Response of Ornamental Plant Aster to Inoculation of Various Inoculant of Soil Microbial Consortia

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ABSTRACT

The aster (*Callistephus chinensis* L.) is an important commodity and cultivated by using NPK compound fertilizer. The other nutrient source in the environmental-friendly agriculture is biofertilizer contained mixed strain of beneficial microbes. The purpose of a field experiment was to observe influence of various biofertilizer composed of mixed strain of nitrogen-fixer bacteria and phosphate-solubilizer microbes on aster plant growth and yield of flower. The research design was randomized block design consisted of four biofertilizer type and one control. The results verified that biofertilizer increased plant height at nine weeks after application but have not influence the initial generative stadia. The consortia of *Pseudomonas diminuta, P. Cepaceae, Penicillium* sp. and *Aspergillus* sp. showed the best effect on the plant healthy, flower number per plot and flower diameter. The experiment confirmed that all biofertilizer could be used as source of biostimulant for aster.
Key words: Biofertilizer, Flower, Microbes, NPK fertilizer.

INTRODUCTION

The ornamental plant aster now become an important commodity grown in mountainous region of Indonesia, including West Java. This ornamental plant is cultivated in the high-altitude mountainous area to fit their need to low temperature; and aster cut flowers are purchased by conventional market as well as e-commerce. Farmers grow the asters in the plastic house from rooted cutting by conventional nutrition system. The nutrient need is supplied by organic matter using applied in soil preparation and NPK compound fertilizer during the cultivation.

Optional nutrient source in the environmental-friendly agriculture is microbial inoculants that usually composed of beneficial soil microbes as described by Aloo *et al*. (2022). In the commercial term, this inoculant is well known as biofertilizer that provide the essential macro nutrients. In fact, the function of Plant Growth Promoting Rhizobacteria (PGPR) does not only supply the nutrients but also stimulate plant growth through phytohormones they produced. The prominent PGPRs formulated as biofertilizer are nitrogen (N)-fixer bacteria (NFB); and phosphate (P)-solubilizer microbes (PSM) which include bacteria and fungi. Phytohormone production by these bacteria have been regarded as the most important factors for plant growth promotion (Timofeeva *et al*., 2023).

The prominent genus of soil microbes broadly used as biofertilizer are, for examples, the NFB Azotobacter, Azospirillum and Bacillus; and the PSM Pseudomonas, Bacillus, Penicillium and Aspergillus. Some bacterial strain produces the phytohormone (Fukami *et al*., 2018; Hindersah *et al*., 2020; Fitriatin *et al*., 2022; Orozco-Mosqueda *et al*., 2023). Biofertilizer is formulated in single or mixed strain. The benefit of mixed strain inoculant permits synergistic interactions to stimulate biochemical activities of microbes together with the improvement of microbial viability (Yu *et al*., 2021); and hence stimulate plant growth and available soil nutrients (Wang *et al*., 2021).

The application of various phytohormone-producer bacteria has been reported to increase growth of ornamental plants including the aster (Sajjad *et al*., 2017; Vidya *et al*., 2022). However, little is known about the effectiveness of periodic inoculations of mixer biofertilizer on growth of aster in Indonesia. Therefore, the aim of the field was to observe the aster plant growth and their flower yield following various biofertilizer consortia application.

MATERIALS AND METHODS

The research was conducted in the farmer's horticultural area in Mekarwangi village of Cisarua District, West Bandung Regency, West Java on March-May 2021. The altitude of the field is 1.260 m above sea level with the average temperature of 17 ^{0}C-28 ^{0}C. The soil is Inceptisols with the pH of 5.87; the chemical properties of soil was low in organic C (1.62%), total N (0.19%) and C/N (8); while soil was very high in total P_2O_5 (60.78 mg/100 g); and high in available P_2O_5 (10.75 mg/kg) and potassium (44.21mg/kg). The cation exchange capacity (CEC) of soil was as high as 27.58 cmol/kg.

Experimental Design

The experiment was arranged in Randomized Block Design consisted of five treatments and four replications. The treatments comprised of on control and four inoculant treatments included:

A: Diluted NPK Fertilizer (Control treatment)

B: Mixed of N-fixer bacteria and P-solubilizer microbes

C: Consortia of *Bacillus* spp.

D: Consortia of *Bacillus* spp. dan *Azotobacter* spp.

E: P-solubilizer bacteria under the patent name of BIOP

The application dose of each biofertilizer inoculant was 10 L ha^{-1}, which was by spraying the soil and plants canopy at the same time. All inoculant was diluted 5% by using ground water before being sprayed.

Soil Biology Laboratory, Faculty of Agriculture, Universitas Padjadjaran provided All inoculants of beneficial microbes including:

a. Consortia of N-fixer bacteria of *A. chroococcum, A. vinelandii, Azospirillum* sp., and *Acinetobacter* sp.; and P-solubilizer bacteria *Pseudomonas cepaceae* and *Penicillium*. sp. registered as BION-UP
b. Bacillus consortia composed of *B. safensis*, *B. altitudinis, B. subtilis* and *B. megaterium*.
c. Mixed of *Bacillus* and *Azotobacter* that is formulated by using *A. choroccoccum*, *A. vinelandii*, *B. subtilis* and *B. megaterium*.
d. Consortia of P-solubilizer microbes of *P. mallei, P. cepaceae, Penicillium* sp. and *Aspergillus* sp. with patent name of BIOP.

The bacterial population in each inoculant was >10^8 CFU mL^{-1}, while the fungal count was approximately 10^5 CFU mL^{-1}. Each liquid biofertilizer were formulated in organic-based solution. All biofertilizer contained the phytohormones of Indole Acetic Acid (IAA), Cytokinin includes Zeatin and Kinetin, and/or Gibberellin of GA3.

The 10 cm stem cuttings of aster plants were provided by farmers; the cuttings made from previous plantation consisted of mixed color of aster. The stem cuttings were grown in the nursery for one month until rooting prior to transplanting.

Experiment Implementation

The one-month aster transplants were grown in the field soil of the plastics-covered conventional greenhouse. The dimensions of each bed were 3 m x 1 m with the distance between plots was 30 cm (Figure 1). The 30 cm surface soil in all beds was thoroughly mixed with 20 t ha^{-1}, of chicken manure a week before transplanting. Single rooted-cuttings of aster were grown in 8 cm deep holes with the space between hole of 10 cm x 10 cm. A total of 250 rooted cutting was grown in each bed.

Liquid biofertilizers were applied at three weeks after planting (WAP), 5 WAP and 6 WAP by spraying 3.75 L of diluted inoculant/bed. The control beds treated by diluted NPK fertilizer (16-16-16); which is10 g of fertilizer was mixed evenly in 3.75 L of water while the treated plots did not receive the NPK fertilizer. The application method of NPK fertilizer was similar to the biofertilizer.

The plant height was measured once a week during four months. The number of healthy and deteriorated plant (dead, unhealthy, and stunted plants) were counted from 20 plants/bed, (2 rows each contained 10 plants) in triplicate bed. Flowers are harvested at 13 WAP; due to the C-19 pandemic, the harvest only performed once. Flower number and diameter was counted from 30 plants in one plot which were determined purposively without border plants.

Statistical Analysis

Analysis of variance ($p<0.05$) was performed to the entire data set, and then Duncan's Multiple Range Test with $p<0.05$ was performed. Data analysis was carried out using IBM SPSS version 24 software.

RESULT AND DISCUSSION

In general, rooted cuttings of multi-colored aster grow well in all bed from the beginning of planting until their blooming time (Figure 1). The seedlings were mixed of various color of aster so the experimental data did not distinguish the color. Farmers did not grown aster based on the color; for economical purpose, the mixed color of aster was better sell.

At the first week, the height of young plants in all bed were similar; indicated that the stem seedlings were uniform. Plants in all treatments in general grew normally; the stem height was increased until the initial of blooming stage at 12 WAP. The plants height was varied at 6 WAP and 9 WAP, and determined by the sort of Biofertilizer (Table 1). The mixed of NFB and PSM (BION-UP) did not affect plant height at 6 WAP but did at 9 WAP compared to the control plants; mean while the consortia of Bacillus, Bacillus + Azotobacter, and PSM were significantly enhanced the plant height at 6 WAP and 9 WAP. At 12 WAP, the plant height in all treatments was similar. However, plants treated with the mixed of Bacillus and Azotobacter has lower stem than plants received another biofertilizer.

Figure 1. a. Plants in the planting day; b. two weeks after planting, and c. blooming flower at the first harvest.

The majority of 250 plants in bed was survived even though the planting distance was short (10 cm). The lower and higher deteriorated plants was found in beds treated with the PSM and control bed respectively (Figure 3).

Table 1. Effect of various biofertilizer on the plant height of aster at 3-12 WAP

Biofertilizer treatments	Plant height (cm) at			
	3 WAP	6 WAP	9 WAP	12 WAP
A: Diluted NPK fertilizer	21.7 a	47.2 a	107.4 a	121.3 a
B: NFB[a] dan PSM[b]	22.6 a	62.7 b	116.0 c	120.2 a
C: Bacillus	22.3 a	70.2 c	116.1 c	122.7 a
D: Bacillus dan Azotobacter	22.5 a	70.5 c	113.9 b	121.2 a
E: PSM[b]	22.9 a	72.1 c	117.5 c	122.4 a

explanation: Number in each column followed by the same letters are not significantly difference bases on Duncan Multiple Range Test ($p<0.05$). [a]NFB N-Fixer Bacteria; [b]PSM P-Solubilizer Microbes

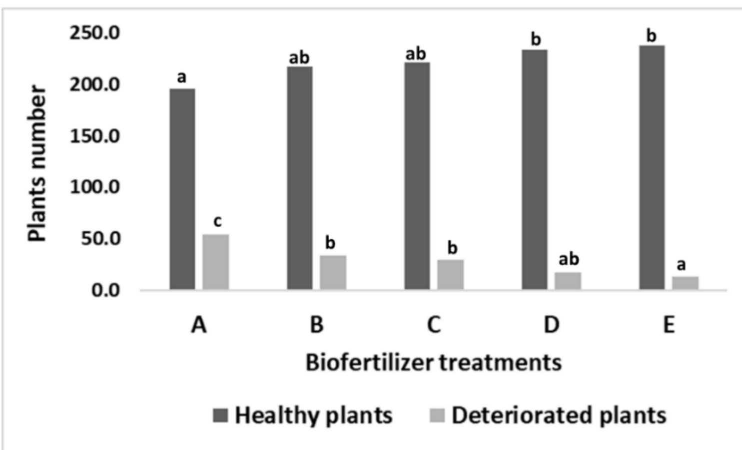

Figure 3. The number of healthy and deteriorated plants in each bed following biofertilizer application

Comparing to the control, spraying the biofertilizer enhanced the healthy plant number by 15.9%. The increment of healthy flower number (21.3%) was found in plots with PSM inoculation. Surprisingly, the beds with mixed of NFB-PSB (BION-UP) had higher deteriorated plants compared to another treatments. The BION-UP have not yet applied in the ornamental plants, but the role of BION-UP to induce nutmeg seedlings and rice production was recorded (Hindersah *et al*., 2021; Hindersah *et al*., 2022). It is likely that the dose of BION-UP did not fit with the growth requirement of aster. However, more research is needed to observe their impact to aster.

The first harvest was at 13 WAP; which is one week after the 4th fertilizer application. Due to Covid-19 situation, the yield measurement of existing plants beds and flower parameters was only taken once. Harvest in experimental area was 10 days faster than farmer's beds that was treated with other type of NPK compound fertilizer (25-9-9) enriched with the trace element. Moreover, the farmer sprayed those NPK once a month until blooming stadia. Higher N content of applied fertilizer prolonged the vegetative stage.

The flower diameter was increased by biofertilizer; the average diameter of flower picked from treated plant was 5.97 cm; which is 0.56 larger than the control plants. The flower number per plants of treated beds was not different with control plant (Table 2). The lowest flower diameter was found in beds with the Bacillus consortia. Nonetheless, higher healthy plants number in biofertilizer-treated beds would be related to the total flower number in a bad, resulted in the increase of flower in a bed received biofertilizer.

Table 2. Effect of various biofertilizer on the number of full-open flower and flower diameter at 13 WAP

Biofertilizer treatments	Flower number		Flower
	per plant	per bed	diameter (mm)
A: Diluted NPK fertilizer	1.91	374.7	5.41 a
B: NFB[a] dan PSM[b]	1.88	406.6	6.26 b
C: Bacillus	1.61	356.3	5.79 ab
D: Bacillus dan Azotobacter	1.61	374.9	5.87 b
E: PSM[b]	1.88	447.3	5.98 b

explanation: Number in each column followed by the same letters are not significantly difference bases on Duncan Multiple Range Test ($p<0.05$). [a]NFB N-Fixer Bacteria; [b]PSM P-Solubilizer Microbes

The significant enhancement of biofertilizer-treated plants verified the ability of microbes to provide essential macronutrient. The NFB and PSM were reported elsewhere to provide N and P for a root uptake. Moreover, all biofertilizer contained phytohormone-producing bacteria; that secondary metabolites are prominent to control cell development and tissue generation (Taiz and Zeiger, 2010). The phytohormones that present in all sort of biofertilizer were IAA, Cytokinin (Kinetin and Zeatin) and/or Gibberellin (GA3); the key growth hormones. The roles of IAA and cytokinin are root development regulation, root vascular differentiation and gravitropism (Aloni *et al*., 2006). The auxin-cytokinin antagonisms regulate the shoot/roots ratio that is essential for terrestrial plant survival and adaption to nutrient and soil availability in soil (Kurepa and Smalle, 2023). The optimal level of

Gibberellin controls longitudinal and radial dynamics in roots development (Shtin *et al*., 2022).

The asters are cultivated in soil contained high total N and available P; the function of NFB to fix the N is inhibited by high available N in soil (Sepp *et al*., 2023). High available P also limited the function of PSB to change available P form unavailable inorganic P through microbial organic acid secretion (Fitriatin *et al*., 2022). Therefore, the increase of plant height (Table 1) as well as the survival of plant (Table 2) in beds with biofertilizer was possibly related to the phytohormones production by rhizobacteria. Even though the root traits did not analyze, considerably biofertilizer-treated aster had better root growth. Researchers reported that the root system plays a key role in the shoot growth of development of horticultural ornamental plants (Shekar, 2014; Song *et al*., 2017). The root system adsorbs the water and nutrient from the soil and supply it to the plant shoot.

The different plant response on various biofertilizer on plant is verified. All biofertilizer had the same effect on plant growth, but the diverse effect was shown in plant population and hence flower number. Each consortium in this study had different composition of microbial species and strain. Regardless the statistics, the results showed that PSM consortium had better impact on aster growth and flower production. Both *P. mallei* and *P. cepaceae* of PSM consortium (BIOP) produce organic acid, phosphatase and IAA (Fitriatin *et al*., 2022). The phosphatase catalyzed the organic P degradation to provide available P; while the organic acid is possibly reduced the pH and hence increase the uptake of essential metal.

CONCLUSION

All biofertilizer clearly enhanced the weight and number of healthy plant of aster, as well as the flower number and flower diameter. The biofertilizers increase plant height at 6 WAP and 9 WAP but did not at 12 weeks when the plants entering the generative stadia. The mixed of NFB and PSB showed the weaker effect on height of 6-weeks old plant compared to another biofertilizer but at 9 weeks the height of all treated plants was similar. Even though each biofertilizer had distinct role in plant growth and flower production, the PSM consortia composed of *P. mallei*, *P. cepaceae*, *Penicillium* sp. and *Aspergillus* sp. was better to enhance the number of healthy plants and reduce the deteriorated plants in beds than other treatment. This experiment suggested that the biofertilizer composed of mixed strain of microbes might be suggested for cut flower aster production in the field.

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