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Nutritional qualities and heavy metals accumulation in grains: A study on lowland irrigated rice with different fertilizer inputs and growing seasons

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ABSTRACT

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Keywords

Arsenic Cadmium Conventional system Grain quality Mineral fertilizer Organic system.

Integrated and organic nutrient management has become a focal point of current production systems seeking better perspectives on environmental friendliness. The food quality in such a scenario requires special consideration for ensuring safety during consumption. This study was conducted to understand the grain quality of the Bg300 rice variety grown under three fertilizer input systems: conventional (100% N supply with the recommended by the Department of Agriculture (DOA), integrated (50% of N provided with DOA recommended fertilizer + 25% of N supply with organic fertilizer), and 50% of N provided with organic fertilizer in dry tropical irrigated lowland systems in Sri Lanka. The grains were analyzed for proximate composition (moisture content, ash, protein, fat, fiber, and carbohydrate), micronutrients (Fe, Cu, Zn, and Mn), and heavy metals (As, Cd, and Pb). The experiment was arranged as a randomized complete block design and conducted during five seasons, from the 2018-19 wet seasons to the 2020-21 wet seasons. The highest moisture content and carbohydrate content were reported with the organic system. The ash content and protein content significantly (p < 0.05) changed with the respective levels of fertilizer in the three input systems. Cadmium and arsenic micronutrients were detected below the permissible level (0.4 and 0.2 ppm), while lead was detected above the permissible level (0.2 ppm). Integrated and organic systems can be used instead of the conventional fertilizer application method without compromising the quality of the rice grain.

Contribution/Originality: Rice, when treated solely with organic amendments, is perceived to be low in grain and nutritional qualities due to unintentional deficiencies and heavy metals. Three contrasting nutrient regimes in five continuous seasons illustrated depletion in protein and mineral contents and were associated with probable risks of heavy metal accumulation under sole organic systems.

1. INTRODUCTION

Rice, as the staple food of the 18.6 million people in Sri Lanka, contributes approximately 18% to the gross domestic product. Rice with 12% water, 75-80% starch, and 7% protein (Verma & Srivastav, 2017) levels plays a major role in the nutritional status of the whole population in the country (Adhikarinayaka, 2005). Rice provides 45% of the total calorie requirement and 40% of the protein requirement of an average Sri Lankan (Weerakoon et al., 2011). Apart from that, rice is a great source of complex carbohydrates (Umadevi, Pushpa, Sampathkumar, & Bhowmik, 2012), which is an important source of energy our body needs. Also, it is rich in vitamins such as Thiamin (B1), Riboflavin (B2), Niacin (B3), Vitamin D, and Calcium-like minerals (Umadevi et al., 2012). Rice grain quality characteristics are determined by its physical and physiochemical properties (Cruz & Khush, 2000). Those various quality characteristics are valuable to growers, processors, traders, and consumers. In general, rice grain quality is affected by many factors corresponding to cultivation conditions, fertilizer application methods, and water management (Balindong et al., 2018).

According to FAO (Food and Agriculture Organization) estimates, more than two billion people worldwide do not receive enough energy from their diets to meet their daily nutritional requirements and are affected by micronutrient deficiencies (Kuppusamy, Yoon, Kim, Kim, & Lee, 2017). Thus, food is a means through which nutrition is delivered, and staple crops are of particular importance in improving consumer health. Therefore, biofortification of these crops is a potential measure to alleviate micronutrient malnutrition (Cuevas, Takhar, & Sreenivasulu, 2019; Hao, Wei, Yang, Ying, & Wu, 2007; Kuppusamy et al., 2017). Therefore, more consideration is needed in balancing fertilizer application for future improvements.

Concerning fertilizer applications, paddy cultivation consumes the largest part of chemical fertilizers which accounts for approximately 50% of the overall use of chemical fertilizers in Sri Lanka. Disproportionate and indiscriminate application of the chemical fertilizer influences the different hazard conditions related to environmental pollution, human health, and global warming (Ma, Wang, Tang, & Yang, 2017). Due to massive industrialization, an increase in the use of chemical fertilizer and heavy metal pollution has become serious threats to food safety and health at present. Heavy metals are often classified as those metals with a specific density greater than 5g/cm³, and they become noxious at high concentrations (Ugochukwu, Eneh, Igwilo, & Aloh, 2017). Heavy metals can be transmitted from agricultural soils to crops growing in the field and provide detrimental effects on human health, especially cardiovascular, kidney, nervous system, blood, and bone diseases, through the food chains (Ma et al., 2017). Therefore, it is essential to understand and identify the various alternative fertilizer input systems with the appropriate level of application.

The application of organic fertilizer combined with chemical fertilizer is an important approach that is implemented worldwide to improve soil fertility, fertilizer use efficiency, and environmental protection rather than the application of chemical fertilizer alone (Xu et al., 2008). However, there was a lack of evidence and research studies to understand the effect of different fertilizer levels and different fertilizer sources on the quality and nutritional status of rice grains. Limited research has been documented, particularly in the context of low-irrigated rice cultivation, which is the primary method of paddy production in Sri Lanka. Therefore, this study was designed to hypothesize that the dynamics of nutrients with different fertilizer input application systems have a significant effect on the quality of rice grains. Thus, this study was conducted to examine the effect of different fertilizer input systems and growing seasons on quality attributes, the accumulation of macro- and micronutrients, and the content of heavy metals in rice grains under the lowland irrigated rice cultivation system in Sri Lanka.

2. MATERIALS AND METHODS

This study was conducted within the farm premises of the Faculty of Agriculture, Rajarata University of Sri Lanka. The site is located at Puliyankulama in the Anuradhapura district of Sri Lanka, which belongs to the agroecological region of DL1b (Punyawardena, 2020) with 8°25'18.12" *latitude and* 80°24'9.37" *longitude*. This area

consists of the undulated catenary landscape (Thenabadu, 1988) and imperfectly drained reddish-brown earth soils (Soil Taxonomic Order: Alfisols, Suborder: Ustalfs, Great Group: (Hapludalfs) (Mapa, Somasiri, & Dassanayake, 2010). The study was undertaken during the 2018-2019 wet (Maha), 2019 dry (Yala), 2019-2020 wet, 2020 dry, and 2020-2021 wet seasons of the long-term sustainable strategic research project. Total rainfall across five seasons varied from 962 mm to 181.6 mm; mean monthly maximum and minimum temperatures, respectively, were 35.7°C and 21.6°C during the wet and dry seasons Table 1.

The experiment consisted of three main fertilizer input systems, which were: Conventional: 100% N applied as chemical fertilizer application based on recommendations by the Department of Agriculture Sri Lanka (DOA) (2015), Integrated: 50% N supply with chemical fertilizer and 25% N supply with organic fertilizer application: T_{3} : Organic: No chemical fertilizer was added and organic manure was applied to satisfy the 50% N amount of the DOA chemical fertilizer application. Organic fertilizers (compost prepared by incorporating buffalo manure, poultry manure, gliricidia leaves, and sunnhemp plants), which previously calculated the nitrogen content, determined the relevant rate to get the required nitrogen content, and thereby managed the soil fertility. These N rates were decided considering the losses of N from urea and organic matter while aiming to provide adequate N for crop growth. Using this concept, we aimed to enhance the efficiency of nutrients applied and explore a sustainable nutrient management plan for rice-based cropping systems. The standardization of phosphorus and potassium rates was not implemented in both the integrated and organic systems. The amount of these two elements depended on the quantity and quality of organic materials used to supply N to both integrated and organic systems Table 2. The size of the plot was 90 m². A randomized complete block design was used as an experimental design with three replicates.

For rice cultivation, the land was prepared by impounding water after ploughing for two weeks and harrowing, followed by fine leveling with the help of a wooden leveler. Pre-germinated seeds of the Bg300 rice variety were broadcasted at a rate of 120 kg/ha on the puddled and leveled plots. All the management practices were done according to the guidelines given by the Department of Agriculture Sri Lanka (DOA) (2015). Irrigation was started one week after the seed sowing and impounded with a 5 cm depth of water to keep the soil sufficiently moist throughout the cultivation period. Flood irrigation methods were followed to keep the soil moist for rice.

Climate attribute	Wet 2018/19	Dry 2019	Wet 2019/20	Dry 2020	Wet 2020/21
Cumulative seasonal rainfall (mm)	364.6	181.6	962	621.7	751.1
Minimum T (°C)	23.4	25.4	23.4	25.1	23.4
Maximum T (°C)	32.4	34.1	31.6	32.7	30.8
Day RH (%)	67.6	64.6	71.2	70.4	74.8
Night RH (%)	90.6	87.4	91.8	89.2	93.1

2.1. Proximate Analysis

Grounded rice grain samples from five seasons were analyzed separately to determine the proximate composition of the rice grains. Moisture content, crude protein content, crude fat content, total ash content, and crude fiber content were determined by the oven-dry method described in standard official methods of analysis of the Association of Official Agricultural Chemists (AOAC) (method 14:004), and the Kjeldahl method (Nielsen, 2017), the Soxhlet extraction method described by Oko and Ugwu (2011), furnace incineration described by AOAC (method 14:006) and the method of AOAC (method 14:020), respectively. Carbohydrate content will be determined by the difference method using the equation defined by Oko and Ugwu (2011).

 $Carbohydrate \ content = \{100-(ash+moisture+ \ protein+ \ fat+ \ total \ crude \ fiber)\}$

Fertilizer	Mineral nutrient	Synthetic fertilizer	Nutrients from	Organic
input system	(kg/ha)	rate (DOA 2013)	organic fertilizer	fertilizer rate
		(kg/ha)	(kg/ha)	(Mg/ha)
Conventional	N - 103.5 (Urea 46%)	225 (Urea)	N - 0	0
	$P - 3.9 (P_2O_5 43.7\%)$	55 (TSP*)	P - 0	0
	K - 30.0 (K ₂ O 60%)	60 (MOP**)	K – 0	0
Integrated	N – 51.8 (Urea 46%)	112.5 (Urea)	N - 25.9	6
	$P - 1.9 (P_2O_5 43.7\%)$	27.5 (TSP)	P - 0.65	6
	K – 15 (K ₂ O 60%)	30 (MOP)	K - 52.5	6
Organic	N – 0 (Urea 46%)	0	N – 51.8	12
	$P - 0 (P_2O_5 43.7\%)$	0	P – 1.9	12
	$K = 0 (K_2 O 60\%)$	0	K – 15	12

Table 2. Fertilizer input systems and their respective nutrient conten
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Note: * TSP - Triple super phosphate; ** MOP - Muriate of potash.

2.2. Microelements and Heavy Metals

All five seasonal grounded rice samples were used to determine the micronutrient and heavy metal content of the rice grains. We used an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (iCAP7400 Duo MFC) to look at heavy metals (Lead (Pb), Arsenic (As), and Cadmium (Cd)) along with micronutrients (Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn)) (Ghazanfarirad et al., 2014).

2.3. Statistical Analysis

Data from five seasons for proximate analysis and microelements and heavy metals were analyzed to identify the impact of different fertilizer input systems and cropping seasons. The SAS computer program version 9.0 was used for statistical analysis. Percentages were tested for normality and heteroscedasticity by identifying the seasons, and then the data were log-transformed to maintain the homogeneity and normality of residuals. Analysis of variance (ANOVA) was carried out using the repeated measures MIXED model to determine the effect of fertilizer input systems and season. The season was considered a repeated factor. The means were separated using the least significant difference (LSD) method at the 5% probability level.

3. RESULTS AND DISCUSSION

3.1. Determination of the Proximate Composition of Dried Grain Powder

The moisture content of rice grains was significantly influenced (p > 0.05) by the interaction between the fertilizer input system and the season Table 3. Grains of the wet seasons resulted in high moisture content Figure 1 under conventional drying due to higher cumulative rainfall than in dry seasons Table 1. The inclusion of grains inside the organic system led to somewhat elevated levels of grain moisture. However, it was not only the climatic factors; varietal characteristics, agronomic factors, temperature of the season, duration of drying, and, storage conditions were also affecting the moisture content (Wireko-Manu & Amamoo, 2017). The moisture content of grains in all systems was around 12%, which is the accepted standard for long-term storage and to avoid insect infestation and microbial growth (Verma & Srivastav, 2017). Nutrient content in some commercial rice in Malaysia and moisture content in aromatic and non-aromatic Indian rice also reported close correspondence value to this research, despite the selected rice variety being in indica types (Mohd et al., 2015; Verma & Srivastav, 2017).

Table 3. The probability values for the effect of different fertilizer input systems, crop rotation with the season on proximate composition of rice grains.

Effect	Moisture content	Ash	Protein	Fat	Fiber	Carbohydrate
Input system (IS)	ns	**	**	ns	ns	**
Season (SE)	**	**	**	ns	**	**
IS×SE	**	ns	ns	ns	ns	**

Note: **-Significant at p<0.05 level, ns-Not significant at p<0.05 level.

The total ash contents of harvested grains from conventional systems were recorded as the highest compared to integrated and organic system Table 3 and Figure 2 and only the dry season of 2019 was low in ash content Figure 2.



Figure 1. Variation of moisture content of rice grains with cropping seasons and fertilizer input systems.

The difference in the ash content between conventional and organic is approximately 1.15% Figure 2, the quantities might directly relate to the inputs of fertilizers. Similar to the findings by Wenela (2013), this study also reflected that changes in soil quality have an impact on the ash content of the rice. The seasonality showed an insignificant impact on the ash content, while the dry season of 2019 resulted in ash content lower than 1% Figure 3.



The reduction might be more associated with weather and water management regimes than with fertilizer inputs. The range of ash content in this study is comparable to the value range obtained by Wenela (2013), and Wireko-Manu and Amamoo (2017) for white rice, and Zubair, Rahman, Islam, Abedin, and Sikder (2015) for brown rice.



Figure 5. Variation of protein content with cropping seasons.

Starch is the most abundant nutritional element in rice grain, yet the protein has become important in terms of defining the quality of the milled rice. The protein content of rice grains during this study was significantly dependent on fertilizer input systems and seasons Table 3. Protein contents of the rice crop ranged between 7.8–6.7%. Figure 4 shows that the protein content of the Bg300 rice variety was found to be approximately 7.8% (Fari, Rajapaksa, & Ranaweera, 2011). Thus, the lower level of protein could be proportionate to the nitrogen inputs in the Table 2, as very similarly explained by Verma and Srivastav (2017). Particularly, the highest level of protein resulted from the conventional fertilizer input system, while the lowest resulted from the organic systems, similar to previous studies (Fari et al., 2011; Prasantha, Hafeel, Wimalasiri, & Pathirana, 2014; Saha et al., 2007). A study conducted by Hossain, Bhuiya, Ahmed, and Mian (2009) explains that the use of mineral fertilizers reduces the physical properties and microbiological activities of soil while supplying several elements in high quantities, resulting in an improvement in the quality of rice grains. Hence, the almost 1% protein gain could be a result of high N inputs. The recommended N input can be the theoretical upper limit of N inputs to show a positive impact on net protein (Firouzi, 2015). The dry seasons of 2020 resulted in a significantly higher protein content compared to other seasons Figure 5. The difference could be attributed to the environment and management interactions,

which modified the N use efficiencies during this season. Further, a slight variation in protein content might happen due to water supply, handling, degree of milling, environmental stress, location of the growing area, growing conditions, and time of the season (Verma & Srivastav, 2017). The fat content of rice grain from three fertilizer input systems was low and similar; thus, fat was not changed by the sole use of mineral or organic inputs Table 3. However, the mean fat content of 2.8% was found to be lower than the findings by Prasantha et al. (2014) and higher than the findings by Fari et al. (2011) and Lansakara et al. (2016) for variety Bg300. The fat content may be different from cultivar to cultivar under different fertilizer inputs, yet variety Bg300 was not sensitive in this study, which was in line with the study by Kanageswaran (2015), who observed the same level of fat content for the rice cultivars in Sri Lanka. Further, the degree of milling might influence the fat content; hence, there is a possibility of changing the fat content during milling to result in approximately the same level of fat.

Interestingly, the grain fiber content was significantly impacted by the season, despite no differences between fertilizer input systems Table 3. Mainly, this difference was attributed to the high fiber content in the dry season of 2019, which resulted in more than 1% high fiber content compared to other seasons Figure 6. The potential cause for the reduced ash content observed during the dry season of 2019 could be attributed to the elevated fibre content. The grain fiber content can also be dependent on weather and climate, nutrient content in the soil, growing conditions for plants, use of fertilizer, and degree of milling (Verma & Srivastav, 2017). The findings were slightly lower than the fiber content reported by Lansakara et al. (2016) and Hafeel, Bulugahapitiya, de Zoysa, and Bentota (2020) for Bg300, which were grown in different environments.



Figure 6. Variation of fiber content with cropping seasons.

Grain carbohydrate was significantly affected (p<0.05) by the interaction between the fertilizer input system and season Table 3. The grain carbohydrate contents were higher in the organic system, and even during the last season, the carbohydrate content of the organic system was higher than the other two systems. The carbohydrate content was more than 70%, which is general for modern rice cultivars; however, compared to conventional and integrated organic systems, it resulted in a higher range of 77.6-75.2%. The carbohydrate content might have altered, resembling the nutrient regime, where high N input allows the crop to derive more protein, compensating for lower carbohydrate levels. The protein content may be the primary factor, given that the fat concentrations were comparable across the various fertilizer input scheme Figure 7. The percentage of carbohydrates can be influenced by environmental factors under which rice is grown, like soil type, rainfall, solar radiation, and the growth temperature (Wireko-Manu & Amamoo, 2017), which were similar in this study; thus, the fertilizer input might have generated interaction with the growing environment. The results obtained by this study are in line with the carbohydrate contents reported by Kariyawasam, Godakumbura, Prashantha, and Premakumara (2016) for Sri Lankan traditional rice varieties.





3.2. Determination of Micronutrient Content of Dried Grain Powder

Among the tested micronutrients, Mn content was significantly (p < 0.05) affected by the fertilizer input system and season, while Fe, Cu, and Zn were changed due to the effect of seasons Table 4. Manganese content ranged between 1 and 1.2 mg/kg across nutrient management in fertilizer input systems and Mn content in conventional and integrated systems illustrating the impact of mineral fertilizers Figure 8. The seasonal effect was pronounced mainly due to high Mn content in the dry season of 2019, which resulted in 1.8 mg/kg) compared to the lowest of 0.77 mg/kg in the dry season of 2020 Figure 9.

Table 4. The probability values for the effect of different fertilizer input systems with crop rotation on the micronutrients.

Effect	Mn	Fe	Cu	Zn
Input system (IS)	**	ns	ns	ns
Season (SE)	**	**	**	**
IS×SE	ns	ns	ns	ns



Note: **-Significant at p < 0.05 level, ns – Not significant at p < 0.05 level.

Figure 8. Variation of Mn content with fertilizer input systems.





Similarly, Fe, Cu, and Zn contents were also higher in the dry season of 2019 and showed a substantial difference between all other seasons. Differences between dry 2019 and other seasons were recorded with Zn, Fe, and Cu content, respectively. (2018/19 wet season: 2.11 mg/kg; 0.2 mg/kg; 0.69 mg/kg, 2019/20 wet season: 2.53 mg/kg; 0.25 mg/kg; 0.63 mg/kg, 2020 dry season: 1.89 mg/kg; 0.13 mg/kg; 0.72 mg/kg, 2020/21 wet season: 2.41 mg/kg; 0.22 mg/kg; 0.21 mg/kg; 0.21 mg/kg) (Figures 10,11,12). Mineral fertilizer and irrigation water could be the major reasons for the seasonal dynamics of micronutrients. Elements like Mn, Zn, Fe, and Cu are highly likely to change with the source of phosphorus fertilizer. In the Sri Lankan context, phosphorus fertilizers are imported from multiple origins, so microelements can be added to the crops in different concentrations in different seasons (Lai et al., 2018). The 2019 season exhibited a comparatively decreased precipitation, resulting in a notable depletion of water resources from prominent irrigation systems. The water generally came through many paddy fields within the cascade or irrigation network. Therefore, there is a possibility of observing higher Mn, Fe, and Cu concentrations in grains. Such observations were also reported by Lai et al. (2018). Akinyele and Shokunbi (2015) in contrasting environments. Lower Mn values for the same Bg300 variety recorded with Herath, Chandrasekara, Pulenthiraj, Chandrasekara, and Wijesinghe (2019) study compared to this study in the same environment.



Figure 10. Variation of Fe content with cropping seasons.



Figure 11. Variation of Cu content with cropping seasons.

Zinc content resulted in a different dynamic to rest as the wet season 2020-2021 resulted in the highest content Figure 10. The observation might have been linked to organic fertilizers which are rich in micronutrient content and support the sequestration of elements in the long run. Yet, dissimilarity in microelement (Mn, Fe, Cu, Zn) content could be possible due to different soil characteristics, genetic characteristics, fertilizer, different geographical areas, environmental factors, and degree of polishing (Verma & Srivastav, 2017). The seasonal changes in the quality of applied mineral fertilizers (particularly P and K) and soil physicochemical properties such as low pH, high organic matter, and high total/bioavailable micronutrients enhance micronutrient uptake by rice (Kuppusamy et al., 2017).



Figure 12. Variation of Zn content with cropping seasons.

3.3. Determination of Heavy Metals in Dried Grain Powder

Grain Cd content was changed significantly due to fertilizer input systems and seasons, while Pb and As were affected by the effect of seasons only Table 5. The integrated systems exhibited the highest concentration of Cd, while the conventional system yielded the lower concentration. Interestingly, the organic system showed an intermediate level of Cd compared to the other two systems Figure 13. None of the samples had exceeded the permissible level of 0.4 ppm for Cd Figures 13 and 14 recommended by FAO/WHO joint CODEX Alimentarius. The Pb content of the wet season 2018/19 and the dry season 2019 exceeded the permissible level for Pb in rice (0.2 ppm), according to CODEX STAN 193-1995 Figure 15. However, concerning as content, all samples resulted

below the recommended minimum level (0.2 ppm) according to CODEX STAN 193-1995, hence were safe for consumption Figure 16.

Effect	Cd	Pb	AS
Input system (IS)	**	ns	ns
Season (SE)	**	**	**
IS×SE	ns	ns	ns

Table 5. The probability values for the effect of different fertilizer input systems with crop rotation on the heavy metal of rice.

Note: **-Significant at p<0.05 level, ns-Not significant at p<0.05 level.

Similar to many studies (Chandrajith, Ariyaratna, & Dissanayake, 2012; Mataveli et al., 2016) and Mataveli et al. (2016), the heavy metal content of milled grains during this study was also not found to be unsafe. However, the Cd content was highly similar to the studies by Mohapatra and Bal (2006) and Pirsaheb, Fattahi, Sharafi, Khamotian, and Atafar (2016) for Indian rice samples, which showed connectivity to the inherent Cd content changes of added P fertilizers. Pb-contaminated P fertilizers resulted in higher grain Pb contents (Ghazanfarirad et al., 2014; Mataveli et al., 2016); however, they can be lower depending on the source, and substantially higher values were obtained by Wu et al (2020). The grain As content can be lower since rice plants concentrate As in the outer layer of the grain, in the region corresponding to the pericarp. Hence, a great amount of As is removed during rice polishing. A lower As content is possible in polished rice grains compared to brown rice (Mataveli et al., 2016). In addition to fertilizers, water management might have a greater impact on the heavy metal accumulation in rice (Lai et al., 2018). Cross-contamination through surrounding fields is possible; in large-scale farming, using a common source of irrigation is possible; and even in the study, the water used was from a major irrigation scheme. They used irrigation water from other rice fields; thus, seasonal differences were observed which were linked to the fertilizer amount and quality. Hence, the variation in the heavy metal concentration was observed, due to different cultivars, geography, environment, water quality, and growth conditions.



Figure 13. Variation of Cd content with fertilizer input systems.





Figure 16. Variation of As content with cropping seasons.

Cropping season

4. CONCLUSIONS

The organic system resulted in higher grain moisture and higher grain carbohydrate contents at the end of each season. Ash and protein content of the rice grain were changed with the respective effect of the fertilizer

-0.05

а

application. Among microelements, Mn was the only element that changed parallel to the fertilizer application level. Mn, Fe, and Cu were changed with the effect of irrigation management. Cd and As contents were detected below the permissible level (0.04 and 0.2 ppm), while Pb was detected above the permissible level (0.2 ppm). Therefore, integrated and organic alternative fertilizer application systems can be used instead of the conventional fertilizer application method without compromising the quality of the rice grain.

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Authors' Contributions: Carried out the experiment, data collection and wrote sections of the manuscript, D.M.O.E.U. & A.M.T.T.A.; carried out the experiment, laboratory analysis, data analysis and wrote the manuscript, W.M.D.M.W.; research supervision, D.W.M.M.M.K.; financial handling, D.A.U.D.D.; supervision and review, L.D.B.; conceptualization, designed the experiment supervision, review and editing, D.I.D.S.B; conceptualization, designed the experiment, review and editing, W.C.P. All authors have read and agreed to the published version of the manuscript.

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