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# The effect of *Azotobacter* inoculation on shallot plants (*Allium cepa*) and availability of phosphate in the saline soil

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**Abstract.** *Widawati S, Suliasih. 2017. The effect of* Azotobacter *inoculation on shallot plants (*Allium cepa*) and availability of phosphate in the saline soil. Biodiversitas 18: 86-94. Azotobacter* is diazotrophic bacteria having character as Plant Growth Promoting Rhizobacteria (PGPR). It provides growth hormones such as IAA, ACC-deaminase, N and P nutrients. The objective of the research was to determine the effect of *Azotobacter* inoculation on the growth and yield of shallot crops and the availability of P in saline soil. The experiment design was a Completely Randomized Design with the factorial pattern. The first factor was the source of water, freshwater and sea water. The second factor was the source of inoculants: (i). No inoculant (control), (ii). NPK fertilizer, (iii). *Azotobacter paspali*, (iv). *Azotobacter chroococcum*, (v). *Azotobacter* sp.1, (vi). *Azotobacter* sp.2, and (vii). Mixed inoculants consisted of *Azotobacter paspali*, *Azotobacter chroococcum*, *Azotobacter* sp.1, and *Azotobacter* sp.2. All treatments were repeated four times. The results showed that mixed inoculation, caused better growth and higher yield of shallot crops compared to single *Azotobacter* in saline soil. Inoculation of *Azotobacter chroococcum* on "Tuk Tuk" cultivar shallot reduced plant sensitivity to salinity up to 4.19 dS/m, improved the growth and shallot bulbs (101.28 g/pot). The *Azotobacter* maintained its population up to $10^6$ cfu/g of soil during PMEase activity at 0.170 µg/mL p-nitrophenol/h and dissolved Phosphate at 0.898 ppm until post-harvest. The highest activity of PMEase and highest availability of P in the soil were during flowering and post-harvest and were obtained from the treatment of mixed *Azotobacter* at a population density of $10^6$ cfu/g of soil.

**Keywords:** *Azotobacter*, shallot, salinity, dissolved P, PMEase

## INTRODUCTION

Salinity caused low productivity along the coastline area of Indonesia. In other situation, the rice fields and open lands are increasingly narrowed in line with the increasing population. Salinity stress will seriously lead environmental degradation in the future due to its negative effect on soil fertility and productivity. Falkenmark (2013) reported that by 2050, the extent of saline soils would continue to get higher and higher, accompanied by declining of water resources.

Salinity is an abiotic stress indicated by the high content of NaCl (3.5%) as the cause of the destruction of soil structure and low aeration and permeability (Candrabarata 2011). Abiotic stress also affects soil productivity for the survival of functional bacteria in the soil and the growth and yield of the plant (Hussain et al. 2008). Saline condition supply a soil with high dissolve ionic compounds ($Na^+$, $Mg_2^+$, $K^+$, $Cl^+$, $SO_4^{2-}$, $HC0_3^-$, and $CO_3^{2}$), decrease or lower of osmotic pressure, and result in the deficiency of some metal elements Fe, Cu, Zn, and Mn elements (FAO 2005). The phenomenon causes an imbalance of nutrients and the occurrence of oxidative stress (Hussain et al. 2008). Another indication of salty soil is the increasing of calcium (Ca) element which effects in soil P availability (Malik et al. 2013) and N mineral deficiency (Zahran 1999). In addition, the salinity also affects soil enzyme activities (Siddikee et al. 2011), which could indirectly affect plant growth. Considering the importance of N and P nutrient for growth and crop production, field crops and paddy fields with these minerals deficiency are faced an obstacle for optimization. Nutrients are absorbed only in the form of soluble phosphate ion (Pi), $HPO_4^{2-}$or $H_2PO^{4-}$. Most of P is provided in the form of an organic and inorganic compound. Thus only a few part of soluble P (1 mg/kg or less) is available for plant growth (Richardson et al. 2009).

One of the solutions to optimize plant growth in the saline soil is to develop activity of soil bacteria that produces N, P, ACC-deaminase, and *Indole-3-Acetic Acid* (IAA). The soil bacteria usually live independently, and a large number of bacteria normally live in the rhizosphere, and are often called as a group of *Plant Growth Promoting Rhizobacteria* (PGPR) (Biswas et al. 2000). Bacteria which are included in PGPR and used throughout the world to increase crop productivity are *Bacillus, Enterobacter, Burkholderia, Acinetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Beijerinckia, Erwinia, Flavobacterium, Rhizobium* and *Serratia* (Bharti et al. 2013). One of the bacteria (*Azotobacter*) from PGPR group is being fertilized in the saline land. The method to use *Azotobacter* conventionally is an effective technology (Higa and Parr, 1994; Sergey et al. 2013). Early, Wang et al. (2003) tried to use it by using genetic engineering methods, but the results were not optimal. Furthermore, other researchers like Sapsirisopa et al. (2009), Singh et al. (2011), Nia et al. (2012), Dodd and Perez-Alfocea (2012),

Nautiyal et al. (2013), and Ramadoss et al. (2013) re-utilized functional bacteria with PGPR quality and as potential *biofertilizer* on crops in the saline field.

*Azotobacter* is diazotrophic bacteria of Gammaproteobacteria classes with aerobic quality. It is free-living bacteria and able to fixate nitrogen from the atmosphere. The bacteria can thrive on many mediums having no nitrogen by utilizing natural nitrogen for synthesizing its cells protein (Hindersah and Simarmata 2004). The another ability of *Azotobacter* is to produce hormones of gibberellins, cytokinins (Hindersah and Simarmata 2004), IAA (Egamberdieva et al. 2013), and compounds of antibiotic and siderophore that are useful to suppress pathogenic activity (Glick, 1995). Furthermore, it can also produce enzyme of 1-aminocyclopropane-1-carboxylase ACC-deaminase to reduce the level of ethylene growth in plant root (Yildirim et al. 2006). *Azotobacter* can also be useful as a plant growth promoters (Maor et al. 2004) and help the plant to be tolerant to extreme soil conditions such as the soil with high salinity (Pliego et al. 2011).

*Azotobacter* can be developed to facilitate the growth of crops in saline soils (Hayat et al. 2010) and may help the plants resistance to the high salinity environment (Berg et al. 2013). The group of bacteria has activity in the rhizosphere and they share a common spatial interface with various plants (Turan et al. 2006). The effect of this *Azotobacter*-plant connection in the soil is a beneficial mutual interaction.

*Azotobacter* will mediate the decomposing process of organic materials such as cellulose, amylose, and other fat and protein-rich substances into smaller organic and inorganic substances used by plants (Nurosid 2008). They supply plants by synthesizing some specific compounds (Dobbelaere et al. 2003) and facilitates absorption of certain nutrients from the soil (Çakmakçi et al. 2006). It also reduces or prevents the plant from soil-borne disease (Guo et al. 2010) as well as assist decomposition, nutrient mobilization, mineralization, storage of nutrients and water, nitrogen fixation and denitrification. Sometimes, it can dissolve phosphate and converts insoluble soil phosphate compound into dissolved form (Pradhan and Sukla 2005).

*Azotobacter* inoculation has succeeded in restoring unproductive environment into productive one in various places, thus increasing the growth and results of various plants (Li et al. 1999). It was proved that the application of *Azotobacter* in the field had saved 20 kg N/ha to increase the yield up to 1.24 tons/ha (Sattar 2010). The application of *Azotobacter* on "Tuk Tuk" shallot cultivars in saline environments is still lacking. Thus the recent research used the cultivar as the plant material. Shallot (*Allium cepa* L.) is one of the featured horticultural crops and much needed by the human in their daily lives. The need of shallot is increasing in line with rising world population in recent times. Almost all cuisines need it, that it becomes a commodity with good prospects at present and in the future. Another reason is that shallot production from some production centers like Brebes, Tegal, and Cirebon has been declining. Thus Indonesia must import the commodity from China. This situation happened because the shallot fields suffering from saturated chemical fertilizer, so the soil health and productivity decreases.

The activity of *Azotobacter* which is saline tolerant is expected to help to solve the problem of shallot growth in a saline environment. The objective of the study was to determine the effect of *Azotobacter* inoculation on the growth and yield of shallot crops and the availability of P in saline soil. The hypothesis is that the mixture of *Azotobacter* can provide better growth and yield of shallot crop rather than that of single *Azotobacter* in the saline soil environment.

## MATERIALS AND METHODS

### Preparation of *Azotobacter* inoculant

The inoculants are *Azotobacter* isolated from the rice fields in Panikel beach area, Kampung Laut Sub-district, Cilacap District, Central Java, Indonesia (S 7º 40' 0"; E 108º 55' 0") (Table 1). The isolation process to get the *Azotobacter* from sample ground was by leveled soil dilution technique ($10^1$-$10^7$). Furthermore, soil extract (0.2 mL) in dilution of $10^3$, $10^5$, and $10^7$ was planted in Petri dish containing selective Mannitol Ashby media (Subba Rao 1994). Based on Bergey's Guidelines for Systematic Bacteriology (Tchan and New 1984), the obtained bacteria was purified and identified by observing all morphological characters (cell form: coccus, rod, short rod), positive/negative Gram, movement, and cells (motile, spore formation, single, paired or chain). A careful investigation has successfully identified some bacteria in sample soil consisting of *Azotobacter paspali* bacteria, *Azotobacter chroococcum*, *Azotobacter* sp.1, and *Azotobacter* sp.2. Furthermore, all bacteria were tested in the Laboratory of Environmental Microbiology, Research Center for Biology, Indonesian Institute of Sciences. The test results showed that four *Azotobacter* turn out to be tolerant to NaCl 3% and positively produce IAA and ACC deaminase. Furthermore, each bacterium was made to become inoculant by culturing them in liquid Mannitol Ashby medium. Inoculated medium was incubated on a rotary shaker at room temperature for five days. Inoculants were harvested after bacteria density reached $10^8$ cfu/mL. It was injected into carrier material in the form of sterile compost with a ratio of 100 g of compost to 60 mL of liquid inoculants. Furthermore, the bacteria were incubated until the cell density reaching $10^8$ cfu/g of sterile compost.

### Inoculating *Azotobacter* on shallot plants

The shallot seeds of "Tuk-tuk" non-hybrid cultivar were sown in trays with planting medium by a mixture of soil and sterile compost with a ratio of 1: 1. Seeds were spread in the tray and covered with a mixture of soil and sterile compost, then doused with water slowly until moist. The tray was placed in the shade for one month. Furthermore, planting was conducted using polybag filled with 5 kg of soil. One-month-old shallot seed was transferred into the polybag and each polybag planted with three plants. As they became vigorous, and then screened them out until

only a plant/pot remains. Watering the plant with fresh water (300 mL/pot) should be done on a daily basis throughout the pot and the pot was watered with sea water (salinity = 3.5%) every two days by treatment with a volume of 300 mL/pot. *Azotobacter* inoculation was given two times at the planting and flowering period according to the treatment, namely 20 g/pot. Fertilizer of NPK (16-16-16) was given to plants with fertilizer schedule (4 g/pot), then was given again (1 g/pot) on 15 dap and 30 dap (days after planting) embedded into the ground. A pot experiment was arranged and placed in Research Center garden for Biology, Indonesian Institute of Sciences, Cibinong. The experiment was conducted using Completely Randomized Design (CRD) with a factorial pattern. The first factor was Watering, consisting of (i). Freshwater, (ii). Sea water. The second factor was inoculation consisted of (i). Non-inoculant, (ii). NPK fertilizer, (iii). *Azotobacter paspali* inoculant, (iv) *Azotobacter chroococcum* inoculants, (v). *Azotobacter* sp.1 inoculants, (vi). *Azotobacter* sp.2 inoculants, (vii). Mixed inoculants (all *Azotobacter*). All treatments were repeated 4 times. The observed parameters were plant height, leaf number, dried weight of leaves, and dried weight of shallots bulbs. Yields were calculated statistically by using SPSS software and significant differences of treatment were determined by Duncan's test ($p<0.05$).

**Soil analysis in flowering and post-harvest period**

Soil analysis was performed in the Laboratory of Environmental Microbiology, Research Center for Biology, Indonesian Institute of Sciences, Cibinong, Bogor, West Java, Indonesia. The analysis included the activities of PMEase enzyme, soil P solubilization, chemical analysis and soil bacteria population in the period of flowering and post-harvest. Analysis of PMEase activity and P solubilization (dissolved P) in the soil were done in accordance with the method of Tabatabai et al. (1969) and method of Olsen et al. (1954). While chemical analysis of the soil (C, N, C/N ratio, P, Na), salinity level, and sums of the bacterial population in the soil follows the method of Rowell (1994), Follett et al. (1981), and Vincent. (1992). Yields were calculated statistically by using ANOVA within SPSS software and significant differences of treatment were determined by Duncan's test ($p<0.05$)

## RESULTS AND DISCUSSION

**Inoculating *Azotobacter* on shallot plants**

Single and mixed *Azotobacter* inoculants, as well as NPK fertilizer on the growth and yield of shallot in the soil environment flushed with fresh water (non-saline) and the soil watered by sea water (saline), are statistically significantly different (Table 1).

Compared to controls, there were significant differences ($p<0.05$) in many parameters except for the plant height, i.e. the number of leaves, dried weight of leaves and dried weight of shallot bulbs. The number of leaves and dried weight of leaves respectively ranged between 5.00-11.75 leaves/pot and 1.12 to 3.15 g/pot, while dried weight of shallot bulbs ranged from 53.73-109.76 g/pot. The highest number of leaves, dried weight of leaves, and dried weight of shallot bulbs were obtained when shallot plants inoculated with mixed *Azotobacter* inoculant and watered with fresh water, 11.75 leaves/pot; 3.15 g/pot; and 109.76 g/pot. It was followed by the plants inoculated by *A. chroococcum* flushed with fresh water (9.80 pieces/pot; 3.08 g/pot; 100.09 g/pot) and next, by *A chroococcum* watered with sea water (9.75 pieces/pot; 3.10 g/pot; 101.28 g/pot), and the last, by mixed *Azotobacter* watered with sea water (10.75 pieces/pot; 3.13 g/pot; 102.00 g/pot).

The data summary indicates that compared to control plants which watered with fresh water, mixed *Azotobacter* inoculation with a sprinkling of fresh water to the shallot crop has an increased percentage of the number of leaves, dried weight of leaves and dried weight of shallots bulbs amounted to 80.8%, 59.1%, and 42.6%. While compared to control plants watered with sea water, mixed *Azotobacter* inoculation watered with sea water on shallot crop had an increased percentage of the number of leaves, dried weight of leaves and dried weight of shallot bulbs by 115%, 179.5%, and 89.8%. Other parameters such as the dried weight of shallots result obtained the balanced result in between treatments in the saline and non-saline environments. The result of this study showed that, compared to control plants and plants fertilized with NPK, inoculation with saline-resistant bacteria such as *Azotobacter* have improved the growth quality of shallot plants in non-saline (flushing with fresh water) and saline (flushing with sea water) soil environment. The results also indicate that providing mixed *Azotobacter* inoculation was more profitable than the individual one. This may be caused by inoculation of each *Azotobacter* contained in the mixed *Azotobacter* that can work simultaneously to jointly improve dried weight of plants and shallot crops in non-saline and saline soil. Nevertheless, the results of this research have discovered a single bacterial activity (*Azotobacter chroococcum*) that positively helps the plants' growth and yield. Those bacteria are saline-tolerant bacteria, capable of producing IAA and ACC-deaminase and the PGPR bacteria group. *A. chroococcum* bacterium is capable of associating with the roots of shallot crop, thus reducing negative impact caused by the saline soil environment. Jamil et al. (2006) reported that plants with saline-sensitive roots would hamper the growth of the plants. Some shallot plants will suffer from irregular growth due to salinity (Putri 2008). This is due to disruption of work systems (photosynthesis, stomatal conductance) in the plant (Golpayegani and Tilebeni 2011).

Koswara (2007) assumed that shallot bulbs productivity is affected by the environmental condition, its variety, and seeds quality being used. Actually, "Tuk-tuk" shallot which had been treated with inoculation by *A. chroococcum* in the saline environment (3.09 dS/m) was quite reliable. It could be seen from the net dry weight of shallot bulbs at saline soil, i.e., 101.28 g/pot. This result was much higher that the research result proposed by Akhwan et al. (2012) which used inoculation + guano on sand media and salinized with

sea water (21.4-22.8 dS/m) and resulted shallot net dry weight by 17.21 g; It was also higher than the result of the research on Bima variety carried out by Koswara (2007) by 22.3 g/crop; on Ampenan variety by 24.6 g/crop; on Garut variety by 18.40 g/crop; and on Bangkok variety by 16.90 g/crop. This number was even higher when being compared to the other plant which were treated with the inoculation by using *Azotobacter* at saline soil, such as eggplant which was watered with NaCl 1000 mg/L (65%) during the research performed by Bintoro (1983) and eggplant crop which was watered with sea water, of which the result showed that these crops resulted in nothing (Suliasih and Widawati 2016).

According to Christensen et al. (2007), the growth rate and the crop harvest on the saline environment depend on crop/plant variety and saline stress period. The success of the other researchers on the different crop treated with PGPR inoculation around saline environment, can be found on mustard green (Asghar et al. 2002), peanut (Khalid et al. 2004), cucumber, peppers, and tobacco (Kidoglu et al. 2008), rice (Ashrafuzzaman et al. 2009); wheat (Egamberdieva and Kucharova 2009), tomato (Tank dan Saraf 2010), black pepper, and banana (Maleki et al. 2010), chickpea (Patel et al. 2012), corn (Rojas-Tapias et al. 2012). However, contrary result were obtained by such typical PGPR inoculation in saline soil which could not boost the crop growth rate when applied on soybean plant (Essa 2002), string bean (Al Mutawa 2003), kidney bean (Rabie dan Almadini 2005), peanut (Mensah et al. 2006), wheat (Egamberdieva 2009), rice (Xu et al. 2011), and corn (Khodarahmpour et al. 2012).

The increase and the decrease in the plant growth and harvest rate, indeed, were influenced by the role of the inoculation of *Azotobacter* as PGPR, but its work mechanism cannot be completely understood. However, the service provide by *nitrogen-fixing bacteria* activity, especially genus of *Azotobacter* holds their important role in soil fertility (Fischer 2007), since they can improve N nutrient substance (Tejera et al. 2005) and P nutrient substance (Farajzadeh et al. 2012) which may be absorbed by plant root, thus it can boost the plant growth (Richardson et al. 2009), especially shallot. The other reason, *Azotobacter*, especially *Azotobacter chroococcum* can produce phytohormones, such as gibberellin, auxin, cytokinin, IAA in saline environment (Hayat et al. 2010), and active metabolic cyst which can be formed under unprofitable environment and it also becomes an essential part of the implementation of *Azotobacter* strain in various environment (Daiz et al. 2010). Therefore, the use of rhizobacteria which are saline tolerant, such as *Azotobacter paspali*, *Azotobacter chroococcum* and *Azotobacter* sp.1 and *Azotobacter* sp.2 bacteria have given such advantage in boosting the plant growth in Saline environment, as the inoculation on saline soil shows positive impact towards plant growth and output.

**Soil analysis during flowering and post-harvest**

The analysis of the *Azotobacter* inoculation on dissolved phosphate, PMEase enzyme activity, soil chemical properties, and also a population of bacteria during flowering and post-harvest was shown in Tables 2, 3 and 4. The activities of PMEase enzyme and dissolved P in soil being planted with shallot were measured twice, during the flowering period and post-harvest period (Table2).

The bacteria activities of *Azotobacter* sp.1, *Azotobacter* sp.2, *Azotobacter paspali*, and *Azotobacter* mix in soil being watered with fresh water and sea water during the flowering period and post-harvest period, resulted in higher level of PMEase enzyme and dissolved P contents in the soil when being compared to controlled treatment. There were significant differences at $p<0.05$.

The highest soil PMEase activity is generated by soil being treated with inoculation of *Azotobacter* mix bacteria and watered with sea water, i.e. 0.522 µg/mL p-nitrophenol/h (on flowering period) and decreased into 0.316 µg/mL p-nitrophenol/h (on post-harvest period), while, in soil had been watered with fresh water, the number became 0.507 µg/mL p-nitrophenol/h (on flowering period) and then decreased into 0.261 µg/mL p-nitrophenol/h (on post-harvest period). The same results also happened on dissolved P content in soil, of which it resulted in average dissolved P value between 0.01-1.461 ppm (on flowering period) and 0.237-1.679 ppm (on post-harvest period). The highest dissolved P value was reached on soil being treated with inoculation of *Azotobacter* mix and watered with both sea water and fresh water by 1.465 ppm during the flowering period and by 1,461 ppm and 1,146 ppm during post-harvest period. These research results were very low when being compared to the research result offered by Widawati and Suliasih (2016), in which the activities of *Azotobacter chroococcum* B4 have a capability in producing PMEase enzyme and dissolved P by 455.85 µg/mL p-nitrophenol/h and 3.4 ppm in saline soil. The results may be affected by the different ability of *Azotobacter* in producing organic acid such as succinate, acetate, propionate, glyoxylic, fumaric, oxalic, lactic, and ketoglutarate (Illmer and Schinner, 1992). The organic acid production produced by this kind of bacteria highly affects the production level of PMEase enzyme and then the production level of PMEase enzyme influences the production level of dissolved P (Linu et al. 2009). PMEase enzyme plays an important role towards the mineralization of organic P into inorganic P which is needed by crops, as well as by soil microorganism to conduct their activity (Linu et al. 2009).

The research result showed that the content of PMEase enzyme and dissolved P would increase gradually during the flowering period and decrease gradually during maturation period (fertilization period) and it continued to happen until post-harvest period (Table 2). This result was supported by the research result generated by Linu et al. (2009), which showed that the decreased activities of PMEase enzyme and dissolved P during maturation period (towards the harvest period). So, it was proven that the tolerant saline nitrogen-fixing bacteria (*Azotobacter paspali*, *Azotobacter chroococcum*, *Azotobacter* sp.1, dan *Azotobacter* sp.2) being isolated from the saline environment (Panikel, Kampung Laut, Cilacap) was effective in the PMEase enzyme activity and the dissolving of P-bound into dissolved P for the crops.

**Table 1.** Effect of *Azotobacter* activity on the growth and yield of onion in freshwater and saline environment

| Treatments | | Parameters | | | |
|---|---|---|---|---|---|
| **Source of water** | **Inoculants** | **Plant height (cm)** | **Number of leaves (leaf/pot)** | **Leaf dry weight (g/pot)** | **Dry weight of shallot bulbs (g/pot)** |
| Freshwater | Control | 37.75 a | 6.50 ab | 1.98 abcd | 76.99 abc |
| | Fertilizer of NPK | 45.50 a | 9.50 abc | 2.63 bcd | 100.38 bc |
| | *Azotobacter paspali* | 41.25 a | 7.75 abc | 2.29 abcd | 92.97 bc |
| | *Azotobacter chroococcum* | 44.00 a | 9.80 bc | 3.08 cd | 100.09 bc |
| | *Azotobacter* sp.1 | 38.25 a | 6.75 ab | 2.32 abcd | 93.68 bc |
| | *Azotobacter* sp.2 | 44.00 a | 9.50 abc | 3.04 bcd | 90.88 bc |
| | Mix inoculants | 40.50 a | 11.75 c | 3.15 d | 109.76 c |
| Sea water | Control | 36.00 a | 5.00 a | 1.12 a | 53.73 a |
| | Fertilizer of NPK | 42.75 a | 9.50 abc | 2.65 bcd | 96.60 bc |
| | *Azotobacter paspali* | 38.50 a | 7.25 abc | 1.75 abc | 79.99 abc |
| | *Azotobacter chroococcum* | 41.00 a | 9.75 bc | 3.10 cd | 101.28 bc |
| | *Azotobacter* sp.1 | 37.75 a | 9.00 abc | 1.67 ab | 66.80 ab |
| | *Azotobacter* sp.2 | 39.00 a | 6.25 ab | 1.99 abcd | 89.96 bc |
| | Mix inoculants | 44.75 a | 10.75 bc | 3.13 cd | 102.00 bc |

Note: The number followed by the same letter are not significantly different at ($p<0.05$) level of Duncan's test

**Table 2.** Effect of *Azotobacter* inoculant on phosphatase enzyme (PMEase) and soluble P in soil

| Treatments | | PMEase enzyme (µg/mL p-nitrophenol/h) | | Dissolved P (ppm) | |
|---|---|---|---|---|---|
| **Source of water** | **Inoculants** | **Flowering** | **After harvest** | **Flowering** | **After harvest** |
| Freshwater | Control | 0.352 b | 0.111 b | 0.447 b | 0.326 c |
| | Fertilizer of NPK | 0.448 cdef | 0.224 fg | 0.886 g | 0.543 e |
| | *Azotobacter paspali* | 0.465 cfg | 0.184 f | 0.884 fg | 0.437 d |
| | *Azotobacter chroococcum* | 0.477 fg | 0.232 g | 1.212 h | 0.837 g |
| | *Azotobacter* sp.1 | 0.403 c | 0.170 ef | 0.960 g | 0.148 b |
| | *Azotobacter* sp.2 | 0.446 cdef | 0.157 de | 0.813 f | 0.371 cd |
| | Mix inoculants | 0.507 gh | 0.261 h | 1.365 i | 1.146 h |
| Sea water | Control | 0.224 a | 0.112 a | 0.237 a | 0.016 a |
| | Fertilizer of NPK | 0.407 cd | 0.136 cd | 0.706 e | 0.729 f |
| | *Azotobacter paspali* | 0.422 cde | 0.103 ab | 0.526 bc | 0.557 e |
| | *Azotobacter chroococcum* | 0.477 fg | 0.170 ef | 1.169 h | 0.898 g |
| | *Azotobacter* sp.1 | 0.453 def | 0.119 bc | 0.614 d | 0.657 f |
| | *Azotobacter* sp.2 | 0.440 def | 0.103 ab | 0.534 cd | 0.830 g |
| | Mix inoculants | 0.522 h | 0.316 i | 1.365 i | 1.461 i |

**Note:** The number followed by the same letter are not significantly different at ($p<0.05$) level of Duncan's test

*Azotobacter paspali*, *Azotobacter chroococcum*, *Azotobacter* sp.1, and *Azotobacter* sp.2 bacteria were able to dissolve P-bound from calcium phosphate by 17.67%, 161.52%, 37.36%, 19.46%, respectively. Seshadri (2002) reported that phosphate solubilizing bacteria being isolated from the wet ecosystem and has high salinity (coastal ecosystem) was able to dissolve P by 30% from the phosphate zinc bound, 19% from calcium phosphate, and 18% from tricalcium phosphate. Farajzadeh et al. (2012) reported that *Azotobacter* has the capability to dissolve inorganic phosphate compound into its organic form, especially for *Azotobacter chroococcum* bacteria which has a potency to dissolve P (Tejera et al. 2005). Those reports strongly support the evidence of this research result, which showed that *Azotobacter*, especially for *Azotobacter chroococcum* bacteria, had effective PMEase activity thus it can provide dissolved P in soil. According to Kothari et al. (1990), the increase and the decrease of the activity of PMEase enzyme and dissolved P depend on the bacteria population in soil (Table 3).

Based on Table 3, it could be seen that the *Azotobacter* inoculation in saline soil with bacteria population density of $10^8$ cfu/g of soil, was evidently effective in increasing initial number of soil bacteria of $10^1$ cfu/g of soil (initial population) into $10^5$ cfu/g of soil (after fostered by NPK) and into $10^5$-$10^6$ cfu/g of soil (after inoculated by *Azotobacter*) during post-harvest period. These results showed that the bacteria being treated with inoculation was able to adapt and survive saline soil with the higher population number of $7.6 \times 10^5$ cfu/g of soil (on flowering period) and $7.6 \times 10^6$ cfu/g of soil (on post-harvest period). The bacteria population in soil, indeed, had not met soil

fertility index. According to Obaton (1977), fertile soil, at least, must contain bacteria population by $10^7$ cfu/g of soil. Decreased bacteria population in sample pot of shallot might be caused by higher soil salinity level which limits *Azotobacter* life. Another possibility is the association of *Azotobacter* has not fitted with its crop type. Whipps (2001) reported that the bacteria population is influenced by type and number of plant growing in certain habitat since the plant roots will release certain nutrition which will benefit in boosting the growth of active bacteria population in the rhizosphere of the plant. The similar result was also reported during the experiment performed by Kaushik and Sethi (2005), which suggested that the increase of soil salinity will decrease nitrification bacteria population (*Azotobacter*) in the soil of rice plant experiment pot. Meanwhile, Garcia and Hernandez (1996) in Yildrim et al. (2006) stated that salinity may lead into higher osmotic pressure. Thus it will influence microbe growth activity, except saline tolerant bacteria.

Four *Azotobacter* isolates being inoculated into shallot and being examined in the saline environment, showed the decrease of bacteria population, but they still effectively boosted shallot growth and PMEase and dissolved P activities. Hayat et al. (2010) stated that the population of saline tolerant bacteria in soil holds prominent role to improve soil quality, since this kind of bacteria functioned as the facilitator of plant growth in saline soil. These bacteria may also build the molecular mechanism to survive and grow along with the increasing salinity level (Tripathi et al. 2002).

The above statements considerably support the results generated from this study. It proved that *Azotobacter* endured the salinity with the population density of $10^6$cfu/g of soil and could still boost shallot growth as well as PMEase and dissolved P activities in the saline soil environment.

It is not only the salinity and other factors which have been mentioned above that limit bacteria population growth in soil, but according to Nihorimbere et al. (2011) and Heydarnezhad et al. (2012), the availability of organic materials such as macro and micro elements in soil also limit the bacteria growth in soil (Table 4).

Table 4 shows the result of soil chemical analysis on macronutrient such as C, $P_2O_5$ which categorize very high soil chemical criteria, N and CN ratio which in medium position and Na as the micronutrient which had a very low percentage. While the content of non-saline and saline soils during post-harvest period on nutrient C, N, CN ratio, $P_2O_5$, and Na were 3.71-8.40% (high-very high), 0.31-0.49% (moderate), 10-19 (moderate-very high), 0,71-0.98 ppm (very high), 0.02-0.08% (very low). According to Buckman and Brady (1982), the content of C, N, $P_2O_5$, and Na elements in normal soil should not exceed 5%, 0.75%, 0.1 ppm, and 1%. High salinity soil will contain high ion $Na^+$ and $Cl^-$and it can end in the lack of micronutrient availability (FAO 2005) such as Na. Kucey (1983) assumed that high and low level of macro and micro nutrients (inorganic) in saline soil, especially C, had very high impact on the activity level of phosphatase enzyme (PMEase) which may lead to the high level of availability of P in soil. Nitrogen (N total) and dissolved P ($P_2O_5$) elements may be available in saline soil due to *Azotobacter* activity which is inoculated into the saline soil. Rojas-Tapias et al. (2012) reported that PGPR from *Azotobacter* strain will increase the phosphorus intake in various salinity stress level and reduce $Na^+$. It can be shown by that four saline tolerant, that *Azotobacter* isolates were able to survive on sea water watering (containing 35 g/L NaCl) as well as on low salinity level (3.23 dS/m) and moderate salinity level (4.58 dS/m) during the post-harvest period (Table 4). The same results were also generated by Ravikumar et al. (2004), in which *Azotobacter Chroococcum* bacteria could survive salinity up to the concentration level of 35 g/L NaCl. Kaushik and Sethi (2005) reported that halophiles and halo tolerance microbes can flourish in the environment with moderate salinity and high salinity (EC 5, 10. 15 dS/m).

**Table 3.** *Azotobacter* population in selective medium (mannitol Ashby)

| Treatments | | Bacteria population (cfu/g soil) | |
|---|---|---|---|
| **Source of water** | **Inoculants** | **Flowering** | **After harvest** |
| Freshwater | Control | $2.5 \times 10^3$ a | $3.0 \times 10^4$ abc |
| | Fertilizer of NPK | $8.5 \times 10^3$ b | $2.5 \times 10^5$ ab |
| | *Azotobacter paspali* | $2.9 \times 10^4$ i | $9.0 \times 10^5$ f |
| | *Azotobacter chroococcum* | $1.6 \times 10^4$ e | $1.5 \times 10^6$ g |
| | *Azotobacter* sp.1 | $1.4 \times 10^4$ de | $8.0 \times 10^5$ ef |
| | *Azotobacter* sp.2 | $1.1 \times 10^4$ bc | $7.5 \times 10^5$ ef |
| | Mix inoculants | $3.3 \times 10^4$ j | $1.9 \times 10^6$ h |
| Sea water | Control | $4.8 \times 10^3$ a | $2.0 \times 10^4$ a |
| | Fertilizer of NPK | $1.3 \times 10^4$ cd | $4.5 \times 10^5$ bcd |
| | *Azotobacter paspali* | $1.1 \times 10^4$ bc | $7.5 \times 10^5$ ef |
| | *Azotobacter chroococcum* | $2.6 \times 10^4$ h | $2.0 \times 10^6$ g |
| | *Azotobacter* sp.1 | $1.9 \times 10^4$ f | $4.8 \times 10^5$ cd |
| | *Azotobacter* sp.2 | $2.2 \times 10^4$ g | $6.5 \times 10^5$ de |
| | Mix inoculants | $7.6 \times 10^5$ k | $7.6 \times 10^6$ i |

**Note:** The number followed by the same letter are not significantly different at ($p<0.05$) level of Duncan's test

**Table 4.** Soil chemical properties in samples from trial polybag per treatment

| Source of water | Soil samples (per pot treatment) | C organic (%) | N total (%) | CN ratio | $P_2O_5$ (ppm) | Na (%) | Salinity dS/m |
|---|---|---|---|---|---|---|---|
| Freshwater | Control | 5.37 de | 0.35 abc | 10.00 a | 0.78 abc | 0.02 a | 1.22 a |
| | | Very high | Moderate | Moderate | Very high | Very low | Non-saline |
| | Fertilizer of NPK | 3.71 a | 0.43 defg | 11.00 ab | 0.85 c | 0.02 a | 1.25 a |
| | | High | Moderate | Moderate | Very high | Very low | Non-saline |
| | *A. paspali* | 4.71 bcd | 0.44 defg | 13.00 bcd | 0.71 a | 0.02 a | 1.21 a |
| | | High | Moderate | High | Very high | Very low | Non-saline |
| | *A. chroococcum* | 8.40 h | 0.45 efg | 19.00 f | 0.84 c | 0.02 a | 1.22 a |
| | | Very high | Moderate | Very high | Very high | Very low | Non-saline |
| | *Azotobacter* sp.1 | 5.90 efg | 0.46 fg | 17.00 e | 0.73 ab | 0.02 a | 1.19 a |
| | | Very high | Moderate | Very high | Very high | Very low | Non-saline |
| | *Azotobacter* sp.2 | 6.15 f | 0.46 fg | 14.00 cd | 0.78 abc | 0.02 a | 1.25 a |
| | | Very high | Moderate | High | Very high | Very low | Non-saline |
| | Mix inoculants | 6.42 g | 0.49 g | 15.00 d | 0.78 abc | 0.02 a | 1.25 a |
| | | Very high | Moderate | High | Very high | Very low | Non-saline |
| Sea water | Control | 4.10 ab | 0.31 a | 14.00 cd | 0.76 abc | 0.07 cd | 4.58 c |
| | | High | Moderate | High | Very high | Very low | Moderate |
| | Fertilizer of NPK | 4.83 bcd | 0.34 ab | 12.50 bc | 0.96 d | 0.05 b | 4.16 c |
| | | High | Moderate | High | Very high | Very low | Moderate |
| | *A. paspali* | 4.14 ab | 0.35 abc | 12.00 abc | 0.82 bc | 0.06 bc | 3.19 b |
| | | High | Moderate | Moderate | Very high | Very low | Low |
| | *A. chroococcum* | 6.12 efg | 0.35 abc | 14.00 cd | 0.76 abc | 0.08 d | 4.19 c |
| | | Very high | Moderate | High | Very high | Very low | Moderate |
| | *Azotobacter* sp.1 | 5.02 bcd | 0.42 cdef | 11.00 ab | 0.79 abc | 0.05 bc | 4.11 c |
| | | Very high | Moderate | Moderate | Very high | Very low | Moderate |
| | *Azotobacter* sp.2 | 5.44def | 0.38 bcd | 12.00 abc | 0.79 abc | 0.07 cd | 3.23 b |
| | | Very high | Moderate | Moderate | Very high | Very low | Low |
| | Mix inoculants | 4.39 abc | 0.39 bcde | 12.00 abc | 0.76abc | 0.06 bc | 3.45 b |
| | | High | Moderate | Moderate | Very high | Very low | Low |

**Note:** The number followed by the same letter are not significantly different at ($p<0.05$) level of Duncan's test

This result (Table 1 to 4) implied that the contribution of microbes or biofertilizer, especially related to nitrogen fixer and phosphate solubilizing bacteria with saline tolerance was needed to improve plant growth in saline soil. This research was still at the beginning stage, thus it needed to be continued at the field scale, especially in the area that had salinity problems.

In summary, the results revealed that inoculation with mixed *Azotobacter* (*Azotobacter paspali*, *Azotobacter chroococcum*, *Azotobacter* spp.1, and *Azotobacter* spp.2) caused better growth and yield of shallot crops compared to single *Azotobacter* in saline soil. Inoculation of *Azotobacter chroococcum* on "Tuk Tuk" shallot cultivar reduced plant sensitivity to salinity up to 4.19 dS/m and improved the growth and the bulbs of shallot (101.28 g/pot). *Azotobacter* maintained its population up to $10^6$ cfu/g of soil during PMEase activity at 0.170 µg/mL p-nitrophenol hour and dissolved Phosphate at 0.898 ppm until post-harvest. The highest activity of PMEase and dissolved P in the soil during flowering and post-harvest was obtained from the treatment of mixed *Azotobacter* at a population density of $10^6$ cfu/g of soil.

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