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Test some concentration of *Bacillus subtilis* to increase the resistance of tomato plants to *Xanthomonas campestris pv. vesicatoria*

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ABSTRACT

The aim of this study is to know the effect of several concentrations of *Bacillus subtilis* and to obtain a concentration capable of inducing tomatoes from attack by *Xanthomonas campestris pv. vesicatoria*. This study was conducted experimentally using a completely randomized design (RAL) with 5 treatments and 4 replications. The treatment used was the concentration of *Bacillus subtilis* which consisted of B0 = no use of *Bacillus subtilis*, B1 = concentration of *Bacillus subtilis* 106 cells.ml-1, B2 = concentration of *Bacillus subtilis* 107 cells.ml-1, B3 = concentration of *Bacillus subtilis* 108 cells.ml-1, B4 = concentration of *Bacillus subtilis* 109 cells.ml-1. The parameters observed were the number of colonies of *Bacillus subtilis* in the growth medium, the concentration of salicylic acid content, disease intensity, plant height, stem diameter, and weight of fruit. The results showed that the concentration of *Bacillus subtilis* in each treatment was able to induce tomato plant resistance to *Xanthomonas campestris pv. vesicatoria* and gave significantly different results on the number of colonies on soil medium, the concentration of salicylic acid content, disease intensity, plant height, steam diameter, and weight of fruit.
Keyword: tomato, concentration, *Bacillus subtilis, Xanthomonas campestris pv. vesicatoria*

ABSTRAK

Penelitian ini bertujuan untuk mengetahui pengaruh beberapa konsentrasi *Bacillus subtilis* dan mendapatkan konsentrasi yang mampu dalam menginduksi tomat dari serangan *Xanthomonas campestris* pv. *vesicatoria*. Penelitian dilaksanakan secara eksperimen dengan menggunakan rancangan acak lengkap (RAL) yang terdiri dari 5 perlakuan dan 4 ulangan. Perlakuan yang digunakan adalah konsentrasi *Bacillus subtilis* yang terdiri dari B0 = tanpa pemberian *Bacillus subtilis*, B1 = konsentrasi *Bacillus subtilis* 106 sel.ml-1, B2 = konsentrasi *Bacillus subtilis* 107 sel.ml-1, B3 = konsentrasi *Bacillus subtilis* 108 sel.ml-1, B4 = konsentrasi *Bacillus subtilis* 109 sel.ml-1. Parameter yang diamati adalah jumlah koloni *Bacillus subtilis* pada media tanam, konsentrasi kandungan asam salisilat, intensitas penyakit, tinggi tanaman, diameter batang dan bobot per buah. Hasil penelitian menunjukkan bahwa pemberian konsentrasi *Bacillus subtilis* pada setiap perlakuan mampu menginduksi ketahanan tanaman tomat terhadap *Xanthomonas campestris* pv. *vesicatoria* dan memberikan hasil berbeda nyata terhadap jumlah koloni pada medium tanah, konsentrasi kandungan asam salisilat, intensitas penyakit, tinggi tanaman, diameter batang dan bobot per buah.
Kata kunci: tomat, konsentrasi, *Bacillus subtilis, Xanthomonas campestris* pv. *Vesicatoria*

1. Introduction

Tomato (Solanum lycopersicum L.) is an important horticultural commodity in Indonesia and is one of the most popular types of vegetables (Sihotang, 2008) also multipurpose because it can be used as a seasoning, beverages, food coloring, cosmetic ingredients, and medicine. Harvest production of tomatoes in Riau in 2019 was 116,5 tons with a land area of 62 hectares. This number decreased significantly from the previous year,

namely in 2018 production reached 239.6 tons with a land area of 76 hectares (Badan Pusat Statistika, 2020). Based on these data, it can be seen that there is a decrease in tomato production in Riau. The decline in production was caused by several factors including unsuitable varieties planted, poor technical culture, and pest and disease disturbances.

One of the important diseases in tomato plants is bacterial spot disease caused by *Xanthomonas campestris* pv. *vesicatoria* (Villareal, 1980). *Xanthomonas campestris* pv. *vesicatoria* is the cause of the leaf spot which is very detrimental because it can attack tomato plants from the seedling phase to maturity. The attack of *Xanthomonas campestris* pv. *vesicatoria* is one of the limiting factors that cause a decrease in tomato production, therefore it is necessary to control the attack of *Xanthomonas campestris* pv. *vesicatoria*. Control efforts that have been carried out have not shown optimal results, therefore other control alternatives are needed, namely biological agents. Biological control of plant pathogens includes the use of antagonistic organisms and the induction of plant resistance. One of the bacteria that can be used to control plant pathogens is *Bacillus subtilis*. The results of research by Rajendran and Samiyappan (2008), found that Bacillus sp. can induce the resistance of cotton plants to sprouting disease caused by Rhizoctonia solani through an increase in plant defense enzymes. The results of Sufiyanti's research (2019), it was also found that the biocontrol test of endophytic bacteria isolates Micrococcus endopyticus and Bacillus sp. can inhibit the growth of soft rot disease caused by Dickey sp. on potato plants. The aim of this study is to know the effect of several concentrations of *Bacillus subtilis* and to obtain a concentration capable of inducing tomatoes from attack by *Xanthomonas campestris* pv. *vesicatoria*.

2. Materials and Methods

The research was carried out from June 2021 to December 2021 at the Plant Disease Laboratory, Soil Science Laboratory, and Technical Management Unit Experimental Garden, Faculty of Agriculture, Riau University, Bina Widya Campus Km 12.5, Binawidya Village, Binawidya District, Pekanbaru.

The materials used are tomato variety servo F1, isolate *Bacillus subtilis* (Fifi Puspita collection, University of Riau), isolate *Xanthomonas campestris* pv. *vesicatoria* (Sukma Mukhti Collection, University of Jember), Nutrient Agar (NA) media, sterile distilled water, 70% alcohol, plastic wrap, aluminum foil, tissue, duct tape, polybag, plastic, manure, urea fertilizer, TSP fertilizer, KCl fertilizer, Regent 50sc, and paranet. While the tools used are the microscope, petri dish, Erlenmeyer, measuring cup, beaker, test tube, dropper, vortex mixer, incubator, oven, Laminar Air Flow Cabinet (LAFC), shaker, refrigerator, analytical balance, needle loop, knife, colony counter, lamp bunsen, lighter, hand sprayer, camera, and stationery.

This research was conducted using a completely randomized design with 5 treatments:
B0 = No use of *Bacillus subtilis*,
B1 = Concentration of *Bacillus subtilis* 106 cells.ml-1
B2 = Concentration of *Bacillus subtilis* 107 cells.ml-1
B3 = Concentration of *Bacillus subtilis* 108 cells.ml-1
B4 = Concentration of *Bacillus subtilis* 109 cells.ml-1

Each treatment was repeated 4 times. Each treatment was tested on 3 plants, therefore the total number of plants for the experiment was 60 plants. Research methods include the Rejuvenation of *Bacillus subtilis* isolate, Rejuvenation of *Xanthomonas campestris pv. vesicatoria*, land preparation, Planting, Application of *Bacillus subtilis* treatment to tomatoes, Inoculation of *Xanthomonas campestris* pv. *vesicatoria* on tomato plants, Plant Care and Harvest. The variables observed were the number of colonies of *Bacillus subtilis* on growing media (cfu.ml-1), the concentration of salicylic acid content (%), disease intensity (%), plant height (cm), stem diameter (mm), the weight of each fruit (g).

3. Results and Discussion

3.1. Number of colonies of Bacillus subtilis on growth medium (cfu.ml^{-1})

The results of variance showed that the treatment of several concentrations of *Bacillus subtilis* had a significant effect on the number of colonies of *Bacillus subtilis* on growth medium. The number of colonies after further testing using BNJ at a level of 5% can be seen in Table 1.

Table 1. Number of colonies of *Bacillus subtilis* 4 MSA

Concentrations of *Bacillus subtilis* (cell.ml^{-1})	Number of colonies
0	0,00 d
10^6	136,00 c
10^7	177,00 b
10^8	202,00 b
10^9	268,00 a

Numbers followed by the same letter in the same column are not significantly different at the 5% level (BNJ) after transforming √y + 0,5

Table 1 shows the number of colonies of *Bacillus subtilis* on a tomato growth medium with *Bacillus subtilis* treatment at a concentration of 109 cells.ml-1 at the fourth week was significantly different from other treatments. This is presumably because the higher the concentration of *Bacillus subtilis* given, then the higher the number of bacterial colonies of *Bacillus subtilis* on the growth medium. The highest number of colonies was found at the concentration of *Bacillus subtilis* 109 cell.ml-1, which was 268, significantly different from other treatments. The number of colonies at a concentration of *Bacillus subtilis* 108 and 107 cells.ml-1, namely 202 and 177 were not significantly different, but it was significantly different from the treatment with the concentration of *Bacillus subtilis* 106 cells.ml-1, which was 136. This indicates that different concentrations of *Bacillus subtilis* will result in different increases in the ability to colonize bacteria on a growth medium. The number of colonies in the treatment with the highest concentration of *Bacillus subtilis* 109 cells.ml-1 was because the concentrations of *Bacillus subtilis* 109 cells.ml-1 was the best in colonizing bacteria in the soil. Campbell (1989) stated that the higher the concentration of microbes incubated, the greater the ability to colonize the area around plant roots.

3.2. The concentration of salicylic acid content

The results of variance showed that the treatment of several concentrations of *Bacillus subtilis* had a significant effect on the concentration of salicylic acid content in tomato plants. The concentration of salicylic acid content after further testing using BNJ at a level of 5% can be seen in Table 2.

Table 2. Concentration of salicylic acid content treated with *Bacillus subtilis* concentration (cell.ml^{-1})

Concentrations of *Bacillus subtilis* (cell.ml^{-1})	Concentration of salicylic acid content
0	0,00049716 d
10^6	0,00089075 c
10^7	0,00091146 bc
10^8	0,00093218 ab
10^9	0,00095289 a

Note: Numbers followed by the same letter in the same column are not significantly different at the 5% level (BNJ) after transforming √y + 0,5

Table 2 shows that the concentration of *Bacillus subtilis* 109 cells.ml-1 had the highest salicylic acid content and this treatment was significantly different with all concentrations, except for the concentration of *Bacillus subtilis* 108 cells.ml-1, it can be concluded that the treatment of *Bacillus subtilis* concentration of 109 cells.ml-1 was more capable of inducing plants from pathogens than other concentrations having lower salicylic acid content. This is supported by Siddiqui & Shaukat (2004), that salicylic acid activity is one indicator that systemic resistance is induced in plants.

Table 2 also shows that the higher the concentration of *Bacillus subtilis*, then the higher the concentration of salicylic acid contained in plants. This is due to the influence of pathogen inoculation *Xanthomonas campestris* pv. *vesicatoria* so as stimulate the increase in salicylic acid in plants, as well as the application of different concentrations of *Bacillus subtilis* bacteria which caused differences in the increase in the content of salicylic acid in these plants so that the plants became more resistant. According to Nurcahyani et al. (2013), an increase in salicylic acid is one indicator of the effect of plant resistance on pathogens. The increase was thought to be caused by the activity of *Bacillus subtilis* and Pseudomonas fluorescens bacteria *Bacillus subtilis* and Pseudomonas fluorescens bacteria in plant tissues. Phenolic compounds are the result of plant metabolism which are formed as a system of plant chemical resistance to prevent the development of plant pathogens.

3.3. Disease intensity (%)

The results showed that the treatment of several concentrations of *Bacillus subtilis* had a significant effect on the intensity of the disease caused by the bacterium *Xanthomonas campestris* pv. *vesicatoria* on tomato plants after analysis of variance. The intensity of the disease after further testing using BNJ at a level of 5% can be seen in Table 3.

Table 3. Disease intensity by *Xanthomonas campestris pv. vesicatoria*

Concentration of *Bacillus subtilis* (cell.ml^{-1})	Disease intensity (%)
0	68.75 e
10^6	43.75 d
10^7	37.50 c
10^8	25.00 b
10^9	18.75 a

Note; Numbers followed by the same letter in the same column are not significantly different at the 5% level (BNJ)

The data in Table 3 shows the attack intensity of *Xanthomonas campestris* pv. *vesicatoria* on tomato plants to all treatments were significantly different. The highest intensity of disease attack was found in the treatment without *Bacillus subtilis* treatment, which was 68.75%. Meanwhile, the lowest intensity of disease attack was in the treatment of *Bacillus subtilis* concentration of 109 cells.ml-1, which was 18.75%. It can be seen that the low intensity of attack on plants is due to the effect of resistance induction by the *Bacillus subtilis* treatment given, namely the higher the concentration of *Bacillus subtilis*, the intensity of disease attacks caused by the bacterium *Xanthomonas campestris* pv. *vesicatoria* is also getting lower. This is in accordance with Suganda (2016), the lower the intensity of the disease, it means that the treatment is effective in suppressing the development of pathogens. According to Istiqomah and Kusumawati (2018), *Bacillus subtilis* can induce tomato plant resistance to bacterial wilt disease (R. solanacearum). This is also supported by Hersanti et al. (2019), *Bacillus subtilis* and Lysinibacillus sp. can induce resistance of shallot plants to purple spot disease caused by Alternaria porri.

3.4. Plant height (cm)

The results of variance showed that the treatment of several concentrations of *Bacillus subtilis* had a significant effect on the average height of tomato plants. Plant height after further testing using BNJ at a level of 5% can be seen in Table 4.

Table 4. Height of tomato plants treated with *Bacillus subtilis* concentration (cell.ml^{-1})

Concentrations of *Bacillus subtilis* (cell.ml^{-1})	Plant height (cm)
0	69.67 d
10^6	79.55 c
10^7	80.65 c
10^8	82.20 b
10^9	85.47 a

Note : Numbers followed by the same letter in the same column are not significantly different at the 5% level (BNJ)

Table 4 shows that the tomato plant height given the concentration of *Bacillus subtilis* 109 cell.ml-1 had the highest plant height and showed significantly different results with all treatments. The results of plant height observations in Table 4 show that the higher the concentration given, then the higher the plant height growth. This is related to the results of observations of attack intensity which shows that the lower the intensity of disease attacks, the better plant growth. This is presumably because the role of *Bacillus subtilis* as a biological agent is not only seen in the decrease in the intensity of bacterial spot disease (Table 3), but also has an impact on increasing plant growth in the form of an increase in plant height. The increase in growth was caused by the role of Bacillus sp. as PGPR (Plant Growth Promoting Rhizobacteria). Yazdani (2009) stated that *Bacillus subtilis* is a bacterium that is Plant Growth Promoting Rhizobacter (PGPR). This is also in accordance with Ismawati and Rida (2012), that the higher the concentration of PGPR, then the higher the plant growth. PGPR also protects plants from diseases caused by bacteria, fungi, and nematodes. PGPR affects plant growth by means of nitrogen fixation, hormone synthesis, dissolution of mineral substances and synthesis of enzymes that can regulate hormones in plants (Siddiqui, 2005).

3.5. Stem diameter (mm)

The results of variance showed that the treatment of several concentrations of *Bacillus subtilis* had a significant effect on the average stem diameter of tomato plants. The diameter of the stem after further testing using BNJ at a level of 5% can be seen in Table 5.

Table 5. Average stem diameter of tomato plants concentrated *Bacillus subtilis* (cell.ml^{-1})

Concentration of *Bacillus subtilis* (cell.ml^{-1})	Stem diameter (mm)
0	6.63 c
10^6	7.07 bc
10^7	7.27 b
10^8	7.54 b
10^9	8.19 a

Note : Numbers followed by the same letter in the same column are not significantly different at the 5% level (BNJ)

Table 5 shows that the effect of giving concentration *Bacillus subtilis* 109 cells.ml-1 was significantly different from all other concentrations. This is presumably because the concentration of *Bacillus subtilis* 109 cells.ml^{-1} can produce more IAA in the form of the auxin hormone in tomato plants to increase the stem diameter. Auxin hormone will encourage cell elongation by influencing cell wall metabolism in tomato plants. This is in accordance with the statement of Tarabily et al. (2003), that auxin is one type of hormone that can stimulate plant growth by increasing the process of cell elongation and stem elongation as well as cell differentiation. Sarief (1985) also said that *Bacillus subtilis* can increase plant growth, which is also known as a plant growth promoter because it produces a compound driving or plant growth hormone IAA. This hormone produced can stimulate the growth of the plant as a whole.

The results also showed that the concentration of *Bacillus subtilis* 109 cells.ml-1 was able to optimize the growth of tomato plants compared to other treatments because the nutrient requirements for plant growth were more fulfilled so that the plant's ability to photosynthesize was higher and the results of photosynthesis were more. More carbohydrates were translocated to tomato plant stems and used to stimulate secondary growth, namely the expansion of stem cells which was indicated by a wider stem diameter. Bustamam (2006) stated that *Bacillus subtilis* can decompose organic matter in the soil so that nutrients are more easily absorbed by plants for photosynthetic activities in producing photosynthate as plant growth increases. Therefore, it can be said that B. subtilis can play a role in helping the decomposition of organic matter. Djamaluddin (1983) also stated that the increase in stem diameter was caused by good plant growth because the nutrients needed were quite available.

3.6. Weight of Fruit (g)

The results of variance showed that the treatment of several concentrations of *Bacillus subtilis* had a significant effect on the weight of fruit of tomato plants. The weight of fruit after further testing using BNJ at a level of 5% can be seen in Table 6.

Table 6. Weight of fruit of tomato plants given concentration *Bacillus subtilis* (cell.ml^{-1})

Concentrations of *Bacillus subtilis* (cell.ml^{-1})	Weight of fruit (g)
0	27,27 c
10^6	30,89 bc
10^7	33,66 ab
10^8	34,07 ab
10^9	37,21 a

Note : Numbers followed by the same letter in the same column are not significantly different at the 5% level (BNJ)

Table 6 shows that the concentration of *Bacillus subtilis* 109 cells.ml-1 had the largest weight of fruit and this treatment was significantly different for all concentrations, except for *Bacillus subtilis* 108 and 107 cells.ml-1. Treatment without *Bacillus subtilis* showed the smallest weight of fruit, this could be due to the higher the concentration given, then the more colonies produced in the growing medium (Table 1) which resulted in a better ability to decompose organic matter in the soil, so that the availability of elements for plant growth will be better. Kumar et al. (2011) stated that Bacillus sp as PGPR can act as a solvent for phosphorus to make it more available to plants. The availability of sufficient nutrients will increase plant metabolic activities which

have an impact on increasing the number of assimilation in plants so that the weight of fruit increases. This is in line with the opinion of Bustaman (2006), that Bacillus sp. can play a role in helping the decomposition of organic matter in the soil so that nutrients are available for plants.

Table 6 also shows that the high concentration of *Bacillus subtilis* tended to give better results in increasing the weight of fruit of tomato plants. This could be because the intensity of disease attack at this concentration was lower than without *Bacillus subtilis* concentration. In accordance with the results of research by Sudarsono and Malik (2006), a decrease in disease intensity can increase plant wet weight because the presence of disease can reduce plant growth.

4. Conslusion

The concentration of *Bacillus subtilis* in each treatment was able to induce tomato plant resistance to *Xanthomonas campestris pv. vesicatoria* and gave significantly different results on the number of colonies in soil medium, concentration of salicylic acid content, disease intensity, plant height, stem diameter and weight of fruit. The concentration of *Bacillus subtilis* 109 cell.ml-1 was the best treatment for inducing tomato plants from *Xanthomonas campestris* pv. *vesicatoria*.

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