Shading and vermicompost effect on growth and flavonoid content of Tapak Liman (*Elephantopus scaber* L.)

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Abstract. Tapak Liman (*Elephantopus scaber* L) is one of Indonesian medicinal plants which is well known as weed. In Thailand, Tapak Limanthis plant is used for traditional medicine due to its flavonoids contains. Flavonoid is compound with red, yellow, purple and blue pigments, used for cancer, aphrodisiac and anti-radical treatments. One obstacle of Tapak liman cultivation is the effort to increase its flavonoids compound. There is a bridge between flavonoids compound with growth and yield of Tapak Liman. For that, this research aims to find out the effect of shade intensity combined with vermicompost dosage on Tapak Liman growth and yield. This research was conducted in Mei to August 2016 at Medicinal Plantation of BPTO, Tanjungsari Village, Tegal Gede, Karanganyar. Complete Randomized Design compiled with split plot and two factors: shade intensity (0%, 50%, 75%) and vermicompost dosage per plant (0 g, 250 g, 500 g, 750 g) used as the experimental design. The variables observed are leaves number, leaves length, canopy diameter, fresh weight, dry weight, root length, chlorophyl analysis and flavonoid identification. Data were analyzed using ANOVA, any significant treatments followed with Duncan’s Multiple Range Test (DMRT) at α = 10%. Result showed that 75% shade intensity and 750 g of vermicompost have gave highest yield of leaf and total simplicia of Tapak Liman. Shade intensity of 50% with 250 g and 500 g/plant of vermicompost dosage showed highest flavonoid rendement (Rf 0,5) with highly contrasting spot colors.

1. Introduction

Tapak Liman (*Elephantopus scaber*) is a plant containing several compounds such as dihydro deoxyelephantopus, lupeol, stigmasterol and flavonoids [1] which can be used as medicine for various diseases. Types of diseases that can be treated with Tapak Liman include various inflammations such as inflammation of the tonsils, influenza, sore throat, inflammation of the eyes, acute inflammation of the kidney and inflammation of the uterus and vaginal discharge [2].

The study shows that flavonoids are affected by different light intensity [3]. A similar trend to increase flavonoid content by light intensity is also seen in some medicinal plants, as shown in [4] research. Many flavonoid compounds contained in the leaves, so the simplicia leaves of Tapak Liman often used as a medicinal. To increase the quantity and quality of simplicia leaf of Tapak Liman, it require intensive cultivation.
One effort to increase the simplicia of the Tapak Liman is by applying vermicompost to the soil. Vermicompost is a high quality fertilizer due to its various nutrients content which needed by plants. Vermicompost contains a lot of N in available form that is used for the formation of plant organs especially leaves, but also N influential in flavonoid formation process [5]. Thus, the application of shade treatment and vermicompost expected to give effect to the flavonoids content in Tapak Liman.

2. Methods
The research was conducted in BPTO Medicinal Plant Garden, Tanjungsari Village, Tegal Gede, Karanganyar. The field experiment was conducted from June to August 2016. The geographical location of the research site was -7° 36’ 687” S and 110° 57’ 766” E. The altitude of the site was approximately 215 meters above sea level. Daily temperature ranging from 27°C - 30°C. The materials used in this research were the seeds of Tapak Liman (72 seedlings), planting medium in the form of soil, vermicompost fertilizer, polybag, bamboo, 50% and 75% parernet, treatment board, ethanol 0.1N, Tapak Liman leaf extract and aquades.

This research was performed using CRD experimental design which arranged in split plot. Treatment consists of shade as main plot and vermicompost dose as sub plot. Shade treatments consists of 0% (without shade), 50% and 75%. The dose of vermicompost (per polybag) consists of 0 g, 250 g, 500 g and 750 g. Each treatment was repeated 3 times and each experimental unit consist of 2 sample plants. The data obtained were analyzed using ANOVA, any significant treatments were followed with Duncan Test at α = 10%.

3. Results and Discussion

3.1. Tapak Liman leaves simplicia

3.1.1. Leaves weight The result of shade and vermicompost treatment level on leaf weight of Tapak Liman is shown in Figure 1 and 2.

![Figure 1. Effect of shade on Tapak Liman leaves weight](image)

Plants with a shade treatment of 75% gave significant difference results compared to plants without shade and 50% shade. The 75% shade treatment has the highest average leaves weight value of 19.5 grams/plant, whereas without shade and 50% shade treatment produce 10.29 gram/plant leaf weight. [6]. Shade serves to reduce the radiation received leaves and reduce water loss so dehydration can be avoided. This causes the weight of the leaves to increase in the shaded condition, because the leaves store more water.
Plants with 750 g of vermicompost treatment shows the highest average leaves weight of 16 g/plant, while the dose of 250 g/plant and 500 g/plant treatment resulted in average leaves weight of 13.55 g/plant. The lowest average leaves weight produced by plants without vermicompost which is 10.44 g/plant [7]. The formation of leaves by plants is strongly influenced by the availability of nutrients N and P on planting medium. In the treatment without vermicompost the plants undergo nutrient deficiency, because the planting media lack of nutrients.

3.1.2. Number of leaves

The effect of shade intensity and vermicompost treatment on leaves number shown in Figure 3 and 4.

Plants with 75% shading treatment gave the highest average yield of leaves of 15.67 pieces, while the plants with shade treatment of 50% and without shade resulted in average leaves number of 13.04 pieces.
Plants without vermicompost produce the lowest average of 12.78 sheets, while the highest average leaves number of 14.72 pieces was produced by the treatment of vermicompost with a dose of 500 g/plant and 750 g/plant. Plants with a dose of vermicompost 250 g/plant yields a total of 13.44 pieces of leaves. Tapak Liman on the treatment without the application of vermicompost fertilizer experienced obstacles in the formation of plant leaves [8]. The availability of N in sufficient quantities will facilitate plant metabolism and will eventually affect the growth of organs such as stems, leaves, roots for the better. Leaves number variables have significantly different correlation (r = 0.342) with root length. That was, the greater length of the root, the more the number of leaves produced.

3.1.3. Leaf length and canopy diameter The result of analysis of variation of shade intensity and vermicompost dose to leaf length and canopy diameter showed no significant difference to Tapak Liman leaf length. Treatment of shade and vermicompost on leaf length variables also did not cause interaction. The influence of shade on the leaf length and vermicompost of Tapak Liman can be seen that the average length of leaf Tapak Liman was 13.31 cm. The average canopy diameter produced by the shade and vermicompost treatment was 25 cm.

3.1.4. Chlorophyl content The result of shade treatment effect on chlorophyll content is shown in Figure 5.

Plants without shade showed highest average yield of the chlorophyll content while the 75% shade treatment resulted in the lowest average chlorophyll content of 27.81%. 50% shade treatment resulted in average chlorophyll content of 29.85%. The higher shade level, the less chlorophyll produced. This happens because, in a state of light with low intensity, protein synthesis for chlorophyll formation decreases and more leads to the occurrence of leaf senescence [9].
3.2. Tapak Liman root simplicia

3.2.1. Root weight and root length The result of analysis of variance (anova) of shade and vermicompost effect showed no significant difference to the root weight and root length of the Tapak Liman. It is known that the average root weight of the Tapak Liman produced by the shading treatment and vermicompost treatment is 5.79 grams. The result of shading treatment and vermicompost treatment yielded an average root length of 35.80 cm.

3.3. Total simplicia of Tapak Liman

3.3.1. Fresh weight The result of analysis of variance (anova) on the effect of shade and dose of vermicompost to fresh weight of plant showed significant difference (Figure 6).

![Figure 6. Effect of shade on the fresh weight of Tapak Liman](image)

Plants with 75% shading treatment showed the highest average fresh weight yield of 24.88 g/plant. While without shade treatment showed the lowest average fresh weight of 14.48 g/plant. The shade treatment of 50% shows the average yield of fresh weight of 19.45 g/plant.

![Figure 7. Effect of vermicompost on the fresh weight of Tapak Liman](image)

The highest average fresh weight produced by plants with treatment of 500 g and 750 g vermicompost is 22.9 g/plant, while the lowest average fresh weight produced by plants without vermicompost of 16.59 g/plant (Figure 7). Plants with a vermicompost treatment of 250 g/plant yield average fresh weight of 17.71 g/plant. The fresh weight of the plant is influenced by the increase in the length and volume of the plant caused by expansion of plant cells [10]. This is what causes the increase in fresh weight of the plant.
3.3.2. Dry weight  The results of variance analysis showed that only shade treatment had a significant effect on the dry weight of Tapak Liman (Figure 8).

![Figure 8](image1.png)

**Figure 8.** Effect of shade on the fresh weight of Tapak Liman

The highest average dry weight was produced by plants with 75% shade treatment of 5.87 g/plant, while the lowest average dry weight was shown in no shade treatment which is 3.9 g/plant. Plants with a shade of 50% produce an average dry weight of 4.58 g / plant. [11] says that light with high intensity can lead to growth inhibition associated with the balance of photosynthesis and respiration. Light with high intensity, increase temperature, so that respiration increased and the lower dry weight produced. The lower light intensity results in a decrease in photosynthesis rate so that the dry weight is lower.

3.4. Flavonoid analysis

3.4.1. Extract content  It can be seen the highest extract level of Tapak Liman is produced by 50% shade treatment with 500 g vermicompost of 13.33% (Figure 9). The highest extract content was then shown by 50% shade treatment with 750 gram vermicompost of 10%. From the 12 treatment combination, 10 treatment combinations were on average yielding an extract level of 6.67%. The content of this extract shows the secondary metabolite content present in plant. The greater level of extract, the more secondary metabolite content in the plant, whereas the fewer extract content, the lower secondary metabolite content in the plant will be. These active compounds will later be identified as flavonoid compounds.

![Figure 9](image2.png)

**Figure 9.** Effect of shade intensity and vermicompost dose treatment on Tapak Liman extract concentration of active compound.

3.5. Flavonoid of Tapak Liman

Flavonoids are accumulated or secreted in the vacuole, as in the roots, but are more often produced in flowers and leaves. These compounds are usually formed due to stressful environmental conditions
and to protect themselves against pathogens and pests. Flavonoid of Tapak Liman analyzed using KLT test and the results shown in Figure 10.

![Figure 10. Test result of KLT of flavonoid content](image)

The result of TLC analysis of flavonoids content (Table 1) shown that there are other flavonoid group compounds at Rf 0.5. The compound of flavonoids successfully isolated in the range of Rf 0.45

Table 1. Result of flavonoid test of Tapak Liman

| Code | Treatment                                              | Spot Diameter | Color | Flavonoid | Harkat       |
|------|--------------------------------------------------------|---------------|-------|-----------|--------------|
| 1    | Standard                                               | 4             | 3     | 7         | Average      |
| 2    | Without shade intensity and without vermicompost       | 3             | 3     | 6         | Average      |
| 3    | Without shade intensity and 250 g vermicompost         | 2             | 3     | 5         | Low          |
| 4    | Without shade intensity and 500 g vermicompost         | 3             | 4     | 7         | Average      |
| 5    | Without shade intensity and 750 g vermicompost         | 3             | 2     | 5         | Low          |
| 6    | Shade intensity 50% and without vermicompost           | 3             | 4     | 7         | Average      |
| 7    | Shade intensity 50% and 250 g vermicompost            | 2             | 1     | 3         | Very low     |
| 8    | Shade intensity 50% and 500 g vermicompost            | 3             | 4     | 7         | Average      |
| 9    | Shade intensity 50% and 750 g vermicompost            | 1             | 2     | 3         | Very low     |
| 10   | Shade intensity 75% and without vermicompost           | 2             | 1     | 3         | Very low     |
| 11   | Shade intensity 75% and 250 g vermicompost            | 1             | 2     | 3         | Very low     |
| 12   | Shade intensity 75% and 500 g vermicompost            | 2             | 2     | 5         | Low          |
| 13   | Shade intensity 75% and 750 g vermicompost            | 2             | 2     | 4         | Low          |

Information:

- Spot diameter score:
  1: diameter 0 – 24%
  2: diameter 25 – 54%
  3: diameter 55 – 74%
  4: diameter 75 – 100%

- Color score:
  1: nuclear
  2: not contrast
  3: contrast
  4: very contrast

- Flavonoid score:
  2 – 3: Very low
  4 – 5: Low
  6 – 7: Average
  8: High

The result of TLC analysis of flavonoids content (Table 1) shown that there are other flavonoid group compounds at Rf 0.5. The compound of flavonoids successfully isolated in the range of Rf 0.45.
which is quercetin (Figure 10 No. 14) to Rf 0.68 (Figure 10 Number 1) which is isodeoxyelephantopin [12]. So the spot that appears on Rf 0.5 can be expressed as a class of flavonoids. To know the name of the compound is needed further quantitative analysis. The spots generated from the TLC test were performed on 12 treatment combinations, most of them in the same Rf. This indicates that the flavonoid group found in the 12 treatments were the same. These spots can be measured by scores so that the data obtained into semi-quantitative to find out how much spot generated by each treatment to know whether the amount exceeds the standard value or not. The table of flavonoid scales of Tapak Liman shown as follows.

Without shade treatment with 500 g of vermicompost produce a spot of 66.6% with very bright color brightness in a flavonoid score of 7 (medium) equal to standard (isodeoxy elephantopin), whereas without shade treatment with 750 g of vermicompost produced a spot of equal magnitude of 66.6% but the pale color brightness belonged to a flavonoid score of 5 (low). Differences in the types of flavonoids according to [13] occurs because of the type missing/not appearing due to the possibility of shade. This is evident from the type of flavonoids that decrease in number along with increasing shade levels.

Such spots contained in a 50% shade treatment with 250 g vermicompost and a 75% shade without vermicompost resulted in an almost invisible spot or with very pale color brightness resulting in flavonoid scoring of 3 (very low). Flavonoids have a correlation with dry weight (r = 0.723). This shows that there is a relationship between dry weight and flavonoids. The process of photosynthesis produces a primary metabolite in the form of photosynthate, then some of the photosynthate processed for secondary metabolites, one of the materials for the formation of flavonoid formation.

4. Conclusion
Level of shade of 75% yield the highest result on leaves simplicia variable and total simplicia of Tapak Liman with leaves weight of 19.5 gram, number of leaves of 24.85 piece, and fresh weight of 24.85 gram. The dosage of vermicompost of 750 grams/plant produce the highest yield on the simplicia varieties of leaves and the total simplicia of Tapak Liman with 22.9 grams leaves weight. The shade treatment of 50% with 250 g/plant vermicompost dosage yields the highest extract content of 13%. Without shade treatment and 50% shade with 500 g/plant vermicompost produced the greatest spot of 66.6% with very bright red brightness defined as flavonoids at Rf 0.5 with flavonoid score 7 defined as moderate.

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