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The Influence of Integrated Fertilizer on Physiological and Agronomic Character of *Aloe Vera* L. Plant in Sandy Soil

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ABSTRACT: Abiotic environment usually influence the physiological properties of plants, which consequently affect its growth and yield. The research aims to determine the effect of combined integrated fertilizers on the physiological and agronomic character of *Aloe vera* plants. The experiment was laid out in RCBD, with three replications. Treatments were factorial combinations of cow manure rate (30 and 45 t ha^{-1}) and humic nitrogen of eight levels i.e. urea, AS, NPK fertilizer, KNO_3, humic urea, humic AS, humic NPK, and humic KNO_3. Observed variables include physiological and agronomic component of plants. Data were subjected to ANOVA followed by DMRT at 5% significance level. There were significant interaction effect between manure rates and nitrogen sources upon all variables measured. Highest values of stomata index and density were achieved by the combined effect of manure rate of 30 t ha^{-1} with urea, while manure rate of 45 t ha^{-1} with humic urea resulted the highest value of stomata aperture. Increase in all physiological as well as agronomical characters were achieved by manure of 30 and 45 t ha^{-1} with the addition of humic urea.

KEYWORDS: Ammonium sulphate, chlorophyll, proline, stomata.

Abbreviations: ANOVA- analysis of variance; DMRT - Duncan's Multiple Range Test; KNO_3 – potassium nitrate; AS - ammonium sulphate; RCBD - randomized complete block design; NPK - nitrogen phosphor potassium; NRA - nitrogen reductase activity.

INTRODUCTION

Aloe vera L. is known as miracle plant, a multifunctional plant which belongs to horticulture. Aloe has diverse morphologies and functions. The leaves contain carbohydrates, proteins, fat compounds, 17 essential amino acids, minerals, four different kinds of vitamin and six different kinds of enzyme. Secondary metabolites like alkaloids, aloins, lectins, lignin, saponins, tannins, phenolic and glukomannan are also present in it. It plays an important functional role as an ingredient of phytotherapeutics (Nandal et al., 2012; Rajeswari et al., 2012). Aloe is a medicinally and economically important genus. Many uncommon species of aloe were endangered because of plant destruction and destroyed of natural habitat (Attia and Sodany, 2019).

According to Ciccarelli et al. (2009), plants respond to stress condition by bending leaves, increasing glandular trichome, epidermis, stomata, hydathode, aerenchyma, and water holding parenchyma. In salt stress, there was an increase in osmotic potential, chlorophyll, Na, Ca, and Mg content, but a decrease in protein and phosphorous content was also reported by Abou-Leila (2012). Owing to its range of benefits, cultivation of aloe plant is very important. Unfortunately, arable land in Yogyakarta Special Territory was converted into non-agricultural land, at the rate of approximately 200 hectares per year (Yanti et al., 2016), therefore, the available land is in the form of sandy soil. The characteristics of sandy soil are porous, high soil temperature and high evaporation rate, low water and nutrient content, and high wind speed will directly affects plant structure and cell activity, which eventually affecting plant growth.

Humic acid could affect plant physiology by improving the soil structure, nutrient absorption and influencing its root orientation. Humic acid contains auxin, a plant regulator substance (Trevisan et al., 2010). Prakash et al. (2012) reported that application of 4% humic potassium increased in chlorophyll and steviocide content of *Stevia rebaudiana*, while in *Morus alba*, 4% humic potassium increased total chlorophyll and carbohydrate content and ultimately improved plant growth and yield (Prakash et al., 2013). Futher research has to be conducted to assess the effects of manure and various humic nitrogen on physiological and yield characters of *Aloe vera* L. plant grown in coastal sandy soil, which will affect the growth and yield of plants.

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MATERIALS AND METHODS

A field experiment was carried out during May 2018 to June 2019, in Cangkring village, Bantul Regency, Yogyakarta Special Territory, Indonesia. The area is 15 meters above sea level, has sandy type of soil, and 100% sun light. The temperature of site was 30-36^oC with annual rainfall of 1625 mm year^{-1}.

It was a factorial experiment, laid out in Randomized Complete Block Design, with three replications. The first factor was cow manure rate of 30 and 45 t ha^{-1} and the second factor was nitrogen source i.e. urea, ammonium sulphate, NPK fertilizer, potassium nitrate (KNO_3), and with addition of humic substance to each nitrogen source.

Seedlings were prepared in polybags before transplanted in the field to conduct the experiments. Soil tillage was conducted using hoe, then 48 plots and planting holes were made. Cow manure was applied in each plot as basic fertilizer, equivalent to manure rate of the treatment. Manure was spread along the row, one week before transplanting.

After two months grown in polybags, seedlings were transplanted in the field. One third of nitrogen source was applied one month later, while the second and third nitrogen application was done after two to three months. Watering was done every afternoon, using plastic hoses. Weed control was done manually by removing the weeds grew around the plants and the whole field experiment as needed. The crop was harvested after 12 months. Data collected were fresh weight and dry weight of leaves, physiological characters namely density, index, and aperture of stomata, chlorophyll a and b content, Nitrogen Reductase Activity, and proline content. Stomata determination was done using transparant nail polish and then observed under microscope. Chlorophyll content was determined by spectrophotometric characters (Wintermans and De Mots, 1965), while Bates et al. (1973) used rapid determination to obtain proline content. Analysis of variance (ANOVA) were used to analyze all data and followed with Duncan's Multiple Range Test at 5% level of significance for means comparison (SAS, 1988).

RESULTS AND DISCUSSION

There were significant interaction effects between manure rate and humic nitrogen sources on all variables observed.

Density, Index, and Aperture of Stomata

The highest stomata density was obtained at 30 t ha^{-1} manure combined with urea, while lower values was obtained when combined with KNO_3 (Table 1). High values of Stomata Index was obtained by the combination 30 t ha^{-1} manure with urea or AS, whereas highest values of stomata aperture was obtained when 45 t ha^{-1} manure combined with humic urea. The result revealed that combination of manure with humic nitrogen gave lower values of stomata conductivity which was the opposite to Abbas *et al*. (2012) work, that stomata conductivity increased by combination of manure with humic acid at the rate of 40 ml^{-1} per plant on kinnow mandarin. However, Kahraman (2017) reported that application of 70 kg ha^{-1} humic acid could increase stomata conductivity of string bean.

Chlorophyll a and b content and Nitrogen Reductase Activity

Highest values of chlorophyll a content was achieved when 30 t ha^{-1} manure combined with humic NPK, on the other hand lower values obtained when 45 t ha^{-1} manure combined with humic urea (Table 2). Sindu et al. (2014) reported that the application of farmyard manure increase the quality of glycoside content of *Indigofera tinctoria*. Chlorophyll a content lowered when there is no addition of humic acid. This showed that humic acid play important role in nutrient absorption by plant root. This result showed great resemblance with Prakash et al. (2012) and Prakash et al. (2013) who stated that chlorophyll content of *Stevia rebaudiana* and *Morus alba* increased when 7% humic acid was applied. Kandil et al. (2017) also reported the same result by the addition of 5% humic acid to wheat.

High values of chlorophyll b was obtained by the application of 30 and 45 t ha^{-1} manure combined with humic urea and humic NPK, while lowest values was obtained if combined with ammonium sulphate (Table 2). Increase in chlorophyll b content was observed by the addition of humic acid and highest values was obtained when applied as NPK and urea. This showed that humic acid could improve root's nutrient absorption. This result was in line with Ameri and Tehraniar (2014), who reported that addition of 20 ppm plant^{-1} humic acid to *Fragaria ananassa* increased its chlorophyll content. Application of 1 ppm putrescine and 1 ppm humic acid to cotton increased in chlorophyll b content (Hernandes et al., 2014).

The highest NRA was found at the application of 45 t ha^{-1} manure combined with humic NPK, whereas the lowest values obtained when application of manure combined with ammonium sulphate. In general, higher values was achieved when humic acid applied. Boogar et al. (2014) reported that addition of humic acid to *Petunia hybrida* at the rate of 400 ppm plant^{-1} increased its micronutrient absorption that consequently increased enzymes synthesis in it, whereas Hernandez et al. (2014) found that humic acid of 1.5% increased nitrate reductase in caisim. Shahein et al. (2015) also reported that humic acid increase nitrogenase enzyme in caisim cultivar, and Danyaei et al. (2017) reported that humic acid of 25 kg ha^{-1} increased micronutrient absorption in Olive.

Proline Content, Fresh Weight of Leaf and Dry Weight of Leaf

Highest values of proline content was obtained when 30 t ha^{-1} manure combined with ammonium sulphate, and lower values achieved when combined with any other nitrogen sources (Table 3). Proline content decrease by the addition of humic nitrogen. Lowest values obtained when 45 t ha^{-1} manure with addition humic NPK, humic KNO_3, or humic ammonium sulphate. This showed that sandy soil stress can be reduced by the addition of humic substances. Aydin et al. (2012) found that application of 1% humic acid decreased proline content of string bean (*Phaseolus vulgaris* L.) leaves, and 1500 ppm humic acid also resulted in reduction of proline content of linseed (Neware and Bobade, 2018). Furthermore, it was supported by Kandil et al. (2017) that addition of 5% humic acid decreased proline content of wheat leaves.

Highest values of leaf fresh and dry weight was achieved when manure rate of 30 and 45 t ha^{-1} applied with humic urea, but lower values obtained when manure applied with nitrogen source such as urea, AS, NPK fertilizer and KNO_3 (Table 3). Different from the report of Sukartono et al. (2011) that the highest maize yield in the rainy season was noted for cattle manure, while Subaedah et al. (2019) reported that application of organic fertilizer 20 t ha^{-1} increase growth and yield of soybean. Lower values with no significant difference of leaf fresh weight was obtained by manure combination with humic AS, humic NPK, and humic KNO_3. This indicated that addition of humic acid gave better effects, such as better growth was obtained by the application of manure combined with humic nitrogen. Danyaei et al. (2017) found that application of 25 kg ha^{-1} humic acid increased Olive fruit weight, while Khan et al. (2017) stated that application of 300 L humic compound could increase potato yield. This findings were also supported by Neware and Bobade (2018), that 300 – 500 ppm humic acid increased yield of Linseed. Different from the report by Li et al. (2019) that rapeseed sowing method with nitrogen 180 kg ha^{-1} was proved to be more effective.

CONCLUSIONS

It is concluded that all physiological character and yield variables of *Aloe vera* were significantly affected by the interaction effect of manure rate and nitrogen sources, 30 t ha^{-1} manure combine with urea fertilizer gave highest value of stomata density and index, while humic urea when combined with 45 t ha^{-1} manure gave highest value of stomatal aperture. The highest value of chlorophyll a content was obtained by the combination of humic NPK fertilizer with manure dosage of 30 t ha^{-1}, as well as chlorophyll b content, while the highest nitrogen reductase activity was obtained by a combination of humic NPK fertilizer with manure rate of 45 t ha^{-1}. The combination of ammonium sulphate fertilizer with 30 t ha^{-1} manure, increased the proline content, while the highest fresh and dry weight of leaves was obtained at the application of humic urea with manure rate 30-45 t ha^{-1}.

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TABLES

Table 1. Density, Index, and Aperture of Stomata

Humic nitrogen source	Stomata Density		Stomata Index (%)		Stomata Aperture (mμ)	
	Manure Rate		Manure Rate		Manure Rate	
	30 t ha^{-1}	45 t ha^{-1}	30 t ha^{-1}	45 t ha^{-1}	30 t ha^{-1}	45 t ha^{-1}
Urea	47,51 a	31,67 c	0,115 a	0,080 cd	261,41 ef	375,40 b
AS	31,67 c	31,67 c	0,122 a	0,093 b	347,80 d	344,47 c
NPK	31,67 c	15,84 d	0,064 f	0,046 g	288,83 ef	347,70 c
KNO_3	36,95 b	31,67 c	0,077 cd	0,061 f	244,66 f	359,86 c
Urea +H	31,67 c	15,84 d	0,074 de	0,044 f	360,06 c	467,45 a
AS +H	31,67 c	31,67 c	0,069 ef	0,078 cd	302,94 ef	373,88 b
NPK +H	31,67 c	31,67 c	0,085 bc	0,085 de	350,83 cd	379,29 b
KNO_3+H	31,67 c	31,67 c	0,057 f	0,068 ef	334,41 de	373,37 b
Interaction	($P \leq 0.05$)		($P \leq 0.05$)		($P \leq 0.05$)	

Note: means followed by same letter in the same column are not significantly different based on Duncan's Multiple Range Test at 5% level of significant.

Table 2. Chlorophyll a and b Content**,** and Nitrogen Reductase Activity

Humic nitrogen source	Chlorophyl a (%)		Chlorophyl b (%)		NRA (%)	
	Manure Rate		Manure Rate		Manure Rate	
	30 t ha^{-1}	45 t ha^{-1}	30 t ha^{-1}	45 t ha^{-1}	30 t ha^{-1}	45 t ha^{-1}
Urea	0,101 c	0,089 e	0,057 de	0,059d	2,41 d	2,82 cd
AS	0,109 c	0,095 de	0,052 f	0,052f	2,14 e	2,16 e
NPK	0,085 e	0,095 de	0,056 e	0,055 e	3,32 c	2,44 d
KNO_3	0,095 de	0,095 de	0,057 de	0,058 de	2,77 d	2,54 d
Urea +H	0,124 b	0,108 bc	0,074 ab	0,072 b	3,42 c	3,95 b
AS +H	0,127 b	0,105 c	0,065 c	0,065 c	3,85 b	3,36 c
NPK +H	0,143 a	0,122 b	0,082 a	0,072 b	3,84 b	5,24 a
KNO_3 +H	0,099 c	0,102 c	0,065 c	0,066 c	3,90 b	3,61 c
Interaction	($P \leq 0.05$)		($P \leq 0.05$)		($P \leq 0.05$)	

Note: means followed by same letter in the same column are not significantly different based on Duncan's Multiple Range Test at 5% level of significant.

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Table 3. Proline Content, Fresh Weight of Leaf and Dry Weight of Leaf

Humic nitrogen source	Proline Content (%)		Fresh Weight of Leaf (g)		Dry Weight Leaf (g)	
	Manure Rate		Manure Rate		Manure Rate	
	30 t ha^{-1}	45 t ha^{-1}	30 t ha^{-1}	45 t ha^{-1}	30 t ha^{-1}	45 t ha^{-1}
Urea	4,60 d	4,46 d	336,44 b	331,12 b	42.47 c	43.29 c
AS	9,92 a	3,83 e	286,16 c	292,32c	44.82 c	44.97 c
NPK	8,01 b	3,81 e	296,66 c	286,14 c	45.44 d	42.18 d
KNO_3	5,33 c	3,12 e	218,84 f	232,24 e	37.16 e	34.97 e
Urea +H	2,15 fg	2,67 f	426,44 a	438,66 a	66.07 a	62.19 a
AS +H	2,03 g	1,61 h	328,24 b	302,36 b	52.52 b	58.38 b
NPK +H	2,87 f	1,07 h	304,22 b	326,44 b	58.67 b	58.97 b
KNO_3+H	2,37 g	0,99 h	322,66 b	328,27 b	57.23 b	47.72 bc
Interaction	($P \leq 0.05$)		($P \leq 0.05$)		($P \leq 0.05$)	

Note: means followed by same letter in the same column are not significantly different based on Duncan's Multiple Range Test at 5% level of significant.

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