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# Research Article
# Characteristics of Soil Transmission of *Ralstonia syzygii* subsp. *syzygii*, the Cause of Sumatra Disease of Clove in Indonesia

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## Abstract

**Background and Objective:** Transmission of Sumatra disease of cloves is known to occur through the insect vectors from sick plants to healthy plants. This research studied the transmission of *Ralstonia syzygii* subsp. *syzygii*, the cause of Sumatra disease through the soil. **Materials and Methods:** Observations were made on two-year-old seedling plants planted in Kendal, Central Java, Indonesia infected with Sumatra disease. Detection of rhizosphere soil, infected plant residues and symptomatic plants was carried out using PCR method. The transmission experiment of Sumatra disease in clove seedlings was applied by artificial inoculation with the method of shoot titration, root immersion, root watering and root wounding conducted at greenhouse. Symptom appearance, incubation period, disease severity and Area Under Disease Progress Curve (AUDPC) were recorded periodically. **Results:** *Ralstonia syzygii* subsp. *syzygii* was detected in symptomatic replanting plants, rhizosphere soil layers and infected plant debris in the field. In the greenhouse experiment, all inoculation methods were able to cause Sumatra disease symptoms. The incubation period for the root immersion, root watering and root wounding treatment was shorter (12 DAI) than the titration treatment (17 DAI). The root wounding treatment gave the highest value of the disease severity (37.04%) and AUDPC (201.85) compared to other treatments. **Conclusion:** *Ralstonia syzygii* subsp. *syzygii* can be detected in infected soil and plant debris and can be transmitted through the soil.

Key words: Cloves, *Ralstonia syzygii* subsp. *syzygii*, soilborne, Sumatra disease, transmission

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

*Ralstonia syzygii* subsp. *syzygii,* the cause of Sumatra disease in cloves, belongs to the *Ralstonia solanacearum* species complex (RSCC) phylotype IV along with *Ralstonia syzygii* subsp. *celebesensis*, the cause of blood disease in bananas and *Ralstonia syzygii* subsp. *Indonesiensis*, the cause of wilt disease in several Solanaceae plants. Yield losses due to Sumatra disease can reach up to 40% annually due to reduced production of flowers and leaves that can be harvested. *Ralstonia syzygii* subsp. *syzygii* is classified as xylem-limited bacteria that live and develop in xylem tissue. This is because xylem tissue not only contains low concentrations of organic components as energy for plants, but also consists of various amino acid compounds, organic acids and inorganic ions in the form of monomers which are essential nutrients for bacteria[1].

Externally, the symptoms of infection by *R. syzygii* subsp. *syzygii* is indicated by leaves that turn yellow and eventually fall at the ends of the branches to the crown of the tree. Symptoms appear from the lower branches to the crown and plants die 6-18 months after the infection. The internal symptoms are characterized by a change in the color of the wood adjacent to the cambium to pale grayish brown and if the infected branch is cut crosswise it will show bacterial ooze of milky white to pale brown[2-4].

So far, it is known that the spread of Sumatra disease occurs through transmission by vector insects, namely *Hindola* spp., which feed from the xylem tissue of plants. *Hindola fulva* is an insect species naturally found as a vector of Sumatra disease in Sumatra and *H. striata* is the vector in Java[5]. Sumatra disease develops in a broad spectrum with an estimated distribution acceleration of 1-2 km per year[2]. The pattern of the spread of Sumatra disease occurs randomly (jump spread patterns) in an area of land, which is thought to be due to the mobility of *Hindola* spp.

Isolation of *R. syzygii* subsp. *syzygii* of the insect vector *Hindola* spp., is only successful on 13 out of 65 *Hindola fulva* insects found in Solok and only 1 out of 20 *Hindola striata* insects found in Sukamaju[5]. In addition, field observations show that planting healthy seedlings in planting locations previously infected with Sumatra disease can cause the seedlings to experience external symptoms resembling Sumatra disease, including plant leaves that become fewer due to falling and leaf color that persists on branching to gradually turn brown like scorched.

Plant pathogens that cause wilt belonging to the genus *Ralstonia* such as *R. solanacearum* and *R. pseudosolanacearum* can be transmitted through soil[6-8]. Likewise, *R. syzygii* subsp. *celebesensis* can be transmitted through the soil and agricultural tools too[4]. Therefore, *R. syzygii* subsp. *syzygii* has the potential to persist and transmit through the soil. To enhance understanding related to the disease cycle of *R. syzygii* subsp. *syzygii* and efforts to mitigate its spread, the information regarding the transmission of this pathogen is urgently needed. The objective of this study was to detect *R. syzygii* subsp. *syzygii* on soil and clove plant residue, figure out the transmission of Sumatra disease through the soil and prove soilborne transmission using various methods of artificial inoculation.

## MATERIALS AND METHODS

**Study area:** Observation of symptoms of Sumatra disease was carried out in the clove plantation of PT. Cengkeh, Kendal, Central Java, Indonesia. Molecular detection and transmission experiment of *R. syzygii* subsp. *syzygii* was conducted at the Laboratory of Plant Pathology and Greenhouse, Department of Plant Protection, Faculty of Agriculture, University of Gadjah Mada. The research was carried out from July, 2022 to June, 2023.

**Observation and sampling of plants showing symptoms of Sumatra disease in the field:** Observations were made on two-year-old seedling plants planted in areas infected with Sumatra disease (replanting). Sampling included three plants with size of 50-80 cm height showing symptoms of Sumatra disease, rhizosphere soil in the infected area and remaining infected plant roots. Detection of *R. syzygii* subsp. *syzygii* in the sample was carried out using the Polymerase Chain Reaction (PCR) technique[9].

**Hypersensitivity and pathogenicity test of *Ralstonia syzygii* subsp. *syzygii*:** Hypersensitivity and pathogenicity tests were conducted on isolates suspected to be *R. syzygii* subsp. *syzygii.* A hypersensitivity test was carried out on 3 month-old tobacco plants using the method referred to Sharma[10]. The observation of hypersensitive reactions was carried out until there were necrotic symptoms on the injected tobacco leaves using the isolates obtained. Pathogenicity assay was carried out on one-year old Zanzibar clove seedlings. As much as 10-20 µL of bacterial suspension with a density of $1\times10^8$ CFU $mL^{-1}$ was injected into the part of the plant stem close to the shoot that had been perforated using a microtip[11]. The inoculated plants were incubated in a suitable environment for 2 months and the symptoms and development of Sumatra disease were observed.

**Molecular detection of *Ralstonia syzygii* subsp. *syzygii* :** Extraction of isolates suspected as *R. syzygii* subsp. *syzygii* was carried out using the CTAB method (Cetyltrimethylammonium bromide). Confirmation of isolates of *R. syzygii* subsp. *syzygii* applied a specific primer UGMRss-F/UGMRss-R (5'-GCTCACCATCGCCAAGGACAGCG-3'/5'TTCGATCGAAC GCCTGGTTGAGC-3') with a total reaction volume of 10 µL consisting of 2 µL DNA template, 1 µL reverse primer, 1 µL forward primer, 1 µL Nuclease Free Water and 5 µL 2x MyTaq Red Mix (Bioline) on a PCR machine (Bio-Rad T100, Germany). The cycle used consisted of pre-denaturation at 96°C for 5 min, followed by denaturation at 94°C for 15 sec, annealing at 59°C for 30 sec, extension at 72°C for 30 sec and equipped with a final extension at 72°C for 10 min, the cycle was repeated 35 times. A total of 4 µL of PCR product was visualized using electrophoresis (Bio-Rad DNA Electrophoresis Cell) at 60 volts for 50 min on 1% agarose gel stained with ethidium bromide. Gel visualization was carried out under UV transilluminator[9].

**Sumatra disease transmission experiment on clove seedlings:** The experiment was carried out using a completely randomized design (CRD) with 5 treatments and 3 replications, each consisting of 3 plant units. The treatments used consisted of P1 = Control, P2 = Shoot titration, P3 = Root immersion, P4 = Root watering and P5 = Root wounding. The clove seedlings used were one-year-old Zanzibar variety with uniform height (±50 cm). The *R. syzygii* subsp. *syzygii* was cultured on casein peptone glucose (CPG) broth medium and the density was measured using a spectrophotometer (Spectronic GENESYS 10 UV-Vis) at $OD_{600}$ nm until the value OD = 0.1 ($1\times10^8$ CFU $mL^{-1}$) was obtained.

The shoot titration inoculation method was carried out by punching holes in the internode of the clove plant (d = 1 mm) using a sterile needle and then injecting 20 µL of suspension of *R. syzygii* subsp. *syzygii*. The suspension was allowed to settle and enter slowly into the exposed vascular tissue with the help of microtips. Clove seedlings were covered using a clear plastic for 24 hrs. The inoculation method by root immersion was carried out by soaking the clove plant seedlings (including the roots and planting medium in polybags) in a suspension of *R. syzygii* subsp. *syzygii* for 2 hrs. The root watering method was carried out by watering ±500 mL suspension of *R. syzygii* subsp. *syzygii* on the media and clove plant roots until the media conditions turned into field capacity or saturated. The root wounding method was carried out by wounding the clove plant roots using a sterile scalpel and then a suspension of *R. syzygii* subsp. *syzygii* was poured until the media became saturated.

**Observation of incubation period, disease severity and Area Under the Disease Progress Curve (AUDPC) of Sumatra disease**

**Incubation period:** The incubation period indicates the length of time it takes for the pathogen to cause symptoms in plants. The incubation period of Sumatra disease in each treatment was observed every day. The initial emergence of Sumatra disease symptoms was a benchmark in determining the incubation period of *R. syzygii* subsp. *syzygii* in each treatment.

**Disease severity:** Disease severity of Sumatra disease was observed every 7 days since the emergence of the first symptom in one of the treatments. The results of the disease severity assessment were calculated using the following formula[12]:

$$DS\ (\%) = \frac{\sum(n_i \times v_i)}{(N \times Z)} \times 100$$

Where:

DS = Disease severity
$n_i$ = Number of sample plants with i value
$v_i$ = Disease score in each sample plant with i value
N = Total sample plants observed in one treatment
Z = Highest score used as a reference

The scoring method was based on the condition of the clove canopy which referred to the assessment by Widodo *et al.*[13] with slight modification (Table 1).

Table 1: A score of Sumatra disease based on canopy condition

| Score | Percentage | Description |
|---|---|---|
| 0 | x = 0 | Healthy, no dieback symptom |
| 1 | x≤20 | Early symptoms indicate infection in less than 20% of the branches or less than 20% of the canopy showing symptoms such as leaf drop, yellowing, or browning resembling a scorched appearance |
| 3 | 21≥x≤30 | Advanced symptoms: 21-30% of the plant's branches are infected, or 21-30% of the canopy showing leaf drop, yellowing, scorched appearance and twig drying |
| 5 | 31≥x≤45 | Moderate symptoms: 31-45% of the branches are infected, canopy shows leaf drop and branch breakage |
| 7 | 46≥x≤65 | Severe symptoms: 46-65% of the plant's branches are infected and branches and twigs experience death and breakage |
| 8 | >65 | Very severe symptoms: More than 65% of the plant's branches were infected, the entire canopy withered, scorched appearance, leaf drop and upper to lower branches dried and dead |

**Area Under the Disease Progress Curve (AUDPC) of Sumatra disease:** Disease severity progression was calculated using the AUDPC based formula by Cooke[14] with the following formula:

$$AUDPC = \sum_{i=1}^{n-1}\left(\frac{Y_i + Y_{i+1}}{2}\right)\left(t_{i+1} - t_i\right)$$

Where:

AUDPC = Area Under the Disease Progress Curve
n = Total number of observations
$Y_i$ = Assessment of the disease severity at the 1st observation
$t_i$ = Time at the 1st observation

**Confirmation of plant disease symptoms using the PCR method:** The DNA extraction of *R. syzygii* subsp. *syzygii* was carried out on the roots, stems and leaves of clove seedlings with symptoms of Sumatra disease using the ZymoBIOMICS™ DNA Miniprep KIT based on an existing protocol. Overall, the amplification and visualization program used was the same as the DNA amplification and visualization method of *R. syzygii* subsp. *syzygii* used in this study.

**Statistical analysis:** Data obtained from observations were analyzed using Analysis of Variance (ANOVA) with a significance level of 5%. If there was a significant difference between the treatments, a further test would be carried out using the Duncan's Multiple Range Test (DMRT) with a significance level of 5% with the help of the IBM SPSS Statistics software version 20.

## RESULTS

**Symptoms of Sumatra disease in the field:** Observations in the field showed that there was still root debris from plants that had previously been cut down due to infection with Sumatra disease. Two-year-old plants planted at a distance of 1-2 m from diseased plant sites showed symptoms of Sumatra disease. The incubation period required for symptoms of Sumatra disease to emerge in replanting seedlings varied between 5-7 months after transplanting.

Symptoms of Sumatra disease began with the drying of the branches and leaves located at the very bottom of the plant canopy. Symptoms then developed vertically towards the branches and leaves located at the top. One month after the emergence of the initial symptoms, the entire plant experienced changes in the color of the leaves and stems to brown and dried and the shoots of the plants became scorched (Fig. 1a). After a month, some of the dried leaves were shed, while others remained on the stems and branches of the plants (Fig. 1b).

Detection of *R. syzygii* subsp. *syzygii* using the PCR technique on rhizosphere soil and clove roots aged ±40 years which had been logged for two years showed results in the form of the emergence of a 378 bp DNA band (Fig. 2).

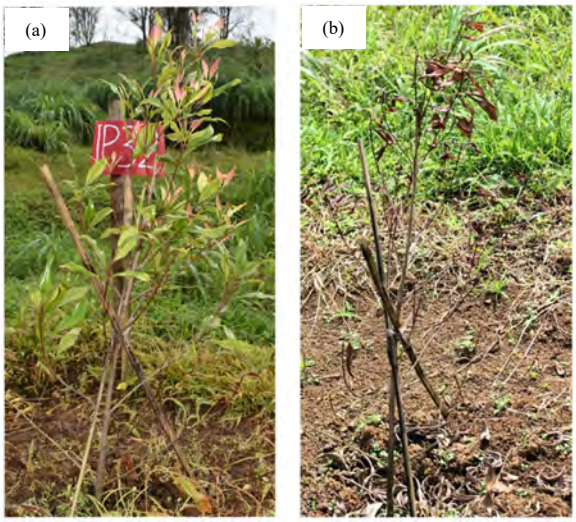


Fig. 1(a-b): Clove seedlings showed initial symptoms of (a) Sumatra disease at 5 months after transplantation and (b) Clove seedlings were died at 6 months after transplantation

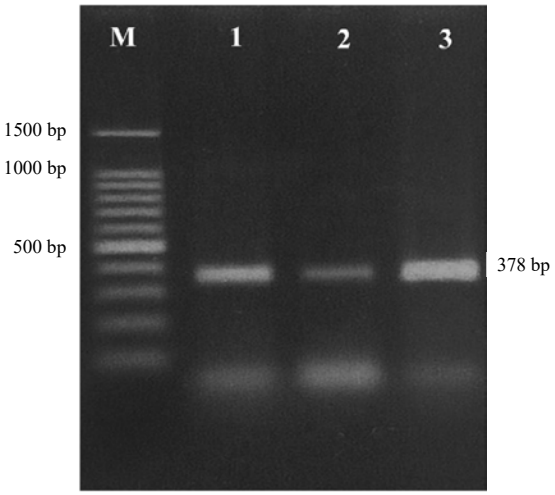


Fig. 2: Visualization of soil and root DNA amplicons using the specific primers UGMRss-F/UGMRss-R
PCR products were analyzed using 1% agarose gel electrophoresis in 1x TBE buffer. (M: 100 bp DNA Marker), Lane 1: Positive control using *Ralstonia syzygii* subsp. *syzygii* isolate, Lane 2: Soil sample and Lane 3: Root sample

Fig. 3: Hypersensitive reaction of *Ralstonia syzygii* subsp. *syzygii* on tobacco leaves (4 days after inoculation) as indicated by an arrow


Fig. 4(a-b): Pathogenicity assay on clove seedlings, (a) Healthy clove seedlings without inoculation (control treatment) and (b) Clove seedlings with *Ralstonia syzygii* subsp. *syzygii* inoculation at 35 days after inoculation

**Hypersensitivity reactions and pathogenicity of *Ralstonia syzygii* subsp. *syzygii*:** The isolate of *R. syzygii* subsp. *syzygii* showed hypersensitivity reactions to tobacco leaves. Areas of necrosis on the leaves were formed 4 days after the pathogen was inoculated (Fig. 3). Pathogenicity assay of *R. syzygii* subsp. *syzygii* performed on one-year-old clove seedlings showed that clove plants inoculated using a suspension of *R. syzygii* subsp. *syzygii* showed symptoms of Sumatra disease in the form of yellowing of the leaves starting from the bones and edges of the leaves and then drying and scorched brown in color (Fig. 4a). Meanwhile, uninoculated clove plants did not develop symptoms until 35 days after inoculation (DAI) (Fig. 4b). Detection of isolates of *R. syzygii* subsp. *syzygii* using PCR showed the formation of a single band of single DNA bands at 378 bp.

**Transmission of Sumatra disease in clove plant seedlings:** The control plants showed no symptoms (Fig. 5a), while symptoms of Sumatra disease occurred in the shoot titration, root immersion, root watering and root wounding treatments (Fig. 5b-e). The clove plants inoculated using the titration method was marked by a change in leaf color to yellowish starting from the leaves that were positioned close to the inoculation point (Fig. 5b). Meanwhile, the treatment with root immersion (Fig. 5c), root watering (Fig. 5d) and root wounding (Fig. 5e) experienced symptoms of Sumatra disease which was indicated by drying of the leaves starting from the bottom as a sign of *R. syzygii* subsp. *syzygii* infection.

Within 70 days after inoculation (DAI), all leaves and stems dried out and turned brown in color with plant shoots showing symptoms like scorched. Furthermore, some of the dried leaves fell and some of the leaves remained on the stems and twigs of the plant.

**Incubation period, disease severity and AUDPC of Sumatra disease:** Treatment using different inoculation methods showed differences in symptoms, incubation period, disease severity and AUDPC of Sumatra disease in clove seedlings in the greenhouse. The inoculation treatment by root immersion, root watering and root wounding had a faster incubation period of disease of 12 days after inoculation compared to the titration treatment which had a longer incubation period of 17 days after inoculation (Table 2).

The percentage of the disease severity in all treatments was directly proportional to the AUDPC value. The highest disease severity and AUDPC value occurred in the inoculation treatment using the root wounding method, namely 37.04% and 201.85, while the lowest disease severity and AUDPC value occurred in the titration treatment, namely 16.05 and 85.81%. All treatments had a percentage of disease severity and AUDPC values that were significantly different from the control treatments (Table 2). Based on this, it can be seen that the inoculation method affects the disease severity and AUDPC.

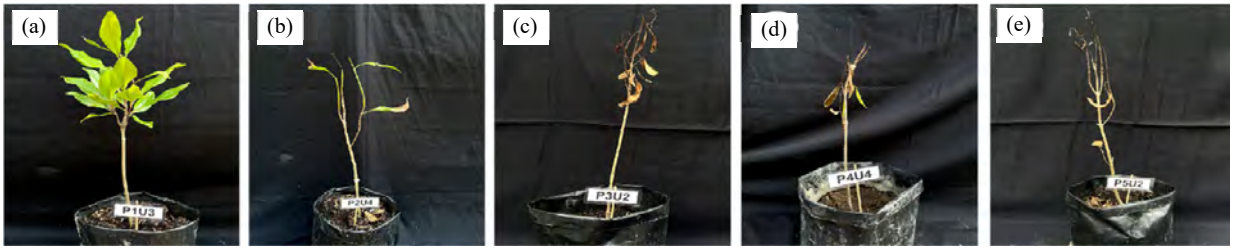


Fig. 5(a-e): Symptoms of Sumatra disease in clove seedlings in the greenhouse, (a) Healthy plant without inoculation, (b) Shoot titration inoculation method, (c) Root immersion inoculation method, (d) Root watering inoculation method and (e) Root wounding inoculation method


Fig. 6: Visualization of DNA amplicons from plant seedling samples using specific primers UGMRss-F/UGMRss-R
PCR products were analyzed using 1% agarose gel electrophoresis in 1x TBE buffer (Lane 1-5: Leaf samples-control, shoot titration, root immersion, root watering, root wounding, Lane 6-10: Stem samples-control, shoot titration, root immersion, root watering, root wounding, Lane 11-15: Control, shoot titration, root immersion, root watering, root wounding and M: 100 bp DNA Marker)

Table 2: Incubation period, disease severity and AUDPC for each treatment

| | | | | Detection using PCR | | |
|---|---|---|---|---|---|---|
| Treatment | Incubation period (DAI) | Disease severity (%) | AUDPC | Leaves | Stem | Root |
| P1 | 0 | 0.00[a] | 0.00 | - | - | - |
| P2 | 17 | 16.05[b] | 85.81 | + | + | + |
| P3 | 12 | 34.57[b] | 160.49 | + | + | + |
| P4 | 12 | 30.86[b] | 183.95 | + | + | + |
| P5 | 12 | 37.04[b] | 201.85 | + | + | + |

Disease severity at 70 days after inoculation (DAI), Data followed by the same letter do not show significant differences at a 95% confidence level, P1: Control, P2: Shoot titration, P3: Root immersion, P4: Root watering and P5: Root wounding

**Confirmation of Sumatra disease symptoms in clove seedlings using PCR:** Detection of *R. syzygii* subsp. *syzygii* in plants with symptoms of Sumatra disease was carried out on all parts of the plant including leaves, stems and roots of each treatment. Detection using PCR showed that the leaves, stems and roots of clove seedlings treated with shoot titration, root immersion, root watering and root wounding showed the formation of DNA bands at 378 bp (Fig. 6), while the control treatment without *R. syzygii* subsp. *syzygii* did not show any formation of DNA bands that matched the target. This indicated that *R. syzygii* subsp. *syzygii* could colonize all parts of the clove plant from root to leaf and vice versa.

## DISCUSSION

Symptoms shown on two-year-old clove plants in the replanting area are typical symptoms of Sumatra disease. In general, the external symptoms of Sumatra disease are infected leaves that turn yellow and fall or suddenly wither, then quickly turn brown and remain attached to stems and branches as if scorched by fire. Infected branches change color from green to reddish brown and progressively die off starting from the tip of the plant[15].

Some of the outbreaks of the Sumatra disease of clove population that occurred in West Sumatra Province caused

population deaths reaching 70% of the total area of 15,000 ha during 1975-1985. The death of this population also included plants that were replanted in the same area (replanting)[3]. This showed that the soil and clove plant roots in the remaining and decayed areas could still be hosts of *R. syzygii* subsp. *syzygii.* Based on the results of this study, as a pathogen belonging to the RSSC group, *R. syzygii* subsp. *syzygii* could be transmitted through the soil. Furthermore, *R. solanacearum* and *R. pseudosolanacearum* can also survive in the soil as saprophytic bacteria[16].

Based on Choudhary *et al.*[17] *R. solanacearum* is a pathogen that can survive in aquatic habitats and irrigation water contaminated by diseased plant tissues as a source of inoculum. According to Navitasari *et al.*[18] when the plant dies, *R. solanacearum* changes its life cycle to become a saprophyte in soil or other environments and can survive until it finds a new host. When there are no host plants, *R. solanacearum* can survive in the natural environment, but its population is influenced by biotic and abiotic factors. Several ways for *R. solanacearum* to survive outside the host plant and in unfavorable environmental conditions include in the form of Viable but Non-Culturable Bacteria (VBNC), starved cells, PC type and biofilms.

Under conditions of status as VBNC bacteria, bacterial metabolic activity continues, but cannot be cultured on solid media that is generally used and forms dormant cells. *Ralstonia solanacearum* can survive in soil as the VBNC form in less than one month at 4°C. In the condition of being starved cells, bacteria survive using population management and do not grow, but can still be cultured. Besides that, *R. solanacearum* can form biofilm-like structures when in contact with biotic and abiotic surfaces.

*Ralstonia syzygii* subsp. *syzygii* exhibits a hypersensitive reaction in tobacco plants. Tobacco leaf cell membranes that come into contact with pathogenic bacteria will experience destruction so that the leaves become dry and necrotic[19]. This shows that bacterial isolates injected into leaf cells have the potential act as plant pathogens. The ability of *R. syzygii* subsp. *syzygii* to show a hypersensitive reaction on tobacco leaves because this pathogen has a Type-III *Hrp* secretion system which has an important role in the pathogenesis process[20]. In the pathogenicity assay, *R. syzygii* subsp. *syzygii* in this study showed a longer emergence of symptoms compared to the study by Danaatmadja *et al.*[21] and Trianom *et al.*[22] due to differences in the age of clove seedlings and the density of bacterial cells used.

The transmission of Sumatra disease experiment in clove seedlings showed that there were differences in the location of the initial symptoms between the titration inoculation method and other methods. This was presumably because the development of Sumatra disease symptoms corresponds to the entry point of the bacterial colony *R. syzygii* subsp. *syzygii*. Generally, *R. solanacearum* enters the host plant tissue through wounds at the ends of the roots and the point where the roots emerge. It then gradually infiltrates the xylem vessels and spreads via the vascular system to the aerial parts of the plant (stems and leaves). Wilting symptoms are typically brought on by the xylem tissues significant bacterial colonization and the excessive production of exopolysaccharide (EPS), which obstructs the passage of water and nutrients[23].

The presence of pathogens in the xylem tissue can cause embolism and reduce the conductivity of the vascular tissue. In plants with wilting symptoms, the association between pathogens, vascular tissue embolism and symptom development is closely related and the formation of bubbles triggers a defensive response of the plant, resulting in occlusion of the vessels due to gel production and tylosis[24]. In general, the main factor that causes symptoms to appear in infected plants is the result of blockade in the xylem tissue due to bacterial biofilm activity as well as due to tylose or sap that is actively produced by plants.

*Ralstonia syzygii* subsp. *syzygii*, which was inoculated from the roots, could be detected on the roots, stems, the tops of the clove plant seedlings. Research conducted by McElrone *et al.*[25] on one of the xylem inhibiting bacteria, *Xylella fastidiosa*, indicates that the movement of pathogens occurs from the base of the plant to the top of the *Parthenocissus quinquefolia* plant. The petioles lying at the base are colonized first and eventually limit all movement of water into the leaf. In addition, cutting off the leaf stalks on vines with symptoms of scorch indicates a blockage by bacterial colonies in the vessels on the leaves.

The same thing happened to the treatment by inoculation using the shoot titration method, which showed that *R. syzygii* subsp. *syzygii* can be detected up to the roots of the clove plant. Based on research by Saponari *et al.*[26] inoculation of *X. fastidiosa* using the stem prick method showed that at 4 weeks after inoculation, *X. fastidiosa* was detected in the roots of the *Olea europaea*. According to Petit *et al.*[27] the presence of a high diffusion signal factor (DSF) can induce bacterial synthesis and produce polygalacturonase, which can degrade cell walls and endo-1,4-b-glucanase, which can enlarge the pores between xylem vessels, allowing the transfer of bacteria between vascular tissues. The presence of these enzymes is thought to play an important role in the distribution of bacterial mass from the leaf shoots as entry points during inoculation, to all parts of the plant including stems and roots[28].

## CONCLUSION

*Ralstonia syzygii* subsp. *syzygii* can be detected in symptomatic plants, as well as in the rhizosphere soil and debris of infected plant roots. Artificial inoculation through root wounding was able to cause Sumatra disease symptoms in clove seedlings. This indicated that the transmission of Sumatra disease can occur through the soil.

## SIGNIFICANT STATEMENT

*Ralstonia syzygii* subsp. *syzygii* is known to be detectable molecularly using PCR techniques on soil and debris from infected plant roots that have been logged for 2 years. In addition, artificial inoculation using root immersion, root watering and root wounding methods was able to show the appearance of Sumatra disease symptoms on clove seedlings. This can be the basis for realizing that the transmission of Sumatra disease can occur through the soil. Knowledge about the transmission of Sumatra disease can be the basis for making decisions related to the management of Sumatra disease in the field so that it is more effective and able to prevent transmission of Sumatra disease in a wider area.

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