The effect of dosages of microbial consortia formulation and synthetic fertilizer on the growth and yield of field-grown chili

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Abstract. Chili (Capsicum annuum, L) is one of important horticultural crop in Indonesia. Formulation of microbial consortia containing Bacillus subtilis, Pseudomonas sp., Azotobacter chroococcum and Trichoderma harzianum has been developed. This study evaluated the effects of dosage of the microbial formulation combined with NPK fertilizer on growth and yield of chili plants in the field experiment. The experiment was arranged in completely randomized design of factorial, in which the first factor was dosage of formulation (0, 2.5, 5.0, 7.5, 10 g per plant) and the second factor was NPK fertilizer dosage (0, 25, 50 and 75% of the standard dosage). The treatments were replicated three times. For application, the formulation was mixed with chicken manure 1:10 (w/v). The results showed that application of microbial formulation solely improved the chili growth. There was interaction between dosages of the microbial formulation and NPK fertilizer in improving plant height, nitrogen availability and the chili yield, while there was no interaction between those dosages in improving the root length. Combination between microbial formulation at the dosage of 5.0-7.5 g per plant combined with NPK fertilizer with the dosage 50 or 75% of the standard dosage support relatively better growth and the chili yield.

1. Introduction
Fertilizers are important agricultural input supporting plant production. Conventional agriculture depends on high input of synthetic fertilizers. The long-term use of such input, however, has led to various negative impacts to the environment such as decreasing soil quality and environmental pollution. Concerning the sustainability of food production, currently agriculture development has been directed toward eco-friendly agricultural systems. One of eco-friendly technology supporting such system is the use of plant growth promoting microorganisms (PGPM) to reduce high input of synthetic or inorganic fertilizers.

Plant growth promoting microorganisms are microorganisms, usually bacteria, fungi, protozoa, inhabiting plant rhizosphere that have abilities to improve plant growth [1, 2]. The abilities of the microorganisms to promote plant growth were through direct or indirect mechanisms. The direct mechanisms comprise the production of plant growth hormone, supporting the nutrient availability such as through nitrogen fixation, phosphorous solubilization, production of iron chelating agents...
(siderophore). The indirect mechanisms encompass the suppression of plant pathogens and deleterious microbes [2, 3, 4].

We have developed microbial formulation containing PGPM consortia which are Bacillus subtilis, Pseudomonas sp. (a fluorescent Pseudomonad), Azotobacter chroococcum and Trichoderma harzianum. Azotobacter has been known as non-symbiotic Nitrogen-fixing bacteria that can also produce plant hormone such as indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK) [5] and also vitamins such as thiamine and riboflavin [6]. Fluorescent Pseudomonads are nonpathogenic Pseudomonas that can promote plant growth through production iron-chelating agent, siderophore [7, 8] plant hormones such as auxin (indole acetic acid, IAA), cytokinins [9] and suppression of plant pathogens [7, 9]. Another plant growth promoting microbes is Bacillus. Bacillus subtilis has been reported to produce cytokinins [7, 10] and mediating synthesis of other hormones such as IAA [10, 11]. Trichoderma harzianum has also ability to increase plant growth through production of phytohormone such as IAA and Giberelic acid [12] and solubilization of phosphate and micronutrients [13].

The abilities of PGPM to support plant growth make them potential to be used as biofertilizer that can reduce the requirement of synthetic or inorganic fertilizers [1, 14]. This paper discussed the effects of dosage of the microbial formulation combined with NPK fertilizer on the growth and yield of chili plants in the field experiment.

2. Methods
The field experiment was conducted in Citespong, Lembang, West Bandung, West Java with andisol soil type. The experiment was arranged in Completely Randomized Design with factorial combination. The first factor was the dosages of microbial consortia (0.0, 2.5, 5.0, 7.5 and 10 g) and the second factor was the dosages of NPK (0, 25, 50 and 75% of standard dosages). Each treatment consisted of 28 plants, which were planted in 60 × 60 cm planting space. Each treatment was replicated three times.

For all treatments, chicken manure was applied with the rate as that usually used by the famers (15 ton per ha including the part used for mixing with the formulation). Formulation was prepared based on the method developed by Istifadah et al. [15]. For application, the formulation was mixed with chicken manures 1:10 (v/v) and incubated for 7 days. The mixture was then applied in the planting holes just before transplanting. The NPK fertilizers (the rate depending on the treatment with the standard dosage as recommended for chili) were applied at 3, 6 and 9 weeks after transplanting. The chili cultivar used was Ciko.

The plant height and stem diameter were observed at 2, 4, 6 and 8 weeks after transplanting. The chili yield was accumulated based on two harvesting times.

3. Results and Discussions
The results showed that in general, the microbial formulation and NPK fertilizer enhanced the chili plant height, even though in two weeks after transplanting (WAT), there was no significant different on the plant height (Table 1). The experiment was conducted in andisol soil in which in all treatments including the check, composted chicken manure was applied in the soil. In the early growth stage, the nutrients supported by organic matters probable were still sufficient to support the growth of young chili plants.

At 4-8 weeks after transplanting there was interaction between dosage of the formulation and NPK fertilizer on the plant height. At fourth weeks after transplanting, the microbial formulation without NPK fertilizer significantly increased the chili height, whilst the NPK fertilizer had significant effect on chili height (Table 2). In general, there is no effect of combination between the microbial formulation and NPK fertilizer on plant height. This means in this stage, application of microbial formulation solely is enough to support chili growth.
Table 1. The effect of microbial formulation and NPK fertilizer in chili plant height (2 WAT)

| Treatments | Plant Height (cm) | Treatments | Plant Height (cm) |
|------------|------------------|------------|------------------|
| ao: Check  | 9.7              | bo: Check  | 9.5              |
| a1: Formulation 2.5 g per plant | 9.9          | b1: NPK 25% of the standard dosage | 9.6 |
| a2: Formulation 5.0 g/ plant | 9.5         | b2: NPK 50% of the standard dosage | 9.9 |
| a3: Formulation 7.5 g per plant | 9.6       | b3: NPK 75% of the standard dosage | 10.1 |
| a4: Formulation 10.0 g per plant | 10.2      |           |                  |

Table 2. The effect of microbial formulation and NPK fertilizer in chili plant height (4 WAT)

| Dosage of Microbial Formulation | B0: without NPK fertilizer | B1: 25% of standard dosage | B2: 50% of standard dosage | B3: 75% of standard dosage |
|--------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| A0: Without Formulation        | 19.5 (a)                   | 22.1 (a)                   | 22.0 (a)                   | 22.6 (a)                   |
|                               | A                          | A                          | A                          | A                          |
| A1: Formulation, 2.5 g per plant | 22.8 (b)                 | 21.3 (a)                   | 21.0 (a)                   | 20.1 (a)                   |
|                               | B                          | AB                         | AB                         | A                          |
| A2: Formulation, 5.0 g per plant | 22.7 (b)                 | 22.1 (a)                   | 22.3 (a)                   | 22.2 (a)                   |
|                               | A                          | A                          | A                          | A                          |
| A3: Formulation, 7.5 g per plant | 22.4 (b)                 | 22.0 (a)                   | 21.1 (a)                   | 22.0 (a)                   |
|                               | A                          | A                          | A                          | A                          |
| A4: Formulation, 10.0 g per plant | 21.7 (b)                 | 21.5 (a)                   | 23.6 (a)                   | 23.2 (a)                   |
|                               | AB                         | A                          | B                          | AB                         |

Data in the same column followed by the same lower case letter is not significantly different based on Tukey’ HSD (p<0.01). Data in the same row followed by the same capital letter is not significantly different based on Tukey’ HSD (p<0.01)

At six weeks after transplanting, the microbial formulation or NPK fertilizer applied solely increased the plant height (Table 3). Combination between them, however, did not increase the plant height, compared to the respective dosage of the formulation or NPK fertilizer, applied solely. Combination between microbial formulation and NPK that supported better plant growth were combination between microbial formulation at dosage of 5.0 g per plant and NPK fertilizer 25% of the standard dosage or combination formulation at the dosage 2.5 g per plant and NPK fertilizer 75% of standard dosages.

Table 3. The effect of microbial formulation and NPK fertilizer in chili plant height (6 WAT)

| Dosage of Microbial Formulation | B0: without NPK fertilizer | B1: 25% of standard dosage | B2: 50% of standard dosage | B3: 75% of standard dosage |
|--------------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| A0: Without Formulation        | 24.1 (a)                   | 29.2 (ab)                  | 31.2 (a)                   | 30.3 (ab)                  |
|                               | A                          | B                          | B                          | B                          |
| A1: Formulation, 2.5 g per plant | 30.5 (c)                 | 30.3 (b)                   | 27.7 (a)                   | **34.4 (b)**               |
|                               | AB                         | AB                         | A                          | B                          |
| A2: Formulation, 5.0 g per plant | 26.8 (ab)                | **31 (b)**                | 28.1 (a)                   | 29.5 (ab)                  |
|                               | A                          | C                          | AB                         | BC                         |
| A3: Formulation, 7.5 g per plant | 29.4 (bc)                | 27.4 (a)                   | 28.1 (a)                   | 26.3 (a)                   |
|                               | A                          | A                          | A                          | A                          |
| A4: Formulation, 10.0 g per plant | 28.1 (bc)                | 29.9 (ab)                  | 29.5 (a)                   | 29.5 (ab)                  |
|                               | A                          | A                          | A                          | A                          |

Data in the same column followed by the same lower case letter is not significantly different based on Tukey’ HSD (p<0.01). Data in the same row followed by the same capital letter is not significantly different based on Tukey’ HSD (p<0.01)
At eight weeks after transplanting, the microbial formulation and NPK fertilizer applied solely also increased the plant height (Table 4). Combination between them generally did not increase in plant height. The combination that resulted in significant increase was combination between microbial formulation at the dosage of 7.5 g per plant and NPK fertilizer at the dosage 50% of standard dosage.

### Table 4. The effect of microbial formulation and NPK fertilizer in chili plant height (8 WAT)

| Dosage of Microbial formulation | B0: without NPK fertilizer | B1: 25% of standard dosage | B2: 50% of standard dosage | B3: 75% of standard dosage |
|---------------------------------|----------------------------|-----------------------------|---------------------------|---------------------------|
| A0: Without Formulation         | 48.7 (a)                  | 52.3 (ab)                   | 52.0 (a)                  | 51.6 (a)                  |
| A1: Formulation, 2.5 g per plant| 51.4 (ab)                  | 51.1 (a)                   | 52.3 (a)                  | 53.2 (a)                  |
| A2: Formulation, 5.0 g per plant| 52.7 (b)                  | 51.5 (a)                   | 52.3 (a)                  | 52.6 (a)                  |
| A3: Formulation, 7.5 g per plant| 53.3 (b)                  | 52.1 (a)                   | 55.8 (b)                  | 52.9 (a)                  |
| A4: Formulation, 10.0 g per plant| 53.0 (b)                  | 52.4 (a)                   | 51.3 (a)                  | 52.2 (a)                  |

Data in the same column followed by the same lower case letter is not significantly different based on Tukey’ HSD (p<0.01). Data in the same row followed by the same capital letter is not significantly different based on Tukey’ HSD (p<0.01).

Based on other parameters such as stem diameter and root development, there was no interaction between the dosages of microbial formulation and NPK fertilizer, and therefore the analysis was based on the main factors. Based on stem diameter of chili plants, the application of microbial formulation or NPK fertilizer did not provide any significant effects. The diameter in any treatments was not significantly different to that of the control plants (Table 5).

Based on chili root fresh weight, the application of microbial formulation of 5.0 g per plant increased the root growth (Table 5). Meanwhile the application of NPK fertilizer at the dosage 50% of standard dosage increased the fresh root weight (Table 6).

### Table 5. The effect of microbial formulation on stem diameter and root development

| Treatments                  | Average of Stem Diameter (cm) | Average of Root Length (cm) |
|-----------------------------|-------------------------------|-----------------------------|
| a0 : Check                  | 0.76 a                        | 26.98 a                     |
| a1 : Formulation 2.5 g per plant | 0.76 a                      | 28.22 ab                    |
| a2 : Formulation 5.0 g per plant | 0.76 a                      | 29.36 b                     |
| a3 : Formulation 7.5 g per plant | 0.77 a                      | 28.08 ab                    |
| a4 : Formulation 10 g per plant | 0.77 a                      | 28.27 ab                    |

Data in the same column followed by the same letter is not significantly different based on Tukey’ HSD (p<0.01).

### Table 6. The effect of microbial formulation and NPK fertilizer in chili plant height

| Treatments                  | Average of Stem Diameter (cm) | Average of Root Length (cm) |
|-----------------------------|-------------------------------|-----------------------------|
| b0 : Check                  | 0.76 a                        | 27.14 a                     |
| b1 : NPK Dosage 25% of the standard | 0.77 a                      | 28.24 ab                    |
| b2 : NPK Dosage 50% of the standard | 0.76 a                      | 29.23 b                     |
| b3 : NPK Dosage 75% of the standard | 0.76 a                      | 28.11 ab                    |

Data in the same column followed by the same letter is not significantly different based on Tukey’ HSD (p<0.01).

The application of microbial formulation and NPK fertilizer solely increased the nitrogen availability in the chili rhizosphere. The increase in nitrogen availability in the chili rhizosphere may be due to the existence of nitrogen-fixing bacteria, *A. chroococcum*, in the formulation. There was an
interaction between the dosage of formulation and NPK fertilizer. The combination that led to highest nitrogen availability in the soil was combination between microbial formulation at the dosage of 10 g per plant and NPK fertilizer with dosage 25% of standard dosage (Table 7).

**Table 7.** The effect of microbial formulation and NPK fertilizer on the nitrogen availability in the chili rhizosphere

| Dosage of Microbial Formulation | B0: without NPK fertilizer | B1: 25% of standard dosage | B2: 50 % of standard dosage | B3: 75% of standard dosage |
|--------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| A0: Without Formulation        | 15.75 (a)                 | 21.76 (a)                 | 21.96 (a)                 | 24.93 (ab)                |
| A1: Formulation, 2.5 g per plant | 19.62 (ab)               | 28.25 (bc)               | 22.84 (a)                 | 21.02 (a)                |
| A2: Formulation, 5.0 g per plant | 21.35 (bc)               | 22.63 (ab)               | 26.53 (a)                 | 23.80 (ab)               |
| A3: Formulation, 7.5 g per plant | 26.37 (c)                | 23.89 (ab)               | 20.61 (a)                 | 26.53 (b)                |
| A4: Formulation, 10.0 g per plant | 23.33 (bc)              | **30.85 (c)**           | 27.11 (a)                 | 24.59 (ab)               |

Data in the same column followed by the same lower case letter is not significantly different based on Tukey’ HSD (p<0.01). Data in the same row followed by the same capital letter is not significantly different based on Tukey’ HSD (p<0.01)

There was interaction between application of microbial formulation and NPK fertilizer on the healthy chili yield (Table 8). Many chili fruits were drop due to lack of water. Many harvested chilies also showed cracked skin, and therefore they were not considered as healthy fruits. The application of microbial formulation at dosage of 7.5 g per plant increased the healthy chili yield. Meanwhile, the application of NPK fertilizer at reduced dosage (25-75% of the standard dosage) also increased the healthy yield (Table 8). Even though in many combinations the weight of healthy chili accumulated from two harvest times was not significantly different to that of the control plants, there were certain combinations such as application of microbial formulation 7.5 g per plant and NPK fertilizer 75% of standard dosage, that significantly higher than that of the check plants. In this treatment, the healthy chili yield increased 61.4%.

**Table 8.** The effect of microbial formulation and NPK fertilizer on the chili yields per plant

| Dosage of Microbial Formulation | B0: without NPK fertilizer | B1: 25% of standard dosage | B2: 50 % of standard dosage | B3: 75% of standard dosage |
|--------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| A0: Without Formulation        | 144.9 (a)                 | 191.8 (a)                 | 191.9 (a)                 | 161.6(a)                  |
| A1: Formulation, 2.5 g per plant | 174.7 (ab)               | 214.9(a)                  | 208.8 (a)                 | 178.1 (a)                |
| A2: Formulation, 5.0 g per plant | 133.6 (a)                | 143.6 (a)                 | 206.8 (a)                 | 189.03 (ab)              |
| A3: Formulation, 7.5 g per plant | 207.1 (b)                | 183.0(a)                  | 207.0 (a)                 | **233.8 (b)**            |
| A4: Formulation, 10.0 g per plant | 162.0 (ab)               | 152.5 (a)                 | 207.8(a)                  | 143.6 (a)                |

Data in the same column followed by the same lower case letter is not significantly different based on Tukey’ HSD (p<0.01). Data in the same row followed by the same capital letter is not significantly different based on Tukey’ HSD (p<0.01)

The overall results showed that in early vegetative growth (four weeks after transplanting), application of microbial formulation or NPK fertilizer solely increased the chili vegetative growth. At 6 – 8 weeks after transplanting, there was an increase in the plant growth if formulation and NPK
fertilizer combined at appropriate dosages. There was tendency that the lower dosage of microbial formulation required relatively higher dosages of NPK fertilizer and vice versa. This means that the microbial formulation and NPK fertilizer can be complementary. In this study, the application of microbial formulation could reduce the requirements of the NPK dosage up to 75% reduction. For supporting roots system, the dosage of NPK fertilizer could reduce to 50% of the recommended dosage.

The abilities of microbial formulation to increase the chili growth and reduced the requirement of NPK fertilizer seems due to the abilities of microbes in the formulation to produce plant hormone and support nutrient availability. *Azotobacter chroococcum* used in this study is nitrogen-fixing bacteria that also produced phytohormon [16]. The abilities of *A. chroococcum* to support the nitrogen availability was confirmed by the increase on the nitrogen availability in the chili rhizosphere treated by the microbial formulation. *Pseudomonas* sp. used in this study also can produce phytohormone kinetin, zeatin, IAA [17] and phosphatase enzyme leading to solubilization of soil phosphate [17, 18]. Other microbes, *T. harzianum* [12] and *B. subtilis* [7,10] are also known as plant growth promoting microbes that capable of producing plant hormone.

Even though the microbial formulation could support the early vegetative growth of chili, it should be combined with synthetic fertilizer when the plants were in the end of vegetative stages (7-8 weeks after transplanting). Combination that sufficient to support the growth and yield of chili were application of 7.5 g formulation combined with NPK 50% or 75% of the standard dosage. This means that microbial formulation could reduce the requirement of synthetic fertilizers or improve their efficiency [1,19]. The use of PGPM in plant production system is very beneficial as they are not only support the nutrients management, but they can also play important role in plant protection as many of PGPM are antagonistic to plant pathogens [20].

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