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Research Article

Potential to Increase the Agronomic Character and Phytochemical Content of *Aloe vera* Plant by Application of Integrated Fertilizers in Sandy Soil

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Abstract

Background and Objective: *Aloe vera* plant provides a variety of compounds, minerals, phytochemicals and secondary metabolites content in the leaves. Therefore, this research was aimed to determine character agronomy and phytochemical content of *Aloe vera* L. plant by application of nitrogen fertilizer dosage and biofertilizers sources in sandy soil. **Materials and Methods:** 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 was two levels of nitrogen fertilizer dosages namely 200 and 300 kg ha^{-1}. The second factor was eight sources of biofertilizer and control (400 kg urea ha^{-1}, without biofertilizer) treatment. The variables observed were growth, yield, vitamin, amino acid content and activity antioxidant of *Aloe vera* L. plant. Statistical analysis of data was tested by analysis of variance (ANOVA) and Duncan's Multiple Range Test at $p<0.05$. **Results:** The best results were obtained with a combination of 200-300 kg of urea and mycorrhiza, which had the same effect as the combination of 200-300 kg of urea with bamboo rhizobacteria on endophytic populations, growth, yield and amino acid content of *Aloe vera* leaves. Meanwhile, the highest concentration of antioxidant compounds (provitamin A, provitamin E and vitamin C) was obtained at a combination of urea dosage of 300 kg ha^{-1} with mycorrhiza or PGPR. **Conclusion:** The result showed that there was an interaction between nitrogen fertilizer dosage and biofertilizer sources on all of the observed variables. Mycorrhiza could be used as a biofertilizer in an integrated management system to increase growth, yield and phytochemical content of *Aloe vera* plants in sandy soil.

Key words: Amino acid, mycorrhiza, provitamin E, yield of leaves, antioxidant, vitamin C

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

INTRODUCTION

Aloe vera leaves contain fat compounds, carbohydrates, proteins, 18 essential amino acids, four kinds of vitamins, minerals and six kinds of enzymes. They also contain secondary metabolites including alkaloids, aloins, lectins, lignin, saponins, tannins, phenolic, glucomannan, vitamins A, B_1, B_2, C, E and minerals, which were all synergically interacted[1]. The *Aloe vera* leaf has been used as an ingredient in health foods, for the cosmetic industry and an ingredient in phytotherapeutics[2]. The study of Firdous *et al*.[3] that *Aloe vera* gel could be an excellent edible coating material should be used commercially as a technologically viable postharvest preservation technique for tomato fresh product.

The available land at the location is a marginal one in the form of sandy soil. Jensen *et al*.[4] reported that sandy soil is often dry, nutrient deficiency and fast draining. Generally increased growth and yield of plants including *Aloe vera* L. plant is relied on the high dosages of synthetic fertilizer to obtain high yield but tends to cause environmental pollution.

Biofertilizer is a microorganism that mainly play role in nitrogen fixation, phosphate dissolution, biocontrol of soil pathogens and produce growth regulators that can increase the growth and yield of the crop. Oliveira *et al*.[5] reported that biofertilizer plays an important role for modern agriculture, as it is environmentally friendly, harmless, non-toxic and also can be used to reduce the level of soil and water pollution[6]. 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*. Karnwal[7] stated that mechanisms of PGPR in increasing the plant growth by various mechanisms which are hormonal regulation, nutrient balance, dissolving nutrients facilitating plant absorption and increasing the resistance of pathogenic attacks[8]. Biofertilizers can play a key role in the development of integrated management system in the productivity of sustainable agricultural cultivation with low environmental effects[9]. Zrnic and Siric[10] reported that plants with mycorrhiza are more tolerant to nutrients, water stress, soil salinity and high heavy metals concentrations. It has been shown that mycorrhizal symbiosis positively affects plants during attacks of foliar pathogens and plant-parasitic nematodes. These effects propose the possibility use of mycorrhiza in sustainable agroecosystems. *Azotobacter* is a N_2 fixer bacterium that can produce gibberellin, cytokinin and indole acetic acid, which can stimulate root growth[11]. Enebe and Babalola[12] reported that Rhizobacteria played a role as stimulant of growth and plant production. Likewise, the report of Itelima *et al*.[13] that biofertilizer is the key player in enhancing soil fertility, mineral absorption, growth, yield and quality of plant. PGPR potential is to promote the plant growth in various ways through phosphate solubilization, production of phytohormone, nutrient cycling and siderophore production. Wen *et al*.[14] reported that amino acid can be grouped into different categories.

The application of organic manures and bioinoculants could minimize the problems over chemical fertilizers in improving soil fertility. Thus, it is necessary to integrate three different sources of nutrients, viz., organics, chemicals and bioinoculants, for a more efficient and economical productive system in the long run. Bioinoculants are environmentally safer and a cost-effective supplement to chemical fertilizers. Hence, there is an urgent need to study the influence of straight or integrated application of organic manure and biofertilizers on the yield and quality of tea plant[15].

Based on the description above, this study determined the effect of urea fertilizer dosage with biofertilizer of different sources on root endophytic infection, agronomic characteristics and phytochemical content of *Aloe vera* L. plants.

MATERIALS AND METHODS

Experiment site and plant material: The research was carried out in Poncosari, Srandakan, Bantul Special Region of Yogyakarta, April, 2019 to March, 2020. Condition of agri climate is a temperature of 28-36°C, 100% light intensity, 64-75% humidity and 1672.5 mm year^{-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, gliricidia), PGPR, mycorrhiza and 1.250 two months old seedlings.

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., 200 and 300 kg ha^{-1}. The second factor was biofertilizer different sources consisting of eight types namely Indigenous Rhizobacteria gliricidia, Rhizobacteria bamboo, *Rhizobacteria gliricidia*+PGPR, Rhizobacteria bamboo+PGPR, Rhizobacteria gliricidia+ mycorrhiza, Rhizobacteria bamboo+Mycorrhiza, PGPR and Mycorrhiza, so there were 16 combined treatments and control (urea 400 kg ha^{-1} without Rhizobacteria).

Experimental procedure: Experimental procedures consisted of: Seedling preparations of aloe plants grown in a polybag. Soil tillage using hoe, land plotting and making planting holes. Application of manure as a basic fertilizer at 20 t ha^{-1}. Planting the seedlings. Application of urea dosage fertilizer namely 200 and 300 kg ha^{-1}. Each biofertilizer sources are given 3 times with 1 month interval, the volume of application is 100 mL and 2% concentration, at 1 month after planting up to 3 months after planting. Irrigation was done every day in the afternoon using a sprayer. Weeding was done manually. Final observation of endophyte root infection, fresh and dry weight of leave, provitamin A, provitamin E, vitamin C, activity antioxidant and amino acid content of *Aloe vera* leave, were done 12 months after planting.

Data collection and statistical analysis: The variables for the growth component were observed, included the percentage of *Pseudomonas* and *Azotobacter* infection, yield of fresh and dry weight of the leaves, provitamin A, provitamin E, vitamin C (A.A. Spectrophotography method)[16], antioxidant activity with 2, 2-Di Phenyl-1-Picryl Hydrazyl (DPPH) method[17] and amino acid content Liquid Chromatography Mass Spectrometry (LCMS) method[18]. All data were analyzed 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[19].

RESULTS

There were significant effects of the combination treatment of urea fertilizer dosage and biofertilizer source on the bacterial infection (Table 1), agronomic characteristics (Table 1), antioxidant activity (Table 1) and phytochemical content of *Aloe vera* plants (Table 2-4 and Fig. 1-3).

Root bacterial infection: The application of urea fertilizer at a dosage of both 200 and 300 kg ha^{-1} combined with rhizobacteria from gliricidia resulted in the highest rate of root bacterial infection, reaching a percentage of 78.33 and 82%, respectively. Meanwhile, the lowest rate of root bacterial infection was observed in the plants treated with urea fertilizer at a dosage of both 200 and 300 kg ha^{-1} combined with rhizobacteria from bamboo and PGPR, showing a percentage of 51.50 and 54.93%, consecutively (Table 1).

Leaf fresh weight of *Aloe vera* plants: According to the results presented in Table 1, the highest value of fresh weight of leaves was obtained in the plants treated with 200 and 300 kg ha^{-1} urea fertilizer combined with PGPR or with Mycorrhiza, ranging from 491.18-505.36 g. Conversely, the combination of 300 kg ha^{-1} urea and rhizobacteria from bamboo resulted the lowest value of leaves' fresh weight (410.44 g).

Table 1: Effects of urea fertilizer dosages and biofertilizer sources on the bacterial infection, fresh and dry weight of leaves and antioxidant activity

	Variable of observation			
Urea dosages and sources of biofertilizer	Bacterial infection (%)	Dry weight of leaves (g)	Fresh weight of leaves (g)	Antioxidant activity (%)
Urea 200, Rh. Bamboo	61.26^{e}	71.14^{b}	424.26^{c}	28.60^{c}
Urea 200, Rh. Glir	78.33^{a}	72.55^{b}	420.62^{c}	30.30^{b}
Urea 200, Rh. Bamb+PGPR	51.50^{f}	71.29^{b}	450.21^{b}	30.60^{b}
Urea 200, Rh. Glir+PGPR	77.07^{b}	75.12^{b}	460.42^{b}	36.60^{b}
Urea 200, Rh. Bamb+Myco	71.50^{c}	72.21^{b}	462.23^{b}	31.20^{b}
Urea 200, Rh. Glir+Myco	71.05^{c}	71.51^{b}	444.33^{b}	36.10^{b}
Urea 200, PGPR	69.53^{c}	78.52^{a}	495.32^{a}	40.00^{a}
Urea 200, Mycorrhiza	60.10^{e}	80.72^{a}	491.18^{a}	40.80^{a}
Urea 300, Rh. Bamboo	67.47^{d}	64.98^{d}	410.44^{d}	33.16^{b}
Urea 300, Rh. Glir	82.00^{a}	72.37^{b}	450.26^{b}	31.66^{b}
Urea 300, Rh. Bamb+PGPR	54.93^{f}	72.31^{b}	455.28^{b}	30.86^{b}
Urea 300, Rh. Glir+PGPR	70.20^{c}	72.98^{b}	451.16^{b}	34.22^{b}
Urea 300, Rh. Bamb+Myco	70.60^{c}	69.71^{c}	437.44^{b}	33.28^{b}
Urea 300, Rh. Glir+Myco	76.00^{b}	73.05^{b}	453.26^{b}	36.42^{b}
Urea 300, PGPR	71.33^{c}	82.91^{a}	501.44^{a}	40.22^{a}
Urea 300, Mycorrhiza	63.27^{e}	83.66^{a}	505.36^{a}	41.06^{a}
Interaction	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
Mean	68.82^{x}	79.04^{x}	355.24^{x}	33.34^{x}
Control (Urea 400 kg ha^{-1})	38.93^{y}	60.87^{y}	272.38^{y}	23.60^{y}

Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level. Rh: Rhizobacteria, Myco: Mycorrhiza, Glir: Gliricidia

Table 2: Effects of urea fertilizer dosages and biofertilizer sources on the content of arginine, histidine, lysine, phenylalanine, isoleucine and leucine

	Type of amino acid (ppm)					
Urea dosages and sources of biofertilizer	Arginine	Histidine	Lysine	Phenylalanine	Isoleucine	Leucine
Urea 200, Rh. Bamboo	209.87^{c}	89.87^{b}	319.39^{a}	202.02^{a}	189.77^{a}	369.34^{a}
Urea 200, Rh. Gliri	81.76^{f}	15.66^{e}	273.96^{b}	124.65^{c}	118.50^{c}	236.65^{b}
Urea 200, Rh. Bamb+PGPR	176.25^{d}	51.44^{d}	288.43^{b}	132.30^{b}	164.54^{b}	321.01^{a}
Urea 200, Rh. Glir+PGPR	109.24^{e}	29.77^{e}	173.46^{c}	105.51^{c}	109.41^{c}	213.99^{b}
Urea 200, Rh. Bamb+Myco	170.25^{d}	91.78^{b}	254.32^{b}	171.25^{b}	159.41^{b}	343.25^{a}
Urea 200, Rh. Glir+Myco	188.17^{d}	72.24^{c}	278.11^{b}	120.48^{c}	120.66^{c}	312.24^{a}
Urea 200, PGPR	102.29^{e}	52.97^{d}	239.74^{b}	160.53^{b}	95.43^{d}	210.00^{c}
Urea 200, Mycorrhiza	243.66^{a}	104.56^{a}	313.21^{a}	177.93^{b}	181.85^{a}	329.68^{a}
Urea 300, Rh. Bamboo	202.02^{c}	94.44^{b}	302.63^{a}	199.68^{a}	201.24^{a}	346.44^{a}
Urea 300, Rh. Gliri	88.46^{f}	24.36^{e}	182.66^{c}	119.44^{c}	126.22^{c}	241.88^{b}
Urea 300, Rh. Bamb+PGPR	179.88^{d}	48.68^{d}	282.34^{b}	139.49^{b}	196.33^{a}	318.44^{a}
Urea 300, Rh. Glir+PGPR	212.46^{b}	79.34^{c}	183.47^{c}	110.36^{c}	112.38^{c}	244.28^{b}
Urea 300, Rh. Bamb+Myco	179.34^{d}	66.42^{c}	267.12^{b}	112.14^{c}	164.66^{b}	334.12^{a}
Urea 300, Rh. Glir+Myco	126.46^{e}	62.78^{c}	266.87^{b}	128.66^{c}	126.24^{c}	289.11^{b}
Urea 300, PGPR	111.79^{e}	57.26^{c}	248.33^{b}	116.48^{c}	102.84^{d}	243.46^{b}
Urea 300, Mycorrhiza	235.79^{a}	124.58^{a}	332.45^{a}	184.24^{a}	189.66^{a}	331.00^{a}
Interaction	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
Mean	140.88^{x}	59.22^{x}	217.67^{x}	128.06^{x}	131.06^{x}	260.27^{x}
Control	83.66^{y}	13.24^{y}	144.42^{y}	78.66^{y}	74.82^{y}	142.36^{y}

Means within a column followed by the same letters are not significantly different according to Duncan’s Multiple Range Test at the 0.05 significance level

Table 3: Effects of urea fertilizer dosages and biofertilizer sources on the content of tyrosine, methionine, valine, proline, glutamic acid and aspartic acid

	Type of amino acid (ppm)					
Urea dosages and sources of biofertilizer	Tyrosine	Methionine	Valine	Proline	Glutamic acid	Aspartic acid
Urea 200, Rh. Bamboo	64.03^{a}	25.86^{a}	238.37^{a}	158.61^{a}	344.36^{b}	317.24^{a}
Urea 200, Rh. Gliri	77.25^{a}	7.11^{c}	143.57^{c}	20.29^{d}	305.24^{c}	175.26^{d}
Urea 200, Rh. Bamb+PGPR	42.50^{b}	8.09^{c}	200.11^{b}	88.77^{c}	321.40^{c}	291.19^{b}
Urea 200, Rh. Glir+PGPR	26.69^{c}	10.56^{b}	130.89^{c}	70.20^{c}	278.36^{d}	172.01^{d}
Urea 200, Rh. Bamb+Myco	28.41^{c}	28.41^{a}	198.44^{b}	131.09^{a}	317.74^{c}	271.79^{c}
Urea 200, Rh. Glir+Myco	36.22^{c}	10.44^{b}	192.16^{b}	121.24^{b}	283.32^{d}	196.24^{d}
Urea 200, PGPR	75.46^{a}	10.56^{b}	117.01^{c}	116.42^{b}	266.80^{d}	211.65^{c}
Urea 200, Mycorrhiza	43.69^{b}	11.15^{b}	244.62^{a}	142.43^{a}	580.53^{a}	365.96^{a}
Urea 300, Rh. Bamboo	54.03^{b}	27.16^{a}	241.29^{a}	160.41^{a}	324.16^{b}	321.14^{a}
Urea 300, Rh. Gliri	77.25^{a}	8.66^{c}	145.55^{c}	21.69^{d}	315.14^{c}	171.26^{d}
Urea 300, Rh. Bamb+PGPR	42.50^{b}	8.69^{c}	202.21^{b}	86.74^{c}	311.40^{c}	281.29^{b}
Urea 300, Rh. Glir+PGPR	26.69^{c}	10.84^{b}	133.66^{c}	74.28^{c}	274.86^{d}	170.61^{d}
Urea 300, Rh. Bamb+Myco	28.41^{c}	28.56^{a}	200.48^{b}	146.19^{a}	312.78^{c}	218.79^{c}
Urea 300, Rh. Glir+Myco	36.22^{c}	11.54^{b}	196.26^{b}	120.84^{b}	273.82^{d}	194.34^{d}
Urea 300, PGPR	75.46^{a}	11.68^{b}	115.41^{c}	119.22^{b}	261.60^{d}	210.95^{c}
Urea 300, Mycorrhiza	63.69^{a}	21.15^{a}	249.42^{a}	152.23^{a}	610.53^{a}	369.16^{a}
Interaction	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
Mean	44.36^{x}	13.36^{x}	150.43^{x}	96.15^{x}	298.45^{x}	218.83^{x}
Control	28.72^{y}	8.46^{y}	98.22^{y}	68.24^{y}	165.88^{y}	162.24^{y}

Means within a column followed by the same letters are not significantly different according to Duncan’s Multiple Range Test at the 0.05 significance level

Leaf dry weight of *Aloe vera* plants: Similar to the results on the fresh weight of leaves, the application of urea fertilizer at a dosage of both 200 and 300 kg ha^{-1} combined with PGPR or Mycorrhiza resulted the highest value of leaf dry weight, ranging from 78.52-83.66 g, consecutively. Meanwhile, the lowest value of leaf dry weight (64.98 g) was obtained in the plants treated with the combination of urea fertilizer and rhizobacteria obtained from bamboo (Table 1).

Antioxidant activity: The application of urea fertilizer at a dosage of 200 and 300 kg ha^{-1} combined with both PGPR and Mycorrhiza resulted in the highest antioxidant activity, ranging from 40-41.06% (Table 1). Meanwhile, the application of 200 kg ha^{-1} urea fertilizer combined with indigenous rhizobacteria from bamboo resulted in the lowest antioxidant activity (28.60%).

Table 4: Effects of urea fertilizer dosages and biofertilizer sources on the content of cysteine, threonine, serine, alanine, glycine and tryptophan

Urea dosages and sources of biofertilizer	Type of amino acid (ppm)					
	Cysteine	Threonine	Serine	Alanine	Glycine	Tryptophan
Urea 200, Rh. Bamboo	13.24[a]	149.31[a]	157.64[a]	233.17[a]	169.65[a]	Undetected
Urea 200, Rh. Gliri	12.15[a]	89.75[c]	117.36[b]	110.93[c]	107.93[b]	Undetected
Urea 200, Rh. Bamb+PGPR	12.34[a]	114.04[b]	135.72[b]	178.77[b]	133.76[b]	Undetected
Urea 200, Rh. Glir+PGPR	12.74[a]	73.27[c]	91.53[c]	112.17[c]	98.27[c]	Undetected
Urea 200, Rh. Bamb+Myco	12.30[a]	145.02[a]	163.35[a]	190.50[a]	191.15[a]	Undetected
Urea 200, Rh. Glir+Myco	10.40[b]	112.16[b]	112.16[b]	185.32[b]	161.32[a]	Undetected
Urea 200, PGPR	12.40[a]	87.04[c]	107.42[b]	142.09[b]	101.09[b]	Undetected
Urea 200, Mycorrhiza _	12.80[a]	132.22[a]	151.28[a]	195.44[a]	168.48[a]	Undetected
Urea 300, Rh. Bamboo	13.24[a]	146.31[a]	164.64[a]	237.17[a]	172.67[a]	Undetected
Urea 300, Rh. Gliri	12.15[a]	88.71[c]	127.36[b]	110.93[c]	102.73[b]	Undetected
Urea 300, Rh. Bamb+PGPR	12.34[a]	117.14[b]	139.72[b]	168.77[b]	113.76[b]	Undetected
Urea 300, Rh. Glir+PGPR	12.74[a]	78.47[c]	98.43[c]	116.17[c]	88.37[c]	Undetected
Urea 300, Rh. Bamb+Myco	12.30[a]	147.12[a]	167.65[a]	196.50[a]	197.19[a]	Undetected
Urea 300, Rh. Glir+Myco	10.40[b]	116.26[b]	112.16[b]	189.32[b]	171.42[a]	Undetected
Urea 300, PGPR	12.40[a]	86.84[c]	117.47[b]	152.09[b]	106.19[b]	Undetected
Urea 300, Mycorrhiza	12.80[a]	146.22[a]	159.68[a]	206.44[a]	178.18[a]	Undetected
Interaction	$p\leq 0.05$	$p\leq 0.05$	$p\leq 0.05$	$p\leq 0.05$	$p\leq 0.05$	$p\leq 0.05$
Mean	12.27[x]	121.99	141.57[x]	181.65[x]	150.81[x]	-
Control	8.36[y]	72.66[y]	89.74[y]	112.04[y]	87.66[y]	-

Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test at the 0.05 significance level

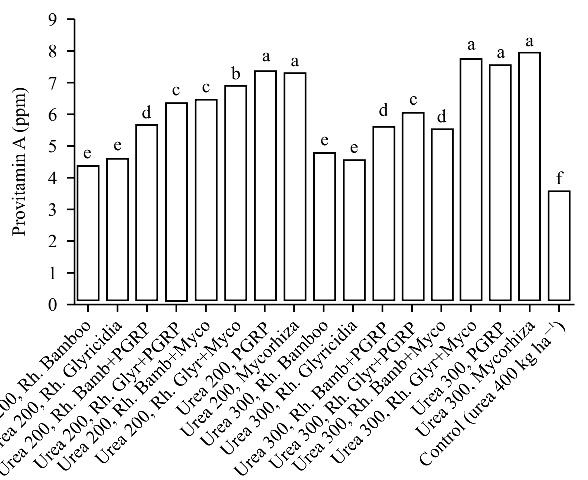

Fig. 1: Effects of urea fertilizer dosages and biofertilizer sources on the content of provitamin A

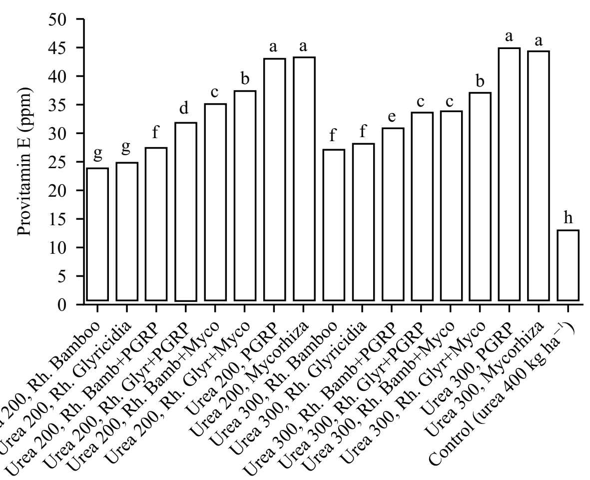

Fig. 2: Effects of urea fertilizer dosages and biofertilizer sources on the content of Provitamin E

Arginine, histidine, lysine, phenylalanine, isoleucine and leucine content: According to Table 2, the highest content of arginine, histidine, lysine, isoleucine and leucine was resulted by the application of 200 and 300 kg ha^{-1} urea fertilizer combined with Mycorrhiza, producing 235.79, 124.58, 332.45, 189.66 and 331 ppm, respectively. Meanwhile, the highest content of phenylalanine was observed in the plants treated with 300 kg ha^{-1} urea fertilizer combined with rhizobacteria from bamboo and with Mycorrhiza, resulting in a value of 199.68 and 184.24 ppm, consecutively. The combination of urea fertilizer, either at 200 or 300 kg ha^{-1} and rhizobacteria from gliricidia, either alone or with PGPR and Mycorrhiza, tended to result in the lowest content of arginine, histidine, lysine, phenylalanine, isoleucine and leucine (Table 2).

Tyrosine, methionine, valine, proline, glutamic acid and aspartic acid content: The application of urea fertilizer at a dosage of 300 kg ha^{-1} combined with Mycorrhiza resulted in the highest content of tyrosine, methionine, valine, proline, glutamic acid and aspartic acid, reaching a value of 63.69, 21.15, 249.42, 152.23, 610.53 and 369.16 ppm, respectively

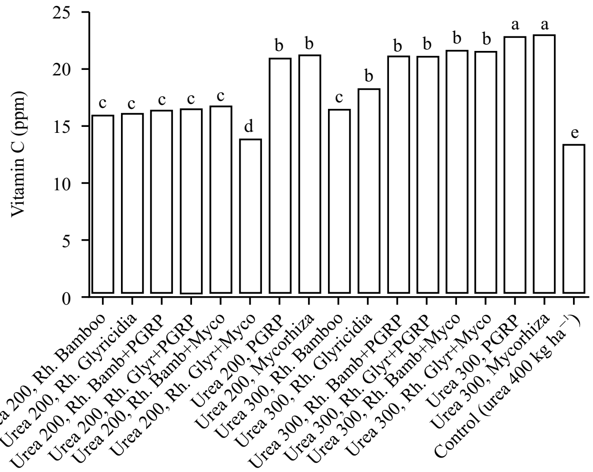

Fig. 3: Effects of urea fertilizer dosages and biofertilizer sources on the content of vitamin C

(Table 3). The interaction of the urea fertilizer dosages and the biofertilizer sources showed various effects on the content of tyrosine, methionine, valine, proline, glutamic acid and aspartic acid, which can be seen in detail in Table 3.

Cysteine, threonine, serine, alanine, glycine and tryptophan content: The application of urea fertilizer, either at 200 or 300 kg ha^{-1}, combined with rhizobacteria from gliricidia+Mycorrhiza tended to result in the lower content of cysteine compared to other treatment combinations (Table 4). Almost similar to the effect on cysteine content, the application of urea fertilizer, either at 200 or 300 kg ha^{-1}, combined with rhizobacteria from gliricidia or PGPR tended to result in the lower content of threonine, serine and alanine. Meanwhile, the glycine content was the lowest due to the application of urea fertilizer, both at 200 and 300 kg ha^{-1}, combined with rhizobacteria from gliricidia+PGPR, showing a value of 133.76 and 88.37 ppm, consecutively. Among all of amino acids analyzed in this study, only tryptophan was undetected due to its extremely low content (Table 4).

Provitamin A content: High provitamin A (ranging from 7.41-8.04 ppm) was obtained in the combination of urea 200-300 kg ha^{-1}, either with PGPR or Mycorrhiza. In contrast, the combination of urea with indigenous rhizobacteria from bamboo or gliricidia resulted in the lowest provitamin A content, ranging from 4.48-4.88 ppm (Fig. 1).

Provitamin E content: High provitamin E, was also obtained from combination of 200-300 kg ha^{-1} urea, either with PGPR or Mycorrhiza. Meanwhile, the lowest provitamin E content was resulted by the application of 200 kg ha^{-1} urea combined with indigenous rhizobacteria from bamboo and PGPR, showing a value of 27.81 ppm and the application of 300 kg ha^{-1} urea combined with rhizobacteria from bamboo or gliricidia, showing a value of 27.66 and 28.63 ppm, respectively (Fig. 2).

Vitamin C content: High vitamin C was also obtained from the application of 300 kg ha^{-1} urea combined with either indigenous rhizobacteria from gliricidia+Mycorrhiza (23.42 ppm) or PGPR (23.82 ppm). Meanwhile, the lowest vitamin C content was found at the application of 400 kg ha^{-1} urea, showing a value of 11.66 ppm (Fig. 3).

DISCUSSION

The application of urea fertilizer at 200 and 300 kg ha^{-1} combined with indigenous rhizobacteria from gliricidia resulted in the highest percentage of root microbial infection. Meanwhile, the combination of urea, both at 200 and 300 kg ha^{-1}, with PGPR and Mycorrhiza resulted in the lower percentage of infection. At the same time, the most moderate was the application of urea combined with indigenous rhizobacteria from bamboo or PGPR (Table 1). All treatment combinations of urea fertilizer dosages and the sources of indigenous rhizobacteria with PGPR or Mycorrhiza resulted in higher bacterial infection compared to the control treatment. This result is consistent with Reyes-Tena *et al.*[20], who reported that the combination of arbuscular mycorrhizal fungi and Actinomycetes increased the percentage of *Phytophthora capsici* root infection and Gao *et al.*[21], who reported that the combination of biofertilizers and organic fertilizers increased the activity and the number of microbes. The highest value of leaf fresh weight was obtained from the combination of urea fertilizer with PGPR and Mycorrhiza. Conversely, the combination of urea, indigenous rhizobacteria and PGPR resulted in the lower leaf fresh weight and the combination of urea fertilizer and indigenous rhizobacteria resulted in the lowest leaf fresh weight. The application of urea fertilizer at all dosages combined with the sources of indigenous rhizobacteria, with PGPR or Mycorrhiza, produced higher leaf fresh weights compared to the control treatment. This result is in line with El-Azab and El-Deviny[22], stating that the application of nitrogen minerals combined with *Azotobacter* microbes could increase growth, yield and quality of corn, while Aziez *et al.*[23] reported that the application of Mycorrhiza could increase the growth of rainfed lowland rice plants. Based on the report of Doan and Cao[24], the combination of chemical fertilizers and biofertilizer increased the weight of *Angelica dahurica* tubers. Pelealu *et al*.[25] also reported that biofertilizer significantly increased the yield of fresh chilies (*Capsicum frutescent*) to 13.61 ton ha^{-1}

The highest value of leaf dry weight was obtained from the combination of urea fertilizer with PGPR and Mycorrhiza. On the contrary, the combination of urea fertilizer, indigenous rhizobacteria and PGPR or Mycorrhiza produced lower leaf dry weight and the combination of urea fertilizer and indigenous rhizobacteria from bamboo results the lowest leaf dry weight. The application of urea fertilizer at all dosages combined with the sources of indigenous rhizobacteria, with PGPR or Mycorrhiza, resulted in the higher dry leaf weight compared to the control treatment. This result is in line with Singh[26], who reported that the combination of RDF N fertilizer and *Azospirillum* PGPR increased the number of pods and yields of fenugreek (*Trigonella foeningraecum* L.). Another research reporting the same result is the study of Nalawde and Bhalerao[27], stating that adding *Rhizobium* sp. and *Azotobacter* sp. could increase the growth of *Vigna mungo* L. Hepper.

The highest percentage of antioxidant activity was resulted by the application of 200 and 300 kg ha^{-1} urea fertilizer combined with PGPR or with mycorrhiza. Meanwhile, a combination of urea fertilizer at 200-300 kg ha^{-1}, indigenous rhizobacteria and PGPR or mycorrhiza produced lower antioxidant activity and the application of only 200 kg ha^{-1} urea fertilizer combined with indigenous rhizobacteria from bamboo resulted in the most moderate antioxidant activity. The application of urea fertilizer at all dosages combined with the sources of indigenous rhizobacteria, with PGPR or Mycorrhiza, resulted in the higher antioxidant activity compared to the control treatment. This result is not in accordance with Ordookhani *et al*.[28], who reported that the application of PGPR and Arbuscular Mycorrhiza Fungi (AMF) could increase lycopene and antioxidant activity in tomatoes, while Kuan *et al*.[29] reported that the application of mineral fertilizers and biofertilizer *Trichoderma* sp. could increase the antioxidant content in tomatoes.

There were 17 types of amino acids obtained in this study and 7 of them were essential amino acids. This result is not in accordance with the report by Pugazhendi and Sekar[30], mentioning that *Aloe vera* leaves contain 22 types of amino acids and 8 types of which are essential amino acids. Heng *et al*.[31] also reported that *Aloe vera* leaves contain 20 amino acids and 7 of them are essential amino acids.

The combination of various dosages of urea fertilizer and biofertilizer sources resulted in various concentrations of the amino acids types contained in *Aloe vera* leaves. The highest concentration of amino acid type of glutamic acid was obtained in the application of urea fertilizer at 200 kg ha^{-1} combined with rhizobacteria from bamboo or with mycorrhiza, while the lowest was observed in the application of a combination of urea fertilizer at 200 kg ha^{-1} combined with indigenous rhizobacteria from gliricidia. All treatment combinations resulted in undetectable content of tryptophan (Table 4). This result is not in accordance with the report of Ekinci *et al*.[32], mentioning that the application of Plant Growth Promoting Rhizobacteria increased nutrient, organic acid, amino acid and hormone content of cauliflower (*Brassica oleracea* L.). Likewise, Hussain *et al*.[33] reported that *Rhizobacteria*, *Bacillus* sp. AZ 6 (PGPR) was able to provide tryptophan amino acid of 35.30 ppm, while Dabbghi *et al*.[34] reported that giving biofertilizer foliar fertilizers increased phytohormones and amino acids of leaves and roots of *Olea europaea* plant. Ahmad *et al*.[35] also reported that the application of *Bacillus* strains increased the protein content in mung bean and corn seeds.

The highest content of provitamin A was observed in the application of urea fertilizer at 200-300 kg ha^{-1} combined with PGPR or with Mycorrhiza. Meanwhile, a lower content of provitamin A was resulted by the combination of urea fertilizer, indigenous rhizobacteria from gliricidia, PGPR or Mycorrhiza and the lowest content was observed in the application of urea fertilizer combined with indigenous rhizobacteria. The application of urea fertilizer at all dosages combined with the sources of indigenous rhizobacteria, with PGPR or Mycorrhiza, resulted in the higher provitamin A content compared to the control treatment. This result is not following the research by Shaymaa *et al*.[36], reporting that the use of organic fertilizers and biofertilizers could increase the carotenoid content of garlic bulbs. In contrast, Kuan *et al*.[29] reported that the application of mineral fertilizers and biofertilizer *Trichoderma* sp. could increase the content of provitamin A (carotenoids) in tomatoes.

The highest provitamin E content was obtained in the application of 200-300 kg ha^{-1} urea fertilizer combined with PGPR or with mycorrhiza. Conversely, the combination of urea fertilizer at a dosage of 200-300 kg ha^{-1}, indigenous rhizobacteria from gliricidia and mycorrhiza resulted in the lower content of provitamin E. Meanwhile, the application of only 300 kg ha^{-1} urea fertilizer combined with indigenous rhizobacteria from bamboo or gliricidia produced the lowest provitamin E content. The application of urea fertilizer at all dosages combined with the sources of indigenous rhizobacteria, with PGPR or Mycorrhiza, resulted in the higher provitamin E content compared to the control treatment. This result is consistent with the report of Shaymaa *et al.*[36], mentioning that the application of PGPR could increase organic compounds as precursors of provitamin E in cauliflower plants (*Brassica oleracea*). Nalawde and Bhalerao[27] also reported that the application of *Rhizobium* sp. and *Azotobacter* sp. improved the quality (carbohydrate, protein and fat content) of *Vigna mungo* L. Hepper.

The highest content of vitamin C in *Aloe vera* leaves was resulted by the application of of 300 kg ha^{-1} urea fertilizer combined with PGPR or with Mycorrhiza. Meanwhile, the combination of urea fertilizer, indigenous rhizobacteria, PGPR or Mycorrhiza obtained a lower vitamin C content and the combination of urea fertilizer and indigenous rhizobacteria resulted in the lowest vitamin C content. The application of urea fertilizer at all dosages combined with the sources of indigenous rhizobacteria, with PGPR or Mycorrhiza, resulted in the higher vitamin C content compared to the control treatment. This result is not in line with the report of Nalawde and Bhalerao[27], stating that the application of PGPR and Mycorrhiza could increase the vitamin C content in tomatoes. Khan *et al.*[37] also reported that the application of mineral fertilizers and biofertilizer *Trichoderma* sp. could increase the content of vitamin C (ascorbic acid) in tomatoes. Likewise, Bona *et al.*[38] reported that the application of mycorrhiza and PGPR could increase nutrition value of tomato and Altnatas[39] reported that the application of organic manure and biofertilizer could increase broccoli ascorbic acid content.

CONCLUSION

Based on the analysis results, the combination of 200-300 kg ha^{-1} urea with mycorrhiza had the best effect on the growth, yield and essential amino acid content of *Aloe vera* leaves. Meanwhile, the highest concentration of antioxidant compounds (provitamin A, provitamin E and vitamin C) was obtained at the combination of 300 kg ha^{-1} of urea with mycorrhiza or PGPR. The addition of rhizobacteria from bamboo gave good results on the valine and threonine content of *Aloe vera* leaves.

SIGNIFICANCE STATEMENT

This study discovered that mycorrhiza could increase the yield and phytochemical content of *Aloe vera* leaves that can be beneficial to use aloe leaves as healthy food. The result showed that bamboo rhizobacteria had the effect as good as mycorrhiza, especially on methionine, valine and threonine. It needs to explore whether bamboo rhizobacteria can improve the phytochemical content of aloe leaves and it will be a cost-effective supplement.

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