Optimization of curcumin temulawak (*Curcuma xanthorrhiza* Roxb.) on calcareous marginal land under teak

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**Abstract.** Temulawak produces bioactive compounds that have antioxidant activity and changes in its chemical composition are caused by environmental pH. Sulphur is an essential nutrient for plants and a constituent of several coenzymes and vitamins that play a role in plant metabolism. Marginal calcareous land in Tuban is generally used as teak production forest, because food crops cannot grow optimally in this land. Temulawak is a shade-tolerant plant that can grow well in conditions of low light intensity. The objective of this research is to obtain the appropriate goat manure and sulphur fertilizer dosages for temulawak planted beneath teak stands on calcareous marginal soil in order to maximize its yield and quality. The field experiment was RCBD with three replications and two factors: manure level (10, 15 and 20 t ha⁻¹) and ZA level (0, 40, 80 and 120 kg ha⁻¹). The results showed that a higher fresh weight of rhizomes per clump was obtained in combination of 10 and 15 t ha⁻¹ manures and 40 kg ha⁻¹ sulphur fertilizer. Similar high yield is obtained when 20 t ha⁻¹ of manure is applied in combination with 80 kg ha⁻¹ of sulphur. In addition, a higher curcumin content and antioxidant activity were obtained in plant treated with 10 t ha⁻¹ manure without S fertilizer. Similar high curcumin content and antioxidant activity of the rhizome were also obtained from rhizome treated with 15 t ha⁻¹ of manure in combination with 40 kg ha⁻¹ sulphur fertilizer and 20 t ha⁻¹ manure without sulphur fertilizer.

**Keywords:** Curcuma, Manure, Sulphur, Curcumin, Antioxidant

1. **Introduction**

Temulawak (*Curcuma xanthorrhiza* Roxb.) is medicinal native plants of Indonesia and widely grown in Java Island. Temulawak rhizomes contain curcumin and xantorrhizol as bioactive compounds that are useful as free radical scavengers [1]. Temulawak is a plant that is generally cultivated under the shade. In natural habitats, curcuma grows under bamboo and teak trees. It has fairly high levels of adaptation to shade [2] and temulawak had higher yields under shade of up to 50% [3].

Agricultural land in the Tuban area, especially in Montong district is comprised of calcareous hills with soil pH from 6.9 to 7.3 and has a low organic matter of 0-1.04% [4]. These conditions caused a lot of land in this area are planted with teak. The area is not suitable for food crop plantation resulted in a lower yield of food crops. Teak production forest is spread over an area of 14,000 ha in almost all sub-districts in Tuban district [5]. The extent of teak production forest supports the use of land under teak stands for...
the cultivation of shade-tolerant plants that can adapt to unfavourable environmental conditions, such as temulawak.

According to Neina [6], soil pH significantly influences the biological, chemical, and physical qualities of soil, as well as the processes that influence plant growth and yield. The availability of all mineral nutrients is affected by soil pH [7]. Based on research by Gunarti and Nurificahyanti [8], curcuminoids have antioxidant activity whose chemical composition changes, especially the color, by environmental pH. Research by Hossain and Ishimine [9] showed that soil with pH 5.2 produced 0.20% higher curcumin content than soil with pH 7.48 which produced only 0.10% curcumin content.

The use of soil amendment such as manure can improve soil structure, soil chemistry and soil organisms (microbes and macro fauna). Research by Graham et al., [10] showed that organic matter increased microbial activity that can reduce pH due to microbial degradation activity and organic acid production, stabilize soil aggregates, influencing soil moisture status, nutrient dynamics and soil porosity in a positive way, soil physical qualities are improved by increasing the aggregate's porosity and stability. In addition to reduction of soil pH by organic manure, according to Taiz and Zeiger [11] chemical fertilizers can also be applied to change soil pH. Alkaline soils with high pH require the application of a soil enhancer containing sulphur to lower soil pH [12]. Microorganisms absorb sulphur and release sulphate and hydrogen ions which can acidify the soil. In addition to the impact of sulphur to soil pH. Sulphur is needed by the plant as it is a constituent of several co enzymes and essential vitamins require for plant metabolism [11]. The formation of acetyl Co-A is also influenced by sulphur, where acetyl Co-A will be involved in the biosynthesis of curcumin. Therefore, this research examine the effect of of the dosages of goat manure and sulphur fertilizer on yield and yield quality of temulawak grown in calcareous land under teak. The study was undertaken as an effort to increase the yield and quality of temulawak planted on calcareous marginal land under teak.

2. Materials and methods
The research was conducted from November 2020 until June 2021 on Singobarong street, Pakel Village, Montong District, Tuban Regency and Plant Physiology Laboratory, Faculty of Agriculture, Brawijaya University. This study used the secondary rhizome of local varieties temulawak (Curcuma xanthorrhiza Roxb.) which is planted on calcareous land under teak tree. The field experiment was laid out in RCB (Randomized Complete Block Design) with three replications and two factors: the first factors were manure level (10 t ha⁻¹, 15 t ha⁻¹ and 20 t ha⁻¹) and the second factors were sulphur fertilizer level (0 kg ha⁻¹, 40 kg ha⁻¹, 80 kg ha⁻¹ and 120 kg ha⁻¹). Temulawak seedlings were planted in 1.5 m × 3.3 m beds. Goat manure was applied once during tillage (10 t ha⁻¹, 15 t ha⁻¹ and 20 t ha⁻¹). ZA fertilizer (S 24% and N 21%) applied once on 30 days after planting (0 kg ha⁻¹, 40 kg ha⁻¹, 80 kg ha⁻¹ and 120 kg ha⁻¹). Urea 300 kg ha⁻¹, SP36 100 kg ha⁻¹ and KCl 150 kg ha⁻¹ applied once on 30 days after planting. Fertilization methods for all inorganic fertilizer using localized placement (to applying fertilizer in a hole near root area).

2.1. Preparation for curcumin analysis
The dried curcumin sample that has been mashed is taken as much as 1 g and macerated with 10 ml of methanol for 24 hours, after 24 hours the solution was filtered through Whatman filter paper 41, it is repeated until a solution of 50 ml is obtained. Curcumin was tested by adding 9 ml of curcumin standard to 1 ml of the solution obtained from maceration. A UV-VIS spectrophotometer was used to measure the absorbance at a wavelength of 421 nm.

2.2. Preparation for antioxidant analysis
Antioxidant testing was carried out by free radical scavenging method using DPPH (1,1-diphenyl-2-picrylhydrazil) using 0.5 mL each of methanol extract. To each sample a 2 mL DPPH solution was added and the mixture was vortexed for 2 minutes. The efficiency of free radicals is indicated by the change in color of the solution from purple to yellow. In addition, the absorbance was measured with a UV-VIS spectrophotometer at a wavelength of 517 nm in the last 5 minutes before 30 minutes incubation.
2.3. Data analysis
The effect of treatment on the yield and quality of temulawak plants was investigated using analysis of variance (ANOVA). The HSD test was performed at the 5% level for parameters with a significant different in ANOVA test.

3. Results and discussion
Table 1. shows that there was an interaction between manure and sulphur fertilizer on the fresh weight of temulawak rhizomes per clump at 180 days after planting. The application of 10 t ha$^{-1}$ and 15 t ha$^{-1}$ manure required an additional of 40 kg ha$^{-1}$ sulphur fertilizer to achieve a higher average fresh weight of rhizomes per clump. When manure is applied at a rate of 20 t ha$^{-1}$, sulphur fertilizer is required at a rate of 80 kg ha$^{-1}$ to obtain the similar high yield.

| Treatment       | Sulfur Fertilizer | Rhizome Fresh Weight (per clump) at 180 dap (days after planting) |
|-----------------|-------------------|--------------------------------------------------------------------|
|                | 0 kg ha$^{-1}$    | 40 kg ha$^{-1}$          | 80 kg ha$^{-1}$          | 120 kg ha$^{-1}$          |
| 10 t ha$^{-1}$  | 70.41 a           | 101.39 bc               | 113.41 c                | 114.18 c                 |
| 15 t ha$^{-1}$  | 76.91 ab          | 104.43 bc               | 82.04 ab                | 80.27 ab                 |
| 20 t ha$^{-1}$  | 78.62 ab          | 75.73 ab                | 119.73 c                | 127.71 c                 |
| HSD 5 %         |                   |                       | 30.76                   |
| CV (%)          |                   |                       | 18.60                   |

Notes: Numbers followed by the same letter in the same column and row indicated no significant difference, based on the 5% HSD test; HSD = Honest Significant Difference.

According to Munawar [13], manure contains NPK nutrients, the major nutrient required for vegetative growth and development both stems and leaves growth require adequate availability of nitrogen. Phosphorus is required for root development, cell division, propagation and to form energy required for plant growth and metabolism processes, while potassium is required for stem development, cell division, carbohydrate production and translocation. Turmeric, in particular, needs potassium for rhizome development [14].

Sulphur is not readily available for translocation in plants, so all plants require a continuous supply of sulphur from the early stage of growth to the maturity of the plant [15]. McKenzie [15] pointed out that higher doses of sulphur fertilizer are expected to provide a continuous supply of sulphur to meet crop needs. Sulphur is an important component of plant growth and development. Amino acids, chloroplasts, vitamins, coenzymes, and prosthetic groups are all sulphur-containing substances [16], and therefore S plays a vital role in plant photosynthesis. With the development of chlorophyll and suitable environmental conditions, the photosynthesis process may take place properly, resulting in optimal photosynthetic production.

Table 2 shows that without the addition of sulphur fertilizer, the curcumin content of 10 t ha$^{-1}$ manure was greater. If 15 t ha$^{-1}$ of manure is used, a sulphur fertilizer application of 40 kg ha$^{-1}$ is necessary to obtain a high curcumin content or in the 20 t ha$^{-1}$ of manure application without sulphur fertilization.
Table 2. The Interaction of Manure and Sulphur Fertilizer on Curcumin Content of The Temulawak Rhizome Grown in Calcareous Soil Under Teak.

| Sulfur Fertilizer | 0 kg ha\(^{-1}\) | 40 kg ha\(^{-1}\) | 80 kg ha\(^{-1}\) | 120 kg ha\(^{-1}\) |
|-------------------|-----------------|----------------|----------------|----------------|
| Goat Manure 10 t ha\(^{-1}\) | 82.45 c | 64.11 abc | 61.87 abc | 54.10 ab |
| 15 t ha\(^{-1}\) | 41.72 a | 84.73 c | 64.68 abc | 65.81 bc |
| 20 t ha\(^{-1}\) | 63.86 abc | 63.99 abc | 46.55 ab | 44.39 ab |
| HSD 5% | 23.26 | | | |
| CV (%) | 21.81 | | | |

Notes: Numbers followed by the same letter in the same column and row indicated no significant difference, based on the 5% HSD test; HSD = Honest Significant Difference.

Table 3 shows that the antioxidant activity of rhizome was higher when 10 t ha\(^{-1}\) manure was applied without S fertilization or in the 15 t ha\(^{-1}\) of manure application in combination with 40 kg ha\(^{-1}\) and in the 20 t ha\(^{-1}\) or manure in combination sulphur fertilizer of 40 kg ha\(^{-1}\).

Table 3. The Interaction of Manure and Sulphur Fertilizer on The Antioxidant Activity of The Temulawak Rhizome Grown in Calcareous Land Under Teak.

| Antioxidant Activity (%) at 180 (days after planting) | Sulfur Fertilizer | 0 kg ha\(^{-1}\) | 40 kg ha\(^{-1}\) | 80 kg ha\(^{-1}\) | 120 kg ha\(^{-1}\) |
|-----------------------------------------------------|------------------|----------------|----------------|----------------|----------------|
| Goat Manure 10 t ha\(^{-1}\) | 92.87 cd | 89.88 abc | 90.57 abc | 89.15 abc |
| 15 t ha\(^{-1}\) | 87.29 a | 94.34 d | 91.27 bc | 91.56 bc |
| 20 t ha\(^{-1}\) | 89.52 abc | 91.35 bcd | 88.81 ab | 88.63 ab |
| HSD 5% | 3.74 | | | |
| CV (%) | 2.39 | | | |

Notes: Numbers followed by the same letter in the same column and row indicated no significant difference, based on the 5% HSD test; HSD = Honest Significant Difference.

On calcareous marginal soil, application of 10 t ha\(^{-1}\) manure without sulphur fertilization produced the rhizome with similar high curcumin and antioxidant activity as 15 t ha\(^{-1}\) manure in combination with 40 kg ha\(^{-1}\) sulphur fertilizer, 80 kg ha\(^{-1}\), and 120 kg ha\(^{-1}\) sulphur fertilizer. The findings were equally similar when 20 t ha\(^{-1}\) manure was applied without sulphur fertilization and when 20 t ha\(^{-1}\) manure was applied with 40 kg ha\(^{-1}\) sulphur fertilizer. This condition could be caused by the availability of nutrients being limited in the usage of manure 10 t ha\(^{-1}\) without the addition of sulphur fertilizer compared to when sulphur fertilizer is added, resulting in a higher curcumin content than in other manure treatments. The activity of important enzymes such as PAL, regulates phenolic production under a stressful environment [17]. PAL activity increases in extreme environmental conditions, including low nutrient levels and low soil water content [18]. The curcumin content and antioxidant activity in the 10 t ha\(^{-1}\) manure treatment without sulfur fertilizer are increased as a result of this condition.

Organic fertilizers contribute to the soil's chemical characteristics by supplying macro and micronutrients. The macronutrients that can be provided by organic fertilizers are N, P, K, Ca, Mg and S [19]. Phenylalanine ammonia lyase enzyme (PAL) functions as a catalyst in the formation (biosynthesis) of curcumin which is very sensitive to environmental factors. PAL activity will increase in extreme environmental conditions [20]. Curcumin is a secondary metabolite of the polyphenol group. PAL is one of the enzymes that regulate phenolic activity [21]. Although PAL does not synthesis phenol exclusively from phenylalanine, its enzymatic activity may be related to phenol concentration. [22]. Curcumin content and antioxidant activity in temulawak will increase as PAL activity rise.
4. Conclusion
A higher fresh weight of rhizomes per clump was obtained in combination of 10 t ha\(^{-1}\) and 15 ha\(^{-1}\) manures and 40 kg ha\(^{-1}\) sulphur fertilizer. Similar high yield is obtained when 20 t ha\(^{-1}\) of manure is applied in combination with 80 kg ha\(^{-1}\) of sulphur. In addition, a higher curcumin content and antioxidant activity of the rhizome cultivated in calcareous land under the teak were obtained in plant treated with 10 t ha\(^{-1}\) manure without S fertilizer. Similar high curcumin content and antioxidant activity of the rhizome were also obtained from rhizome treated with 15 t ha\(^{-1}\) of manure in combination with 40 kg ha\(^{-1}\) sulphur fertilizer and 20 t ha\(^{-1}\) manure without sulphur fertilizer.

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