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Application of Livestock Waste Liquid Fertilizer and Mychorriza Biofertilizer on The Growth of Biduri (*Calotropis gigantea*)

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Abstarct
Biduri (*Calotropis gigantea*) was a plant with several benefits, namely as a medicinal plant and textile material. Biduri was a wild plant that could grow in various soil conditions. The existence of biduri still needed to be fully utilized by many people. So it was necessary to develop the cultivation technology of Biduri to take advantage of this plant. This research was conducted at the field laboratory of Jumantono, Faculty of Agriculture, Universitas Sebelas Maret, Karanganyar, and used a factorial Randomized Completely Block Design (RCBD), which consisted of 2 factors, namely organic liquid fertilizer from livestock waste and mycorrhizae. Livestock waste liquid fertilizer consists of 40ml/kg soil cow urine, 40ml/kg soil goat urine, 40ml/kg soil rabbit urine, and 40ml/kg soil fish waste. Mycorrhizae consisted of 0 grams/plant, 5 grams/plant, 10 grams/plant, and 15 grams/plant. There were 20 treatment combinations repeated three times, so the total was 60 treatment combinations. The interaction between livestock waste liquid fertilizer and mycorrhizae did not significantly affect all the physiological observation variables. The results showed that the application of livestock waste liquid fertilizer had no significant effect on all observations of physiological variables. Giving mycorrhizae shows that it can increase the number of stomata and the width of the stomata aperture.

Keyword: Biduri; urine of cattle; fish waste; mycorrhizae; physiological

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Introduction

Biduri (*Calotropis gigantea*) is a herbaceous plant that can grow wild and reproduce quickly. (1) stated that this plant can grow freely in various places such as calcareous soil, hard soil, highlands, and beaches, so the existence of biduri can be found in various places. Biduri is also a medicinal plant that has many benefits. Leaves, stems, flowers, sap, and roots can be utilized. According to (2), biduri has benefits as herbal medicine that can relieve coughs, toothaches, diarrhea, and itching.

The biduri plant has been used by most people and has begun to be sought for some

requirements, so it needs to be developed with good cultivation. One essential action in cultivation is adding nutrients to the soil by applying fertilizer. An excellent fertilizer, especially for medicinal plants, is organic because it has a complete nutrient content and is safer than chemical fertilizers. Organic fertilizers are fertilizers that come from the remains of plants, animals, and humans, for example, green manure, compost, and manure, or we can also use livestock waste.

Livestock waste fertilizer is a by-product of farmed animals. The livestock waste produced may be in the form of solid and liquid waste or feces and urine. The stool or feces is one of the livestock business outputs primarily used in agriculture. However, feces require a decomposition time of 2-3 months to provide nutrients for plants. As stated by (3), using organic fertilizer for livestock urine is one of the attempts to repair soil damage due to the excessive use of chemical fertilizers. Using liquid fertilizer for livestock urine can increase plant growth because it contains high nitrogen and potassium. In addition, plants absorb livestock urine quickly, so the application does not require a long time. Livestock urine is often used as liquid organic fertilizer for cows, goats, and rabbits.

The adverse impact of using excessive chemical fertilizers can be overcome by applying Arbuscular Mycorrhizal Fungi (AMF) because these fungi can improve the soil structure that has been damaged. AMF can help the process of nutrient absorption so that it can increase plant growth and the result. (4) explained that the role of mycorrhizal fungi in increasing plant growth is to help plants absorb water and increase the ability to absorb nutrients from natural phosphate, which is phosphorus (P). The activity of mycorrhizal fungi can produce phosphatase enzymes and organic acids that can help plants to absorb P nutrients.

In this study, it is necessary to treat livestock waste in the form of urine and mycorrhizal fungi to produce biduri cultivation. This treatment is purposed to ensure the biduri plant still receives sufficient nutrition. The addition of liquid waste from livestock urine and mycorrhizal fungi is expected to increase the optimal growth of biduri.

Materials and Methods

The research was conducted from April to October 2021 at the field laboratory of Jumantono, Faculty of Agriculture, Universitas Sebelas Maret, Karanganyar, and used a factorial Randomized Complete Block Design (RCBD). The altitude of this location is 180 meters above sea level (masl), located at coordinates 7° 30' South Latitude and 110° 50' East Longitude. There are two treatment factors: liquid fertilizer from livestock waste and mycorrhizae. Livestock waste liquid fertilizer consists of 40ml/kg soil of cow urine, 40ml/kg soil of goat urine, 40ml/kg soil of rabbit urine, and 40ml/kg of fish waste. Mycorrhizae consisted of doses of 0 grams/plant, 5 grams/plant, 10 grams/plant, and 15 grams/plant. Twenty treatment combinations were repeated three times, so the total was 60 treatment combinations. This research consisted of physiological observations, namely the number of stomata, the width of the stomata aperture, the rate of photosynthesis, the rate of transpiration, and the chlorophyll content. The data was collected, then analyzed using ANOVA, and continued using the DMRT test at a significance level of 5%.

Result and Discussion

Based on the results of chemical laboratory analysis and soil fertility in table 1, it has a total N content of 0.17% (low), available P 7.07 ppm (low), K exchanged 0.26 me% (low), C-organic 1, 39% (low), organic matter 2.39% (medium), and soil pH 5.82 (slightly acidic). (5) added that alfisol soils have low organic matter content, low availability of nutrients, especially nitrogen and potassium, and have a reddish-brown to dark red color. In addition, (6) explained that the low K in alfisol soils is because the soil has agile properties, so it is easily lost through the washing process or carried away by the current of water movement.

Table 1. Results of preliminary soil chemical analysis

Soil Chemical Properties	Method	Result	Dignity
N total	Kjeldhal	0,17%	Low
P available	Bray III	7,07 ppm	Low
K changed	Ekstraksi HNO_3 dan $HClO_4$	0,26 me %	Low
C organic	Walkley & Black	1,39%	Low
Organic material	Walkley & Black	2,39%	Medium
pH	Elektrode glass	5,82	Slightly acidic

Source: Laboratory analysis of chemistry and soil fertility Faculty of Agriculture UNS

Number of stomata

The number of stomata is the number of stomata that can be counted on the plant leaf's top, middle, and bottom. Stomata are components of leaf epidermal cells that function as respiratory organs in plants. According to (7), stomata function as a place for CO_2 exchange in photosynthesis and as a place for the evaporation of water for transpiration. Based on the analysis of variance, giving mycorrhizae had a significant effect on the number of biduri stomata (*Calotropis gigantea*), followed by a DMRT follow-up test at a level of 5%.

Table 2. The effect of mycorrhizae on the number of stomata (number/mm²) of biduri (*Calotropis gigantea*) at 8 WAP

Mycorrhizae Dose	The number of stomata (number/mm²)
0 gram/plant	112,33 a
5 gram/plant	121,00 ab
10 gram/plant	132,13 b
15 gram/plant	122,93 ab

Note: Numbers followed by the same letter in each treatment showed no significant difference based on DMRT at a level of 5%.

Based on the results of DMRT in table 2, the mycorrhizal treatment at a dose of 10 grams/plant was significantly different than without giving mycorrhizae but not significantly different than the mycorrhizal treatment at a dose of 5 grams/plant and 15 grams/plant on the number of stomata. This is presumably because mycorrhizae have infected plant roots, so they can absorb nutrients optimally. As stated by (8), mycorrhizae that have infected plant roots will produce hyphae that can increase the absorption capacity of both macro and micronutrients and water absorption. (9) added that adequate macro and micro nutrients and water can help plant growth through photosynthesis. Also, (10) said that giving mycorrhizae can provide a higher number of stomata. The intensity of the light also affects the number of stomata, and the density of stomata depends on the concentration of CO_2. If CO_2 increases, the number of stomata per unit area will decrease.

The width of the stomata aperture

The width of the stomata aperture can be seen using a binocular microscope with a magnification of 40x and measured using the image master application. Stomata aperture can be affected by light, temperature, and humidity. During the day, the stomata can be opened wide because it is exposed to sunlight. According to (11), the wider the stomata aperture, the higher the transpiration process. Based on the analysis of variance, mycorrhizae had a significant effect on the width of the stomata aperture. It continued with a DMRT follow-up test at the level of 5%.

Table 3. The effect of mycorrhizae on stomata aperture width (µm) biduri (*Calotropis gigantea*) at 8 WAP

Mycorrhizae dose	The width of stomata aperture (µm)
0 gram/plant	4,39 a
5 gram/plant	4,91 b
10 gram/plant	4,93 b
15 gram/plant	5,10 b

Note: Numbers followed by the same letter in each treatment showed no significant difference based on DMRT at a level of 5%.

The results of DMRT in table 3 show that the mycorrhizal treatment at a dose of 15 grams/plant gave the best width of stomata aperture, and it's significantly different than the control treatment but not significantly different from the mycorrhizal treatment at a dose of 5 grams/plant and 10 grams/plant. This is presumably because of the condition of the growing media that became better after being given a mycorrhizal biofertilizer with a dose according to the plant's needs. (12) explained that mycorrhizae could help accelerate plant growth by increasing the absorption of nutrients from the soil by expanding the root system. According to (13), mycorrhizae that have infected the root system of plants can produce a wide range of external hyphae, thereby increasing the ability of roots to absorb nutrients, especially phosphorus.

Rate of photosynthesis

Photosynthesis is the process of making food plants with the help of sunlight. According to (14), photosynthesis requires chlorophyll in the leaves to increase the photosynthetic process in plants. The method of photosynthesis occurs when the chlorophyll in the leaves captures sunlight which is used to convert water and carbon dioxide into sugar and oxygen. Based on the analysis of variance, the application of liquid fertilizer from livestock waste, mycorrhizae, and their interaction did not significantly affect the rate of photosynthesis.

Table 4. The effect of livestock waste liquid fertilizer on the rate of photosynthesis (µmol/m²/s) of biduri (*Calotropis gigantea*) at 8 MST

Type of Fertilizer	Rate of photosynthesis (µmol/m²/s)
Without Fertilizer	5,42 a
Cow urine 40ml/kg soil	6,17 a
Goats urine 40ml/kg soil	5,20 a
Rabbit urine 40ml/kg soil	3,90 a
Fish waste 40ml/kg soil	5,79 a

Note: Numbers followed by the same letter in each treatment showed no significant difference based on DMRT at a level of 5%.

The results of DMRT in table 4 show that the application of liquid fertilizer from livestock waste, cow urine, goat urine, rabbit urine, and fish waste was not significantly different than without livestock waste liquid fertilizer application on the rate of photosynthesis. This is presumably because the application of liquid fertilizer from livestock waste has yet to meet the needs of plants in the photosynthesis process. According to (15), the element N is needed by plants in the formation of chlorophyll because chlorophyll plays a role in the photosynthesis process by absorbing sunlight, changing water (H_2O), carbon dioxide (CO_2), glucose ($C_2H_{12}O_6$) and oxygen (O_2). (16) added that photosynthesis requires water and CO_2, which is assisted by sufficient sunlight. Also, (17) stated that sufficient water needs in plants can make the photosynthesis process run well and produce photosynthate to support plant growth.

Table 5. The effect of mycorrhizae on the rate of photosynthesis (µmol/m²/s) of biduri (*Calotropis gigantea*) at 8 MST

Mycorrhizae Dose	Rate of photosynthesis (µmol/m²/s)
0 gram/plant	4,72 a
5 gram/plant	5,86 a
10 gram/plant	6,01 a
15 gram/plant	4,69 a

Note: Numbers followed by the same letter in each treatment showed no significant difference based on DMRT at a level of 5%.

Based on the results of DMRT, table 5 shows that giving mycorrhizae doses of 5 grams/plant, 10 grams/plant, and 15 grams/plant was not significantly different than without giving

mycorrhizae on the rate of photosynthesis. However, giving mycorrhizae at a dose of 15 grams/plant gave the least rate of photosynthesis. This is presumably because the more significant the dose of mycorrhizae given, the number of inoculants is also too much, so there is competition between inoculants and mycorrhizae, and it can't infect plant roots optimally. According to (18), the decrease in mycorrhizal infections in plant roots also reduces mycorrhizae's role in helping increase plant roots' ability to absorb nutrients.

The content of chlorophyll

Chlorophyll is a green plant substance that absorbs energy from sunlight in photosynthesis. The content of chlorophyll in a plant is affected by the age of the plant. According to (19), the chlorophyll content will increase in the early growth or vegetative phase and decrease in the aging stage. The analysis of variance showed that the application of liquid fertilizer from livestock waste, mycorrhizae, and their interaction did not significantly affect the chlorophyll content of biduri (*Calotropis gigantea*).

Table 6. The effect of liquid fertilizer from livestock waste on chlorophyll content (mg/g) of biduri (*Calotropis gigantea*) at 8 MST

Type of fertilizer	The content of Chlorophyll (mg/g)
Without fertilizer	0,73 a
Cow urine 40ml/kg soil	0,73 a
Goat urine 40ml/kg soil	0,67 a
Rabbit urine 40ml/kg soil	0,71 a
Fish waste 40ml/kg soil	0,70 a

Note: Numbers followed by the same letter in each treatment showed no significant difference based on DMRT at a level of 5%.

The DMRT results in table 6 show that the application of liquid fertilizer from livestock waste, cow urine, goat urine, rabbit urine, and fish waste was not significantly different than the application of liquid fertilizer from livestock waste on the chlorophyll content. This is presumably due to the presence of shade in the research area. According to (20), shade can cause a reduction in sunlight received by plants so that chlorophyll is degraded. (21) added that chlorophyll degradation can also occur due to exposure to sunlight with high intensity for a long time. Chlorophyll degradation also causes color differences in the leaves. Also, (22) stated that the more chlorophyll content, the greener the color of the leaves. Plants in the process of photosynthesis use leaves; the greener the color of the leaves, the higher the rate of photosynthesis.

Table 7. The effect of mycorrhizae on the chlorophyll content (mg/g) of biduri (*Calotropis gigantea*) at 8 MST

Mycorrhizae Dose	The content of Chlorophyll (mg/g)
0 gram/plant	0,69 a
5 gram/plant	0,68 a
10 gram/plant	0,76 a
15 gram/plant	0,70 a

Note: Numbers followed by the same letter in each treatment showed no significant difference based on DMRT at a level of 5%.

The results of DMRT in table 7 show that the inoculation of mycorrhizal doses of 5 grams/plant, 10 grams/plant, and 15 grams/plant was not significantly different than without inoculation of mycorrhizal on chlorophyll content. This follows the research results of (23) that the administration of several doses of mycorrhizae on purple pulut corn (*Zea mays* L. var *ceratina* Kulesh) showed results that were not significantly different from the lowest dose of chlorophyll content. This is presumably because the environmental conditions in the growing media are less supportive of mycorrhizal propagation. According to (24), soil environmental factors that affect the proliferation of mycorrhizae include temperature, pH, nutrients, organic matter, root residues also water content in the soil. (25) added that mycorrhizae

can adapt to acid soils. Acid soil causes the availability of P elements to become low, so mycorrhizae's role in helping absorb low P elements in acid soils is more optimal.

Rate of Transpiration

Transpiration is the process of releasing water vapor from the surface of the plant body through the stomata, cuticle, and lenticels. (26) explained that transpiration could also occur through wounds and epidermal tissue on leaves, stems, branches, flowers, fruits, and roots. Based on the analysis of variance, the application of livestock waste liquid fertilizer, mycorrhizae, and their interaction did not significantly affect the chlorophyll content of the transpiration rate of biduri (*Calotropis gigantea*).

Table 8. The effect of livestock-waste liquid fertilizer on transpiration rate (µmol/m²/s) of biduri (*Calotropis gigantea*) at 8 MST

Type of Fertilizer	Rate of Transpiration (µmol/m²/s)
Without Fretilizer	0,83 a
Cow urine 40ml/kg soil	0,92 a
Goat urine 40ml/kg soil	0,67 a
Rabbit urine 40ml/kg soil	0,50 a
Fish waste 40ml/kg soil	0,55 a

Note: Numbers followed by the same letter in each treatment showed no significant difference based on DMRT at a level of 5%.

Based on the DMRT results in table 8, it shows that the application of livestock waste liquid fertilizer from cow urine is not significantly different than the application of livestock waste liquid fertilizer from goat urine, rabbit urine, fish waste, and without livestock waste liquid fertilizer application on the rate of transpiration. This is likely because it involved three leaves per plant under different conditions, so the average is much different too. According to (27), leaf thickness and mesophyll cells in leaves can reduce the transpiration rate. Apart from the effect of leaf thickness, the low availability of water in the soil can also reduce transpiration. Transpiration is higher than the water absorbed by plant roots and can cause the stomata on the leaves to close. (28) added that closing stomata can reduce the transpiration rate and result in a reduced supply of nutrients from the soil to plants because the transpiration process helps plants absorb water. Then nutrients are absorbed by plants through the flow of water.

Table 9. The effect of mycorrhizae on the rate of transpiration (µmol/m²/s) of biduri *(Calotropis gigantea*) at 8 MST

Mycorrhizae dose	Rate of transpiration (µmol/m²/s)
0 gram/plant	0,68 a
5 gram/ plant	0,74 a
10 gram/ plant	0,69 a
15 gram/ plant	0,66 a

Note: Numbers followed by the same letter in each treatment showed no significant difference based on DMRT at a level of 5%.

The results of DMRT table 9 show that giving mycorrhizae did not have a significant effect. Giving mycorrhizae at a dose of 15 grams/plant gave the most negligible transpiration rate value compared to a dose of 5 grams/plant and a dose of 10 grams/plant. This is presumably because the planting medium soil has a high fertility level, so mycorrhizae cannot optimally infect plant roots. By (29), mycorrhizae have a symbiotic relationship with plant roots, usually in less fertile soil and dry conditions. (30) added that mycorrhizae that have been in symbiosis with plants could stimulate the process of increasing root length. The roots of plants that have been inoculated with mycorrhizae will form external hyphae, which can increase the capacity of the roots to absorb nutrients in the soil.

Conclusion

The conclusion that can be drawn based on the research that has been done it follows:

a. The interaction between livestock waste liquid fertilizer and mycorrhizae did not increase the physiology of biduri (*Calotropis gigantea*).
b. The application of liquid fertilizer from livestock waste does not increase the physiology of biduri (*Calotropis gigantea*)
c. Giving mycorrhizae can increase the number of stomata and the width of the stomata aperture.

Conflict of Interest

All authors declare no conflicts of interest in this section.

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