p-ISSN 0854-9028 e-ISSN 2549-9343
AGRIC
JURNAL ILMU PERTANIAN
Fakultas Pertanian dan Bisnis Universitas Kristen Satya Wacana
Jl. Diponegoro 52-60 SALATIGA 50711 - Telp. 0298-321212 ext 354
email: jurnal.agric@adm.uksw.edu, website: ejournal.uksw.edu/agric
Terakreditasi Kementrian Riset, Teknologi dan Pendidikan Tinggi berdasarkan SK No 200/M/KPT/2020

BIOFERTILIZER-COATED UREA FOR PROMOTING YIELD OF LETTUCE AND REDUCING NITROGEN FERTILIZER DOSE IN FIELD EXPERIMENT

Reginawanti Hindersah[1*], Dhia Rhasajati[2], Rara Rahmatika Risanti[3], Mieke Rochimi Setiawati[1], Betty Natalie Fitriatin[1]
[1] Faculty of Agriculture Universitas Padjadjaran, Indonesia.
[2] Agrotechnology Undergraduate Program, Faculty of Agriculture Universitas Padjadjaran, Indonesia.
[3] Graduate of Soil Science Master Program, Faculty of Agriculture Universitas Padjadjaran, Indonesia
e-mail correspondence: reginawanti@unpad.ac.id

Received: 13 July 2022 | Accepted: 31 October 2023

ABSTRACT

Urea intensive application is considered to reduce soil quality, and food quality. The constraints of urea application in tropics are low effectivity due to intensive leaching and volatilization. In order to decrease the urea dose, cheap and renewable biofertilizer application is suggested. Coating urea with biofertilizer become an easy way to apply both biofertilizer. In the previous research, biofertilizer-coated urea (BCU) had been formulated by using Bacillus subtilis, B. megaterium, Azotobacter chroococcum and A. vinelandii. The objective of this research was to verify the effect of BCU on the growth and yield of lettuce (Lactuca sativa L.) cv. Romaine; and decrease of urea dose in a planting season. The field experiment was arranged in randomized block design with five treatments and five replications. Lettuces were treated with a dose and half dose of two BCU formulations. The results showed that BCU didn't affect growth, P uptake and plant yield but half dose of BCU produced the same lettuce productivity with one dose of urea. Half dose of BCU resulted in the same shoot N uptake as one dose of urea but a dose of particular BCU formulation possibly decreased N uptake. The population of total Bacillus and Azotobacter in the rhizosphere was determined by dose and BCU formulation but they didn't change abruptly after BCU application. The experiment suggested that application of urea coated by carrier-based biofertilizer was reliable to support lettuce cultivation and reduce the urea dose.

Keywords: Azotobacter; Bacillus; Carrier-based biofertilizer; Nitrogen uptake, Phosphor uptake

INTRODUCTION

Urea is an important input for agriculture but they are easily lost through volatilization resulted in reduced efficiency of nitrogen uses mainly in dry land. Loss of ammonia from urea in dry land is reported up to 24 percent in sugar cane plantation and 12-24 percent in sorghum (Mariano et al., 2012; Wallace et al., 2020). A way to prevent ammonia volatilization is coating the urea with materials such as polymers and organic matter (Geng et al., 2016; Sigurdarson et al., 2018). Coating the urea by organic matter can be accompanied with beneficial-microbe enrichment. The water holding capacity and negative charge of organic matter maintain the adaptability and viability of microbial cells (Abd El-Fattah et al., 2013).

Nowadays, biofertilizer contained Plant Growth Promoting Rhizobacteria (PGPR) is suggested as an integral part of balance fertilizers in agriculture. The PGPR inoculation in horticultural crops is suggested to enhance the yield, reduce the diseases intensity (Constantia et al., 2023; Hindersah et al., 2021; Kalay et al., 2020). Despite of the positive effect of PGPR on plant growth, only a few Indonesia farmers are willing to apply biofertilizers. Otherwise, they introduce inorganic fertilizers including urea in food crops production. Coating urea with organic carrier-based biofertilizers is suggested to increase beneficial-microbe application and minimize urea volatilization. In order to achieve that objective, urea coating microbes must be resistant to drought because the water content of urea is only 0.5 percent. Soil microbes with that characteristics are the PGPR Bacillus and Azotobacter which respectively form spores and cysts in dry conditions (Toyota, 2015; Sivapriya and Priya, 2017). Both PGPR is widely formulated as commercialized biofertilizer.

In soil, several Bacillus species enable to change insoluble phosphate (P) to available P for root uptake (Saeid et al., 2018). Azotobacter are non-symbiotically nitrogen fixer that convert dinitrogen to ammonia which is then transformed to nitrate by nitrifying bacteria (Barth et al., 2020). Phytohormone production of both soil bacteria are widely examined (Rubio et al., 2013; Saxena et al., 2020). Furthermore, in some circumstances, they tolerate biotic and abiotic stress in soil (Bal et al., 2013; Syed and Chinthala, 2015; Hindersah et al., 2019). Researchers elsewhere have been reported plant growth and yield increment of food crops following Bacillus as well as Azotobacter inoculation. The ability of both PGPR to reduce inorganic fertilizer level in plant production also described. The biofertilizer comprised of *B. subtilis, B. pumilus*, and *B. amyloliquefaciens* enhance leaves number and weight of lettuce heads under reduced inorganic fertilizer by 50 percent (Venancio et al., 2019). The *B. subtilis* 21-1 increased yield of lettuce and Chinese cabbage along with reduced soft rot disease incidence to 23.5 percent and 45 percent respectively (Lee et al., 2014). Azotobacter inoculation in cauliflower substituted 50 percent NPK fertilizers while increasing the yield and improved morphological traits (Subedi et al., 2019). Introducing 50 percent NPK with microbial inoculation included Azotobacter promoted highest yield compared to 100 percent of NPK (Shahein et al., 2013).

We have been formulated solid inoculant of mixed Bacillus and Azotobacter species. Organic-based liquid inoculant contained two species of Bacillus as well as Azotobacter have been formulated to enhance cell count up to 10^{10} CFU/mL (Hindersah et al., 2020). The formulation of compost-based inoculant biofertilizers by using liquid inoculant with compost enriched with 1 percent or 5 percent zeolite might be effective to promote population of vegetative cell of both PGPR as well as Bacillus spore. The two formulations described above were used for coating the prilled urea.

In order to verify the ability of BCU to substitute urea and maintain plant growth, a field experiment has been performed for lettuce cv. Romaine, a high-priced vegetable in Indonesia urban community's diet. The objective of this experiment was to determine the growth and yield of lettuce cv. Romaine by introducing biofertilizer-coated urea (BCU); as well as the decreased of urea fertilizer doses in a field experiment.

MATERIALS AND METHODS

The field experiment has been conducted on October 2020 to December 2020 in the farmer's field. The site is located in Mekarwangi village of Cisarua District, Bandung Barat Regency in West Java. The altitude of this tropical mountainous area is 1,260 m above sea level with the average temperature of 17 ^{0}C – 28 ^{0}C.

The volcanic soil in the field experiment before experiment was a slightly acid (pH of 5.87) and silty clay Andisols The soil was low in organic C (1.62 percent), total N (0.19) and C/N (8); very high in total P_2O_5 (60.78 mg/100 g); and high in available phosphate (10.75 mg/kg) as well as potassium (44.21mg/kg). The cation exchange capacity of soil was as high as 27.58 cmol/kg. The chemical status of those parameters was based on soil fertility evaluation released by Pusat Penelitian Tanah (1995)

Two formulations of BCU have been prepared a week before application in the Laboratory of Soil Biology, Faculty of Agriculture, Universitas Padjadjaran. The compost-based solid biofertilizer has been made from liquid inoculant with certain concentration of zeolites. Liquid inoculant described above was composed of two species of phosphate-solubilizing Bacillus and two species of nitrogen-fixing Azotobacter. The coating material for A formula (FA) consisted of 200 mesh composted cow manure enriched with 1 percent of 100-mesh zeolite and inoculated with 10 percent (v/v) mixed liquid inoculant; while the B formula (FB) was coated by using composted cow manure with 5 percent zeolite and 5 percent liquid inoculant.

Experimental Design

The experimental research has been setup in randomized block design to test single experimental factor i.e the formulations and the doses of BCU. The control treatment was application of recommended dose of conventional prilled Urea provided by Indonesian fertilizer company, PT Pupuk Kujang in Cikampek of West Java. The treatments were:

1. Control (recommended doses of Urea)
2. FA-1 (Full-dose of A formula)
3. FB-1 (Full-dose of B formula)
4. FA-0.5 (Half-dose A formula)
5. FB-0.5 (Half-dose B formula)

All treatments were replicated five times. The individual BCU treatment was applied in 1 m x 3 m bed with 20 cm in height. According to Technical lettuce cultivation released by Horticultural Plant Research Institute, the recommended dose of N fertilizer for lettuce is 60 kg/ha which is equal to 130 kg/ha of urea. The A formulation of BCU was made by coating urea with solid biofertilizer consisted of 200-mesh manure enriched with 1percent zeolite with 10 percent of liquid inoculant of mixed Bacillus and Azotobacter; while the biofertilizer in B formulation contained 5 percent zeolite with 5 percent initial liquid inoculant.

Field Experiment Establishment

A total of 5 beds with the dimension of 1 m x 16 m were prepared by fields hoeing and tilling at 20 cm depth. That plowing has been carried out along with chicken manure application at the rate of 20 t/ha. Each single bed was divided into 5 smaller beds of 1m x 3m with 0.25 m free space between beds

The 3-weeks old lettuce seedlings were grown in 3 m^2 bed with the planting distances of 20 cm (Figure 1). Two days after planting, half doses of BCU and Urea (control plots) and a dose of SP-36 and KCl have been introduced on the 2-cm depth hole at the distance of 2 cm from the lettuce stem. Another half dose of both nitrogen fertilizers was applied 12 days after based on the treatments.

Experimental Traits and Statistical Analysis

The growth traits included plant height, leaves number as well as fresh and dry weight of above ground plant have been measured at 4 weeks after transplanting. At the same time, vegetative cell and spore of Bacillus as well as vegetative cell of Azotobacter were enumerated in the rhizosphere by serial

Figure 1 Lettuce seedling at transplanting day in experimental beds (a) and the crop at the harvest time, 4 weeks after planting (b).

dilution plate method (Ben-David and Davidson, 2014). The N and P content of the shoot was analyzed by AOAC method (AOAC, 2012); and each essential nutrient uptakes by plant shoots were calculated by multiplying the nutrient content with the dry weight.

Plant and soil samples were taken up from the three-row column in the middle of bed. Soil samples were collected from 10-cm deep around the plant roots. No samples had been obtained from the border of plot. A total of 10 samples were recovered from the plant in the middle area (Figure 1) for plant growth and nutrient uptake measurement. All plant material was wrapped in paper bag and put in the oven at 70 ^{0}C for two days in order to dry weight determination. That data depicted in the results section were the average of 10 samples. The rhizosphere soil from 10 plant samples were mixed evenly, pour into the sealed white polyethylene bag, and put in the cold box before transporting to laboratory for bacterial enumeration. The population of Bacillus vegetative cell and spores as well as Azotobacter population of rhizosphere have been performed by serial dilution plate method.

All data was analyzed by variance analysis to verify the effect of treatment on all measured traits. If the sum square of the treatments on certain parameters was significant, the Duncan's Multiple Range Test ($p<0.05$) was done. All statistical analysis has been carried out by Sigmastat 3.1

RESULTS AND DISCUSSION

Plant Growth

According to the analysis of variance, BCU didn't affect shoot height, leaves number, as well as shoot dry weight. Both formulation and doses of BCU resulted in the equal growth traits compared to the plant with conventional Urea (Fig 2). Regardless of statistical analysis, plants received B formula of BCU (FB-0.5) had a potency to have a similar shoot dry weight with the control one (Fig 2c). Moreover, application of half-dose FB-0.5 resulted in better plant growth traits although the values were not significantly different based on Duncan's Multiple Range Test ($p<0.05$).

Application of BCU didn't change all bacterial cells (Table 1). After heating soil suspension by 80^{0}C, the spore counts were only 0.1 percent of vegetative cell. Despite

Table 1 Population of Bacillus and Azotobacter in the rhizosphere of 4-week old lettuce treated by different formulations and doses of biofertilizer-coated Urea

Biofertilizer-coated Urea	Bacillus		Azotobacter (10^6 CFU/g)
	Vegetative cells (10^8 CFU/g)	Spores (10^5 CFU/g)	
Control	3.98	0.88	3.36
FA-1	5.22	1.14	2.48
FB-1	5.38	1.08	2.62
FA-0.5	6.66	1.02	3.94
FB-0.5	5.74	1.00	3.18

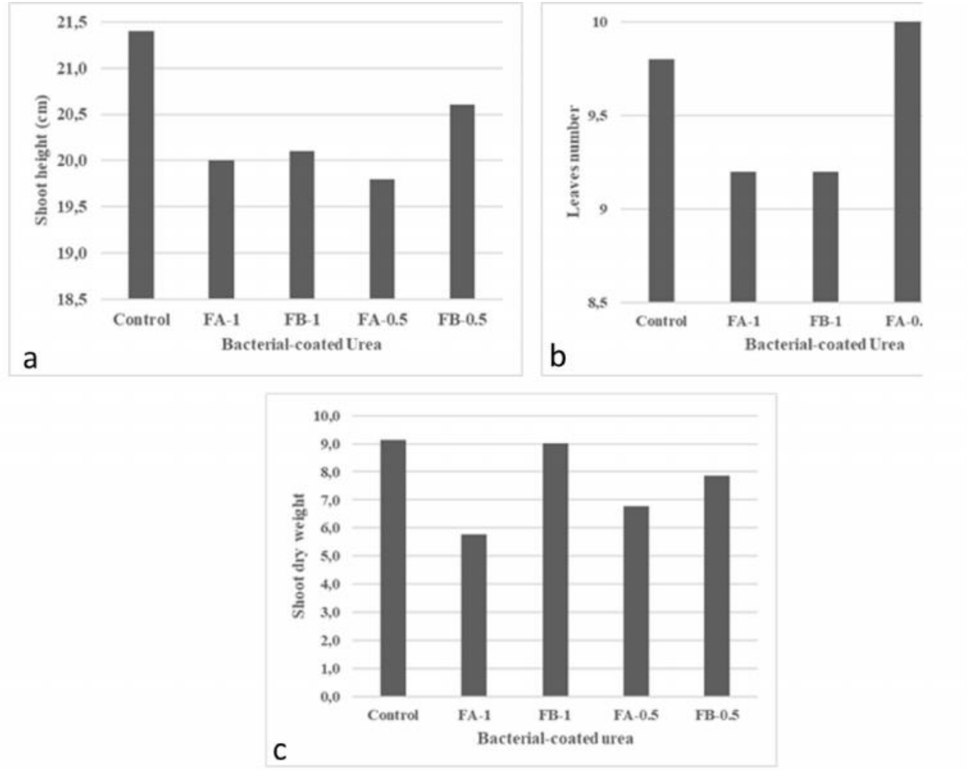

Figure 2. Effect of two formulation and doses of biofertilizer-coated urea on shoot height (a), leaves number (b) and dry weight (c) of lettuce at 4 weeks after transplanting.

of no significant difference between treatments, plant with half doses of BCU (FA-0.5 and FB-0.5) showed highest vegetative cell of Bacillus and Azotobacter in the rhizosphere compared to another treatment. In overall, the population of Azotobacter was lower than Bacillus vegetative cell count.

The N content of above ground plants was not influence by any formulations and doses of BCU (Table 2). Despite of similar dry weight of all plants treated with BCU, the N uptake of plant shoot was clearly depending on the urea application. All BCU application resulted in relatively lower N uptake compared to the control but B formulation of BCU (FB) consistently resulted in the higher N uptake than A formulation (FA).

Means followed by the same letter are not significantly different based on Duncan Test ($p<0.05$).

Table 2 Nitrogen (N) content and uptake of lettuce shoot after biofertilizer-coated Urea application

Biofertilizer-coated Urea	N content (%)	N uptake (mg/plant)
Control	4.39 a	402.07 c
FA-1	4.55 a	260.92 a
FB-1	4.49 a	406.81 bc
FA-0.5	4.64 a	314.93 ab
FB-0.5	4.38 a	347.26 abc

Table 3 Phosphorous (P) content and uptake of lettuce shoot after biofertilizer-coated urea application

Biofertilizer-coated Urea	P content (%)	P uptake (mg/plant)
Control	1.47 a	134.98 a
FA-1	1.85 a	111.34 a
FB-1	1.61 a	143.50 a
FA-0.5	1.37 a	93.34 a
FB-0.5	1.81 a	144.01a

In contrast, the P uptake of above ground plants have not affected by BCU application (Table 3). Regardless of statistical analysis, application of B formulation of BCU increased P uptake of shoot compared to that of plant shoot with A formulation.

Yield as measured by the shoot weight of individual plant and in plot were not affected by BCU application compared to the control (Table 4). Plot received half-dose of B formulation (FB-0.5) had the higher yield per hectare compared to the control and BCU.

Table 4 The yield of lettuce with different formulation and dose of biofertilizer-coated Urea application

Biofertilizer-coated Urea	Yield		
	g/plant	kg/plot	t/ha
Control	38.9 a	2.92 a	9.74
FA-1	33.3 a	2.53 a	8.34
FB-1	39.2 a	2.94 a	9.81
FA-0.5	39.2 a	2.91 a	9.80
FB-0.5	44.4 a	3.33 a	11.10

Means followed by the same letter are not significantly different based on Duncan Test ($p<0.05$)

In general, BCU application didn't influence lettuce growth traits during 4 weeks after transplanting. The similar effect was also recorded for yield traits. Certain BCU treatments clearly decreased the N uptake of shoot and potentially reduced the P uptake compared to prilled urea application. At the harvest time we verified that the yield (shoot fresh weight) in every treated plot were not different statistically. However, the population of Bacillus and Azotobacter in the rhizosphere of lettuce treated by BCU were similar to the control.

The bacterial count data verified that both exogenous Bacillus and Azotobacter proliferated in the rhizosphere of lettuce. Slight increase of each bacterial population was recorded after BCU application although it was not significantly different with the control. Both PGPR were well adapted to the lettuce rhizosphere and likely enable to use the nutrient from roots. In general, exudate of rhizosphere composed of secondary metabolites, amino acid and carbohydrate (Kiers et al., 2011; Oku et al., 2012; Nguema-Ona et al., 2013) which is an essential for heterotrophic metabolism of both bacteria. Root exudate substances play a role in the plant–microbe and plant–microbiome interaction in the rhizosphere (Huang et al., 2014). The establishment of exogenous bacteria in the rhizosphere is initial step to start their activity for promoting plant growth.

The clear decrease in N uptake and slight decrease in P uptake by lettuce shoot showed less nutrient sufficiency for plant with BCU. It is possibly due to the competition for N and P between roots and bacteria. However, the N uptake of lettuce grown with FB-1 and FB-0.5 is similar with the control. The FB is BCU coated with composted cow manure enriched with 5 percent zeolite and 5% liquid inoculant. The urea in FB was drier than FA that contain only 1 percent zeolite. It resulted in slower N release from FB. Since the initial population of Bacillus and Azotobacter in FB was lower than FA, competition with native soil bacteria might be reduced and hence increase N fixation by introduced bacteria. Both Bacillus and Azotobacter enable to fix N_2 and solubilize P (Saeid et al., 2018; Barth et al., 2020). In soil inoculated with full either half dose of FB along with Azotobacter, the population of bacteria maybe enough to reach quorum sensing to start nitrogen fixation.

The results showed that all treated plant have sufficient N and P content in fully developed leaves. The N content of treated plants were 4.39-4.64 percent (Table 2) which is categorized as adequate N content (Jones et al., 1991) for lettuce in general. Increased N fertilizer applied in lettuce cv Romaine without and with P and K fertilizer resulted in nitrate as well as nitrite accumulation in romaine leaves grown in soil and irrigated hydroponic system (Hoque et al., 2010; Mendoza-Tafolla et al., 2021).

Regardless of statistical analysis; in this current study, the higher P content of shoot were shown by plant with FA-1 (1.85 percent) dan FB-0.5 (1.81percent). According to Jones et al. (1991), the P content of 28-day old leaves lettuce in general is 0.4-0.76 percent. Reuter and Robinson (1997) stated that the leaves contain 1.61 percent P categorized as high in P. This pot experiment verified that application of biofertilizer-coated urea resulted in the increase of P content in shoot. Nitrogen fertilizer with or without P fertilizer didn’t change the P status in leaves significantly but 337 kg N/ha fertilization caused the increasing of P content in leaves up to 0.68-0.71 percent (Hoque et al., 2010).

Surprisingly, the dry mass of shoot of lettuce with BCU didn’t surpass that of control. Dry matter of plant come from photosynthesis. Nitrogen and P play an important role in photosynthesis but soil and climatic factors determine the extent of photosynthesis. The plants grown in the field during rainy season. On October-November, reduced sunlight exposure was happening in Cisarua area. Limiting solar radiation reduced the photosynthesis and the dry mass subsequently

Nonetheless full-dose of B formula has a potency to have equal shoot dry weight compared with the control. At the end of experiment, introducing BCU with B formula (FB) has a potency to surpass the yield of lettuce grown with prilled conventional urea. Despite of nonsignificant effect of BCU on yield, half doses of BCU of any formulation have the potency to improve plant growth as well as P uptake and bacterial population at the end of experiment. This field experiment showed that a half dose of BCU, mainly B formula might be replace a dose of conventional urea. At the end of experiment, the similar effect was also recorded for yield traits. Since the use of biofertilizers will be increasingly important, the results of this first experiment concerning BCU might be considered for increasing the use of biofertilizer and reducing the dose of urea in leafy vegetable.

CONCLUSION

Introducing the urea coated with solid inoculant of Bacillus and Azotobacter has no effect on shoot height, leaves number and dry weight of lettuce romaine at 4 weeks after transplanting.

The BCU did not affect plant growth, P uptake and plant yield but the application of a half dose of BCU gave the similar lettuce productivity compared to one dose of urea. Half dose of B formulation of BCU resulted in the same shoot N uptake compared to one dose of urea but a dose of A formulation of BCU possibly decreased N uptake. The population of Bacillus and Azotobacter in the rhizosphere of lettuce treated by BCU were slightly increased compare to the control. This field experiment demonstrated that the yield of plant treated with BCU was statistically similar with the control., introducing BCU, mainly B formula which is urea coated with solid biofertilizer consisted of manure, 5 percent zeolite and 5 percent initial liquid bacterial inoculant, might be considered to replace a dose of conventional urea. The Andisols used in this current study is a fertile soil. Therefore, application of biofertilizer-coated urea either a dose or half dose had no effect on yield. The results indicated that the fertilizer dose applied by farmers might be reduced since a half dose of urea in the form of BCU gave a comparable yield with the common urea.

Acknowledgements

The research was funded by Higher Education General, Indonesia Ministry of Eduction year 2020.

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