



## Effect of *Azotobacter* sp. and Cow Manure on Nitrogen Availability in Saline Soil, Root Length, and Vitamin C Content of Tomato

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### ABSTRACT

*Nitrogen is a macronutrient for plants, but its availability in saline soil is a limiting factor, making it difficult to cultivate plants. This study aims to examine the effect of the combination of *Azotobacter* sp. and cow manure in increasing the available N of saline soil, and to obtain the best combination in increasing the growth of tomato plants in saline soil. This research was conducted in the greenhouse and laboratory of UPN "Veteran" Jawa Timur. Saline soil samples came from the Wonorejo Mangrove land, Surabaya. *Azotobacter* sp. isolates came from the roots of Wonorejo mangrove trees. The study used a Completely Randomized Design with 2 factors with 3 replications. First factor was addition of *Azotobacter* sp., consisted of A0 (no addition of *Azotobacter*), and A1 (addition *Azotobacter* at  $10^7$  CFU/mL). Second factor was cow manure (K) involved 4 levels (in ton/ha): A0 (0); K1 (20); K2 (30); K3 (40). The results of this research showed that application of *Azotobacter* sp. and cow manure affected the parameters of available N, EC, pH and vitamin C content, but did not affect the parameters of plant root length. Combination of *Azotobacter*  $10^7$  CFU/mL + 40 tons/ha of cow manure produced the best available N of 246.48 ppm. The highest vitamin C content was 36.75 mg/g in the treatment of  $10^7$  CFU/mL *Azotobacter* + 30 ton/ha of cow manure. Cow manure decreased soil EC and increased soil pH.*

### 1. INTRODUCTION

Salinity is one of a problem and threat to agriculture in almost every country, including Indonesia. As an archipelagic state, Indonesia's saline land area is estimated at around 0.6 million hectares (Maspanti *et al.*, 2022). Soil salinity can be caused by the high input of salt-containing water (Rachman *et al.*, 2018). The presence of dissolved salt content in saline soil means that saline land has not been used optimally due to nutrient deficiencies, especially nitrogen.

Nitrogen is one of the macronutrients for plants, but its availability in saline soil is a limiting factor, making it difficult to cultivate horticultural crops, including tomatoes. Tomatoes (*Solanum lycopersicum*) are a versatile crop and a source of essential vitamin C. Tomato plants have a moderate level of tolerance under saline conditions. Tomato plants have a moderate level of tolerance to saline conditions. The solution to this problem is to use soil microorganisms and organic matter applied to saline soil.

Soil microorganisms that have the ability to adapt to saline soils can be utilized for agricultural production. One type of soil microorganism is *Azotobacter* sp., a non-symbiotic nitrogen-fixing bacterium that can live freely in the rhizosphere (Widawati, 2015). *Azotobacter* has the ability to fixing nitrogen from the air freely into nitrogen available to plants (Angraeni *et al.*, 2023). As an organic material, cow manure that applied to the soil can function as a nutrient source for microorganisms, especially *Azotobacter*, and increase bacterial activity (Perdana, 2022). Organic matter also plays a dual role in improving saline soil. Manure application of 10 tons/ha has been shown to reduce the soil EC value from 4.1 dS/m to 2 dS/m (Tolib *et al.*, 2017).

Palupi (2015) reported that the application of organic fertilizer in the form of vegetable waste with microorganisms increased the total N content of the soil by 2.13% compared to the control which was only 1.63. The application of poultry manure fertilizer with *Azotobacter* increased the total N content to 26.2 g/kg better than the control which was 21.3 g/kg (Rodrigues *et al.*, 2018). *Azotobacter* can work effectively on soil that is given organic materials such as fertilizer.

Although *Azotobacter* sp. is known to increase nitrogen availability, little research has examined its effectiveness in combination with cow manure on saline soils and its effects on the physiological quality of tomatoes. This study aims to examine the effect of *Azotobacter* combined with cow manure on the availability of nitrogen in saline soil and the growth of tomato plants. Results of this study is expected to help optimize the role of soil microorganisms and improve the fertility of saline land by increasing soil nitrogen availability.

## 2. MATERIALS AND METHODS

### 2.1. Location

This research was located in Surabaya, specifically at the Wonorejo Mangrove, to collect soil samples. Planting, *Azotobacter* isolation, and soil analysis were conducted in the greenhouse and laboratory of the Universitas Pembangunan "Nasional" Jawa Timur. The research began in January 2024 and ended in May 2024.

### 2.2. Materials

The equipment used was a hoe to take soil samples, 35 cm x 35 cm polybags and a shovel for planting, a microscope, a petri dish and a test tube for *Azotobacter* isolation, as well as a digital scale, an EC meter, a pH meter, and a destructive tube for soil analysis. The materials used were saline soil samples from the Wonorejo Mangrove land, cow manure, tomato seeds of the servo variety, liquid glucose media (for *Azotobacter* growth) and aquades.

### 2.3. Design of Experimental

The design used was a factorial Completely Randomized Design involving 2 factors: *Azotobacter* and cow manure. The first factor *Azotobacter* consisted of 2 levels, namely A0 (no *Azotobacter*), and A1 (*Azotobacter*  $10^7$  CFU/mL). The second factor cow manure, consisted 4 levels: K0 (without cow manure), K1 (20 tons/ha), K2 (30 tons/ha), and K3 (40 tons/ha). The 8 treatment combinations were repeated 3 times so that the total number of experimental units obtained was 24 units.

The data results were analyzed using analysis of variance (ANOVA) and continued with an F test with a 5% error rate. Next, an Honestly Significant Difference Test (HSD) was conducted at the 5% level to determine the differences in the effects of each treatment.

### 2.4. Soil Sampling

This research began with a preliminary analysis to determine the initial characteristics of the soil before treatment, including soil EC, soil pH, and soil available nitrogen. The next step is collecting soil from the topsoil of the mangrove area to a depth of 0–20 cm using a hoe placed in a sack. The soil samples were then air-dried for approximately 7 days until no moisture remained. Afterward, the soil was loosened and sieved to achieve a homogeneous size. The dried soil was weighed at 10 kg, equivalent to BTKO, and placed into polybags.

### 2.5. *Azotobacter* Isolation

The soil sample used for *Azotobacter* isolation was taken from the roots of mangrove plants. A total of 1 g of soil was weighed and then a series of dilution solutions were made from  $10^{-2}$  CFU/mL to  $10^{-7}$  CFU/mL. The diluted suspension is then poured onto the LG medium. After the bacteria have grown on the LG medium, they are purified on fresh medium and incubated for 2–3 days. This is followed by identification of the bacteria based on their morphological characteristics and Gram staining.

## 2.6. Planting and Treatment

Planting began with preparing cow manure and *Azotobacter* liquid inoculant. The soil in polybags was filled with cow manure according to the dosage for each treatment. The *Azotobacter* isolate was dissolved with aquades and vortexed until dissolved, and the inoculum was ready for use. Inoculation was carried out the day before planting. Inoculation was done by pouring the appropriate dosage into each polybag. Afterward, the tomato seeds were planted to a depth of approximately 2 cm. The seeds were the Servo variety, and was observed for 60 days after planting (DAP).

## 2.7. Plant Maintenance

Plants were watered regularly in the afternoon to prevent evaporation during the day, especially during the early stages of plant growth, and adjusted to the soil conditions in the polybags. Watering was carried out sufficiently until the soil was moist in all treatments.

## 2.8. Observation Parameters

There were two types of observations observed, namely soil and plant characteristics. Soil analysis was carried out at four time intervals of 60 days every 15 days so that there were four observations. Soil analysis included soil EC using an EC meter, soil pH using a pH meter, and soil available N using the destructive distillation method. Plant analysis observed included root length and vitamin C content of tomatoes. The length of tomato plant roots was observed after harvest using a ruler or meter with cm units. The vitamin C content test of tomatoes used a spectrophotometer method.

## 3. RESULTS AND DISCUSSION

### 3.1. Initial Soil Characteristics

The initial analysis intend to determine the initial properties of the soil before conducting research and treatment. The initial soil characteristics before treatment are presented in Table 1 below.

Table 1. Initial soil characteristics based on laboratory test results

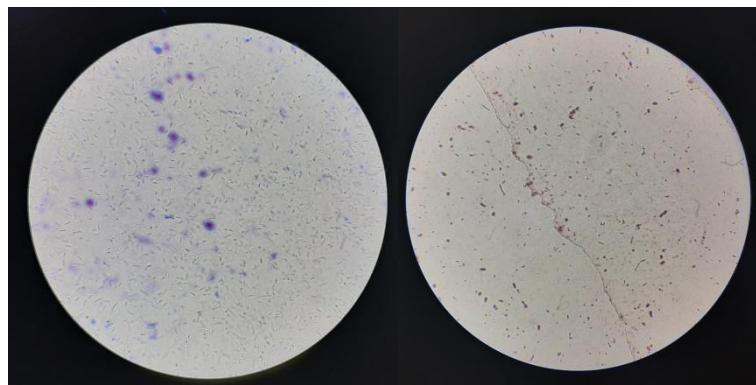
Parameter	Unit	Result	Category (*)
EC Tanah	dS m <sup>-1</sup>	7.58	Very high
pH H <sub>2</sub> O	-	7.75	Slightly alkaline
pH KCl	-	7.20	Neutral
N-NH <sub>4</sub> <sup>+</sup>	Ppm	85.95	-
N-NO <sub>3</sub> <sup>-</sup>	Ppm	49.53	-

Note (\*) : The criteria are based on (BPSITP, 2023)

Laboratory test results showed that the EC of the mangrove soil used in this study was 7.58 dS/m, categorized as very high. Saline soil is formed due to the presence of free salt levels dissolved by groundwater and exchangeable ions on the surface of soil particles (Suud *et al.*, 2015). The soil pH of H<sub>2</sub>O is at 7.78 categorized as slightly alkaline, while the pH of KCl at 7.20 categorized as neutral. The high of EC and pH indicate that the soil is categorized as saline soil. The levels of ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) in mangrove soil showed 85.95 ppm and 49.53 ppm. Despite the high availability of ammonium and nitrate in the soil, the salt content in saline soil can affect the absorption of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> by plants, resulting in nitrogen deficiency (Tarigan *et al.*, 2024).

### 3.2. *Azotobacter* Morphology

The purification and Gram staining results of the *Azotobacter* isolate are shown in Figure 1. The *Azotobacter* bacteria explored from the Wonorejo mangrove roots were microscopically rod-shaped with rounded ends and red in color. This bacterium is classified as gram-negative. This isolation result is consistent with the *Azotobacter* identified by Maatoke *et al.* (2024) which is rod-shaped and has a red color.

Figure 1. Microscopic of *Azotobacter* sp Isolate.

### 3.3. Available Nitrogen

Nitrogen is an essential macro nutrient that is needed by plants in large quantities during the vegetative period. Plants can absorb nitrogen from the soil in the form of ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ) (Khotimah *et al.*, 2020). The combination of *Azotobacter* sp. and cow manure had a very significant impact on  $\text{NH}_4^+$  of soil levels at 30 days after planting, as shown in Table 2. The decrease at  $\text{NH}_4^+$  in the soil at 45 and 60 days after planting (DAP) is suspected according to Patti *et al.* (2018), to be due to the nitrification process and plant uptake. The highest  $\text{NH}_4^+$  content was found in the A1K3 treatment (*Azotobacter*  $10^7$  CFU/mL + 40 tons/ha cow manure) at 182.03 ppm, while the other treatments showed no significant difference from the control (A0K0). Gan *et al.* (2020) stated that organic matter contains a lot of nitrogen, and rapid nitrogen release due to the presence of microorganisms. This statement indicates that the rapid decomposition of cow manure is due to the activity of *Azotobacter* sp..

Table 2. Average of  $\text{NH}_4^+$  Levels on Combination of *Azotobacter* sp. and Cow Manure

Treatment	NH <sub>4</sub> <sup>+</sup> (ppm)			
	15 DAP	30 DAP	45 DAP	60 DAP
A0K0	71.97	92.78 a	97.33	48.93
A0K1	146.37	168.53 ab	129.65	54.96
A0K2	116.58	151.97 ab	125.31	80.74
A0K3	77.93	101.59 ab	156.77	94.74
A1K0	110.10	124.27 ab	95.81	125.05
A1K1	115.41	112.67 ab	138.64	83.98
A1K2	114.69	169.55 ab	84.97	116.23
A1K3	152.46	182.02 b	164.44	122.79
HSD 5%	ns	82.92**	ns	ns

Note: Numbers followed by the same letter in the same column indicate no significant difference in the 5% HSD test. ns = no significant, \*significant, \*\*very significant.

The combination of *Azotobacter* sp. and cow manure had no significant impact on increasing  $\text{NO}_3^-$  at all time intervals. The single factor of *Azotobacter* sp. and cow manure significantly influenced the increase in  $\text{NO}_3^-$ . The availability of inorganic nitrogen in the form of nitrate is partly due to the results of microbial activity in the mineralization process. Table 3 shows the single effect of cow manure on increasing soil  $\text{NO}_3^-$  levels at 30 days after planting. The K1 treatment (20 tons/ha) produced the highest  $\text{NO}_3^-$  of 72.81 ppm and was followed by the K3 treatment (40 tons  $\text{ha}^{-1}$ ) which produced levels of 60.97 ppm. Increased nitrate was also found in the study Nurmi *et al.* (2024) by providing manure, the nitrate content increased to 0.89 ppm compared to the control of only 0.42 ppm. The availability of nitrogen in the form of nitrate is the result of microbial activity in the mineralization process. (Meiliyansari *et al.*, 2023).

The results showed that the release of nitrogen available to plants increased from 15 days after planting and reached a maximum at 30 days after planting, then decreased until 60 days after planting. Parjono (2019) said that the

properties of  $\text{NH}_4^+$  can be quickly lost and changed, therefore at 45 DAP until 60 DAP the availability of nitrogen begins to decrease because it has been used by the plants.

Table 3. The Single Effect of *Azotobacter* sp. and Cow Manure on Average of  $\text{NO}_3^-$ 

Treatment	NO <sub>3</sub> <sup>-</sup> (ppm)			
	15 DAP	30 DAP	45 DAP	60 DAP
<i>Azotobacter</i> sp.				
A0 (0 CFU/ml)	45.43	53.85	54.91	105.11
A1 ( $10^7$ CFU/ml)	41.69	65.57	61.84	122.49
HSD 5%	ns	ns	ns	ns
Cow Manure				
K0 (0 ton/ha)	37.80	46.80 a	56.13	117.56
K1 (20 tons/ha)	56.05	72.81 b	49.61	102.55
K2 (30 tons/ha)	33.06	58.26 ab	65.45	117.08
K3 (40 tons/ha)	47.32	60.97 ab	62.31	118.01
HSD 5%	ns	23.50*	ns	ns

Note: Numbers followed by the same letter in the same column indicate no significant difference in the 5% HSD test; ns = no significant, \*significant, \*\*very significant.

There was a significant interaction between *Azotobacter* sp and manure on the total available nitrogen of saline soil ( $\text{NH}_4^+ + \text{NO}_3^-$ ) at 30 DAP. Table 4 shows that the best treatment that can increase the average total available N content at 15 DAP is A1K3 (*Azotobacter*  $10^7$  CFU/mL + 40 tons/ha tons cow manure) which reached 246.48 ppm. The results obtained are in line with research by [Ichwan \*et al.\* \(2022\)](#), which stated that the application of plant growth promoting rhizobacteria (PGPR) with chicken manure fertilizer had a positive effect on increasing soil nitrogen content. The average of  $\text{NO}_3^-$  content increased with each time interval, with the highest content occurring 60 days after planting tomatoes. This is thought to be because the availability of  $\text{NO}_3^-$  is influenced by the availability of  $\text{NH}_4^+$ , which then undergoes nitrification to  $\text{NO}_3^-$ , thus increasing the availability of  $\text{NO}_3^-$  ([Damanik \*et al.\*, 2014](#)).

Table 4. Average Available Total of N Levels on Combination of *Azotobacter* sp. and Cow Manure

Treatments	Available Total of Nitrogen $\text{NH}_4^+ + \text{NO}_3^-$ (ppm)			
	15 DAP	30 DAP	45 DAP	60 DAP
A0K0	107.12	130.65 a	152.34	152.66
A0K1	204.55	232.27 bc	166.66	149.70
A0K2	152.19	208.27 bc	194.20	195.17
A0K3	151.92	159.07 ab	215.50	202.28
A1K0	150.55	179.99 abc	195.88	215.36
A1K1	169.33	194.44 abc	158.02	235.41
A1K2	145.21	229.77 bc	166.52	235.97
A1K3	194.34	246.48 c	230.33	251.26
HSD 5%	ns	76.16**	Ns	Ns

Note: Numbers followed by the same letter in the same column indicate no significant difference in the 5% HSD test; ns = no significant, \*significant, \*\*very significant.

The increase in available N in the soil was caused by the application of cow manure as a nitrogen contributor to the soil. The addition of organic matter allows soil microbes that decompose organic matter to mineralize the cow manure and produce  $\text{NH}_4^+$  and  $\text{NO}_3^-$  with the result increasing the total N content of the soil ([Indriani \*et al.\*, 2019](#)).

### 3.4. Soil pH

The combination of *Azotobacter* sp. and cow manure had no significant effect on soil pH from 15 to 60 days after planting. However, further testing of the HSD 5% with a single application of 5% cow manure significantly affected soil pH at 60 days after planting, but no significant difference was observed between 15 and 45 days after planting. This is because the soil is still adjusting its pH after being treated with *Azotobacter* sp. and cow manure. The average soil pH value at 60 days after planting is shown in Table 5. The applied dose of 40 tons/ha (K3) of cow manure significantly increased the soil pH reaching 7.94.

Table 5. The single effect of *Azotobacter* sp. and cow manure on average of pH

Treatment	Soil pH			
	15 DAP	30 DAP	45 DAP	60 DAP
<b><i>Azotobacter</i> sp.</b>				
A0 (0 CFU/ml)	7.73	7.77	7.79	7.83
A1 ( $10^7$ CFU/ml)	7.79	7.80	7.93	7.88
HSD %%	ns	ns	ns	ns
Cow Manure				
K0 (0 ton/ha)	7.73	7.78	7.84	7.75 a
K1 (20 tons/ha)	7.78	7.80	7.87	7.86 ab
K2 (30 tons/ha)	7.70	7.73	7.91	7.88 ab
K3 (40 tons/ha)	7.82	7.84	7.92	7.94 b
HSD 5%	ns	ns	ns	0.18*

Note: Numbers followed by the same letter in the same column indicate no significant difference in the 5% HSD test; ns = no significant, \*significant, \*\*very significant.

The K3 treatment at 60 days after planting resulted in the highest soil pH compared to the other treatments. The increase in soil pH was caused by the release of minerals by organic matter that had undergone further decomposition. Organic matter will increase  $\text{OH}^-$  ions from carboxyl groups (-COOH) and hydroxyl groups ( $\text{OH}^-$ ).  $\text{OH}^-$  ions will neutralize  $\text{H}^+$  ions in the soil so that the soil pH will increase (Salewan *et al.*, 2022). These results are in accordance with the findings of the Nasamsir & Huffia (2020) research that reported applying 170 g of cow manure alone to sugarcane plants can increase pH of soil from 5.77 to 6.99.

### 3.5. Soil EC

The combination of *Azotobacter* sp. and cow manure did not significantly affect on soil EC from 15 to 60 days after planting. Both the single *Azotobacter* sp. and single cow manure of treatments significantly affected soil EC at 15 DAP. Overall, the EC values in Table 6 tended to decrease up to 60 DAP. Soil EC decreased to 4.60 dS/m after applying a dose of 30 tons/ha of cow manure. The results of this study are supported by research conducted by David *et al.* (2021) that the manure application of 15 tons/ha has been proven to have an impact on reducing soil EC from 4.24 dS/m to 3.13 dS/m. Rahmawati & Khumairah (2023) research also reported that providing organic material in the form of straw compost had an impact on reducing soil EC with a percentage reduction of 2.75%. In this study, single application of cow manure can reduce soil EC because cow manure as an organic material can bind dissolved salt ions such as sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ) so that it will reduce its toxicity to plants (Ratna *et al.*, 2024).

Table 6. The single effect of *Azotobacter* sp. and cow manure on average of EC

Treatment	Soil EC (dS/m)			
	15 DAP	30 DAP	45 DAP	60 DAP
<b><i>Azotobacter</i> sp.</b>				
A0 (0 CFU/ml)	5.62 b	4.39	4.03	3.45
A1 ( $10^7$ CFU/ml)	4.89 a	4.40	4.16	3.27
HSD 5%	0.64*	ns	ns	ns
Cow Manure				
K0 (0 ton/ha)	6.20 b	4.90	4.58	3.38 a
K1 (20 ton/ha)	5.51 ab	4.32	3.83	3.53 a
K2 (30 ton/ha)	4.60 a	4.19	4.15	3.57 a
K3 (40 ton/ha)	4.72 a	4.16	3.83	3.83 a
HSD 5%	1.22**	ns	ns	ns

Note: Numbers followed by the same letter in the same column are not significant in the 5% HSD test; ns = no significant, \* = significant, \*\* = very significant.

### 3.6. Root Length

The addition of *Azotobacter* sp. along with cow manure had no significant effect on the root length of tomato plants. This is assumed to be because plant root meristems are sensitive to mineral salts found in saline soil. Low auxin

production can also disrupt optimal root growth (Kusumiyati *et al.*, 2017). However, with the provision of *Azotobacter* sp. and cow manure treatment, there was an increase in root length as shown in Figure 2. Treatment A1K3 (*Azotobacter*  $10^7$  CFU/mL + 40 tons/ha cow manure) tended to produce the highest plant root length, namely 26.83 cm, higher than the treatment of 12.50 cm. This increase in root length was due to *Azotobacter* and cow manure as nitrogen contributors for plants. The nitrogen element forms proteins, nucleic acids and protoplasm which can stimulate vegetative growth such as root growth (Lizawati *et al.*, 2017). So that the presence of *Azotobacter* sp. and cow manure can help plants be more resistant to the impact of salinity.

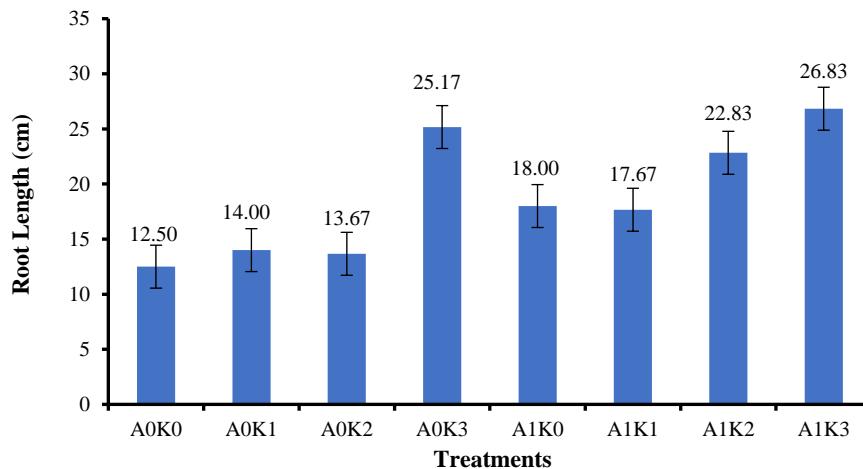


Figure 2. The effect of *Azotobacter* sp and cow manure on root length of tomatoes

### 3.7. Vitamin C Content of Tomatoes

The combination of *Azotobacter* sp. and cow manure showed a significant effect on the vitamin C content of tomatoes in Table 7. Treatment A1K2 (*Azotobacter*  $10^7$  CFU/mL + 30 tons/ha cow manure) gave a result of 36.75 mg, which was the best treatment and significantly different compared to other treatments in increasing the vitamin C content of tomatoes. The A0K0 or control treatment produced the lowest average, namely 19.25 mg. These results are supported by research (Febriyanto *et al.*, 2023) that the addition of 150 g cow manure + 6 mL/L POC has an effect on increasing the vitamin C content of green mustard plants to 138.00 mg/100 ml.

Table 7. Average of Vitamin C content of tomatoes on combination of *Azotobacter* sp. and cow manure

Treatment	Vitamin C Content of Tomatoes (mg/g)
A0K0	19.25 a
A0K1	25.99 b
A0K2	24.69 b
A0K3	23.94 b
A1K0	20.78 a
A1K1	25.03 b
A1K2	36.75 c
A1K3	25.42 b
HSD5%	2.11**

Note: Numbers followed by the same letter in the same column indicate no significant difference in the 5% HSD test; ns = no significant, \*significant, \*\*very significant.

Tomatoes are one of the fruits that contain a lot of vitamin C, besides citrus fruits. Ripe tomatoes contain vitamin C up to 40 mg per 100 g (Byard & Maxwell-Stewart, 2018). Nutrients are very influential in the forming of the vitamin C content of fruit and vegetables, especially the nutrients N and K. Excess of the nutrient N will reduce the vitamin C

content while excess of the nutrient K will increase the vitamin C content (Natanael & Banjarnahor, 2021). Based on this statement, it can be seen that the ideal dose for the vitamin C content of tomatoes in this study was the A1K2 treatment, namely *Azotobacter* 10<sup>7</sup> CFU/ml with 30 tons/ha of manure.

#### 4. CONCLUSION

The combination of *Azotobacter* sp. and cow manure has been shown to increase the available N (NH<sub>4</sub><sup>+</sup> + NO<sub>3</sub><sup>-</sup>) and vitamin C content of tomatoes in saline soil. The A1K3 treatment produced the highest available nitrogen of 246.48 ppm. Application of 30 tons/ha of manure decreased soil EC to 4.60 dS/m, while 40 tons/ha of cow manure increased soil pH by 7.94. The combination of *Azotobacter* sp. and cow manure had no significant effect on tomato root length, but the average root length tended to be better than the control. The A1K2 treatment produced the highest vitamin C content, at 36.75 mg/g. It is recommended that further research increase the fertilizer dose to 30-50 tons/ha or the dose of *Azotobacter* sp.

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