Distribution of bacterial wilt disease (*Ralstonia solanacearum*) on tobacco in Temanggung

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**Abstract** Bacterial wilt caused by *Ralstonia solanacearum* is one of major diseases on tobacco worldwide. This research aimed to determine the distribution of bacterial wilt disease on tobacco plantation in Temanggung District, Central Java. Observation of the incidence of bacterial wilt disease was carried out on 53 sampling areas determined based on land system. The observation was conducted on two-month old tobacco in the field. At the same time, soil samples were collected for detection of bacterial population. The last activity was carried out three different times i.e. before, when, and after tobacco planting in the field. The results revealed that the incidence of bacterial wilt diseases ranged from 0-28% which means that the distribution of bacterial wilt across tobacco plantation was categorized from very low to moderate. The highest incidence of bacterial wilt was found in sampling point 23 located in Tegalurung, Bulu. We also found zero incidence of bacterial wilt in thirteen locations. The population of bacteria was determined in 11 sampling locations which are generally known as “lahan linca”. The results showed that the highest population was reached at after planting time followed by when and before planting, they were 3.67-80.67x10⁴, 0-10.67 x10⁴ and 0-1 x10⁴ cfu/g soil, respectively.

Key words: bacterial wilt, *Ralstonia solanacearum*, tobacco

1. **Introduction**  
Bacterial wilt caused by *Ralstonia solanacearum* is one of major diseases on tobacco. It has broad range of plant host including tomato and eggplant and could cause considerable yield loss on those two plants. Initially, the bacterial wilt disease was reported to infect tobacco in Deli, North Sumatera, Indonesia. Farmers tend to deny as it only caused little incidence. However, in 1880s, the disease was spread across cigar tobacco plantation in Deli and most of plants were collapsed. In 1931, about 11% cigar tobacco seedlings were destroyed because of bacterial wilt infection [1].

The infected plant would be wilting at the day but then seem to be recovered at the evening. There are external and internal symptoms of bacterial wilt on tobacco. Wilting of the upper part of plant is the most recognized external sympton, which then followed with inhibited growth and yellow leaves at one side of the plant. The internal symptom was determined by the existence of dark-small line which then develop along plant vascular. If the infected stalk slashed, the milky-like substrate would be produced on the stalk surfaces [2]. [3] revealed that the susceptibility of host plant and pathogen aggressiveness affected on disease development.

Infection started when the bacteria invaded plant root then penetrated host tissues through natural or artificial wound caused by a nematode. During infection, the bacteria were motile and distributed...
into vascular bundle through water translocation [4], [5] also revealed that when the bacterial concentration increased, it would activate virulent genes and produce non-motile extracellular polysaccharides which were able to secrete pectin-degraded enzymes and would result in plant died.

Temanggung is recognized as one of tobacco center production in Indonesia. It produces Temanggung tobacco which are known as sliced tobacco with specific characters such as aromatic and high nicotine content. There is limited information on the distribution of bacterial wilt disease on tobacco plantation. Therefore, this research aimed to determine bacterial wilt disease distribution across Temanggung tobacco plantation. This information will be useful to determine proper control methods for the disease.

2. Research Methods
The survey was conducted during February to October 2019 in 53 sampling areas of tobacco plantation in Temanggung determined by land system method. The sampling areas were distributed into various elevation and land types. The survey on bacterial wilt disease incidence in the field was carried out on two-month old tobacco. For each sampling area, observation was conducted on 25 plants, then number of infected plants was recorded. The incidence of bacterial wilt disease was calculated according to the formulae below:

\[ \text{KP} = \frac{a}{b} \times 100\% \]

KP = Bacterial wilt incidence (%)

a = Number of infected plants

b = Total number of observed plants

In order to study bacterial population, soil sampling was also carried out for each sampling area. Bacterial population was isolated using selective media SMSA-E (Selective Medium South Africa-Elphinstone) [9,10] with three replicates.

3. Result and Discussion
Symptom of bacterial wilt disease on tobacco in the field can be identified by wilting on one side of infected host plant dan asymmetric growth (Figure 1). Bacteria penetrated plant tissues through natural wound within plant tissues or artificial wound because of nematode infection. This can assist bacteria to penetrate plant tissues. When the environmental condition is favorable for the pathogen, symptom will emerge on infected plant.

![Figure 1. Bacterial wilt disease on tobacco](image-url)
In our survey, we found that the incidence of bacterial wilt disease was low ranged from 0 to 28%. It might be influenced by environmental condition when the survey took place. It was very dry at that time, as there has not been rain for three months. On the other hand, for disease development, bacteria need enough humidity. Growth of R. solanacearum highly depends on environmental conditions, especially the humidity, in which, high humidity might affect the level of incidence as well as bacterial multiplication would also increase on infected plants [6].

In Temanggung, there are some areas generally known as “lahan lincat”. This is a local term illustrates a non-productive land for tobacco. This condition was because of land degradation which resulted in decrease of soil fertility and high population of soilborne pathogens including R. solanacearum, Phytophthora nicotianae, dan Meloidogyne spp. [7]. Our observation on 11 sampling areas which were known as lahan lincat revealed that the incidence of bacterial wilt disease could be categorized into low to moderately low ranged from 0 to 28% (Table 1).

**Table 1. The incidence of bacterial wilt disease on lahan lincat areas**

| Sampling number | Village    | Sub-district | Bacterial wilt incidence (%) | Level of category |
|-----------------|------------|--------------|------------------------------|-------------------|
| 6               | Legoksari  | Tlogomulyo   | 12,93                        | Low               |
| 16              | Wonotirto  | Bulu         | 8,00                         | Very low          |
| 20              | Wonotirto  | Bulu         | 20,00                        | Moderate          |
| 23              | Tegalurung | Bulu         | 28,00                        | Moderate          |
| 28              | Wonotirto  | Bulu         | 20,00                        | Moderate          |
| 32              | Pagergunung| Bulu         | 11,31                        | Low               |
| 35              | Tlahap     | Kledung      | 12,00                        | Low               |
| 39              | Campursari | Bulu         | 8,00                         | Very low          |
| 43              | Giripurno  | Ngadirejo    | 16,00                        | Low               |
| 50              | Tawangsari | Wonoboyo     | 0,00                         | Very low          |
| 53              | Kedu       | Kedu         | 12,00                        | Low               |

We did assume that low incidence of bacterial wilt disease during our survey might be caused by dry condition and low humidity. Those conditions were not favorable for bacterial growth and development, consequently it influenced on the ability of the pathogen to infect host plant. In addition, at unfavorable environment, the bacteria enable to produce external polysaccharide as a survival structure to adapt with abnormal condition. Soil water content also influenced on the bacterial wilt infection and interaction between pathogen and its host after infection occurred [6].

### 3.1 Distribution of bacterial wilt disease

Our survey revealed that the bacterial wilt disease has been distributed on most of 53 sampling areas except on 13 points including village of Kemloko, Ngaditirto, Tlahap, Tuksari, Caneggal, Katengsari, Mangunsari, Purwosari, Cemoro, Tawangsari, Kebonsari, Gandurejo and Ngimbang. All those villages distributed into 8 sub-districts involving Tembarak, Selopampang, Kledung, Candiroto, Ngadirejo, Wonoboyo, Temanggung and Bulu (Figure 2).
Figure 2. Distribution of bacterial wilt disease in Temanggung

3.2 Bacterial wilt population within soil samples
Beside observation of bacterial wilt incidence, we did also soil sampling to determine bacterial wilt population. Soil samples on lahan lincat areas were collected at three different times, they were before, when and after planting of tobacco. Lahan lincat is traditional term for a land if planted with tobacco it would not be able to grow well. When the soil was isolated it was found that population of soil borne pathogen including nemathodes Meloidogyne sp, R. solanacearum and Phytophthora nicotianae [7]. The results revealed that bacterial population significantly increased after planting time (Table 2).

| Sampling number | Village | Sub-district | Bacterial wilt population (cfu/g soil) |
|-----------------|---------|--------------|---------------------------------------|
|                 |         |              | Before planting | When planting | After planting |
| 6               | Legoksari | Tlogomulyo | 0            | 2,00 x 10⁴  | 36,33 x 10⁴  |
| 16              | Wonotirto | Bulu        | 0            | 2,00 x 10⁴  | 46,67 x 10⁴  |
| 20              | Wonotirto | Bulu        | 0            | 10,67 x 10⁴ | 80,67 x 10⁴  |
| 23              | Tegalurung | Bulu       | 0            | 3,67 x 10⁴  | 70,00 x 10⁴  |
| 28              | Wonotirto | Bulu        | 1x 10⁴       | 1,33 x 10⁴  | 25,67 x 10⁴  |
| 32              | Pagergunung | Bulu    | 0            | 1,00 x 10⁴  | 70,00 x 10⁴  |
| 35              | Tlahap   | Kledung     | 0            | 3,33 x 10⁴  | 23,00 x 10⁴  |
| 39              | Campursari | Bulu    | 0            | 0,00 x 10⁴  | 3,67 x 10⁴   |
The population of bacterial wilt was very low at before planting time (0-1x10⁴ cfu/g soil). At this time, the bacterial was only found on both sampling areas of 28 and 43. None of bacteria was found at the rest of sampling points. Then the bacterial population gradually increased (0-10.67x10⁴ cfu/g soil) when tobacco was about two-month old in the field. Surprisingly, the bacterial population was significantly increased into 3.67-80.67x10⁴ cfu/g soil after planting time. This result in line with the incidence of bacterial wilt disease, in which, low bacterial population resulted in low incidence of bacterial wilt disease in the field. According to [8], minimum bacterial population for infection should reach up to 6.6x10⁶ cfu/g soil. Beside bacterial population, environmental condition also influences the incidence of bacterial wilt disease especially temperature and relative humidity. The optimum temperature and relative humidity needed for the disease was 30 °C and >81.42%, respectively [8].

**4. Conclusion**

Bacterial wilt disease has been distributed into 40 of 53 sampling areas of tobacco plantation. However, the incidence of the disease was ranged from very low to moderately low. In addition to low incidence of bacterial wilt disease, the bacterial population was also very low at before planting time but then gradually increased when tobacco existed in the field and significantly increased after planting.

**Acknowledgement**

Authors acknowledge to Badan Perencanaan Pembangunan Daerah (BAPPLEDA) Temanggung, Central Java who provided funding for this research.

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