Effects of Dosage and Application Frequency of Microbial Consortium Mixed with Animal Manure on Bacterial Wilt and Late Blight Diseases of Potato

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Abstract. Major constraints in potato production includes bacterial wilt disease (Ralstonia solanacearum) and late blight disease (Phytophthora infestans). To support environmentally-friendly agricultural system, a microbial consortium containing Trichoderma harzianum, Bacillus subtilis and non pathogenic Pseudomonas sp. has been studied for biocontrol of plant diseases in horticultural crops. For application, the microbial consortium was mixed with organic matters (10%, v/v). The objective of this research was to obtain dosage and application frequency of microbial consortia mixed with chicken manure for controlling bacterial wilt disease and their effects on airborne disease, late blight disease, in potato. The experimental design used was Randomized complete design with 10 treatments and three replications. The treatments consisted of application of the mixture in planting hole at the dosage of 25, 50 or 100 g solely or in combination with drenching the water suspension of the mixture weekly or every two weeks. The results showed that the dosage and application frequency of the microbial consortia mixed with chicken manure influenced their abilities in suppressing the bacterial wilt disease in potato. The application of the mixture at planting hole with the dosage of 50 g per plant followed by drenching the water suspension of the mixture every week suppressed the bacterial wilt disease by 75.0 - 81.9%.

1. Introduction

Potato is one of important horticultural crop that are commonly cultivated in many highland areas in West Java, Indonesia. The main constraints in potato production include plant diseases. Bacterial wilt disease caused by Ralstonia solanacearum is a devastating soil-borne disease in many plants including potato. The infected plants become wilted and finally died. The latent infection of seed potatoes can spread the disease to other field and areas [1]. In addition to soil-borne disease, airborne disease such as late blight disease caused by Phytophthora infestans is also destructive disease in potato worldwide that may lead to significant yield loss. The disease can infect almost all parts of the potato such as the leaves, petiole, stem and potato tubers [2].

The most common control measure for plant diseases is the use of pesticides. However, the continuous use of synthetic pesticides may lead to various negative impacts to the human health and the environment. Concerning this, environmentally-friendly control measures, such as the use of organic matters and biological control need to be developed.

Microbes commonly used for biological control of plant diseases include genera Trichoderma [3], Bacillus [4,5] and non-pathogenic Pseudomonas [6]. Combination of several compatible microbes can improve the efficacy of the control [7,8]. Microbial consortium containing Trichoderma harzianum, Bacillus subtilis and Pseudomonas sp. has been developed for biocontrol of plant diseases in horticultural crops. The microbes were compatible and the consortium of the microbes provided better disease control rather than individual microbe [8] For application, the consortium was mixed with organic matters such as compost or animal manure. The mixture has been found to be effective in
controlling damping off disease caused by *Rhizoctonia solani* [8] and root knot nematode (*Meloidogyne* spp.) [9] in chili. *Trichoderma harzianum* and *B. subtilis* mixed with chicken manure was also reported to suppress the disease incidence of bacterial wilt disease and reduced the late blight disease intensity in potato [10].

The efficacy of biocontrol agents is influenced by several factors such as the dosage and frequency of application. To control soil-borne disease, biocontrol agents can be applied at the nursery, or at the planting holes. Repeated application of biocontrol agents after planting can improve their effectiveness in controlling the disease [11,12]. [10] also found that repeated application of *T. harzianum* and *B. subtilis* mixed with chicken manure could reduce the dosage of NPK fertilizer up to 50% of the standard dosage. The objective of this study was to obtain dosage and frequency of applications of microbial consortium mixed with chicken manure that lead to the best control of bacterial wilt and to examine if that application can reduce late blight disease in potato.

2. Materials and methods
The experiment was conducted in the field located at Citenspong, Lembang, West Bandung, West Java, Indonesia. The experiment was arranged in Randomized Complete Block Design with 10 treatments and three replications. The treatments examined were the application of microbial consortia mixed with chicken manure in planting hole with the dosage of 25 g, 50 g and 75 g and drenching its water suspension every one or two weeks solely or in combination. Each treatment was repeated three times and in each replication there were 20 potato plants. In all treatments chicken manure was applied at the rate of 15 tons per hectare. Concerning that the use of microbial consortium could reduce the dosage of NPK fertilizer up to 50% of the standard dosage [10] in this study, all treatments using the microbial consortium NPK fertilizer was applied at the rate 50% of the standard dosage. In these treatments, part of the chicken manure was mixed with the microbial consortia (in molasses 2% medium) with proportion of 10% (v/v) and incubated for one week. The mixture was applied at planting holes at the time of planting with the dosage depended on the treatment. For drenching the plants, the mixture was added to the water (1:4. v/v) and incubated in the lid-closed container for one week. The plants were drenching with the suspension 50 ml per plant every one or two weeks.

The variables observed were the percentage of plant infected by bacterial wilt disease (disease incidence) and disease intensity of late blight disease. Disease index used for calculating disease intensity of late blight disease were: 0: no symptom; 1: percentage of plant infected 0 < x ≤ 12%; 2: percentage of plant infected 12% < x ≤ 25%; 3: percentage of plant infected 25% < x ≤ 50%; 4: percentage of plant infected 50% < x ≤ 75%; 5: percentage of plant infected 75% < x ≤ 100%. The disease incidence or severity was observed weekly. Data disease intensity and incidence were used for calculating *Area Under Disease Progress Curve* (AUDPC). Another variable observed was plant height which was measured every week. Data was statistically analyzed by analysis of variance using SPSS program 20 version. Data were checked for their normal distribution and transformed when it was necessary. The differences between treatments were analyzed using Tuckey’s HSD test (p<0.05).

3. Results and Discussion

3.1 The effects of dosage and application frequency of microbial consortia mixed with chicken manure on bacterial wilt disease

The results showed that application of microbial consortium mixed with chicken manure significantly suppressed the bacterial wilt in potato by 31.0 - 82.2 %. The dosages and frequency of application influenced the disease reduction. The microbial consortium mixed with animal manure was effective when it was applied at the appropriate dosage or frequency of application. Based on the disease incidence at 7 weeks after planting, the application of the microbial consortium mixed with chicken manure at planting hole the dosage of 25 g or 50 g and or drenching the plants with the mixture suspension every week were effective to reduce bacterial wilt disease in potato by 71.4-75%. However, based on the progress or development of disease incidence during 5 weeks observation, the
treatment that led to a relatively high disease control (81.9% suppression) was application of the microbe-manure mixture at the rate of 50 g at planting holes and drenching its water suspension weekly (Table 1).

The application of the mixture only once at the time of planting also reduced the bacterial wilt disease by 57.1-61.2%, but the rate of application was 100 g per planting hole. It seems that if the dosage of the mixture application in planting hole was low (i.e. 25 g per planting hole) the repeated application by drenching its water suspension could enhance its efficacy. The weekly application of the water suspension of the mixture itself also led to effective bacterial wilt disease control (75.0 - 78.4% disease reduction). [14] also found that drenching the tomato plant by the suspension of *Trichoderma* spp. and *Pseudomonas fluorescens* weekly resulted in 97% plants recovered from bacterial wilt (*R. solanacearum*) infection.

### Table 1. The effects of dosage and application frequency of the microbe-chicken manure mixture on bacterial wilt disease (8 Weeks After Planting)

| Treatments                                                      | Disease incidence (%) | Disease suppression (%) | AUDPC value | Disease suppression (%) |
|-----------------------------------------------------------------|-----------------------|-------------------------|-------------|-------------------------|
| A Control                                                       | 93.3 d                | 0.0                     | 1353.3 d    | 0.0                     |
| B Drenching the plant with water suspension of the mixture of microbial consortia and manure every week | 23.3 a                | 75.0                    | 291.7 ab    | 78.4                    |
| C Application of the mixture in planting hole 25g and drenching its suspension every week | 26.7 a                | 71.4                    | 443.3 ab    | 67.2                    |
| D Application of the mixture in planting hole 50g and drenching its suspension every week | 23.3 a                | 75.0                    | 245.0 a     | 81.9                    |
| E Application of the mixture in planting hole 75g and drenching its suspension every week | 46.7 abc              | 50.0                    | 711.7 bc    | 47.4                    |
| F Drenching the plant with water suspension of the mixture of microbial consortia and manure every two weeks | 30.0 ab               | 67.8                    | 443.3 ab    | 67.2                    |
| G Application of the mixture in planting hole 25g and drenching its suspension every two weeks | 63.3 c                | 32.1                    | 921.7 c     | 31.9                    |
| H Application of the mixture in planting hole 50g and drenching its suspension every two weeks | 33.3 ab               | 64.3                    | 443.3 ab    | 67.2                    |
| I Application of the mixture in planting hole 75g and drenching its suspension every two weeks | 60.0 bc               | 35.7                    | 898.3 c     | 33.6                    |
| J Application of the mixture in planting hole 100 g | 40.0 abc              | 57.1                    | 525.0 abc   | 61.2                    |

Note: Data in one column followed by different letters were significantly different (*p* < 0.05) based on Tuckey HSD test.

### 3.2 The effects of dosage and application frequency of microbial consortia mixed with chicken manure on late blight disease

In addition to their effects on soilborne disease, application of the microbial consortia mixed with chicken manure also reduced the airborne disease, potato late blight, by 44.4%- 56.8% (Table 2). This
results was in line with previous study which also found that application of *B. subtilis* and *T. harzianum* mixed with chicken manure reduced late blight disease by 44.0- 45.4% [10]. In this current study, the dosage and application frequency did not affected the abilities of the microbes-manure mixture in reducing the late blight disease. The AUDPC values, that reflected the disease development during the observation, in all treatments using the mixture were not significantly different (Table 2).

| Treatments                                                                 | AUDPC value | Disease suppression (%) |
|---------------------------------------------------------------------------|-------------|-------------------------|
| A  Control                                                                | 595.9 b     | 0.0                     |
| B  Drenching the plant with the suspension of the microbial consortia mixed with manure every week | 299.8 a     | 49.7                    |
| C  Application of the mixture in planting hole 25g and drenching its suspension every week | 261.0 a     | 56.2                    |
| D  Application of the mixture in planting hole 50g and drenching its suspension every week | 276.4 a     | 53.6                    |
| E  Application of the mixture in planting hole 75g and drenching its suspension every week | 331.3 a     | 44.4                    |
| F  Drenching the plant with suspension of the microbial consortia and manure mixture every two weeks | 280.7 a     | 52.9                    |
| G  Application of the mixture in planting hole 25g and drenching its suspension every two weeks | 296.1 a     | 50.3                    |
| H  Application of the mixture in planting hole 50g and drenching its suspension every two weeks | 285.6 a     | 52.1                    |
| I  Application of the mixture in planting hole 75g and drenching its suspension every two weeks | 260.5 a     | 56.3                    |
| J  Application of the mixture in planting hole 100 g                       | 257.3 a     | 56.8                    |

Note: Data in one column followed by different letters were significantly different (p < 0.05) based on Tuckey HSD test

Even though the microbial consortium mixed with chicken manure was applied in the soil, but it was also able to reduce late blight disease in the leaves. This result indicated the abilities of the mixture to enhance the potato resistance to the disease. Biocontrol agents such as *T. harzianum* and *B. subtilis* have been reported to induce resistance to early blight (*Alternaria solani*) and late blight (*P. infestans*) diseases in tomato [15] as well as to late blight disease in potato [16].

### 3.3 The effects of dosage and application frequency of the microbes-manure mixture on the potato growth

The potato growth was not only influenced by the availability of nutrient but also affected by the diseases infected the plants. In this study, the plant height was measured on the plants that were not wilted. The growth of the plants was still even after two weeks after planting (WAP), as the disease infection was still low. In this stage the effect of the mixture of microbe-manure on potato growth could be observed more clearly. At 2 WAP, the application of the microbes mixed with chicken manure enhanced the potato growth (Table 3).
Table 3. Effects of dosage and application frequency of the microbial consortia mixed with chicken manure on the potato growth

| Treatments                                                                 | Average of Plant Height (cm) |
|---------------------------------------------------------------------------|-------------------------------|
|                                                                          | 2 WAP | 3 WAP | 4 WAP |
| A  Control                                                               | 43.3 a | 53.8 a | 63.4 a |
| B  Drenching the plant with of the suspension of the microbial consortia and manure mixture every week | 47.4 ab | 65.2 a | 73.9 a |
| C  Application of the mixture in planting hole 25g and drenching its suspension every week | 52.8 bc | 63.8 a | 71.6 a |
| D  Application of the mixture in planting hole 50g and drenching its suspension every week | 56.6 c | 67.6 a | 73.6 a |
| E  Application of the mixture in planting hole 75g and drenching its suspension every week | 53.7 bc | 61.4 a | 71.7 a |
| F  Drenching the plant with suspension of the microbial consortia and manure mixture every two weeks | 53.9 bc | 54.8 a | 59.5 a |
| G  Application of the mixture in planting hole 25g and drenching its suspension every two weeks | 52.0 abc | 67.8 a | 78.1 a |
| H  Application of the mixture in planting hole 50g and drenching its suspension every two weeks | 55.0 bc | 58.7 a | 66.7 a |
| I  Application of the mixture in planting hole 75g and drenching its suspension every two weeks | 53.1 bc | 68.1 a | 74.7 a |
| J  Application of the mixture in planting hole 100 g                        | 50.7 abc | 52.6 a | 61.6 a |

Note : Data in one column followed by different letters were significantly different ($P < 0.05$) based on Tuckey HSD test

At two week after planting, application of the microbial consortium mixed with chicken manure 50 g at planting hole, followed by drenching its water suspension weekly resulted in better potato growth. [10] also found that combination of application of the microbial-manure mixture at planting hole and drenching the plant with its suspension led to better potato growth.

At 3 and 4 WAP, the potato heights in all treatments were not significantly different (Table 3). At that time, the number of plants infected by late blight disease started to increase; therefore, this might affected the plant growth. The heights of the plants were varied, even in the same treatments, and hence there were no significant difference among the treatments. The observation on potato growth was only up to 4 WAP as after that ≥ 50 % of the plants in the control has already infected by bacterial wilt disease. At that time the plant height was varied considerably so that it was not be observed anymore.

Overall results showed that the microbial consortium containing T. harzianum, B. subtilis, Pseudomonas sp. that was mixed with chicken manure suppressed bacterial wilt disease by 31.0 - 82.2 %. The effectiveness of the mixture can be achieved if the rate or frequency of application is appropriate. Repeated application of the mixture tended to result in better disease control rather than one application, only at the time of planting. [12] found that weekly application of bacterial antagonist (Pseudomonas putida 06909-rif/nal) through water irrigation led to significant increase on its population in the soil. The repetitive application could also maintain the high population of biocontrol agent in the soil over time. Application of the mixture 50 g in planting hole followed by drenching its suspension tended to result in better disease control. [17] also found that combination of application of spent mushroom substrate in planting hole and drenching its water extract every two weeks resulted in relatively better suppression of basal rot disease and development of the onion bulb rather than solely method.
Application of microbial consortium mixed with chicken manure in the soil was not only able to control soil borne disease, but it also reduced the destructive airborne disease, the potato late blight disease. This result was in line with another study that also found that *T. harzianum* and *B. subtilis* mixed with chicken manure reduced the late blight disease in potato [10].

To determine the appropriate rate and frequency of application, it needs to consider NPK dosage applied. [10] found the trend that if the application of microbial-manure mixture only once at the time of planting (100 g per planting hole), the NPK fertilizer was needed to be applied at standard dosage. However, if the mixture was applied several times (at planting hole and as regular soil drenching) the NPK dosage could be reduced up to 50% of the standard dosage. Based on this result, the NPK dosage in this study was only 50% of the standard dosage. It means that the mixture of microbial consortium and chicken manure can reduce the synthetic fertilizer and hence it can support Low External Input Sustainable Agriculture (LEISA) system.

4. Conclusion

Based on the results of this study it can be concluded that the mixture of microbial consortium (*T. harzianum*, *B. subtilis*, *Pseudomonas* sp.) and chicken manure was able to suppress bacterial wilt disease in potato. The rate and frequency of application influenced the efficacy of the mixture in reducing the bacterial wilt disease, but it did not affect its ability to reduce late blight disease intensity. Application of the mixture 50 g at planting hole followed by drenching its water suspension weekly resulted in the highest reduction (by 75.0%-81.0 %) of bacterial wilt disease incidence. The application of the mixture also reduced late blight disease by 44.4%-56.8%.

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