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Sustainable use of eco-enzyme as biostimulants for enhancing yield and curcumin content in turmeric

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ABSTRACT
Turmeric (*Curcuma longa* L.) is widely cultivated for its medicinal properties and is used in the food industry. However, agricultural practices often prioritize rhizome yields over phytochemical richness. Efficacy studies have been conducted on turmeric harvest quality using organic fertilizers. However, there are no reports on the curcumin content of turmeric grown organically using ecoenzymes. This study introduces an innovative application of ecoenzymes as biostimulants in sustainable turmeric cultivation to enhance both rhizome quality and yield. A Completely Randomized Design (CRD) with three replicates was employed, involving four treatments: control, 0.5%, 1%, and 1.5% eco-enzyme concentrations. Data were analyzed using ANOVA and Duncan's Multiple Range Test at a 5% significance level. The results showed that while eco-enzyme application had no significant effect on tiller number and dry biomass at four months, it significantly increased rhizome weight per plant ($p<0.05$). Phytochemical screening confirmed the presence of curcumin in all the samples, with significantly higher concentrations in the eco-enzyme-treated groups. Additional statistical tests indicated positive correlations between eco-enzyme concentration and curcumin levels. This study highlights the potential of eco-enzymes as a sustainable alternative for turmeric cultivation, producing rhizomes rich in bioactive compounds. Compared to other sustainable practices, such as organic fertilization, eco-enzymes offer the dual benefit of enhancing yield and phytochemical content, with minimal environmental impact. Future research should investigate their composition, impact on soil microflora, and long-term ecological safety. These findings contribute to a growing body of knowledge on sustainable agriculture and biostimulants.

Contribution/Originality: This study presents a new use of eco-enzymes as natural boosters in growing turmeric. This method increases both the weight of the turmeric and its curcumin content. It is a major step forward in sustainable farming in Indonesia. The results provide a green solution by using organic waste (eco-enzymes) in an environmentally friendly way. This is important for the pharmaceutical and food industries that rely on turmeric's active compounds.

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1. INTRODUCTION

Turmeric is a plant used as medicine and a spice. It contains curcumin, which helps reduce inflammation, fights cancer, and acts as an antioxidant. Using chemical fertilizers harms the environment, so people are moving towards eco-friendly farming. Sustainable farming is important to meet global needs without harming nature. Eco-enzymes, made from fermented organic waste, help protect the environment and improve crop growth. This study looked at using eco-enzymes in growing turmeric to boost curcumin levels, supporting the global trend of eco-friendly farming.

Growing turmeric is hard because the soil is getting worse, and farmers use too much chemical fertilizers (Zhu, Sanidad, Sukamtoh, & Zhang, 2017). Studies show that using organic materials like compost and biofertilizers can help plants grow more, but they affect quality differently (Libutti, Trotta, & Rivelli, 2020). Eco-enzymes, which contain nutrients and enzymes, can help plants grow better, but they are not used much in turmeric farming (Khan et al., 2023). Regular farming methods often reduce the organic matter in soil, which is bad for plant growth (Montgomery & Biklé, 2021). We need better ways to keep the curcumin levels high and protect the soil.

This study is important for addressing the global challenge of turmeric with high curcumin content through sustainable agricultural practices, which contribute to the fields of agriculture and pharmacy. By evaluating the effectiveness of eco-enzyme use in agriculture, this study introduces a new organic fertilizer to increase curcumin levels, potentially reducing dependence on chemical inputs (Kariada et al., 2023). These findings can inform large-scale agricultural practices that are aligned with environmental sustainability goals. Additionally, this study provides insights into the interaction between eco-enzymes and secondary metabolite production in medicinal plants.

According to Hemalatha and Visantini (2020) eco-enzymes produced through the fermentation of organic kitchen waste, such as fruit and vegetable scraps, brown sugar, palm sugar, or cane sugar, along with water, is a process that requires approximately three months (Barman, Hazarika, Gogoi, & Talukdar, 2022; Nazim & Meera, 2013). Eco-enzymes have a wide range of applications, particularly during the pandemic, as they can be utilized as disinfectants and hand sanitizers. In agriculture, they can function as fertilizers, pesticides, and germicides, as well as purify contaminated river water.

In the early 1900s, research mainly aimed to boost turmeric production using chemical fertilizers, often ignoring curcumin levels. In the late 1900s, organic farming became popular, highlighting the importance of biofertilizers and their impact on plant compounds (Gao et al., 2020; Panday, Bhusal, Das, & Ghalehgolabbehbahani, 2024). Eco-enzymes, a type of biofertilizer, use microorganisms such as bacteria, fungi, and algae to improve soil and plant health. These biofertilizers are beneficial because they can fix nitrogen from the air, make nutrients like phosphorus and potassium available, and boost soil microbial activity (Dhir, 2017). Recent studies are examining the use of eco-enzymes in medicinal plants, which could facilitate their application in cultivating turmeric.

Ecoenzymes are important for increasing soil microbes, which help us understand how carbon and nutrients move in land ecosystems (Gao et al., 2022). These enzymes help rice plants take in more nitrogen by affecting the genes and processes that control how well plants use nitrogen (Lee et al., 2020). Ecoenzymes also help good microbes grow around plant roots, which keeps plants healthy (Velmourougane et al., 2017). Spinach treated with enzymes has more antioxidants, which can be good for health by lowering stress in the body (Simon et al., 2023). But, using these treatments on turmeric is not common, so more research is needed (Kaur, Al-Khazaleh, Bhuyan, Li, & Li, 2024). While ecoenzymes can improve crop yield and quality, their effects on turmeric need more study.

These eco-friendly solutions are popular because they can be used in sustainable farming and caring for the environment (Osman et al., 2024; Shanka, 2020). They contain many important nutrients, making them great as organic fertilizers (Dhaliwal, Naresh, Mandal, Singh, & Dhaliwal, 2019). They are also studied for cleaning household wastewater and industrial waste, such as from tofu production. In short, eco-enzymes are a new, cost-effective, and environmentally friendly way to manage waste and support sustainable practices (Das et al., 2024; Salsabila, 2024). Making them helps reduce organic waste and creates a useful product for farming, animal health, and cleaning the environment (John, Sharma, & Venkatesh, 2023).

Curcumin in turmeric is affected by the environment, like soil quality and microbes (Khan et al., 2023). Nitrogen and phosphorus levels greatly affect curcumin gene activity (Oyarce et al., 2019). But the effect of microbial substances, like those in eco-enzymes, on curcumin production is not yet known. Turmeric and its roots are used in traditional medicine, herbal products, cosmetics, and cooking spices (Ibáñez & Blázquez, 2020; Sharifi-Rad et al., 2020).

Curcumin is produced through a process called the phenylpropanoid pathway. This process is influenced by soil nutrients and microbes (Shofia, Anggraeni, & Nurlaila, 2024). Eco-enzymes result from the breakdown of organic waste without oxygen. They are rich in enzymes, organic acids, and beneficial microbes that enhance soil health (Blair, Dickson, & O'Malley, 2021). Different types of soil microbes are important for recycling nutrients and affect how plants make certain chemicals (Saghai, Wittorf, Philippot, & Hallin, 2022). Sustainable farming uses organic materials to reduce harm to the environment and improve crop quality. This study examines how eco-enzymes interact with the curcumin-making process in turmeric.

Soil nutrient imbalances affect curcumin production, so good management is needed (Beganovic & Wittmann, 2024). Chemical fertilizers can harm soil microbes, reducing curcumin over time (Dincă, Grenni, Onet, & Onet, 2022). Organic amendments should be optimized to boost activity without lowering yield (Kracmarova et al., 2020). Ecoenzymes might help, but they need thorough testing in turmeric farming. Using ecoenzymes could increase curcumin and keep the soil healthy. More research is needed to find the best ways to use them for different turmeric types and soils. Working together, experts in farming, biochemistry, and soil science can help develop sustainable ways to boost curcumin in turmeric.

Ecoenzymes help improve soil health, make nutrients more available, and increase plant growth using eco-friendly farming methods (Mishra, Maurya, & Sharma, 2024; Srivastava et al., 2023). Organic materials like biofertilizers also boost plant yield (Dzvene & Chiduza, 2024). Ecoenzymes have been successful in many crops but are not well-studied in turmeric. More research is needed to use ecoenzymes in medicinal plants. Studies show that ecoenzymes can improve crop yields and soil health, but their use in turmeric is still limited (Purwaningsih, Hi Wahid, & Pamungkas, 2023). The effect of ecoenzymes on curcumin production is not well understood.

This study aimed to assess the quality of turmeric rhizomes cultivated in an environmentally friendly manner using eco-enzymes. Additionally, the agronomic characteristics of turmeric plants cultivated in an environmentally friendly manner were examined.

This study contributes to a novel eco-enzyme approach to enhance curcumin biosynthesis, advancing sustainable agriculture and medicinal plant production.

2. MATERIALS AND METHODS

2.1. Study Site

This study took place from February to July 2024 at the Experimental Garden of the Faculty of Agriculture at Universitas PGRI Yogyakarta (UPY) in Bantul, Yogyakarta Special Region, Indonesia. The area has a tropical climate, with temperatures ranging from 25 to 30°C and humidity levels of 78 to 84%. The study site is approximately 70 meters above sea level.

2.2. Materials and Methodology

The research utilized turmeric roots, eco-enzymes, polybags, Regusol soil, rice husks, ethanol, digital scales, a spectrophotometer, and an oven. Regusol is sandy soil with over 80% sand and less than 10% clay, and its pH ranges between 6 and 7. It exhibits good air and water flow but has low organic matter content (approximately 0.95%) and retains little water and nutrients. The study employed a Completely Randomized Design with three replicates. There were four treatments: no eco-enzymes, 0.5% eco-enzymes, 1% eco-enzymes, and 1.5% eco-enzymes. Each treatment consisted of 16 polybags (Figure 1).

Figure 1. Cultivation and care of *Curcuma longa* plants with eco-enzyme.

2.3. Sustainable Cultivation

Turmeric was grown by sprouting healthy rhizomes in a box with soil and rice husks. After about a month, when sprouts appeared, the rhizomes were moved to bags filled with the same mix. The plants were watered daily unless it rained. Eco-enzymes were given every two weeks, with 100 mL added to each bag. Eco-enzymes are made by fermenting fruit and vegetable waste, molasses, and water in a 3:1:10 ratio. This process took four months. The waste came from papaya, orange, apple, banana, watermelon, melon, dragon fruit, pear, carrot, spinach, cabbage, kale, and mustard greens. The eco-enzyme used in this study had N 0.04%, P 0.008%, K 0.34%, Fe 17.72 ppm, Zn 0.84 ppm, Mn 3.82 ppm, pH 4.01 (Purwaningsih et al., 2023).

2.4. Observation and Data Analyzed

Observations included measurements of dry plant weight, number of tillers, rhizome weight, and the quantified phytochemical content of the rhizomes. Data from the study were analyzed using analysis of variance at the 5% significance level. Duncan's multiple range test was conducted to determine significant differences between treatments at the same significance level.

2.5. Qualitative and Quantitative Analysis of Curcumin

Qualitative analysis followed the method by Harborne et al. For quantitative analysis, a spectrophotometric method was used. A standard curve was prepared by dissolving 1 mg of curcumin in 95% ethanol. It was then diluted to obtain concentrations of 0, 2, 4, 6, 8, 10, and 12 ppm. The absorbance of each solution was measured at a wavelength of 420 nm using a spectrophotometer. To measure curcumin in the turmeric extract, 1 mg of the extract was dissolved in 95% ethanol. Its absorbance was recorded at 420 nm using the same method. The curcumin content in the extract was calculated using the standard curve and expressed as the amount of curcumin in the sample (Tanesib, Rahmawati, & Darusman, 2023).

3. RESULT AND DISCUSSION

3.1. Analysis of Plant Growth and Yield

The observed growth and yield variables of the turmeric plants included the number of tillers, dry shoot weight, dry root weight, and rhizome weight. Data collection for these variables was conducted when the turmeric plants reached four months of age. The results of the analysis of variance (ANOVA) and Duncan's multiple range test are presented in Table 1 and 2.

Table 1. Tiller number, dry root weight (g), and dry shoot weight (g) at various eco-enzyme concentrations.

Eco-enzyme concentrations	Tiller number	Dry root weight (g)	Dry Shoot weight (g)
0 (Control)	3.44 a	18.78 a	62.05 a
0.5% (Eco-enzyme)	3.50 a	17.27 a	57.56 a
1% (Eco-enzyme)	3.56 a	19.94 a	68.17 a
1.5% (Eco-enzyme)	3.83 a	17.11 a	64.78 a

Note: Numbers followed by the same letter indicate not significantly different based on Duncan's multiple range test at the 5% significance level.

Table 1 demonstrates that the application of eco-enzymes at various concentrations did not significantly influence the growth of turmeric plants, as evidenced by the variables of tiller number, dry root weight, and dry shoot weight at four months after planting. Turmeric plants not subjected to eco-enzyme treatment (control) exhibited no significant difference in growth compared to plants treated with eco-enzymes at all tested concentrations.

In this study, the researchers examined three plant growth factors: dry root weight, dry shoot weight, and tiller number. These factors indicate how well plants grow. Roots assist plants in absorbing water and nutrients, which are essential for growth (Fageria & Moreira, 2011). Plants with strong roots can absorb more water and nutrients, leading to better growth, as shown by the dry root weight. Roots also produce hormones that affect how plants grow by controlling nutrient intake (Sathiyavani, Prabaharan, & Surendar, 2017). The eco-enzymes used in this study contain the nutrients necessary for root growth, so plants treated with eco-enzymes exhibit improved root development. Figure 2 illustrates turmeric rhizomes cultivated using eco-enzymes.

Figure 2. Turmeric rhizomes after harvesting time.

Dry shoot weight shows how much food a plant makes and stores in its shoots. More dry shoot weight means the plant is making more food, which indicates it is efficient at photosynthesis (Li et al., 2022). Photosynthesis is needed to make plant material (Zou et al., 2022). Some enzymes help in photosynthesis by balancing how food is made and used. The food produced by photosynthesis supports plant growth, including leaf and flower development (Singh, Nath, Marboh, & Sharma, 2017), like forming new shoots and developing turmeric roots..

In this study, there were no significant differences in dry root weight, dry shoot weight, and tiller number between plants with and without eco-enzyme treatment. This may be because the plants were only four months old, and their growth was still developing. At this stage, both vegetative and generative parts of the plant were still growing.

Vegetative growth influences generative growth and plant yield (Barłóg, Grzebisz, & Łukowiak, 2018). In this study, turmeric rhizome weight was observed to assess yield. The results of the analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) for rhizome weight are shown in Table 2.

Table 2. Turmeric rhizome weight (g) at various eco enzyme concentrations.

Eco-enzyme concentrations	Rhizome weight per clump (g)	Rhizome weight per plant (g)
0 (Control)	209.11 a	82.16 b
0.5% (Eco-enzyme)	220.01 a	107.71 a
1% (Eco-enzyme)	206.11 a	105.62 a
1.5% (Eco-enzyme)	242.28 a	110.64 a

Note: The letters a and b are notations to show real differences between treatments. Numbers followed by the same letter indicate not significantly different based on Duncan's multiple range test at the 5% real level.

Table 2 shows that using an eco-enzyme does not greatly increase the rhizome weight per clump, but it does increase the rhizome weight per plant. There is a strong positive link between the number of tillers and the rhizome weight per clump (r = 0.65*). The rhizome weight was measured when the turmeric plants were four months old, so not all tillers had grown rhizomes, or the rhizomes were not fully developed. The number of tillers was counted based on having opened leaves. The eco-enzyme likely helped because it contains enough N, P, and K nutrients needed for rhizome growth (Noor, Akhter, Islam, Hasan, & Hamidullah, 2014; Samanhudi, Nugroho, Santosa, & Harjanto, 2014).

3.2. Curcumin Content Analysis of Turmeric Rhizomes

In this study, turmeric plants were picked four months after planting. The rhizomes were checked for curcumin levels. Wahid, Harahap, Rambe, and Nasution (2023) found that using eco-enzyme treatments significantly increases the amount of beneficial compounds in ginger, making it a more effective antioxidant. The positive effects of eco-enzymes on medicinal plants come from the way they work together with the plant's natural growth processes. This interaction helps initiate certain processes in the plant, leading to more beneficial compounds and enhancing the plant's effectiveness for health.

Table 3. Average curcumin content in fresh turmeric rhizomes at various eco-enzyme concentrations.

Eco-enzyme concentrations	Curcumin content (ppm ± SD)
0 (Control)	2.312 ± 0.009 d
0.5% (Eco-enzym)	4.524 ± 0.002 a
1% (Eco-enzym)	4.135 ± 0.008 b
1.5% (Eco-enzym)	3.005 ± 0.001 c

Note: The letters a, b, c, and d are notations to show real differences between treatments. Numbers followed by the same letter indicate not significantly different based on Duncan's multiple range test at the 5% significance level.

ANOVA results in Table 3 show that using eco-enzyme at different levels increased curcumin in turmeric rhizomes compared to no eco-enzyme (0%). The most curcumin was at 0.5% eco-enzyme, which was much more than with no eco-enzyme, and more than with 1% and 1.5% eco-enzyme. Using more eco-enzymes did not increase curcumin. In fact, more eco-enzymes reduced curcumin levels.

The elevated curcumin content at 0.5% eco-enzyme level can be attributed to the enzymatic action of the eco-enzyme, which may have increased curcumin solubility or release. Eco-enzyme, containing enzymes such as proteases, amylases, and lipases, may cause the breakdown of cell walls or other structures, thereby promoting the extraction of bioactive materials (curcumin).

The positive impact of low pH (acidic nature of eco-enzyme) might reinforce this effect, as an acidic environment could enhance curcumin solubility (Hewlings & Kalman, 2017; Kępińska-Pacelik & Biel, 2023).

The decrease in curcumin content at 1 and 1.5% eco-enzyme indicates an inhibitory or saturation effect. At 1.5%, curcumin content was found to be significantly lower compared to 0.5% and 1%. This phenomenon may be due to curcumin degradation resulting from higher enzymatic activity and unfavorable pH changes that occur at elevated eco-enzyme concentrations.

Curcumin is susceptible to degradation by oxidation or hydrolysis under certain conditions, which can be triggered by high enzyme concentrations (Zhu et al., 2017). Comparison with the control validated the significance of the eco-enzyme in increasing curcumin content, and a 95.5% increase was observed at 0.5% concentration. The use of multiple enzymes resulted in higher extraction yields of phenolic compounds. However, when curcumin was at 1.5%, the results began to decline, which indicates that the use of eco-enzymes has an upper limit that must be considered at certain concentrations.

The results of the regression analysis of eco-enzyme concentration and curcumin levels are expressed in a regression equation, $y = 0.338x + 3.2405$. The regression equation graph can be seen in Figure 3.

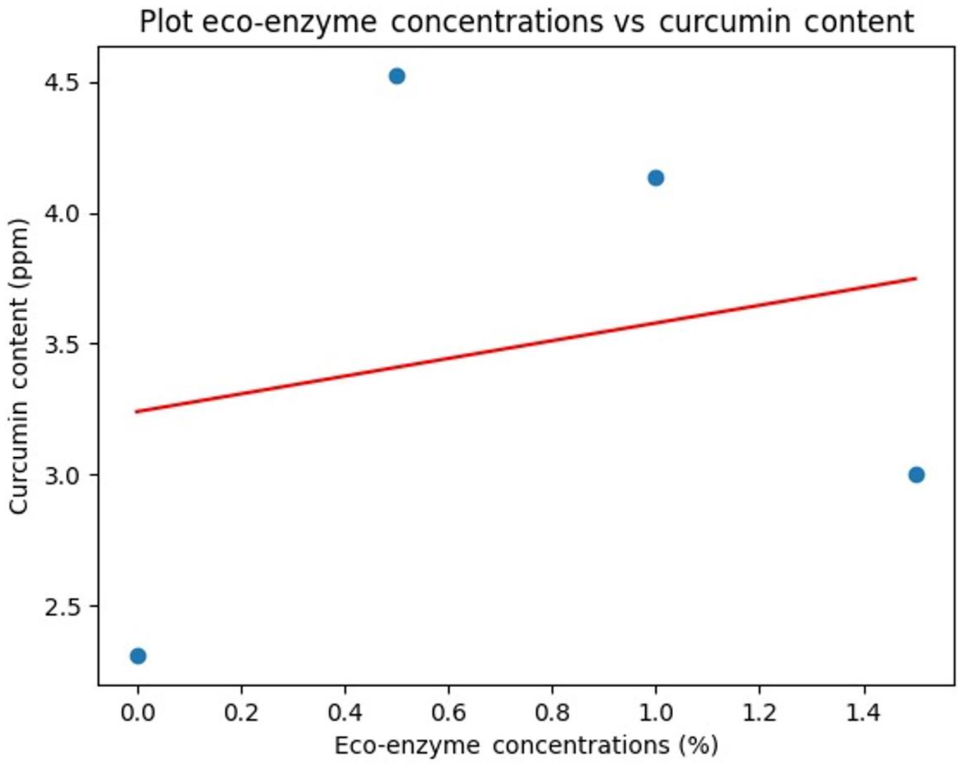

Figure 3. Regression analysis graph of the eco-enzyme concentration and curcumin levels.

4. CONCLUSIONS

The application of eco-enzyme in turmeric cultivation did not result in statistically significant increases in tiller quantity, dry root mass, or dry shoot mass in four-month-old turmeric plants; however, it did lead to a significant increase in rhizome mass per plant.

Phytochemical analysis revealed the presence of curcumin compounds in turmeric rhizomes across both the control and all eco-enzyme concentrations. The eco-enzyme treatment significantly elevated the curcumin content in turmeric rhizomes ($p<0.05$. Using ecoenzymes to grow turmeric can help increase curcumin, which is important for making traditional medicine. This can lower production costs. Research on ecoenzymes to boost natural compounds is suggested to advance the use of natural materials in medicine and health products.

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