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The role of *azotobacter* sp. In reducing inorganic fertilizer of nitrogen on growth of local maize (*zea mays* l.) In ultisol

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Biological N_2 fixation technology can play a vital role as substitution to commercially available N-fertilizer in crop production and reduction of environmental problem to some extent. This study aimed to determine the effect of *Azotobacter* sp. in improving the local maize vegetative growth and reducing the inorganic fertilizer of urea usage on ultisol soil. The experiment was arranged in a randomized block design (RBD) with six treatments and four replications. The treatments were as six *Azotobacter* sp combinations with urea fertilizer dosage. The treatments were as follow: A0, without *Azotobacter* sp. and without inorganic fertilizer of nitrogen (control); A1, *Azotobacter* sp. without inorganic fertilizer of nitrogen; A2, *Azotobacter* sp. with 25% recommended inorganic fertilizer of nitrogen; A3, *Azotobacter* sp. with 50% recommended inorganic fertilizer of nitrogen; A4, *Azotobacter* sp. with 75% recommended inorganic fertilizer of nitrogen; and A 5,100% recommended inorganic fertilizer of nitrogen. The data collected consist of plant height, leaf number, stem diameter, and leaf area of plant. The results showed that the application of biological fertilizers *Azotobacter* sp. combined by inorganic fertilizer nitrogen could increase the vegetative growth of maize on Ultisols soil. Treatment of biological fertilizers *Azotobacte*r sp. with 25% recommended inorganic fertilizers of nitrogen were the best treatment to improve the vegetative growth of maize plant on ultisol. Utilization of *Azotobacter* sp could reduce inorganic fertilizer of urea usage until 75% from recommendation dosage.

Keywords: *Azotobacter* sp., Local maize, Inorganic fertilizer of urea, Ultisol.

INTRODUCTION

The use of inorganic fertilizers is one of the efforts made by farmers to increase crop production in Ultisol, but the use of inorganic fertilizers continuously in the long term will have a negative impact on the environment due to the accumulation of inorganic materials in soil that is not matched by the rate of absorption of nutrients by crop optimally. Environmental pollution caused by chemical fertilizers is one of human problems recently. Application of biological fertilizers non-symbiotic fixing-nitrogen as *Azotobacter* sp. and *Azospirillum* sp. were able to reduce the use of urea, preventing a decrease in soil organic matter and reduce pollution. Inoculation *Azotobacter* sp. can raise results between 15-100% and reduces the use of artificial fertilizers by up to 30% on dry land ecosystems (Simarmata, 1994; Kader et al., 2002; Sattar et al., 2008, Syaiful et al., 2013). An intensive use of chemical fertilizers and costly in recent years resulted in environmental pollution problems so that the focus of current research on the possible use of biological fertilizers as an alternative or as a complement of chemical fertilizer.

The application of bio fertilizers can decrease the adverse effect on environment (Javaid, 2011; Carvajal-Muñoz and Carmona-Garcia, 2012; Rai et al., 2014; El Sabagh et al., 2016) and reducing the chemical fertilizers use (Muhammadi et al., 2013; Roychowdhury et al., 2014; Saeed et al., 2015; Monda et al., 2017; Angadi et al. 2017). Biofertilizer has significant role in promoting sustainable agriculture, alleviating environmental pollution and deterioration of nature (Namvar et al., 2012; Rana et al., 2012). *Azotobacter* sp. and *Azospirillum* sp. are used as biofertilizers in the cultivation of many agricultural crops (Karakut and Aslantas, 2010; Erturk et al., 2011; Datta et al., 2011). The microbial communities like *Azotobacter* sp. and *Azospirillum* sp. in the increase the roots health and the absorption of nutrients and water (Diaz-Zorita and Fernandez-Canigia, 2009; Daneshmand et al., 2012). The application of biofertilizer It is very important to increase crop production (Simarmata et al., 2016; Zaremanesh et al., 2017) and reduction the environmental effect.

Southeast Sulawesi is one of the maize producing areas of considerable potential, because besides having dry lands which has not been utilized extensive enough at around 202,973 ha (CBS, 2012), also because the corn crop in this region enough important considering the populated especially who resided in region with many islands some still use corn as a staple food. the productivity of maize in Southeast Sulawesi around 2.49 tons ha^{-1} (CBS, 2013), decreased compared to productivity in 2012 which reached 2.87 tons ha^{-1}, and only reached 2.85 tons ha^{-1} in 2017 (CBS, 2017). Corn productivity in southeast Sulawesi is still much lower than the national average productivity of around 4.84 tons ha^{-1}. One of the causes of low productivity of maize crop in Southeast Sulawesi is a land cultivation is generally concentrated in the Ultisol.

The acid soils characterized in by the presence of Al, Fe and Mn which high, these substances are toxic to plants (White et al., 2010; Nurmas, 2015; Nurmas et al. 2015a, 2015b; Bojórquez-Quintal et al., 2017). At the lands dour occurs that plants need nutrient deficiency such as N, P, Ca, Mg, Mo (Wuana and Okieimen, 2011; Rout et al., 2015). The use of inorganic fertilizers is one of the efforts made by farmer to increase the crop production in Ultisol, but the use of inorganic fertilizers are continually in the long term will have a negative effect on the environment due to the accumulation of inorganic materials in soil which is not offset by the level of nutrient absorption by crop maximally. The use of microorganisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture (Freitas et al., 2007). During the past couple of decades, the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased in various parts of the world.

Nitrogen as the most important vital elements of plant in quality and quantity production of cultivation products plays important role. *Azotobacter* and *Azospirillum*as fixing bacteria of nitrogen can freely fix molecular nitrogen and be considered as biological fertilizer (Amiri and Rafiee, 2013). Free-living nitrogen-fixing bacteria *eg Azotobacter chroococcum* and *Azospirillum lipoferum,* were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Essam and Lattief,2013). *Azotobacter* have a full range of enzymes needed to perform the nitrogen fixation: ferredoxin, hydrogenase and an important enzyme nitrogenase. (Karunakaran et al., 2014). Nitrogen fixation is achieved by the enzyme nitrogenase, which reduces N to ammonia. However, this enzyme is extremely sensitive to oxygen in Azotobacter species.

Efforts to maintain soil health and productivity of the plant needs to be done inoculation Azotobacter sp. because the rhizobacteria role as agents for improving plant growth through the production of phytohormones which can be utilized by plants. Fitohormon function as a substance that regulates all physiological processes, my growth and development in plants. The result of research Nurmas et al., (2014) showed that isolates of Azotobacter, potential as a biological fertilizer and stimulate plant growth because it has capability in produce IAA, dissolving phosphate and capable of adapting in marginal lands. Based on the above background it is necessary to conducted research on the role of *Azotobacter* sp. in efforts to reduce inorganic fertilizer of urea at the growth of local maize (*Zea mays* L.) on Ultisol.

MATERIALS AND METHODS

Main materials

The main materials used were: indigenous Azotobacter isolates (LP7a and KU6e isolates) (Nurmas, 2015; Nurmas et al. 2014, 2015a, 2015b), Nutrient agar medium, local corn seeds, animal manure, inorganic fertilizers: nitrogen (Urea), phosphor (SP-36), kalium (KCl), and Ultisol soil.

Purification and formulation of Azotobacter

Azotobacter isolates purified and grown in nutrient Agar media (NA) for 48 hours and then suspended in sterile distilled water to achieve the concentration of 10^8-10^{10} CFU ml^{-1}, and used as Azotobacter liquid formulations. The liquid formulation directly applied to seeds and plants.

Preparation of planting medium.

Ultisol soil for growth medium taken from Lamomea village, Konda, South Konawe Regency, Province of Southeast Sulawesi, Indonesia. Soil mixed with animal manure (4:1 v/v) and sterilized by steam sterilization. Soil medium put into a polybag in size 40cm x 20cm and placed at Experimental Farm according to the layout of experiment.

Treatment of seeds and planting

Maize seed are soaked (priming) in Azotobacter liquid formulations in a ratio 1: 2 (v/v) and incubated in a shaker rotary with a speed 150 rpm for a day. Seed for control treatment was seed just soaked in sterile water at the same time and conditions. Furthermore, the seed dried in a laminar airflow for an hour and ready to be planted in polybags.

Application of Azotobacter and inorganic fertilizer

Azotobacter formulation was applied twice by pouring around the plant roots, with the dose of 10 mL/plant, the first application was conducted 2 weeks after planting, and the second at 4 weeks after planting. Inorganic fertilizer used were nitrogen (Urea), phosphor (SP36), and KCl. Urea fertilizer was applied twice at planting time and four weeks after planting in dosage according to the treatments. While the SP-36 and KCL fertilizers were applied at planting time according to the recommendations dosage.

Experimental design

This study used a Randomized Complete Block Design (RCBD) consisted of six treatment, namely: A0 (without both Azotobacter formulation and inorganic fertilizers of urea/control); A1 (Azotobacter formulation only); A2 (Azotobacter formulation + 25% inorganic fertilizers of Urea); A3 (Azotobacter formulation + 50% inorganic fertilizers of urea); A4 (Azotobacter formulation + 75% inorganic fertilizer of urea), and A5 (100% inorganic fertilizer of urea). Each unit of experiments using five plants, repeated four times, to make overall so 120 plant sample units.

Plant Observation

a. Azotobacter formulation was applied twice by pouring around the plant roots, with the dose of 10 mL/plant, the first application was conducted 2 weeks after planting, and the second Plant height (cm), measured from the ground to the tip of the leaf polybag longest at ages 2, 4 and 6 MST.
b. Leaf number (pieces), calculated all perfectly formed leaves at the age of 2, 4, and 6 MST.
c. Stem diameter (cm), measured at the lower stem near the root collar at ages 2, 4 and 6 MST
d. Leaf area at the age of 2, 4 and 6 MST done by measuring the length and width of leaves that have been opened perfectly and still green. Leaf area can be calculated using the formula:

Statistical analysis

The data were analyzed using analysis of variance, followed with Duncan's Multiple Range Test (DMRT) at 95% confidence level

RESULTS

Results of analysis of variance showed that the application of biological fertilizers Azotobacter formulation + Inorganic fertilizer of Urea very significant effect on plant height at 2 and 4 weeks after planting (WAP) (Table 1), very significant effect on stem diameter and leaves number at 2, 4, and 6 WAP (Table 2 and 3). Results of the analysis of a wide variety of leaves showed that application of biological fertilizers Azotobacter formulation + Inorganic fertilizer of Urea significantly at 2 WAP and highly significant at the age of 4 and 6 WAP (Table 4) and Fig.

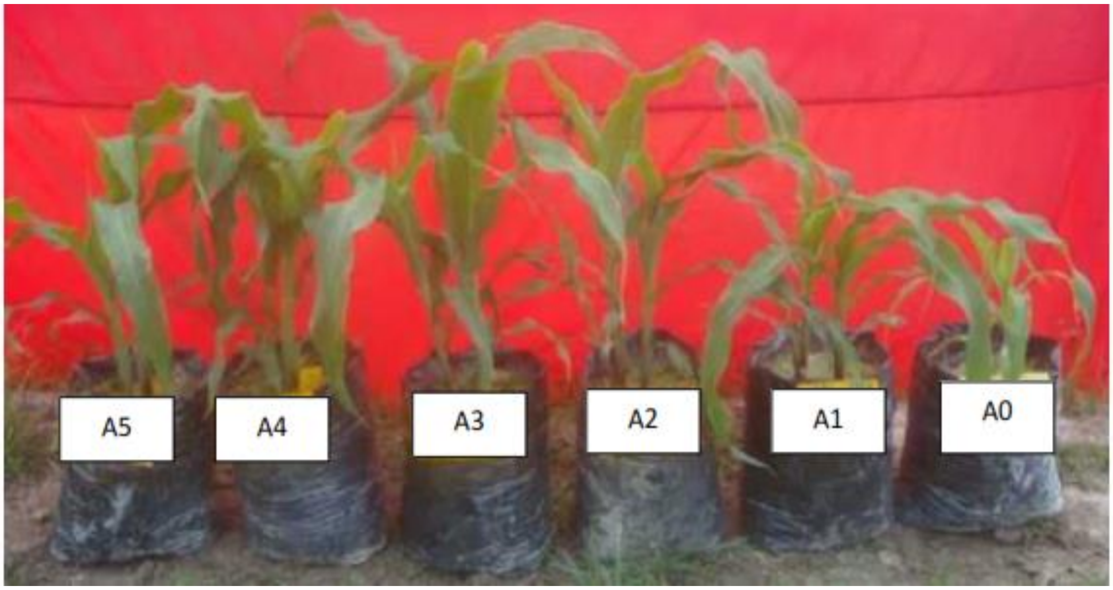

Table 1. Effect application of Azotobacter formulation + inorganic fertilizer of Urea on the plant height of local corn

Treatments	The averages plant height (cm	
	2 WAP	4 WAP
A0= control	4.553[c]	43.225[c]
A1=*Azotobacter* sp.+ without urea fertilizer	10.240[a]	122.475[ab]
A2=*Azotobacter* sp.+ 25% dose of Urea fertilizer	10.775[a]	119.513[ab]
A3= *Azotobacter* sp.+50% dose of Urea fertilizer	10.183[a]	127.280[a]
A4= *Azotobacter* sp.+75% dose of Urea fertilizer	9.573[a]	119.750[ab]
A5=100% dose of Urea fertilizer	8.035[b]	111.125[b]

Note: Values followed by different letter at the same column (a-c) were significant differences at DMRT of 95 percent confidence level

Table 2. Effect application of Azotobacter formulation + inorganic fertilizer urea on stem diameter of local corn

Treatments	The average stem diameter (cm)		
	2 WAP	4 WAP	6 WAP
A0= control	0.378[c]	0.603[c]	0.838[b]
A1=*Azotobacter* sp.+ without Urea fertilizer	0.913[a]	1.675[b]	2.085[a]
A2=*Azotobacter* sp.+ 25% dose of Urea fertilizer	0.932[a]	1.822[a]	2.332[a]
A3= *Azotobacter* sp.+50% dose of Urea fertilizer	0.908[a]	1.805[ab]	2.310[a]
A4= *Azotobacter* sp.+75% dose of Urea fertilizer	0.795[ab]	1.880[a]	2.360[a]
A5=100% dose of Urea fertilizer	0.717[b]	1.837[a]	1.975[a]

Note: Values followed by different letter at the same column (a-c) were significant differences at DMRT of 95 percent confidence level.

Table 3. Effect application of Azotobacter formulation + Inorganic fertilizer Urea on the leaves number of local corn

Treatments	The average leaf number (cm)		
	2 WAP	4 WAP	6 WAP
A0= control	0.838[b]	4.460[b]	6.040[d]
A1=*Azotobacter* sp.+ without Urea fertilizer	1.693[a]	6.752[a]	8.958[c]
A2=*Azotobacter* sp.+ 25% dose of Urea fertilizer	1.823[a]	8.543[a]	10.835[b]
A3= *Azotobacter* sp.+50% dose of Urea fertilizer	1.805[a]	8.503[a]	11.083[b]
A4= *Azotobacter* sp.+75% dose of Urea fertilizer	1.880[a]	8.833[a]	12.878[ab]
A5=100% dose of Urea fertilizer	1.838[a]	8.455[a]	14.210[a]

Note: Values followed by different letter at the same column (a-d) were significant at DMRT of 95 percent confidence level

Table 4. Effect application of Azotobacter formulation + Inorganic Urea fertilizer on plant leaves area of local corn

Treatments	The mean leaves area (cm)		
	2 WAP	4 WAP	6 WAP
A0= control	12.175[b]	56.950[b]	94.110[b]
A1=*Azotobacter* sp.+ without Urea fertilizer	26.560[a]	230.790[a]	326.730[a]
A2=*Azotobacter* sp.+ 25% dose of Urea fertilizer	32.810[a]	249.410[a]	344.020[a]
A3= *Azotobacter* sp.+50% dose of Urea fertilizer	27.445[a]	267.670[a]	352.220[a]
A4= *Azotobacter* sp.+75% dose of Urea fertilizer	26.135[a]	237.900[a]	366.820[a]
A5=100% dose of Urea fertilizer	26.583[a]	240.640[a]	359.830[a]
Note: Values followed by different letter at the same column (a-d) were significant differences at DMRT of 95 percent confidence level			

DISCUSSION

Research results of variance analysis showed that the application of biological fertilizers Azotobacter formulation + Inorganic fertilizer Urea on local maize growth resulted significantly in plant height, stem diameter, leaf number and leaf area at 2, 4 and 6 WAP. Hellal et al., (2011) reported that application of biological fertilizer in single or combined with chemical fertilizer N increases the growth, yield and chemical content fennel plant compared with plants control treatment. The highest value of vegetative growth, the percentage of oil, chlorophyll and NPK fertilizers obtained in the treatment of biological fertilizer plus two-thirds of the Urea fertilizer dose is recommended.

Similar results were reported by Abdel-Kader et al., (2012) and Gendy et al., (2013.) on the Guar and Roselle plants. El Gendy et al., (2013) report that the applications of Urea and biological fertilizers in the soil have a significant impact on characters vegetative growth of plant *Cymbopogon Citratus*. Interaction of biological fertilizer and chemical fertilizer Urea causing a significant increase in essential oil yield, the content of polyphenols and flavonoids compared with the control of the two season. Furthermore Ghilavizadeh et al., (2013) showed an increase in seed yield, essential oil content in plants ajowan. Said-Al Ahl et al., (2015) showed that Urea fertilizer and / or treatment of a biological fertilizer causes an increase in total carbohydrate, the amount of chlorophyll and the content of N, P, K on *Anethum graveolens*.

Inoculation Azotobacter and Pseudomonas plus reduce the application of chemical fertilizer 25 to 50% in the field. Wheat farmers can get the same results if their apply the half of recommended doses of chemical fertilizer with Azotobacter and Pseudomonas (Yousefi and Barzeger, 2014). Applications Azotobacter and Azospirillum bacteria in various levels of Urea in plant of sunflowers showed that combined application of these two types of bacteria improve plant growth characteristics and reduce Urea fertilizer applications by 50% (Mirzaei et al., 2010). *Azotobacter* sp. and *Azospirillum* sp. contribution on the fixing nitrogen (Jiménez et al., 2011) and increase plant tolerance (Curá et al., 2017).

Aazadi et al., (2014) reported that the application of bacteria *Azotobacter* spp. and *Azospirillum* spp. on the wheat cultivation could reduce the use of nitrogen fertilizers. The combination of biological fertilizer and chemical fertilizer could precisely achieve the expected results and reduce the negative impact on the environment. The results of this research confirmed the important of agricultural systems sustainably and environmentally friendly. Applications 75% of the recommended dose of NPK plus biological fertilizer managed to lose 25% of the recommendation dose of N, P and K and significantly increased productivity of barley plant in saline soils and reduce environmental pollution by reducing the extensive use of chemical fertilizers (El-Shahat, et al., 2014).

CONCLUSION

Application of biological fertilizers *Azotobacter* sp. combined with inorganic fertilizer nitrogen could improve the vegetative growth of corn plants in the Ultisol. Treatment of biological fertilizers *Azotobacter* sp. + Inorganic fertilizers nitrogen 25% from the dosage recommendation is the best treatment to improve the vegetative growth of local maize in Ultisol.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AN designed and performleed the experiments and also wrote the manuscript. Anwar collected the data. LK, LS and AK performed field experiment, data analysis and wrote the manuscript. MHD reviewed the manuscript. All authors read and approved the final version..

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