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## Effect of manure and plants spacing on yield and flavonoid content of *Elephantopus scaber* L.

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**Abstract.** This experiment is aimed to observe the growth and flavonoid contain of Tapak Liman (*Elephantopus scaber* L.) with different manure types and plants spacing treatment. This experiment is conducted at Tegal Gede Village, Karanganyar District on June until August 2016. The analysis of secondary metabolism was done in B2P2TOOT, Tawangmangu. This experiment is conducted with Randomized Complete Block Design (RCBD) with two treatment factors, those are manure and plants spacing. Animal manure treatment had 3 levels, those are without manure, cow manure with 20 ton/ha dose, and chicken manure with 20 ton/ha dose. Plants spacing treatment had 3 phrase, those are 20 cm x 20 cm; 30 x 30 cm; 40 cm x 40 cm. The result of this experiment shows that chicken manure with 20 ton/ha dosage increase the development of leaves' lengthiness, header's diameter, plant's fresh weight, and plant's dry weight. Plants spacing 40 cm x 40 cm increase for the development of leaves' lengthiness, header's diameter, plant's wet weight, and plant's dry weight. The combination between chicken manure with 20 ton/ha dose and plants spacing 40 cm x 40cm treatments show the highest amount of tapak liman extract and alleged having the biggest amount of flavonoid substance.

### 1. Introduction

Organic fertilizer is a fertilizer composed of living matter such as the remains of plants, animals and humans. The use of organic fertilizers should be encouraged to restore soil conditions as well as increase land productivity and human health. It is also beneficial to increase agricultural production. The use of organic fertilizer is very important as a buffer of physical, chemical, and biological properties so that soil can improve fertilizer efficiency and land productivity [1]. In addition to nutrient factors, plant spacing also plays an important role in increasing agricultural production. Plant spacing determines the population of plants in a certain area [2], so a good arrangement can reduce the competition of water, light and nutrients.

Tapak Liman (*Elephantopus scaber* L.) including in the family of Asteraceae which was often regarded as a weed in cultivated plants. Even so, the elephant's foot contains chemicals that have pharmacological effects. The tapak liman contains chemicals which one of them is flavonoid [3]. Flavonoids are lactagogous substances in which they can increase the hormone prolactin and oxytocin as a trigger exclusive breastfeeding [4]. The efficacy of tapak liman is undoubtedly primarily for the development of herbal medicine, but its excessive use will lead to scarcity if not balanced with



cultivation. The good and right tapak liman procedure of cultivation such as the use of organic fertilizer and plant spacing arrangement was expected to increase the production both quality and quantity.

## 2. Methods

The research was conducted in June to August 2016 in experimental garden of Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT) of Tegal Gede Village, Karanganyar Regency. Flavonoid content analysis was performed at the B2P2TOOT Phytochemical Laboratory. Materials in this study are including of tapak liman seeds, soil media, manure (chicken and cow), ethanol, methanol, hexan, and ethyl acetate. The tools used in this study including of hoes, gauges, ovens, knives or scissors, analytical scales, kilogram scales, rulers, cameras, plastic tubs/plastic trays, stationery, grinders, thin layer chromatography (TLC), chamber, porcelain, 50 ml beaker, waterbath, sonicator, dropper, tweezers and filter paper.

This research use Randomized Complete Block Design (RCBD) factorial pattern with two factors (manure and plant spacing). Different types of manure as the first factor consists of 3 levels: control/no manure, cow manure, and chicken manure. While the second factor of plant spacing consists of 3 levels of plant spacing (20 cm x 20 cm, 30 cm x 30 cm, and 40 cm x 40 cm). From these two factors, there were 9 treatment combinations and each treatment combination was repeated 3 times. Observation variables such as leaf length, crown diameter, leaves number, root length, fresh stover weight, dry stover weight, total extract content, flavonoid content analysis. Data analysis was used variance analysis if the real effect continued with DMRT (Duncan Multiple Range Test) test at the level of 10%.

## 3. Results and Discussion

### 3.1 General condition of Research

This research was conducted at B2P2TOOT Medicinal Plant Garden, Tanjungsari, Tegal Gede Village, Karanganyar. The geographical location of the research site is 7°36.687' S and 110° 57.766' E. The height study site is about 215 meters above sea level. The temperature at the study site ranged from 27°C to 30°C. The soil type in this area is red latosol land, textured clay and with the medium nutrients content.

### 3.2 Leaves Number

The result of variance analysis was showed that manure has no significant effect on the tapak liman leaves number (Table 1). The average number of tapak liman leaves without manure is 32.03, with cow manure is 32.42 and with chicken manure are 36.61.

**Table 1.** Influence of manure on the leaves number

Manure	Leaves number
Without manure	32.03 ± 1.32
Cow	32.42 ± 2.05
Chicken	36.61 ± 2.11

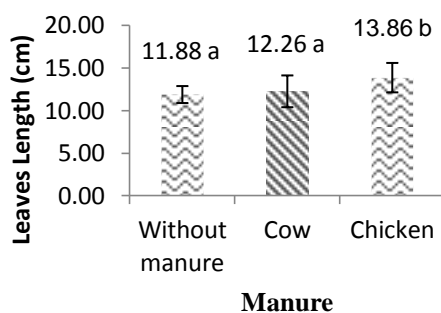
The result of variance analysis on plant spacing treatment did not give significant effect to the leaves number (Table 2). The average of leaves number on 20 cm x 20 cm of plant spacing treatment is 32.25, at 30 cm x 30 cm of plant spacing is 34, and at 30 cm x 40 cm plant spacing is 36.80. The closer the spacing between plants will be the lower rate of photosynthesis, because there is competition in obtaining water, nutrients, and sunlight needed for the photosynthesis process [5].

**Table 2.** The effect of plant spacing on the leaves number

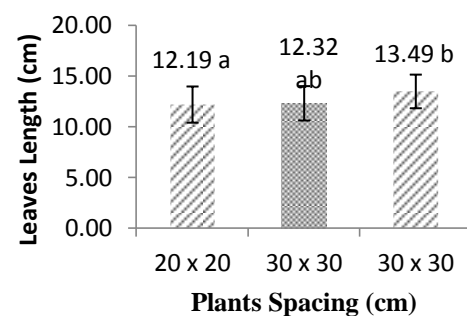
Plants spacing (cm)	Leaves number
20 x 20	32.25 $\pm$ 2.46
30 x 30	34.00 $\pm$ 2.10
40 x 40	36.80 $\pm$ 1.98

### 3.3 Leaves length

Leaves length observation was done by measuring the longest leaves of each sample. The result of variance analysis was showed that manure significantly affected leaves length (Figure 1). The average leaves length on the without manure treatment is 11.8 cm, cow manure is 12.26 cm, and chicken manure is 13.86 cm. It was stated that chicken manure has more nutrients and is easily decomposed so that roots can absorb more nutrients ions to be used in photosynthesis [6].



Numbers were followed by the same letter does not differat the real level of DMRT test of 10%

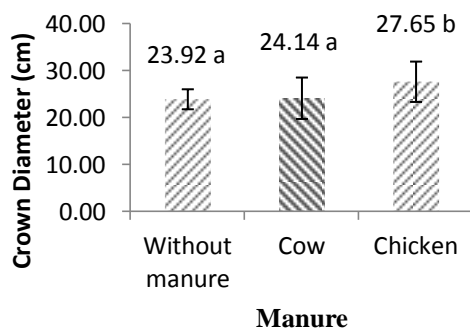
**Figure 1.** Effect of manure on leaves length**Figure 2.** Effect of plant spacing on leaf length

Treatment of plant spacing had significant effect on the leaves length of the tapak liman (Figure 2). Average of leaves length at plant spacing of 20 cm x 20 cm is 12.19 cm, 30 cm x 30 cm plant spacing is 12.32 cm, and 40cm x 40cm plant spacing is 13.49 cm. This is an accordance to the research [7] which says the more spaced of plants more solar energy captured by the plants used for photosynthesis.

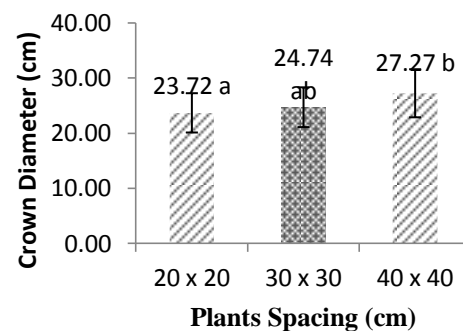
### 3.4 Crown Diameter

The result of variance analysis showed that manure significantly affect the crown diameter (Figure 3). The average of crown diameter on the treatment without manure is 23.92 cm is not significantly different with cow manure of 24.14 cm, but the real difference in chicken manure of 27.65 cm. This is because chicken manure contains a higher N element. [8] stated nitrogen is necessary to support vegetative growth of plants [8]. In a research also stated that the provision of chicken manure can increase growth such as plant height and leaf area [9].

The result of variance analysis was showed that the plant spacing treatment had significant effect on crown diameter (Figure 4). At plant spacing of 20 cm x 20 cm has a crown diameter average of 23.72 cm, plant spacing of 30 cm x 30 cm is 24.74 cm and plant spacing of 40 cm x 40 cm is 27.27 cm. According to [10] the more planting spacing will reduce competition in using nutrients and sunlight.



Numbers were followed by the same letter does not differat the real level of DMRT test of 10%  
**Figure 3.** The effect of manure on crown diameter



**Figure 4.** The effect of plant spacing on crown diameter

### 3.5 Root Length

The result of variance analysis was showed that manure has no significant effect on root length (Table 3). The roots length average on the treatment without manure is 26.69 cm, cow manure is 28.54 cm and chicken manure is 27.09 cm.

**Table 3.** The effect of manure to root length (cm)

Manure	Root Length (cm)
Without manure	26.69 a $\pm$ 2.83
Cow	28.54 a $\pm$ 3.13
Chicken	27.09 a $\pm$ 1.74

Description: The numbers followed by the same letters show no significant difference in the Duncan test level of 10%

Plant spacing treatment did not have a significant effect on root length (Table 4). The average of root length of tapak liman at plant spacing of 20 cm x 20 cm is 26.62 cm, plant spacing of 30 cm x 30 cm is 27.08 cm and plant spacing of 40 cm x 40 cm that is 28.62 cm. [11] was argued that root growth was influenced by many things, one of which is the soil properties. In addition, soil water availability also affected root growth.

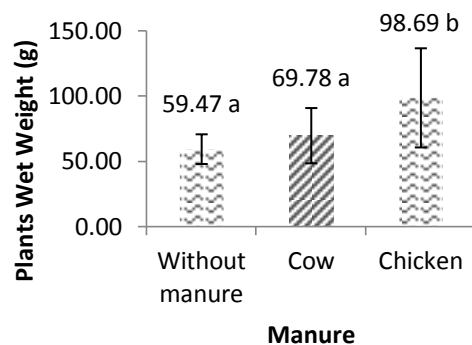
**Table 4.** The effect of plant spacing on root length (cm)

Plants spacing	Root Length (cm)
20 x 20	26.62 a $\pm$ 2.02
30 x 30	27.08 a $\pm$ 2.74
40 x 40	28.62 a $\pm$ 2.98

Description: The numbers followed by the same letters show no significant difference in the Duncan test level of 10%

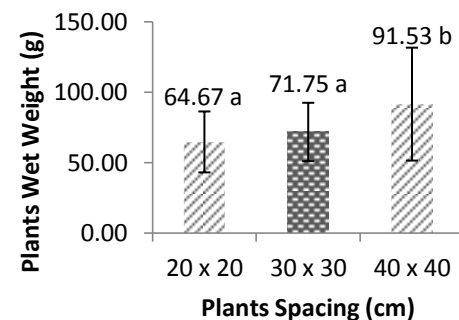
### 3.6 Plants Wet Weight

The result of variance analysis showed that manure significantly affected the plants wet weight (Figure 5). Plants wet weight on the without manure treatment is 59.47 g, cow manure is 69.78 g, and chicken manure is 98.69 g. According by opinion of [12], the fresh weight of the plant is influenced by the increase in the length and volume of plants caused by the increase of plant cells. N elements contained in chicken fertilizer can stimulate the formation of auksin which serves to soften the cell wall so as to increase the ability of plants to absorb water. This is what causes the increase in fresh weight of the plant.



Numbers were followed by the same letter does not differat the real level of DMRT test of 10%

**Figure 5.** The effect of manure on plants wet weight



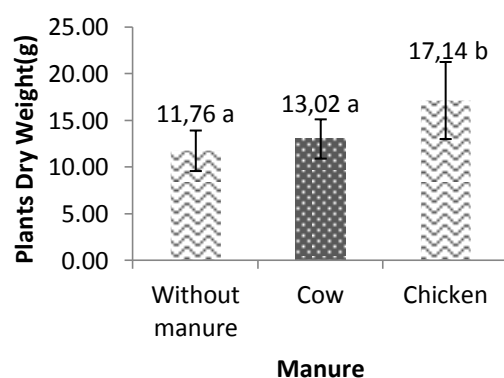
**Figure 6.** The effect of plant spacing on plants wet weight

The result of variance analysis showed that plant spacing had significant effect on fresh stover weight (Figure 6). Plant spacing of 40 cm x 40 cm have a significant effect on the fresh stover weight, in line with the research results of [13], the wider plant spacing, the greater the fresh forage production. In the treatment of plant spacing 40 cm x 40 cm was resulted a highest fresh stover weight of 64.67 g, significantly different with the plant spacing of 30 cm x 30 cm of 71.75 g, and also the plants spacing of 20 cm x 20 cm of 91.53g.

### 3.7 Plants Dry Weight

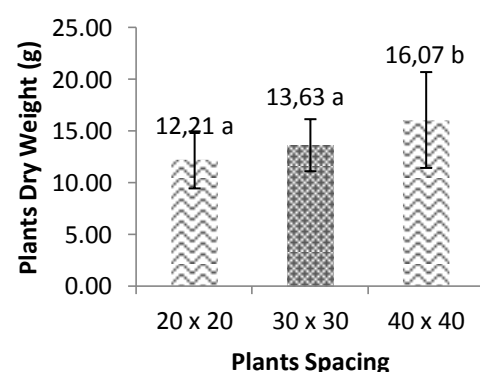
The result of variance analysis was showed that the application of manure significantly affected the dry stover weight (Figure 7). The treatment of chicken manure resulted in average dry stover weight higher than cow manure and without manure which amounted to 17.14 g. In cow manure only produce average of dry stover weight of 13.02 g, while without manure is 11.76 g. This is in accordance with the opinion [14] that the high N elements in chicken manure can spur the growth of plant biomass. [15] also said that N elements in chicken manure cause formation of flavonoid.

The plant spacing treatment was showed a significant effect on the dry stover weight (Figure 8). The average of dry stover weight at 20cm x 20 cm plant spacing is 12.21 g and plant spacing of 30 cm x 30 cm is 13.63 g. Different with plant spacing of 40 cm x 40 cm is to 16.07 g. According to [16] it is caused by the large percentage of light that can be forwarded by plants at a wider range so that plants can take advantage of growth and light for its development.



Numbers were followed by the same letter does not differat the real level of DMRT test of 10%

**Figure 7.** The effect of manure on plants dry weight

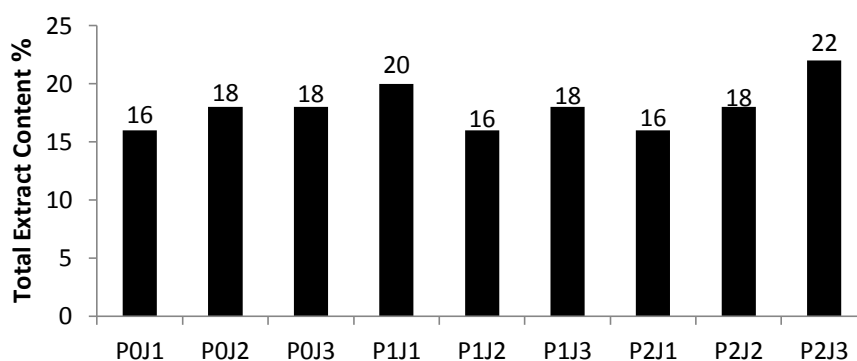


**Figure 8.** The effect of plant spacing on plants dry weight



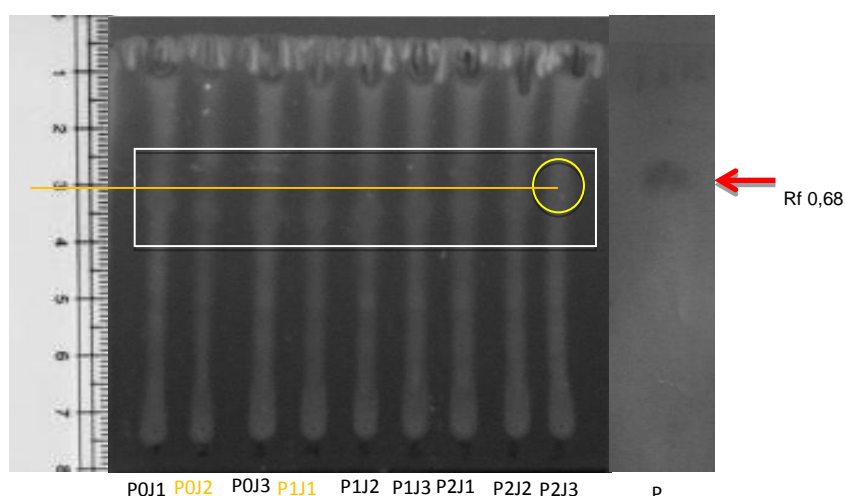
### 3.8 Flavonoid Analysis

The first step before doing flavonoid analysis is calculated the percentage of total extract content of the tapak liman. The highest percentage of total extract content (Figure 9) resulted in the manure treatment at the plant spacing of 40 cm x 40 cm, that is 22%, while the lowest extract level was produced at the treatment without the manure at the plant spacing of 20 cm x 20 cm, cow manure at plant spacing of 30 cm x 30 cm and chicken manure at plant spacing of 20 cm x 20 cm is 16%. The percentage of extract content was indicated the presence of secondarymetabolite content within the plant.



**Figure 9.** The influence of manure and plant spacing to the total extract content

Analysis of flavonoid compounds was used the method of Pharmacopoeia Herbal Indonesia Edition I Year of 2008 with TLC. TLC plates were used made of silica gel with size of 8 cm x 9 cm. The TLC plates of silica gel were activated by oven at 100° C for 1 hour. The viscous extract of the extraction was then dissolved with hexane as much as  $\pm 5$  ml. Hexane works to dissolve fat and fatty acids. It is then bottled along the plate using a micro pipette at a distance of 1 cm from the bottom line and each treatment is 1 cm apart. Then eluted to give the best separation result on TLC, i.e. n-Hexane-atil acetate-methanol by comparison of 5: 5: 1. After the formation of propagation then TLC plat aerated and examined under UV light at a wavelength of 366 nm (Figure 10).



**Figure 10.** The result of TLC test of secondary metabolite compound of tapak liman

The brighter the spot color was indicated the higher the concentration of certain compounds. So in the flavonoid compounds, the brighter the spot color the higher the level of flavonoid compound. When plants containing flavonoids will produce orange, pink, and red spot colors. From Figure 10, the spot color of chicken manure treatment and plant spacing of 40cm x 40cm is at Rf 0,68 which is closest to comparative compounds and has a red-orange color indicated as a flavonoid compound in the tapak liman.

#### 4. Conclusion

Based on the research results, can be concluded that, chicken manure has a good yield potential for the growth of tapak liman seen from leaves length component reaches to 13,86 cm, the highest crown diameter of 27,65 Cm, fresh stover weight of 98,69 g and dry stover weight of 17,14 g. Plant spacing treatment of 40 cm x 40 cm can increase the leaves length of tapak liman of 13.49 cm, crown diameter of 27,27 cm, fresh stover weight of 91,53 g, and the dry stover weight of 16,07g. The combination of chicken manure and plant spacing of 40 cm x 40 cm can increase the total extract content by 22% and indicated flavonoid compound

Based on this research, it is necessary to do research by using other types of fertilizers in order to determine which the best fertilizer in improving the growth and production of tapak liman. Need to do the analysis until the quantitative stage to known how much flavonoid levels in the tapak liman. Another secondary metabolite analysis was required to know what compounds are contained in the tapak liman.

#### Acknowledgement

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