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Prevalence and Incidence of Bacterial Wilt Disease (*Ralstonia syzygii* subsp. *indonesiensis*) on Tomato in Simpang Empat District Karo

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Abstract. The major disease known as bacterial wilt, which affects tomato plants in Indonesia, is brought on by *Ralstonia syzygii* subsp. *indonesiensis*. In Simpang Empat District, Karo Regency, Indonesia, *R. syzygii* subsp. *indonesiensis* causes bacterial withery in tomato plants. The purpose of this study is to map its spread. This study is an experimental one that used the survey method to determine the prevalence of disease, the incidence of disease, and the pathogenic isolation of tomato plants at nine different locations throughout Simpang Empat District. The pathogen was isolated from the sample and identified in the lab as *R. syzygii* subsp. *indonesiensis*, according to the results. The outcome of the presence of bacterial wilt brought on by *R. syzygii* subsp. *indonesiensis* bacteria with various illness occurrences. At a proportion of disease incidence of 57.07%, Ndokum Siroga Location 1 in the community experienced the most disease.

Keywords: bacterial wilt disease, *Ralstonia syzygii* subsp. *indonesiensis*, tomato plants, survey

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1. Introduction

One fruit that is well-liked in society is Tomato (*Lycopersicon esculentum* Mill) which is a major vegetable crop along with chili, onion, and potato. People eat tomatoes every day in a variety of serving sizes. Fresh tomatoes are frequently served as a side dish with salad or vegetables. The sauce frequently includes tomatoes, which enhances the food's appealing color and flavor. Vitamins A and C are known to be found in tomatoes. Today, tomatoes are regarded as the primary source of lycopene, an active component that functions as an antioxidant, in addition to being a source of vitamins A and C [1].

In North Sumatra, the largest contributor to tomato production is Karo District. Karo Regency contributes 78.68% to the total production of tomatoes in North Sumatra. Like other plants, tomatoes cannot be separated from plant pests and diseases (OPT). Some of the emerging pests

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and diseases in tomato are tomato caterpillar (*Helicoverpa armigera* Hubn.), leaf or fruit rot (*Phytophthora infestans*), fusarium wilt (*Fusarium* sp), bacterial wilt (*Ralstonia syzygii* subsp. *indonesiensis*) and *Meloidogyne* spp. [2].

Bacterial wilt disease *R. syzygii* subsp. *indonesiensis,* previously named *Ralstonia solanacearum* (phylotype IV) [3] is one of the limiting factors for tomato production in North Sumatra. It is possible for pathogens to spread through soil, irrigation water, agricultural machinery, and human aid. The upper portion of the plant first shows signs of the disease before entirely withering after a few days. When sliced crosswise, the vessels near the base of infected plant stems are brown, and when submerged in water, they release oose [4].

By damaging plant cells, these infections prevent the transfer of nutrients and water. Cellulase and pectinase enzymes are two of the enzymes involved in this process. This enzyme breaks down the cellulose and pectin-containing plant cell walls. This attack causes physiological abnormalities in plants, disrupting the transfer of water and other nutrients, which causes the plants to wither and eventually die [5]. The spread of bacterial wilt in tomatoes in the Karo Region of North Sumatra has not yet been studied. This research is purposed to see the distribution of *R. syzygii* subsp. *indonesiensis* on tomatoes in Karo District.

2. Materials and Methods

2.1. Survey and Sampling

At Simpang Empat District, Karo District, Indonesia, the survey was carried out at nine tomato planting sites (Table 1). Each area had a single farmer's plot where observations of disease prevalence were made on various kinds of crops. Following the observation, questionnaire-based interviews were conducted with the farmers who owned the land at each location. The questions covered the respondent's identity as well as details about the land, including the variety of crop planted, the age of the plant, the source of the tomato seeds planted, the plants that were present before the tomatoes, the type of crop used (monoculture/polyculture), and any treatments applied.

Sampling was done by looking for existing symptoms, such as wilting and stunted development, as well as the existence of bacterial masses if the plant stems were cut. From each site, three symptomatic plants were selected as samples, and each sample was put into a polyethylene bag and labeled with the date of collection, the location, the height, and the age of the plants. The samples were brought to the laboratory for additional processing following field observations.

Table 1. Survey Locations in Simpang Empat Sub-district, Karo

No	Villages	Altitudes (masl[1])	Coordinates
1.	Surbakti	Location 1 : 1213	N 3°8'29" E 98°27'15"
		Location 2 : 1216	N 3°8'18" E 98°27'21"
		Location 3 : 1217	N 3°8'32" E 98°27'16"
2.	Ndokum Siroga	Location 1 : 1283	N 3°8'59" E 98°28'18"
		Location 2 : 1257	N 3°8'46" E 98°27'58"
		Location 3 : 1257	N 3°8'52" E 98°28'3"
3.	Bulan Baru	Location 1 : 1313	N 3°9'39" E 98°28'44"
		Location 2 : 1315	N 3°9'55" E 98°29'0"
		Location 3 : 1337	N 3°10'13" E 98°29'16"

Note: [1]: masl= meter above sea level

2.2. Isolation of Pathogen from Collected Samples

The tomato plants affected by bacterial wilt were rinsed under running water before being separated. To see the ooze, the roots were sliced in half crosswise and placed in distilled water (bacterial mass). If you notice a large amount of bacteria emerging, clean the stalk near the tomato plants' roots and cut it into pieces measuring 5 cm each at the stem's base. The parts were cleaned in 70% alcohol, rinsed three times with sterile distilled water, then aerated and ground. 10 ml of sterile, distilled water was added to the scouring and allowed for 30 minutes. The bacteria-containing suspension was then spread with a needle onto semi-selective Tetrazolium chloride (TZC) medium and incubated for 1x24 hours at 27°C. Additionally, the outcomes of the bacterial isolation were repeated in order to acquire single colony. After obtaining a single colony, then the bacterial isolates were characterized by observing colony morphology, Gram staining and performing biochemical tests.

Macroscopic observations of bacteria were carried out by observing the morphology of bacterial colonies with observations from pure cultures until they became single colonies. Observations were made in the form of shape, color, edge and elevation. Microscopic observations were carried out by Gram testing to determine the type of Gram of this bacterium. By adding one ose isolate to the glass preparations and fixing them over the fire, the gram staining technique is carried out. In addition, bacteria are dyed with violet crystals and then washed with running water after 1 minute. Iodine is then added, and the process is repeated after 1 minute. In addition, bacteria are exposed to ethyl alcohol for 30 seconds, washed with running water, and then given safranin. All of these treatments are repeated with running water. Furthermore, absorbent paper and emersion oil are used to dry the preparation glass. Under a 1000-fold

magnifying microscope, the shape and color of bacterial cells may be seen. Gram -negative bacteria are red and gram -positive bacteria in blue [6].

2.3. Catalase Test

The catalase test was carried out by dropping 3% hydrogen peroxide (H_2O_2) on a clean object glass. The culture was smeared on an object glass that had been dripped with hydrogen peroxide. The suspension was mixed slowly using a loop, the formation of oxygen bubbles was evidence that resulted in a positive catalase test result, no bubbles produced after 20 seconds were interpreted as a negative test result [7].

2.4. KOH Test

The KOH test was carried out by placing 1 drop of 3% KOH on a glass slide then the culture was taken with a sterile loop and rubbed in 3% KOH solution and the suspension was stirred continuously for one minute and then the loop was gently pulled. The test is considered positive if mucus (thick thread) is seen within the first 30 seconds after mixing in the KOH solution [8].

2.5. Disease Incidence and Prevalence

Observations of disease incidence were carried out on all plants by looking at the symptoms of visually. Disease incidence is calculated using the formula [9] as follows:

$$DI = \frac{a}{b} \times 100\%$$

where DI = Incidence of bacterial wilt disease; a = Number of plants affected by bacterial wilt; b = Number of plants observed

The prevalence of disease was calculated in all locations that have been observed:

Disease prevalence = (number of infected locations / total location) x 100%.

3. Result and Discussion

3.1. Characterization and Identification of Bacterial Wilt Pathogen of Tomato

The bacterial isolates of the samples is a Gram negative bacteria. When grown on TZC media, it is dark red with a round colony shape and has a convex elevation and flat side of the colony (Figure 1). Champoiseau and Timur [10] stated that the change from virulent to non-virulent bacterial cells occurred during storage or oxygen stress in liquid media. Tetrazolium chloride (TZC) medium was developed to differentiate between two types of colonies, where virulent colonies appear white with a pink center and non-virulent colonies appear dark red. There are nine isolates of bacteria from nine locations, which an isolate represents each location.

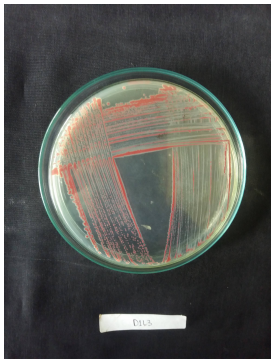

Figure 1. Morphology of the Isolated Colonies

The results of morphological observations showed that the bacterial isolates is rod-shaped and belongs to the type of Gram-negative bacteria which is characterized by a red color of the bacterial cells. The catalase test was observed from 9 bacterial isolates showed a positive result with the formation of air waves (Figure 2). In the KOH test, 9 bacterial isolates showed the presence of mucus (positive reaction) (Figure 3).

Figure 2. Catalase test; positive reaction showed the presence of bubble.

Figure 3. KOH test; positive reaction showed the presence of slimes.

Based on the morphological and physiological characteristics as well as the symptoms of the plant samples, the bacterial isolates were identified as *R. syzygii* subsp. *indonesiensis*. Rahayu [11] stated that *R. syzygii* subsp. *indonesiensis* belongs to a group of Gram-negative bacteria, cell morphology is short rod-shaped, single cells measuring 0.5–0.7 x 1.5–2.0 m.

3.2. Disease Prevalence

Based on observations made at nine different sites around the Simpang Empat district, it was determined that the disease was present at every single site. This was due to the fact that the disease, which had an incidence rate of between 31.58% and 57.07%, affected all sites where it had been identified with varied signs and symptoms (Table 2).

Table 2. Disease Incidence of Bacterial Wilt Caused by *Ralstonia syzygii* subsp.*indonesiensis* (%)

Villages	Plant's age(wap[1])	Disease Incidence (%)
Surbakti Location 1	6	31.58
Surbakti Location 2	16	52.45
Surbakti Location 3	12	50.26
Ndokum Siroga Location 1	16	57.07
Ndokum Siroga Location 2	12	47.80
Ndokum Siroga Location 3	20	52.85
Bulan Baru Location 1	16	41.44
Bulan Baru Location 2	20	40.00
Bulan Baru Location 3	20	39.17

Note: [1]: WAP = week after planting

Overall, each place has a similar disease incidence value that is evenly distributed throughout all locations. The bacterial wilt disease often affects tomato plants at every stage of development, from the vegetative until generative stage. All areas with various plant ages are affected by the bacterial wilt disease's symptoms. According to Champoiseau and Timur [10] bacterial wilt disease symptoms can manifest as seedlings and during the plant growth phase. Stunted plants are another common symptom present in the field. The state of the leaves is still green and the plants are still producing fruit, though not as well, while wilting like they have been scalded by hot water (Figure 4). Pradhanang *et al.* [12] stated that in mature plants, the first signs in hot weather are wilting of the upper leaves, which is followed by recovery during the night and early morning. As the condition worsens, withered leaves keep their green tint. The plant will completely wilt and die under hot, humid conditions that are conducive to the disease. One of the abiotic factors that influence the development of bacterial wilt disease *R. syzygii* subsp. *indonesiensis* is the rainfall. At the time of data collection at each location, it was observed that the conditions were entering the beginning of the dry season and the environment was humid. Bacterial wilt disease usually occurs in the early dry season or the end of the rainy season, with land conditions still moist and the weather warm [13].

Figure 4. The Symptom of Bacterial Wilt Disease on Tomato in the Field

3.3. Disease Incidence

Observation of disease incidence in 9 (nine) tomato planting locations in Simpang Empat District, Karo Regency, obtained disease incidence between 31.58-57.07% (Table 2). The highest percentage of disease incidence was in Ndokum Siroga Village location 1 with a plant age of 16 weeks, while the lowest disease incidence was in Surbakti Village location 1 with a plant age of 6 weeks. The high incidence of *R. syzygii* subsp *indonesiensis* disease in Ndokum Siroga Village location 1 could be due to the fact that at the time the survey was conducted the sanitation conditions of the land were not good, namely the land was filled with weeds (Figure 5). This condition causes the pathogen to have an alternative host, and if the main host is not present after harvest, the pathogen will be able to survive with asymptomatic weed hosts and in the soil for long periods of time. *R. syzygii* subsp. *indonesiensis* can survive in plant tissue or associate with alternative hosts such as *Ageratum conyzoides*, *Crassocephalum crepidiodes*, *Crotalaria juncea* and *Croton hirtus* [14]. On the other hand, the lowest percentage of incidence was found in Surbakti Village location 1, this could be due to the very good land sanitation condition during the survey.

Figure 5. Land Sanitation at the Ndokum Siroga Village Location 1

From the results of the interview, it can be seen that in the village of Ndokum Siroga location 1 using the servo F1 tomato plant variety (Table 3) is the location with the highest percentage of disease incidence. This is because the servo F1 variety is one of the varieties that are susceptible to *R. syzygii* subsp. *indonesiensis*. According to Navitasari *et al*. [15] stated that high yielding tomato varieties (Servo) were susceptible to *R. syzygii* subsp. *indonesiensis* which showed high intensity of bacterial wilt disease and reduced high plant productivity.

Table 3. Farmer' Interview Output at the Nine Sample Locations

Location (Village)	Questions				
	Plant's age (WAP[1])	**Plant variety**	**Plant material**	**Previous plant**	**Type of planting**
Surbakti Location 1	6	Royal 58	Purchase	Orange and Carrot	Polyculture (Chili dan Broccoli)
Surbakti Location 2	16	Royal 58	Purchase	Carrot	Polyculture (Chili)
Surbakti Location 3	12	Royal 58	Purchase	Carrot	Polyculture (Chili)
Ndokum Siroga Location 1	16	Servo F1	Own seeds	Cabbage and Napa cabbage	Polyculture (Chili)
Ndokum Siroga Location 2	12	Kani F1	Purchase	Egg plant	Polyculture (Chili)
Ndokum Siroga Location 3	20	Delta	Purchase	Tomato	Polyculture (Chili dan Broccoli)
Bulan Baru Location 1	16	Natavi	Purchase	Chryssantemum	Polyculture (Chili)
Bulan Baru Location 2	20	Delta	Purchase	Coffee	Polyculture (Chili)
Bulan Baru Location 3	20	Kani F1	Benih beli	Tomato, Chili and Napa cabbage	Polyculture (Chili)

Note: [1]: WAP = week after planting

4. Conclusion

From nine point locations in Simpang Empat District, it was found that the prevalence of the disease from all locations was 100%. The highest percentage of disease incidence was found in Ndokum Siroga Village location 1 with 57.07% disease incidence while the lowest disease incidence was found in Surbakti Village location 1 with 31.58% disease incidence.

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