The Effect of a combination of growing media and biological agents to control bacterial wilt disease of potato (Ralstoniasolanacearum) in greenhouse

Mukhtar Iskandar Pinem, Laimuddin Lubis, Marheni, Dedi Maradi Zuhdi
Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan, Indonesia, 20155

Email: mi_pinem@yahoo.com

Abstract. Potato (Solanumtuberosum L.) in Indonesia is a commodity that priorities todevelop, and has the potential to be marketed in the domestic as well as export. One of the obstacles is important that faced is pathogen soil-borne which is caused by the Ralstoniasolanacearum. This pathogen can causing loss of yield of potato 40-100%. Bacillus sp. and Pseudomonasfluorescens are antagonistic microbes that are proven to be able to control bacterial wilt disease by 69,95 % and 61,67%. This study aimed to determine the effect of the comparison composition of growing media and the ability of some agents of biological within suppress the growth of the disease wilt bacteria of potatoes which is caused by R. solanacearum. This research using completely randomized design (CRD) nonfactorial with 10 treatments, namely: M0(top soil), M1(top soil, R. solanacearum), M2(top soil, R. solanacearum, P. fluorescens), M3(top soil, R. solanacearum, Bacillus subtilis), M4(top soil, Chicken manure, R. solanacearum, P. fluorescens), M5 until M9 are combination of them. The results showed that the application of biological agents was able to suppress the growth of the pathogen R. solanacearum, as well as significantly affect plant height, number of shoots, and production.

1. Introduction
Potato (Solanumtuberosum L.) in Indonesia is a horticultural commodity that has priority to be developed, and has the potential to be marketed domestically and exported[1]. In the cultivation business of potatoes encountered various obstacles that can hamper its productivity. One important obstacle is soil-borne pathogens caused by the bacterium Ralstoniasolanacearum E. F. Smith. This bacterial attack causes the plants to wither followed by root rot. Field observations show that this pathogen can cause loss the results plant potato 40-100%[2].

This pathogen is a disease-causing bacterium that is quite important in the tropics, subtropics and warm temperatures[3] and attacks more than fifty plant families[4], such as tomatoes, potatoes, pepper, tobacco, eggplant, bananas, ginger and beans[5].

Bacillus sp. and P. fluorescens are antagonistic microbes that have the potential to be developed as pathogen control agents in potato plants. Laboratory, greenhouse and field scale test results show that the application of Bacillus sp. And P. fluorescens in emulsion formulations can control Fusariumoxysporumf. dianthi at carnations up to 63,63%[6]. P. fluorescens and B. subtilis proved to be able to control bacterial wilt disease (R. solanacearum) by 69,95 % and 61,67%[7].
Manure has natural properties and does not damage the soil, providing macro and micro elements. In addition, manure works to increase water holding capacity, soil microbiological activity, cation exchange capacity value and improve soil structure. The type of fertilizer used in this study is organic fertilizer from chicken manure and humus [8]. The biological function of organic matter is as energy and food sources of soil microorganisms so as to increase the activity of soil microorganisms which are very useful in the supply of plant nutrients. As such the provision of organic fertilizer will ultimately increase plant growth and production [9].

To determine the effect of comparison of the composition of growing media and the ability of some biological agents in suppressing the growth of bacterial wilt disease in potato plants caused by *Ralstoniasolanacearum*.

2 Material and Method

2.1 Place and time of research
The study was conducted at the Laboratory and Greenhouse at the Vegetable Crops Research Institute, Tongkoh Experimental Field, Berastagi, North Sumatra. This research was conducted from June to October 2019.

2.2 Research methods
The research method used was using a non factorial complete randomized design (CRD) with 10 treatments, namely:

- M0 = Top Soil (Control)
- M1 = Top Soil + *Ralstoniasolanacearum*
- M2 = Top Soil + *R. solanacearum* + *Pseudomonasfluorescens*
- M3 = Top Soil + *R. solanacearum* + *Bacillus subtilis*
- M4 = Top Soil + Chicken manure + *R. solanacearum* + *P. fluorescens*
- M5 = Top Soil + Chicken manure + *R. solanacearum* + *Bacillus subtilis*
- M6 = Top Soil + Humus + *R. solanacearum* + *Pseudomonasfluorescens*
- M7 = Top Soil + Humus + *R. solanacearum* + *Bacillus subtilis*
- M8 = Top Soil + Chicken manure + Humus + *R. solanacearum* + *P. fluorescens*
- M9 = Top Soil + Chicken manure + Humus + *R. solanacearum* + *B. subtilis*

2.3 Research Implementation

2.3.1 Mixing Planting Media
For treatments M0, M1, M2, M3 only use top soil, M4 and M5 use top soil media and chicken manure with a ratio of 1: 1, M6 and M7 use top soil and humus media with a ratio of 1: 1, and M8 and M9 using top soil media, chicken manure and humus with a ratio of 1: 1, then sifted. Mixing of planting media is carried out in a greenhouse by first being given a plastic base at the bottom, so that the planting media does not come in direct contact with the greenhouse floor and to minimize contamination of the planting media. After all the media is mixed evenly and already filled into polybags, then arranged according to the treatment made.

2.3.2 Preparation of planting material
The planting material was obtained from the Vegetable Crops Research Institute of the Tongkoh Experimental Field, Berastagi, namely the zero- generation (G0) Granola variety.

2.3.3 Inoculation of Ralstoniasolanacearum

*R. solanacearum* pure isolates originated from potato plantations which were isolated directly from the tubers and stems of potato plants in the field. Pure Isolate *R. solanacearum* is infested into the planting
medium by spraying it into the planting hole (done 2 hours before planting). Population density of R. solanacearum was applied at 10^8 CFU/ml in each treatment of 20 ml (except M0 / control).

Isolates of P. fluorescens and biological agents Bacillus subtilis was obtained from BALITSA, Lembang, Bandung through an intermediary of the Vegetable Crops Research Institute, Tongkoh Experimental Garden, Berastagi. Application P. fluorescens and Bacillus subtilis was carried out by immersing G0 potato tuber seedlings with a population density of 10^8 CFU/ml for 2 hours in each treatment[10].

2.4. Observation Parameters

2.4.1. Plant Height (cm)

Plant height measurements are carried out every week using a meter measuring centimeters (cm). The purpose of measuring plant height is to determine the growth of plant height every week.

2.4.2. Number of Shoots

Observation of the number of shoots is done by manually counting the number of shoots that grow every week.

2.4.3. Tuber Production (g)

The calculation of potato plant production is carried out when the plant is physiologically mature. This is indicated by the yellowing of the potato plants after passing through the flowering process. Production weighing processes are carried out using analytical scales.

3. Results and Discussion

3.1. Plant height

Based on the observation of plant height after the application of R. solanacearum and biological agents (P. fluorescens and B. subtilis) in the 8th week after application. The data is listed as follows:

| Treatment | Replication I (cm) | Replication II (cm) | Replication III (cm) | Total (cm) | Average (cm) | Notation |
|-----------|-------------------|---------------------|----------------------|------------|--------------|----------|
| M0        | 58.67             | 66.67               | 67.67                | 193.00     | 64.33        | b        |
| M1        | 53.67             | 61.00               | 71.33                | 186.00     | 62.00        | cd       |
| M2        | 62.33             | 76.67               | 61.33                | 200.33     | 66.78        | a        |
| M3        | 68.00             | 59.33               | 71.33                | 198.67     | 66.22        | ab       |
| M4        | 28.33             | 50.67               | 40.00                | 119.00     | 39.67        | e        |
| M5        | 30.00             | 32.33               | 53.00                | 115.33     | 38.44        | e        |
| M6        | 69.00             | 65.33               | 58.00                | 192.33     | 64.11        | bc       |
| M7        | 63.67             | 58.67               | 69.33                | 191.67     | 63.89        | c        |
| M8        | 55.67             | 42.00               | 56.00                | 153.67     | 51.22        | d        |
| M9        | 42.67             | 37.00               | 49.67                | 129.33     | 43.11        | de       |
| TOTAL     |                   |                     |                      | 1679.33    |              |          |

The treatment that has the highest plant height is the M2 treatment. This happened because P. fluorescens have a good adaptability to growing media such as soil/top soil. Thus, plants with M2 treatment (66.78 cm) can grow well. The lowest plant height is M4(39.67 cm) and
M5 (38.44 cm), it is happened because both of that treatment have too humid condition of growing media. This is consistent with [11] which states that P. fluorescens that has been adapted is able to colonize plant roots so that it induces plants to increase the production of secondary metabolites of salicylic acid and phytoalexin which play a role in plant resistance and has an effective effect as a rhizobacteria that induces resistance and plant growth booster. In addition, P. fluorescens also produces growth regulators, including auxins, gibberellins, cytokinins, and IAA in plants [12]. P. fluorescens able to adapt and use various substrates as a source of nutrition and its growth is much faster compared to pathogenic bacteria so that it can maintain optimal population in plant roots [13].

3.2. Number of Shoots

Based on observations of the number of shoots after the application of R. solanacearum and biological agents (P. folurescens and B. subtilis) in the 8th week after application. Data is displayed as follows:

**Table 2. Number of Shoots With Comparison of Growing Media and Biological Agents**

| Treatment | I   | II  | III | Total | Average | Notation |
|-----------|-----|-----|-----|-------|---------|----------|
| M0        | 4.67| 5.00| 4.67| 14.33 | 4.78    | a        |
| M1        | 5.33| 3.33| 4.67| 13.33 | 4.44    | ab       |
| M2        | 4.33| 3.33| 4.00| 11.66 | 3.89    | a b c    |
| M3        | 3.33| 3.00| 3.67| 10.00 | 3.33    | a b c    |
| M4        | 1.00| 1.67| 2.33| 5.00  | 1.67    | c        |
| M5        | 1.67| 2.00| 3.00| 6.66  | 2.22    | c        |
| M6        | 5.67| 7.00| 2.33| 15.00 | 5.00    | a        |
| M7        | 5.33| 3.00| 5.33| 13.66 | 4.56    | a        |
| M8        | 3.33| 1.67| 2.00| 7.00  | 2.33    | c        |
| M9        | 2.00| 3.00| 2.33| 7.33  | 2.44    | bc       |
| TOTAL     |     |     |     | 104.00|         |          |

The treatments that had the most number of shoots were M6 (5.00), M0 (4.78) and M7 (4.56). The treatments that had lowest number of shoots were M4 (1.67) and M5 (2.22). This gives an indication that the provision of organic material, especially humus, will be able to suppress the growth of wilting bacteria in potato plants. In addition, the provision of humus also has an effect on enlarging the pores in the planting media, so that the water does not last too long which can cause disruption of plant metabolism and facilitate the growth of new shoots later. This is in accordance with the statement of [14] which states that the provision of humus can increase soil suppressibility, suppress the population of R. solanacearum thereby reducing the intensity of wilting disease. Soil suppressibility is related to organic matter content or organic management. Suppressivesoils that have the capacity to prevent and suppress disease are often applied to suppress soil borne pathogens such as R. solanacearum.

3.3. Tuber Production (g)

Production data is taken when the harvest takes place, when the age of the plant 90-115 days after planting (DAP). To see the results of crop production every treatment. The purpose of weighing the yields of potato tuber as well as the treatment is to compare the total production of all treatments and to get the best treatment.

**Table 3. Tuber Production With Comparison of Growing Media and Biological Agents(g)**
Treatment | Replication | Total | Average | Notation | (DMRT 5%)
---|---|---|---|---|---
M0 | 512.89 | 436.60 | 487.89 | 1437.38 | 479.13 | a
M1 | 473.83 | 390.61 | 514.41 | 1418.65 | 406.22 | cd
M2 | 452.37 | 472.73 | 410.15 | 1321.25 | 440.42 | ab
M3 | 450.23 | 379.37 | 472.73 | 1465.34 | 495.11 | bc
M4 | 40.63 | 36.41 | 137.72 | 214.76 | 71.59 | g
M5 | 422.31 | 538.43 | 351.65 | 1101.74 | 367.25 | d
M6 | 420.79 | 281.08 | 399.86 | 1101.74 | 367.25 | d
M7 | 188.72 | 43.17 | 165.69 | 397.58 | 132.53 | e
M8 | 95.40 | 202.32 | 26.23 | 323.94 | 107.98 | ef

**TOTAL** | | | | 8790.34 |

The treatment that has the highest amount of production is M0(479.13g) and the treatment that has the lowest of production is M4(49.87g). This is because the M0 treatment does not get the treatment of *R. solanacearum* pathogen, so that all plant organs in this treatment can work normally and can produce optimally. This is in accordance with [15], that the branch of the plant is where the leaves grow. The leaves of plants are small in branches which are also small in number, and it can be assumed that the implication for leaf area of whole plants is also lower. As a plant organ that functions to harvest light, leaf area plays an important role. Plant leaves as photosynthetic organs are very influential on the results of photosynthesis. The results of photosynthesis in the form of reducing sugars are used as an energy source to maintain plant life, are formed as plant bodies (roots, stems, leaves) and are accumulated in fruit, seeds or other hoarding organs.

### 4. Conclusion

1) The treatment that has the highest plant height is M2(Top Soil + *R. solanacearum* + *P. fluorescens*) treatment with number 66.78cm can grow well and the lowest plant height is M4(Top Soil + Chicken manure + *R. solanacearum* + *P. fluorescens*) with number 39.67 cm and M5(Top Soil + Chicken manure + *R. solanacearum* + *Bacillus subtilis*) with number 38.44 cm.

2) The treatments that had the most number of shoots were M6 (Top Soil Humus+ *R. solanacearum*+ *P. fluorescens*)5.00, M0(Top Soil/Control)4.78 and M7(Top Soil + Humus + *R. solanacearum*+ *Bacillus subtilis*) 4.56. The treatments that had lowest number of shoots were M4(Top Soil + Chicken manure + *R. solanacearum* + *P. fluorescens*)1.67 and M5(Top Soil + Chicken manure + *R. solanacearum* + *Bacillus subtilis*)2.22.

3) The treatment that has the highest amount of tuber production is M0(Top Soil/Control)479.13g and the treatment that has the lowest of tuber production is M4M4(Top Soil + Chicken manure + *R. solanacearum* + *P. fluorescens*) 49.87g.

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