Acta Agrobotanica

Article ID: 185310
DOI: 10.5586/aa/185310

Publication History
Received: 2023-12-13
Accepted: 2024-02-24
Published: 2024-05-09

Handling Editor
Agata Konarska; University of Life Sciences in Lublin, Poland; https://orcid.org/0000-0003-2174-7608

Authors' Contributions
TS: Research concept and design; TS, TT, SK, WC, PC, SS, AS: Collection and/or assembly of data; TS, TT, SK, PC: Data analysis and interpretation; TS: Writing the article; TS: Critical revision of the article; TS: Final approval of the article

Funding
This research was partly funded by the Prince of Songkla University, Surat Thani campus (Student research project, 2022).

Competing Interests
No competing interests have been declared.

ORIGINAL RESEARCH

Natural farming negatively influences the growth of Sangyod Muang Phatthalung rice (*Oryza sativa* L.) but not its grain production or quality in preliminary comparison to conventional farming

Theera Srisawat [1*], Tanapa Tarasuk [1], Siriwan Kaosuwan [1], Warapong Chimpud [2], Parinuch Chumkaew [3], Sainiya Samala [4], Apinya Sukolrat [5]

[1] Department of Agricultural Science and Technology, Faculty of Innovative Agriculture and Fisheries, Prince of Songkla University, 31 Moo 6, Muang, Surat Thani 84000, Thailand
[2] Rice Department of Thailand, Phatthalung Rice Seed Center, 244 Moo 15 Thumbol Khuan Maphrao, Muang, Phatthalung 93000, Thailand
[3] Department of Chemistry for Industry, Faculty of Science and Industrial Technology, Prince of Songkla University, 31 Moo 6, Muang, Surat Thani 84000, Thailand
[4] Department of Biology, Faculty of Science, Suratthani Rajabhat University, 272 Moo 9, Muang, Surat Thani 84100, Thailand
[5] Office of Scientific Instrument and Testing, Prince of Songkla University, 15 Hat Yai, Songkhla 90112, Thailand

[*] To whom correspondence should be addressed. Email: theera.s@psu.ac.th

Abstract

Sangyod Muang Phatthalung (SMP) rice is a great source of various nutritional ingredients, but it is only grown in chemical-dependent systems with synthetic fertilizers and insecticides. Farming practices for food security and environmental conservation in the paddy field are highly controversial, but most practices have focused on either chemical-dependent or organic farming. In this study, we compared the growth, yield, quality, phytochemical profiles, and data on grain elements (EDX associated with SEM analysis) of SMP rice along with its production costs when using natural farming (NF) [*Azolla* and leaf compost applied] with those when using conventional farming (CF) [chemical fertilizer use (18-8-8 and 46-0-0) and high dose fertilizer (HF) as recommended by the supplier (18-4-5, 20-8-20, and 15-15-15)]. Our data show negative results in terms of plant growth and positive results in terms of yield, physical grain qualities, chemical grain qualities, and chemical composition for SMP rice grown under a natural farming system. A total of six important inorganic elements were present in the kernel (C and O) and the aleurone layer (P, K, Mg, and S) of rice grain. This study claims a high performance for natural farming, based on rice yield and quality with decreased production costs and a possible increase in the market price of high-value rice. These findings have significant implications for applying natural farming practices in the context of food security and environmental conservation, without risk to farmers' finances and health or the agroecosystem.

Keywords

indigenous rice; natural practice; SEM-EDX; Sangyod

1. Introduction

Rice is a major energy source to 65% of the people in the world (Bharali et al., 2021), and it is mainly derived from paddy lands in most of Asia, especially in Southeast Asia, which is the dominant rice producing area globally (Yuan et al., 2022). Although Thai commercial Jasmine rice has been considered the top-quality rice in the world (Rahman et al., 2009), the Thai rice consumer has increasingly emphasized traditional rice varieties because the current rice farming practices excessively rely on chemical fertilizers and pesticides that pose a significant threat to consumer health and an environmental risk (Nguyen et al., 2022). By the conventional practices of rice production, the average yield of a high-yield variety is up to 500 kg/rai or 3,125 kg ha^{-1} (Rahman et al., 2009) (rai is the Thai land property unit, equal to 0.16 hectares). In contrast, the yield of a traditional rice variety is still very low (Boualaphanh et al., 2011; Sinha & Mishra, 2015).

Sangyod Muang Phatthalung (SMP) rice is a traditional rice variety grown in the lowland paddy fields of southern Thailand, especially in Nakhon Si Thammarat, Songkhla, and Phatthalung provinces, which are the main SMP rice producing areas. The SMP grain yield is relatively very low because this native species with an average yield of 64–2,500 kg ha^{-1} relies on the area and the type of standard (Petruang & Napasintuwong, 2022; Tongkaemkaew et al., 2022). Although SMP rice has been among the top five rice types popular in Southeast Asia (Tasteatlas, 2021), being a brown rice with high levels of iron, vitamin B, niacin, and anthocyanin antioxidants (Banchuen et al., 2009), with an increasingly popular demand, the SMP rice production still encounters problems associated with climate change, decrease in paddy fields, and overuse of chemical fertilizers and pesticides (Duong et al., 2023; Irawan & Antriyandart, 2021; Pame et al., 2023; Phitthayaphinant & Tongkaemkaew, 2018; Taratima et al., 2023). With the overuse of chemical fertilizers, the productivity of SMP rice can be increased to 2,187–2,375 kg ha^{-1} (Phattanavibak, 2012). This practice has expanded dramatically, while increasing greenhouse gas emissions and decreasing soil fertility (Irawan & Antriyandart, 2021). On the other hand, to avoid the effects of synthetic chemicals, organic farming has been promoted as an alternative way of rice production with potential to reduce water consumption and being a fit with the context of sustainable agriculture in its economic, social, and environmental aspects (de Carvalho et al., 2022; Johannes et al., 2019). Most organic paddy field practices have been reported as having rice-duck-*Azolla* and fish integration (Baigi et al., 2013; Cagauan et al., 2000; Chisembe et al., 2020; Safriyani et al., 2020).

Although the organic farming practices in rice production have been claimed to potentially meet the requirements of sustainable agriculture, organic farming can suffer from a crop yield decrease (Ponisio et al., 2015), and a failure in transition to organic agriculture occurred recently in Sri Lanka inducing an economic crisis and a local famine (Duddigan et al., 2023). Natural farming, the original pattern of organic practice (Miyake & Kohsaka, 2020), emphasizes an agroecosystem approach that follows the ways of nature in farming, and this is believed to be an effective way to address all of these problems (Kumar et al., 2023). Many positive results from natural farming practices have been recently reported as regards the yield, soil quality, soil organism abundance, and biodiversity in agroecosystems (Duddigan et al., 2023; Lin et al., 2021). The agroecosystem concept is believed to have been initiated by two Japanese philosophers named Mokichi Okada and Masanobu Fukuoka, following only a set of practices in natural ways (Miyake & Kohsaka, 2020): synthetic chemicals, raw waste from animals, and insect and weed control substances are prohibited, while only leaf compost and green manure are recommended to promote the natural ecosystem in agricultural lands (Xu, 2008). Moreover, UNEP (2022) recommended that workers (farmers) should practice protection, conservation, restoration, and sustainable use to manage natural terrestrial, freshwater, and agricultural ecosystems for addressing sustainable development. Therefore, in the context of sustainable grain production, a transformation of rice farming practices from conventional to natural farming is necessary, and additional market development for safe and healthy rice products is needed (Erisman et al., 2017).

Nevertheless, there is a lack of evaluated data supporting natural farming in paddy fields, especially as regards successful sustainable agriculture of SMP rice without livestock integration. Thus, this study sought to answer the following core question: How are the natural farming inputs helping the growth, yield, quality, and phytochemical and elemental composition measures of SMP rice grain? The present study is based on an outdoor experiment conducted in an LDPE-pond plot where the adoption of inputs in each plot is believed to directly affect and not interact with outside factors.

2. Material and methods

2.1. Grain materials and seedling preparation

Grains of Sangyod Muang Phatthalung (SMP) rice (code-KGTC82239-2) were officially prepared at the Phatthalung Rice Seed Center, the Rice Department of Thailand, Muang district, Phatthalung province. All grains were soaked in sterile water for 72 h before selecting germinated grains. After that, the germinated grains were spread on a silty clay loam tray for seedling induction. Fourteen days after sowing, the seedlings were transplanted into the sub-plot of each experiment that was filled with silty clay loam, with the distance of planting set at 20 × 20 cm and a depth of 3 cm. The silty clay loam was derived from topsoil in the natural lowland area of Chaiya district (the main rice production area in Surat Thani province).

2.2. Plot

This work was conducted in the farm area of Prince of Songkla University, Surat Thani campus. The plot was prepared by turning the soil, and it was wrapped with a 200 μm LDPE black sheet. The preparation of the experimental plot with three replications per treatment was performed for 30 days before planting, with a plot size of 2.0 × 1.0 × 0.9 m (L × W × H) each, with the distance between plots of 0.5 m. The total area of each treatment was 6.0 m^2. The silk clay loam in each plot was plowed, mixed, and leveled (45 cm depth) before being irrigated (shallow water level at 10 cm was maintained).

2.3. *Azolla* and leaf compost preparation

The distribution of 100 g *Azolla* per m^2 was conducted in a pond for 4 weeks before transfer to a natural treatment plot. For leaf compost preparation, dried leaves on the ground from a natural tree were collected from around the university and accumulated in a 3.0 × 3.0 m steel mesh storage tank. The leaves were exposed to sunlight and rain for three months before moving to a new place covered with a 2.5 m high roof. The soft black-colored leaves were removed from sunlight and any water for 5 months, and within this time period, the decomposition of the leaves was facilitated by turning over once a month until fine black particles formed. The leaf compost was sieved through a 0.5 cm mesh-size basket before use. The physical and chemical characteristics of the obtained leaf compost are summarized in Table 1.

2.4. Experimental design

This research used a Randomized Completely Block Design (RCBD) with four treatments: (1) control treatment (without any input applied), (2) high fertilizer practice (HF; 18-4-5, 20-8-20, and 15-15-15 applied for 156.25, 125, and 62.5 kg ha^{-1}, respectively), (3) conventional practice (CF; 18-8-8 and 46-0-0 applied for 187.5 and 93.75 kg ha^{-1}, respectively), and (4) natural farming practice (NF; *Azolla* applied for 625 kg ha^{-1} and leaf compost applied for 25,000 kg ha^{-1}). The doses and times of fertilizer application and day (s) of practice are listed in Table 2.

2.5. Growth and yield measurements

The variables determined the indicated plant growth parameters [plant height, number of leaves, leaf length, leaf width, number of tillers/hill, dry straw weight (g), and leaf chlorophyll content (observed using a SPAD 502 chlorophyll meter 70 days

Table 1 Physical and chemical characteristics of the leaf compost*.

Analyte	Unit	Value
Organic carbon	Percent	60.68
C/N ratio	-	11
EC	decisemens (dS/m)	4.52
pH	-	6.35
Nitrogen	Percent	3.20
Phosphorus	Percent	0.91
Potassium	Percent	2.31
Sodium	Percent	0.07
Moisture	Percent	45.86

* Leaf compost analysis was done by the Surat Thani Land Development Regional Office 11 using standard certified methods.

Table 2 Fertilizer practice in frequency and dosing by treatment group.

Practice of treatment	Day(s) of inputs applied	Type of inputs (N-P-K)	kg ha^{-1}	g $plot^{-1}$
Conventional farming (CF)	30th	18-8-8	187.5	37.5
	60th	18-8-8	187.5	37.5
	105th	46-0-0	93.75	18.75
High fertilizer (HF)	25th	18-4-5	156.25	31.25
	45th	18-4-5	156.25	31.25
	60th	20-8-20	125	25.0
	105th	15-15-15	62.5	12.5
Natural farming (NF)	7 before planting	*Azolla*	625	125
	7 before planting	Leaf compost	25,000	5,000
	60th	Leaf compost	25,000	5,000
	105th	Leaf compost	25,000	5,000

after planting)], yield components [number of panicles/hill, number of grains/panicle, number of full and empty grains/panicle, number of grains/hill, and yield (kg)/plot and hectare (ha)], and grain physical properties [100-grain weight, grain length, paddy color, brown rice color, and germination score]. These were assessed following the methods specified in the Standards of Thai Fragrant Rice (Rice Department of Thailand). The grain chemical/functional properties were determined via an analysis of anthocyanin (Ranganna, 1986), amylose content (Juliano, 1971), cooking time, elongation ratio, water uptake ratio and volume expansion ratio (Mohapatra & Bal, 2006), gel consistency (Verma et al., 2015), alkali spreading value and gelatinization temperature (Kongseree & Juliano, 1972), and proline test (Bates et al., 1973) following standardized measurements.

2.6. Phytochemical analysis

For testing the phytochemical constituents in SMP grains, the chemical components, i.e. alkaloids, flavonoids, phenols, steroids, terpenoids, glycosides, cardiac glycosides, saponins, tannins, and anthraquinones, were determined following methods described previously (Chumkaew & Srisawat, 2014; Kamba & Hassan, 2010).

2.7. Analysis of grain elements using Energy Dispersive X-ray (EDX) analysis associated with Scanning Electron Microscopy (SEM)

The morphology and elemental composition of each SMP grain sample were characterized using a SEM coupled with an EDX system. To detect the presence of particular elements present in the SMP grain, the EDX analysis was performed with an Oxford Instruments Xmax80 detector analyzing the presence of carbon (C), oxygen (O),

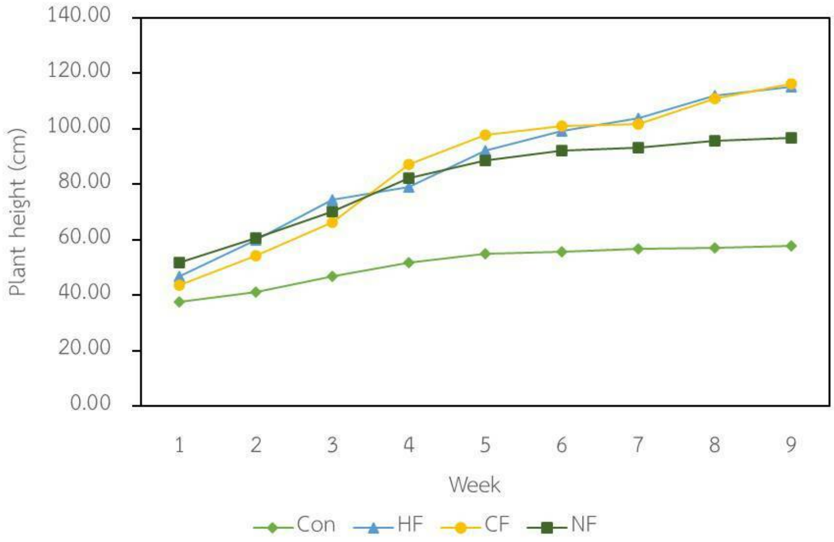

Figure 1 SMP rice plant development in height evaluated for 9 weeks before the booting stage of development - Control (**Con**), High fertilizer (**HF**), Conventional fertilizer (**CF**), and Natural farming (**NF**) treatments.

phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S) in the SMP grains. The SEM imaging was done with Quanta 400 (The Czech Republic) with microscope control software and a mode of operation having a voltage of 20 kV. The brown seeds of SMP rice were gently transversely fractured by hand. The central part and the border of a transversely cracked kernel were mounted on aluminum stubs with a carbon tape and coated with an approximately 150 Å thick gold layer before observation under SEM.

2.8. Statistical analysis

The data of the growth and yield parameters and the grain physical and chemical qualities were subjected to one-way ANOVA using SPSS 11 software (IBM Corp., Armonk, New York, USA). When the farming practice effects occurred, Duncan's Multiple Range Test (DMRT) was determined for significant differences at a 5% probability.

3. Results

3.1. Growth and yield of Sangyod Muang Phatthalung (SMP) rice

The application of the chemical fertilizer had positively affected the plant growth at 77 days after planting, whereas the application of *Azolla* and the leaf compost had low but statistically significant effects (Table 3 and Figure 1). The CF treatment produced the highest averages of plant height, number of tillers/hill, dry weight of straw, and leaf chlorophyll content, followed by the HF treatment. Similarly, the CF treatment produced the highest average yield at 110 days after planting. The high number of panicles and rice grains in the CF treatment resulted in the highest yield in kg ha^{-1} (2,082.81 kg ha^{-1}), followed by the NF treatment (1,885.69 kg ha^{-1}) (Table 4 and Figure 2). The further increase in the chemical fertilizer dose did not increase the SMP grain yield, but increased the leaf length when applied in the high-dose fertilizer treatment (HF-treatment).

3.2. Grain physical qualities

The positive effects of *Azolla* and the leaf compost on grain quality were reflected in the increases in grain weight and grain length (Table 5). The largest 100-grain weight and the longest grain were found in the CF treatment. The paddy color did

Table 3 Growth parameters of Sangyod Muang Phatthalung rice grown in conventional and natural farming systems (Mean ± SD).

Practice	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	No. of tillers/hill	Dry straw weight (g)	Leaf chlorophyll content
Control	57.83 ± 8.12^{c}	13.33 ± 5.86^{c}	44.61 ± 5.51^{c}	0.70 ± 0.03^{c}	2.33 ± 1.53^{b}	3.94 ± 0.85^{b}	15.86 ± 1.87^{c}
HF	115.04 ± 1.88^{a}	56.67 ± 5.03^{a}	80.79 ± 1.64^{a}	1.22 ± 0.02^{a}	11.00 ± 1.73^{a}	38.17 ± 8.31^{a}	23.73 ± 1.55^{b}
CF	116.40 ± 3.13^{a}	65.67 ± 8.14^{a}	77.57 ± 1.32^{a}	1.28 ± 0.02^{a}	12.33 ± 2.52^{a}	51.06 ± 10.49^{a}	28.28 ± 2.43^{a}
NF	96.87 ± 3.80^{b}	40.67 ± 10.97^{b}	68.33 ± 2.93^{b}	1.06 ± 0.08^{b}	9.00 ± 5.29^{a}	33.89 ± 16.69^{a}	25.49 ± 1.87^{ab}
p-value	0.000	0.000	0.000	0.000	0.019	0.004	0.000

HF and CF are conventional farming systems using a high-dose fertilizer and a conventional dose of chemical fertilizer, respectively, whereas NF means natural farming. In each column, means followed by the same superscript are not significantly different at the 5% level of DMRT.

Table 4 Yield parameters of Sangyod Muang Phatthalung rice grown in conventional and natural farming systems (Mean ± SD).

Practice	No. of panicles/hill	No. of grains/panicle	Full grains/panicle	Empty grains/panicle	No. of grains/hill	Yield (g) plot^{-1}	Yield (kg) ha^{-1}
Control	2.33 ± 0.58^{b}	168.67 ± 9.29^{b}	163.67 ± 10.02^{b}	5.33 ± 0.58	339.33 ± 75.84^{b}	185.73 ± 24.74^{c}	928.69 ± 123.69^{c}
HF	6.33 ± 0.58^{a}	224.33 ± 24.91^{a}	213.67 ± 21.13^{a}	10.67 ± 3.79	1458.33 ± 351.76^{a}	296.87 ± 26.21^{b}	1484.31 ± 131.06^{b}
CF	7.67 ± 1.15^{a}	231.00 ± 17.09^{a}	218.67 ± 16.62^{a}	12.33 ± 0.58	1824.00 ± 268.74^{a}	416.57 ± 16.52^{a}	2082.81 ± 82.56^{a}
NF	6.67 ± 3.06^{a}	217.00 ± 10.82^{a}	198.33 ± 15.95^{a}	18.67 ± 8.96	1441.67 ± 771.64^{a}	377.13 ± 28.52^{a}	1885.69 ± 142.63^{a}
p-value	0.021	0.007	0.0013	ns	0.017	0.000	0.000

HF and CF are conventional farming systems using a high-dose fertilizer and a conventional dose of chemical fertilizer, respectively, whereas NF means natural farming. In each column, means followed by the same superscript are not significantly different at the 5% level of DMRT.

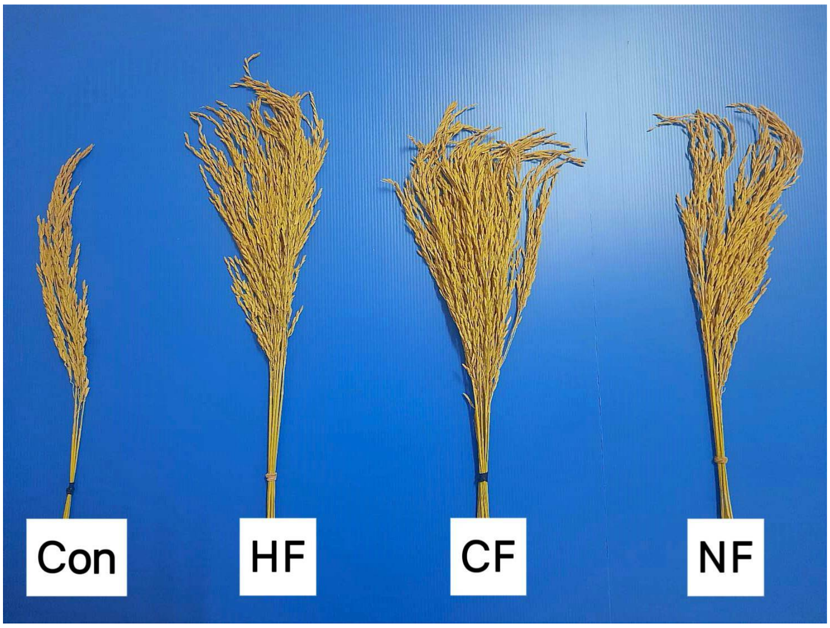

Figure 2 Subjectively clear differences in panicles of rice/hill between the Control (**Con**), High fertilizer (**HF**), Conventional fertilizer (**CF**), and Natural farming (**NF**) treatments of the SMP rice variety.

Table 5 Grain physical qualities of Sangyod Muang Phatthalung rice grown in conventional and natural farming systems (Mean ± SD).

Practice	100-grain weight (g)	Grain length (mm)	Paddy color (score)	Brown rice color (score)	Germination (score)
Control	1.30 ± 0.01^{c}	6.07 ± 0.26^{c}	1.88 ± 1.00	2.96 ± 0.70^{c}	0.33 ± 0.58^{b}
HF	1.32 ± 0.00^{bc}	6.17 ± 0.26^{b}	2.16 ± 1.06	3.12 ± 0.90^{bc}	3.00 ± 1.00^{a}
CF	1.38 ± 0.01^{a}	6.28 ± 0.26^{a}	2.22 ± 1.20	3.38 ± 0.64^{b}	0.33 ± 0.58^{b}
NF	1.34 ± 0.02^{b}	6.18 ± 0.26^{b}	1.92 ± 0.94	3.76 ± 0.89^{a}	1.67 ± 0.58^{b}
p-value	0.000	0.001	ns	0.000	0.005

HF and CF are conventional farming systems using a high-dose fertilizer and a conventional dose of chemical fertilizer, respectively, whereas NF means natural farming. In each column, means followed by the same superscript are not significantly different at the 5% level of DMRT.

not differ significantly between the treatments, but the highest score of brown color was recorded in the NF treatment (Figure 3).

3.3. Grain chemical quality

Table 6 presents a summary of the positive effects on grain chemical characteristics in the NF treatment. The natural farming of SMP rice required a suitable integration between *Azolla* and the leaf compost for a high level of rice grain quality measures. The highest anthocyanin and amylose contents were found in the grains from the NF treatment. More than 2 mm of elongation was achieved when SMP grains were produced with the chemical-free treatment. The gel consistency and alkali spreading of SMP grains were positively influenced when the rice was grown in the NF treatment (Figure 4).

3.4. Phytochemical analysis

The phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, steroids, terpenoids, cardiac glycosides, and anthraquinones in the SMP rice grain. The NF-treated grain was comparatively richer in phytochemicals (flavonoids, phenols, terpenoids, cardiac glycosides, and anthraquinones) than those with the use of the chemicals. Interestingly, flavonoids and anthraquinones were dominant in the NF treatment only, while they were not detected in CF and HF (Table 7).

Table 6 Grain chemical quality measures of Sangyod Muang Phatthalung rice grown in conventional and natural farming systems.

Practice	Anthocyanin (g/100 mL)*	Amylose (%)	Cooking time (min)	Elongation ratio (mm)		Water uptake ratio (g)	Volume expansion ratio (cm)		Gel Consistency (cm)	Alkali Spreading (score)	Gelatinization temperature (°C)	Proline (OD_{520})
				Before	After		Before	After				
Con	0.05 ± 0.02^{b}	3.08 ± 0.00	29.67 ± 0.58^{d}	6.01 ± 0.11	8.26 ± 0.24	4.20 ± 0.24	1.33 ± 0.06	3.70 ± 0.10	0.93 ± 0.67^{ab}	3.17 ± 0.21^{b}	>74	0.04 ± 0.00
HF	0.22 ± 0.03^{a}	3.09 ± 0.00	25.00 ± 1.00^{b}	6.06 ± 0.07	7.84 ± 0.85	4.54 ± 0.28	1.40 ± 0.10	4.10 ± 0.30	1.10 ± 0.66^{ab}	3.40 ± 0.26^{ab}	>74	0.06 ± 0.02
CF	0.19 ± 0.04^{a}	3.09 ± 0.01	26.67 ± 0.58^{c}	6.22 ± 0.08	7.88 ± 0.26	4.34 ± 0.19	1.40 ± 0.10	3.90 ± 0.26	0.37 ± 0.35^{b}	3.63 ± 0.06^{a}	>74	0.05 ± 0.01
NF	0.25 ± 0.02^{a}	3.10 ± 0.01	19.33 ± 0.58^{a}	6.10 ± 0.11	8.21 ± 0.65	4.65 ± 0.34	1.43 ± 0.06	4.30 ± 0.52	1.97 ± 0.35^{a}	3.63 ± 0.12^{a}	>74	0.05 ± 0.00
p-value	0.000	ns	0.000	-	ns	ns	-	ns	0.036	0.037	ns	ns

* 1.0% HCl in methyl alcohol; Control (Con), High fertilizer (HF), Conventional fertilizer (CF), and Natural farming (NF) treatments. In each column, means followed by the same superscript are not significantly different at the 5% level of DMRT.

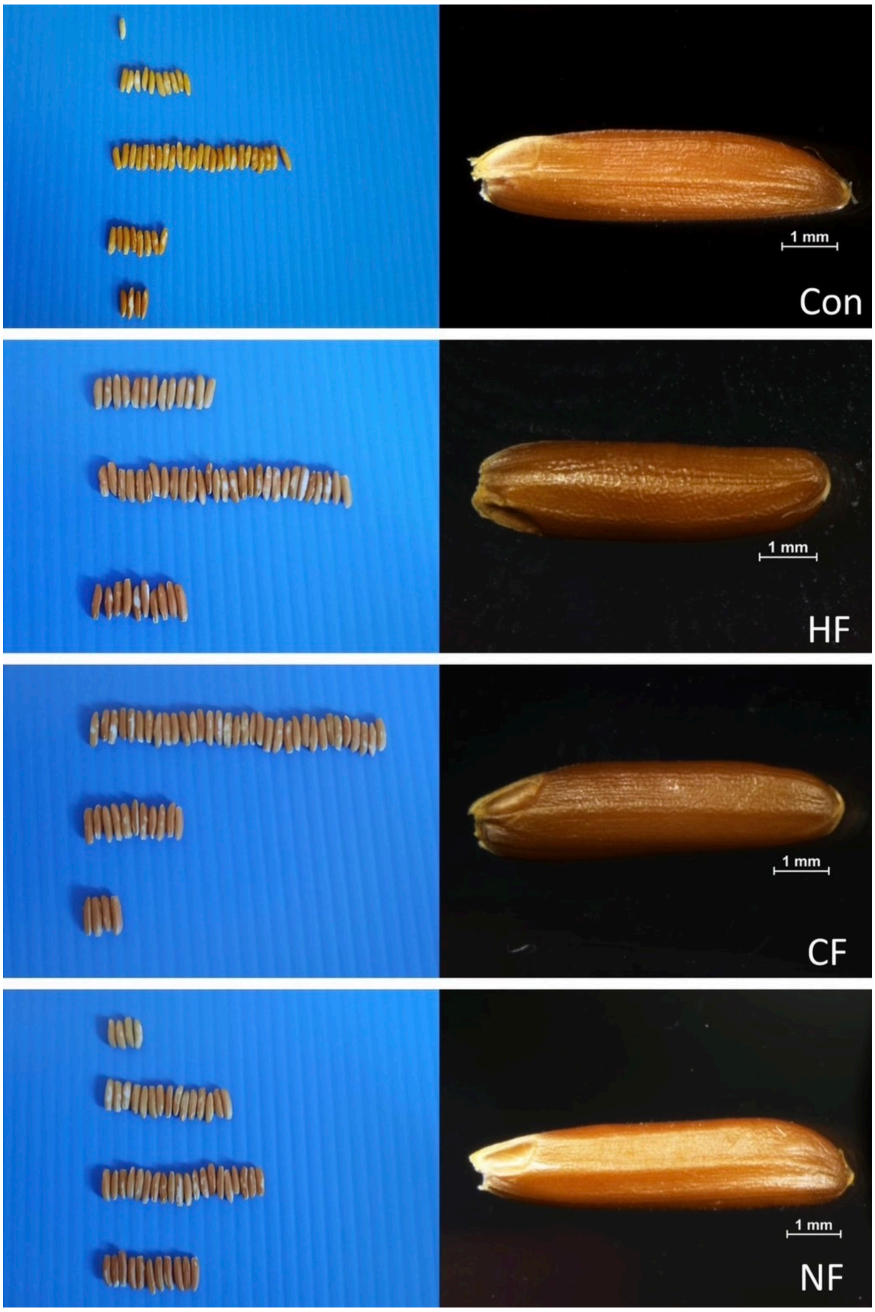

Figure 3 Brown rice scores of SMP rice from the Control (**Con**), High fertilizer (**HF**), Conventional fertilizer (**CF**), and Natural farming (**NF**) treatments.

3.5. Analysis of grain elements using Energy Dispersive X-ray (EDX) analysis associated with Scanning Electron Microscopy (SEM)

As shown by the SEM observations, the SMP grain had a one-cell aleurone layer with a uniform shape of cells that were fully filled by aleurone grains (Figure 5). Across all types of farming, no variation of the aleurone layer was observed. However, the nucellus of the SMP grain from the NF treatment had the highest thickness, being slightly thicker than that from the other types of farming (data not shown). Starch granules were visible singly and as clumped compound granules, and were polygonal in shape. Most starch granules were found in the center of the grain endosperm, giving the appearance of chalkiness. Some spherical granules seen between the starch granules may have been protein bodies. The spectrum and map of elemental compositions in the SMP grain are shown in Figure 6 and Figure 7, respectively. The dominant component in the SMP grains was carbon followed by oxygen, contributing 60–62% and

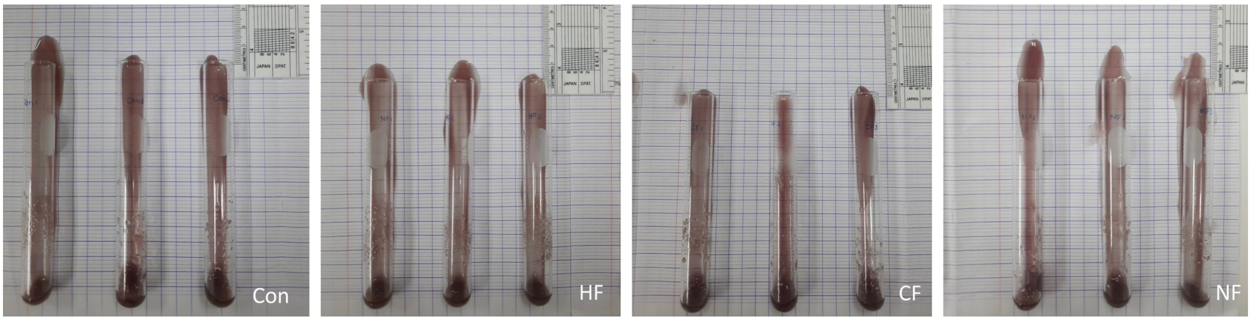

Figure 4 Gel consistency of the SMP rice samples evaluated from gel flow on paper. Shelly SMP rice with hard gel, medium gel, and soft gel consistency were displayed by gel length as follows: <0.5 cm, 0.5–1.0 cm and >1.0 cm, respectively. Control (**Con**), High fertilizer (**HF**), Conventional fertilizer (**CF**), and Natural farming (**NF**) treatments.

Table 7 Phytochemical constituents screened in Sangyod Muang Phatthalung (SMP) rice grain grown in high fertilizer, conventional, and natural farming systems.

Phytochemical type	Type of farming practiced		
	High Fertilizer Farming	Conventional Farming	Natural Farming
Alkaloids	+	—	—
Flavonoids	—	—	+
Phenols	—	+	+
Steroids	—	+	—
Terpenoids	+	+	+
Glycosides	—	—	—
Cardiac glycosides	+	—	+
Saponins	—	—	—
Tannins	—	—	—
Anthraquinones	—	—	+

+ indicates the presence of the phytochemical; — indicates its absence (i.e., not detected).

36–38% by weight, respectively, dispersed around the kernel. All the farmed grains also contained small percentages of phosphorus, potassium, magnesium, and sulfur accumulated at the aleurone layer. Small percentages of silicone (Si) and aluminum (Al) were only seen in the SMP grain produced in the HF treatment, which was sprayed at the vegetative stage. Interestingly, the highest percentage of carbon was found in the SMP grain from the NF treatment (62.2%), whereas high oxygen content (approximately 38% by weight) was recorded in cases from the chemical fertilizer farming (HF and CF).

4. Discussion

Although the results of variance analysis indicated a negative effect of interaction from the treatments with *Azolla* and the leaf compost on the growth of the SMP rice plants, beneficial impacts of both inputs on promoting SMP rice grain yield and quality were demonstrated. Rice grain yield per unit area is the most important indicator for evaluating the effects of different rice farming practices (Yang et al., 2021). The NF treatment in this study, which consisted in application of 625 kg ha^{-1} *Azolla* and 25,000 kg ha^{-1} leaf compost, enhanced the rice grain yield (1,885 kg ha^{-1}) and its quality (highest anthocyanin and amylose contents, and detected flavonoids and anthraquinones). This might be due to the increase in the organic carbon (60.68% w/w) and the N-P-K proportions in the leaf compost, integrated with the *Azolla*-nutrition content. Moreover, the application of a biofertilizers, such as leaf compost (high nutrient content as shown in Table 1), is the newest and most technically advanced way of supplying mineral nutrients that improve the phytochemical constituent activity and enhance the content of antioxidants in many plant species (Ibrahim et al., 2013; Manikandan

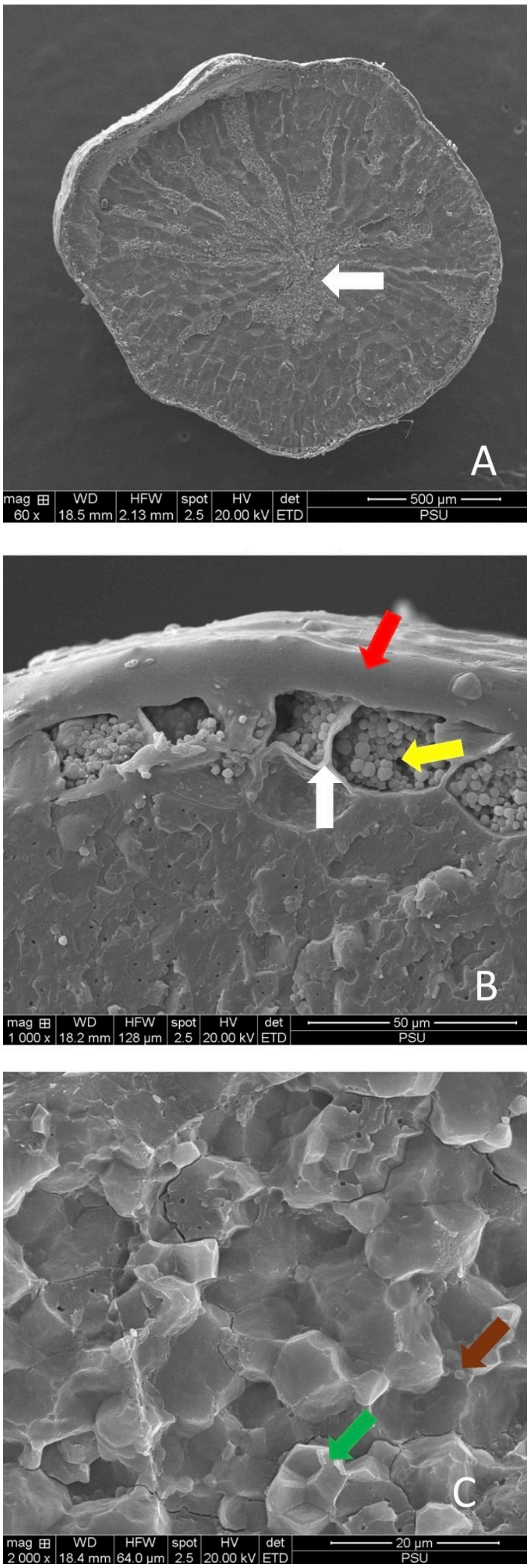

Figure 5 (**A**) SEM micrograph of a transverse-fractured field of SMP rice grain; the arrow indicates chalkiness, which is a starch granule-rich zone; (**B**) at the ventral side of SMP rice grain, one cell of the aleurone layer wall of cell parenchyma (white arrow), aleurone grain (yellow arrow), and thick nucellar tissue (red arrow); (**C**) center of the endosperm of SMP grain showing a polygonal form of starch (green arrow) and a few spherical protein bodies (brown arrow).

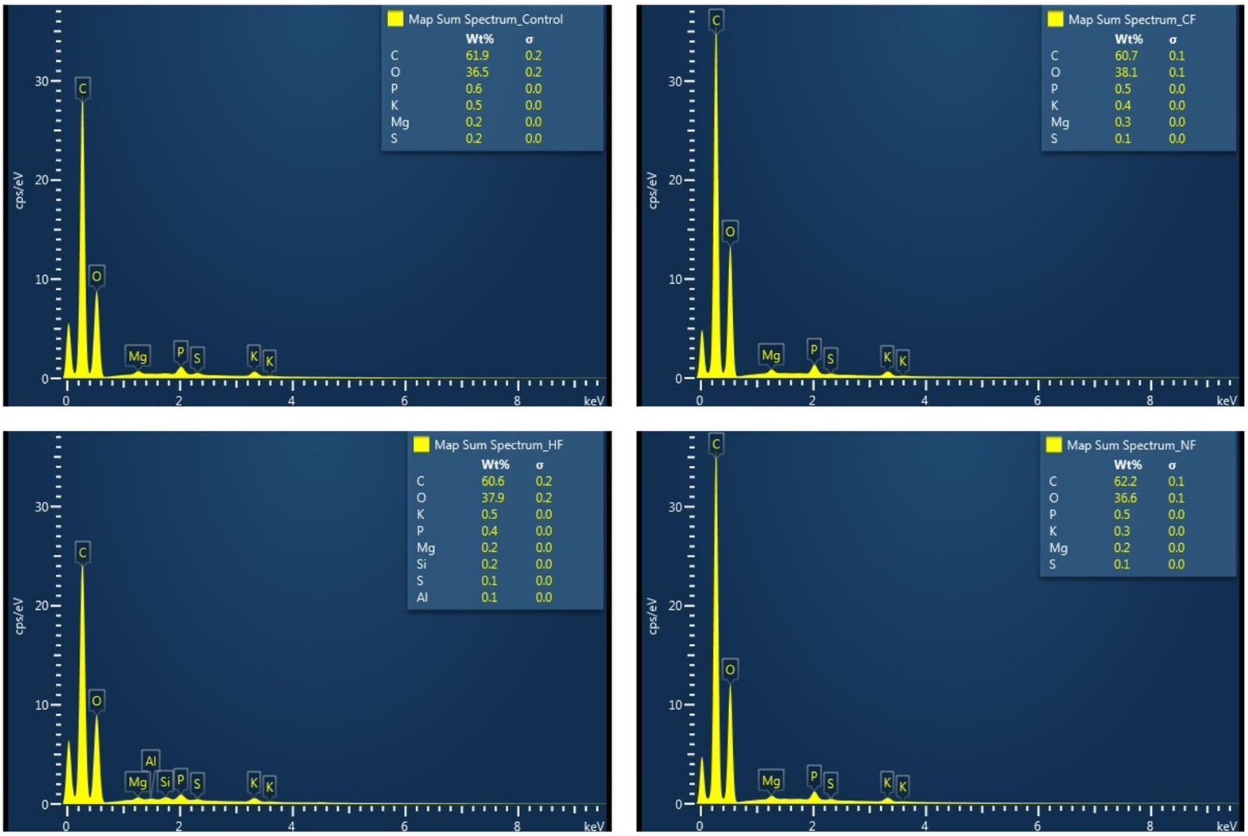

Figure 6 EDX analysis of SMP rice grains from different farming practices. Control, High fertilizer (**HF**), Conventional fertilizer (**CF**), and Natural farming (**NF**) treatments.

& Thamizhiniyan, 2016; Salehi et al., 2019). In previous work, the application of *Azolla* significantly affected the growth and production of rice plants (Safriyani et al., 2020), because it increased nitrogen availability in paddy fields. The nitrogen content in *Azolla* varies from 0.63% to 4.0% (wet weight) (Bhaskaran & Kannapan, 2015) and is highly correlated with its phosphorus content (0.45%) (Watanabe & Ramirez, 1990). The application of only *Azolla* without livestock integration was not able to provide the nitrogen required by rice plants in paddy fields, causing rice plant growth to be reduced. Thus, previous studies have indicated that the integrated use of livestock, such as duck or fish, with *Azolla* cover markedly improved rice growth and production for many varieties (Baigi et al., 2013; Cagauan et al., 2000; Chisembe et al., 2020; Safriyani et al., 2020).

Regarding organic practices in rice cultivation, the application of organic fertilizers to soil can rapidly increase the availability of nutrients and nitrogen as macro-elements, which are needed by rice plants for growth and grain production (Safriyani et al., 2020). Although nitrogen availability plays a leading role in increasing rice growth and grain yields, providing up to 80% of the yield increase (Tumanian et al., 2020), the SMP rice plants in the NF treatment showed lower growth and higher yield than those obtained from conventional farming (with a high dose of the 46-0-0 formula). This may be due to an appropriate dose of nutrient elements in the NF treated soil compared to a high dose (overuse) of the fertilizer in the chemical treatments. Interestingly, the removal of chemical toxins, such as methane (CH_4), from the soil in organic paddy farming can significantly improve soil quality (Safriyani et al., 2020). On the other hand, the biofertilizer has positive impacts on soil and water quality and biodiversity, contributing to the growth and productivity of terrestrial crops (Abdou et al., 2023; Omara et al., 2022). Thus, we conserved natural biodiversity in the distance between the plots in a natural way for domesticated weeds and pest-controlling species and in the plot with soil animals and soil microorganisms living in the leaf compost to complete the relationship in the agroecosystem (Bambaradeniya & Amarasinghe, 2003). Unfortunately, little is known about the impacts of leaf compost on rice growth

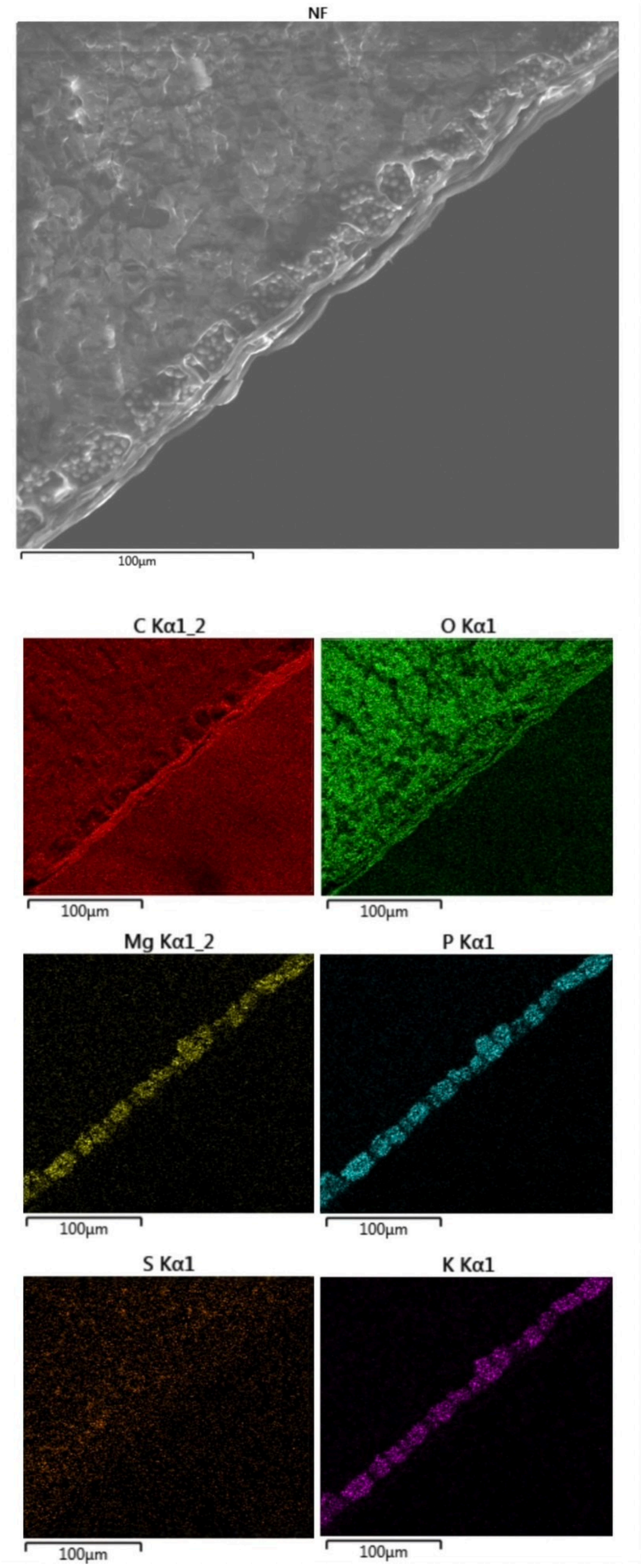

Figure 7 SEM and EDX/map analysis of transverse sections of SMP rice grains derived from natural farming (**NF**). (**A**) SEM image of the border of a SMP kernel showing the aleurone layer with aleurone grains. (**B–G**) EDX maps for carbon (**C**), oxygen (**O**), magnesium (**Mg**), phosphorus (**P**), sulfur (**H**), and potassium (**K**).

and grain production in paddy fields. Hawlader et al. (2020) only reported the positive influence of *Cassava* leaf compost on the growth and yield of rice caused by the upregulation of nutrient uptake by rice plants. Thus, this is the first report on leaf compost application in paddy farming and represents a new challenge to sustainable agriculture for high-quality SMP rice grain production.

Pigment accumulation in *Azolla* can be induced by biotic and abiotic stresses, such as pathogen attack, UV-B radiation, or low temperature (Dai et al., 2012). Application of *Azolla* with high content of anthocyanin to paddy fields was found to induce a high level of anthocyanin content in black rice grain (Sulandjari & Yunindanova, 2018). On the other hand, leaf compost induced anthocyanin in many fruit species when the dose of leaf compost increases (Pinto-Morales et al., 2022), whereas an increase in the level of nitrogen nutrition in the plot decreased the amylose content in rice grain (Barus et al., 2022; Tumanian et al., 2020). Bahmaniar and Ranjbar (2007) reported that the maximum amylose content was achieved when using none of the fertilizers or no nitrogen fertilizer. Regarding the endosperm morphology of SMP grains, the endosperm had a single-cell layer covering the grain throughout, with cubic shaped aleurone cells. It may differ from one to five layers, depending on the position being either dorsal, ventral, or lateral in other rice varieties (An et al., 2020; Kasem et al., 2011). On the other hand, as shown by the EDX analysis, the main elements were carbon and oxygen, which were mapped in the full kernel tissue of rice grain and may have contributed to forming the starch granules in a central area of chalkiness. Accordingly, the results of the high amylose content in the SMF rice grain from the NF treatment might be due to the high level of organic carbon in the leaf compost, which in turn provided high carbon content in the kernel tissue. As mentioned, it might be concluded that the natural farming increases the percentage of carbon in SMP rice grains but reduces the amount of other inorganic elements in the kernel, which mostly come from the fertilizer (excepted for P, which may come from both the leaf compost and the fertilizer). Interestingly, the characteristic X-ray dispersion generated by ionization of Mg, P, S, and K was mapped only in the aleurone layer, indicating a high level of accumulation of these elements at the protein bodies of the aleurone cells, especially in the chemical treatment. Aleurone cells have a high nutritional value with various antioxidant compounds (Gubatz & Weschke, 2014); thus, accumulation of the highest levels of inorganic elements from a chemical fertilizer may affect the type and amount of phytochemical constituents in brown rice (Ma et al., 2023). In studies on agriculture, the EDX is an important tool to detect elements (generally used to improve the performance of major elements and microelements used to promote the growth and quality of an agricultural product), and this is the first report exploring the elemental composition of rice grains as affected by the choice of farming practices.

Therefore, the high dose of chemical fertilizers in the CF and HF treatments gave no significant improvements in grain yield and quality, compared to the use of natural fertilizers only. The application of chemical fertilizers (in the CF and HF treatments) and the related daily farming practices increased the approximate costs of production by up to 446.43–535.71 USD ha^{-1} compared to the NF treatment (Zero USD ha^{-1}). Currently, the exchange rate of USD is about 1 USD per 35 baht (December 2023). We thus conclude that the increases in SMP grain yield and quality can just be attributed to the leaf compost and *Azolla* application, at least as regards anthocyanin and amylose contents that tended to increase under the balancing of energy flow and material cycles in the agroecosystem of the paddy field.

5. Conclusion

In summary, as a preliminary finding, the integration of *Azolla* and the leaf compost in the SMP paddy farming had positive effects encouraging further development of natural farming in rice production based on grain yield and quality. The chemical fertilizer increased the plant growth, but decreased the SMP grain production and quality. Natural farming that utilizes *Azolla* and composted leaf materials should be considered as a promising alternative towards sustainable agriculture, especially in rice farming.

Acknowledgments

Special thanks to Dr. Seppo Juhani Karrila for assistance during manuscript preparation, the Phatthalung Rice Seed Center for supplying the official rice grain variety for the research, and the Surat Thani Land Development Regional Office 11 for the analysis of the leaf compost composition.

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