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Endophytic Root Dynamics, Yield and Quality of Aloe vera L. Plants by Application of Bio-Mineral Fertilizer Combined in Sandy Soil

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Abstract. This research was aimed to determine endophytic root population dynamics, yield and quality of Aloe vera L. by application of nitrogen fertilizer dosage and biofertilizers sources in sandy soil. The research was conducted in Bantul, Special Region of Yogyakarta Indonesia and arranged in a Randomized Complete Block Design with three replications. The first factor were two level of nitrogen fertilizer dosages namely 300 and 400 kg ha⁻¹. The second factor were eight sources of biofertilizer and control (200 kg urea ha⁻¹, without biofertilizer) treatment. The variables observed were endophytic root population, yield and quality of Aloe vera L plant. Statistic analysis of data was test by analysis of variant (ANOVA) and Duncan's Multiple Range Test at $P < 0.05$. The results showed that there was interaction between nitrogen fertilizer dosage and biofertilizer sources on all of observation variable. The highest endophyt population was obtained at a combination of a 300 kg urea fertilizer dosage and indigenous Rhizobacteria bamboo roots with PGPR, while hight yield and quality of Aloe vera plant obtained at a combination of a 300 kg urea fertilizer dosage and mycorrhiza source. All observational variables showed better application of a combination of urea fertilizer with biofertilizer sources than control treatment.

1. Introduction

Generally increased growth and yield of plants including Aloe vera L. plant is relied on the synthetic fertilizer to obtain a high yield but tends to cause an environmental pollution. Biofertilizer is microorganism which mainly play a role in nitrogen fixation, phosphate dissolution, biocontrol of soil pathogens and produce growth regulators that can increase the growth and yield of the crop. Biofertilizer becomes more important as it is environmentally friendly, harmless, non-toxic, and also can be used to reduce the level of soil and water pollution [1].

According to Ref. [2] reported that the biological fertilizer plays an important role for modern agriculture as it is environmentally friendly and sustainable. The bacteria that aggressively colonize with the plant roots will produce the growth regulating substances which are capable to increasing the plant growth (Plant Growth Promoting Rhizobacteria / PGPR), including Pseudomonas fluorescent and Bacillus subtilis. These bacteria can increase the plant growth by various mechanisms which are hormonal regulation, nutrient balance, dissolving nutrients facilitating plant absorption, and increasing the resistance of pathogenic attacks [3]. Biofertilizers can play a key role in the development of integrated management system in the productivity of sustainable agricultural cultivation with low environmental effects [4]. Ref.[5] reported that plants with mycorrhiza are more tolerant to nutrients and water stress, soil salinity and high heavy metals concentrations. Also, it has been shown that mycorrhizal symbiosis positively affects plants during attacks of foliar pathogens and plant-parasitic



nematodes. These effects propose the possibility of use of mycorrhizas in sustainable agroecosystems. Also, Ref. [6] stated that the mechanisms of PGPR in increasing the plant growth are through the phosphate dissolution; producing the growth hormone (Indole acetic acid/IAA, ammonia, and siderophore); producing an enzyme activity that can degrade cell walls such as cellulase (chitinase and proteases), producing HCN and as a defence against the environment. According to Ref. [7, 8], potential applicability of PGPR is steadily increasing in agriculture because it offers a promising approach to replace the use of chemical fertilizers, pesticides and other supplements. Recent progress in our understanding enhances on the diversity of PGPR in the rhizosphere along with their colonization ability and mechanism of action that would facilitate their wider application in the management of sustainable agricultural crop production. Azotobacter is a N₂ fixer bacteria that can produce gibberellin, cytokinin and indole acetic acid, the compounds can stimulate root growth [9], as well of Ref. [10] reported that Rhizobacteria played a role as stimulant of growth and plant production increasing through several mechanism such as N fixation, phosphate solvent and produced plant growth hormone (IAA, gibberellin and cytokinin). Likewise the report of Ref. [11] that biofertilizer (Rhizobacteria and mycorrhiza) a key player in enhancing soil fertility, mineral absorption, growth, yield and quality of plant. PGPR possess potential to promote the plant growth in various ways through phosphate solubilization, production of phytohormone, nutrient cycling and siderophore production. Based on the description above, it is necessary to conduct research to determine the effect of urea fertilizer dosage with biofertilizer sources on endophytic population dynamics and agronomic characteristics of Aloe vera L. plants.

2. Material and Methods

2.1. Experiment site and plant material

Research has been conducted in Poncosari, Srandakan, subdistrict Bantul Regency, Special Region of Yogyakarta, with a temperature of 28 - 36°C, 100% light intensity, 64-75% humidity, and 1672.5 mmyear⁻¹ rainfall. The research of observation was conducted in the Crop Production Laboratory of Agriculture Faculty of Universitas Sarjanawiyata Tamansiswa, Biotechnology Laboratory of Agricultural Technology Faculty and Integrated Research and Testing Laboratory of Universitas Gadjah Mada. Materials used in the experiment were cow manure, urea fertilizer, sources of biofertilizers (indigenous Rhizobacteria of bamboo root, glyricidia root), PGPR, mycorrhiza and 1.250 two months old seedlings.

2.2. Experiment Design

The experiment was arranged in a Randomized Complete Block Design factorial with 3 replications. The first factor was dosages of urea fertilizer consisting of two levels, i.e. 300 and 400 kg ha⁻¹. The second factor was biofertilizer sources consisting of eight types namely Indigenous Rhizobacteria from glyricidia, bamboo, glyricidia + PGPR, bamboo + PGPR, glyricidia + mycorrhiza, bamboo + Mycorrhiza, PGPR and Mycorrhiza, so there were 16 combined treatments.

2.3. Experimental Procedure

Experimental procedures consisted of: (1) Seedling preparations of aloe plants grown in a polybag. (2) Soil tillage using hoe, land plotting and making planting holes. (3) Application of manure as a basic fertilizer at 20 t. ha⁻¹. (4) Planting the seedlings. (5) Application of nitrogen dosage fertilizer namely 300 and 400 kg ha⁻¹. (6) Biofertilizer sources are given 3 times with 1 month interval, the volume of application is 100 mL, at 1 month after planting up to 3 months after planting. (7) Irrigation was done every day in the afternoon using a sprayer. (8) Weeding was done manually. (8) Final observation of population of endophyt root and infection, fresh and dry weight of leave, provitamin A, vitamin C and activity antioxidant of Aloe vera leave, were done 12 months after planting.

2.4. Data Collection and Statistical Analysis

The variables for the growth component included the percentage of bacterial infection, Pseudomonas and Azotobacter population, yield of fresh and dry weight of the leaves, provitamin A, provitamin E, vitamin C content, antioxidant activity with DPPH method . All data were analysed using analysis of

variance at a significance level of 5%, continued by Duncan's Multiple Range Test at a significance level of 5% for mean comparison.

3. Result and Discussion

Based on the analysis results, urea fertilizer doses with bio-fertilizer sources. Bio-fertilizer such as indigenous rhizobacteria gliricidia, indigenous bamboo rhizobacteria, PGPR, and Mycorrhiza had some relationship. Those indicators were on all observational variables.

High population dynamics of *Pseudomonas* sp root endophytes was a combination of urea fertilizer 300 kg with indigenous rhizobacteria gliricidia and PGPR, as well as a combination of urea fertilizer 400 kg with indigenous rhizobacteria gliricidia and PGPR. The lowest population was a combination of 300 kg of urea, indigenous rhizobacteria, and Mycorrhiza or 400 kg of urea with Mycorrhiza (Table 1). Based on the analysis, showed that the indigenous rhizobacteria gliricidia produce a higher bacterial population than indigenous bamboo, as well as PGPR, which showed a higher bacterial population than Mycorrhiza. It also showed that the application of PGPR and Mycorrhiza increased bacterial communities compared to controls.

Table 1. Application of urea dosage fertilizer and biofertilizer source on *Pseudomonas* and *Azotobacter* bacteria population

No.	Urea dosage (300, 400) kg ha ⁻¹ & biofertilizer (Rhizobacteria source, PGPR, Mycorrhiza) sources	Variable of observation	
		<i>Pseudomonas</i> sp. Population	<i>Azotobacter</i> sp. population
1.	Urea 300, Rh. Bamboo (N ₁ B ₁)	2.330.000 f	2.627 e
2.	Urea 300, Rh. Glyricidia (N ₁ B ₂)	3.270.000 bc	5.820 d
3.	Urea 300, Rh. Bamb + PGPR (N ₁ B ₃)	3.010.000 bc	17.400 c
4.	Urea 300, Rh. Glyr + PGPR (N ₁ B ₄)	3.690.000 a	164.667 a
5.	Urea 300, Rh. Bamb + Myco (N ₁ B ₅)	2.930.000 cd	330 f
6.	Urea 300, Rh. Glyr + Myco (N ₁ B ₆)	2.320.000 f	5.640 d
7.	Urea 300, PGPR (N ₁ B ₇)	2.730.000 de	41.733 b
8.	Urea 300, Mycorrhiza (N ₁ B ₈)	2.140.000 g	216 f
9.	Urea 400, Rh. Bamboo (N ₂ B ₁)	2.680.000 e	262 f
10.	Urea 400, Rh. Glyricidia (N ₂ B ₂)	2.200.000 fg	6.720 d
11.	Urea 400, Rh. Bamb + PGPR (N ₂ B ₃)	2.660.000 e	17.467 c
12.	Urea 400, Rh. Glyr + PGPR (N ₂ B ₄)	3.660.000 a	85.290 ab
13.	Urea 400, Rh. Bamb + Myco (N ₂ B ₅)	2.420.000 fg	5.778 d
14.	Urea 400, Rh. Glyr + Myco (N ₂ B ₆)	3.250.000 bc	16.330 c
15.	Urea 400, PGPR (N ₂ B ₇)	2.320.000 f	3.663 e
16.	Urea 400, Mycorrhiza (N ₂ B ₈)	2.120.000 g	268 f
	Interaction	(P ≤ 0,05)	(P ≤ 0,05)
	Mean	2.675.625 x	27.575 x
	Control (Urea 200 kg ha ⁻¹)	1.980.000 y	27 6 y

Note: Mean within a column followed by the same letter are not significantly different using Duncan's Multiple Range Test at the 0.05 significance level.

Reference [12] reported that the application of Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhiza Fungi (AMF) increased the population of *Pseudomonas* sp, *Azotobacter* sp. and *Azospirillum* sp which can increase lycopene and antioxidant activity in tomatoes. Similarly, based on the report of [13] by giving PGPR can increase bacterial colonization both in the root area or as root endophyte. *Azotobacter* sp. Endophyte population high was obtained at a combination of urea fertilizer at 300 kg dose with indigenous rhizobacteria gliricidia and PGPR, as well as a combination of urea fertilizer at 400 kg, indigenous rhizobacteria gliricidia and PGPR. In contrast, a deficient endophyte population was 400 kg urea dose with indigenous bamboo combination, and fertilizer dosage: 300 kg of urea with Mycorrhiza and 400 kg of urea with Mycorrhiza.

High percentage of Aloe vera root bacterial infection from the combination of 300 kg of urea with indigenous rhizobacteria gliricidia and urea 400 kg with indigenous rhizobacteria gliricidia. The combination of urea with PGPR and urea with Mycorrhiza showed a lower percentage of infection. At the same time, the most moderate was a combination of urea and bamboo rhizobacteria with PGPR (Table 2). All were giving a combined dose of urea fertilizer, an indigenous source of rhizobacteria with PGPR or Mycorrhiza obtained a bacterial infection that was higher than the control.

High fresh weight of Aloe vera leaves was obtained from the combination of urea fertilizer with PGPR and Mycorrhiza, while the combination of urea, indigenous rhizobacteria, and PGPR resulted from the lower leaf fresh weight of, and the combination of urea fertilizer with indigenous rhizobacteria obtained the lowest fresh weight. All giving combined a dose of urea fertilizer, an indigenous source of indigenous rhizobacteria with PGPR or Mycorrhiza, got fresh leaf weights that were higher than controls. This was in line with Ref. [14] stated that the provision of a combination of nitrogen minerals with Azotobacter microbes could increase growth, yield, and quality of corn, while of Ref. [15] reported that the application of Mycorrhiza could increase the growth of rainfed lowland rice plants.

The high dry weight of Aloe vera leaf was obtained from the combination of urea fertilizer with PGPR and Mycorrhiza, while the combination of urea, indigenous rhizobacteria, and PGPR or Mycorrhiza gained lower leaf fresh weight, and the combination of urea fertilizer with bamboo indigenous rhizobacteria obtained the lowest fresh leaf weight. All were giving a combined dose of urea fertilizer, an indigenous source of indigenous rhizobacteria with PGPR or Mycorrhiza, obtained leaf dry weight higher than the control. This was in line with Ref. [16] reported the combination of RDF N fertilizer with Azospirillum PGPR increased the number of pods and yields of fenugreek (*Trigonella foenimgraecum* L.). Another report which had the same line was the study of Ref. [17], who reported that adding *Rhizobium* sp. and *Azotobacter* sp. could increase the growth of *Vigna mungo* L. Hepper.

Table 2. Application of urea dosage fertilizer and biofertilizer source infection bacteria, fres and dry weight of leave and vitamin C content

No.	Urea dosage & Sources of biofertilizer	Variable of observation			
		Infection Bacteri(%)	Fresh weight of leave (kg)	Dry weight of leave (g)	Vitamin C (ppm)
1.	Urea 300, Rh. Bamboo	61.26 e	0.324 cd	71.14 b	15.09 d
2.	Urea 300, Rh. Glyri	78.33 ab	0.320 cd	72.55 b	15.20 d
3.	Urea 300, Rh. Bamb + PGPR	51.50 f	0.350 b	71.29 b	15.49 d
4.	Urea 300, Rh. Glyr + PGPR	77.07 b	0.360 b	75.12 b	16.64 c
5.	Urea 300, Rh. Bamb + Myco	71.50 c	0.362 b	72.21 b	15.85 d
6.	Urea 300, Rh. Glyr + Myco	71.05 cd	0.344 bc	71.51 b	14.05 d
7.	Urea 300, PGPR	69.53 cd	0.395 a	78.52 ab	20.00 b
8.	Urea 300, Mycorhiza	60.10 e	0.391 a	80.72 a	21.27 b
9.	Urea 400, Rh. Bamboo	67.47 d	0.310 d	64.98 d	16.57 c
10.	Urea 400, Rh. Glyri	82.00 a	0.350 b	72.37 b	18.41 b
11.	Urea 400, Rh. Bamb + PGPR	54.93 f	0.355 b	72.31 b	21.21 b
12.	Urea 400, Rh. Glyr + PGPR	70.20 cd	0.351 b	72.98 b	21.17 b
13.	Urea 400, Rh. Bamb + Myco	70.60 cd	0.337 bc	69.71 c	21.66 b
14.	Urea 400, Rh. Glyr + Myco	76.00 b	0.353 b	73.05 b	21.65 b
15.	Urea 400, PGPR	71.33 c	0.401 a	82.91 a	22.85 a
16.	Urea 400, Mycorhiza	63.27 e	0.405 a	83.66 a	23.00 a
	Interaction	(P ≤ 0,05)	(P ≤ 0,05)	(P ≤ 0,05)	(P ≤ 0,05)
	Mean	68.82 x	0.355 x	79.04 x	18,81 x
	Control (Urea 200 kg ha ⁻¹)	38.93 y	0.272 y	60.87 y	13.48 y

Note: Mean within a column followed by the same letter are not significantly different using Duncan's Multiple Range Test at the 0.05 significance level.

High vitamin C content of Aloe vera leaves were obtained from a combination of 400 kg of urea fertilizer with PGPR or with Mycorrhiza, while the combination of urea fertilizer, indigenous rhizobacteria, and PGPR or Mycorrhiza obtained a lower vitamin C content, and the combination of urea fertilizer with indigenous rhizobacteria contains vitamin content C was the lowest. All administering combined a dose of urea fertilizer, an indigenous source of rhizobacteria with PGPR or Mycorrhiza obtained higher vitamin C content than controls. This was not in line with the report of Ref. [17] that the application of PGPR and Mycorrhiza could increase the vitamin content in tomatoes, as well as of Ref. [19], reported that the application of mineral fertilizers and biofertilizer *Trichoderma* sp could increase the content of vitamin C (ascorbic acid) in tomatoes.

High provitamin A content was a combination of urea fertilizer 300-400 kg with PGPR or urea fertilizer 300-400 kg with Mycorrhiza. In contrast, the combination of urea fertilizer, indigenous rhizobacteria *gliricidia*, PGPR, or with Mycorrhiza had a lower provitamin A content, and the mixture only urea fertilizer with indigenous rhizobacteria obtained the lowest provitamin A content (Table 3). All were administering a combined dose of urea fertilizer, an indigenous source of indigenous rhizobacteria with PGPR or Mycorrhiza, obtained a higher provitamin A content than the control. This was not following the report of reference [20] reported that the use of organic fertilizers and biofertilizers could increase the carotenoid content of garlic bulbs. In contrast with Ref. [13] reported that the administration of mineral fertilizers and biofertilizer *Trichoderma* sp could increase the content of provitamin A (carotenoids) in tomatoes.

Table 3. Application of urea dosage fertilizer and biofertilizer source on content of provitamin A, provitamin E and antioxidant activity

No.	Urea dosage & sources of biofertilizer	Variable of observation		
		Provitamin A (ppm)	Provitamin E (ppm)	Antioxidant Activity (%)
1.	Urea 300, Rh. Bamboo	4.48 e	24.26 g	28.60 c
2.	Urea 300, Rh. Glyricidia	4.71 e	25.24 g	30.30 b
3.	Urea 300, Rh. Bamb + PGPR	5.79 d	27.81 f	30.60 b
4.	Urea 300, Rh. Glyn + PGPR	6.46 c	32.23 d	46.60 a
5.	Urea 300, Rh. Bamb + Myco	6.57 c	35.48 c	31.20 b
6.	Urea 300, Rh. Glyn + Myco	7.01 b	37.85 b	36.10 b
7.	Urea 300, PGPR	7.48 a	43.34 a	40.00 a
8.	Urea 300, Mycorrhiza	7.41 a	43.63 a	40.80 a
9.	Urea 400, Rh. Bamboo	4.88 e	27.66 f	33.16 b
10.	Urea 400, Rh. Glyricidia	4.66 e	28.63 f	31.66 b
11.	Urea 400, Rh. Bamb + PGPR	5.74 d	31.35 e	30.86 b
12.	Urea 400, Rh. Glyn + PGPR	6.16 c	34.23 cd	34.22 b
13.	Urea 400, Rh. Bamb + Myco	5.66 d	34.28 cd	33.28 b
14.	Urea 400, Rh. Glyn + Myco	7.86 a	37.53 b	36.42 b
15.	Urea 400, PGPR	7.66 a	45.26 a	40.22 a
16.	Urea 400, Mycorrhiza	8.04 a	44.85 a	41.06 a
	Interaction	($P \leq 0,05$)	($P \leq 0,05$)	($P \leq 0,05$)
	Mean		34.60 x	33.34 x
	Control (Urea 200 kg ha ⁻¹)		13.48 y	23.60 y

Note: Mean within a column followed by the same letter are not significantly different using Duncan's Multiple Range Test at the 0.05 probability level.

High provitamin E content was obtained from a combination of 300-400 kg urea fertilizer with PGPR or a combination of 300-400 kg urea fertilizer with mycorrhiza. In contrast, the combination of urea fertilizer with a dose of 300-400 kg, indigenous rhizobacteria *gliricidia*, and mycorrhiza, the content of provitamin E was obtained low, and the combination of only 400 kg urea fertilizer with indigenous rhizobacteria of bamboo or *gliricidia* obtained the lowest provitamin E content. All giving combined a

dose of urea fertilizer, an indigenous source of rhizobacteria with PGPR or mycorrhiza obtained higher provitamin E content than controls. This was consistent with the report of Ref. [20] that the application of PGPR can increase organic compounds as precursors of provitamin E in cauliflower plants (*Brassica oleracea*), this was consistent with the statement of Ref. [17] reported that the administration of *Rhizobium* sp. and *Azotobacter* sp. improve quality (carbohydrate, protein, and fat content) *Vigna mungo* L. Hepper.

High antioxidant activity was obtained from 300-400 kg of urea fertilizer with PGPR or a combination of urea fertilizer 300-400 kg with mycorrhiza, while a combination of urea fertilizer at 300-400 kg, indigenous rhizobacteria, PGPR or mycorrhiza had lower antioxidant activity, and the combination of only 300 kg urea fertilizer with bamboo indigenous rhizobacteria obtained the most moderate antioxidant activity. All were giving a combined dose of urea fertilizer, an source of indigenous rhizobacteria with PGPR or mycorrhiza obtained higher antioxidant activity than controls. This was not in accordance Ref. [12] that the application of PGPR and Arbuscular Mycorrhiza Fungi (AMF) could increase lycopene and antioxidant activity in tomatoes, while reference [13] reported that the application of mineral fertilizers and biofertilizer *Trichoderma* sp could increase the antioxidant content of tomatoes.

4. Conclusion

Based on the analysis results, there was an interaction between the application of ammonium sulphate doses with all sources of biological fertilizers. The endophytic population of *Pseudomonas* sp. and *Azotobacteria* sp. was obtained at a combination of 300 kg of urea, indigenous bamboo rhizobacteria, and PGPR. Fresh leaf weights and high quality obtained in conjunction with urea dose of 300 kg with mycorrhiza. All components of the observed variables with the combination of urea, indigenous Rhizobacteria, and PGPR or mycorrhizae showed better results than controls.

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