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# Applications Trichoderma *Koningii Oudem.* to Control Bacterial Wilt (*Ralstonia Solanacearum Smith.)* In Potato (*Solanum Tuberosum L.)*

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## Abstract

Bacterial wilt disease caused by Ralstonia solanacearum Smith. with yield losses reaching 50–100% in potato plants. The application of chemical pesticides can indeed reduce

the intensity of the disease quickly but can cause losses in the form of environmental pollution and can kill beneficial microorganisms in the soil. An alternative control is needed with the concept of Integrated Pest Management, one of which is using the biological agent Trichoderma koningii Oudem. This study aims to determine the effect of the application of T. koningii to reduce the intensity of bacterial wilt ( R. solanacearum ) on potatoes ( Solanum tuberosum L.). The research was conducted from November 2022-February 2023 at the UPTD Potato Seed Center, Food Crops and Horticulture Service, West Java Province. The study was conducted using the experimental method of randomized block design with 5 treatments and 5 repetitions. The treatment consists of: Application of T. koningii with a concentration of 5 grams/plant, 10 grams/plant, 15 grams/plant, 20 grams/plant and without application. The results showed that the use of the application of T. koningii had the best effect on suppressing the intensity of the disease of R. solanacearum with a concentration of 20 grams/plant which had the best effect by suppressing the disease intensity of 89%.

**Keywords**

Trichoderma koningii Oudem., disease intensity, Ralstonia solanacearum Smith., potato plants, applications.

## Introduction

The potato plant (*Solanum tuberosum* L.) is a horticultural plant belonging to the order *Solanales* because it produces tubers for consumption. *S. tuberosum* is also the third main food crop in the world after rice and wheat (Astarini, et al., 2018). This plant is one of the food crops that have high economic value so potato farming is very feasible to develop ( Ahmadi, 2023 ). In addition, *S. tuberosum tubers* also have high nutritional value and contain complex carbohydrates so that they can be used as an alternative food to replace rice (Lutfi, et al., 2020). The demand for potato commodities is increasing increased along with changes in the diet of an increasingly modern society. Potato consumption in Indonesia has fluctuated tending to increase from 2018-2022, in 2018 potato consumption was 2.282 kg/cap/year, and in 2022 potato consumption reached 3.167 kg/cap/year (Pusdatin, 2022). Therefore, good production is needed to meet the needs of society. The production *of S. tuberosum* in Indonesia from 2017-2021 is shown in the following table:

Table 1. Potato Crop Production 2017-2021 in Indonesia

| Year | Harvested Area (ha) | Production (tons) | Yield per Hectare (tonnes/ha) |
|---|---|---|---|
| 2017 | 75,611 | 1,164,738 | 15,404 |
| 2018 | 11.377.934.44 | 59,200,533.72 | 52.03 |
| 2019 | 10,677,887.15 | 54.604.033.34 | 51.14 |
| 2020 | 10.657.274.96 | 54.649.202.24 | 51.28 |
| 2021 | 10,411,801.22 | 54.415.294.22 | 52.26 |

Source: Central Bureau of Statistics (2022)

The potato crop production data above shows that potato crop production

from 2017–2021 has fluctuated. Fluctuations are a limiting factor in increasing production in potato plants which are influenced by several constraints such as land area, labor, availability of quality seeds, provision of nutrients for plants, and control of Plant Destruction Organisms (OPT) (Agatha, 2018; Adah, 2020). Bacterial wilt disease is one of the main pests that attack potato plants caused by *Ralstonia solanacearum* Smith. In Indonesia, the disease has a major impact on food crops (Setiawan, AW, 2019). Yield losses due to bacterial wilt can reach 50–100% and can cause plant death (Muthoni, et al., 2010 in Nur, P., et al., 2016).

Symptoms that appear in infected plants start with wilting of the leaves at the top then spread to all leaves and eventually the plant dies ( Aisyah, et al., 2022). To find out if the plant is infected, you can cut the stem and then put it in the water so that the mass of bacteria will appear to come out of the stem. The pathogen can survive on potato tubers, weeds or in the soil. Not a few farmers use chemical pesticides to control bacterial wilt because it is considered faster to control. But often farmers use chemical pesticides not by the recommended doses given. So the application of chemical pesticides can be at risk of polluting the environment and killing microorganisms that benefit the soil. Therefore, it needs to be implemented control that pays attention to the environment (Noor, I., et al., 2015).

*Trichoderma koningii* Oudem. can control bacterial wilt (Rofika Sari, 2022). The ability of *Trichoderma koningii* Oudem. able to parasitize plant pathogens and has antagonistic properties because it can kill or inhibit the growth of other pathogens. Secondary metabolites owned by Trichoderma *koningii* Oudem. is antimicrobial against *Ralstonia solanacearum* Smith. with the antibiotic compound that belongs to *Trichoderma koningii* Oudem. in the form of viridin and trichomidine, which two compounds are antibiotics (Trisnawati. E., et al., 2019). Use of the biological agent *Trichoderma koningii* Oudem. to suppress bacterial wilt disease caused by *Ralstonia solanacearum* Smith. at UPTD Balai Seed Potatoes located in West Java which is domiciled in Kp. Baru Ibun, Sukamanah Village, Pangalengan District, Bandung Regency has never been researched. So it is necessary to research the effect of the application of *Trichoderma koningii* Odem. to control *Ralstonia solanacearum* Smith. on potato ( *Solanum tuberosum* L. *)*

*Trichoderma koningii* Oudem. with a dose of 10 grams/polybag before planting can control bacterial wilt in potatoes (Karamina, et al. 2018). According to research by Trisnawati, et al. (2019) showed that giving *Trichoderma* sp. with a dose of 60 grams at the time of planting can inhibit the growth of bacterial wilt in banana plants. According to research by Rofika Sari, et al. (2022) *T. koningii* can produce gliotoxin and glioviridine antibiotic compounds. *T. koningii* also produce trichoconins which have anti-microbial activity.

Research on the application of *T. koningii t*o control bacterial wilt (*R. solanacearum*) on potato plants (*S. tuberosum*) has never been implemented at the UPTD Potato Seed Center located in West Java, it is necessary to research to reduce the intensity of bacterial wilt (*R. solanacearum*). The purpose of this study

was to determine the effect of the application of *T. koningii* and to find out the best dose for controlling bacterial wilt (*R. solanacearum*) on potato (*S. tuberosum*).

**Research Methods**

The study was conducted using a randomized block design experiment (RBD) with five treatments and five replications. Totaling 25 plots, formed from 12 plants per plot. There are five application treatments of *T. koningii* used for the test as follows:

1. A : Without treatment ( *Trichoderma koningii* Oudem.)
2. B : *Trichoderma koningii* Oudem. dose 5 gram/plant
3. C : *Trichoderma koningii* Oudem. dose 10 grams/plant
4. D: *Trichoderma koningii* Oudem. Dose of 15 grams/plant
5. E: *Trichoderma koningii* Oudem. dose of 20 grams/plant
6. The linear model for RAK is based on Freund and Wilson (1996) in Vina, R.F., et al 2014 as follows:

$Y_{ij} = \mu + t_i + \beta_j + \Sigma_{ij}$

If the results of the F test show a significant difference, then to be able to distinguish the average of each treatment, a follow-up test is carried out using Duncan's multiple range test method at the 5% level with the following formula:

LSR = SSR x $S\overline{x}$

**Preliminary Test**

**1. Calculating the Spore Density of Trichoderma koningii Oudem**

Testing the spore density was first carried out by dilution. Dilution was carried out 4 times depending on the test to be performed. However, 5 *test tubes are needed* because one *test tube* is used as the mother liquor. The results of calculating the spore density *of T. koningii* namely as follows:

$$K = \frac{\bar{X}}{L \times T \times DX} \; x \; 10^3$$

$$= \frac{8{,}6}{2x10^{-1} x \; 10^{-1} \; x \; 10^{-4}} \; x10^3$$

$$= \frac{8{,}6}{2 \; x \; 10^{-6}} \; x \; 10^3$$

$$= 4.3 \times 10^{9}$$

Box 1 = 3 conidia, box 2 = 10 conidia, box 3 = 6 conidia, box 4 = 7 conidia, and box 5 = 17 conidia with a value of $\bar{\chi}$ totaling 8.6. The results of calculating the density of conidia of *Trichoderma koningii* Oudem. is 4.3 x 10 9 conidia/ml -1 .

**2.Identify Ralstonia solanacearum Smith**

Identification of the disease was carried out on the potato plants being researched. Samples were taken randomly by selecting plants showing symptoms of bacterial wilt (*R. solanacearum*). The samples taken are shown in Figure 1.

 .

Figure 1. Symptoms of bacterial wilt
(Source: Personal documentation, 2023).

Laboratory tests were carried out using potato plants which showed positive symptoms of bacterial wilt disease. This is evidenced by the symptoms that appear on infected plants starting with wilting of the leaves at the top then spreading to all the leaves and finally the plant dies ( Aisyah, et al., 2022). To find out if the plant is infected, you can cut the stem and then put it in the water so that the mass of bacteria will appear to come out of the stem.

The exudate solution in the form of a bacterial mass in Erlenmeyer was then stirred until homogeneous for 3 minutes. The exudate containing the bacterial mass was streaked onto NA (Nutrient Agar) medium.


Figure 2. Macroscopic Identification of Bacterial Wilt
(Source : Personal Documentation, 2023).

The results of observations made on bacterial colonies on NA media indicated that potato plants showing symptoms of bacterial wilt were thought to be caused by bacteria because it could be seen based on the characteristics showing a slimy surface, slightly wet texture, foul smell and also no mycelium found as in mold. Bacteria do not take long for their development because bacteria carry out cell division while fungi need more time to be able to produce spores, hyphae and mycelum (Monica, W. et al., 2016).

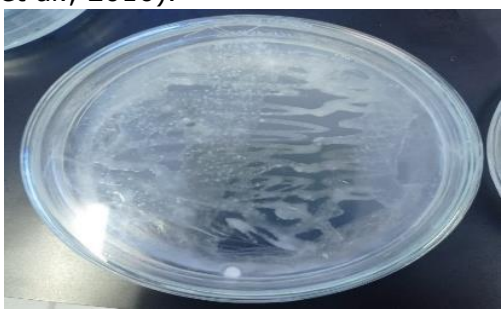

Figure 3. Colony of *Ralstonia solanacearum* Smith.
(Source: Personal documentation, 2022).

**Implementation of Activities in the Screen House**

**Land Preparation**

Activities are carried out in *a screen house* with an area of 528 m 2 . Land preparation used through several stages. The first stage is sanitation in the form of cleaning *the screen house* by cleaning the weeds around the land. Furthermore, the activity of preparing the plant media, cocopeat mixed with manure using a ratio of 4:1, after the planting media is ready then put it into a polybag measuring 35 cm x 35 cm. The spacing used between plants was 30 x 30 cm, the distance for each treatment was 30 cm and the distance for each replicate was 50 cm.

**Seed Preparation**

The seeds used need to be selected first, separate the seeds that look damaged or sick. The seeds used were the seeds of the Granola L variety of quality and qualified potato seeds.

**Planting and Fertilization**

Potato seeds are best planted in the morning or evening , this is done to avoid wilting of the plants. The position of the seed when planting is facing upwards, with a depth of 7-10 cm. The polybags used were 35 cm × 35 cm in size, with a spacing of 30 cm × 30 cm. Basic fertilization using organic fertilizer that is well ripe. In addition, inorganic fertilizers are also given.

**Sprinkling**

Watering the plants is done 2 times a week according to the humidity of the planting medium. Watering is done by paying attention to the condition of the planting media, if the planting media looks dry, watering can be done.

**Stitching**

Seeds that do not grow must be replaced with embroidery or done use new seeds with the same plant and age. Stitching is done by taking dead seeds, then placing new tuber seeds and backfilling to a depth of about 8 cm. Embroidery is done in the morning or evening.

**Propagation of Trichoderma koningii Oudem**

Propagation of *T. koningii* implemented as a medium that will be used for application in the field in suppressing the intensity of *R. solanacearum* in potato

plants. Steps in the propagation of *T. koningii* according to Sumitro, et al. (2020) namely as follows:

1. Corn is washed as much as 3 kg, and soaked in water for 12 hours
2. Then the corn is dried at room temperature
3. After the corn is dried, then the corn is put into a heat-resistant plastic with a size of 100 grams/plastic, then sterilized for 15 minutes using *an autoclave* .
4. The loop needle is sterilized by spraying 70% alcohol and heated over a bunsen. *T. koningii* isolate . taken to taste then put into the corn and then the corn is mixed until homogeneous and stored in a clean place with minimal lighting.
6. The corn was observed for a change in color until the 14th day.

**hoarding**

Bumping is done to strengthen the condition of the stems so that the plants do not fall over easily. Besides that, it also aims to cover the roots of plants that grow a lot upwards. Pembumbunan is done when the plant is 40 MST. Pembumbunan is done by filling the base of the plant with planting media to cover the roots that are popping up.

**Installing Ajir**

Ajir has the function of supporting plants to get optimal sunlight and prevent drooping. Ajir can be made of wood or bamboo with a length of 70-80 cm and a width of 2-3 cm. Staggers were installed on potato plants starting from 3 WAP.

**Weeding**

Weeding is done after weed growth is seen around the plantation. Weeding is done by pulling weeds or cleaning with tools.

**Trichoderma koningii Oudem. application**

Application of the biological agent *T. koningii* done by sprinkling *T. koningii* according to the dose of each treatment. The application is done in the morning and starts from 7 MST, 8 MST, 9 MST, 10 MST, 11 MST, and 12 MST.

**Sampling technique**

Sampling was carried out 2 days after the application of *T. koningii s*ampling this study used a probability sample technique, with the *cluster sampling* method. 1 plot consisting of 12 plants with a total sampling of 10 plant samples aims to minimize the error rate in data collection. The data collection pattern uses the *double cross technique*.

**Harvest**

*S. tuberosum* can be harvested when it is 100 days old. Harvesting is done in the afternoon or early morning and when the weather is sunny. Wet tubers cause rot so that when stored they can trigger other diseases. One week before harvesting the stems of the potato plants are trimmed to leave the base of the stem, roots, and tubers. Pruning is done with the aim that the potato tuber skin becomes stronger to avoid damage during harvesting and lifting.

**Observation Parameters**

Parameters observed included bacterial wilt disease intensity, plant height and production S. *tuberosum*.

**1. Bacterial Wilt Disease Intensity ( Ralstonia solanacearum Smith.)**

The intensity of R. *solanacearum* was observed to determine the intensity of bacterial wilt after the application of *Trichoderma koningii* Oudem. Observations were made by looking at the symptoms of an attack R*. solanacearum* on potato plants, calculated using the absolute formula. Observations were made after the application of *Trichoderma* koningii Oudem. to determine the effect of *Trichoderma koninggi* Oudem application. in controlling the intensity of bacterial wilt (*R. solanacearum*) on *S. tuberosum plants* . Observation of disease intensity was carried out by taking a sample of 10 plants/plot. Observation after application, starting at 7 MST, 8 MST, 9 MST, 10 MST, 11 MST, and 12 MST. Parameters of bacterial wilt damage are included in the absolute damage, using the following formula:

IP = $\frac{n}{N}$x 100 %

Information:

IP = Absolute damage

N = Number of damaged leaves

N = Total observed leaves

**2. Plant height**

Observation of plant height aims to determine the effect of the application of *T. koningii* on the growth of potato plants. Plant height was measured from the base of the rootstock to the growing point using a tape measure and observed every 1 week for each observation starting from 7 WAP, 8 MST, 9 MST, 10 MST, 11 MST, and 12 MST. This observation was carried out by taking a sample of 10 plants/plot.

**3. Potato Production Results**

Observations on potato production were carried out to determine the effect of the application of *T. koningii* on potato production. Observations were made at the end of the planting or harvesting period, observations were made to determine the effect of the application of *T. koningii* in controlling *R. solanacearum* on potato

plants. Observation of the production of potato plants was carried out by taking 10 plants per plot as samples and then weighing them.

## Results and Discussion

### 1. Disease Intensity of Ralstonia solanacearum Smith

Observing the disease intensity *of R. solanacearum* is carried out once a week, which is 2 days after the application of *T. koningii.* Observations were made by counting the wilted leaves and the total leaves on the plants were then recorded. The following table is the average intensity with percent units for each observation:

Table 3. Average Ralstonia solanacearum Smith Disease Intensity. Every Observation.

| Treatment | 7 MST | 8 MST | 9 MST | 10 MST | 11 MST | 12 MST |
|---|---|---|---|---|---|---|
| A | 2.09a – | 6.41 a | 12.63 a | 16.92 a | 27.97a – | 35.75a |
| B | 0.60a – | 1.90 ab | 2.63 b | 3.69 b | 6,14 b | 8.83 b |
| C | 0.40a – | 0.56b – | 0.66b – | 2.69 b | 4.77 b | 7.89 b |
| D | 0.59a – | 0.60 b | 1.15 b | 2.36 b | 4.24 b | 5.24 b |
| E | 0.64 a | 0.70 b | 0.70 b | 1.68 b | 2.40 b | 3.82 b |

**Information**

1. Numbers followed by the same letter in the same column are not significantly different according to Duncan's multiple range test at the 5% level
2. MST = Week after Planting
3. A = control,
4. B = *Trichoderma koningii* Oudem. 5 gram/plant
5. C = *Trichoderma koningii* Oudem. 10 grams/plant
6. D = *Trichoderma koningii* Oudem. 15 gram/plant
7. E = *Trichoderma koningii* Oudem. 20 grams/plant.

Based on the analysis of Duncan's test at the 5% level, the results of the analysis of the average intensity of the attack by bacterial wilt disease caused in potato plants, it was observed that at 7 WAP, all treatments were not significantly different from treatment A (control). It is suspected that the seeds of the Granola L variety are resistant to *R. solanacearum* with an environment that supports the growth of potato plants. Observations at 8 WAP all treatments were significantly different from treatment A (control) except treatment B (*T. koningii* 5 grams/plant). Observations 9 to 12 WAP all treatments were significantly different from treatment A (control). Based on the average results at 12 MST observations, the lowest intensity was in treatment E ( *T. koningii* 20 grams/plant) namely 3.82% which was able to reduce the intensity of bacterial wilt disease (*R. solanacearum*) by 89.31% compared to treatment A (control). According to Karamina, et al., (2018) that *T. koningii* dose of 10 grams/polybag before planting can control bacterial wilt disease in potato plants. According to Lehar, et al., (2018) administration of *Trichoderma* sp directly benefits plants with sufficient organic matter from the given organic fertilizer, *Trichoderma* sp can colonize and penetrate the root system which creates a plant defense system

which can cause certain plant defense mechanisms that induce resistance systemic in the whole plant, it can strengthen the plant defense system against pathogen attack. This is under the opinion of Rofika Sari. , et al., (2022) *T. koningii* can produce gliotoxin and glioviridine antibiotic compounds. *T. koningii* also produces trichoconins which have anti-microbial activity.

**2. Plant height**

Observation of plant height was carried out once a week, ie 2 days after the application of *T. koningii* observations using measuring devices such as meters and counting starts from the base of the stem to the growing point. The following is a table of the average potato plant height in each observation.

Table 4. Average Potato Plant Height ( Solanum tuberosum L.)

| Treatment | 7 MST | 8 MST | 9 MST | 10 MST | 11 MST | 12 MST |
|---|---|---|---|---|---|---|
| A | 2,09[a] | 6,41[a] | 12,63[a] | 16,92[a] | 27,97[a] | 35,75[a] |
| B | 0,60[a] | 1,90[ab] | 2,63[b] | 3,69[b] | 6,14[b] | 8,83[b] |
| C | 0,40[a] | 0,56[b] | 0,66[b] | 2,69[b] | 4,77[b] | 7,89[b] |
| D | 0,59[a] | 0,60[b] | 1,15[b] | 2,36[b] | 4,24[b] | 5,24[b] |
| E | 0,64[a] | 0,70[b] | 0,70[b] | 1,68[b] | 2,40[b] | 3,82[b] |

**Information**

1. Numbers followed by the same letter in the same column are not significantly different according to Duncan's multiple range test at the 5% level
2. MST = Week After Planting
3. A = control
4. B = *Trichoderma koningii* Oudem. 5 gram/plant
5. C = *Trichoderma koningii* Oudem. 10 grams/plant
6. D = *Trichoderma koningii* Oudem. 15 gram/plant
7. E = *Trichoderma koningii* Oudem. 20 grams/plant

Based on the analysis of Duncan's test at the 5% level, the results of the analysis of the average height of potato plants at 7 WAP to 9 WAP showed that all treatments were not significantly different from treatment A (control). It is suspected that the nutrient content contained in the planting medium has been sufficient so that the intensity of *R. solanacearum* disease. did not affect the growth of potato plant height. Observations from 10 to 12 MST showed that all treatments were significantly different from treatment A (control). Treatment A (control) had the lowest overall plant height because *Trichoderma* sp. acts as a plant growth booster, namely, it can secrete growth hormones that are useful for plants by producing the IAA hormone which belongs to the auxin group which works by stimulating shoot growth and root elongation (Charisma, et al., 2012 in Nira, N., et al., 2021 ). According to Guo, et al., (2021) that *T. koningii* produced the lowest yield in terms of the effect on plant height and root length in tomato plants compared to *Trichoderma harzianum* Rifai. and *Trichoderma viride.*

**3. Potato Tuber Weight Yield**

The yield of potato tuber weight is one of the main parameters in this study. The process of observing the yield of potato tuber weight was carried out 100 days after planting by calculating the yield of tuber per sample. Based on the results of Duncan's multiple range test analysis at 5% level using *Trichoderma koningii* Oudem. showed an effect on the weight yield of potato tubers. The following results of the average weight of potato tubers are presented in Table 5 below:

Table 5. Average weight analysis results of potato tubers ( Solanum tuberosum L.)

| **Treatment** | **Average Weight of Potato Tuber (grams)/plant** |
|---|---|
| A (Control without treatment) | 1216$^{bc}$ |
| B ( *Trichoderma koningii* Oudem 5 grams/plant) | 1453$^{b}$ |
| C ( *Trichoderma koningii* Oudem 10 grams/plant) | 2002$^{a}$ |
| D ( *Trichoderma koningii* Oudem 15 grams/plant) | 2050$^{a}$ |
| E ( *Trichoderma koningii* Oudem 20 grams/plant) | 2271$^{a}$ |

**Information**

1. Numbers followed by the same letter in the same column are not significantly different according to Duncan's multiple range test at the 5% level
2. MST = Week After Planting
3. A = control
4. B = *Trichoderma koningii* Oudem. 5 gram/plant
5. C = *Trichoderma koningii* Oudem. 10 grams/plant
6. D = *Trichoderma koningii* Oudem. 15 gram/plant
7. E = *Trichoderma koningii* Oudem. 20 grams/plant

Based on the analysis of Duncan's test at a 5% level on production, the average weight of potato tubers showed that treatments C, D, and E were significantly different from treatment A (control). Treatment A (control) was not significantly different from treatment B ( *Trichoderma koningii* Oudem. 5 grams/plant). The highest average yield of potato tuber weight was shown in treatment E ( *Trichoderma koningii* Oudem. 20 grams/plant) but not significantly different from treatments C and E. Treatment A (control) had the lowest tuber yield compared to other treatments. According to Lehar, et al., (2018), *Trichoderma sp* microorganisms can help degrade organic matter and expedite metabolic processes in the soil so that the soil becomes better able to provide the nutrients needed by plants. Giving *Trichoderma* sp can produce growth hormones that stimulate growth and yield. The availability of nutrients in the soil will be absorbed by the roots as compensation for increased light energy to produce photosynthates which are translocated to the roots so that they can increase the size and volume of root cells (Ferliati, et al., 2014 in Lehar, et al., 2018). According to Baswarsiati, et al., 2001 in Lehar, et al., (2018) the ability of plants to form tubers is adjusted to their ability to absorb food and the availability of food around them.

**Conclusion**

## Conclusion

Based on the results of the study, the conclusions are as follows.

1. Application of *T. koningii* able to reduce the intensity of bacterial wilt disease caused by *R. solanacearum* on potato (*S tuberosum*).
2. *T. koningii* with a concentration of 20 grams/plant had the best effect on suppressing the intensity of bacterial wilt disease caused by *R. solanacearum* on potato plants (S. *tuberosum*) with an emphasis of 89%.

## Suggestion

Based on the results and discussion of the research that has been done, it is suggested to carry out further research with a preventive application so that *T. koningii* has time in suppressing the intensity of bacterial wilt (*R. solanacearum*).

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