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Growth and Flavonoids Content of Black Rice (*Oryza sativa* L. *indica*) with Compost Tea of Oyster Mushroom Waste

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Abstract

The flavonoid compounds in black rice (*Oryza sativa* L. *indica*) are very important for health, they serve, for instance, as antioxidants and anticancer. Giving compost tea of oyster mushroom waste (COMW) can increase plant growth and flavonoid content to black rice. This research aims to determine the effectiveness of COMW to increase the growth and flavonoid content of black rice. This experiment using completely randomized design with single factor of five COMW concentrations, four replicates respectively. The COMW was given at five different concentrations (0, 25, 35, 45 and 55%) from 25 to 45 days after planting. Parameters observed including plant height, fresh and dry weight of plants, fresh and dry weight of roots, fresh weight of grain, fresh and dry weight of total and flavonoid content. The results showed that COMW significantly increased black rice growth, the best growth was obtained by the concentration of 45% and the highest flavonoid content is by 35% concentration. No significant effect on crown height and canopy dry weight. There were significant effects on root fresh weight, shoot fresh weight, grain fresh weight, total fresh weight, root dry weight, dry weight grain, total dry weight and increased levels of flavonoids in black rice. From the research, it can be concluded that COMW increased both growth and flavonoids content of black rice.

Keywords: antioxidant activity; compost tea; flavonoids; pigmented rice; secondary metabolit

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INTRODUCTION

Rice is the staple food for the Indonesians; and it is, thus, the main agricultural commodity (Zaeroni and Rustariyuni, 2016). Various types of rice can be cultivated in Indonesia, one of which is black rice (*Oryza sativa* L. *indica*). Black rice has several advantages compared to white rice and red rice. It is rich in fiber, has low glycemic index and contains several important amino acids. The needs of the Indonesian people for black rice have recently continued to increase along with the increasing population (Kantikowati et al., 2018; Munir et al., 2018).

The increasing demand for black rice is due to the benefits when compared to red rice or white rice (Yuniarti et al., 2020). Black rice is widely consumed by diabetics patients because of its low sugar content (Munir et al., 2018). In addition, black rice is also useful for increasing body resistance, repairing liver cell damage, preventing kidney disorders, slowing aging and preventing anemia (Yuniarti et al., 2020). Black rice contains high levels of flavonoids,

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anthocyanins, fiber and iron (Warman et al., 2016).

Flavonoids are secondary metabolites found in plants. Flavonoids are derived from polyphenols which have 15 carbon atoms, composed of two C6 groups (substituted benzene rings) connected by a three-carbon aliphatic chain. Flavonoids are phytochemical compounds that are classified as polar compounds so that they will dissolve in polar solvents such as ethanol, methanol, butanol, acetone and dimethylformamide. The bioactive activity of flavonoids has broad biological benefits for humans (Arifin and Ibrahim, 2018). Flavonoids have several benefits, namely increasing bioactivity in animals, antioxidant activity and anticancer activity. Based on Abidin (2016), ethanol extract of black rice containing flavonoids has potential as anticancer because it can reduce the activity of protein kinase B (PKB) and CA 15-3 serum in white rats. The levels of flavonoids in black rice need to be increased because it has various benefits for humans (Munir et al., 2018).

Compost tea from oyster mushroom waste (COMW) is an oyster mushroom growing medium that has expired and cannot be used anymore. The abundance of baglog waste will cause air and soil pollution around the waste disposal. Therefore, the oyster mushroom baglog waste needs to be utilized. Mushroom baglog waste can be used to increase soil fertility and plant growth because it contains macronutrients including N (0.6%), P (0.7%), K (0.02%) and organic C (49%) (Bellapama et al., 2015; Rahmah et al., 2016; Hunaepi et al., 2018).

The use of organic fertilizers for rice production is constrained by the nature of the material which has a low nutrient content and causes additional procurement costs, such as the unit cost of the nutrients contained are quite expensive and the results of the effect are not visible. One of the technological developments in the production of fertilizers is through the application of extracting fertilizers into liquid fertilizers. This liquid organic fertilizer product is a simple and inexpensive fertilizer (Mudmainah et al., 2019), so it is environmentally safe and economically viable alternatives for crop production especially in developing countries (Gebrewold, 2018).

Compost tea is an alternative to organic fertilizers that can reduce the use of chemical fertilizers. Compost tea or commonly known as compost extract has the role of controlling pests and diseases, increasing rice production and improving the condition of agricultural land (Mudmainah et al., 2019; Solikhatin et al., 2021). Compost tea comes from microbial-enriched essence so that it provides hormones for rice growth (Purnomo et al., 2021). Compost tea as potential alternatives is viewed to replace the use of synthetic chemical fungicides. There are diseases suppressive effects in numerous agricultural system, their efficacy remain variable. Compost tea is multi-purpose so it can be used in several different fields (Shaaban et al., 2015). In addition, compost tea contains beneficial microorganisms such as fungi and bacteria (Banu and Tefa, 2018). The content of microorganisms in compost tea are *Rhizobacteria*, *Trichoderma*, *Bacillus* and *Pseudomonas*. These microorganisms are able to increase plant growth and production, plant hormones and chemical compounds such as tannins, phenols and flavonoids (Munthe et al., 2014).

According to Soesanto et al. (2010), the bacterium *Pseudomonas fluorescens* produces auxin compounds that can stimulate plant growth. *Bacillus subtilis* is a Plant Growth Promoting Rhizobacteria (PGPR) that produces siderophores and IAA so that it can increase plant growth (Prihatiningsih et al., 2017). Based on research by Rachman et al. (2020), *Trichoderma* sp. is able to increase the content of glycoside compounds and the activity of peroxidase (POX), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) enzymes in tomato plants. Istiqomah and Kusumawati (2018), also stated that the treatment with *B. subtilis* and *P. fluorescens* was able to induce an increase in the activity of the PAL enzyme in plants. The activity of the PAL enzyme is closely related to the increase in the total flavonoid content in plants. This is because PAL is the main precursor in the biosynthesis of the phenylpropanoid pathway (Ekawati, 2018).

So far, oyster mushroom baglog waste in Indonesia has not been used optimally and is only left as organic waste. In addition, information regarding the use of COMW is also very limited. Therefore, research on the utilization of COMW needs to be carried out to determine its contribution in increasing growth and flavonoid levels in black rice. The aim of this study was to determine the effectiveness of COMW in increasing growth and flavonoid content of black rice. The results of this study are expected to provide information about the utilization of oyster mushroom baglog waste for black rice.

MATERIALS AND METHOD

Study location

This research was conducted by experiment using a completely randomized design from June to August 2021. The experiment was carried out at the Plant Physiology Laboratory and Green House, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Banyumas, Central Java, Indonesia. The latitude of experiment site is 7°24'33.3"S, the longitude is 109°15'18.0"E and the elevation is 118.9 m above sea level (m asl).

Materials

The materials and tools used in this study were black rice seeds, spray bottle, compost tea oyster mushroom baglog waste mixed with water, EM4 (effective microorganisms 4), molasses, rice husks, goat manure, soil, polybag, tarpaulin, aquarium pump and cloth strainer, treatment of plants by polybag and measuring cup. *In vivo* test required analytical scale and oven; flavonoid test used methanol, ethanol 96%, standard quercetin, AlCl3, potassium acetate 1 M, aquabidestillata, aquades, HCl and magnesium with the following tools erlenmeyer, test tube, pipette tip, hotplate, UV mini -1240 Spectrophotometer and blender.

Methods

Mushroom waste composting

Composting in anaerobic system was start by mixing 3 kg baglog waste, 2 kg goat manure and 500 g husk thoroughly. The materials were doused by 0.1% EM4 solution and mixed evenly, then covered with plastic tarpaulin. In the process of composting, the material pilewas turned every 3 days until maturation. Mature compost was characterized by a brownish black color, loose texture, odorless.

Tea of mushroom waste composting

Composting of oyster mushroom baglog waste tea refers to the research of Mudmainah et al. (2019), the compost was placed in a bucket and given water with compost and water ratio of 1:10. The mixture was then aerated using an aquarium pump and incubated for 14 days. The results of the compost extract were filtered using cheesecloth. Composting was conducted by adding EM4 to the oyster mushroom baglog waste tea compost until evenly distributed (Hasbiah et al., 2018).

In vivo test

The *in vivo* compost tea test refers to the research of Putri et al. (2017), black rice seedlings were planted in polybags with a size of 35 cm × 35 cm which were filled with soil and manure in a ratio of 2:1. Compost tea was given using the drench media method, when the plants were 25, 30, 35, 40, 45 DAP (days after planting) with 5 concentration levels, which were 0, 25, 35, 45 and 55% per treatment. In total there were 20 plants, each treatment was replicated 4 times. Observations started at 6 weeks after treatment with parameters including height, weight of crown, weight of roots, dry weight of grain, weight of total and flavonoid content.

Sample extraction

Sample extraction was conducted by the maceration method Rejeki et al. (2020), where black rice grains were sorted, separated from the husks, washed, aerated, mashed with a blender and sieved. The maceration method using 300 g of black rice powder in a beaker glass, then immersed with 750 ml of 96% ethanol. The immersion was done for 2 days while stirring repeatedly. After the immersion process completed, the mixture was strained with flannel until the filtrate was obtained. The process was repeated one more time and the resulting filtrates were combined, evaporated on a hotplate until a thick extract was obtained.

Flavonoid qualitative test

Qualitative analysis of flavonoid compounds from black rice ethanol extract were tested by comparing it with quercetin, with the Willstätter cyanidin test method.

Flavonoid quantitative test

Quantitative analysis of flavonoid compounds was tested by total flavonoid content. Determination of total flavonoid levels by colorimetric method using Quercetin equivalent (QE) as standard. The total flavonoid content (mg ml^{-1}) was determined using aluminum chloride (AlCl3) method. The total flavonoid content refers to Ahmad et al. (2015), 25 mg of black rice grain methanolic extract was taken and dissolved in 25 ml of methanol. From the stock solution, 1 ml of the stock solution was pipetted and 10 ml of methanol was added. Then 1 ml of solution was added with 3 ml of methanol, 0.2 ml of AlCl3, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. After that, it was incubated for 30 minutes at room temperature and the absorbance was measured on a 440 nm spectrophotometer. After that, the flavonoid content was calculated.

Experiment design and data analysis

The experiment was carried out at Plant Physiology Laboratory and Green House, Faculty of Biology, Universitas Jenderal Soedirman in Banyumas. The plants used in this experiment were 25, 30, 35, 40, 45 DAP with 5 treatment levels. Each of treatment was replicated 4 times, so there were in total of 20 plants. This study uses two variables, including the dependent and independent variables. The dependent variables are plant height, fresh and dry weight of crown, fresh and dry weight of roots, fresh and dry weight of grain, fresh and dry weight of total and flavonoid content. The independent variable was concentration levels of compost tea. The data obtained of each parameter were analyzed using Analysis of variance (ANOVA) ($\alpha = 0.05$) SPSS 13 software, if results showed significant differences, it was continued by Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

The height of the black rice plant was not significantly affected by the COMW treatment which means that the COMW treatment on black rice had no effect on the yield of the black rice plant height.

Fresh weight of black rice with COMW

The weight of wet black rice showed different results between the treatment with compost tea from baglog oyster mushroom waste and the control. Giving compost tea from baglog oyster mushroom waste had a significant effect on root wet weight with the best concentration was of 45%, shoot wet weight with the best concentration was of 45%, grain wet weight with the best concentration of all treatments except control and total wet weight with the best concentration was of 45% (Table 1).

The difference in the fresh weight of the roots can be influenced by the nitrogen content. Based on the research of Rahmah et al. (2015), oyster mushroom baglog compost contains nitrogen ranging from 1.14% to 1.82%. According to Gardner et al. (1991) higher nitrogen content will increase auxin. Inhibition is influenced by ethylene, because auxin will trigger various types of plant cells to produce ethylene, especially if auxin is added in large quantities. According to Desti et al. (2019) the increase in adventitious roots is related to ethylene production. Ethylene provides a stimulus to form aerenchyma in roots and the formation of new roots and increase plant resistance. The aerenchyma network facilitates the movement of O_2, CO_2, CH_4 and C_2H_2 (Arsana et al., 2003).

Table 1. Fresh weight of root, plant, grain and total

Compost of tea concentration (%)	Fresh weight (g) of			
	Root	Plant	Grain	Total
0	$(50.98\pm20.43)^{ab}$	$(70.13\pm7.13)^{a}$	$(26.69\pm5.08)^{a}$	$(181.82\pm32.32)^{a}$
25	$(44.15\pm17.75)^{a}$	$(87.54\pm11.65)^{ab}$	$(39.07\pm5.83)^{b}$	$(191.95\pm16.46)^{ab}$
35	$(57.80\pm21.13)^{ab}$	$(90.49\pm11.67)^{b}$	$(42.93\pm3.18)^{b}$	$(208.93\pm36.95)^{ab}$
45	$(73.73\pm4.32)^{b}$	$(120.49\pm8.04)^{c}$	$(41.12\pm7.29)^{b}$	$(249.58\pm11.63)^{b}$
55	$(67.53\pm13.94)^{ab}$	$(104.65\pm16.78)^{bc}$	$(46.30\pm9.96)^{b}$	$(189.59\pm62.93)^{a}$

Note: Different superscripts in the same column shows significant difference at the 5% level according to the DMRT

The results of the canopy fresh weight test were related to the relationship between root growth and crown growth. According to Simbolon and Tyasmoro (2020) the better root growth will affect the growth of the crown. Fast root growth affects the absorption of nutrients and water for optimal photosynthesis. The optimal photosynthesis process results in an increase in assimilate yields, thereby accelerating plant development and increasing plant wet weight (Farid, 2011). According to Sitompul and Guritno (1995) the increased production of photosynthate will allow for better formation of all plant organs. The formation of these organs can occur in leaves, stems and roots, resulting in greater production weight.

The average yield of the fresh weight of the grains in the compost tea treatment resulted in the fresh weight of the grains being heavier than the control. This is related to the macro and micronutrients provided by the compost tea during the vegetative phase so that it affects the good growth of leaves, roots and tillers thereby increasing grain production. The amount of grain can be associated with root growth and number of tillers. The low average wet weight of grains in the control was caused by less optimal nutrient absorption so that the grains were still empty. In addition, it could also be caused by the lack of nitrogen and phosphorus (Asifah et al., 2019). Maintang et al. (2010) stated that grain filling was influenced by the results of photosynthesis in stem and leaf. Then translocated and accumulated into the grain. The condition of the leaves that are erect, thick, narrow, dark green and do not fall off quickly are important in the process of maximum grain filling. According to Sugeng (2008) if the condition of the rice leaves has turned yellow but the panicles have not turned yellow, the leaves cannot translocate the photosynthetic results to the rice grains so that the grains will become empty.

The application of compost tea from baglog oyster mushroom waste had a significant effect on the total fresh weight of black rice. Based on previous research of Pant et al. (2012), it also showed the same result that compost tea had a significant effect on the fresh weight of pakcoy plants. This is because compost tea can increase the absorption of nutrients for plants. In addition, oyster mushroom baglog waste compost tea contains nitrogen which can increase plant vegetative growth, thereby increasing plant's wet weight (Pamuji et al., 2018).

Dry weight of black rice with COMW

The dry weight of black rice showed different results between the treatment with compost tea from baglog oyster mushroom waste and the control (Table 2). Giving compost tea from baglog oyster mushroom waste had a significant effect on root dry weight with the best concentration was of 45%, grain dry weight where all treatments were better than the control and total dry weight with the best concentration were of 45% and 55%. Meanwhile, giving compost tea of oyster mushroom baglog waste did not affect the dry weight of the canopy.

The difference in root dry weight between control and treatment was due to phosphorus content in the oyster mushroom baglog compost tea. Phosphorus has a role in stimulating the growth of young plant roots and also strengthening the growth of young plants (Suryawaty and Wijaya, 2012). Dry weight of roots and shoots became the benchmark to determine the metabolic rate of plants. According to Afrillah et al. (2015) that the total dry matter of shoots and roots describes the ability of plants to capture sunlight through the process of photosynthesis. The dry weight of the plant becomes a benchmark in seeing the effect of giving oyster mushroom compost tea baglog treatment on plant quality.

Table 2. Dry weight of black rice

Compost tea concentration (%)	Dry weight (g) of			
	Root	Crown	Grain	Total
0	$(11.57±5.26)^{ab}$	$(5.66±1.18)^{a}$	$(17.74±3.53)^{a}$	$(51.09±8.79)^{a}$
25	$(7.06±0.94)^{a}$	$(7.22±1.10)^{a}$	$(25.69±4.60)^{b}$	$(57.89±6.84)^{ab}$
35	$(10.27±3.61)^{ab}$	$(8.89±3.58)^{a}$	$(29.25±2.15)^{b}$	$(68.20±10.97)^{b}$
45	$(23.01±3.83)^{c}$	$(7.30±1.64)^{a}$	$(27.97±4.14)^{b}$	$(82.19± 8.98)^{c}$
55	$(17.21±11.54)^{bc}$	$(7.99±1.75)^{a}$	$(30.65±5.70)^{b}$	$(81.57±14.76)^{c}$

Note: Different superscripts in the same column show a significant difference at the 5% level according to the DMRT

The results of data analysis showed that the compost tea of oyster mushroom baglog waste had no significant effect on the dry weight of the canopy. Previous research Indrianto (2021), also showed the same result that the addition of compost tea had no effect on the dry weight of the mung bean crown. This can be caused by the availability of nutrients that are not sufficient for optimal photosynthesis so that plant biomass is not optimal. Environmental factors such as the intensity of sunlight also affect the dry weight of the plant because the more light intensity received by the plant will increase photosynthesis (Febriyono et al., 2017).

The difference in dry weight of black rice grains between control and treatment was due to the contents in the oyster mushroom baglog waste compost tea. Compost tea from baglog oyster mushrooms contains macronutrients including N (0.6%), P (0.7%), K (0.02%) and organic-C (49%) which can increase soil fertility and growth. Crops (Bellapama et al., 2015), including dry weight of rice grains. In addition, compost tea also contains bacteria that can act as plant growth promoting rhizobacteria (PGPR) such as *B. subtilis* and *P. fluorescents* which can increase plant growth and production (Munthe et al., 2014). According to Sivasakthi et al. (2014), *B. subtilis* and *P. fluorescents* can increase plant growth through the production of plant growth hormones such as IAA, siderophore production and increased phosphate solubility.

The average dry weight of the total treatment concentration was 45% and 55% resulted in a total dry weight that were heavier than the other treatments. According to Bellapama et al. (2015), oyster mushroom baglog waste can also increase the dry weight of pakcoy plants because it contains nutrients that are used for the formation of cell number and cell wall thickness. Dry weight in plants is strongly influenced by optimal photosynthesis. Photosynthesis is the process of accumulating carbon dioxide assimilation during the growth phase. Photosynthesis can increase plant dry weight because assimilate produced from photosynthesis can increase plant biomass so that it can also increase plant dry weight (Sobari et al., 2018; Indrianto, 2021). The availability of nutrients in oyster mushroom baglog waste can increase photosynthetic activity. Nitrogen, phosphorus and potassium are able to increase chlorophyll so that photosynthesis also increases (Solikhatin et al., 2021).

Effect of COMW on flavonoid levels

In the qualitative test of black rice flavonoids, the Willstätter cyanidin test was used. A total of 0.5 ml of rice sample extract was dripped with 2 to 3 drops of HCl and Mg powder showed a dark red color change (Figure 1). This shows that black rice contains flavonoids. Based on research by Mariana et al. (2013), positive results for flavonoids were indicated by a color change to red. The color change occurs due to the reduction of Mg and HCl which forms a complex compound so that the reaction causes a color change to red or orange. The addition of Mg aims to make the flavonoid carbonyl group bind to Mg and the addition of HCl aims to form a red to orange flavylium salt (Mariana et al.,2013; Afriani et al., 2016). In addition, HCl in this test also aims to hydrolyze flavonoids into their aglycones by hydrolyzing O-glycosyl and glycosyl will be replaced by H^+ from hydrochloric acid because it is electrophilic (Mariana et al., 2013).

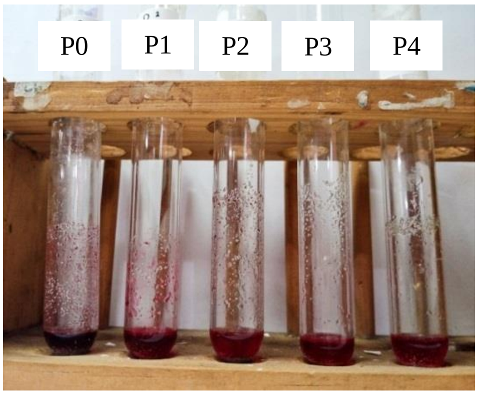

Figure 1. Flavonoid test results

Total flavonoid content

Giving compost tea from baglog oyster mushroom waste had a significant effect on the flavonoid content of black rice. Based on the results of the analysis that can be seen in Table 3, the best concentration of compost tea that can increase flavonoids is 35%.

Table 3. Total flavonoid content of black rice

Compost tea concentration (%)	Total flavonoid (mg ml^{-1})
0	(0.14639±0.00012)^{ab}
25	(0.14634±0.00004)^{ab}
35	(0.14643±0.00015)^{b}
45	(0.14630±0.00007)^{ab}
55	(0.14626±0.00003)^{a}

Note: Different superscripts in the same column show a significant difference at the 5% level according to the DMRT

The increase in flavonoid levels in black rice was caused by the influence of the compost tea content used. According to Munthe et al. (2014) compost tea contains microorganisms such as *Rhizobacteria*, *Trichoderma*, *Bacillus* and *Pseudomonas*. *Trichoderma* sp., *Bacillus* and *Psedomonas* which can increase PAL enzyme activity so as to increase phenol content in plants (Istiqomah and Kusumawati, 2018; Rachman et al., 2020). In addition, Rhizobacteria can also activate PAL enzymes to increase resistance against pathogens (Agustiansyah et al., 2014).

Increased flavonoid content in plants is closely related to PAL enzyme activity. The PAL enzyme has a role as a major precursor in the biosynthesis of phenylpropanoid for the formation of secondary metabolites. Through the phenylpropanoid pathway, flavonoid and anthocyanin compounds will be formed (Taufika et al., 2016; Ekawati, 2018). Flavonoids are pigments that are usually found in plants, for example anthocyanins. In plants, flavonoids function to protect chlorophyll from exposure to ultraviolet light so that it can continue to photosynthesize and grow (Pratiwi, 2017).

CONCLUSIONS

Based on the results of the study, it can be concluded that compost tea from baglog oyster mushroom waste had no significant effect on crown height and canopy dry weight, but had a significant effect on root wet weight, shoot wet weight, grain wet weight, total wet weight, root dry weight, dry weight grain, total dry weight and increased levels of flavonoids in black rice. The best concentration for black rice growth was 45% and for increasing flavonoid content was 35%.

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