EFFECT OF AZOLLA PINNATA-BASED BIOFERTILIZER ON N-UPTAKE, PH, AZOTOBACTER SP. POPULATION, AND YIELD OF PAKCOY (BRASICA RAPA L.) ON INCEPTISOLS FROM JATINANGOR

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ABSTRACT
Inceptisol Jatinangor West Java soil conditions have a low fertility. Actions that can be made to improve the soil conditions of Inceptisols are by applying organic fertilizers to improve the physical, biological, and chemical properties of the soil and adding available nutrients to increase the quantity of crop yields. The purpose of the study was to determine the concentration of Azolla pinnata-based biofertilizer that could produce the best results against Azotobacter sp. population, N-uptake, pH, and yield of pakcoy plant. The research was carried out at the experimental garden, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, West Java. The research design used in the experiment was a Randomized Block Design (RBD) which consisted of thirteen treatments, namely: control (without the application of Azolla biofertilizer), Azolla-based biofertilizer with a concentration of 5 ml L-1, 10 ml L-1, 15 ml L-1, 20 ml L-1, each concentration treatment was combined with 3 application times, namely 1, 2, and 3 times. The experimental results showed that the treatment of Azolla-based biofertilizer with a concentration of 10 ml L-1 with 2 times applications increased the N-uptake, pH, and yield of pakcoy plants 184 g of fresh weight pakcoy/polybag. The population of Azotobacter sp. was not affected by the application of Azolla pinnata-based biofertilizer.

Keywords: Azotobacter, Inceptisols, N-Uptake and Pakcoy.

1. INTRODUCTION
Pakcoy (*Brassica rapa* L.) is one type of vegetable plant that has commercial value economically because it is widely needed by the community as food for green vegetables. Pakoy plants contain a lot of fiber, various vitamins A, B, B2, B6, C, in addition to containing calcium, phosphorus, copper, protein, and iron (Tania et al., 2012). Production of vegetable crops in Indonesia is starting to increase, due to the good selling value and supported by the short age of leaf vegetable plants which are the main characteristics of leaf vegetable plants from the *Brassicaeae* group. One of the widely known Brassicaeae plants is the pakcoy plant (*Brassica rapa* L.) (Perwitasari et al., 2012).

In supporting the success of pakcoy cultivation, in addition to using superior varieties, it is important to pay attention to soil quality, both physical, chemical, and biological properties of the soil. One of the causes of low soil quality is the continuous use of inorganic fertilizers, causing the population of microbes and organic matter in the soil to decrease (Nurhasannah et al., 2015).

Inceptisol soil is one of the soil orders in Indonesia with an area of about 70.5 million hectares or 37% of the total land area in Indonesia. Inceptisols belong to soils that experience

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moderate weathering and are easily leaching (Sancez, 1992). Inceptisol soil characteristics have a thickness of 1-2 meters solum, black or gray to dark brown, texture of sand, dust, and clay. Loose consistency with a crumb soil structure, in general, Inceptisol soil fertility is quite low, productivity is moderate to high. The results of the analysis of Inceptisol soil from Jatinangor, West Java, have a slightly acidic pH of 5.58; C-organic 1.89% low; C/N 8% low; N-total 0.24% moderate; P_2O_5 108.86 mg/100g very high; and K_2O 29.17 mg/100g moderate. Disadvantages of Inceptisols soil are low organic matter content so that it can be increased through balanced fertilization using organic and inorganic fertilizers. Efforts to increase Inceptisol soil fertility through microbial activity can be through the provision of biofertilizers. The use of biofertilizers is one way to restore soil fertility because it contains soil microbes that are functional as nutrient providers in the soil, thereby increasing fertilization efficiency.

The role of nitrogen is important for pakcoy plants because it can stimulate the growth of vegetative parts of plants, especially leaves. Biofertilizers containing N-fixing bacteria such as Azotobacter are environmentally friendly. N-fixing bacteria also produce growth hormones that are useful for accelerating plant growth.

Azolla-based liquid biofertilizer is a type of liquid biofertilizer that uses Azolla extract as a carrier. The water fern *Azolla pinnata* is known to have a symbiotic relationship with the cyanobacteria *Anabaena azollae* which can fix free N_2 in the atmosphere. *Azolla pinnata* can act as a carrier for liquid fertilizer because it contains complete micro and macro nutrients. Nitrogen fixing bacteria Azotobacter is a bacterium commonly used in biofertilizers and plays a role in providing N nutrients for plants. Nitrogen is the main nutrient, as well as a growth limiting factor, so that it becomes one of the keys to the success of plant growth (Purwaningsih, 2004).

Azolla has great potential as a natural nitrogen source. The fulfillment of nitrogen needs in pakcoy plants can be done by applying biofertilizers with appropriate concentrations. The application time and concentration of different biofertilizers can affect different yields on pakcoy plants. However, the concentration of biofertilizers with the right application time can increase the growth and yield of plants and pakcoy plants.

2.MATERIALS AND METHODS

The research was carried out from August to November 2021 in the Ciparanje experimental garden, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor District, Sumedang Regency, West Java. The materials used in this study included Inceptisol soil from Jatinanor, pakcoy seeds, urea fertilizer, SP-36, KCl, materials for soil analysis, soil microorganism analysis materials, Azolla-based biofertilizers.

This experiment was conducted using a Randomized Block Design (RBD). The treatment consisted of a combination of concentration and time of application of Azolla-based biofertilizers. The concentrations of biofertilizers given were without biofertilizers (control), 5 ml L^{-1}, 10 ml L^{-1}, 15 ml L^{-1}, and 20 ml L^{-1}. The application times were 1 time, 2 times, and 3 times the application, the interval of each treatment was 1 week. Each treatment was repeated 3 times.

Observations whose data were statistically analyzed were observed at harvest age of 30 DAP, the total population of *Azotobacter* sp. taken from the plant rhizosphere and analyze using the plate count method. Plant N uptake was analyzed using the Kjeldahl method, soil pH was analyzed using a glass electrode. The yield of pakcoy plants was in the form of fresh weight of pakcoy plants without roots (g/pot).

3.RESULTS AND DISCUSSION

Plant N-Uptake

Table 1 shows that the control treatment (P0) and Azolla-based biofertilizer 10 mL L^{-1} 2 times (P5) showed higher N-uptake results than the P9 treatment. Other treatments did not give better results than the control and P5 treatment.

Table 1. Effect of Azolla-based biofertilizer on Plant N-Uptake

Code	Treatments	N-Uptake (g/plant)
P0	Control	41.66 b
P1	Azolla-based biofertilizer 5 mL L^{-1}, 1 time application	33.53 ab
P2	Azolla-based biofertilizer 5 mL L^{-1}, 2 times application	38.10 ab
P3	Azolla-based biofertilizer 5 mL L^{-1}, 3 times application	35.49 ab
P4	Azolla-based biofertilizer 10 mL L^{-1}, 1 time application	37.04 ab
P5	Azolla-based biofertilizer 10 mL L^{-1}, 2 times application	41.66 b
P6	Azolla-based biofertilizer 10 mL L^{-1}, 3 times application	37.50 ab
P7	Azolla-based biofertilizer 15 mL L^{-1}, 1 time application	29.61 ab
P8	Azolla-based biofertilizer 15 mL L^{-1}, 2 times application	29.37 ab
P9	Azolla-based biofertilizer 15 mL L^{-1}, 3 times application	24.95 a
P10	Azolla-based biofertilizer 20 mL L^{-1}, 1 time application	32.90 ab
P11	Azolla-based biofertilizer 20 mL L^{-1}, 2 times application	33.14 ab
P12	Azolla-based biofertilizer 20 mL L^{-1}, 3 times application	29.84 ab

Note: Numbers followed by the same letter are not significantly different at the 5% Duncan test level.

The movement of nitrogen, especially in the form of NH_4^+, is slow in the soil solution, which can cause low N uptake. The average nitrogen content in plant tissue is 2- 4% dry weight (Tisdale et al., 1990). In dry land, it is possible for water shortages to occur which result in changes at the molecular, cellular, physiological and morphological levels. Changes that occur can be a reduction in cell volume, a decrease in leaf area, leaf thickening, the presence of hairs on the leaves, changes in gene expression, changes in carbon and nitrogen metabolism, changes in the production and activity of enzymes and hormones, increased sensitivity of stomata, decreased photosynthetic rate. The condition of rapid water evaporation causes the soil to become volatile or the soil lacks nitrogen in the form of NH_3. In addition, the high and low content of N in the soil is influenced by the amount of input and loss of N in the N cycle. The low N content of the soil can occur because N has been absorbed by plants or due to leaching and evaporation (Khalif et al., 2014). In addition, it is caused by the large number of soil microbes using nitrogen as a nutrient. Soepardi (1983), stated that microorganisms can harm plants by competing for nutrients, one of the most contested is nitrogen. The growth of microorganisms requires two important elements, namely carbon (C) as an energy source and nitrogen (N) as a constituent of cells.

Soil pH

The acidity (pH) of Inceptisol soil from Jatinangor for each treatment can be seen in Table 2. The pH value is a value that indicates the concentration of hydrogen ions (H^+) in the soil, the more H^+ ions in the soil, the more acidic the soil. In addition to the presence of H^+ ions, there are also OH- ions which must be opposite in number to H+ ions, if the concentration of OH^- ions in the soil is more, then the soil has an alkaline pH. If the concentrations of H^+ and OH^- are balanced, the soil has a neutral pH or pH = 7 (Hardjowigeno, 2010).

Table 2. Effect of Azolla-based biofertilizer on Soil pH

Code	Treatments	pH
P0	Control	6.44 ab
P1	Azolla-based biofertilizer 5 mL L^{-1}, 1 time application	6.30 ab
P2	Azolla-based biofertilizer 5 mL L^{-1}, 2 times application	6.57 ab
P3	Azolla-based biofertilizer 5 mL L^{-1}, 3 times application	6.41 ab
P4	Azolla-based biofertilizer 10 mL L^{-1}, 1 time application	6.60 b
P5	Azolla-based biofertilizer 10 mL L^{-1}, 2 times application	6.30 ab
P6	Azolla-based biofertilizer 10 mL L^{-1}, 3 times application	6.45 ab
P7	Azolla-based biofertilizer 15 mL L^{-1}, 1 time application	6.17 a
P8	Azolla-based biofertilizer 15 mL L^{-1},	6.45 ab
P9	2 times application	6.61 b
P10	Azolla-based biofertilizer 15 mL L^{-1},	6.54 ab
P11	3 times application	6.32 ab
P12	Azolla-based biofertilizer 20 mL L^{-1},	6.28 ab
	1 time application	
	Azolla-based biofertilizer 20 mL L^{-1},	
	2 times application	
	Azolla-based biofertilizer 20 mL L^{-1},	
	3 times application	

Note: Numbers followed by the same letter are not significantly different at the 5% Duncan test level.

Table 2 shows that the treatment of Azolla-based biofertilizer 10 mL L^{-1} 1 application (P4) and Azolla-based biofertilizer 15 mL L^{-1} 3 times application (P9) was not significantly different but significantly different from P7. Both treatments had a pH of 6.60 and 6.61 higher than the treatment of Azolla-based biofertilizer 15 mL L^{-1} 1 application. Treatments P4 and P9 showed that the application of biofertilizers could increase soil pH. In addition, this study used cow manure which can bind H^+ ions in the soil, so that the concentration of H^+ ions is less than OH^- ions. According to Widyati (2013), the process of nitrogen absorption in the form of nitrate by plant roots produces hydroxyl compounds that make the rhizosphere more alkaline. Organic matter (cow manure) undergoes a decomposition process to produce humus and increase the affinity of OH^- ions originating from carboxyl groups (-COOH) and phenolic compounds. The presence of OH^- will neutralize H^+ ions in the soil solution so that the concentration of H^+ ions can be exchanged to decrease.

Azotobacter population

In Table 3, the control treatment (P0) compared with other treatments did not show any difference to the Azotobacter population. Biofertilizer containing *Azotobacter* sp. which is applied is expected to increase the population of *Azotobacter* sp. on the growing medium.

Table 3. Effect of Azolla-based Biofertilizer on *Azotobacter* sp. population

Code	Treatments	Population x 10^5 (CFU ml^{-1})
P0	Control	74,22 a
P1	Azolla-based biofertilizer 5 mL L^{-1}, 1 time application	60,36 a
P2	Azolla-based biofertilizer 5 mL L^{-1}, 2 times application	76,61 a
P3	Azolla-based biofertilizer 5 mL L^{-1}, 3 times application	77,52 a
P4	Azolla-based biofertilizer 10 mL L^{-1}, 1 time application	68,66 a
P5	Azolla-based biofertilizer 10 mL L^{-1}, 2 times application	66,50 a
P6	Azolla-based biofertilizer 10 mL L^{-1}, 3 times application	65,50 a
P7	Azolla-based biofertilizer 15 mL L^{-1}, 1 time application	66,16 a

P8	Azolla-based biofertilizer 15 mL L^{-1}, 2 times application	110,25 a
P9	Azolla-based biofertilizer 15 mL L^{-1}, 3 times application	94,66 a
P10	Azolla-based biofertilizer 20 mL L^{-1}, 1 time application	84,33 a
P11	Azolla-based biofertilizer 20 mL L^{-1}, 2 times application	101,16 a
P12	Azolla-based biofertilizer 20 mL L^{-1}, 3 times application	62,66 a

Note: Numbers followed by the same letter are not significantly different at the 5% Duncan test level.

The results that are not significantly different in the table above are thought to be due to the presence of indigenous microbes native to the habitat in the growing media. This causes the Azotobacter bacteria in the given biofertilizer to experience competition with other microbes in obtaining the nutrients needed, thereby inhibiting bacterial growth (Fitriani et al., 2017). According to Sumathi et al. (2012), indigenous microbes have relatively better adaptability, efficiency and competitiveness than introduced microbes and in addition to competition, the consistency of introduced microbial will affect the success of the introduction of biofertilizers (Triplett & Sadowsky, 1992). Shantharam & Mattoo (1997) suggested that microbes for biofertilizers should be isolated from certain areas and inoculated back into the same environment to ensure successful inoculation. Diagnosis of the quality of biofertilizers in the evaluation of their use has not been widely carried out, including viability during the dormant period in storage and microbial activity after application.

In general, the increase in concentration of biofertilizers increases the population of *Azotobacter* sp. The increase in the average population of *Azotobacter* sp. influenced by the growing media environment. According to Munawar (2011) growing environmental conditions such as pH and temperature that support will increase the population of *Azotobacter* sp.

Pakcoy Plant Yield

The maximum growth of pakcoy plants can be supported by optimal soil pH, N-uptake, and Azotobacter population. Table 4 shows that the fresh weight of pakcoy plants that were given Azolla-based biofertilizer 10 mL L^{-1} 2 times (P5) yielded 184 g and was significantly different from the treatment with higher concentration of biological fertilizers (P7, P9, P11). This proves that the addition of low concentration of azolla-based biofertilizer can increase the yield of pakcoy.

The application of lower concentrations and intervals of biofertilizers showed higher yields than the application of higher concentrations and intervals. The nutrient content contained in the P5 treatment was more adequate than P7, P9, and P11. This is due to the rapid increase in the microbial population in the soil which causes competition for space and nutrients, which in turn reduces the microbial population. In the growing media, microbes from biofertilizers will compete, so that in the end only certain microbes live dominantly. In Table 2 the N-absorption value of P9 treatment has a low value compared to other treatments, causing the growth of pakcoy fresh weight to be low.

Table 4. Effect of Azolla-based Biofertilizer on Fresh Weight of Pakcoy

Code	Treatments	Fresh Weight (g)
P0	Control	178.66 bc
P1	Azolla-based biofertilizer 5 mL L^{-1}, 1 time application	140.66 abc
P2	Azolla-based biofertilizer 5 mL L^{-1}, 2 times application	156.33 bc
P3	Azolla-based biofertilizer 5 mL L^{-1}, 3 times application	148.66 abc
P4	Azolla-based biofertilizer 10 mL L^{-1}, 1 time application	158.33 bc
P5	Azolla-based biofertilizer 10 mL L^{-1}, 2 times application	184.00 c
P6	Azolla-based biofertilizer 10 mL L^{-1}, 3 times application	152.33 abc
P7	Azolla-based biofertilizer 15 mL L^{-1}, 1 time application	120.66 ab
P8	Azolla-based biofertilizer 15 mL L^{-1}, 2 times application	128.33 abc
P9	Azolla-based biofertilizer 15 mL L^{-1}, 3 times application	94.66 a
P10	Azolla-based biofertilizer 20 mL L^{-1}, 1 time application	135.00 abc
P11	Azolla-based biofertilizer 20 mL L^{-1}, 2 times application	170.66 ab
P12	Azolla-based biofertilizer 20 mL L^{-1}, 3 times application	132.00 abc

Note: Numbers followed by the same letter are not significantly different at the 5% Duncan test level.

The use of Azolla as a carrier for N-fixing biofertilizers increases the intake of N into the soil. Colnaghi et al. (1997) stated that ammonium excreted out of cells by N-fixing bacteria can be directly utilized by plants and other organisms. The presence of N-fixing bacteria around plant roots can increase the contribution of these bacteria in providing N compounds for plants. The added Azolla can also increase the N content of the soil because Azolla organic fertilizer contains N 4.92% N of its dry weight (Bhuvaneshwari and Kumar, 2013).

4.CONCLUSION

Based on the results of the research that has been done, the following conclusions can be drawn:

1. The application of Azolla pinnata-based biofertilizer with various concentrations and application time had a significant effect on increasing Inceptisol plant N-uptake, soil pH, and pakcoy crop yields.
2. Application of biofertilizer with a concentration of 10 mL L^{-1} 2 times was a treatment that showed better yield and N-uptake of pakcoy plants than the treatment with a higher concentration and interval of application.

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