Enhancement of antioxidant activity of kencur rhizome in the shade by potassium fertilizer

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Abstracts. Kencur has been traditionally used as a medical plant for a long time. The potential content of kencur rhizomes as a medical plant lead to the idea of conducting a study that aims to determine the yield and antioxidant content of rhizomes cultivated in the shade and the effect of potassium fertilizer application. The research was conducted in two stages. The first stage was kencur cultivation in the AgroTechno-Park UB in Jatikerto from November 2020 to June 2021 designed according to the Split-split plot design (3 factor treatments). The main factor was the shade, sub plots were accession of kencur, and t doses of potassium fertilizer. The second stage was conducted in the Plant Physiology Laboratory to analyze the antioxidant content after the field experiment. The results showed that there was increased antioxidant activity in kencur rhizomes due to the use of potassium fertilizer and shade. The 25% shade produced higher antioxidants than 50% shade in both Lumajang and Nganjuk’s accessions.

1. Introduction
Kencur (Kaempferia galanga L.) is a medicinal plant in tropical and subtropical areas. The use of kencur both by industry and households is not only for medicine but also as ingredients in foods and drinks with various health benefits. The benefits of kencur plants usually come from the rhizome. In general, the kencur rhizome can be divided into 2 parts, namely the main rhizome which is larger in size and the branch rhizome which is smaller. Both parts of the rhizome can be used as seed material for propagation or other materials to produce diverse products. The rhizome of the kencur plant can also be used as a raw material for the traditional medicine industry, kitchen spices, food ingredients, vinegar fragrance, hair wash and other refreshing drinks [1].

Kencur plants can grow well under full sun intensity or shade with a percentage of 25-30% when the plant is in the vegetative phase, while for the generative phase kencur plants do not require full light or shade up to 50-65%. According to[2], kencur can also be used as a ground cover plant under coconut tree stands or forestry plants such as sengon and teak trees. Shade greatly affects light intensity. The light intensity is one of the factors affecting plant growth and yield. [3]. Shade treatment will affect plant
growth and yield parameters such as plant height, apparent stem diameter, leaf length, leaf width, number of leaves, canopy dry weight, number of buds on primary rhizomes, number and length of rhizomes [4].

Potassium(K) plays a role in the process of opening and closing stomata. When K moves, the guards cells around the stomata will swell so that the stomata will open and gas exchange will occur. Based on the results of the study by[5] type of rhizome (main rhizome and subsidiary rhizome) used as seeds had no effect on the nutritional content of the rhizome but had a significant effect on nutrient absorption of K elements. [6] explained that if the K content is inadequate, the process of opening and closing of the stomata will be slow down and a lot of water vapor will loss so that the plant becomes susceptible to drought, and can also result in stunted plant growth, soluble and N compounds accumulate [6].

Low sunlight intensity will decrease the rate of nutrition absorption because it decreases transpiration rate. It will also decrase nutrients absorption through mass flow so that, in this condition nutrients in the soil must be supplied more especially potassium nutrients. According to[7], ginger plants that grow in shaded conditions can only grow ideally with high levels of K fertilization. This is because potassium plays an important role in the transportation of water and plant nutrients. [6] explain that when the potassium content is low, the absorption of other nutrients such as N, P, Ca, Mg and amino acids will be suppressed, so that plants will be deprived of nutrients.

In addition to the selection of accessions with high rhizome production, another important component that needs to be considered is the quality of metabolites in the rhizomes. The main components of kencur rhizome are essential oils, starch and juice content. Large kencur rhizome generally has a lower quality than small rhizome. The size of kencur rhizome from population grown in Subang and Sumedang regencies is smaller than the kencur rhizome from Bogor. However, the skin color is darker, which is an indicator of the high content of rhizome essential oil [2]. Efforts to improve production and quality of kencur is needed, and there are several aspects that need to be considered such as the intensity of light received and appropriate dosages of fertilizers to support the growth of kencur plants. Based on the results of [2], there are 5 potential accession of kencur with four superior kencur accession (V1, V3, V4, V5) had the ability to produce the same fresh and dry rhizomes.

Kencur is widely used as raw material for the biopharmaceutical industry because of its compound content. The high demand for kencur has not been matched by high production and quality. The harvested area and production of kencur during the last three years has decreased. The efforts to increase production with land extensification and intensification are possible alternatives, given the increasing conversion of productive agricultural land to the non-agricultural sector. The intensification efforts such as using Paranet for shading, but it is necessary to study the optimal level of shade so that in choosing the right shade intensity. The intensification efforts such as the addition of K fertilizer are also important. K fertilizer is required to improve the quality of kencur rhizomes. On the other hand, the level of shade plays a very important role in the nutrient absorption process, so this study examined the level of shade and the appropriate dose of K fertilization in the cultivation of kencur. Thus, this study aimed to analyze the effect of potassium fertilization at various levels of shade on the yield and quality of two accessions of kencur, namely Lumajang and Nganjuk’s accessions.

2. Materials and method
The research was conducted from October 2020 to May 2021 at the Agro Techno Park experimental garden, Faculty of Agriculture, Universitas Brawijaya, Jatikerto village, Kromengan District, Malang Regency with an altitude of 400 meters above sea level.

2.1 The research design
This study used a split-split plot design with three replications according to the research plan. The main plot consisted of two levels of shade, namely 50% shade (S25) and 50% shade (S50). Sub-plots are doses of Potassium fertilizer and kencur accession. There are 4 levels of dose that is fertilizer by 120 kg ha⁻¹ K₂O (K120), fertilizer by 180 kg ha⁻¹ K₂O (K180) and fertilizer 240 kg ha⁻¹ K₂O (K240), while the accession sub-sub plot consisting of 2 accessions, Lumajang accession and Ngajuk accession.

2.2 The observation of dry weight
The dry weight observation of kencur was carried out destructively by measuring the total weight of plants such as leaves, roots, stems and rhizomes after harvesting and drying in an oven at 80 °C for 2 x 24 hours constantly. The samples used were 8 plants per treatment.

2.3 The observation of TAA (total antioxidant activity)
TAA analysis was carried out in the Laboratory of Plant Physiology, majoring in agricultural cultivation, Faculty of Agriculture, Universitas Brawijaya. The level of antioxidant activity (TAA) or radical scavenging rate, which is the free radical scavenging activity of DPPH (DPPH$^+$ + AH → DPPH-H + A$^-$), is calculated by the following equation: 

$$\text{TAA}(\%) = \frac{A_0 - A_t}{A_0} \times 100\%$$

Where $A_0$ is the absorbance rate of blank solution (DPPH) at the beginning (t = 0 minutes), $A_t$ is the absorbance of DPPH with antioxidants from plant samples at t = 30 minutes.

2.4 The statistical analysis
The results of observations in this study were analyzed for variance (F test) at the level of 5% and 1%. If there are significantly different results, it will be continued with the LSD test at the 5% level. If the analysis of the follow-up test is significantly different, then further analysis will be carried out, namely the regression test is the relationship or degree of association between two variables, while regression is a mathematical model.

3. Results and discussion

| Shade | Total dry weight of plants (g plant$^{-1}$) | | | | |
| --- | --- | --- | --- | --- |
| | $K_0$ | $K_{120}$ | $K_{180}$ | $K_{240}$ |
| Lumajang accession | | | | |
| S25 | 11.44 a | 14.53 ab | 12.18 a | 12.03 a |
| S50 | 15.97 bc | 20.13 de | 17.86 bcde | 17.91 cde |
| Nganjuk accession | | | | |
| S25 | 16.98 bcd | 25.77 f | 18.73 cde | 17.97 cde |
| S50 | 26.84 f | 34.12 g | 30.77 g | 20.80 e |

LSD 5% 3.36

%CV Shade 5.44
%CV Accession 14.56
%CV Potassium 10.15

Remarks: (S25) = Shade 25%; (S50) Shade 50%; $K_0 =$ Potassium 0 kg ha$^{-1}$; $K_{120} =$ Potassium 120 kg ha$^{-1}$; $K_{180} =$ Potassium 240 kg ha$^{-1}$; $K_{240} =$ Potassium 240 kg ha$^{-1}$; Numbers accompanied by the same letter in the same column and row show that they are not significantly different, based on the 5% LSD test.

The total dry weight of plants in the Lumajang’s accessions in 50% shade was higher than 25% shade. In the Lumajang’s accessions planted in the 25% shade did not show a different total dry weight of the plant at different doses of potassium fertilizer while in the Lumajang’s accessions grown in the 50% shade, the dry weight of plants in $K_0$ was lower than $K_{120}$ but there was no significant different between $K_{120}$, $K_{180}$ and $K_{240}$ and also $K_0$, $K_{180}$ and $K_{240}$ had the total dry weight of plants that were not significantly different.

The total dry weight of plants in the Nganjuk’s accessions, in all doses of Potassium grown in 25% shade was lower than thesgrown in 50% shade. For the Nganjuk’s accession planted at 25% shade, the Kdose120 had the highest total dry weight compared to other fertilizer doses, while the Nganjuk’s accession planted at 25% shade with a dose of $K_{240}$ yielded lower dry weight than $K_0$, $K_{120}$ and $K_{180}$. 
Table 2. Interaction effect due to shade, accession and potassium fertilizer on total fresh weight of kencur rhizome.

| Shade | Total Fresh Weight of Kencur Rhizome (g plant⁻¹) |
|-------|--------------------------------------------------|
|       | K₀     | K₁₂₀   | K₁₈₀   | K₂₄₀   |
| Lumajang Accession |
| S₂₅ | 15.17 a | 22.74 cd | 17.66 b | 17.14 ab |
| S₅₀ | 21.09 c | 23.44 d | 21.05 c | 21.56 cd |
| Nganjuk Accession |
| S₂₅ | 21.84 cd | 23.11 cd | 22.72 cd | 21.09 c |
| S₅₀ | 28.92 e | 36.23 f | 30.03 e | 29.80 e |

LSD 5% = 2.09
%CV Shade = 7.34
%CV Accession = 10.51
%CV Potassium = 5.31

Remarks: (S 25) = Shade 25%; (S 50) Shade 50%; K₀ = Potassium 0 kg ha⁻¹; K₁₂₀ = Potassium 120 kg ha⁻¹; K₁₈₀ = Potassium 240 kg ha⁻¹; Numbers accompanied by the same letter in the same column and row show that they are not significantly different, based on the 5% LSD test.

Lumajang’s accession planted in 25% and 50% shade in combination with the application of fertilizer doses of K₁₂₀ gave a significant higher fresh rhizome weight of the plant with an average of 22.74-23.44 g plant⁻¹. The average weight of fresh rhizome of K₁₂₀ treatment is 22.74 g plant⁻¹, and it is increased by 33.28% compared to the K₀ treatment with a fresh rhizome weight of only 15.17 g plant⁻¹. While the application of K₁₂₀ fertilizer resulted in the fresh rhizome weight of 22.33% and 24.62% higher than the fertilizer treatments K₁₈₀ and K₂₄₀ with the fresh rhizome weight of 17.66 g plant⁻¹ and 17.44 g plant⁻¹. While the Lumajang’s accession planted in 50% shade with application of K₁₂₀ fertilizer had a higher rhizome fresh weight of 23.44 g plant⁻¹ compared to the K₀, K₁₈₀ and K₂₄₀ fertilizer treatments. Meanwhile, the application of K₁₂₀ fertilizer resulted in a 10.02%, 10.19% and 8.02% increase of rhizome fresh weight compared to the application of K₀, K₁₈₀ and K₂₄₀ fertilizers, respectively. The application of K₀, K₁₈₀ and K₂₄₀ fertilizers in 50% of shade resulted in no significant change on the fresh weight of rhizomes with average gained of 21.23 g plant⁻¹.

Nganjuk’s accession planted in 25% applied with K₀, K₁₂₀, K₁₈₀ and K₂₄₀ had no significant different in the total fresh weight of rhizome weight with mean of 29.58 g plant⁻¹. The application of K₂₄₀ fertilizer resulted in a higher rhizome weights by 20.17%, 17.11% and 17.74%, respectively, compared to K₀, K₁₈₀ and K₂₄₀. The results of the comparison showed that, fresh weight of rhizome obtained from kencur planted in 50% shade was 24.48% and 36.21% higher than in the 25% shade with K₀ and K₂₄₀ fertilizers. Meanwhile, plants with fertilization of K₁₈₀ and K₂₄₀ and planted in 50% shade showed the total rhizome weight of 24.34% and 29.22% higher than in the 25% shade.

The total antioxidant activity of the Nganjuk and Lumajang’s accessions was higher when planted under 25% shade compared to 50% shade (Figure 1). In addition, the data showed an increase in TAA in kencur rhizomes with increasing doses of potassium fertilizer (Figure 2).
Regression analysis to show the effect of potassium fertilization dosages on the percentage of the TAA in the rhizomes of kencur grown in 25% shade was presented in Figure 2, with $R^2$ of 0.98, and $R^2$ of 0.93 for shade 50%, whereas in Lumajang’s accession the $R^2$ for 25% shade was 0.94 and 50% shade was 0.87. This shows a high correlation between potassium dosages with the TAA of kencur, at which increasing the dose of potassium fertilizer can increase TAA in kecur rhizomes grown in 25% and 50% of shades.

Kencur plants that grow in shaded conditions can only grow ideally with high level of K fertilization because of their important role in water and nutrient transportation [7]. When the K content is low, the absorption of other nutrients such as N, P, Ca, Mg and amino acids will be suppressed, so that the plant will deficient in nutrients. In addition, K also plays an important role in regulating cell turgidity and activation of enzymes in the formation of ATP. If the K element is low, the process of opening and closing stomata becomes sluggish and the rate of respiration increases so that the plant becomes susceptible to drought. The presence of shade can create suitable environmental conditions for ginger plants, so that when K needs is met, ATP production can be optimized, respiration is controlled and the photosynthetic process is optimal [6].

According to [6], that if the plant lacks of K element, the starch content decreases, carbohydrates will dissolve and N compounds accumulated. [8] Constraints that are often encountered on plantation due to the high level of erosion are the low level of soil fertility which is characterized by, among other things, the low availability of macro nutrients such K. The application of KCI fertilizer on ginger plants needs to consider the dosage. Excess of KCI fertilizer affects the absorption of nutrients, the formation of meristems, and stomata activity, so that it can reduce the sensitivity of plants to drought and cold air, as...
well as affect the number of leaves. The deficiency of K elements affects rate of photosynthesis, formation and transportation of carbohydrates, resistance to disease, and affects growth and quality of the yield[9].

Potassium plays a role in the process of opening and closing stomata, when K available the guard cells around the stomata will swell so that the stomata open and gas exchange occurred. Based on the results of the study by [5] that the type of rhizome (main rhizome and subsidiary rhizome) that used as seeds had no effect on the nutritional content of K, but had a significant effect on nutrient absorption of K elements. Moreover, [6] explains that if the K content is inadequate, the process of opening and closing of the stomata will be slow and a lot of water vapor is lost so that the plant becomes susceptible to drought, and can also result in stunted plant growth.

Potassium activates enzymes in plants, one of which is enzymes that play a role in the production of ATP, where ATP acts as an energy source in plant metabolic processes, while the balance of charge at the ATP production site can be maintained in the presence of K ions, so if the availability of K elements is less, the rate of photosynthesis and the rate of photosynthesis is low. ATP production will decrease and all chemical processes involving ATP will slow down. Some plants cope with this by directing the leaves closer to the light source to increase light interception, but in some plants avoid light to prevent damage from excess light [References needed in here]. Data on the nutritional content of 100 grams of dried ginger showed that the rhizome contained more K (1,342 mg) compared to other nutrients such as P (148 mg) and Mg (184 mg) [7].

Low sunlight intensity will affect the absorption rate of nutrients because it affects transpiration rate. Nutrients absorbed through mass flow, and low transpiration will also decrease the mass flow rate. To compensate this nutrients in the soil must be more available, especially potassium nutrients. According to [7] ginger plants that grow in shaded conditions can only grow ideally with high levels of K fertilization. This is because potassium plays an important role in the transportation of water and plant nutrients, as explained by [6]. Several environmental factors that affect the growth of kencur plants include water stress, light intensity, CO₂ concentration, and salinity. When the light intensity received is low, then the amount of light received per unit area of the leaf within a certain period of time will be low. As stated by [10] that too high light can suppress auxin work and conversely low light stimulates auxin work. The stress action of high auxin will cause reduced plant height. By using shade, the light received by plants can be reduced and air temperature will also reduce and thus maintain soil moisture [11]. If the temperature is too low, the plant will grow longer [References]. Previous studies has shown that the use of paranet shade with shade intensity of 25 and 50% affected the growth and result of red ginger while emprit ginger grew well at a shade intensity of 50% [3]. The intensity of light will also affect several vegetative and generative factors of plants such as plant leaf area, morphology, and weed growth around plants. The environmental conditions around the plants greatly affect plant growth and rhizome formation. The intensity of light received by plants affects the process of photosynthesis in plants. Each plant has different light intensity requirements. Ginger plants are shade-tolerant plants, according to [12]. Usually plants that are able to adapt to shade often have limitations on high light intensity. Therefore, cultivation of kencur plants in the shade is an alternative in increasing the production of kencur plants. According to [13], kencur agroforestry systems are usually carried out under stands of pine trees, sugar palms, coconuts and fruit trees. [14] explained that the provision of shade significantly affected the growth and rhizome weight of red ginger plants. [15] stated that the distribution of the spectrum of sunlight received by leaves on the canopy surface was greater than that of leaves under shade. In shaded conditions, little light can be used for photosynthesis. Meanwhile, shade can reduce photosynthetic enzymes that function as catalysts in CO₂ fixation and reduce the light compensation point [12], shade reduces the main light radiation that is active in photosynthesis, resulting in decreased net assimilation.

The treatment of different light intensities will result in several changes experienced by plants. The plant adaptation to stress conditions of low light intensity can be seen from the morphological, anatomical, and physiological characters of the plant [3]. One of the changes in plant morphology is the occurrence of etiolation events, this is due to reduced levels of auxin in plants. Low light generally causes stunted growth, while too high light causes plants to dry out [16]. One way to adjust the intensity of sunlight to suit the needs of the plant is to provide shade. Providing shade can be done using paranet as artificial shade or intercropped with trees [8].
Potassium also plays an important role in regulating plant cell turgority, when potassium levels are low, the process of opening and closing stomata will be slow and respiration is high so that plants become susceptible to drought. According to [6], when the element of K is lacking, the rate of photosynthesis and the rate of ATP production decreases and all chemical processes involving ATP will slow down, while respiration in plants increases which can also result in stunted plant growth. The presence of shade can create suitable environmental conditions for ginger plants, so that when K needs are sufficient, ATP production can be optimized, respiration is low and the photosynthetic process is optimal. Potassium also plays a role in the translocation of starch and carbohydrates where in ginger is needed to translocate photosynthate to the vegetative part, namely the rhizome.

4. Conclusion
It can be concluded that the yield of Lumajang and Nganjuk accessions were higher in the 50% shade than the 25% shade, while the the total antioxidant activity of rhizome kencur in the Lumajang and Nganjuk accessions at 25% shade was higher than the 50% shade. An increase of potassium fertilizer dose increases the total antioxidant activity of kencur rhizome accession of Lumajang and Nganjuk, not only in the 25% but also in the 50% shade.

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