

Introduction

The effectiveness of the use of microbes is determined by their ability to adapt to the environment, but there are also microbes that have a very specific dependence on certain environmental conditions such as extremophile microbes. A microbial consortium is a collection of microbes that live together and interact with each other and with their host plants The success of the microbial consortium in the field is strongly influenced by the strains used and the interaction between the microbes and their ecology (Zhang et al., 2022; Santoyo et al., 2021; Duncker et al., 2021; Hassani et al., 2018). This obstacle is answered by utilizing microbes in the form of a consortium. A group of microorganisms (bacteria, viruses, fungi, and other microbes) working together to perform a specific task is called a consortium. One of the constraints is that microbial consortia can become unstable due to imbalances in the microbial population or sudden environmental changes. Changes in the environment, such as changes in temperature or nutrient availability,

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can upset the balance of the consortium and reduce the efficiency of its work.

The application of microbes in the form of a consortium can reduce the risk of failure to use microbes in the field (O'Callaghan et al., 2022). Microbes cannot be separated from the biotic and abiotic environment of an ecosystem (land, water, forest, ocean), because they act as decomposers (Griffiths et al., 2021; Wu et al., 2022, Saleh et al., 2022). Microbes that are in the soil (rhizosphere) play an important role in the processes of decomposition, humification and mineralization (Amaria et al., 2019; Woźniak & Gałązka, 2019). Microbes that inhabit leaves (phylosphere) whose existence was initially doubted, are now believed by researchers to have real implications for increasing crop production. The microbe's consortium needs to be developed in the framework of sustainable soil management in the future (Reisberg et al., 2013; He et al., 2021; Ahmad et al., 2019).

The Fm48 philospheric microbial consortium was explored from the forest ecosystem of Lembang Tongoa Village, Kamarora District, Sigi, Central Sulawesi. Microbes isolated from young leaves of *Emmerrilia ovalis* Miq. Dandy which flora is commonly found in the Sulawesi region so that it is a typical Sulawesi endemic flora with a tree height that can reach ± 45 meters (Pas et al., 2018; Tomas et al., 2021). As for the constituent members of the Fm48 consortium, *namely Serattia* sp, *Enterobacter* sp, *Klebsiella oxytoca* sp, *Acinetobacter* sp. Various types of microbes that make up the consortium have an important role in increasing plant growth and yield. *Serattia* sp is one of the bacteria is capable of producing a red pigment (prodigiosin) which is a secondary metabolite (Virgianti, 2021; Rodríguez et al., 2023). *Serratia* sp belongs to the Gram-negative bacteria, can function as a biocontrol agent, promote plant growth and has the potential to manage biotic stress in plants (Purkayastha et al., 2019; Lee et al., 2023).

Other *Enterobacteriaceae* families that are members of the Fm48 microbial consortium are *Enterobacter* sp and *Klebsiella oxytoca* sp. Both of these bacteria are able to stimulate plant growth so that they can act as PGPR (Roslan et al., 2020). In line with Khalifa & Aldayel (2022) that *Klebsiella oxytoca* sp. isolated from the rhizosphere of the *Lotus corniculatus* plant has potential as a plant biostimulant with its ability to produce IAA hormones, ACC-deaminase enzymes, dissolve phosphate and fix nitrogen. Likewise, *Enterobacter* sp has the potential to be developed as a biocontrol agent and plant growth-promoting bacteria (Sachman-Ruíz et al., 2022). The potential of the *Acitenobacter* sp. community is able to store enzymes that help decolorize dyes and are resistant to antibiotics (Irawati et al., 2022; Kisková et al., 2023).

The R15 bacterial consortium was obtained from exploration of the garden area of Omu Village, Gumbasa District, Sigi, Central Sulawesi (Pas et al., 2018). Microbial isolation come from the rhizosphere of the *Physalis angulata* L., members of the R15 consortium consist of *Stenotrophomonas* sp, *Stenotrophomonas acidaminiphila*, *Bacillus* sp. These three bacteria have the potential to produce IAA hormones, dissolve phosphate bound in the soil and are able to fix nitrogen from the air. In line with the research results of Al-Banna & Arifuddin (2021) that *Stenotrophomonas* sp obtained from the rhizosphere of bamboo plants is capable of producing the growth hormone Indole Acetic Acid (IAA). Phytohormones produced by microbes can stimulate root formation and increase nutrient uptake by roots.

Farmers' efforts to increase production currently rely on the use of synthetic chemical fertilizers. Meanwhile, on the other hand, synthetic chemical fertilizers can cause a decrease in soil and environmental quality. This study aims to study and determine the effect of various consortiums of phyllosphere microbes Fm48 and rhizosphere microbes R15 on rice growth and yields. Microbes have been identified as a promising alternative to minimize dependence on inorganic fertilizers in agriculture. Effective use of microbes can improve the efficiency of inorganic fertilizer use and increase the availability of plant nutrients.

Method

Study Area

This research was conducted from August to November 2021 at the Microbiology Laboratory of the Faculty of Agriculture, Alkhairaat University, as well as in the rice fields of Boya Baliase Village, Marawola District, Sigi Regency, Central Sulawesi Province at the coordinates of 0°94'72.42"S;119°84'75.33"E. At an altitude of 112 m above sea level with an average temperature of 26.9°C and an average rainfall of 434 mm.

Procedures

The materials used were isolates of phylospheric microbes Fm48 and rhizosphere microbes R15, NA media, agar, sterile aquadest, and 70% alcohol, selected red rice seeds, phylospheric isolates Fm48 and rhizosphere isolates R15. Phyllospheric microb consortium (Fm48) was derived from young leaves of *Emmerrilia ovalis* Miq. Dandy which consists of 4 types of bacteria, namely *Seratia* sp strain SE-3, *Enterobacter* sp strain KDP6, *Enterobacter* sp strain MS5, *Klebsiella oxytoca* sp strain LRC162. Rhizosphere microb consortium (R15) derived from ciplukan plant *Physalis angulata* L. which consists of 4 types of bacteria namely *Stenotrophomonas* sp strain U1370-101126-SW 193, *Stenotrophomonas acidaminiphila* strain SZH19, *Bacillus* sp strain SC59 and *Stenotrophomonas* sp strain BCc6 (Pas et al., 2015). The tools used in this study were autoclave, stirrer, Erlenmeyer with a capacity of 1000 ml, aqua bottles with a capacity of 1500 ml, 4 hand sprayers (1 liter and 2 liter capacities), shakers, stoves, eppendorf tube, injection equipment, petri dishes, glass bottles 250 ml, scissors and digital scales, wrapping, sugar plastic, aluminum foil, rubber bands, tissue, label paper and waste paper, handtractors, hoes, machetes, boards, hammers, nails, label boards, handsprayer, wood, shovels, rulers and writing tools.

This research employed the one-factor Randomized Block Design method, organized according to plot height. The experiment involved four treatment levels: Control, which did not include any microbial consortium; Fm48, incorporating the Fm48 phyllospheric microbial consortium; R15, utilizing the Rhizosphere microbial consortium R15; and Fm48R15, where both the Fm48 phyllospheric and Rhizosphere R15 microbial consortia were applied simultaneously. Each treatment was carefully administered to assess its specific impact within the experimental setup.Each treatment was repeated three times so that there were 12 experimental units. To determine the effect of the treatment being tried, an analysis of variance was carried out. The analysis of variance which showed a significant effect then carried out a further test of LSD α 0.5. to determine the effect of treatment, observations were made on the growth and yield of rice plants, which included: Plant height (cm), Number of leaves (sheet), Number of tillers (stem), Number of productive tillers (stem), panicle length (cm), number of filled grains per panicle, number of hollow grains per panicle, grain weight per 1000 seeds (g) and yield per hectare (t).

Data analysis

Data analysis for this research utilized ANOVA to examine the differences among treatment groups. Post hoc comparisons of treatment averages were conducted using the Least Significant Difference (LSD) test, maintaining a significance level of α = 0.05.

Result and Discussion

Plant height (cm)

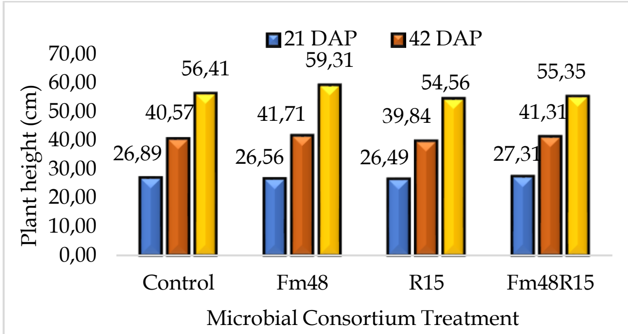

Figure 1. The average height of rice plants at the age of 21, 42 and 63 DAP when given various consortiums of phyllosphere microbes Fm48 and rhizosphere microbes R15

Analysis of variance showed that the treatment of various consortia of phylosphere microbes Fm48 and rhizosphere microbes R15 had no significant effect on plant height at 21, 42 and 63 DAP (Day after planting). The average height of rice plants at the age of 21, 42 and 63 DAP can be seen in Figure 1.

Number of leaves (sheet)

The results of variance showed that the treatment of various consortia of phylosphere microbes Fm48 and rhizosphere microbes R15 had no significant effect on the number of leaves at 21, 42 and 63 DAP. The average number of leaves of rice plants at the age of 21, 42 and 63 DAP can be seen in Figure 2.

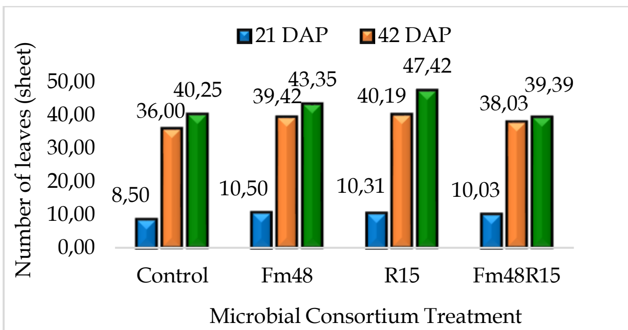

Figure 2. The average number of leaves of rice plants at the age of 21, 42 and 63 DAP when given various consortiums of phyllosphere microbes Fm48 and rhizosphere microbes R15

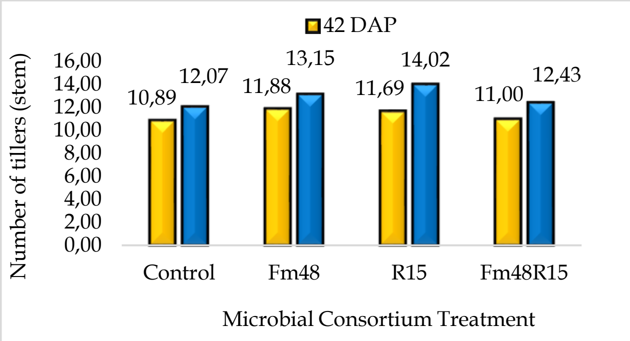

Figure 3. The average number of tillers of rice plants at the age of 42 and 63 DAP when given various consortiums of phyllosphere microbes Fm48 and rhizosphere microbes R15

Number of tillers (stem)

The results of variance showed that the treatment of various consortia of phyllosphere microbes Fm48 and rhizosphere microbes R15 had no significant effect on the number of tillers at 42 and 63 DAP. The average number of tillers of rice plants at the age of 42 and 63 DAP can be seen in Figure 3.

Number of productive tillers (stem)

The results of variance showed that the treatment of various consortia of phyllosphere microbes Fm48 and rhizosphere microbes R15 had no significant effect on the number of productive tillers of rice plants at harvest. The average number of productive tillers of rice plants at harvest can be seen in Figure 4.

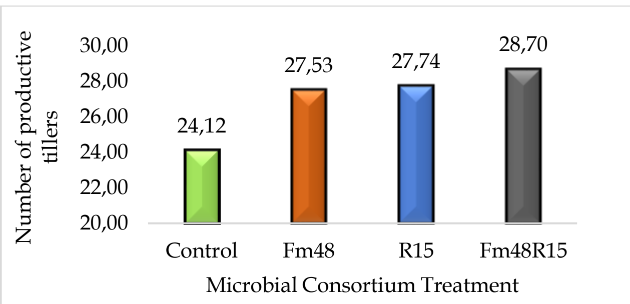

Figure 4. The average number of productive tillers of rice plants at harvest when given various consortiums of phyllosphere microbes Fm48 and rhizosphere microbes R15

Application of microbes consortia which gave insignificant results, presumably due to the limited abundance of bacteria in the soil and the influence of different agroecosystems. This is in line with Afanador-Barajas et al. (2021) and Joshi et al. (2019) who argued that plants cultivated in different ecosystems will provide an abundance of bacteria in different soils.

Panicle length (cm)

The results of the analysis of variance showed that the consortium treatment of various phylospheric microbes Fm48 and rhizosphere microbes R15 had no significant effect on the panicle length of rice plants at harvest. The average panicle length of rice plants at harvest can be seen in Figure 2. However, from the results obtained, there was a tendency for the Fm48 microbial consortium to produce the longest panicles of 17.75 cm. Panicle length is one of the determining parameters in seeing the yield potential of a plant. Long panicles will certainly provide opportunities to produce more and more grain.

Number of grains containing per panicle

The results of variance showed that the treatment of various phyllosphere microbial consortiums Fm48 and rhizosphere microbes R15 had no significant effect on the number of filled grains per panicle of rice plants at harvest. The average number of grains containing rice plants can be seen in Figure 6.

Number of hollow grains per panicle

The results of variance showed that the treatment of various consortia of phyllosphere microbes Fm48 and rhizosphere microbes R15 had a significant effect on the number of hollow grains per panicle of rice plants at harvest. The average number of hollow grains of rice plants at harvest can be seen in Table 1.

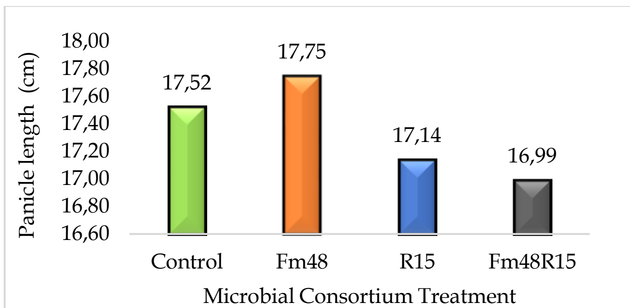

Figure 5. Average panicle length of rice plants at harvest on various consortiums of phyllosphere Fm48 and R15 rhizosphere microbes

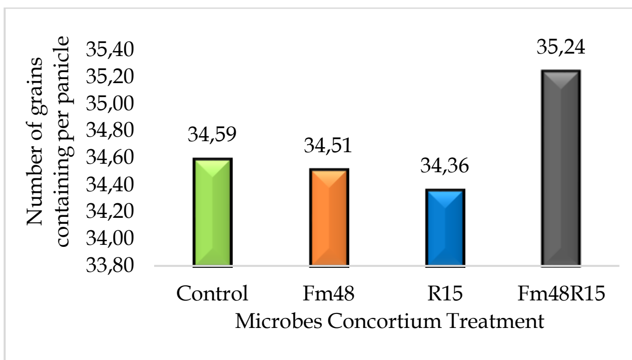

Figure 6. Average number of grains containing rice plants at harvest on various consortium of phyllospheric microbes Fm48 and rhizosphere microbes R15

Table 1. The average number of hollow grains per panicle of rice plants at harvest on various consortiums of phyllosphere microbes Fm48 and rhizosphere microbes R15

Treatment	Number of hollow grains	LSD α 0.5
Kontrol	16.08b	1.99
Fm48	16.40b	
R15	13.10a	
Fm48R15	12.88a	

The results of the study (Table 1) showed that the treatment of the phyllosphere microbial consortium Fm48 and R15 rhizosphere microbes gave the best results with the fewest hollow grains per panicle, averaging 12.88 pieces, although not significantly different from the R15 rhizosphere microbial consortium treatment. However, it was significantly different from the Fm48 phyllosphere microbial consortium treatment and the control. Note: Numbers followed by the same letters in the same column mean that they are not significantly different at the LSD test level α 0.5. The microbial consortium plays a crucial role in influencing plant growth and production, including the production of empty grains. These microbes function as decomposers, enhancing soil environment and root growth, thereby expanding nutrient absorption areas. Nutrients absorbed by rice plant roots contribute to panicle development and seed filling, ultimately reducing the occurrence of hollow grains (Allamah et al., 2018).

Grain weight per 1000 seeds (g)

The results of variance showed that the treatment of various consortiums of phyllosphere microbes Fm48 and R15 rhizosphere microbes had a significant effect on grain weight per 1000 rice grains at harvest. The average grain weight per 1000 rice seeds at harvest can be seen in Table 2.

The results of the study (Table 2) demonstrated that the combination of phyllosphere microbes Fm48 and R15 rhizosphere microbes produced the highest average grain weight per 1000 rice grains, measuring 289.00 g. This result was significantly different from the outcomes observed in other treatments. The microbial consortium's application significantly influenced both grain size and panicle productivity, thereby impacting the weight of 1000 grains. According to Lestari et al. (2021), larger grain size correlates positively with the weight of 1000 grains, highlighting its importance in determining rice yield per unit area. Note: Numbers followed by the same letters in the same column mean that they are not significantly different at the LSD test level α 0.5.

Table 2. Average grain weight per 1000 rice seeds at harvest given various consortiums of philospheric microbes Fm48 and rhizosphere microbes R15

Treatment	Grain weight (g)	LSD α 0.5
Control	272.33b	8.76
Fm48	279.00b	
R15	275.67b	
Fm48R15	289.00a	

Yield per hectare (t)

The results of variance showed that the treatment of various consortiums of phyllosphere microbes Fm48 and R15 rhizosphere microbes had no significant effect on the yield per hectare of rice plants at harvest. The average yield per hectare of rice plants can be seen in Figure 7.

Previous research has succeeded in getting the most effective phyllosphere microbial consortium Fm48 and rhizosphere microbial consortium R15 in increasing the growth and yield of rice plants in experimental pots (Pas et al., 2015), while in the current study the application of consortium Fm48 and R15 had no significant effect on rice plant growth. One of the reasons is because the application of microbial consortium is directly carried out on rice paddy cultivation land where environmental factors cannot be controlled, in contrast to the previous one which was carried out in pots in a green house. The results showed that the consortium of phyllosphere microbes Fm48 and rhizosphere microbes R15 had no significant effect on growth but had a significant effect on lowland rice crop yields (parameters of the number of hollow grains and grain weight per 1000 rice seed). The Fm48R15 treatment gave the best results on the parameters of the number of hollow grains and weight per 1000 rice grains. This shows that the phyllosphere microbes and rhizosphere microbes given together are able to stimulate plant growth which in turn affects the yield of rice plants. Besides being influenced by the microbial consortium Fm48 and R15, the increase in the weight of 1000 grains of grain and the amount of hollow grain produced decreased, which also played a role, namely the rice variety used and the adaptation of microbes to the plants and their growing environment (Wijayanto et al., 2022).

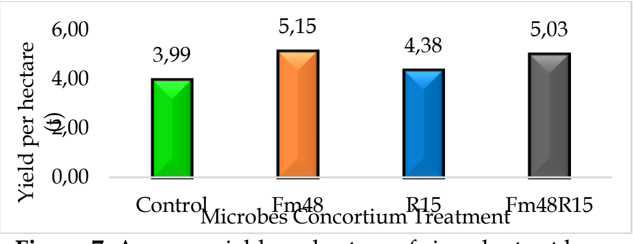

Figure 7. Average yield per hectare of rice plants at harvest on various consortiums of phyllosphere Fm48 and R15 rhizosphere microbes

The provision of Fm48 and R15 microbial consortium resulted in the lowest number of empty grains. This is presumably because the microbes provided are able to interact with plants and supply needed nutrients and protect plants from pathogen attack, as stated by Artawan et al. (2019) that microbes in the rhizosphere are able to protect rice plants against pathogen attacks. Sukars (2018) reported that soil microbes can function as biochemical agents in converting complex organic compounds into mineralized inorganic compounds. Supported by the opinion of Pas et al. (2015) added that the application of microbes in the form of a consortium can reduce the risk of failure to use microbes in the field.

Microbes can trigger plant growth through their ability to fix N2, dissolve P and secrete plant growth hormones. Plants obtain nutrients from the microbes that make up the consortium, especially from phylospheric microbes that can fix N from the air (Zhang et al., 2022; Agbavor et al., 2022). In line with the opinion of Hapsoh et al. (2019) that nutrient N is a constituent component of amino acids, proteins and the formation of cell protoplasm which can function in stimulating plant growth. Nitrogen is a constituent of chlorophyll, so that if chlorophyll increases, photosynthesis will also increase (Sikuku et al., 2016; Fathi, 2022, Zhang et al., 2022).

Pas et al. (2015), in their research concluded that the Fm48 and R15 consortium have the ability to bind N, solvent P and produce plant growth hormones. The application of a consortium of phyllosphere microbes and rhizosphere microbes has the potential to reduce the use of N2 fertilizer by half the dose without reducing yields. There are several microbial consortia such as *Bacillus* sp, *Pseudomonas* sp, *Lactobacillus* sp. which functions in the decomposition of organic compounds to fertilize the soil (Overbeek et al., 2021; Singh et al., 2021; Krestini et al., 2020; Bustamam et al., 2022). *Bacillus* sp, *Bacillus subtilis* can play a role in dissolving phosphate, binding free nitrogen, producing phytohormones (IAA, GA) producing ACC-deaminase enzymes, and synthesizing siderophores (Kalay et al., 2020; Setiawati et al., 2022; Traoré et al., 2016; Hashem et al., 2019; Radhakrishnan et al., 2017; Santos et al., 2021; Misra & Chauhan, 2020; Gamalero & Glick, 2022; Timofeeva et al., 2022).

In addition, the presence of rhizosphere microbes as P solvents plays an important role in supplying P available to plants. The role of these microbes in supplying P causes the weight per 1000 grains of rice to be higher (Liu et al., 2022). This is in line with the opinion of Artawan et al. (2019) that the nutrient phosphorus in plants functions to stimulate root development, increase dry matter yield, seed weight, improve yield quality and accelerate fruit maturity.

Phytohormones produced by the microbial consortium FM48 and R15 play a role in triggering plant growth which in turn can increase crop yields. This result is in line with the opinion of Ikhsani et al. (2018) microbes have the ability to synthesize plant growth hormones such as IAA which are active compounds of auxins and produce cytokinins and gibberellins which play a role in the development and division of plant cells.

The application of the consortium of phyllospheric microbes Fm48 and rhizosphere microbes R15, which have the potential to help the plant growth process has not been able to increase rice yields per unit area but can only improve the quality of rice yields. The use of symbiotic microorganisms with plants can increase metabolism and increase crop production (Elita et al., 2021).

Conclusion

The treatment of various consortiums of phyllosphere microbes Fm48 and R15 rhizosphere microbes had no significant effect on growth parameters but had a significant effect on the number of hollow grains and grain weight per 1000 rice grains. The consortium of phyllosphere microbes Fm48 and rhizosphere microbes R15 gave the best results for the lowest number of hollow grains and the highest grain weight per 1000 rice grains.

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Author Contributions

Conceptualization and design of research work (AA); Implementation of field/laboratory experiments and data collection (A, LIB, Z); Data analysis and interpretation (AA); Manuscript preparation (M).

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Conflicts of Interest

The authors declare no conflict of interest.

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