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The growth and yield responses of two bean cultivars to organic and inorganic nitrogen sources

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Abstract. *Pangaribuan DH, Ermawati, Suryani NMW, Maria WS. 2022. The growth and yield responses of two bean cultivars to organic and inorganic nitrogen sources. Biodiversitas 23: 1677-1682.* The productivity of beans is increased by selecting the best cultivar and applying fertilizers according to the needs of the plant. In addition, the application of high-yielding cultivar and N fertilizers with the right dosage could be used to increase the yield. Therefore, the objective of this study is to determine the effect of nitrogen sources on the growth and yield of two bean cultivars. A 5 × 2 factorial treatment was used in a randomized block design with three replications. The first factor is the Perkasa and Lebat-3 cultivar, the second is organic and inorganic nitrogen fertilizer, namely control, urea 100 kg ha^{-1}, as well as 10, 20, and 30% concentrations of liquid organic fertilizer from leucaena leaves. The results showed that the use of liquid organic fertilizers derived from leucaena could be used as an alternative to nitrogen fertilization. Furthermore, the 20% concentration treatment produced the highest number of trifoliate leaves (8.81 leaves) and dry weight (4.52 g). Based on the results, the use of 20% liquid organic fertilizer with Perkasa cultivar showed the best effect on the number of pods per plant (13.38), and yield (846.67 kg ha^{-1}). The technology of liquid organic fertilizers from leucaena leaves could be recommended for bean organic production.

Keywords: Chlorophyll, liquid organic fertilizers, stomata, urea, yield

INTRODUCTION

Green bean (*Phaseolus vulgaris* L.) is one of the legume plants serving as a source of vegetable protein. The plant is widely consumed by the Indonesians due to its important role in fulfilling health needs as a nutritious food ingredient. Rihana et al. (2013), stated that beans are source of vegetable protein, rich in vitamins A, B, C and have great potential for the treatment of several diseases such as oxidative stress, cardiovascular disease, cancer, and diabetes (Camara et al. 2013). Furthermore, as the population grows, the need for fresh food and vegetables continues to also increase. Therefore, to increase the production of green beans, it is necessary to apply good cultivation techniques such as applying balanced fertilization that fulfills the nutritional needs for growth. The productivity of green beans is also increased by using improved cultivars. Meanwhile, there are numerous green beans cultivars available in the market. Ratnasari et al. (2015), stated that each cultivar has different genetic characteristics, which lead to differences in appearance, character, as well as responses to production factors.

Fertilization is one way to meet the nutritional needs of nitrogen in green beans. Meanwhile, nitrogen is an important nutrient required by green beans for the formation of chlorophyll, protoplasm, protein, and nucleic acids (Fahmi et al. 2010). Fertilization with nitrogen (N) is derived from organic and inorganic fertilizers such as urea. Furthermore, green bean nutritional need for N is higher than any other nutrients. Pahlevi et al. (2016) reported that nitrogen affects the photosynthetic process in plants as well as the photosynthate produced. Maghfoer et al. (2018), also stated that the application of inorganic fertilizers produced large growth and plant yield. However, the continuous use of inorganic fertilizers leads to low soil fertility and productivity. Therefore, an alternative approach is needed to reduce the use of inorganic fertilizers, via organic fertilization.

Nitrogen is commonly supplied alternatively to plants using Liquid Organic Fertilizers (LOF). It is an organic fertilizer available in liquid form and contains nutrients in the form of a solution. Therefore, it is easily absorbed by plants. Furthermore, it is applied via sprinkling or spraying on the leaves or stems of plants. Organic fertilizers are made from plant waste available in the environment. In particular, liquid organic fertilizers are more beneficial because their distribution is adjustable to the needs of plants (Ginandjar et al. 2019). One example of organic matter widely used as a liquid organic fertilizer source of nitrogen is leucaena leaves (*Leucaena leucocephala*). Liquid organic fertilizer derived from this plant contains nitrogen nutrients needed by plants (Jeksen and Mutiara 2017). The N content in leucaena leaves is quite high, namely 3.84%, and is a source of organic nutrients to increase the growth and yield of green beans (Palimbungan et al. 2006). Furthermore, the application of liquid organic fertilizers increased the fresh weight of pods in green beans (Rizqiani et al. 2006), and provided the highest yield of pods and bean per plant in slam weed (*Chromolaena odorata* L.) (Duaja 2013).

Plant cultivar is a factor that greatly determines the quality of agricultural products. The use of high-yielding bean cultivar increases productivity and yield quality. Each cultivar has specific genetic characteristics and traits which lead to differences in the respective character and appearance. Examples of high-yielding beans cultivar include Perkasa and Lebat-3. The advantages of Perkasa cultivar include resistance to leaf rust disease, early maturity as well as the large and long pods (MoA 2002). In contrast, the advantages of Lebat-3 cultivar include being well adapted to the low-highlands, early maturity and has high yield potential (MoA 1999). Sinaga et al. (2017) showed that the use of cultivars had a significant effect on the growth and yield of beans. Furthermore, Duaja (2013) stated that there is an interaction between the use of bean cultivar with liquid organic fertilizer doses on the number of pods and productivity.

The fertilizer requirement by each cultivar is usually different. This is caused by individual genetic characteristics. Hence, the selection of fertilizer types and requirements need to be considered. Besides, the appropriate doses of liquid organic fertilizers also influence plant growth. Ratnasari et al. (2015) stated that variations in genetic traits of cultivar lead to different responses to environmental and production factors. There are only a few studies on the different organic and inorganic nitrogen sources. In this study, two bean cultivars were hypothesized to produce different responses to treatments with various types and doses of inorganic-and-organic-sources-based nitrogen. Therefore, the aim of the study is to determine the effect of inorganic and organic nitrogen sources on the growth and yield of two beans cultivars.

MATERIALS AND METHODS

This study was conducted at the experimental research area of Pemanggilan, Natar, Lampung province, Indonesia from January to March 2020. The soil was first cultivated using hoes, loosened to a depth of 20-30 cm, evenly processed and an experimental plot was made with a size of 3×2.5 m. Furthermore, the Perkasa and Lebat-3 cultivars were planted by drilling the planting holes, while two seeds each were planted with a spacing of 40 cm × 40 cm.

The (2×5) factorial treatment was used in a randomized block design (RBD) with three replications. The first factor was the bean cultivars, namely Perkasa and Lebat-3, while the second was the source of nitrogen, namely control, urea 100 kg ha^{-1}, as well as 10%, 20%, and 30% LOF. Furthermore, the data were analyzed using analysis of variance procedures by using Minitab 17, while the means were compared using the Honestly Significant Difference (HSD) of 5%.

The liquid fertilizer was prepared by mixing chopped leucaena leaves (1 kg of leucaena leaves: 2 liters of water), with 200 mL of molasses and 200 mL of EM4 in a drum, the mixture was then fermented for 15 days and the liquid was filtered. The inorganic fertilizers given include urea 100 kg ha^{-1} (75 g $plot^{-1}$) at the beginning and 4 weeks after planting (WAP), while SP-36 200 kg ha^{-1} (150 g $plot^{-1}$) and KCL 100 kg ha^{-1} (75 g $plot^{-1}$) were given at the beginning of planting. Fertilization was applied in a strip at a distance of 5 cm from the plant.

The liquid organic fertilizer was applied by spraying on the top and bottom of the plant (60:40). This was carried out once a week using a concentration of 10% (100 mL LOF solution + 900 mL water), 20% (200 mL LOF solution + 800 mL water), and 30% (300 mL LOF solution + 700 mL water). The liquid organic fertilizer analysis results showed that the N content in the *Leucaena* leaves was 0.16%, C-organic 3.08%, N-total 0.16%, P-total 0.18% and K 0.56% with a pH of 3.64 and the C/N ratio was 19.25.

Plant maintenance includes weeding manually and thinning, while the harvest was carried out in stages according to the age of each cultivar. The characteristics of beans ready for harvest include young and gloomy pod color, rough skin surface, less prominent seeds in the pods which usually make a popping sound when the pods are broken especially between 2-3 weeks after flowering.

The parameters observed include plant height (6 WAP), number of trifoliate leaves (6 WAP), chlorophyll, carotenoids (5 WAP), number of stomata (5 WAP), soil-plant development analyses, SPAD value (5 WAP), number of pods per plant, dry weight (leaves and stem), and yield. Furthermore, the replica method modified from Paul (2017) was used to observe the stomata on the leaf surface. The leaves were first cleaned with a tissue to remove dust or dirt and then rubbed with transparent nail polish, and allowed to dry for a few minutes. A transparent strip tape measuring 1 cm x 1 cm in size was applied, smoothed, and then peeled off slowly. The sample produced was then attached to the slide and observed under a microscope.

The chlorophyll and carotenoid contents were measured using the spectrophotometric method. The green beans were crushed using a mortar and 100 mL of 70% alcohol solution was added. Furthermore, the extract was filtered and the filtrate was placed in a cuvet to measure the total chlorophyll and carotenoid contents using a spectrophotometer (Cary 100 UV-Vis). The chlorophyll and carotenoid contents were calculated using the formula (Rahimi et al. 2019):

$$\text{Chlorophyll } a = 11{,}24 \times A_{662} - 2{,}04 \times A_{645}$$

$$\text{Chlorophyll } b = 20{,}13 \times A_{645} - 4{,}19 \times A_{662}$$

$$\text{Chlorophyll total} = 7{,}05 \times A_{662} + 18{,}09 \times A_{645}$$

$$\text{Carotenoid} = \frac{(1000 \times A470 - 1{,}90 \text{ x clorofil a} - 63{,}14 \times \text{clorofil b})}{214}$$

RESULTS AND DISCUSSION

The results showed that the Perkasa and Lebat-3 cultivar had no significantly different effect on vegetative growth namely plant height, numbers of leaves, and dry weight, while the application of inorganic-and-organic-sources-based nitrogen affected vegetative growth parameters

(Table 1). Plant height in the control was shorter than the treatment with urea, as well as 10, and 20% LOF, meanwhile, the application of N sources from urea, as well as 10, 20, and 30% LOF showed no significant differences (Table 1). Amin (2011), reported that nitrogen increased plant growth and height to produce numerous internodes which resultantly produce more leaves.

The number of trifoliate leaves in green beans is influenced by the availability of N nutrients. This parameter increased significantly after the application of organic nitrogen sources. The numbers of trifoliate leaves in the control were lesser compared to 10, 20, and 30% LOF. The 10% LOF treatment produced the highest number of trifoliate leaves but was not different from the 20, and 30% LOF, as well as urea treatment. Pramitasari et al. (2016), stated that higher N absorption by plant leads to larger growth of the leaves. Furthermore, the application of LOF from leucaena leaves showed significant growth in plant height, numbers of leaves, and fresh weight of mustard greens (Pary 2015), the highest yield on the number of pakcoy leaves (Hidayat and Suharyana 2019), and increased the growth and number of fruit in tomato plants (Septirosya et al. 2019). The number of trifoliate leaves is a plant characteristic that affects the rate of photosynthesis to capture sunlight. Sab et al. (2020) showed that a higher number of leaves would result in a higher plant dry weight. In this research, leucaena-based liquid organic fertilizers that are rich in nitrogen increased the vegetative growth including number of trifoliate leaves and dry weight.

The application of nitrogen sources affected the dry weight of the plant. Liquid organic fertilizers 20% produced the highest dry weight but were not significantly different from urea and 10% LOF. The plant dry weight was influenced by the initial vegetative growth as this parameter is related to the number of leaves and fresh weight. The availability of sufficient nutrients in the treatment affects plant growth and yield. Arista et al. (2015) stated that nitrogen is an element that functions to increase leaf size and the percentage of protein. Meanwhile, Madusari (2019) reported that liquid organic fertilizers affect plant growth and nutrient absorption by increasing plant stem diameter and dry weight. From these data it could be said that the application of inorganic-and-organic-sources-based nitrogen gave the effect on the plant growth since early or vegetative growth.

The application of inorganic-and-organic-sources-based nitrogen affected physiological characteristics namely chlorophyll, carotenoids, number of stomata and SPAD value (greenness of leaves). However, chlorophyll and leaf carotenoids were not affected by the bean cultivar (Table 2). The chlorophyll content in the control was lower than the urea treatment, and was not different with 30% LOF. Furthermore, the chlorophyll content for the urea treatment did not differ compared to 10 and 20% LOF (Table 2). The application of urea produced a higher carotenoid content compared to the control, but was not different compared to 10 and 20% LOF treatments (Table 2). Also, the application of 10, 20, and 30% LOF showed no differences in the carotenoid content compared to the control. Bojovic et al. (2005), stated that the leaf color of certain cultivars did not directly correlate with chlorophyll content, although Permanasari et al. (2014) stated that chlorophyll formation plays an important role in the process of photosynthesis. Wijiyanti (2019) reported that nitrogen is the main nutrient needed for the formation of chlorophyll and carotenoids. This is also in line with Razaq et al. (2017) who found that chlorophyll and carotenoids synthesis depends on optimal N availability, therefore, nitrogen plays an important role in the formation of photosynthetic pigments. Furthermore, Hendriyani et al. (2018) reported that carotenoids and chlorophyll are complementary pigments, but carotenoids are fewer in number and it play a role in the absorption of light by chlorophyll. It is clear from this research that chlorophyll and carotenoid contents are affected by the availability of N nutrients.

The application of urea produced a higher number of stomata compared to the control, while the application of urea and 20% LOF showed no differences in the number of stomata. Similarly, LOF applications of 10, and 30% also produced no difference in the numbers of stomata (Table 2). The number of stomata in the two cultivars were not affected by the application of nitrogen fertilizers, the two cultivars showed similar responses. Figure 1 showed the number of stomata as a result of treatments in two cultivars investigated. The application of inorganic fertilizers produced more stomata compared to other treatments as shown in Figure 1. Table 2 showed that urea and 20% LOF produced a higher number of stomata compared to the control, as indicated also by Figure 1. Meanwhile, the number of stomata in plants affects metabolic processes in plants such as photosynthesis. The rate of photosynthesis increases as the number of stomata rises; hence, plant productivity also increases. This is supported by Proklamasiningsih et al. (2012), which stated that the rate of photosynthesis in plants is closely related to the yield. Moreover, Putri et al. (2017), reported that stomata play an important role in photosynthesis by acting as a surface for CO_2 exchange in the leaves.

The results showed that the level of SPAD value, number of pods, and bean yield were significantly affected by the interaction of N sources and bean cultivar (Table 3). The treatment without N fertilizer had low SPAD value, while the application of nitrogen fertilizers affected the greenness of the leaves. The 30% LOF treatment with Perkasa cultivar and urea with Lebat-3 produced the highest level of SPAD value compared to the others but were still significantly the same as the other treatments with different N sources. Moreover, the SPAD value of plants treated with organic and inorganic fertilizers was higher than the control. Nitrogen is an important element that increases leaf greenness in plants (Faustina et al. 2015). Pramitasari et al. (2016) also stated that the N nutrient affects plant growth, appearance, and color hence, the plant organs turn green due to the chlorophyll content. Furthermore, Pamungkas and Supijatno (2017), reported that the level of leaf greenness indicates that the plant has sufficient nitrogen levels and good health conditions.

Table 1. Effects of treatment with different nitrogen sources and cultivar on plant height, number of trifoliate leaves, and dry weight

Treatment	Plant height (cm)	Number of trifoliate leaves	Dry weight (g)
Perkasa cultivar	133.60a	7.20a	3.21a
Lebat-3 cultivar	137.63a	8.11a	3.17a
HSD0,05	18.47	1.43	0.92
Control	94.71c	5.57b	1.95b
Urea	171.48a	8.50ab	3.78ab
10% LOF	150.60ab	9.11a	3.13ab
20% LOF	148.02ab	8.81ab	4.52a
30% LOF	113.29bc	6.30ab	2.58ab
HSD0,05	42.11	3.25	2.09

Note: In a column, common values letter(s) do not differ significantly at $p \leq 0{,}05$ as per HSD

Table 2. Effects of treatment with different nitrogen sources and cultivar on chlorophyll, carotenoid, and numbers of stomata

Treatment	Chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)	The number of stomata (mm^{-2})
Perkasa cultivar	12.92a	1.59a	14.40a
Lebat-3 cultivar	13.24a	1.88a	14.67a
$HSD_{0,05}$	1.16	0.29	3.50
Control	9.68b	1.38b	8.50c
Urea	15.93a	2.35a	22.00a
10% LOF	15.56a	1.69ab	11.50bc
20% LOF	13.77a	1.76ab	17.83ab
30% LOF	10.46b	1.47b	12.83bc
$HSD_{0,05}$	2.64	0.67	8.05

Note: In a column, common values letter(s) do not differ significantly at $p \leq 0{,}05$ as per HSD

Table 3. Effects of treatment nitrogen source and cultivar on SPAD value, number of pods, and yield two cultivars bean.

Parameter	Source N	Cultivar		$HSD_{0,05}$
		Perkasa	Lebat-3	
SPAD value	Control	18.31 Ab	17.54Ab	9.34
	Urea	27.79 Aa	28.91 Aa	
	10% LOF	20.13 Aab	28.11 Aa	
	20% LOF	24.06 Aab	24.19 Aa	
	30% LOF	28.96 Aa	21.81 Aab	
Number of pods	Control	5.46 Ac	2.50 Ab	4.40
	Urea	9.13 Aab	9.05 Aa	
	10% LOF	9.96 Aab	12.13 Aa	
	20% LOF	13.38 Aa	4.67 Bab	
	30% LOF	6.92 Abc	3.75 Ab	
Yield (kg ha^{-1})	Control	231.11 Ac	113.33 Ab	220.24
	Urea	720.89 Aab	429.78 Aa	
	10% LOF	565.33 Ab	515.56 Aa	
	20% LOF	846.67 Aa	181.33 Bb	
	30% LOF	333.78 Ac	148.00 Ab	

Note: Mean followed by the same letter (capital letter read horizontally and small letter read vertically) do not differ significantly at $p \leq 0{,}05$ as per HSD

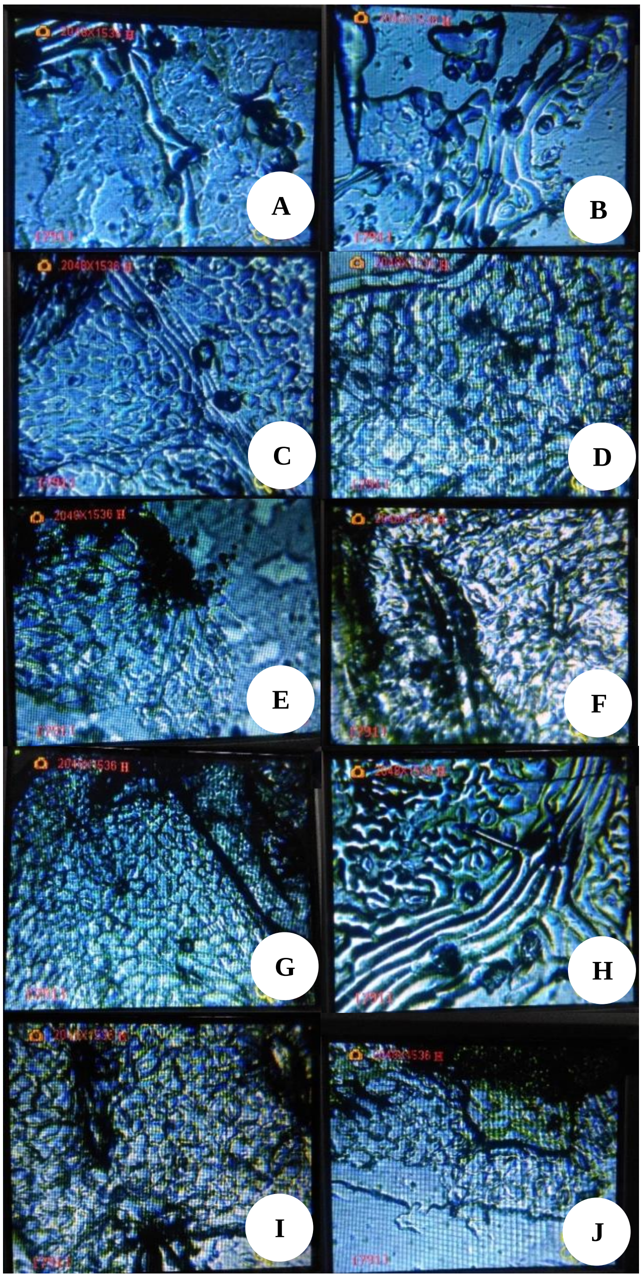

Figure 1. The number of stomata of Perkasa: A. Control, B. Urea, C. 10% LOF, D. 20% LOF, E. 30% LOF. The number of stomata of Lebat-3: F. control, G. Urea, H. 10% LOF, I. 20% LOF, J. 30% LOF

The application of inorganic-and-organic-sources-based nitrogen affected vegetative growth, namely number of pods and yield. Significant increase in yield (Table 3) is confirmed with better vegetative growth (Table 1 and 2). In this study, the application of 10 or 20% LOF was sufficient for the growth of green beans and was not significantly different from urea treatment. Perkasa cultivar with 20% LOF produced a statistically higher number of bean pods compared to the Lebat-3. Similarly, Perkasa cultivar with 20% LOF produced the highest number of bean pods per plant but was not significantly different from the 10% LOF and urea treatments. Furthermore, Lebat-3 cultivar with

LOF 10% produced the highest number of bean pods but was not significantly different from the urea and 20% LOF treatment.

The field observation showed that there was a symptom of N deficiency in green bean leaves without nitrogen fertilizer. This deficiency occurs in the leaves of plants without treatment (control). The symptoms include yellowish-green coloration of leaves compared to normal leaves which are fresh green. Erythrina et al. (2016) stated that nitrogen is the main nutrient in the formation of leaf color due to its important role in increasing leaf green matter and protein. Therefore, the application of nitrogen increases the green color of the leaves but when deficient, the formation of chlorophyll is reduced thereby causing the leaves to appear yellowish-green. Bojovic et al. (2009) stated that nitrogen deficiency causes a reduction in leaf greenish color and area, as well as photosynthesis due to the linear correlation between leaf chlorophyll formation and nitrogen. Furthermore, nitrogen requirement by plants is needed to be in accordance with the demand of the plant. Zainal et al. (2014) stated that the lack of Nitrogen leads to leaf chlorosis indicated by yellowing of the leaves. In contrast, excess nitrogen accelerates plant growth, especially on the stems, the leaves turn dark green.

Lebat-3 cultivar requires a lower LOF concentration compared with Perkasa to produce significant yield. The plant genetic traits in the two cultivars influenced the different responses to liquid organic fertilization. Table 3 shows that Perkasa cultivar with 20% LOF produced a higher yield compared to other treatments but was not significantly different from the 100% recommended urea treatment. The Lebat-3 cultivar with 10% LOF produced a higher yield compared to other treatments but was not significantly different from the recommended 100% urea treatment. Chaturvedi (2005), reported that nitrogen fertilization with the proper dose affects grain yield in rice and other parameters in each cultivar. Furthermore, the use of N sources for plants affects the results of photosynthesis. This is supported by Rathke (2005), which stated that plant yield is improved by increasing the source of N. In addition, nitrogen improves yield by increasing leaf area and the rate of photosynthesis, hence, high carbohydrates are produced by the plants (Chaturvedi 2005). The yield of Lebat-3 cultivar was lower compared to Perkasa but was not significantly different from the recommended 100% urea treatment and 20% LOF. Ratnasari et al. (2015), stated that the differences in the characters possessed by cultivar are caused by the varying genetic composition. Hence, each cultivar responds differently to environmental and production factors. The positive effect of liquid organic fertilizers from leucaena leaves in increasing crop yields was also found by Duaja et al. (2013) on chickpeas, Palimbungan et al. (2006) on mustard plants, Septirosya et al. (2019) on tomato plants, and Ainiya et al. (2019) on sweet corn plants. The experiment showed that the liquid organic fertilizers derived from leucaena leaves are applicable as an alternative nitrogen source in bean cultivation. This low-cost technology is easy to use by farmers and also supports organic bean production.

In conclusion, the use of plant-based nitrogen fertilization from leucaena leaves could be an alternative to nitrogen fertilization in bean plants. The 20% LOF is recommended as an adequate concentration to increase the growth and yield of green beans. Furthermore, each cultivar shows different responses to growth factors. Treatment of Perkasa (with 20% LOF) and Lebat-3 cultivar (with 10% LOF) produced the highest bean yield, respectively.

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