Anthocyanin content, phenolic content, antioxidant activity and yield in indigenous rice as affected by different fertilizers

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Abstract. Prajuntasan R, Ratananikom K, Payoungkiattikun W. 2022. Anthocyanin content, phenolic content, antioxidant activity and yield in indigenous rice as affected by different fertilizers. Biodiversitas 23: 4195-4201. This study was to evaluate the effects of fertilizer management methods (organic and inorganic) on total anthocyanin content (TAC), total phenolic content (TPC), antioxidant activity, and yield in eight local rice varieties. A 2×8 factorial experiment in a randomized complete block design with three replications was set up on a farmer’s farm. Organic fertilizer management increased TAC, TPC and antioxidant activity, but significant increases were found for TAC, and antioxidant activity. The highest TAC was found in Luem Puew, and Kum Puen Baan had the highest TPC. The highest TAC and TPC varieties did not necessarily have the highest antioxidant activity. Sung Yod, Chaow Daeng, Kum Ka Dum and Kum Puen Baan were the highest for this parameter. Luem Puew + organic was the treatment combined with the highest TAC. Still, the result found the highest antioxidant activities in several treatment combinations associated with most local rice varieties except Nang Nuan and Mali Nga Chang.

Keywords: Health products, high yielding varieties, local rice varieties, organic farming, rice production

INTRODUCTION

Thailand is rich in local rice varieties. More than 10,000 local rice varieties had cultivated throughout the country. However, the number of local rice varieties has drastically reduced since the new high-yielding and more palatable varieties have been introduced into cultivation. Local rice is a source of many agronomically important traits such as drought resistance (Pimratch et al. 2019), eating quality (Yan et al. 2016; Indrasari et al. 2019), and phytochemicals-health benefit substances (Yafang et al. 2018; Mbanjo et al. 2019). The variety of rice colors is white rice, black rice, and other colors. Each color results from different pigments located on the outer membrane. Polyphenol, flavonoid, and anthocyanin compounds had reported to be the major pigments. Previous reports demonstrated that rice pigments show antioxidant activities which could prevent many diseases including chronic non-communicable diseases such as cancer, diabetes, ischemic heart disease, stimulating blood circulation, reduction of cholesterol in the blood, and preventing memory loss (Keneswary et al. 2018; Gulladawan et al. 2020). According to Goufo and Trindade (2014) and Wanti et al. (2015), colored rice has higher anthocyanin and antioxidant activity than white rice and is often promoted as healthy food because they contain high amounts of antioxidants (Ghasemzadeh et al. 2018; Sivamaruthi et al. 2018; Chavarin et al. 2019).

Consumers are currently concerned with the food in which they consume and are willing to pay more value for food of higher quality than ordinary food, which is referred to as functional food. To produce high-quality rice, the production method would be organic in which no chemical is allowed for rice production. Production of new rice varieties is dependent heavily on high inputs. On the other hand, low input of the organic production system is more suitable for local rice varieties (Yaowarat et al. 2015; Tin et al. 2017). The slump in rice prices is a recurring problem in the country. Although many government policies had implemented to help the farmers, they are still in the cycle of poverty. Production of local rice varieties with health benefits is an alternative option, which can increase farmers’ income, diversity of rice production, and conservation of local rice varieties at the farm level. Production of local rice varieties with health benefits helps the farmers establish community enterprises to serve the specific segment of the rice market.

However, the information on anthocyanin content, phenolic content, antioxidant activity, growth, and yield are rare in the organic rice production system, and the suitable local rice varieties for the system had not been identified. The objective of this study was to evaluate the effects of fertilizer management methods (organic and inorganic) on total anthocyanin content (TAC), total phenolic content (TPC), antioxidant activity, and yield in eight local varieties. The information obtained in this study has demonstrated the selection of suitable rice varieties for organic rice production, and organic fertilizer management will be transferred to the farmers to establish organic rice production system for the community enterprise.
MATERIALS AND METHODS

Experimental design and crop management

Eight indigenous rice accessions were selected for the experiment because they differed in characteristics such as eating quality, kernel color, and aroma. These varieties were also popular among rice growers before they vanished from cultivation because of the introduction of high-yielding types. Currently, most rice-growing areas in the Northeast of Thailand had replaced by RD 6, a fragrant glutinous rice. In this study, we compared two fertilizer types, including chemical fertilizer and organic fertilizer were compared, for their effects on anthocyanin content, phenolic content, antioxidant activity, and yield, and the treatments were, therefore, set up in a 2x8 factorial experiment with a randomized complete block arrangement of the treatments and three replications. In the rainy season, we undertook the investigation in a farmer’s field at Ban Hua Wua community, Song Plueai sub-district municipality, Na Mon district, Kalasin province.

The soil was plowed and puddled. Rice bunds of 1-meter width construct for each a plot with plot size of 10x40 m. The seeds were sowed on a seedbed for one month before transplanting. The seedlings were transplanted to the plots with about five seedlings per hill with a spacing of 20x20 cm. For conventional fertilizer management, we applied chemical fertilizers to the crop twice at tillering and flower initiation. We used the mixed chemical fertilizer formula 16-16-8 of N-P-K was applied at the rate of 125 kg ha⁻¹ and urea (46-0-0) at the rate of 31.25 kg ha⁻¹ was applied to the tiller crop. We applied chemical fertilizer formula 16-16-8 of N-P-K at 62.5 kg ha⁻¹, and urea (46-0-0) at the rate of 62.5 kg ha⁻¹ to the crop at flowering initiation.

Organic fertilizer management consisted of organic fertilizer, applied once during soil preparation, and two formulae of fermented liquid fertilizer, which we used at tillering, flower initiation, and flowering. The components of organic fertilizer included soybean meal 40 kg, fine rice brand 10 kg, cattle manure 10 kg, rock phosphate 24 kg, milled animal bone 8 kg, bat compost 8 kg, and molasses 26 L. The ingredients were mixed, fermented for 9-12 days, and incorporated into the soil during soil preparation at 625 kg ha⁻¹. Formula I, liquid fermented fertilizer mainly consisted of fruit waste and 8 L of molasses, while formula II mainly consisted of green vegetables and 8 L of fermented molasses. Formula I and formula II are applied using foliar spray at tillering, flower initiation, and flowering. However, the rates of application were different for each spraying. Weeds were controlled manually. Other chemicals did not apply to the crop according to the regulations for organic rice production except for chemical fertilizer treatments for comparison. We harvested the yield at the maturity stage and took the seeds for chemical analysis.

Anthocyanin content

Total anthocyanin content (TAC) was measured by the pH differential method (Maria et al. 2013). The samples were diluted using the appropriate dilution factor. The diluted samples were divided, mixed with pH 1.0 or 4.5 buffer, incubated for 15 min under dark conditions, and absorbance was measured using a UV-vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA) at 510 and 700 nm wavelengths, respectively. The results were calculated with the following equation; \( TAC = A \times MW \times DF \times 1000 \times c \times 1 \), where \( A \) was the absorbance of the diluted sample, calculated from \( A = (A510 - A700) \) pH1.0 - (A510 - A700) pH4.5, MW was the molecular weight of cyanidin-3-glucoside (449.2 g mol⁻¹), DF was the dilution factor, and 1000 was a conversion unit of molar to ppm and the molar absorptivity of 26,900 M⁻¹ cm⁻¹. Anthocyanin levels were expressed as microgram cyanidin-3-glucoside equivalent per gram of dry weight (µg CGE g⁻¹ DW).

Phenolic content

Total phenolic content (TPC) was determined by using Folin-Ciocalteu (F-C) reagent method with a minor modification as described by Kapcum et al. (2016). Briefly, 0.5 mL of 10× of the diluted samples with the extraction solvent were mixed with 2.5 mL of deionized water and 0.5 mL of 1 M F-C reagent. Then, 1.5 mL of a 7.5% Na₂CO₃ solution was added to the mixed samples, stirred until the samples were mixed, and stored at room temperature for 2 h. The optical absorbance was measured at 765 nm with a UV-vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA). Gallic acid (GA) solutions (10-100 µg mL⁻¹) were used to make a standard curve for calibration. The TPC was expressed as milligram GA equivalent per gram dry weight (mg GAE g⁻¹ DW).

Antioxidant activity

Antioxidant activity in indigenous rice was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. The DPPH radical scavenging activity was measured using the method described by Kapcum et al. (2016) with a minor modification. The portion of 0.5 mL of the sample extract 10× diluted in the extraction solvent was mixed with 4.5 mL of the 60 µM DPPH radical solution in methanol. The reaction was stored in dark conditions for 30 min, and the absorbance was measured at 517 nm using a UV-vis spectrophotometer (GENESYS 10S, ThermoScientific, Waltham, MA, USA).

Growth and yield

Data collection was carried out as follows: the stem height, the number of tillers, the number of grains per plant and grain weight.

Data analysis

The data were analyzed statistically according to a factorial experiment in a randomized complete block design. Treatment differences were separated by the least significant difference (LSD) at the 0.05 probability level. Pearson’s correlation was used for calculation of correlation coefficients among parameters under study based on treatment means. All calculations were accomplished using SPSS software (statistic package for social science).
RESULTS AND DISCUSSION

Chemical fertilizer and organic fertilizer were significantly different (P≤0.05) for anthocyanin content and antioxidant activity, but were not significantly different for total phenolic content (Table 1). Organic fertilizer was considerably higher than chemical fertilizer for anthocyanin content and antioxidant activity. Moreover, organic fertilizers tended to increase tilling of rice, the number of grains per plant, and plant height (Figure 1). Kum Ka Dum had the highest tilling and number of grains/plants, which had larger stems and stemmed height when compared to other strains (Figures 2 and 3).

Significant differences (P≤0.05) among rice varieties were observed for total anthocyanin content, phenolic content, and antioxidant activity (Table 2). Total anthocyanin contents ranged between 0.64 and 267.46 mg/100 g, with Nang Nuan being the lowest and Luem Puew being the highest. Total phenolic contents ranged between 27.46 mg/L in Mali Nga Chang and 181.45 mg/L in Kum Puen Baan. Antioxidant activity values were between 20.50% in Mali Nga Chang and 90.93% in Chaow Daeng.

Table 1. Means for total anthocyanin content, total phenolic content, and antioxidant activity determined by DPPH of chemical fertilizer and organic fertilizer treated on indigenous rice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total anthocyanin content (mg/100g)</th>
<th>Total phenolic content (mg/L)</th>
<th>Antioxidant activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical fertilizer</td>
<td>54.19b</td>
<td>85.96</td>
<td>72.19b</td>
</tr>
<tr>
<td>Organic fertilizer</td>
<td>56.32a</td>
<td>86.32</td>
<td>75.05a</td>
</tr>
<tr>
<td>Mean</td>
<td>55.25</td>
<td>86.14</td>
<td>73.62</td>
</tr>
</tbody>
</table>

Note: Means in the same column followed by the same letter are not significantly different by the least significant difference at the 0.05 probability level.

Table 2. Means for total anthocyanin content, total phenolic content, and antioxidant activity determined by DPPH of chemical fertilizer and organic fertilizer treated on indigenous rice

<table>
<thead>
<tr>
<th>Rice variety</th>
<th>Total anthocyanin content (mg/100g)</th>
<th>Total phenolic content (mg/L)</th>
<th>Antioxidant activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mali Daeng</td>
<td>30.20c</td>
<td>111.56d</td>
<td>88.28cd</td>
</tr>
<tr>
<td>Kum Ka Dum</td>
<td>44.86c</td>
<td>91.20d</td>
<td>90.31abc</td>
</tr>
<tr>
<td>Nang Nuan</td>
<td>0.64c</td>
<td>41.63d</td>
<td>24.16d</td>
</tr>
<tr>
<td>Luem Puew</td>
<td>267.46d</td>
<td>83.89e</td>
<td>87.35d</td>
</tr>
<tr>
<td>Sung Yod</td>
<td>8.04c</td>
<td>95.81c</td>
<td>90.96a</td>
</tr>
<tr>
<td>Kum Puen Baan</td>
<td>84.94b</td>
<td>181.45a</td>
<td>89.93ab</td>
</tr>
<tr>
<td>Mali Nga Chang</td>
<td>4.76f</td>
<td>27.46h</td>
<td>20.50f</td>
</tr>
<tr>
<td>Chaow Daeng</td>
<td>1.18f</td>
<td>56.13f</td>
<td>90.93a</td>
</tr>
<tr>
<td>Mean</td>
<td>55.26</td>
<td>86.14</td>
<td>72.80</td>
</tr>
</tbody>
</table>

Note: Means in the same column followed by the same letter are not significantly different by the least significant difference at the 0.05 probability level.
Figure 2. Growth and yield of indigenous rice under chemical fertilizer

Figure 3. Growth and yield of indigenous rice under organic fertilizer
The mean for total phenolic content was 86.14 mg/L, ranging from 26.59 mg/L in Mali Nga Chang + chemical fertilizer to 181.53 mg/L in Kum Puen Baan + organic fertilizer. Most varieties of organic fertilizer were higher than those applied with chemical fertilization. Except for Nang Nuan and Sung Yod, most varieties had intermediate to high total phenolic content except for Nang Nuan and Mali Nga Chang, which had low total phenolic content, ranging from 26.59 to 41.74 mg/L. The mean for antioxidant activity was 72.60%, ranging from 20.33% in Mali Nga Chang + organic fertilizer to 91.20% in Sung Yod + organic fertilizer. Most varieties had high antioxidant activity ranging from 86.61 to 91.20%, except for Nang Nuan and Mali Nga Chang, which had low antioxidant activity, ranging from 20.33 to 23.00%. All correlation coefficients among total anthocyanin content, total phenolic content, and antioxidant activity were positive, ranging from 0.26 between anthocyanin and phenolic content to 0.66** between total phenolic content and antioxidant activity. The correlation coefficient (0.33*) between total anthocyanin content and antioxidant activity was also significantly different (Table 4).

<table>
<thead>
<tr>
<th>Phenolic content</th>
<th>Phosphoric content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic content</td>
<td>0.26</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>0.33*</td>
</tr>
<tr>
<td>Phenolic content</td>
<td>0.66**</td>
</tr>
</tbody>
</table>

Note: ***,** significant at 0.05 and 0.01 probability levels, respectively

Discussion

This study aimed to compare the efficacy of inorganic and organic fertilizers on the growth and yield and to study the effects of fertilizer types on antioxidant activity revealed from 8 indigenous rice. The outcomes of this study may suggest the production system for a community of farmers and select the most suitable local rice varieties for the system. Application of all types of chemicals is prohibited in organic rice production systems except for phosphorus sources in the form of rock phosphate. Fertilizers are obtained solely from organic sources, and only materials from organic sources use for pest and disease management (Ministry of Agriculture and Cooperatives 2021). Weed control is unnecessary because the crop was transplanted in a flooded field. In this study, organic fertilizer management resulted in higher TAC, TPC, and antioxidant activity in local rice varieties. Higher phytochemicals and antioxidant activity in organic rice could be caused by nutrient stress because the organic production system had lower nutrients than the inorganic production system in which we supplied chemical fertilizers to the crop. According to Trojakm and Skowron (2017), anthocyanin accumulation is initiated in response to excessive light higher than photosynthetic capacity in poinsettia leaves. Like a light response, phosphorus deficiency induced anthocyanin accumulation in corn (Vasconcelos et al. 2018). In rice, the application of magnesium and low temperature increased the accumulation of anthocyanins (Tisarum et al. 2018). The increase in phytochemicals and antioxidant activity in organic rice is an advantage to reimburse lower yield because the farmers can obtain a higher price of organic rice.

In addition, drought-tolerant species have an effect on antioxidant production. (Miriam et al. 2019; Sebastiáno et al. 2019) According to Ruslan et al. (2021), drought tolerant rice cultivars of Habo and Sungkul had higher antioxidants content of superoxide dismutase (SOD), peroxide dismutase POD, ascorbic acid (AAred), and alphatocopherol (α-Toch), whereas non-drought tolerant cultivars of Hiwanggum and Lambara exhibited higher production of O2 and H2O2 and lower level of SOD, POD, AAre as well as α-Toch. Moreover, organic fertilizers tended to increase tilling of rice, the number of grains per plant, and grain weight. The result yielded from organic matter of fossil plants contained in organic fertilizer could enhance and restructure the soil (Amjad et al. 2016; Assafe and Tadesse, 2019). In addition, organic fertilizers are able to mitigate problems associated with synthetic fertilizers. They reduce the necessity of repeated application of synthetic fertilizers to maintain soil fertility. They gradually release nutrients into the soil solution and maintain nutrient balance for healthy growth of crop plants. They also act as an energy source for soil microbes, which improve soil structure and crop growth. Microorganisms can degrade organic materials by transforming the organic material into inorganic forms as plants require, thus promoting the increase in tilling and yield of rice (Jannoura et al. 2014; Hitha et al. 2020). According to Abdul-Rahman (2019), organic fertilizers are an attractive source of soil nutrients for small rural farmers because of reducing farming costs as they are cheaper than chemical fertilizers, and farmers can prepare their own if trained.

We evaluated eight local rice varieties for total anthocyanin content, total phenolic content, and antioxidant activity determined by DPPH. Before the release of KDML 105 (also known as Hom Mali) and other high-yielding varieties, these local varieties had been cultivated and popular in many regions of the countries. Most local varieties had high total anthocyanin content, with which Luem Puew was the highest, and these local varieties also had high total phenolic content. However, they had the lowest total anthocyanin and total phenolic content, and their antioxidant activities were also the lowest. Nang Nuan and Mali Nga Chang are white rice varieties, while the rest are colored rice varieties. Most consumers prefer white rice to colored rice. However, colored rice is associated with health benefits (Luo et al. 2014; Siaw et al. 2015; Boue et al. 2016; Mbanjo et al. 2020). Except for the varieties with low antioxidant activity (Nang Nuan and Mali Nga Chang), the other six local varieties had high antioxidant activity, ranging from 87.35 to 90.96%. The varieties with high antioxidant activity will be further selected based on farmers’ preference to establish organic rice production in the community. The most promising local rice varieties for
the highest antioxidant activity and organic production system are Kum Ka Dum and Sung Yod. However, we should consider consumers’ preferences.

In this study, the correlations among total anthocyanin content, total phenolic content, and antioxidant activity were relatively low (0.26 to 0.66). It is interesting to note here that the varieties with the highest total anthocyanin or total phenolic content did not have the highest antioxidant activity. In contrast, the highest antioxidant activities come from the varieties with intermediate total anthocyanin content or intermediate total phenolic content. The phytochemicals other than anthocyanins and phenolic compounds may significantly contribute to antioxidant activity in these local rice varieties. Unfortunately, the measurement of phytochemicals in this study was undertaken for anthocyanins and phenolic compounds. According to Piebiep and Henrique (2014), among the four types of rice ranked by color, black rice varieties emerged as those exhibiting the highest antioxidant activities, followed by purple, red, and brown rice varieties. Furthermore, insoluble compounds constitute the major fraction of rice phenolic acids and proanthocyanidins, but not flavonoids and anthocyanins. It is clear that to maximize the intake of antioxidant compounds. The rice should be preferentially consumed as bran or as whole grain. Studies on other phytochemicals and antioxidant activity in these local rice varieties are still required.

In conclusion, the organic fertilizer promoted antioxidant activity by increasing anthocyanin and phenolic content. Moreover, it enhances the growth and yield of indigenous rice. This result may benefit upstream, midstream, and downstream rice industries.

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REFERENCES


