

## Article

# Effects of Organic and Inorganic Fertilizer Application on Growth, Yield, and Grain Quality of Rice

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**Abstract:** Nutrient management and fertilizer application are influential elements for high yield and preferred grain quality. Negligible information is available regarding fertilizer application in the paddy fields in Afghanistan. This research elucidates the efficacy of different fertilizers' application on growth attributes, yield potential, and grain quality of rice. The treatments included the traditional application rate of nitrogen and phosphorus (RD), animal manure (AM), animal manure with 50% nitrogen and phosphorus of the traditional application rate (AMRD), sawdust (SD), and sawdust with 50% nitrogen and phosphorus of the traditional application rate (SDRD). Growth parameters, grain yield and its components, physicochemical properties, and morphological observation using scanning electron microscopy were recorded. The results revealed that the greatest panicle number, spikelet number, and grain yield were recorded in AMRD and SDRD treatments. Both AMRD and SDRD treatments increased the percentage of protein, amylose, and lipid contents, as well as the percentage of perfect grain compared to the RD treatment. Rice grain in RD treatment had very few protein bodies and their traces (pits), as well as the formation of amyloplasts and starch granules, were normal. However, AMRD and SDRD increased the number of protein bodies and their pits in the rice endosperm. The shapes of the amyloplasts were round and polyhedral with diverse sizes. Starch granules were polygonal with sharply defined edges. This research encourages farmers to adopt the combined application of manures and fertilizers to decrease the dependence on inorganic fertilizers.

**Keywords:** amyloplast; fertilizer; manure; physicochemical properties; quality; rice

## 1. Introduction

Rice is considered to be the major source of calories and staple food worldwide [1]. Paddy fields and rice production have been increased over recent decades in Afghanistan [2,3]. The average paddy rice yield in the country is estimated at 2.8 tons per ha with a total production of 532,000 metric tons during 2010–2011 [1]. Afghan farmers mostly rely upon conventional farming systems and apply a vast amount of fertilizers, particularly inorganic fertilizers such as urea (containing 46% N) and diammonium phosphate (DAP; containing 46% P and 18% N) to enhance paddy rice yield [1,3]. However, the application of inorganic fertilizers is criticized as it poses multiple threats to human health and the environment [4]. Inorganic fertilizers also contaminate groundwater and are not eco-friendly [5–7].

The continuous and steady application of inorganic fertilizers leads plant tissues to frequently absorb and accumulate heavy metals, which consequently decreases the nutritional and grain quality of crops [8–10]. Accordingly, overuse of inorganic fertilizers has caused soil, air, and water pollutions through nutrient leaching, destruction of soil physical characteristics, accumulation of toxic chemicals in water bodies, and so on [4], as well as causing severe environmental problems and loss of biodiversity. Thus, agrochemicals are among the considerable and dominant factors of pollution in developing countries and play a hazardous role in human and livestock health [11].

Application of organic fertilizers such as animal manure, sawdust, and others, or the combination of organic and inorganic fertilizers, can be an alternative option to reduce the utilization of inorganic fertilizers. Organic fertilizers, compared to inorganic fertilizers, maintain soil quality, increase soil organic matter, as well as improve soil physical and chemical properties through the decomposition of its substances [12]. Organic matter enhances soil nutrients, plant growth regulators, and biodiversity [13,14]. Thus, an integrated nutrient management system is required to maintain soil quality as well as to obtain high yield and preferred grain quality. Hence, there is an urgent need to apply numerous sources of organic fertilizers as a substitute to reduce the utilization rate of inorganic fertilizers.

On the other hand, consumer acceptability and the marketability of the rice grain broadly relies upon its quality; hence, it influences the economy of rice producers [15,16]. The physicochemical properties of the rice grain, which consist of physical traits such as chalkiness, shape, size, perfectness, and appearance, as well as chemical composition such as the contents of amylose, protein, and lipid, have an immense influence on rice production, consumption, and consumer preference. Therefore, rice grain quality improvement is increasingly demanded by rice consumers [17,18]. The present research aims to clarify the influence of combined organic and inorganic fertilizers on the growth attributes, yield performance, physicochemical properties, and morphological analysis of rice.

## 2. Materials and Methods

### 2.1. Experimental Site and Design

The research was performed at the research farm of the agriculture faculty of Nangarhar University, Afghanistan, during 2018–2019. The research was conducted with a randomized complete block design in five treatments and four replications. The treatments included a recommended dose of nitrogen and phosphorus (RD), animal manure (AM), animal manure with 50% recommended dose of nitrogen and phosphorus (AMRD), sawdust (SD), and sawdust with 50% recommended dose of nitrogen and phosphorus (SDRD). Detailed information on the treatments is presented in Table 1. The experimental field was plowed with a chisel plow and basins were prepared. Soil samples were collected from the farm by a core sampler method to evaluate soil properties. Soil physical and chemical properties of the research farm at 30 cm depth are illustrated in Table 2. Each plot size was 16 m<sup>2</sup> and was separated from each other by 1 m space within the blocks. Animal manure was a mixture of cow, sheep, and goat dung, and was decomposed well. The sawdust used in this experiment was purchased from a sawmill in Jalalabad city, Afghanistan, and mixed with green leaves to decompose. Animal manure and sawdust were applied ten days before rice seedlings transplantation, while DAP was used during puddling operation as a basal dressing. Urea fertilizer was broadcasted at three stages (50% as a top dress before the maximum tillering stage, 25% as a top dress at the panicle initiation stage, and 25% at the flowering stage).

**Table 1.** Amount of nitrogen and phosphorus in treatments from different fertilizers.

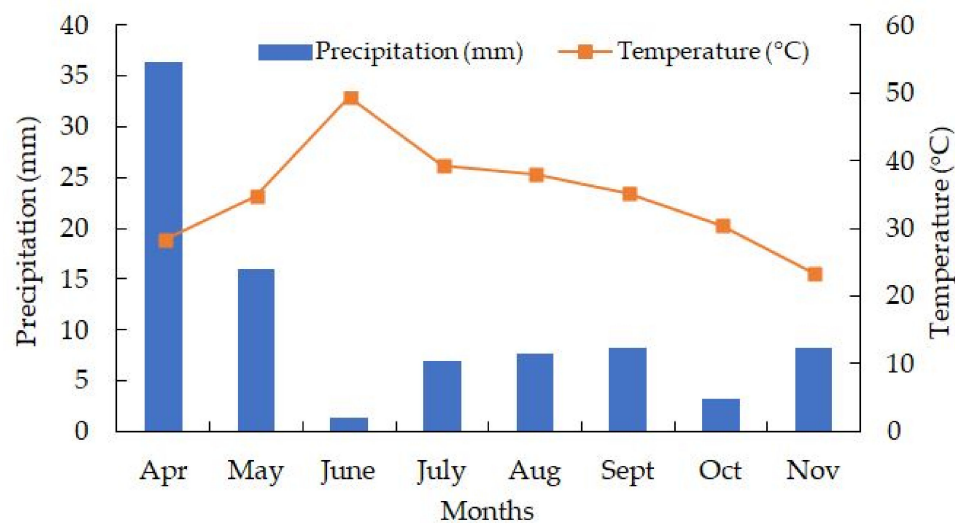
Treatments	Description	Nitrogen (kg/ha)	Phosphorus (kg/ha)
RD	The recommended dose for traditional farming (120 kg/ha urea and 100 kg/ha DAP)	73.2	46.0
AM	Animal manure only (5 tons per ha)	30.0	25.0
AMRD	Animal manure and 50% recommended dose of nitrogen and phosphorus	66.6	48.0
SD	Sawdust only (5 tons per ha)	20.0	10.0
SDRD	Sawdust and 50% recommended dose of nitrogen and phosphorus	56.0	33.0

**Table 2.** Soil physical and chemical properties of the research farm at 30 cm depth.

Soil Properties	Description and Quantity
Texture group	Sandy clay loam
Clay particles	24.82%
Silt particles	27.14%
Sand particles	48.04%
pH	7.61
Electrical conductivity	0.04 dS/m
Total Nitrogen	1.32%
Phosphorus	3.24 mg/kg
Potassium	114.03 mg/kg
Calcium carbonates	22.01%

## 2.2. Plant Materials and Measurements

Rice (*Oryza sativa* L.) cv. Attai-1, one of the most famous rice cultivar in the eastern region of Afghanistan, was selected as a test crop. Seeds were sown in nursery boxes and 27-day old seedlings were transplanted to the prepared fields. The planting density was 15 cm space between crops and 30 cm between rows. Weeds were controlled twice manually by hand and irrigation was conducted through a basin irrigation system based on weather conditions and plant requirements. Monthly precipitation and temperature of the research farm are presented in Figure 1. Ten hills were selected to measure growth and yield attributes consisting of plant length, tiller and leaf number, panicle length, yield components, and grain yield. Plant length was recorded with a common ruler from the surface of the soil to the tip of the plant, and yield and its components were recorded at 18% moisture content by a moisture tester (Wile 55 moisture meter, Farmcomp Oy, Tuusula, Finland) based on a previously reported method [13]. Plants were harvested on 5 October 2019 and the grains were de-hulled by a small husk remover machine (FC2K; Otake Co., Ltd., Aichi, Japan). Grain quality traits, consisting of physical properties (perfect, imperfect, and broken grains) and chemical characters (amylose, protein, and lipid contents) were inspected. Grain physical properties were evaluated by a grain discrimination device (RGQI 10B; Satake Co., Ltd., Hiroshima, Japan), which can automatically measure the percentage of perfect, imperfect, and broken grains. One-hundred gram of brown rice grain sample for each treatment was randomly selected to record grain physical properties; this procedure was repeated three times for each treatment. Chemical characters were measured by a taste analyzer machine (RCTA11A; Satake Co. Ltd., Hiroshima, Japan). The instrument performs a palatability estimation formula based on the combination of near-infrared spectroscopy and physicochemical measurements, with a sensory test by a non-distractive procedure, and calculates the composition based on the present moisture content. To measure chemical properties, 200 g of brown rice grain sample at 12% moisture content was evaluated for each treatment and replicated thrice.



**Figure 1.** Monthly average precipitation and temperature of the research area. Source: <https://www.weather-atlas.com/en/afghanistan/jalalabad>.

### 2.3. Scanning Electron Microscopy Observation

Thirty perfect grains (brown rice) from each treatment were randomly selected to evaluate the cross-cut structures of grains through a scanning electron microscope (JSM6360A model; JEOL, Tokyo, Japan) by a previously described method [3,13]. The selected rice grains were kept in a freeze vacuum dryer (LFD-100NDPS1 version; Nihon Techno Service Co., Ltd., Tokyo, Japan) at  $-60\text{ }^{\circ}\text{C}$  and  $10^{-3}\text{ Pa}$  pressure to be freeze-dried. Then, the grains that were cross-cut with a razor blade and freeze-dried were adjusted on specimens. The grains were covered with platinum by a sputtering machine (JUC-5000 modal; JEOL, Tokyo, Japan) and the micrographs were taken with the scanning electron microscope.

### 2.4. Statistical Analysis

A statistical package for the social sciences (13.0, Prentice-Hall, Upper Saddle River, NJ, USA) was used to analyze the data. One way analysis of variance (ANOVA) was carried out. Means of variance were detached by Tukey's multi comparison test and the significant difference level was expressed at the  $p < 0.05$  probability level.

## 3. Results

### 3.1. Growth and Yield Performances

Growth attributes consisting of plant length, leaf number, tiller number, and panicle length are summarized in Table 3. Significant differences were observed in the tiller number per hill and panicle length among treatments. Tiller number per hill and panicle length, which were 19.0 and 26.1 cm, respectively, were higher in the AMRD treatment compared to others, followed by SDRD, AM, SD, and RD. Plant length, tiller number, leaf number, and panicle length ranged from 110.3–115.6 cm, 14.0–19.0, 15.1–16.9, and 20.3–26.1 cm, respectively. However, all of these parameters were greater in AMRD and were lower in RD treatments.

**Table 3.** Description of growth parameters under different treatments of fertilizer application.

Treatments	Plant Length (cm)	Tiller No. Hill <sup>-1</sup>	Leaf Number Plant <sup>-1</sup>	Panicle Length (cm)
RD	110.3 ± 0.9 a	14.0 ± 0.2 b	15.1 ± 0.7 a	20.3 ± 0.3 b
AM	112.5 ± 0.2 a	16.0 ± 0.6 ab	16.5 ± 0.5 a	24.2 ± 0.8 ab
AMRD	115.6 ± 0.7 a	19.0 ± 0.1 a	16.9 ± 0.2 a	26.1 ± 0.7 a
SD	112.2 ± 0.3 a	15.0 ± 0.9 b	15.4 ± 0.8 a	22.2 ± 0.1 b
SDRD	114.3 ± 0.8 a	18.0 ± 0.4 a	16.8 ± 0.6 a	25.8 ± 0.5 a

Data are presented as means ± standard errors (SE). The same alphabetical letters within a column denote no differences at the  $p < 0.05$  level based on Tukey's multi comparison test.

Grain yield and its components including panicle number per hill, spikelet number per panicle, percentage of ripened grain ratio, and 1000 grain weight are presented in Table 4. Significant differences were found in panicle number per hill, spikelet number per panicle, and grain yield; however, the percentage of ripened grain ratio and 1000 grain weight did not differ significantly. Panicle number per hill, spikelet number per panicle, percentage of ripened grain ratio, 1000 grain weight, and grain yield ranged from 11.4–15.9, 105.3–110.7, 85.9–87.7%, 20.3–20.9 g, and 4.7–7.2 t/ha, respectively. The greatest panicle number per hill, spikelet number per panicle, and grain yield were recorded in AMRD compared to other treatments. Grain yield was higher in AMRD (7.2 t/ha) pursued by SDRD (6.8 t/ha), SD (5.4 t/ha), AM (5.3 t/ha), and RD (4.7 t/ha) treatments, respectively.

**Table 4.** Description of grain yield and its components in different treatments of fertilizer application.

Treatments	Panicle No. Hill <sup>-1</sup>	Spikelet No. Panicle <sup>-1</sup>	Ripened Grain Ratio (%)	1000 Grain Weight (g)	Grain Yield (t ha <sup>-1</sup> )
RD	11.4 ± 0.3 c	106.5 ± 0.6 b	86.2 ± 0.5 a	20.3 ± 0.2 a	4.7 ± 0.7 b
AM	12.7 ± 0.5 b	106.2 ± 0.8 b	86.1 ± 0.8 a	20.5 ± 0.3 a	5.3 ± 0.5 b
AMRD	15.9 ± 0.7 a	110.7 ± 0.1 a	87.7 ± 0.3 a	20.9 ± 0.7 a	7.2 ± 0.9 a
SD	13.0 ± 0.2 b	105.3 ± 0.7 b	85.9 ± 0.7 a	20.4 ± 0.6 a	5.4 ± 0.6 b
SDRD	15.6 ± 0.9 a	109.4 ± 0.3 a	86.7 ± 0.9 a	20.7 ± 0.8 a	6.8 ± 0.7 a

Data are presented as means ± standard errors. The same alphabetical letters within a column denote no differences at the  $p < 0.05$  level based on Tukey's multi comparison test.

### 3.2. Physicochemical Properties

Rice grain physicochemical parameters, including the contents of protein, amylose, and lipid, as well as the percentage of perfect, imperfect, and broken grains are illustrated in Table 5. Significant differences were observed in terms of protein, amylose, and lipid contents, and the percentage of perfect and imperfect grains. AMRD increased the percentage of protein, amylose, and lipid contents as well as the percentage of perfect grain, and decreased the percentage of imperfect grain. Protein, amylose, and lipid contents ranged from 7.6–8.7%, 20.9–23.0%, and 7.8–9.7%; however, the percentage of perfect, imperfect, and broken grains ranged from 54.3–60.7%, 33.7–40.2%, and 0.5–0.9%, respectively. AMRD and SDRD showed significantly higher protein, amylose, and lipid contents than RD treatment.

**Table 5.** Rice grain physicochemical properties in treatments of different fertilizers application.

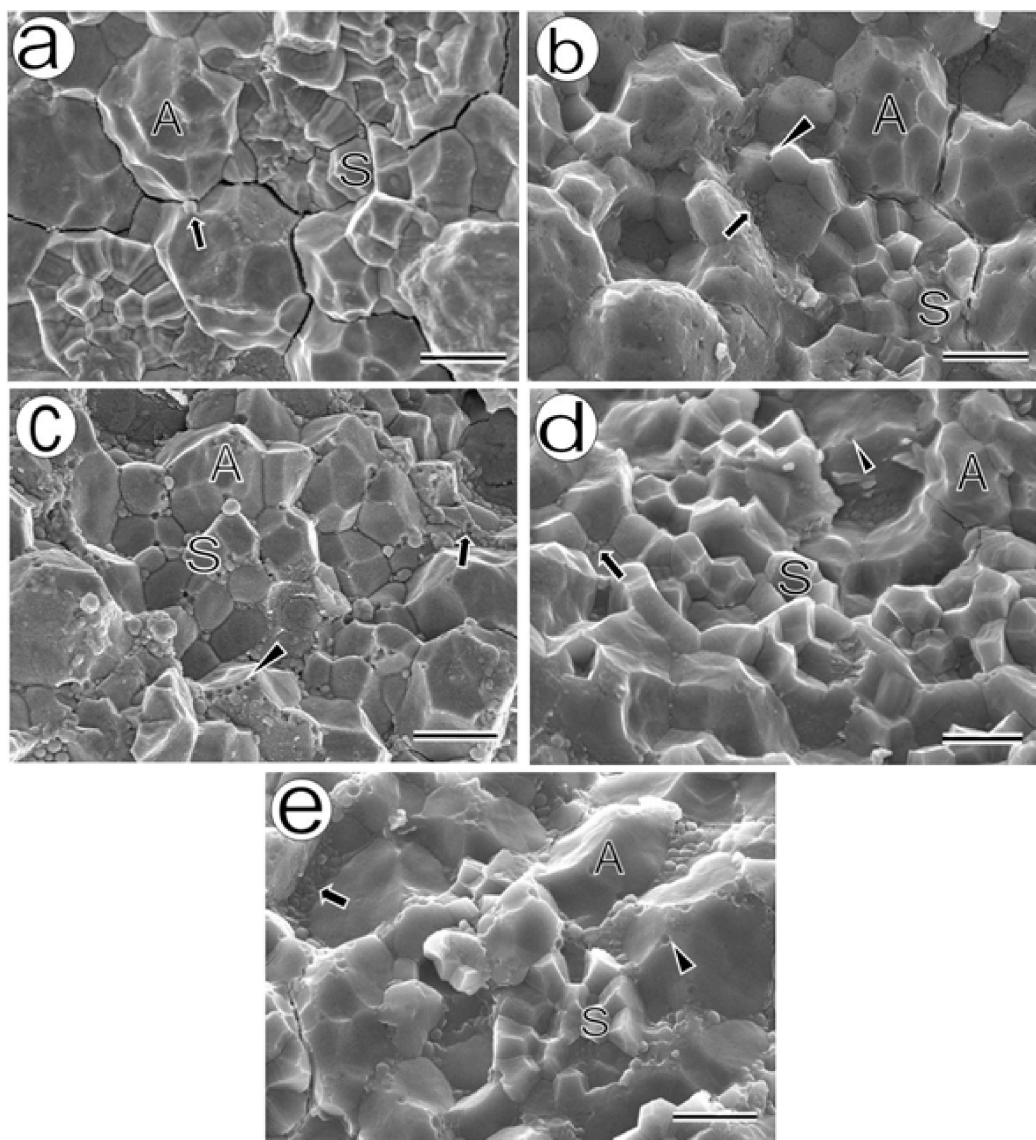
Treatments	Protein Content (%)	Amylose Content (%)	Lipid Content (%)	Perfect Grain (%)	Imperfect Grain (%)	Broken Grain (%)
RD	7.6 ± 0.02 b	20.9 ± 0.02 c	7.8 ± 0.03 c	54.3 ± 0.45 b	40.2 ± 0.08 a	0.9 ± 0.02 a
AM	8.0 ± 0.05 ab	21.5 ± 0.07 b	8.2 ± 0.06 bc	55.1 ± 0.58 b	39.1 ± 0.04 a	0.7 ± 0.05 a
AMRD	8.7 ± 0.01 a	23.0 ± 0.03 a	9.7 ± 0.02 a	60.7 ± 0.36 a	33.7 ± 0.07 b	0.5 ± 0.02 a
SD	7.9 ± 0.06 b	21.9 ± 0.06 b	8.4 ± 0.07 bc	54.9 ± 0.64 b	38.4 ± 0.06 a	0.6 ± 0.06 a
SDRD	8.3 ± 0.03 a	22.6 ± 0.08 a	8.9 ± 0.04 b	57.4 ± 0.45 ab	35.5 ± 0.03 ab	0.6 ± 0.05 a

Data are presented as means ± standard errors. The same alphabetical letters within a column denote no differences at the  $p < 0.05$  level based on Tukey's multi comparison test.



### 3.3. Morphological Observation

The results of the scanning electron microscope revealed that variations were found in the inner structure of rice grain among treatments. Rice grain in RD treatment recorded very few protein particles and their traces (pits) in contrast to the remaining treatments. The structure of amyloplasts and starch granules were normal and without air gaps. Amyloplasts were round in shape and the starch granules were compacted within the amyloplast. Starch granules within the amyloplast were spherical in shape and had no air spaces, which resulted in normal placement and formation in the endosperm (Figure 2a). Amyloplasts in the rice grain of the AM treatment were polygonal in shape and had a greater number of protein particles and their pits than RD treatment. The majority of protein particles were gathered around the amyloplasts while some of them were located on the surface of the amyloplasts (Figure 2b).



**Figure 2.** Accumulated structures and formation of rice grain in different treatments: A, amyloplast; S, starch granules; arrow, protein bodies; arrowhead, pits of protein particles. Bars: 10  $\mu$ m. (a) RD; (b) AM; (c) AMRD; (d) SD; (e) SDRD.

The AMRD treatment increased the amount of protein particles and its pits in the rice grain endosperm. Amyloplasts were round in shape and were polyhedral with diverse sizes. Starch granules were polygonal with sharply defined edges (Figure 2c). SD treatment had almost identical results

as AM but the starch granules were located as single grain rather than combination form within an amyloplast (Figure 2d). There were protein particles and their pits on the surface and around the amyloplast of SDRD treatment, but the amount was less compared to AMRD and greater than the other treatments. The formation of protein particles was spherical in shape and was gathered between or on the surface of the amyloplasts (Figure 2e).

#### 4. Discussion

Unnecessary and excessive application of inorganic fertilizers causes environmental damage, pollution of water sources, and decreases beneficial living organisms [13]. However, organic farming systems avoid the application of inorganic fertilizers and instead rely upon crop rotation, manures, organic amendments, and the biological systems of nutrients mobilization to maximize yield [19,20]. Therefore, it is important to explore the efficacy of organic manures alone or in combination with other fertilizers on growth performance, yield potential, and rice grain quality to reduce dependence on inorganic fertilizers and increase rice yield and to secure food supply and protect the environment by the recycling of organic matter.

In this study, tiller number per hill and panicle length were higher in AMRD treatment than other treatments followed by SDRD, AM, SD, and RD. Additionally, the greatest panicle number per hill, spikelet number per panicle, and grain yield were recorded in AMRD in contrast to other treatments. It is documented that fertilizer application affects rice tillering capacity [21]. Badshah et al. [22] stated that productive tillers are responsible and critical elements for panicle development in rice plants. Duan et al. [23] mentioned that more panicles, spikelet fertility, and 1000-grain weight are among the crucial elements of yield enhancers. Moe et al. [24] reported that the combined application of organic and inorganic fertilizers increased tiller number, panicle number, spikelet number, dry matter production, panicle length, and yield, which are in line with our results.

The physicochemical properties of rice grain are largely related to cultural practices, processing management, and genetic behavior [25,26]. AMRD increased the percentage of protein, amylose, and lipid content, as well as perfect grain, and decreased the percentage of imperfect grain. AMRD and SDRD exhibited significantly higher protein, amylose, and lipid contents than the RD treatment. The differences between amylose, protein, and lipid contents are complicated and mainly depend upon genetic background, cultural practices, and milling processes [27,28]. Improvement in grain quality of rice is the key focusing point among breeders to develop cultivars with better quality [29]. The contents of amylose, protein, and lipid are essential elements to define the grain quality of rice [30]. Low amylose content leads the way for stickiness and softness in rice grains while grains with high amylose content enhance brokenness and hardness [31]. High contents of protein, amylose, and lipid reduce the grain taste quality in Japonica rice cultivars but enhance the taste quality for Indica rice cultivars [32,33]. The amounts of protein and lipids are considered the essential elements for the nutritional value in rice grain [34]. Appearance quality such as chalkiness, grain shape, size, and the frequent presence of perfect grains is a key factor for rice consumption and marketing. Hoshikawa [35] categorized rice grain into perfect (grain with normal ripeness) and imperfect (grain with abnormalities and defective shape) grains. Well-developed and perfect grains lead to a higher head rice yield, while a high percentage of broken grain is an undesirable trait and decrease head rice rate [36]. The yield of perfect grain mainly relies upon genetic background and cultural practices, particularly the application of nitrogenous fertilizers [37].

The rice endosperm shows a superb example of the accumulation and formation of starch granules, protein particles, and amyloplasts [38,39]. Starch granules of rice grain are the smallest particles among the grains of cereal crops and differ from 3 to 8  $\mu\text{m}$  in size [40]. The starch granules are a mixed composition of numerous branched amylopectin or linear amylose molecules. These two portions (amylopectin and amylose) of the starch granules bring variation in the formation and composition of starch as well as lead the way for chain length replication of the molecules and its distribution [41,42], which have a fundamental effect on the physical and chemical attributes of starches [43].

Rice grain in RD treatment had negligible protein particles and their pits in contrast to the other treatments. The development of the amyloplasts and starch granules was normal and without air gaps. Amyloplasts were round in shape and the starch granules were compacted within amyloplast. Starch granules were spherical in shape and observed to be without air spaces; this means that the formation and development of the endosperm and its elements were normal. However, AMRD treatment increased the number of protein particles and their pits in rice grain endosperm. The structure of the amyloplasts was observed to be round and somewhat polyhedral with different sizes. Starch granules were polygonal in shape and their edges were sharply defined. Protein particles and their pits were located on the surface and around the amyloplast of SDRD treatment, but the number was small compared to the AMRD and greater than the other treatments. In the rice grain endosperm, protein content occupies approximately 8% of space between amyloplasts and starch granules [35,44]. These protein particles differ in size from 0.5 to 4  $\mu\text{m}$ , are spherically shaped, and are the major form of protein in the endosperm [45,46]. Rice grains with low protein particles in the periphery section are susceptible to breakage during the grain milling process [47]. Nakamura et al. [48] documented that rice grain caryopsis from inorganic fertilizer treatment has many air spaces, unclear cell walls, and round starch granules while caryopsis from organic fertilizer has a distinct cell wall, polygonal starch granules, and fewer air spaces.

Banerjee [19] demonstrated that high chemical inputs increase the influence of the agriculture sector on the ecosystem and decrease the maintenance and sustainability of agricultural practices. Based on previous results, the utilization of manures alone or in combination with other inorganic fertilizers can decrease soil degradation and enhance soil microbes through less application of agrochemicals [49]. The results of this study will help farmers to adopt manures and to reduce the dependence on inorganic fertilizers, consequently decreasing the economic burden on farmers, reduce agricultural inputs, as well as improve paddy rice production. Hence, this experiment provides useful information related to the application of manure and inorganic fertilizers on rice production and grain quality preferences. The results would take part in the strategies to reduce pollution by high utilization of agrochemicals and will be a good reference for fertilizer-producing companies, ecologists, and researchers.

## 5. Conclusions

In modern agriculture, nutrient management and fertilizer application are the most crucial factors affecting plant growth, yield, and quality performances. The results of this study illustrate that the combined application of manure and inorganic fertilizer enhanced tiller number, panicle length, and yield attributes as well as improved physicochemical properties of the rice grain, compared to the RD treatment. Additionally, the formation and accumulated structures of endosperm were without abnormality in the AMRD treatment. Further research should be undertaken to discover new application methods and an appropriate amount of fertilizer application based on crop type, soil properties, and region conditions to prevent environmental problems.

**Author Contributions:** K.K. assumed the idea, conducted the experiment, and wrote the manuscript. T.D.X. supervised and revised the manuscript. Z.N., S.A., and G.G. collected the data, conducted measurements, and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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