

Effect of Liquid Biofertilizer on Soil Nitrogen And Phosphorous, And Yield of Choy Sum (*Brassica Rapa* L.) Grown in Pot Culture

Reginawanti Hindersah, Pujawati Suryatmana, Betty Natalie Fitriatin,
Mieke Rochimi Setiawati

Faculty of Agriculture Universitas Padjadjaran
Jalan Raya Bandung-Sumedang Km 21 Jatinangor 45363, West Java, Indonesia

ABSTRACT: Biofertilizer is an important agricultural input to reduce the use of inorganic fertilizer and maintain soil health in sustainable agriculture including in leafy vegetable production. The pot experiment to evaluate the change of available nitrogen and phosphorus in soil as well as yield of choy sum (*Brassica rapa*, L.) following inoculation of liquid biofertilizer and NPK fertilizer has been conducted. Experiment was set up in Randomized Complete Block Design which tested some combination of liquid biofertilizer and NPK fertilizer dosages. Biofertilizer contains nonsymbiotic nitrogen fixing bacteria and phosphate solubilizing microbes. This pot experiment verified that consortium of biofertilizer has a significant role to provide major nutrient nitrogen and phosphate in soil. Furthermore, biofertilizer inoculation with NPK fertilizer level up to 75% increased either available nitrogen and phosphate compared to that of 100% NPK fertilizer. It was evidence that fresh shoot weight of plant inoculated with biofertilizer combined with 25% to 75% level of NPK were not differ from that of plant received 100% NPK fertilizer. It was suggested that mixed biofertilizer can lower inorganic fertilizer rate.

Keywords: Biofertilizer; Choy sum; Inorganic fertilizer; Plant Nutrient

I. INTRODUCTION

Application of organic fertilizer, inorganic fertilizer and biofertilizer in appropriate composition is necessary to maintain soil quality and yield in sustainable agriculture. Those fertilizers will keep optimal soil biological and chemical processes which ensure plant nutrients available in sufficient and balanced quantities (Chen, 2008). It has been reported elsewhere that combine used of biofertilizer and inorganic fertilizer is important to maintain plant productivity (Anwar and Gitosuwondo, 2011; Vahed et al., 2012; Namvar and Khandan, 2013).

Soil bacteria and fungi are widely used as biofertilizer as well as biostimulant in sustainable and organic farming. Some different genera of nonsymbiotic Nitrogen-fixing bacteria has been recommended and used as biofertilizer in non leguminous plant production (Bhattacharjee et al., 2008). Nitrogen-fixing bacteria *Azotobacter* and *Azospirillum* which colonize plant rhizosphere might induce plant growth by exerting beneficial effects through dinitrogen (N_2) fixation and phytohormone synthesis (Wani et al., 2013; Spaepen et al., 2008). Considerable amount of phosphate solubilizing bacteria such as *Pseudomonas* and *Bacillus* associated with plant rhizosphere have increased available soil phosphate and exert phytohormones which are beneficial for plant growth (Rodríguez and Fraga, 1999). Many soil fungi also well known as effective phosphate solubilizers, some *Penicillium* species were found to solubilize rock phosphate in liquid or solid culture (Asea et al., 1988; Pandey et al., 2008).

Both nonsymbiotic nitrogen-fixing bacteria and phosphate-solubilizing microbes is well known as Plant Growth Promoting Rhizobacteria (PGPR). The ways by which they promote plant growth is increasing the availability of nitrogen and phosphate respectively to plant. In Indonesia, estimated used of biofertilizer reaches approximately 30,000 to 50,000 t/year for food crops production.

Nowadays, liquid biofertilizer containing microbial consortia is purchased to substitute inorganic fertilizer. We have been isolated some beneficial soil microbes, nonsymbiotic N_2 -fixing *Azospirillum* sp., *Azotobacter chroococcum*, *A. vinelandii*, *Acinetobacter* sp.; phosphate solubilizing microbe (PSM) *Pseudomonas cepacea* and *Penicillium* sp. from the rhizosphere and roots several economically important crops. Beneficial microbes were formulated in liquid biofertilizer by using molasses which is high in organic matter mainly to support carbon and nitrogen availability for heterotroph microbes. To identify the capacity of that liquid biofertilizer on plant production, a pot experiment was conducted by using leafy vegetable choy sum (*Brassica rapa*, L.). The experiment was conducted to evaluate the change in available N and P in soil as well as yield of choy sum following inoculation of liquid biofertilizer consist of nonsymbiotic N-fixing bacteria, phosphate solubilizing bacteria and phosphate solubilizing fungi.

II. MATERIAL AND METHOD

Experiment was conducted in green house at Faculty of Agriculture Universitas Padjadjaran at the altitude of 772.5 m above sea level during end of rainy season in 2014. The site falls under tropical zone. Liquid biofertilizer registered as Bion-UP belong to Soil Biology Laboratory; consist of nitrogen fixing bacteria *Azotobacter chroococcum*, *A.vinelandii* and *Azospirillum* sp., *Acinetobacter* sp. and PSM *Pseudomonas cepacia* and *Pencillium* sp. All microbes were isolated by using certain defined media; the pure culture of three nitrogen-fixing bacteria were maintain in nitrogen-free media while that of PSM in Pikovskaya media. Fertilizer were produced in molasses-based liquid culture for 72 hours in batch fermenter; bacterial and fungal concentration were 10^8 cfu/mL and 10^5 cfu/mL respectively.

The soil was Inceptisols taken from Jatinangor West Java. The soil texture was silty clay loam; low-fertility soil which was slightly acid (pH 5.8). Chemical characteristics of soil was low in organic carbon (1.5%), low in total Nitrogen (0.2%), moderate in available P_2O_5 (33.51 mg/kg) and total P_2O_5 (24.71 mg/100g), and low in total K_2O (15.21 mg/100g).

2.1 Experimental set up

The experiment was set up in Randomized Completed Block Design with three replication which tested seven combinations of biofertilizer and NPK compound fertilizer. The treatments were without biofertilizer nor NPK fertilizer (A), biofertilizer (B), biofertilizer with 100% of NPK fertilizer (C), biofertilizer with 75% NPK fertilizer (D), biofertilizer with 50% NPK fertilizer (E), biofertilizer with 25 % NPK fertilizer (F) and 100 % (recommended) NPK fertilizer (G). Recommended NPK compound fertilizer (N:P:K; 116:16:16) for choy sum was 500 kg/ha.

Soil were collected from top soil of 0-20 cm, air dried for three days and sieved through 5 mm sieve. Polybags was filled with 5 kg soil mixed with 50 g cow manure (pH 6.21, organic C 33.53%, total N 1.8%, C/N 18, total P_2O_5 1.2% and K_2O 3.71%) and incubated at green house for 5 days before planting.

Choy sum seeds was sown on mixture of soil and manure (1:1; v:v) media in nursery box for 4 days. After that, they were transplanted to 2.5 cm height polybag contained the same growth media, and maintained for 12 days. Single transplant was grown on each polybag.

NPK fertilizer is applied at six and 15 days after planting in two equal hole and then thoroughly mixed up with the soil. The depth of individual hole was 5 cm at the distance of 5 cm from plant. Biofertilizer was diluted to final concentration of 10^6 cfu/mL by using destilated water. Plants treated three times with liquid biofertilizer through soil dressing at 4, 13 and 19 days after planting (dap) as much as 50 ml for each application. All plants were maintained in green house for 24 days. Number of leaf was counted at 7, 14 and 21 dap. At the end of experiment; 24 dap, available N ($N-NH_4^+$ and $N-NO_3^-$) and available P were analyzed. Shoots were separated from roots and weigh freshly to obtain plant yield. All data except total leaf were subjected to analysis of variance (F test) at $P < 0.05\%$ followed by Duncan's Multiple Range Test if the effect of treatments on experimental parameters was significant.

2.2 Soil Nutrients Analysis

For ammonium and nitrate analysis, soil immediately crushed and sieved using 2 mm sieve, digested with Morgan-Wolf extract. To determine ammonium, buffer tartarate and Na-fenat were added to soil extract followed by NaOCl 5% prior to analyze ammonium by using spectrophotometer at wavelenght of 636 nm. Brucine solution and 5 mL of concentrated H_2SO_4 were added to soil extract prior to analyze nitrate by using spectrophotometer at wavelenght of 494 nm.

Available P in soil was estimated by calorimetry. After crushed and sieved using 2 mm sieve, soil was shaken with an extracting solution of 0.03 N NH_4F in 0.025 N HCl. Phosphorus is estimated calorimetrically by adding Ammonium Molybdate and thereafter, reducing Molybdenum Phosphate complexes with Stannous Chloride in the acidic medium.

III. RESULTS AND DISCUSSION

3.1. Available N and P in Soil

Nitrogen (N) and phosphorous (P) are most often responsible for limiting crop yields in Indonesia. In low nitrogen soil, role of N fixing bacteria is significant since nitrogenase responsible to change dinitrogen to ammonium is reversibly inhibit by high available nitrogen (Fu and Burris, 1989). Biofertilizer inoculation with and without different level of NPK fertilizer in low fertility Inceptisols clearly increased $N-NO_3^-$ and $N-NH_4^+$ as well as available P (Table 1).

Table 1. Effect of Biofertilizer and NPK fertilizer on soil available N and P of caysim at 24 days after planting

Treatment code	Fertilizer	N (mg kg ⁻¹)*		P ₂ O ₅ * (mg kg ⁻¹)
		NO ₃ ⁻	NH ₄ ⁺	
A	Control	4.12 a	8.09 a	15,72 a
B	Biofertilizer	7.07 d	14.47 d	27,15 d
C	Biofertilizerwith 100% NPK fertilizer	9.6 b	9.36 b	16,18 a
D	Biofertilizerwith 75% NPK fertilizer	7.72 e	16.99 e	32,30 e
E	Biofertilizerwith 50% NPK fertilizer	7.01 d	12,55 c	21,36 b
F	Biofertilizerwith 25% NPK fertilizer	7.37 de	12,16 c	21,49 b
G	100% NPK fertilizer	5.81 c	9,81 b	24,67 c

*Numbers in a column followed by same letters were not significantly differ based on 5% Duncan’s Multiple Range Test

Nonsymbiotic N fixing bacteria is PGPR that provide significant amount of available N in soil and play a key point in sustainable agriculture. Mazinani et al (2012) showed that *Azotobacter* population after inoculation correlate with soil total nitrogen. Efficient strains of *Azotobacter*, *Azospirillum*, *Phosphobacter* and *Rhizobacter* provide significant amount of nitrogen to *Helianthus annus* (Dhanasekar and Dhandapani, 2012). Free living diazotrophic bacteria also showed capacity to increase available P content as well as total N in soil (Berger et al., 2013).

Tropical soil mostly contain high amount of total P but low available P. Phosphate solubilizing microbes (PSM) are sensitive to the presence of high available P due to feed back regulation (Mikanova and Novakova, 2002). So that in this experiment, soil contain moderate available P which still support PSM activity. Quantification of phosphorus solubilized in liquid or solid culture of PSM has been discribed elsewhere. The capacity of 10 Phosphate solubilizing bacteria isolated from vegetable rhizosphere to solubilize phosphorous wasranged from 5.32-151 µg/mL (Alia et al., 2013). *Bacillus* sp. and *Burkholderia* sp., respectively, were the most effective, mobilizing 67% and 58.5% of the total P (Ca₃(PO₄)₂) after 10 days (Oliviera et al., 2009). *Pseudomonas fluorescens* K - 34 solubilized tricalcium phosphate and produced substantial amount of soluble phosphorus up to 968.5 mg/L in Pikovskaya’s media (Parani and Saha, 2012).

Although well known as endophytic N fixing bacteria, *Acinetobacter* was documented to have phosphate solubilizing capacity. N fixing *Azotobacter* strains were also effective to solubilizetricalcium phosphate in agar media; which range from 167-888 µg/ml P due to their ability to synthesis gluconic acid (Ogut and Kandemir, 2010). Seven species of *Penicillium* showed maximumsolubilization after 15 of incubation, followed with decrease in pH of the liquid culture (Pandey et al., 1008).

3.2 Plant Growth and Yield

Effect of mixed biofertilizer on total leaf of choy sum at 7, 14 and 21 dap demonstated no specific pattern (Fig 1). However, plant received biofertilizer with 75% NPK and with 25% NPK had higher total leaf than the other or control treatment.

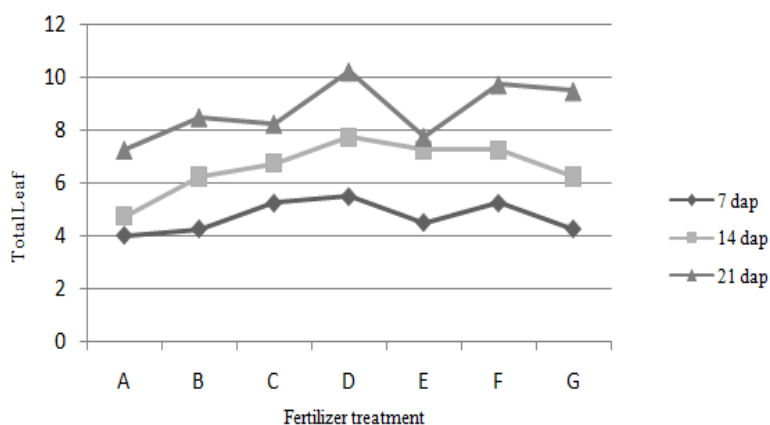


Fig 1. Leaf number of choy sum at 7, 14 and 21 days after planting following biofertilizer and inorganic fertilizer treatment. A: Control; B: Biofertilizer; C: Biofertilizer with 100% NPK fertilizer, D: Biofertilizer with 75% NPK fertilizer; E: Biofertilizer with 50% NPK fertilizer, F: Biofertilizer with 25% NPK fertilizer; G: 100% NPK fertilizer

Plant treated with biofertilizer and 75% NPK fertilizer (D) showed more leaf number compared to another treatment at day 21. It is demonstrated that either biofertilizer and NPK fertilizer had a significant role to enhance leaf number.

Analysis of variance showed that fertilizer treatments had a significant effect on plant height and yield of choy sum (Table 2). Plant height in C, D, F and G treatment has increased in amount of 43%, 46%, 66% and 58% respectively than control. It is clearly showed that combination of biofertilizer and any level of NPK fertilizer increased yield and suggested decreased the uses of NPK fertilizer.

Increased of leaves number indicated the effectivity of this combined biofertilizer to induced plant growth. Various mechanisms have been reported for biofertilizer to increase plant growth and yield. In addition to provide nitrogen, *Azotobacter*, *Azospirillum* and endophytic *Acinetobacter* also recognized to produce phytohormones. Rhizobacteria *Acinetobacter radioresistens* synthesize Indole Acetic Acid (IAA); higher IAA production was observed in the presence of precursor L-TRP (Yasmin et al., 2009). Mali et al. (2011) reported that *Azotobacter chroococcum* isolated from groundnut rhizosphere produced phytohormones IAA and gibberellins (GA). *Azotobacter chroococcum* also was detected to synthesize IAA, Gibberellic acid and Kinetin (Narula et al., 2006). *Azospirillum* have been known for many years as PGPR which induce the plant growth through IAA (auxin) synthesis. Auxin production by *Azospirillum* sp. in liquid media was induced by 0, 2.5, 625 and 5000 ppm tryptophan at 24, 48 and 72 h after incubation (Moghaddam et al., 2012).

Table 2. Effect of Biofertilizer and NPK fertilizer on shoot height and yield at 24 days after planting

Treatment code	Fertilizer	Shoot height (cm)*	Yield (g)*
A	Control	15.1 a	12.2 a
B	Biofertilizer	17.5 a	33.1 ab
C	Biofertilizer with 100% NPK fertilizer	21.7 b	57.9 b
D	Biofertilizer with 75% NPK fertilizer	22.1 b	48.9b
E	Biofertilizer with 50% NPK fertilizer	21.9 b	58.4 b
F	Biofertilizer with 25% NPK fertilizer	25.1 b	49.2 b
G	100% NPK fertilizer	24.0 b	47.1 b

*Numbers in a column followed by same letters were not significantly differ based on 5% Duncan's Multiple Range Test.

Phosphate solubilizing bacteria (PSB) also reported to have capacity in phytohormone synthesis. Some strain of PSB synthesized IAA and GA₃ in significant amount (Sri Ramkumar and Kannapiran, 2011). Increased available nitrogen supposed influence phosphate solubilizing by PSM. Asea et al. (1988) showed that *Penicillium bilaji* and *Penicillium cf. fuscum* that presence of N-NH₄⁺ in the medium was necessary for increased P solubilisation since N-NH₄⁺ affected the duration of the lag phase before the two isolates began to solubilize inorganic P. So that N-fixing bacteria in this experiment indirectly might take an important role to enhance P solubilization.

The beneficial effects of N fixing bacteria and PSM on plant growth and productivity have been widely described. By using dual inoculation *Azotobacter* and *Azospirillum*, higher shoot and seed yield of Black Cumin (*Nigella sativa* L.) were obtained (Ghanepasand et al., 2014). A pot experiment verified that inoculation of *Azotobacter* and *Azospirillum* separately and in combination recorded higher plant growth and yield in *Brassica juncea*; the combination of half dose of both bacteria improved plant growth and yield (Khan et al, 2010).

Inoculation of plants with biofertilizer generally improved plant growth and yield, especially under greenhouse conditions. In this pot experiment, combined biofertilizer significantly increased yield of choy sum. The similar results were showed by 15 PGPR isolates when applied to a local chilli cv 'Suryamukhi' in pots (Datta et al., 2011). A pot experiment conducted by Kannapiran and Sri Ramkumar (2011) verified that single inoculation of nitrogen fixing bacteria and PSB increased shoot height, root lengths, and total dry mass of 15 days old black gram (*Phaseolus mungo* Roxb; Eng).

Effect of biofertilizer on plant development and yield was demonstrated; biofertilizing is a sustainable way in plant production. Anwar and Gitosuwondo (2011) verified that biofertilizer increased the yields of choy sum when combining with recommended but biofertilizer alone did not significantly increases yields in Ultisols, very low fertility soil; they also proved that effectivity of biofertilizer could be improved by reducing quarter dose of NPK fertilizer. In our experiment, without NPK fertilizer, bacterial inoculation could not increase plant height compared to that of control (Table 2). However biofertilizer application with any level of NPK fertilizer increased shoots weight of choy sum compared to control treatment. Either NPK fertilizer without biofertilizer, or lower doses of NPK with biofertilizer significantly increased plant height.

IV. CONCLUSION

This experiment demonstrated significant effect of biofertilizer with and without NPK fertilizer on either available N and P in soil and choy sum biomass. Shoot height of choy sum treated by either biofertilizer with and without NPK and 100% NPK were higher than that of control plant. However at 24 days after planting, nitrate content in soil inoculated with biofertilizer with lower rate of NPK did not differ from that with NPK recommended dosage. The highest ammonium content was in soil inoculated with biofertilizer with recommended rate of NPK fertilizer. In soil with recommended dose of NPK fertilizer, adding biofertilizer enhanced available P clearly but in lower rate of NPK, biofertilizer decreased available P in soil. Regarding the plant yield, result verified that biofertilizer inoculation with lower level of NPK fertilizer did not decrease yield of choy sum. It was evidence that consortium of biofertilizer could substitute NPK fertilizer up to 75%.

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