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Research Article
Influence of Combined NPK and Manure on Improving Growth, Photosynthetic Characteristic and Yield of *Justicia gendarussa* Burm. F.

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Abstract

Background and Objective: *Justicia gendarussa* (family *Acanthaceae*) is regarded as a medicinal plant valued for its beneficial pharmacological activities. A polybag experiment has been conducted at Bogor (Indonesia) to determine the effects on growth, photosynthetic and yield of various NPK ratios and manure fertilizer application in *J. gendarussa*. **Materials and Methods:** Six treatments (control: 50 g/plant manure, NPK (1.1 g/plant N:0.7 g/plant P_2O_5 and 0.6 g/plant K), 50+50%/plant (manure+NPK), 10+50%/plant (manure+NPK) and 50+10%/plant (manure+NPK) were performed in a randomized complete block design. Data of individual plants were extracted from various treatments: plant height, number of leaves, number of branches, chlorophyll content, photosynthetic parameters, yield and sugar content were collected. **Results:** The plant treated with 10+50%/plant (manure+NPK) demonstrated a statistically significant increase in plant height, number of leaves and number of branches with the value of 59.00 cm, 85.00 per plant and 9.50 per plant, respectively. Chlorophyll a (0.538 mg g^{-1} FW), chlorophyll b (0.192 mg g^{-1} FW) and total chlorophyll (0.730 mg g^{-1} FW) were significantly improved by the treatment of NPK fertilizer. The application of manure and NPK at (50+50%) and (50+10%) was significantly increased stomatal conductance and transpiration rate, respectively. Leaves yield (9 g/plant DW) was improved by the application of 10+50%/plant (manure+NPK) fertilizer. The highest sugar content was obtained from *J. gendarussa* growth with 50+50%/plant (manure+NPK) followed by 10+50% plant (manure+NPK) fertilizer. **Conclusion:** The *J. gendarussa* plants could benefit from combined manure and NPK fertilizer application in growth, stomatal conductance, intercellular CO_2 concentration, transpiration rate, leaves yield and sugar content.

Key words: Agronomy performance, *Justicia gendarussa* burm. F., manure, NPK, photosynthetic

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Justicia gendarussa Burm. f., also known as gendarussa in Indonesia, is a small shrub-like medicinal plant from the *Acanthaceae* family that grows to a height of 0.6-1.2 m and is prevalent in Southeast and South Asia's lowlands up to 1500 m above sea level[1]. The *J. gendarussa* has shown a significant amount of pharmacological properties, including anti-HIV[2,3], anti-hypertensive[4], anti-inflammation[5], antioxidant[6] and antimicrobe[7]. The leaves are a part of the *J. gendarussa* plant widely used as traditional medicine[8]. The leaves contain secondary metabolites with pharmacological properties such as apigenin glycosides[8], alkaloids[9] and lignan justiprocumin B[2]. The increased biomass of leaves in these plants is essential in meeting the needs of the herbal medicine industry for raw materials.

The photosynthetic performance of a plant is one of the factors that affect its growth[10]. Starch is a metabolite produced during photosynthesis and stored in the leaves temporarily[11]. Additionally, starch is distributed to all parts of the body that require it or converted into other compounds, including secondary metabolites that are effective as herbs in this case[12,13]. The *J. gendarussa* plants grow naturally in their natural environment but increasing growth optimally can be increased by applying organic and inorganic fertilizers[1]. Fertilizers can supply vital plant nutrients, allowing for optimal growth performance[14]. The literature demonstrates that organic fertilizers or a combination of organic and inorganic fertilizers have been found to have a significant impact on global food production[15]. These fertilizer blends have become ingrained in a large number of global agricultural systems[16]. Plants such as *Actinidia chinesis*[17] and *Moringa oleifera*[14] have been shown to increase growth by applying a combination of organic and inorganic fertilizers. There is currently, regrettably, no research into the impact on growth, photosynthetic and yield of *J. gendarussa* plants by organic manure and the combined application of organic manure and inorganic fertilizer. *J. gendarussa* plants are currently being used directly by farmers from their natural habitat without good standard cultivation procedures, so the quality of the raw materials cannot be standardized[1]. Therefore, the purpose of this study was to assess the effect of applying organic and inorganic fertilizers singly or in combination on growth, photosynthetic character, yield and sugar content in *J. gendarussa* leaves.

MATERIALS AND METHODS

Location and treatments: The research was carried out from March-July, 2020 at the Tropical Biopharmaca Research Center, IPB University, Dramaga Campus, Bogor, West Java, Indonesia, which is located between 6°32'25.47" N and 106°42'53.22" E. The *J. gendarussa* were grown from stem cuttings taken from a parent plant at the Tropical Biopharmaca Research Center. The stem cuttings were raised in a polybag (15×15 cm) for 2 months in 50% shade using media with a soil: Manure: Husk ratio of 1:1:1. In addition, the plants were transferred into a 20×30 cm polybag treatment filled with a soil medium of 5 kg. The experiment consisted of 6 treatments: (T_1) Control: No manure or NPK fertilizer, (T_2) Manure: 50 g/plant manure, (T_3) NPK: 1.1 g/plant N, 0.7 g/plant P_2O_5 and 0.6 g/plant K; (T_4) manure+NPK: 50+50%/plant, (T_5) manure+NPK: 10+50%/plant and (T_6) manure+NPK: 50+10%/plant. The recommended rate of manure used for *J. gendarussa* is 20 t ha^{-1} and recommended rate of NPK used for *J. gendarussa* is 200:100:150 kg ha^{-1}. Therefore, for manure fertilizer, the rate was 10 and 50% of 20 t ha^{-1} and for NPK, it was 10 and 50% of 200, 100 and 150 kg ha^{-1}. The experiment used a three-replicate randomized complete block design.

Growth measurements: Growth parameters measured included plant height, number of leaves and number of branches on plants aged 10 weeks after planting. Plant height measurements were carried out from the base of the stem to the tip of the highest plant leaf. The total number of leaves was determined by counting all leaves except the buds. The number of branches is measured by counting the number of existing branches.

Analysis of leaf chlorophyll content: The method described by Khaleghi *et al.*[18] was adapted to the analysis of leaf chlorophyll content. Fresh leaves have been cleaned to remove contaminants and chlorophyll was extracted using dimethyl sulfoxide (DMSO), with three replicates for each plant treatment. Briefly, fresh leaves samples (0.1 g) in the tube with DMSO (7 mL) were incubated at 65°C for 25 min. The absorbance of blank (DMSO) and DMSO-chlorophyll extraction were measured using a T60 UV-Vis spectrophotometer at 645 and 663 nm. Finally, chlorophyll (a, b and total) was determined using Eq. 1, 2 and 3[19].

$$\text{Chlorophyll a } (\text{mg g}^{-1}) = \frac{12.7(\text{A663}) 2.69(\text{A645}) \times \text{V}}{1000 \times \text{W}} \quad (1)$$

$$\text{Chlorophyll b } (\text{mg g}^{-1}) = \frac{22.9(\text{A645}) 4.68(\text{A663}) \times \text{V}}{1000 \times \text{W}} \quad (2)$$

$$\text{Chlorophyll total } (\text{mg g}^{-1}) = \frac{20.2(A645) + 8.02(A663) \times V}{1000 \times W} \quad (3)$$

where, A645 and A663 are the absorbances of the chlorophyll sample at wavelengths 645 and 663 nm, V: Volume of solvent and W: Weight of the sample.

Photosynthetic analysis: Four months after planting, photosynthesis was determined on plant leaves using a Li-6400XT portable photosynthesis system (LI-COR Inc. Lincoln, NE, USA). The photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and transpiration rate (T_r) were all recorded.

Leaves yield measurement: The leaves of the plants were harvested 4 months after planting. Harvesting is accomplished by pruning up to 15 cm above the soil surface. Following that, the samples were washed, separated from impurities and dried for 2 days at 45°C in an oven. Leaf yield was determined by the number of grams of dry leaves per plant.

Sugar content: The sugar content was determined using the phenol reagent method[20]. Briefly, dry leaves (0.1 g) was mixed with 1 mL of 80% alcohol and then the mixtures were centrifuged for 15 min. One mL extract of the sample was added with 1 mL of 5% phenol and 5 mL of H_2SO_4. Absorbance was measured using a T60 UV-Vis spectrophotometer at 480 nm. Sugar content was determined using a glucose solution standard curve.

Data analysis: SPSS version 25 (IBM Corp USA) was used to analyze all collected data using one-way analysis of variance (ANOVA). To compare means, the Tukey HSD test was used. The result was found to be statistically significant when $p<0.05$.

RESULTS and DISCUSSION

Growth: The plant height, number of leaves and number of branches in *J. gendarussa* plants varied according to fertilizer treatment in Table 1. The plant height ranged from 46.94 cm in the T_1 to 59.00 cm in the T_5 treatment and was significantly higher in T_5 than in the T_1 treatment. There was no significant variation in the number of leaves/plant between treatments. The maximum number of leaves/plant was recorded in the T_5 treatment, followed by T_2, T_1, T_3, T_6 and T_4 treatments. The number of branches per plant in the T_5, T_3, T_1, T_6 and T_4 treatments was significantly higher than in the T_2 treatment. These results indicated that the T_5 treatment, which receiving manure and NPK at the rate of 10+50%, showed higher growth parameters in the *J. gendarussa* plant.

Numerous growth parameters are decreased in a wide variety of herbaceous plants when nutrients are deficient[21,22]. Fertilizer application has been shown to increase growth parameters such as plant height, number of leaves, number of branches and others[23,24]. The current study presented that the combined application of manure and NPK (10+50%) (T_5) displayed a maximum in the growth parameters studied. When organic and inorganic fertilizers are combined, certain plants exhibit a range of growth responses. The study with *Actinidia chinensis* demonstrates that application combined organic and NPK fertilizers do not increase plant growth than when NPK fertilizer[17]. In tomatoes, when chemical and compost fertilizers were combined (30+70%), the growth parameters increased significantly more than when only chemical fertilizer was used[25]. The positive response of plant growth in the mixed (organic and inorganic) fertilizer could be the increased nutrient level and/or the better quality of organic treatments[26].

Chlorophyll content: The amount of chlorophyll in the leaves was affected by the fertilizer treatment in Table 2. NPK (T_3) applied resulted in a significantly higher chlorophyll a, chlorophyll b and chlorophyll total with the value of 0.538, 0.192 and 0.730 mg g^{-1} FW, respectively. All treatments demonstrated that the chlorophyll content in the leaves of the *J. gendarussa* plant was higher than the chlorophyll b content. Similarly, Pal[27] reported that the chlorophyll content in the *J. gendarussa* plant was three times higher than the chlorophyll b content, with a ratio of 3:1.

All fertilizer treatments of combination manure and NPK showed not significantly different with NPK treatment except combination manure and NPK (50+10%) (T_6) fertilizer. Numerous studies have demonstrated that increased nutrient availability has a positive effect on the chlorophyll content of the leaves[17,28]. In this study, a positive effect was also observed, particularly in the NPK (T_3) and manure (T_2) treatments. Recent research has revealed no information on the effect of manure and NPK application, or their combination application, on the chlorophyll content of the *J. gendarussa* plant's leaves.

Table1: Characteristics of plant growth in all types of fertilization

Treatments	Plant height (cm)	Number of leaves/plant	Number of branches/plant
Control (T_1)	50.94±2.79^{ab}	76.28±5.57^{a}	7.67±0.59^{ab}
Manure (M) (T_2)	46.94±2.70^{b}	76.28±6.78^{a}	5.78±1.11^{b}
NPK (T_3)	58.56±2.40^{a}	75.83±6.14^{a}	7.72±0.54^{ab}
50% M+50% NPK (T_4)	53.83±2.57^{ab}	60.72±5.12^{a}	6.72±0.50^{ab}
10% M+50% NPK (T_5)	59.00±2.97^{a}	85.00±9.76^{a}	9.50±1.10^{a}
50% M+10% NPK (T_6)	54.41±2.54^{ab}	71.18±6.65^{a}	7.18±0.98^{ab}

Indicated value is the Mean±SE, n = 3: A significant difference in level p = 0.05 as determined by the Tukey HSD test are indicated by different letters in the same column

Table 2: Chlorophyll content in all fertilization treatments

Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)
Control (T_1)	0.491±0.015^{ab}	0.187±0.003^{ab}	0.678±0.017^{ab}
Manure (M) (T_2)	0.505±0.019^{ab}	0.189±0.004^{ab}	0.694±0.023^{ab}
NPK (T_3)	0.538±0.010^{a}	0.192±0.002^{a}	0.730±0.011^{a}
50% M+50% NPK (T_4)	0.472±0.017^{ab}	0.172±0.005^{bc}	0.643±0.022^{ab}
10% M+50% NPK (T_5)	0.486±0.025^{ab}	0.179±0.006a^{bc}	0.665±0.031^{ab}
50% M+10% NPK (T_6)	0.443±0.022^{b}	0.169±0.005^{bc}	0.612±0.027^{b}

Indicated value is the Mean±SE, n = 3: A significant difference in level p = 0.05 as determined by the Tukey HSD test are indicated by different letters in the same column

Table 3: Photosynthetic characters of leaves in all fertilization treatments

Treatments	P_n (mmol m^{-2} sec^{-1})	G_s (mol m^{-2} sec^{-1})	C_i (mmol mol^{-1})	T_r (mmol m^{-2} sec^{-1})
Control (T_1)	26.07±0.91^{a}	0.30±0.04^{ab}	230.94±17.86^{a}	6.61±0.46^{ab}
Manure (M) (T_2)	25.77±1.21^{a}	0.25±0.02^{b}	205.46±16.41^{a}	5.53±0.36^{b}
NPK (T_3)	26.25±0.89^{a}	0.33±0.07^{ab}	231.23±32.23^{a}	6.85±0.86^{ab}
50% M+50% NPK (T_4)	25.12±1.05^{a}	0.37±0.08^{a}	245.02±30.92^{a}	7.87±1.79^{a}
10% M+50% NPK (T_5)	25.66±0.08^{a}	0.28±0.05^{ab}	217.79±23.04^{a}	6.21±0.65^{ab}
50% M+10% NPK (T_6)	25.22±1.10^{a}	0.34±0.05^{ab}	249.77±26.63^{a}	8.07±1.59^{a}

Indicated value is the Mean±SE, n = 3: A significant difference in level p = 0.05 as determined by the Tukey HSD test are indicated by different letters in the same column, P_n: Photosynthetic rate, G_s: Stomatal conductance, C_i: Intercellular CO_2 concentration, T_r: Transpiration rate

Photosynthetic characteristics: Photosynthetic characters of the *J. gendarussa* plant's leaves were presented in Table 3. The leaf photosynthetic rate (P_n) in all fertilization treatments evaluated in this study showed no significant difference at $p<0.05$. The leaf photosynthetic rate (P_n) ranged between 25.12 (T_4 treatment) and 26.25 mmol m^{-2} sec^{-1} (T_3 treatment). Higher the leaf stomatal conductance (G_s) was significantly recorded in applied of manure and NPK (50+50%) (T_4) treatment followed with T_6, T_3, T_1, T_5 and T_2 treatments. Manure applied in combination with NPK (50+10%) (T_6) showed higher intercellular CO_2 concentration (249.77 mmol mol^{-1}) but no significant than control and other treatments. The maximum transpiration rate of leaves was significantly found in applied manure and NPK in combination (50+10%) (T_6) and (50+50%) (T_4) treatments with values of 8.07 and 7.87 mmol m^{-2} sec^{-1}, respectively. These findings showed that T_6 treatment has positive effects on the *J. gendarussa* leaves photosynthetic properties. There has been no study published to date that examines the effect of fertilization on the photosynthetic characteristics of *J. gendarussa* plants. In comparison, while NPK fertilizer was used to promote plant growth, *Actinidia chinensis* grown with NPK fertilizer was shown to have a significantly higher leaf photosynthetic rate as reported in a work published by Zhang *et al.*[17]. Furthermore, Wei *et al.*[29], discovered that the best photosynthesis rate, stomatal conductance and intercellular CO_2 concentration in sweet potatoes were achieved when organic fertilizer was combined with NPK.

Leaves yield and sugar content: The leaves yield results of the current study revealed that there was no statistically significant difference between the fertilizer treatments in *J. gendarussa* plants in Table 4. The combined application of manure and NPK (10+50%) (T_5) and NPK (T_3) treatments, with values of 9.00 and 8.33 g/plant DW, respectively, resulted in higher leaves yield than the control (7.00 g/plant DW). Organic fertilizer and organic combination with chemical fertilizer have been reported to significantly increase the output of many plants compared to chemical fertilizer[17,25]. Many previous studies suggest that an improvement in yield could benefit from a higher content of organic materials in organic treatments[30-32].

Table 4: Yield (leaves dry weight) and sugar content in all fertilization treatments

Treatments	Yield (g/plant DW)	Sugar content (mg g^{-1} DW)
Control (T_1)	7.00±0.86[a]	344.80±18.66[a]
Manure (M) (T_2)	5.61±1.01[a]	328.35±29.19[a]
NPK (T_3)	8.33±1.74[a]	363.22±69.06[a]
50% M+50% NPK (T_4)	6.22±2.14[a]	445.02±86.41[a]
10% M+50% NPK (T_5)	9.00±3.15[a]	409.93±65.23[a]
50% M+10% NPK (T_6)	6.78±1.55[a]	405.99±56.03[a]

Indicated value is the Mean±SE, n = 3: A significant difference in level p = 0.05 as determined by the Tukey HSD test are indicated by different letters in the same column

The fertilization treatments did not have a significant effect on the sugar content of *J. gendarussa* leaves (Table 4). Applied the manure and NPK combination at the rate of (50+50%) (T_4), (10+50%) (T_5) and (50+10%) (T_6), with values of 445.02, 409.93 and 405.99 mg g^{-1} DW, respectively, showed higher sugar content than the control treatment. These results indicated that the manure and NPK combination can increase the sugar content of *J. gendarussa* plants. Our result was higher than that of Pal[27], which reported an average sugar content of 307 mg g^{-1} in *J. gendarussa* plants that were not fertilized treatment. Plants produce sugars due to primary metabolites produced during the photosynthesis process, which can produce secondary metabolites[33]. The *J. gendarussa* is a medicinal plant whose quality as a raw material for herbal medicines is determined by its secondary metabolite content[34]. Thus, a combination of manure and NPK from this study may be a viable option for improving the quality of *J. gendarussa* raw materials used in herbal medicines.

CONCLUSION

The current study demonstrates several distinct benefits of combining manure and NPK fertilizers. The use of NPK fertilizer has been shown to significantly increase chlorophyll content. Combined manure and NPK fertilizer application could promote the growth, stomatal conductance, intercellular CO_2 concentration, transpiration rate, leaves yield and sugar content of *J. gendarussa* plants.

SIGNIFICANCE STATEMENT

This study discovered the combined NPK and manure fertilizer that improving the growth, photosynthetic characteristic, leaves yield and sugar content of the *J. gendarussa* plant. There are no known reports of scientific studies on this topic as of yet. The results will provide valuable information for future studies and use for commercial purposes in industrial herbs of *J. gendarussa* plants.

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