AN ENHANCEMENT OF PHYTOCHEMICAL CONTENT IN RED GINGER (*ZINGIBER OFFICINALE* VAR. *RUBRUM* RHIZOME) USING ECO-ENZYME

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(Received 3rd Jun 2023; accepted 29th Aug 2023)

Abstract. Red ginger is a biopharmaceutical that is demanded greatly by the public. The quality of ginger rhizome is determined by its phytochemical content. Most of the ginger rhizomes farmers produce are not from environmentally friendly cultivations. This research is essential to determine the increase in the phytochemical content of red ginger cultivated using eco-enzymes. The study was arranged using a completely randomized design (CRD) in three replications with no eco-enzyme treatment (control), and eco-enzyme concentration of 0.25%, 0.50%, and 0.75%. Agronomic character variables of the ginger plants and the phytochemical content of the rhizomes were observed. Data analysis used Analysis of Variance (ANOVA) at the significant level. A Duncan's Multiple Range Test was performed at the significant level to determine the significant differences between treatments. The results showed that applying eco-enzymes to ginger plants increased the number of tillers, root dry weight, and rhizome dry weight. Also, the application of eco-enzymes increases the phenolic content markedly. Therefore, eco-enzymes are recommended for red ginger cultivation to improve the quality and yield of rhizomes.

Keywords: *rhizome, biopharmaceutical, flavonoid, phenolic, eco-friendly farming*

Introduction

Red ginger (*Zingiber officinale* var. rubrum) is a popular spice and traditional medicine used for centuries in Southeast Asia. It is known to contain a variety of phytochemicals, which are biologically active compounds that are naturally present in plants and have been associated with numerous health benefits (Shahrajabian et al., 2019; Simarmata, 2022; Saptiwi et al., 2019; Widadie, 2020). However, the levels of these phytochemicals in red ginger can vary depending on factors such as soil quality, climate, and harvesting practices (Mudau et al., 2022; Id et al., 2017; Bauer, 2015; Vidinamo et al., 2022). Therefore, there is a growing interest in finding ways to enhance the levels of phytochemicals in red ginger to maximize its potential health benefits.

Most farmers in Indonesia cultivate ginger using an inorganic fertilizer. This causes the ginger rhizomes produced to be potentially contaminated with chemicals, thus affecting the quality of the rhizomes as medicinal raw materials (Rusmin et al., 2020). Ginger in Indonesia is widely consumed fresh for beverages and the food industry. The quality and health of the rhizomes will affect the quality of medicine and food made from ginger. Therefore, environmentally friendly ginger cultivation techniques are needed using materials that do not produce harmful residues for the environment and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_54535461

humans. Using eco-enzymes is one alternative in ginger cultivation to improve rhizome quality.

One potential approach for enhancing phytochemical content of red ginger is using eco-enzymes. Eco-enzymes are enzymes that are derived from natural sources and are considered environmentally friendly (Syakdani et al., 2021; Hasanah et al., 2020; Ling et al., 2023; Abu et al., 2022). During the eco-enzyme fermentation process, the following reaction occurs $CO_2 + N_2O + O_2 \rightarrow O_3 + NO_3^- + CO_3^{2-}$ (Hemalatha and Visantini, 2020). Eco-enzymes accelerate biochemical reactions in nature to produce enzymes (lipase, amylase, and protease) that are useful as essential ingredients of fruit or vegetable waste such as fermented pineapple, mango pulp, oranges and tomatoes (Rasit and Mohammad, 2018). They have been widely used in various industries, including food, textile, and pharmaceutical, due to their ability to improve product quality and efficiency. The use of eco-enzymes in enhancing phytochemical levels in red ginger is a relatively new area of research that has the potential to provide a sustainable and cost-effective solution for increasing the health benefits of this plant.

In this study, we aim to investigate eco-enzymes' effectiveness in enhancing the levels of phytochemicals in red ginger. We will explore the concentration of eco-enzymes used. We will then analyze the levels of phytochemicals in the treated red ginger and compare them with those of untreated samples. The results of this study could have important implications for the food and supplement industries and consumers looking to maximize the health benefits of red ginger.

Materials and methods

Study area

The research was conducted at the Experimental Garden of the Faculty of Agriculture, Universitas PGRI Yogyakarta in Kasihan, Bantul, Special Region of Yogyakarta, Indonesia. The altitude is 70 meters above sea level, located at position 115.7.20 South Latitude 8.7.10 East Longitude, Regosol soil type, average rainfall of 70 mm/month.

Experimental design

This research is a pot trial research, and was arranged using a Complete Randomized Design (CRD) in three replications. This study was a single-factor experiment consisting of four treatments: control (without eco-enzyme), the eco-enzyme concentration of 0.25%, 0.5%, and 0.75% (*Fig. 1*).

Research procedures

The planting medium is a mixture of Regosol soil and cow dung manure with a ratio of 2:1. The planting media was put into 35 x 35 cm pots, totaling 8 kg per pot. This study did not use inorganic fertilizers and pesticides, fertilization was carried out using eco-enzymes to meet the nutrient needs of ginger plants.

The eco-enzymes used are fermented from orange peels, peer, dragon fruit, watermelon, apples, papaya, carrots, spinach, kale, cucumber, tobacco leaves, moringa leaves, neem leaves, and molasses which have been fermented for four months. The method of making eco-enzymes is according to the method developed by Dr. Rosukan Poompanvong with some modification. The eco-enzyme concentrations applied include

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DOI: http://dx.doi.org/10.15666/aeer/2106_54535461

without eco-enzyme (control), 0.25% eco-enzyme; 0.5%; and 0.75%. Eco-enzymes were applied to ginger plants at intervals of 1 week, as much as 200 mL/plant. Red ginger seeds are prepared by sowing ginger rhizomes for three weeks. After growing shoots, the rhizome is transferred to the pot. The cultivation of ginger plants is done without chemical fertilizers and pesticides. Plant maintenance is carried out by applying eco-enzymes once a week. Ginger rhizomes are harvested when the plants are 8 months old.

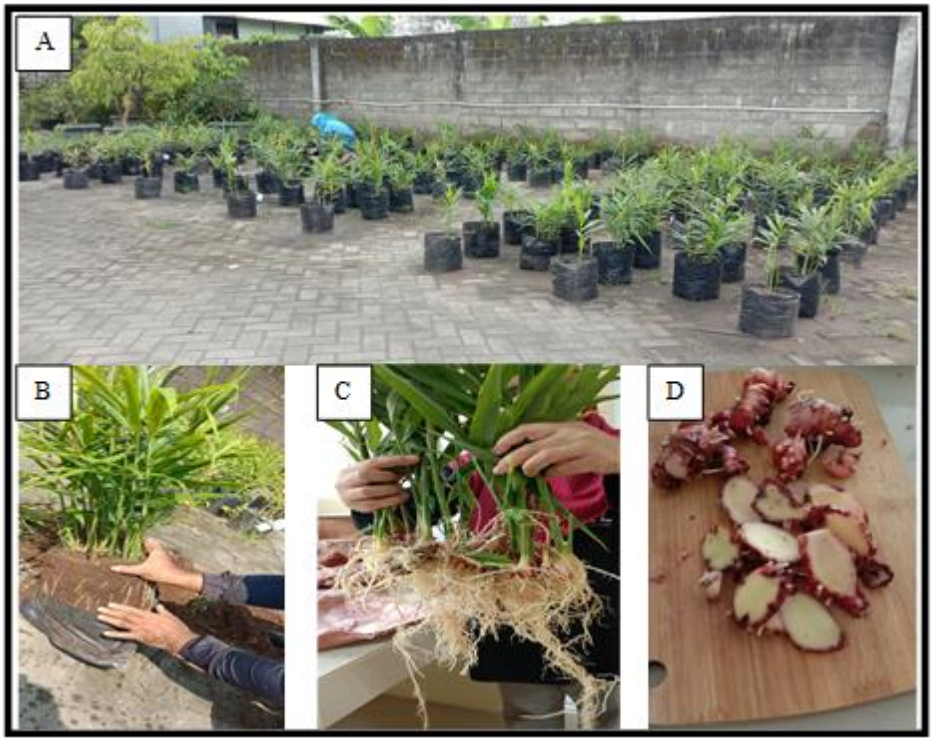

Figure 1. *(A) Cultivation and care of red ginger plants with eco-enzymes, (B) harvesting red ginger (8 months old), (C) washing process, and (D) red ginger rhizomes*

Measurement

Observations were made on the agronomical character of the ginger plant and its phytochemical content. Analysis of the phytochemical content of ginger rhizome using the Folin-Ciocalteu's (FC) phenol reagent obtained from Merck (Darmstadt, Germany), method with UV-Vis Spectrophotometry (Thermo Genesys model 10 S UV–VIS double-beam spectrophotometer-Thermo Fisher, Netherlands), Universal Oven (Memmert). Red ginger flavonoid levels were measured by reacting $AlCl_3$ reagent with quercetin as a marker of flavonoids and readings using a spectrophotometer with a wavelength of 420 nm.

Agronomic character measurements were carried out at harvest (8 months of plant age) including root dry weight, plant dry weight, number of tillers, and rhizome weight. Root dry weight was measured by disassembling the plants, cleaning the roots and baking them at 80°C for 2 × 24 h. Observations of plant dry weight were carried out after the plants were dismantled, baked at 80°C for 3 × 24 h. The number of tillers was measured by counting the number of tillers that grew, with a minimum plant height of 10 cm. Rhizome weight was measured after the plants were disassembled, then cleaned

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_54535461

from the attached soil. All agronomic parameters were measured using digital scales (JB16030-C, Indonesia).

Statistical analysis

The data were analyzed with an Analysis of Variance at a significant level of 5%. To determine the significant difference between treatments using the Duncan Multiple Range Test at the 5% significance level. The data analysis using SPSS.

Results

Analysis of eco-enzyme content

The results of the analysis of nutrient content contained in eco-enzymes: N 0.04%; P 0.008%; K 0.34%; Ca 0.06%; Mg 0.02%; B 11.56 ppm; Fe 17.72 ppm, Cu 1.18 ppm; Zn 0.84 ppm; Mn 3.82 ppm; S 0.05%. eco-enzyme pH of 4.01; total organic carbon 1.04%, organic matter 1.78%, C/N ratio 28.69. Based on these data, eco-enzymes have macro and micronutrients. The largest micronutrient content is Fe. The nutrient element Fe plays a role in influencing the activity of flavonoids by regulating expression and activity as well as regulation. The C/N ratio of the eco-enzymes was within the normal range for organic fertilizers (20-30). The eco-enzyme applied to the ginger plant has a complete nutrient content so that it can be used as a substitute for inorganic fertilizers. Based on these findings, eco-enzymes can substitute inorganic fertilizers due to their complete nutrient content.

Agronomic character analysis of red ginger plants

The agronomic character analysis of red ginger plants focused on the number of tillers, root dry weight, plant dry weight, and rhizome weight. The results of observations of these growth variables can be seen in *Table 1*.

The results showed that the application of eco-enzymes at concentrations of 0.25%, 0.5%, and 0.75% significantly increased the growth of red ginger plants compared to the control group (without eco-enzymes). Eco-enzymes stimulated root growth, likely due to the presence of amylase, protease, and lipase enzymes. These enzymes help in loosening the cell wall and promoting cell expansion. The high potassium levels in eco-enzymes also contributed to increased root growth by supporting protein synthesis and root cell enzyme activity.

Table 1. *The average root dry weight, plants dry weight, number of tillers, and red ginger rhizome weight at various eco-enzyme concentrations*

Eco-enzyme concentration	Root dry weight (g)	Plants dry weight (g)	Number of tillers	Red ginger rhizome weight (g)
Control (0%)	22.33 c	41.00 a	8.67 b	50.00 b
0.25%	38.00 b	61.33 a	7.00 b	51.00 b
0.50%	69.33 a	81.67 a	11.33 a	78.67 ab
0.75%	65.00 a	85.00 a	10.33 a	90.67 a

The mean number followed by the same letter in the same column is not significantly different based on the DMRT test at a 5% significance level

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DOI: http://dx.doi.org/10.15666/aeer/2106_54535461

Eco-enzyme, 0.75% concentration, can increase the production of ginger rhizome and is significantly different from the 0.25% concentration and control. The highest production was obtained at a concentration of 0.75%. This is because the content of nutrients and enzymes contained in eco-enzymes can stimulate plant growth, stimulate cell expansion, and synthesize compounds needed for plant growth (Rasit and Mohammad) (Wen et al., 2020; Siswanto et al., 2023; Ginting and Mirwandhono, 2021; Ling et al., 2023).

Analysis of phytochemical content

The quality of red ginger as a medicinal and cosmetic ingredient is determined by the phytochemical content present in the rhizome. The phytochemical content detected in red ginger rhizomes is flavonoids, terpenoids, and phenolic compounds. The gingerol and shogaol compounds found in ginger rhizome are polyphenolic compounds, so positive reactions to flavonoids, phenolics, and terpenoids indicate the results of the phytochemical test. Gingerol and shogaol compounds are the main components that form the spicy taste of red ginger. These three compounds are also natural antioxidants that work by reducing free radicals depending on the number of hydroxy groups in their molecular structure (Supu et al., 2018; Nurhadi et al., 2020).

The quantitative test results for the flavonoid and phenolic content in red ginger rhizome showed that the administration of eco-enzymes had a significant effect (p-value < 0.001) (*Table 2*). The highest mean of flavonoid content was found in an eco-enzyme concentration of 0.50% and was significantly different from concentrations of 0.75%, 0.25%, and control.

Table 2. *The average content of flavonoids (%) and phenolic (ppm) of red ginger rhizomes at various concentrations of eco-enzymes*

Eco-enzyme concentration	Flavonoids (%)	Phenolic (ppm)
Control	0.065 c	39.74 b
0.25%	0.042 c	22.70 c
0.50%	0.110 a	22.30 c
0.75%	0.086 b	53.15 a

The mean number followed by the same letter in the same column is not significantly different based on the DMRT test at a 5% significance level

Discussion

The analysis of eco-enzyme content revealed that eco-enzymes contain essential macro and micronutrients necessary for plant growth. The presence of Fe, in particular, plays a role in regulating the activity and expression of flavonoids. The C/N ratio of the eco-enzymes falls within the normal range for organic fertilizers, indicating their suitability as a substitute for inorganic fertilizers.

The agronomic character analysis demonstrated that the application of eco-enzymes significantly increased the growth of red ginger plants compared to the control group. The presence of amylase, protease, and lipase enzymes in eco-enzymes promoted root growth by loosening the cell wall and facilitating cell expansion. The high potassium levels in eco-enzymes supported protein synthesis and root cell enzyme activity, further enhancing root growth. The increased root growth subsequently increased the number

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2106_54535461

of tillers and the weight of red ginger rhizomes. Applying 0.75% eco-enzyme concentration resulted in the highest ginger rhizome production.

The application of eco-enzymes stimulates the growth of plant roots because eco-enzymes contain the enzymes amylase, protease, and lipase (Rasit and Mohammad, 2018; Barman et al., 2022). The cell wall composed of polysaccharides and proteins is flexible to environmental conditions, and enzyme hydrolase plays a role in loosening the cell wall for the expansion of plant cells (Ishada and Yokoyama, 2022; Nazipova et al., 2022). Eco-enzymes have high potassium levels to increase root growth; potassium is needed for protein synthesis and root cell enzyme activity (Sustr et al., 2019).

Roots are plant organs that play a role in absorbing water and nutrients plants need. The roots affect the growth and production of the resulting ginger rhizome (Jabborova et al., 2021). A sound root system will allow ginger rhizomes to grow appropriately and produce extensive, high-quality rhizomes (Arif et al., 2022). The results showed that the application of eco-enzymes at concentrations of 0.5% and 0.75% could increase the growth of red ginger roots, as indicated by the dry weight of the roots in the two treatments, which gave significantly different results from the concentration of 0.25% and the control. The increase in root growth was followed by an increase in the number of tillers and the weight of the red ginger rhizome. Fertilization using eco-enzymes 0.5% and 0.75% increased the number of red ginger tillers. The two treatments showed significantly different results with a concentration of 0.25% and control. The ability of eco-enzymes to stimulate cell expansion and synthesize compounds for growth will increase plant growth and the number of tillers. This is evidenced by the large number of tillers in the plants fertilized with 0.5% and 0.75% eco-enzymes.

The analysis of phytochemical content revealed that eco-enzymes significantly increased the flavonoid and phenolic content in red ginger rhizomes. Flavonoids, such as gingerol and shogaol, are essential components that contribute to the spicy taste of red ginger and possess antioxidant properties. The increased flavonoid content indicated improved ginger rhizome quality. The presence of amylase, lipase, and protease enzymes in eco-enzymes likely influenced the secondary metabolism in ginger plants, leading to increased flavonoid synthesis. Additionally, the high iron content in the eco-enzyme further enhanced the antioxidant effect of flavonoids.

The increase in ginger rhizome flavonoid content due to the administration of eco-enzymes will improve the quality of ginger rhizome. As a medicinal plant, ginger has health benefits because it contains many secondary metabolites in its rhizomes. Secondary metabolites in plants are generally flavonoids, alkaloids, steroids, saponins, terpenoids, and tannins. These secondary metabolites are beneficial in pharmacology as antiseptics, antioxidants, anti-inflammatories, anti-coagulants, anti-cancer, and antibiotics (Shukitt-Hale and Lau, 2008). The increased content of flavonoids in the ginger rhizome indicates the increased content of gingerol and shogaol in ginger. The quality of the ginger rhizome can be seen from the content of gingerol and shogaol in the rhizome. The results of this study indicate that the application of eco-enzymes can improve the quality of ginger rhizomes, as indicated by the high content of flavonoids in the rhizome.

1. The application of eco-enzymes to ginger plants can increase the flavonoid content of ginger rhizome, presumably due to the content of amylase, lipase, and protease enzymes present in eco-enzymes. These enzymes affect plant metabolism and play a role in the breakdown of carbohydrates into simple sugars, the breakdown of fats into fatty acids and glycerol, and proteins into amino acids. These compounds will

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DOI: http://dx.doi.org/10.15666/aeer/2106_54535461

affect the secondary metabolism in ginger plants. Flavonoids form stable and robust bonds when interacting with metal ions such as iron to enhance the antioxidant effect. Iron can affect the activity of flavonoids by regulating their expression and activity and regulation (Kejík et al., 2021). The eco-enzyme used in this study has a high iron content, so the red ginger rhizome produced also has a high flavonoid content.

2. Overall, the analysis of eco-enzyme content, agronomic characteristics, and phytochemical content demonstrated the beneficial effects of eco-enzymes on red ginger plants. The eco-enzymes provided essential nutrients, stimulated root growth, increased the number of tillers, and improved the quality of ginger rhizomes by enhancing flavonoid content. These findings support using eco-enzymes as a substitute for inorganic fertilizers in red ginger cultivation.

Conclusions

Fertilization using eco-enzyme concentrations of 0.50% and 0.75% can increase red ginger rhizome's rhizome weight and quality. Eco-enzymes 0.50% and 0.75% can significantly increase the phenolic and flavonoid content in the rhizomes. An eco enzyme concentration of 0.75% is recommended in red ginger cultivation to increase the production and quality of rhizomes.

Acknowledgments. Appreciation and gratitude are conveyed to the Universitas PGRI Yogyakarta for financial support and facilities of this study.

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DOI: http://dx.doi.org/10.15666/aeer/2106_54535461