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Leaf area index, chlorophyll, photosynthesis rate of lettuce (*Lactuca sativa* L) under N-organic fertilizer

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ABSTRACT

The objective of this research is to assess the growth and physiological characteristics of lettuce in the delivery of different N-organics. Research was conducted at Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia. The materials used were seeds of lettuce (*Lettuce sativa* L) on Oxisol soil types, with the total N content of 0.18 %, P (18 mg. g^{-1}), K (23 mg. g^{-1}) and C (1.52 %) were utilized. The organic fertilizers applied were cow-, sheep-, and guano manures, and compost and green manure (*Leucaena leucocephala*). The experimental design was a randomized block design with four replications and five treatments. The treatment dose of fertilizer applied is equivalent to 100 kg N / ha, namely: cow-manure ; sheep-manure; guano manure ; compost and green manure. Green manure, set into the ground is *Leucaena leucocephala*. The variables measured were the number of leaves, leaf area, leaf area index(LAI), the rate of photosynthesis, Chlorophyll total, anti-oxidant activity, fresh weight of lettuce per plot. The data obtained were analyzed using an ANOVA followed by the Duncan test. Guano fertilizer provided the most excellent effect on leaves, LAI, the rate of photosynthesis and biomass lettuce. Fertilizers derived from animal manure (guano, cattle, goats) gave a more excellent effect on the rate of photosynthesis of plants. Compost origin gave the lowest effect on leaf area, LAI, and the rate of photosynthesis. The number of leaves, total chlorophyll, and antioxidant content were not influenced by the different organic N fertilizers.

Key words : Chlorophyll, Leaf Area Index, Lettuce, N-organic, Photosynthesis rate.

INTRODUCTION

Lettuce is an important vegetable crop at the national level of Indonesia. Some bioactive compounds in vegetables are vitamins, minerals, antioxidants, and pigments (chlorophylls and carotenoids) (Limantara *et al,* 2015). Lettuce (*Lactuca sativa* L.) is one of the most widely vegetables consumed in the world since it is a good source of fiber, but has a low in calories, fat, and sodium. More over, it is a good source of iron, folic acid, and C vitamin, also of various bioactive compounds very good for health (Kim *et al*, 2016). Lettuce is an annual herbaceous plant that grows upright with plant height up to 50 cm in length from (12.5 to 25) cm in leaves (Ali *et al,* 2016).

Organic agriculture's contribution to agricultural production in Indonesia and other parts of the world continues to increase. Organic fertilizers not only provide the necessary nutrients but also give positive influences to the overall soil fertility, and can be used efficiently in conventional farming as a constituent of a system of the integrated fertilization (Bharambe, 2015). Organic farming is one of the practices of sustainable production systems with no adverse effects on natural resources and the environment. This production system not only maintain soil fertility, but also save soil moisture (Hasan *et al*, 2014). The application of fertilizers has been known to improve plant growth and development. Nitrogen (N) is one of the largest production inputs and it is an essential nutrient in plant dry matter yield as well as some energy-rich compounds, and crop production. Nitrogen also plays a role in photosynthesis. Comparison between sources of N-inorganic and organic fertilizers is hard to do because there are differences in N availability of both in the N sources (Rashed and Darwesh, 2015).

The supply of nitrogen plays an important role in the balance between vegetative and reproductive growth. Many previous studies have shown that the application of N fertilizer can increase dry matter and protein contents. Organic materials are used to prevent or ameliorate the negative effects on plants, able to reduce soil salinity. The organic fertilizer as one of the best N fertilizer were reported to increase plant height, leaf number, leaf area and tuber yield per plant permeability by roots in the soil. The main objective of this study was to evaluate the effect of different N fertilizer – organics on the growth, physiology and fresh yield of Lettuce.

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MATERIALS AND METHODS

Research conducted on an agricultural farm in Animal and Agricultural Sciences Faculty, Diponegoro University, Indonesia in 2016.The size of each plot is 2 m^2. The spacing of lettuce is 20×20 cm. Oxisol soil types, with a total N content of 0.18 %, P (18 mg. g^{-1}), K (23 mg. g^{-1}) and C(1.52 %).The experimental design employed in the was a randomized block design with four replications and five treatments. The treatment dose of fertilizer applied is equivalent to 100 kg N. ha $^{-1}$ namely: cow-manure is 6 t .ha^{-1} (T1,); sheep-manure 6 t .ha $^{-1}$ (T2); guano manure 1t .ha^{-1} (T3); compost is 7.5 ton. ha $^{-1}$ (T4) and green manure is 3 t. ha $^{-1}$ (T5). Green manure used is *Leucaena leucocephala*, set into the ground (Table 1).

The variables measured were the number of leaves, leaf area, leaf area index, the rate of photosynthesis, total chlorophyll content, anti-oxidant activity, the result of lettuce per plot. All data collected is then analyzed of variance and followed by DMRT (the Duncan's Multiple Range Test) (Steel and Torrie, 1960).

Measuring the chlorophyll content: Measurements of chlorophyll and carotenoid are based on Rajalakshmi and Banu, (2015). Fresh leaves were crushed in a mortar as 500 mg added with 2 ml of acetone 80 %. The result was then added with acetone 80 % of 10 ml solution and then filtered with Whatman paper 42. Insert 3 mL filtrate into the cuvette, then its absorbance was measured with spectrophotometry. Spectrophotometric chlorophyll test was performed with a wavelength of 663 nm (A663) and 645 nm (A645). Chlorophyll concentration was measured by the following formula:

Total chlorophyll = 8,02 (A. 663) + 20,2 (A. 645) mg/l

Measuring the rate o f photosynthesis: Measurement of the rate of photosynthesis (Allott,2001) began by chatching the CO_2 of whole plant when the sun is full (during the day). The photosynthetic rate measurement procedure consists of (1) the initial measurement of the amount of CO_2 and (2) the measurement of the amount of CO_2 for 30 min. from the initial data. Carbon dioxide (CO_2) measurements were made in the following order: plants were covered with translucent plastic (a known volume), in plastic clip, 5 ml of NaOH was prepared. Using a syringe (a known volume), the CO_2 was taken, then it was entered into a plastic clip containing NaOH, and it was a titration with 0.1N HCl was made until the red color disappeared. The same procedure was applied in the measurement of CO_2 with a lag time of 30 min.

Free radical scavenging activity determination: The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical-scavenging activity of the extracts (Koleva *et al.*, 2002). Different concentrations of each herbal extract were added, at an equal volume, to methanolic solution of DPPH (100 M). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Butil hidroxi toluent (BHT) and quercetin were used as the standard control. The concentration of antioxidants, which can reduce or inhibit 50 % free radicals (IC_{50} values) denoted the concentration of sample required to scavenge 50 % of DPPH free radicals.

RESULTS AND DISCUSSION

The number of leaves per plant, leaf area, photosynthetis rate, and the level of chlorophyll are presented in Table 2. Results of analysis of variance showed that there is a real effect between the type of organic fertilizers on the rate of photosynthesis and on the leaf area but not on the real level of total chlorophyll lettuce. The DMRT test showed that the plant treated with guano fertilizer resulted in a significantly wider leaf area than all treatments using other organic fertilizers, where the fertilization with guano manure produced the widest leaf area (1111.75 dm^2), the highest LAI (4.7 dm^2 . dm^{-2}) and the quickest rate of photosynthesis 0.98 (mg CO_2 . dm^{-2} . $second^{-1}$). Photosynthesis in plants is influenced by several factors such as the leaf area, the level of chlorophyll, and the environmental factors. The photosynthesis rate is affected by leaf area, light, water, CO_2, temperature, nutrients and plant age.

Results of analysis of variance showed that fertilization treatments with different types of organic fertilizers significantly affect the fresh weight of lettuce crop. Fresh weight and dry matter yield of lettuce with different types of organic fertilizers is presented in Table 3.

From Table 3 the results of fresh weight affect by guano manure was highest and different from all of the treatment applied are depicited. Guano fertilizer treatment gave DM yield significant difference with cow manure, but significantly higher than those with sheep manure, compost and green manure.

Different organic fertilizers did not significantly affect the number of leaves of lettuce plants because of the dose of fertilizer given same or all N-organic sources. The addition of sufficient nitrogen in the plant will accelerate

Table 1: The Result of analysis of organic fertilizers used in the research

Organic fertilizer	N (%)	P (mg. g^{-1})	K(mg. g^{-1})	C (%)
Cow manure	1,74	66	108	11.43
Sheep manure	1,72	32	112	10.93
Guano manure	8,83	58	111	21.31
Compost	1,32	18	85	11.51
Green manure	3,67	40	149	12.63

Table 2: Leaf area, photosynthesis rate, total chlorophyll content and antioxidant content in different types of organic fertilizer

Organic fertilizer	Leaves number	Leaf area per plot (dm^2) per plant	LAI ($dm^2.dm^{-2}$)	Total Chlorophyll ($mg.g^{-1}$)	Photosynthesis rate	Antioxidant ($ug.g^{-1}$) ($mgCO_2.dm^{-2}.second^{-1}$)
Cow manure	18	611.75 b	3.1ab	0.77	0.518 ab	222.05
Sheep manure	18	640.00 b	3.2ab	0.69	0.476 ab	106.06
Guano manure	19	1111.75a	4.7a	0.77	0.986 a	81.93
Compost	15	329.00 c	2.6 b	0.69	0.198 b	96.71
Green manure	17	559.00 b	3.3ab	0.78	0.183b	92.80

different letters in column indicate significant differences ($p < 0.05$) between treatments

Table 3: Fresh weight and dry matter production of lettuce

Treatment	Fresh weight ($kg \cdot plot^{-1}$)	Dry matter yield · ($kg \cdot plot^{-1}$)
Cow manure	7.20 b	0.41 ab
Sheep manure	6.96 b	0.38 bc
Guano manure	8.21 a	0.49 a
Compost	5.44 c	0.38 c
Green manure	5.25 c	0.31 c

different letters in column indicate significant differences ($p < 0.05$) between treatments

the rate of cell division and elongation, the growth of roots, stems and leaves taking place quickly so that N fertilization of the same dose will give the same effect on plant growth. Previous research on effect of organic manures in enhancing crop have been reported by Sharma *et al,* (2016). They have reported that importance of organic farming is understandable given the important influence of different organic manures on yield and economics of groundnut. These findings are in accordance with the findings of Dadiga *et al*, (2015) In light of above evidences, it seems that application of organic and inorganic sources of nutrients increased growth parameters and yield of coriander. The growth of lettuce due to green manure is similar to the results of Anbukkarasi and Sadasakthi research, 2017, that is the addition of green manure *Leucaena leucocephala* produces the highest okra plant height and also the highest fruit girth compared to other green manure.

The availability of nutrients in the soil can improve soil fertility. Guano fertilizer is basically a good fertilizer to enrich the soil because it contains high phosphorus (P) and nitrogen (N) and can increase the productivity of the soil on the ground that there is a shortage of organic matter. Guano is a source of organic fertilizer or it is a good natural fertilizer for the cultivation of fruits, vegetables and a variety of other crops. Guano fertilizer can stimulate the growth of fruits and seeds, it can stay long in tissue and soil , improve soil productivity and provide nutrients to the plants better than artificial chemical fertilizers. Al Mohameedi *et al* (2014) reported that the stimulant effects of fertilizer guano associated with high levels of N and P were higher and concluded that the land that has been transformed by fertilizer guano affected the nature of plant growth such as plant height, the nitrogen content and uptake of nitrogen through the supply N that is fairly successful in increasing the PEP and RuBSCo to the rate of the photosynthetic physiological processes. In addition, it may increase the hydromechanical properties of the soil.

It is similar to the report by Subaedah *et al* (2016) that the fertilization with 135 kg N. ha^{-1} and 66 kg P_2O_5. ha^{-1} with the application of Crotalaria green manure increased the corn yield of 17.2 % higher than that without crotalaria. Hasan *et al*, (2014) reported the effect of the type of organic material and the method of application of sheep manure on the increase of 3 % chlorophyll compared with the one treated with peat. The content of chlorophyll in the leaves showed significant interactions between organic materials, methods of application and concentration. Because of this interaction, marigold provides the highest level of chlorophyll in the leaves with sheep manure administrated by spraying with 40 % (76.9 mg . 100 g^{-1} wet weight).

Factors affecting the synthesis of chlorophyll include light, carbohydrate, temperature, genetic and nitrogen availability. Chlorophyll is the main pigment in plants of which the main function in photosynthesis is to harness solar energy, trigger CO_2 to produce carbohydrates and provide energy. Different organic fertilizers do not significantly affect the total chlorophyll in lettuce plants because the dose of fertilizer given by crop N needs similar but different sources, so that N needed for the growth of lettuce plants is similar. Based on Hasan *et al*, (2014), organic sources including essential nutrients for plant growth have a positive effect on the formation of chlorophyll and chloroplasts. Organic acids and carbon dioxide have a role in improving the availability of some nutrients such as Mg which plays an important role in the formation of the chlorophyll molecule.

Organic fertilizers derived from animal sources turned out to have a better effect on the rate of photosynthesis. Compost is derived from the campus waste mostly consisting of leaf litter. Photosynthetic rates per unit mass increase significantly in reduced leaf thickness, higher relative surface area, and higher concentrations of pigments and nitrogen in structural and catalytic proteins, including Rubisco. Since metabolism on a dry mass basis with declining leaf thickness increases, photosynthesis expressed per surface

area varies only eight fold among species (Pedersen *et al,* 2013).

The antioxidant content of lettuce has the same effect on a variety of the administrated N-organic source. Positive uses are provided by fruits and vegetables against diseases, reporting a value of 31.5 mg . ml^{-1}. Total phenolic samples ranged from 4.85 mg to 76.05 mg gallic acid equivalent with 100 g fresh weight, where the red coral lettuce has the highest value. Total flavonoid content of the samples ranged from 2.28 mg to 21.96 mg quercetin lettuce equivalent with 100 g fresh weight, and it differed significantly ($p < 0.05$) between samples. Values of IC_{50} as antioxidant activity and Ferric Reducing Antioxidant Power (FRAP) values between the samples were highly correlated with total phenol and flavonoid contents. Among the different varieties of lettuce, red coral lettuce showed the highest total antioxidants and antioxidant activity. Therefore, consumers are encouraged to consume lettuce more regularly to get better health (Gan and Azrina, 2016). The formation of bioactive substances and their content in fruits and vegetables depends on various factors, such as genotypic differences, environmental conditions (climate, temperature, soil) and cultivation practice (Chen *et al,* 2016).

Organic fertilizer including manure gave residual effect on growth and crop those were environmental condition improvement and need of lessening artificial fertilizer cost was the reason to use organic material. Organic fertilizer increased soil fertility by activating microbe biomass (Purbajanti *et al,* 2016). Nutrients in livestock manure can improve soil fertility and increase crop yields. The forms of N and P excreted by livestock have the potential to affect the environment. Urea N and N ammonium are generally considered to be two forms of nitrogen derived from manure that is easily available to plants (Bernier *et al,* 2014). Plant fresh weight is the result of accumulation of photosynthesis in the form of biomass and water content in the leaves. Optimal plant fresh weight can be achieved by plants which need a lot of energy and nutrients order to increase the number and size of the cell in order to be able to achieve its optimal point and to allow an optimal increase in ground water. Both micro nutrients and micro contained in a given organic fertilizer will affect the vegetative growth of lettuce plants. Nitrogen has a role to stimulate overall plant growth. The beneficial effects of organic fertilizers because of their potential to encourage microbial activity, improve nutrient availability and increase in plant photosynthesis. Moreover, it seems that adding organic fertilizer to the soil not only increases the availability of nutrients directly, but also operates as a slow release of a fertilizer which is to provide N, P and K.

Mineralization of organic N in the soil occurs depends on the proportion of biodegradable material which more resistant than organic N, and the rate of mineralization of each type. Provision of green manure crops is easily weathered since by the addition of green manure is ready to support the unlimited mineralization due to the supply of N. The green manure accumulated for two years will result in the higher availability of N needed by any crop. Most N in the accumulation of organic matter not mobile, is easy leached and denitrificated and volatilized. The use of green manure to 'build fertility' has broad applications in the context of the management of agricultural systems (including organic) in which the use of fertilizers is reduced (Stopes *et al,* 1996). Organic matter content increases will increase the ability of soil to retain water and increase the content of soil nutrients, including nitrogen, phosphate and trace nutrients mobilized and concentrated in the top layer of soil that they can be used by plants and therefore the integration of organic material in the cultivation of plants in soil dry experience abiotic stresses is expected to increase the availability of resources needed by plants to increase crop productivity (Subaedah *et al*, 2016). The release of nitrogen from most of these sources is slow and highly dependent on mineralization processes in the soil. Nitrogen supply affects a series of physiological processes, morphological characteristics and yield components (Tiemens-Hulscher *et al*, 2014). Manure significantly increased fruit production, fruit weight, fruit diameter flesh thickness, and sugar content, but it did not increase seeds weight and it reduced the total fruit acid content of *Psidium guajava* (Purbajanti *et al*, 2016). Many data indicate that the use of organic fertilizers such as manure is able to fix the biological properties of soil and nutrients, production, and quality of crops. Manure is a valuable source of plant nutrients because it can improve the quality and productivity of the land (Hariadi *et al*, 2015).

CONCLUSION

It can be concluded that guano fertilizer provides the most excellent effect on leaves, LAI, rate of photosynthesis and biomass lettuce. Fertilizers derived from animal manure (guano, cattle, goats) give better effects on the rate of photosynthesis of plants. Manure compost origin give the lowest effect on leaf area, LAI, the rate of photosynthesis. The number of leaves, total chlorophyll and antioxidant content are not influenced by the different organic N fertilizer.

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