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Increasing Growth Rate and Production of Bioactive Compounds Curcuminoid and Xanthorrhizol in Javanese Turmeric (*Curcuma xanthorrhiza* Roxb.) Rhizomes with Biso Zyme Application

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Abstract

The bioactive compounds from Javanese turmeric (*Curcuma xanthorrhiza* Roxb.), curcuminoid and xanthorrhizol, have been used to treat human diseases. This research aims to study the Javanese turmeric growth, its curcuminoid and xanthorrhizol content using Biso Zyme with different concentration and interval spraying. Biso Zyme is is conjugated enzyme concentrate (CEC) produced by yeast extracts that can be applied to the plant to increase and improve plant yields. The treatments were arranged in a randomized block factorial design with three replications. The concentrations of Biso Zyme were 0.0, 0.1, and 0.05 %, with monthly spraying intervals at 1, 2, 3, 4 and 5 months after planting (MAP). The results showed that the highest leaf area index was 2.71 cm^2g^{-1} followed by leaf area ratio which was 66.47 $cm^2.g^{-1}$ with 0.1% Biso Zyme application. Relative growth rate was 0.048 $g.day^{-1}$ while net assimilation rate was 0.00092 $g.cm^{-2}.day^{-1}$ without Biso Zyme application. The highest fresh and dry weight was those treated with 0.05% Biso Zyme applied at 4 to 5 MAP, i.e., 444.44 g and 68.72 g, respectively. The highest curcuminoid and xanthorrhizol contents were 0.435% and 1.505%, respectively, with 0.05% Biso Zyme spraying interval at 4 to 5 MAP, and 0.1% Biso Zyme with sprayed at 1 to 5 MAP. Thin layer chromatography (TLC) analysis detected standard curcuminoid at Rf 0.23 and xanthorrhizol at Rf 0.66 with dichloromethane:chloroform eluents.

Keywords: curcuminoid, Javanese turmeric, Leaf Area Index, organic fertilizer, xanthorrhizol

Introduction

Turmeric (*Curcuma xanthorrhiza* Roxb.) is a rhizomatous herbaceous perennial plant belonging to the ginger family Zingiberaceae. Javanese turmeric is species native to the forests of Indonesia and the Malaysian peninsula. Javanese turmeric has a long history of medicinal use in Indonesia; the therapeutic effects are caused by curcuminoid and xanthorrhizol derived from turmeric rhizomes. Curcuminoid has anti-inflammatory effects and is a very strong antioxidant as well as anti-hypercholesterolemic (Moemeni et al. 2013), and can be used to prevent cholera (Kim et al., 2014), whereas xanthorrhizol has biological activities such as anticancer, antimicrobial, anti-inflammatory, antioxidant, antihyperglycemic, antihypertensive, antiplatelet, nephroprotective, hepatoprotective, estrogenic and anti-estrogenic effects (Peschel et al. 2006; Oon et al., 2015). An increase in yield and bioactive compound production can be achieved by increasing the biomass of the plants through fertilization. Fertilization can affect the production of bioactive compounds, in this Purnomo et al. (2018) reported that cultivating turmeric in an agroforestry system with one-year-old silk tree with 100 kg NPK 15-15-15 ha^{-1} application produced 139 g fresh rhizome from three-month-old plants. *Curcuma xanthorriza* applied with manures at 20 $t.ha^{-1}$ at one month before planting had the xanthorrhizol content of 43.55% to 47.99%, with "Ngawi" and "Wonogiri" accessions having the lowest and highest values, respectively. IC50 value for α-glucosidase inhibition ranged from 339.05 $g.mL^{-1}$ to 455.01 $g.mL^{-1}$ for "Karanganyar" accession,"Ngawi" had the lowest, and "Wonogiri" had the highest. LC50 values for cytotoxic activities ranged from 33.25 $g.mL^{-1}$ ("Ngawi") to 42.28 $g.mL^{-1}$ ("Karanganyar") with brine shrimp lethality test, 3.10 $g.mL^{-1}$ ("Karanganyar") to 9.85 $g.mL^{-1}$ (cursina-III) in

Vero cell culture, and 1.17 g.mL-1 ("Ngawi") to 6.83 g.mL-1 ("Sukabumi") in MCF-7 cell culture (Nurcholis et al., 2018).

Tropical Biopharmaca Research Center (Trop BRC) with NAIST Japan cooperation examined the effects of Biso Zyme application on growth and bioactive compounds of medicinal plants Wijaya et al. (2016) stated that Biso Zyme is conjugated enzyme concentrate (CEC) produced by yeast extracts and can be applied to the plants to increase and improve plant yields. Conjugated enzyme concentrate contains small molecules, such as nutrient compounds, co-enzymes, and proteins which is helpful for soil management without any contamination of bacteria. Therefore, the CECs are safe to use as it supplies CEC to promote bacterial growth without directly affecting the ecosystem. The type of Biso Zyme used in cultivation of Javanese turmeric is DT1000 (for soil management), which have been produced by extraction of yeasts incubated in a molasses fraction from sugarcane. This product is commercially available in Japan, but not in Indonesia. Biso Zyme may be applied by spraying on the leaf or soil drench. Biso Zyme can improve yields in crops of fruit, vegetables with minimum impact on the environment. Crops that have been tested with the provision of Biso Zyme include grapes, tomatoes, red pepper, cucumber, ginger, melon, mango, onions, and rice. Ahemad and Kibret (2013) stated that mechanism for rhizobacteria growth promotion was by direct, such as through increasing nitrogen fixation, phosphate solubilization, siderophore production, phytohormone production, 1-amino cyclopropane-1-carboxylate (ACC) deaminase, and indirect mechanisms such as acting as biocontrol agent competition for nutrients, niche exclusion, induced systemic resistance and antifungal metabolites production. Wijaya et al. (2016) further validated the repellent effects of CEC for thrips, whitefly, cutworm, *Bradysia difformis* Frey, larva of tea tussock moth (*Euproctis pseudoconspersa*), aphid (plant louse), spider mite, shield bug (stink bug), cucurbit leaf beetle (*Aulacophora femoralis*), handsome fungus beetle (Endomychidae), root-lesion nematode, larva of slug moth, anthracnose, downy mildew and powdery mildews. Similar experiments were conducted in Japan and the results showed that CEC could boost crop yields. Research on Biso Zyme in Indonesia was conducted in Bogor Agricultural University and the University of Andalas. This research aims to study the growth of Javanese turmeric and its curcuminoid and xanthorrhizol content with different Biso Zyme concentrations and spraying intervals.

Material and Methods

Field experiments were conducted in Biopharmaca Cultivation and Conservation Station, Tropical Biopharmaca Research Center on IPB Campus, Bogor, Indonesia, ± 250 m above sea level. The research was conducted from February to August 2016. Curcuminoid and xanthorrhizol content were analyzed at the Tropical BRC Laboratory.

The materials used consisted of Javanese turmeric plants in polybags (Figure 1 A and B), manure, rice-hull ash and Biso Zyme. The equipment used consisted of planting equipment, measuring instrument, hand sprayer, HPLC (High Performance Liquid Chromatography) and Thin Layer Chromatography (TLC).

Figure 1. One-month-old (A) and six-month-old (B) Javanese turmeric plants in the field; Javanese turmeric plants that are ready to harvested before (C) and after (D) separated from their roots.

The experiment was laid out in a randomized block factorial design. The treatments consist of two factors, the Biso Zyme concentrations of 0, 0.1, and 0.05%, and spraying intervals, i.e. 1 to 5, 2 to 5, 3 to 5, 4 to 5 and 5 months after planting. Each treatment was repeated in three replications so that there are 45 experimental units, each consisted of five plants grown individually in polybags (Figure 1 A and B) .

Components of growth and production variables measured were leaf area index (LAI), leaf area ratio (LAR), relative growth rate (RGR), net assimilation rate (NAR), fresh weight, dry weight and content analysis of curcuminoid and xanthorrhizol. Data was analyzed using analysis of variance (ANOVA) with significance levels of α 5%; significant differences between means were further analysed with Duncan's Multiple Range Test (DMRT) at α 5%.

TLC analysis to detect the presence of curcuminoid and xanthorrizol compounds using silica gel 60 F_{254} TLC plate with eluents of Dichloromethane: chloroform of 2: 8. Dried rhizomes (10 g) were analysed for their curcuminoid and xanthorrizol content using HPLC. The stationary phase used in curcuminoid analysis is C 18 column; mobile phase is acetonitrile and acetic acid 2% with 45-55% acetonitrile composition, wavelength 425 nm, flow rate one mL $.min^{-1}$ and oven temperature of 30^{O}C. The stationary phase used in the xanthorrizol analysis is C 18 column; the mobile phase is methanol and phosphoric acid 0.2% with compositions as described in Table 1.

Result and Discussion

Soil Microbial Biomass

The soil microbial analysis before and after Biso Zyme application is presented in Table 2. The amount of phosphate solubilizing microbes before the experiment was not detected and showed no clear pattern after Biso Zyme application. The initial level of Azotobacter was $1.33x10^4$ $CFU.g^{-1}$ and not detected after treatments. The highest phosphate solubilizing microbes were found with spraying interval 1 to 5 MAP, which was 500% higher than spraying once at 5 MAP. These microbes, however, was not detected in the other treatments.

The phosphate solubilizing microbes and Azotobacter mainly live in the rooting zone. Plant roots affect the lives of microorganisms surrounding to the root zone to be more active than those living away from the root zone. The microorganism existence is associated with the amount of organic matter in the soil which directly affects their abundance and activities. In addition, the microbial growth is highly influenced by soil acidity (Ginting et al., 2006). Soil bacteria having the phosphate solubilizing capacity are called Phosphate Solubilising Bacteria (PSB). These bacteria convert the insoluble phosphate into soluble form through the production of organic acids and make it available for plant uptake and nutrition (Satyaprakash et al., 2017).

The soil used in this experiment has a low pH and low organic matter, which is possibly affected by Biso Zyme activity. Enzyme activities play important roles in the biochemical functioning of soils, and information on enzyme activities can be used to describe changes in soil quality (Acosta-Martinez et al., 2007). Soil treatment might alter soil enzymatic activity and subsequently, the growth of the crops; the changes depend on the composition and availability of soil

Table 1. The composition of methanol and phosphoric acid for HPLC*

Time (minutes)	Methanol (%)	Phosphoric acid 0.2% (%)
0	75	25
20	75	25
22	98	2
24	98	2
26	75	25
33	75	25

Note: *at wavelength of 224 nm, flow rate 1 mL $.min^{-1}$ and oven temperature of 40^{O}C.

Table 2. Counts of soil microbes following Biso Zyme treatments

Treatment	Soil microbial (CFU per g)	
	Σ Phosphate solubilizing microbes	Σ Azotobacter
Without Biso Zyme	0	$1.33x10^4$
Spraying interval 1 to 5 MAP	$0.30x10^3$	0
Spraying interval 3 to 5 MAP	0	0
Spraying interval 5 MAP	$1.50x10^3$	0
Biso Zyme at 0.1%	0	0
Biso Zyme at 0.05%	0	0

Note: CFU = colony forming unit; MAP = month after application. The count of soil microbes were conducted once without replication nutrients, pH, and texture of the soil (Kalembasa and Symanowicz, 2012). According to Vurukonda et al. (2016) plant growth promoting rhizobacteria colonize the rhizosphere and endorhizosphere, and induce plant drought tolerance by modulating phytohormone levels and alteration in root morphology. Calvo et al. (2014) stated that there are some commonalities in plant responses to different biostimulants, such as increased root growth, enhanced nutrient uptake, and stress tolerance (Calvo et al., 2014).

Plant Growth Rate

LAI and LAR were not affected by the Biso Zyme concentrations (Table 3). LAI increases with increasing light intensity until the optimum limit crop to intercept light reached. Table 3 showed that there was an increase in LAI up to 4 MAP. Maximum LAI was produced at 0.1% Biso Zyme application and the lowest was with 0.05% Biso Zyme application. The decline in LAI occurred at 5 MAP due to the decrease in the number of leaves, because most of the leaves begin to senesce at this age.

LAR indicates photosynthetic surface area per unit dry weight of a plant and is a measure of the efficiency with which plants deploy their photosynthetic resources. Javanese turmeric showed a declining LAR with increasing age of the plant (Table 3). Similar results were reported on *Gynura pseudochina* in which LAR values decreased with increasing age of the plant (Ghulamahdi et al. 2008). LAR maximum value was obtained without Biso Zyme application (Table 3). Decreased LAR at 3, 4, and 5 MAP was possibly caused by the low increase in leaf area and the higher rate of photosynthesis that results in the high rhizome dry matter.

Table 4 showed that RGR and NAR were not affected by the Biso Zyme application. Increase in RGR and NAR at 2-3 MAP showed rapid growth and decreased with increasing age of the plant at 4 to 5 MAP. The data showed that the Javanese turmeric plants had entered the rhizome filling stage, so most of the photosynthates were allocated to the other organs resulting in the decreased vegetative growth including in leaves and stems. RGR and NAR increased rapidly at the beginning of growth, but declined with increasing age of the plant (Moemeni et al., 2013; Sridevi and Chellamuthu, 2015).

Rhizome Fresh and Dry weight

Rhizome fresh and dry weight at 6 MAP was significantly affected by the Biso Zyme spraying interval. Rhizome fresh and dry weight were the highest with spraying interval of 4 to 5 MAP (444.44 g) whereas spraying interval of 2 to 5 MAP had the lowest (307.41 g) (Table 5).

Rhizome dry weight significantly affected by the Biso Zyme concentration (Table 5). The highest rhizome fresh and dry weight was obtained by applying 0.05% Biso Zyme, which were 18.67% and 15.31% higher than the lowest fresh and the dry weight without Biso Zyme application, respectively. The results are in line with the results of Wijaya et al. (2016) in potato that Biso Zyme increased the production of potato tubers.

The interaction between the Biso Zyme concentration and spraying interval affect rhizome dry weight (Table 6). Biso Zyme concentration at 0.05% and the spraying interval treatment 4 to 5 MAP produced a significantly the higher rhizome weight, whereas application of 0.1% Biso Zyme with 2 to 5 MAP spraying interval had the lowest (Table 6).

No pattern was found on amount of the phosphate

Table 3. Leaf Area Index (LAI) and Leaf Area Ratio (LAR) of Javanese turmeric with Biso Zyme application at 2, 3, 4 and 5 MAP

Treatment	Month after planting			
	2	3	4	5
Biso Zyme concentration (%)	Leaf Area Index (LAI)			
0	0.48[1)]	1.35	2.44	1.95
0.1	0.48[1)]	1.54	2.71	1.45
0.05	0.64[1)]	1.68	2.09	1.26
Biso Zyme concentration (%)	Leaf Area Ratio (LAR) ($cm^2.g^{-1}$)			
0	65.76	50.55	50.50	34.04
0.1	64.81	66.47	51.67	27.41
0.05	57.91	57.23	51.08	33.46

Note: values followed by the same letters within each column were not significantly different at 5 % of DMRT, [1)] = transformation of $\sqrt{x}$ + 1.

Table 4. Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) at various Biso Zyme concentrations treatment at 2 to 5 MAP

Treatment	Plant age (MAP) by month		
	2 to 3	3 to 4	4 to 5
Biso Zyme concentration (%)	Relative growth rate (RGR) (g per day)*		
0	0.048	0.021	-0.009
0.1	0.042	0.026	0.002
0.05	0.036	0.011	0.012
Biso Zyme concentration (%)	Net Assimilation Rate (NAR) ($g.cm^{-2}$ per day)*		
0	0.00092	0.00044	-0.00020
0.1	0.00067	0.00045	0.00005
0.05	0.00062	0.00021	0.00033

Note: values followed by the same letters on the same column were not significantly different at 5 % of DMRT; *= √x + 1 transformation

Table 5. Fresh weight and dry weight of Javanese turmeric rhizome at 6 MAP

Biso Zyme spraying interval	Rhizome fresh weight (g)	Rhizome dry weight (g)
1 to 5 MAP	327.78b	60.22ab
2 to 5 MAP	307.41b	46.20c
3 to 5 MAP	340.74b	55.32bc
4 to 5 MAP	444.44a	68.72a
5 MAP	351.85b	56.50b
Biso Zyme concentration (%)		
0	333.33	53.83b
0.1	334.44	56.28ab
0.05	395.56	62.07a

Note: values followed by the same letters within the same column are not significantly different at 5 % of DMRT

solubilizing microbes, and plant growth variables were not affected by Biso Zyme application. Rhizome dry weight, however, increased with increasing concentrations of Biso Zyme. The highest rhizome fresh and dry weight was with Biso Zyme application at 4 to 5 MAP and 1 to 5 MAP. This data indicated that Biso Zyme could affect Javanese turmeric rhizome when applied at 5 MAP which is synchronized with rhizome filling phase. Without Biso Zyme application the RGR and NAR decreased at 4 to 5 MAP.

Bioactive Compounds in Turmeric Rhizomes

The results of Curcuminoid and xanthorrhizol analysis in turmeric rhizomes are presented in Table 7. Rhizomes from plants applied with Biso Zyme at 0.05% with spraying interval 4 to 5 MAP had the highest curcuminoid content, whereas the highest xanthorrhizol was obtained with Biso Zyme at 0.1% with spraying interval of 1 to 5 MAP. Many factors can affect the content of bioactive compounds, including the time of harvesting. Harvest in this study was conducted at 5 MAP because the leaves had started to senesce. According to Khaerana et al. (2008) harvesting Javanese turmeric at seven months can increase rhizome's xanthorrhizol content.

Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) analysis was conducted to detect the presence of curcuminoid and xanthorrhizol compounds in the Javanese turmeric rhizomes. Curcuminoid and xanthorrhizol detection with 254 nm UV lamp detection was less visible, whereas at 366 nm UV curcuminoid is visible as a green color. Spot xanthorrhizol is visible with anisaldehyde reagents with purple spots. The standard curcuminoid and xanthorrhizol with eluents dichloromethane: chloroform were visible on Rf 0.23 and Rf 0.66, respectively. The spots in the sample were detected on the same Rf, confirming that curcuminoid and xanthorrhizol are present (Figure 2 A, B, C).

Table 6. Effect of the interaction between Biso Zyme concentration and spraying interval of the dry weight of Javanese turmeric rhizome

Biso Zyme Concentration (%)	Spraying interval				
	1 to 5 MAP	2 to 5 MAP	3 to 5 MAP	4 to 5 MAP	5 MAP
	.... g				
0	53.83bcd	53.83bcd	53.83bcd	53.83bcd	53.83bcd
0.1	57.50bc	36.22d	59.56bc	69.44ab	58.67bc
0.05	69.33ab	48.56cd	52.56bcd	82.89a	57.00bc

Note: values followed by the same letters within the same column are not significantly different using DMRT α 5 %

Table 7. Curcuminoid and xanthorrhizol content of 6-month-old Javanese turmeric rhizomes treated with Biso Zyme at different spraying intervals

Biso Zyme concentration (%)	Spraying interval (MAP)	Curcuminoid (%)	Xanthorrhizol (%)
0	1 to 5	0.287	0.988
0.1	2 to 5	0.261	1.505
0.1	3 to 5	0.214	0.764
0.1	4 to 5	0.323	1.081
0.1	5	0.234	1.163
0.1	1 to 5	0.302	1.332
0.05	1 to 5	0.318	1.462
0.05	2 to 5	0.233	1.429
0.05	3 to 5	0.291	1.231
0.05	4 to 5	0.435	1.234
0.05	5	0.332	1.488

Note: MAP = month after planting

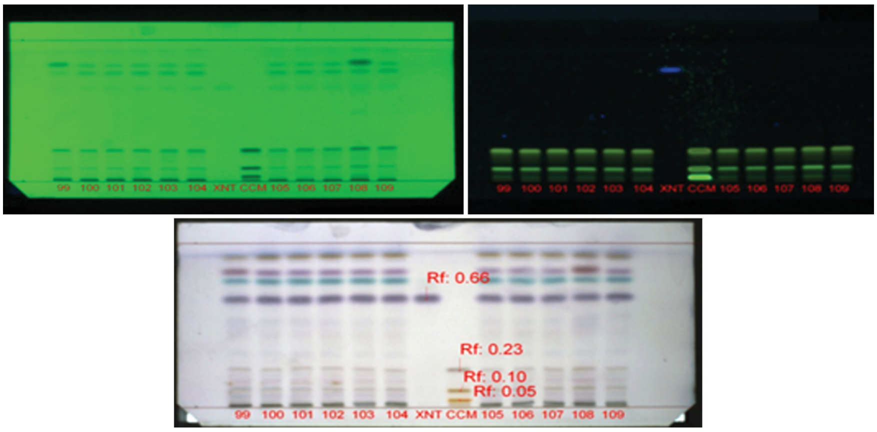

Figure 2. Thin Layer Chromatography (TLC) analysis : (A) detection of curcuminoid and zanthorrizol with UV light at 254 nm; (B) UV light at 366 nm; and (C) anisaldehyde reagents. CCM= curcuminoid standard; XNT= xanthorrhizol standard; 99= without Biso Zyme; 100=Biso Zyme concentration 0.1%/interval spraying 1 to 5 MAP; 101= Biso Zyme concentration 0.1%/interval spraying 2 to 5 MAP; 102= Biso Zyme concentration 0.1%/interval spraying 3 to 5 MAP; 103= Biso Zyme concentration 0.1%/interval spraying 4 to 5 MAP; 104= Biso Zyme concentration 0.1%/interval spraying 5 MAP; 105= Biso Zyme concentration 0.05%/interval spraying 1 to 5 MAP; 106= Biso Zyme concentration 0.05%/interval spraying 2 to 5 MAP; 107= Biso Zyme concentration 0.05%/interval spraying 3 to 5 MAP; 108= Biso Zyme concentration 0.05%/Interval spraying 4 to 5 MAP; 109= Biso Zyme concentration 0.05%/ interval spraying 5 MAP.

Conclusion

LAI, LAR, RGR, and NAR of Javanese turmeric was not affected by Biso Zyme concentrations. The highest rhizome fresh and dry weight were produced with 0.05% Biso Zyme application and spraying interval of 4 to 5 MAP. The interaction of 0.05% Biso Zyme and spraying interval 4 to 5 MAP produced the highest rhizome dry weight. The highest curcuminoid and xanthorrhizol content was obtained from 0.05% Biso Zyme with spraying interval of a 4 to 5 MAP and 0.1% Biso Zyme with spraying interval 1 to 5 MAP. The presence of curcuminoid in the Javanese turmeric rhizome with TLC was visible on Rf 0.23 and xanthorrhizol at Rf 0.66.

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