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Original Research Article

Optimization Growth, Yield and Secondary Metabolites Quality of Kencur (*Kaempferia galanga* L.) with Various Levels of Shade and Sulfur Fertilizer

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ABSTRACT

Kencur can grow well in the lowlands or upper lands with loose soil which does not contain much air. The quality of yield of kencur rhizome is determined by its maturity level. An addition of sulfur and the provision of shading could increase the quality and yield of rhizome. The purpose of this study was to study and determine the response of two accessions to the application of sulfur fertilizer and different shade treatments in increasing yield and quality of the rhizome. The research was conducted from December 2020 to June 2021 in Jatikerto Agro Techno Park of Brawijaya University, Malang. A split-split-plot design was used in this study. The design consisted of main plots at 25% and 50% shades, subplots: Banyuwangi and Blitar accessions, and sub-subplots: 0, 60, 90 and 120 kg ha^{-1} MgSO4. The yields obtained indicated that there were interactions between treatments that occured in dry weight parameters of the rhizome, total plants and kencur productivity. By contrast, other treatments such as fresh weight only interacted in the treatment of accession and sulfur fertilizers without the presence of shade treatment. In both accesions, an increase in sulfur fertilizer doses could increase the percentage of total antioxidant activity. However, the antioxidant content was higher at 25% shade compared to that at 50% shade. The dose of sulfur fertilizer at both the shade and accession used was able to increase ethyl p-methoxycinnamate content.

Keywords: Ethyl p-methoxycinnamate, Magnesium sulfate, Light intensity, Rhizome

Introduction

Kencur (*Kaempferia galanga* L.) belongs to the *Zingiberaceae* family and has a high economic value. One way to obtain high quality of kencur rhizome and its yield is by using the genotype. Kencur rhizome contains a variety of chemical compounds such as ethyl p-methoxycinnamate (EPMC), eucalyptol, carvone, pentadecane and metal cinnamate.[1] EPMC compounds have many uses particularly as a cosmetics ingredient because they can protect the skin from sunlight and can be used as raw materials for perfumes. In addition, the compounds in kencur rhizome are widely used as natural remedies for digestive disorders, respiratory tract and as cosmetics raw materials.[2]
The distribution of kencur in Indonesia is divided into 2 types based on the origin of accessions, namely highland accessions and lowland accessions. Altitude also influences the environmental factors affecting plant growth, especially temperature and light intensity.[3] Light intensity according to the plant species' needs can be classified into minimum, optimum and maximum.[4] The use of shade is suitable to reduce the stress of high light intensity. Shade has an impact on kencur rhizome production, particularly on the amount of high-nutrient content.[5]

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The best growth and highest yield of kencur can be obtained at 60% shade level.[6] The response of plants to shaded conditions could reduce the intensity of light received by plants which can reduce nutrient uptake and photosynthesis. Chansakaow *et al.*[7] found that in addition to plant growth, shade affects the content of phenolic compounds and antioxidants in *Kaempferia parviflora*. The synthesis of phenolic compounds is also affected by the presence of light in most plants.[8]
In the cultivation of kencur, it is necessary to provide nutritional input with the right and optimal dose. Plants need 2 kinds of nutrients: macro and micro nutrients. According to Kavitha and Menon,[9] a deficiency of sulfur and other secondary nutrients will reduce the yield and quality of the kencur plant.
Cultivation of kencur plants by providing shade will reduce light intensity reception which results in a decrease in photosynthesis. Sulfur nutrients make up important metabolic molecules such as amino acids (methionine and cysteine) and secondary metabolites such as glucosinolates and sulfoflavonoids. Sulfur-containing amino acids are involved in the formation of chlorophyll and plant structure and are needed in protein synthesis. The purpose of this study was to study and determine the response of two accessions of kencur plant to the provision of shade and sulfur fertilizer in increasing yield and quality of kencur rhizome.

Materials and Methods

Research Design
This research was carried out from December 2020 to June 2021 in the rainy season at the Jatikerto Agro Techno Park (ATP) of Brawijaya University in Jatikerto Village, Malang Regency with DMS latitude longitude coordinates of 8° 07' 33.8" North and 112° 31' 48.4" East. This research used a split-split-plot design consisting of 2 levels of main plots: N25 with 25% shade and N50 with 50% shade, 2 levels of subplots: BW (Banyuwangi accessions) and BL (Blitar accessions), 4 levels of sub-subplots: S0 with 0 kg ha^{-1} MgSO4, S60 with 60 kg ha^{-1}

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MgSO4, S90 with 90 kg ha^{-1} MgSO4, and S120 with 120 kg ha^{-1} MgSO4. There were 16 treatment combination units with 3 replications, and thus in total there were 48 treatment combination units. As each plot had 160 plants, the total plant population used was 7,680 plants.

Yield Parameter Measurement

The yield parameter was measured when plants reached six months by weighing fresh weight of kencur rhizome and measuring weight loss after harvest in every plot. The weight observations were carried out using a Denver Instrument AA-250 scale. The weight of kencur rhizome was then converted to ton/hectare to get the yield per hectare.

Plant Growth Analysis

Observation of plant growth analysis includes the calculation of crop growth rate (CGR) and net assimilation rate (NAR) values. CGR is the plant's ability to produce biomass per unit time, while NAR is the plant's ability to produce biomass per unit leaf area. CGR and NAR are calculated using the following formula:[10]

$$CGR = \frac{1}{GA} x \frac{W_2 - W_1}{T_2 - T_2}$$

where GA is the spacing, W_2 is the dry weight at the second observation, W_1 is the dry weight at the first observation and T_2 and T_1 are the time periods of W_2 and W_1 observations;

$$NAR = \frac{W_2 - W_1}{T_2 - T_2} x \frac{lnLA_2 - lnLA}{LA_2 - LA_2}$$

where GA is the spacing, W_2 is the dry weight at the second observation, W_1 is the dry weight at the first observation, T_2 and T_1 are the time periods of W_2 and W_1 observations, LA_1 and LA_2 are leaf area at the first and second observations and ln is natural logarithm.

Antioxidant Testing

Antioxidant testing was carried out at the Plant Physiology Laboratory of Brawijaya University. The method used involved reducing free radicals using 0.5 mL DPPH (1,1-diphenyl-2-picrylhydrazyl) added to each of methanol extract and water (dry and wet) added with 2 mL of DPPH solution and vortexed for 2 minutes. The efficiency of free radicals was indicated by a color change of the solution from purple to yellow. Furthermore, in the last 5 minutes before 30 minutes of incubation, the absorbance was measured at a wavelength of 517 nm using a Bio-Rad SmartSpec 3000 UV/Vis Spectrophotometer. Free radical scavenging activity was calculated as the percentage of DPPH color reduction using the following equation:[11]

$$\text{TAA } (\%) = \frac{A_o - A_s}{A_o} x\ 100\%$$

where A_o is the absorbance level of the blank solution (DPPH) at the start (t = 0 minutes), A_s is the absorbance of DPPH with antioxidants from plant samples at t = 30 minutes.

Analysis of Ethyl P-Methoxycinnamate (EPMC)

The EPMC test was carried out using gas chromatography mass spectophotometer (GC-MS) method at the Chemical Engineering Laboratory, State Polytechnic of Malang. Oil analysis was carried out using gas chromatography (GC) and mass spectroscopy (MS) to determine the compounds contained in the oil. The gas chromatography mass spectophotometer (GC-MS) used was the QP2010ULTRA type. The synthesis of p-methoxycinamyl p-methoxycinnamate started by isolating EPMC from kencur rhizome with 96% ethanol. The following steps were transforming EPMC into alcohol (PMS-OH), transforming EPMC to form cinnamic acid followed by reacting this acid with thionyl chloride to form acyl chloride, and the last step was reacting the two products of this transformation into p-methoxycinamyl pmethoxycinnamate ester.[12]

Statistical Analysis

Statistical analysis of the yield data was done through a one-way ANOVA with LSD at a 5% level using SPSS. The least significant difference (LSD) test at 5% level was carried out to determine differences in treatments on variables that have a significance value based on the F test.[10]

Results and Discussion

Effect of Shade and Sulfur Fertilizer on Net Assimilation Rate of Kencur Accession

Table 1 shows that there is a significant difference in net assimilation rate on days 120-160 post planting (days after planting) in the shade treatment and the application of sulfur fertilizer. Shade condition of 50% could produce a higher net assimilation rate than without shade. One factor that contributed to this was leaves as the main organ that function to absorb sunlight. Kencur grown at 50% shade had wide leaves to absorb more sunlight. According to Buntoro *et al*.[13], an increase in leaf area value can increase the rate of assimilation, thus producing high dry weight. This finding is also supported by research on arrowroot (*Maranta arundinacea* L.) plants conducted by Supriyono *et al*.[14] which found that the highest increase in the net assimilation rate occurred in plants grown at 51% shade, which was 0.011 $gram.cm^{-2}.day^{-1}$, or higher than with no shade (45.64%). The second factor that affects the increase in the net assimilation rate is root growth. This is due to the function of roots in absorbing nutrients from the soil. One of essential nutrients that plays a role in photosynthesis is sulfur. According to Kopriva and Rennenberg[15], the availability of sulfur nutrients regulates the efficiency of plant growth and accumulation of plant dry weight because there is a relationship between photosynthesis and the uptake of nitrogen and sulfur. The results of a study by Preeti *et al*.[16] showed an increase in the net assimilation rate of 95.52% in the RH 749 genotype with the addition of 20 kg/acre potassium fertilizer and 60 kg/acre sulfur fertilizer compared to no sulfur fertilizer application. The increase in NAR was probably caused by more vegetative growth due to the addition of sulfur fertilizer.

Effect of Shade and Sulfur Fertilizer on Crop Growth Rate of Kencur Accession

Based on Table 2, the growth rate of kencur plants at 50% shade showed a higher response compared to those at 25% shade. This is because kencur plant is suitable for cultivation under the shade rather than in open conditions.[5] The increase in the growth rate of kencur also occurred along with the addition of sulfur fertilizer. Sulfur nutrients play an important role for plants, which they use for growth and crop production. The results of research by Jamal *et al.*[17] showed a positive correlation between leaf area index, plant growth rate and biomass production, and they will increase by increasing sulfur dose.

Effect of Shading and Sulfur Fertilizer to Yield on Two Kencur Accessions

As shown in Table 3, the yield of kencur shows an interaction between the three treatments. The highest rhizome weight was obtained at 50% shade and 90 kg ha^{-1} of sulfur applied to both accessions where the average yield per hectare was 26.15 t ha^{-1}. The result of the determination of the optimal point of Blitar accession at 25% and 50% shades was 64.63 kg ha^{-1} of MgSO4 fertilizer, while that of Banyuwangi accession at 25% and 50% shades was 71.82 kg ha^{-1} of MgSO4 fertilizer. The optimum growth and formation of kencur rhizomes was at 50% shade because the light intensity was not too high, causing evapotranspiration to run slowly, and water requirements for the rhizomes were fulfilled. Solar radiation as the main source of light for plants is one of the factors that affect the continuity of photosynthesis. According to Monteith[18], solar radiation contains energy that can be used in the process of photosynthesis; therefore, plant production is always limited by the availability of the sun. Environmental changes that occur around cultivated plants may support the determination of high and low yield of each accession of kencur. This is in accordance with Finlay and Wilkinson's[19] finding which shows that varietal adaptation is determined by the interaction between genetic and environmental traits. Sulfur plays an important role in the synthesis of proteins and vitamins in plants. In addition, sulfur is a component of essential amino acids associated with nitrogen in metabolism. As a result, S increases the yield and quality of plants.[20] The addition of sulfur fertilizer has also been proven to increase plant yield in the

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research by Shah *et al*.[21] A sulfur dose of 60 kg ha^{-1} was able to increase the yield of sesame crops by 31.24% when compared to no sulfur fertilizer application.

Total Antioxidant Activity

The relationship between sulfur fertilizer dosage and total antioxidant activity is presented in Figure 1 and Figure 2. Figure 1 shows that the relationship between the dose of sulfur fertilizer and total antioxidant activity in Banyuwangi accession at 25% shade obtained R2 = 0.8582 with the equation 0.1198x + 39.345, while the Banyuwangi accession with 50% shade had an R2 value of 0.993 with the equation polynomial y = 0.0536x + 30.291. In this study, the chemical compounds analyzed for the content of kencur rhizome were total antioxidant activity. Kencur rhizome contains several active compounds. According to Hasanah *et al*.[22], the results of the phytochemical screening of ethanol extract of kencur rhizome revealed the content of flavonoid compounds, polyphenols, tannins, quinones and sesquiterpenes.[23] Flavonoid compounds and polyphenols are secondary metabolite compounds that act as antioxidants. Antioxidants derived from kencur rhizome are classified as natural antioxidants obtained from plant parts.

Table 1. Effect of Shade and Sulfur Fertilizer on Kencur Accession Net Assimilation Rate

Shading	**Net Assimilation Rate (g cm^{-2} day^{-1}) at age- day**		
	0-60	**60-120**	**120-160**
N_{25}	0.00653 ns	0.00108 ns	0.000560 a
N_{50}	0.00735 ns	0.00119 ns	0.000628 b
LSD (%)	ns	ns	0.0000648
Accesion	0-60	60-120	120-160
BW	0.00694 ns	0.00116 ns	0.000584 ns
BL	0.00693 ns	0.00112 ns	0.000604 ns
LSD (5%)	ns	ns	ns
Sulfur	0-60	60-120	120-160
S_0	0.00677 ns	0.00106 ns	0.000446 a
S_{60}	0.00710 ns	0.00118 ns	0.000474 a
S_{90}	0.00703 ns	0.00112 ns	0.000733 b
S_{120}	0.00685 ns	0.00119 ns	0.000722 b
LSD (5%)	ns	ns	0.000197

Description: Means followed by the same letter at the same column showed no significant difference based on LSD test at level 5%; ns: not significant based on LSD test at level 5%. N25: Shade 25%, N50: Shade 50%, BW : Banyuwangi accessions, BL: Blitar accessions, S0: 0 kg ha-1 $MgSO_4$; S60: 60 kg ha-1 MgSO4; S90: 90 kg ha-1 $MgSO_4$; S120: 120 kg ha-1 MgSO4.

Table 2: Effect of Shade and Sulfur Fertilizer on Kencur Accession Crop Growth Rate

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Shade	**Crop Growth Rate (g cm^{-2} day^{-1}) at age- day**		
	0-60	**60-120**	**120-160**
N_{25}	0.1387 a	0.2468 a	0.3565 a
N_{50}	0.1536 b	0.2860 b	0.4374 b
LSD (5%)	0.0065	0.0228	0.0745
Accession	0-60	60-120	120-160
BW	0.1430 ns	0.2515 ns	0.3746 ns
BL	0.1492 ns	0.2830 ns	0.4194 ns
LSD (5%)	ns	ns	ns
Sulfur	0-60	60-120	120-160
S_0	0.1422 ns	0.2713 ns	0.2841 a
S_{60}	0.1493 ns	0.2616 ns	0.3241 a
S_{90}	0.1491 ns	0.2700 ns	0.5147 b
S_{120}	0.1441 ns	0.2662 ns	0.4650 b
LSD (5%)	ns	ns	0.1031

Description: Means followed by the same letter at the same column showed no significant difference based on LSD test at level 5%; ns: not significant based on LSD test at level 5%. N25: Shade 25%, N50: Shade 50%, BW : Banyuwangi accessions, BL: Blitar accessions, S0: 0 kg ha-1 MgSO4; S60: 60 kg ha-1 MgSO4; S90: 90 kg ha-1 MgSO4; S120: 120 kg ha-1 MgSO4.

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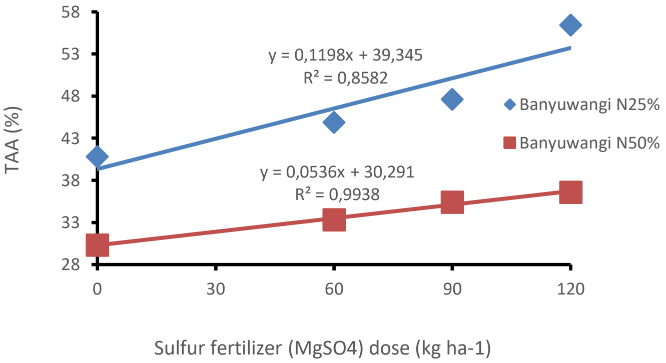

Figure 1: The Relationship between Sulfur Fertilizer Dosage and Total Antioxidant Activity (%) on the Rhizome of the Banyuwangi Accession Kencur Plant at 25% and 50% Shade.
Description: TAA: Total Antioxidant Activity

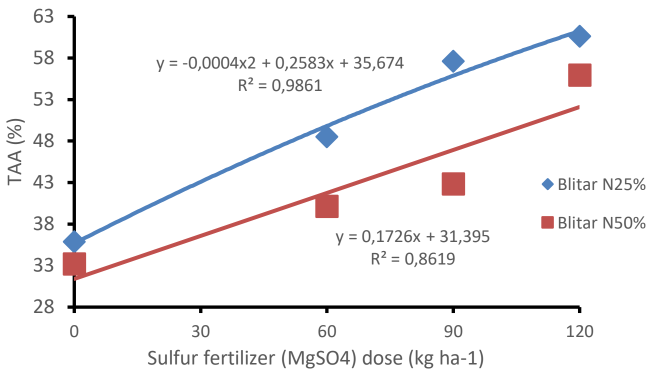

Figure 2: The Relationship between Sulfur Fertilizer Dosage and Total Antioxidant Activity (%) on the Rhizome of the Blitar Accession Kencur Plant at 25% and 50% Shade.

Based on Figure 2, Blitar accessions at 25% shade and a dose of 120 kg ha^{-1} of sulfur fertilizer had the highest TAA percentage of 60.60%. This is in accordance with the research conducted by Hayati and Latifah[24] which found that the antioxidant activity of the ethanol extract of kencur rhizome using the DPPH method resulted in TAA levels with a percentage of 64.93%. This percentage of antioxidant activity indicates the ability of an antioxidant to inhibit free radicals.

In Banyuwangi and Blitar accessions, increasing the dose of sulfur fertilizer could increase the percentage of TAA levels in kecur rhizomes grown at 25% and 50% shades. However, in both accessions the antioxidant content was higher at 25% shade than at 50% shade. Overall, this was particularly a valuable finding as shown by the increase in antioxidants due to the dose of sulfur fertilizer in both accessions at 25% shade and 50% shade. This finding is in accordance with the research of Ghasemzadeh *et al.*[25] which found that shade levels from 20 to 40% with a light intensity of 60-80 can produce optimal antioxidant content. Other research results show that increasing the dose of fertilizer can also increase antioxidant levels in ginger rhizome.[26] High total antioxidant activity in plant rhizomes due to higher light intensity was also found in a study conducted by Ghasemzadeh *et al.*[27] Antioxidants are secondary metabolites produced by most plants in the form of different compounds to protect plants from oxidative damage caused by UV-induced free radicals.[28]

The results of the regression (Figure 2) showed that the effect of giving a dose of sulfur fertilizer on the percentage of TAA in Blitar accessions at 25% shade obtained an R^2 value of 0.9861 with the polynomial equation $y = -0.0004\ x^2 + 0.2583x + 35.674$, while at 50% shade the R^2 value was 0.8619 and the linear equation $y = 0.1726x + 31.395$. Based on the results of this study, Blitar accessions at 25% shade and a dose of 120 kg ha^{-1} sulfur fertilizer had the highest TAA percentage of 60.60%.

In both accessions, this may indicate that increasing the dose of sulfur fertilizer can increase the percentage of TAA levels in kecur rhizomes grown at 25% and 50% shades. However, the antioxidant content was higher at 25% shade compared to that at 50% shade.

Analysis of Ethyl P-Methoxycinnamate (EPMC)

In addition to the percentage of TAA levels in the rhizomes, the levels of ethyl p-methoxycinnamate (EPMC) were also observed. Figure 3 shows the relationship between the dose of sulfur fertilizer and the EPMC content of kencur rhizome of Banyuwangi accession at 25% shade with R^2 of 0.99 and at 25% shade with R^2 of 0.98. Adianingsih *et al.*[29] also conducted research on bioactive content in rhizome organs in 12 kencur accessions in East Java, and the main focus of this study was testing kencur for EPMC content. EPMC compounds are often used as research materials because they have benefits as one of the basic ingredients of cosmetics preparations, such as sunscreen.[30] Plants produce secondary metabolites with various structures, functions, and contents. Kencur plants have a potential as medicinal plants because they have secondary metabolites resulting from the adaptation process of the plants to the environment or stress.[31] Sunlight affects the production of secondary metabolites because plants use sunlight for photosynthesis.

Higher EPMC content was found in kencur plants grown at higher shade level (50%) compared to those at lower shade level (25%) in both study sites. EPMC is a phenol derivative compound that has a role in the distinctive taste and aroma of kencur. The reason for the low total amount of EPMC at higher light exposure (25% shading) was probably because phenolic compounds were more abundant in the leaves than in the rhizome of the kencur plant.[27] Figure 4 shows the relationship between the dose of sulfur fertilizer and the EPMC content of Blitar accession of kencur rhizome at 25% shade with R2 of 0.99 and at 50% shade with R2 of 0.97.

Table 3: Effect of Shade and Sulfur Fertilizer on Kencur Accession Yield

Shading	**Yield (t/ha)**			
	S_0	**S_{60}**	**S_{90}**	**S_{120}**
	BW Accession			
N_{25}	10.91 a	12.30 abc	14.78 d	12.13 ab
N_{50}	20.37 ef	22.23 gh	25.73 i	21.93 fg
	BL Accession			
N_{25}	13.64 bcd	14.00 cd	19.75 e	12.27 abc
N_{50}	23.13 gh	26.33 i	26.57 i	23.79 h
LSD (5%)	1.833			

Description: Means followed by the same letter at the same row and column showed no significant difference based on LSD test at level 5%. N25: Shade 25%, N50: Shade 50%, BW : Banyuwangi accessions, BL: Blitar accessions, S0: 0 kg ha-1 MgSO4; S60: 60 kg ha-1 MgSO4; S90: 90 kg ha-1 MgSO4; S120: 120 kg ha-1 MgSO4.

Conclusion

Shade condition of 50% in various accessions and sulfur fertilizer can increase growth and maintain weight loss of kencur rhizome during storage. Both accessions at 50% shade showed higher yields of 42.55% compared to those at 25% shade. The use of 90 kg ha^{-1} of sulfur fertilizer resulted in higher fresh weight of rhizomes and lower weight loss compared to other doses. The addition of sulfur fertilizer also affected the antioxidant content of ethyl p-methoxycinnamate (EPMC). An increase in the dose of sulfur fertilizer also led to higher antioxidant value.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

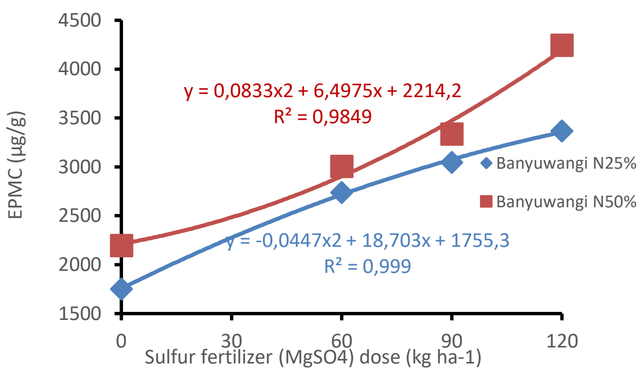

Figure 3: Relationship between Dosage of Sulfur Fertilizer and Ethyl p-methoxycinnamate (EPMC) on Rhizome of Kencur Plant Accession Banyuwangi at 25% and 50% Shade
Description: EPMC: Ethyl p-methoxycinnamate

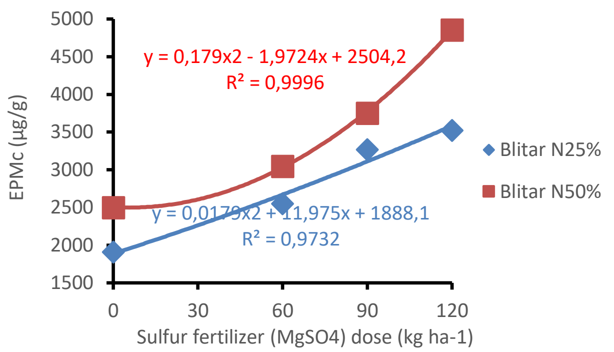

Figure 4: Relationship between Dosage of Sulfur Fertilizer and Ethyl p-methoxycinnamate (EPMC) on Rhizome of Kencur Plant Accession Blitar at 25% and 50% Shade

References

1. Julianti TB, Bakar MFA, and Wikantyasning. Phytochemical, Antioxidant Analysis and In Vitro Xanthine Oxidase Inhibitory Activity of Kaempferia parviflora and Kaempferia galanga. Trop. J. Nat. Prod. Res. 2022, 6(12): 1981-1985. http://www.doi.org/10.26538/tjnpr/v6i12.14
2. Laksemi DAAS, Sukrama DM, Sudarmaja M, Damayanti PAA, Swastika K, Diarthini NLPE, Astawa NM, and Tunas,K. Medicinal Plants as Recent Complementary and Alternative Therapy for COVID-19. Trop. J. Nat. Prod. Res. 2020, 4(12): 1025–1032. doi.org/10.26538/tjnpr/v4i12.1
3. Adianingsih OR, Widaryanto E, Saitama A, and Zaini AH. Analysis of bioactive compounds present in *Kaempferia galanga* rhizome collected from different regions of East Java, Indonesia. IOP Conf. Ser.: Earth Environ. Sci. 2021, 913(21210); 012074.
4. Rezai S, Etemadi N, Nikbakht A, Yousefi M, and Majidi MM. Effect of Light Intensity on Leaf Morphology, Photosynthetic Capacity, and Chlorophyll Content in Sage (*Salvia officinali*s L.). Hortic. Sci. Technol. 2018, 36(1); 46-57.
5. Zaini F, Friska AR, Mustika DM, Tyasmoro SY, Saitama A, Zaini AH, and Widaryanto E. Enhancement of antioxidant activity of kencur rhizome in the shade by potassium fertilizer. IOP Conf. Ser.: Earth Environ. Sci. 2021, 913: 012006.
6. Setiawan TW, Widaryanto E, Saitama A, and Zaini AH. Uji pertumbuhan enam aksesi Kencur (*Kaempferia galanga* L.) di bawah tegakan Jati. J. Agr. Sci. 2021, 5(2); 136-143.
7. Ferreyra S, Bottini R, and Fontana A. Temperature and light conditions affect stability of phenolic compounds of stored grape cane extracts. Food Chem. 2023, 405: 134718.
8. Ghasemzadeh A, Jaafar HZ, Rahmat A, Wahab PE, Halim MR. Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). Int J Mol Sci. 2010, 11(10):3885-3897. 10.3390/ijms11103885.
9. Kavitha PR, and Menon M. Effect of potassium and secondary nutrients on the essential oil and oleoresin contents in kacholam (*Kaempferia galanga* L.). J. Trop. Agric. 2013, *51*(2); 105-110. http://jtropag.kau.in/index.php/ojs2/article/view/288/288
10. Widaryanto E, and Saitama A. Analysis of Plant Growth of Ten Varieties of Sweet Potato (*Ipomoea batatas* L.) Cultivated in Rainy Season. Asian J. Plant Sci. 2017, 16(4): 193- 199.
11. Rosidi A, Khomsan A, Setiawan B, Riyadi H, and Briawan D. Potensi Temulawak (*Curcuma xanthorrhiza* Roxb) Sebagai Antioksidan. Universitas Muhammadiyah Semarang. Semarang. 2014, p. 1-8.
12. Taufikkurohmah T. Sintesis p-Metoksisinamil p-Metoksisinamat dari Etil p-Metoksisinamat Hasil Isolasi Rimpang Kencur (*Kaempferia galanga* L) sebagai Kandidat Tabir Surya. Indo. J. Chem. 2005, *5*(3); 193-197.
13. Buntoro BH, Rogomulyo R, and Trsinowati S. Pengaruh Takaran Pupuk Kandang dan Intensitas Cahaya Terhadap Pertumbuhan dan Hasil Temu Putih (*Curcuma zedoaria* L.). Vegetalika. 2014, *3*(4); 29-39.
14. Supriyono S, Arni PRB, and WijayantiR. Analisis Pertumbuhan Garut (*Marantha arundinaceae*) Pada Beberapa Tingkat Naungan. Agrosains: Jurnal Penelitian Agronomi. 2017, 19(1), 22-27. https://doi.org/10.20961/agsjpa.v19i1.20926
15. Kopriva S, and Rennenberg H. Control of sulphate assimilation and glutathione synthesis: Interaction with N and C metabolism. J. Exp. Bot. 2004, *55*(404); 1831-1842. https://doi.org/10.1093/jxb/erh203
16. Preeti A, Kumar N, Singh, and Jha SP. Effect of Potassium and Sulphur Application on Growth Parameters of Indian Mustard (*Brassica juncea* L.) Genotype at Vegetative Stage under Water Deficit Conditions. Ind. J. Pure Appl. Biosci. 2019, 7 (6); 301-306.
17. Jamal A, Fazli IS, Ahmad S, Abdin MZ, and SongJoong Y. Effect of sulphur and nitrogen application on growth characteristics, seed and oil yields of soybean cultivars. Korean J. Crop Sci. 2005, *50*(5), 34-45.
18. Monteith JL. Does Light Limit Crop Production, In C. B. Johnson (Ed) Physiological Processes Limiting Plant Productivity, Dept. Of Botany, Univ.of Reading-London. 1981, p. 23-28.

19. Finlay KW, and Wilkinson GN. The Analysis of Adaptation in a Plant-Breeding Programme. Aust. J. Agric. Res. 1963, 14, 742-754. http://dx.doi.org/10.1071/AR9630742
20. Danapriatna, N. Peranan Sulfur bagi Pertumbuhan Tanaman. Paradigma: J. Ilmu Pengetahuan Agama dan Budaya. 2008. 9 (1): 39–52.
21. Shah MA, Manaf A, Hussain M, Farooq S, and Zafar-ul-Hye M. Sulphur fertilization improves the sesame productivity and economic returns under rainfed conditions. Int J Agric Biol. 2013, *15*(6); 1301-1306.
22. Hasanah A, Nazaruddin F, Febrina E, and Zuhrotun A. Analisis Kandungan Minyak Atsiri dan Uji Aktivitas Antiinflamasi Ekstrak Rimpang Kencur (*Kaempferia galanga* L.) Analysis of Essential Oil Contents and Anti-Imflammatory Activity Test of Kencur (*Kaempferia galanga* L.). J. Matematika and Sains. 2011, *16*(3); 147-152.
23. Dwiloka B, Setiani BE, and Purwitasari L. The changes in the antioxidant activities, total phenol, curcumin and hedonic quality of first and second brewing spiced drinks. IOP Conf. Ser.: Earth Environ. Sci. 2020, 443: 012108.
24. Hayati EK, and Latifah RN. Antioxidant Activity of Flavonoid from Rhizome *Kaempferia galanga* L. Extract. J. of Chem. 2015, 4 (2); 127-137. https://doi.org/10.18860/al.v4i2.3203
25. Ghasemzadeh A, and Hawa Z. Effect of CO2 Enrichment on Synthesis of Some Primary and Secondary Metabolites in Ginger (*Zingiber officinale* Rosc.). J. Mol. Sci. 2011, 12 (2); 1101-1114. https://doi.org/10.3390/ijms12021101
26. Jabborova D, Sayyed RZ, Azimov A, Jabbarov Z, Matchanov A, Enakiev Y, Baazeem A, El Sabagh A, Danish S, and Datta R. Impact of mineral fertilizers on mineral nutrients in the ginger rhizome and on soil enzymes activities and soil properties. Saudi J. Biol. Sci. 2021 *28*(9), 5268-5274. https://doi.org/10.1016/j.sjbs.2021.05.037
27. Ghasemzadeh A, Jaafar HZE, Rahmat A, Wahab PEM, and Halim MRA. Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). Int. J. Mol. Sci. 2010, *11*(10); 3885–3897. https://doi.org/10.3390/ijms11103885
28. Mpalantinos MA, Soares de Moura R, Parente JP, and Kuster RM. Biologically active flavonoids and kava pyrones from the aqueous extract ofAlpinia zerumbet. Phytother. Res. 1998, 12(6); 442–444. https://doi.org/10.1002/(SICI)1099-1573(199809)12:6<442::AID-PTR320>3.0.CO;2-Y
29. Adianingsih OR, Widaryanto E, Saitama A, and Zaini AH. Analysis of bioactive compounds present in Kaempferia galanga rhizome collected from different regions of East Java, Indonesia. In IOP Conference Series: Earth Environ. Sci. 2021, 913(1). https://doi.org/0.1088/1755-1315/913/1/012074
30. Fareza MS. Transformasi Etil -P-Metoksisinamat menjadi Asam P-Metoksisinamat dari Kencur (*Kaempheria galanga* L.) beserta Uji Aktivitas Antibakterinya. ALCHEMY Jurnal Penelitian Kimia. 2017 *13*(2), 176 -190. https://doi.org/10.20961/alchemy.v13i2.8472
31. Subaryanti, Sulistyaningsih YC, Iswantini D, and Triadiati T. Essential oil components, metabolite profiles, and idioblast cell densities in galangal (*Kaempferia galanga* L.) at different agroecology. Agrivita. 2021, *43*(2); 245-261. https://doi.org/10.17503/agrivita.v43i2.2631