Control of xylem limited bacterium (XLB) disease of clove and its insect vector with biocontrol agents

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Abstract. An experiment was conducted under field condition in Wonogiri, Central Java in 2018 to evaluate the effectiveness of biological control agents against xylem limited bacterium disease and its insect vector (Hindola striata). The experiment was arranged in a completely randomized block design with 12 treatments and 4 replicates. Treatments used were (1) fipronil, (2) Beauveria bassiana, (3) bioprotector, (4) oxytetracycline, (5) Bacillus sp.-Pseudomonas, (6) oxytetracycline + fipronil, (7) oxytetracycline + bioprotector, (8) oxytetracycline + B. bassiana, (9) Bacillus sp.-Pseudomonas + fipronil, (10) Bacillus sp.-Pseudomonas + bioprotector), (11) Bacillus sp.-Pseudomonas + B. bassiana, and (12) control (water). Infected clove plants were treated twice with monthly application interval. Variables observed were the population of H. striata, percentage of plant damage, and the plant shoots growth. Treatments of oxytetracycline, fipronil, oxytetracycline + fipronil, Bacillus sp.-Pseudomonas, oxytetracycline + B. bassiana, and Bacillus sp.-Pseudomonas + B. bassiana could reduce 100% population of H. striata. Treatments of Bacillus sp.-Pseudomonas + B. bassiana could inhibit the plant damage percentage up to 91.94%. While, treatments of Bacillus sp.-Pseudomonas + B. bassiana, Bacillus sp.-Pseudomonas + fipronil, and oxytetracycline + fipronil stimulated the plant shoots growth.

1. Introduction

Clove (Syzygium aromaticum) is one of the most important spice plants usually used for many industries, pharmaceuticals, and foods. This plant is highly demanded in Indonesia due to its role as a source of income for the country and farmers, as well as, provide labour. Indonesia is one of the highest clove production country. However, the national clove production still very low (260–360 kg/ha) as compared to its potential production (500–600 kg/ha). The highest production of clove in Indonesia in the period of 1980-2013 was 374.77 kg/ha in 2003 [1].

The development of many clove-based industries, such as clove cigarettes that occurred after the economic crisis in 1998 had an impact on the increasing need for cloves and start improving clove prices in Indonesia. This caused farmers in some areas to be interested in doing rejuvenation of clove plants that have been damaged/dead. Therefore, since 2001, farmers have been expanding clove planting in some areas [1].

One of the main diseases that cause the decrease of clove production in Indonesia is xylem limited bacteria (XLB). The disease was firstly reported in West Sumatera in the 1960s [2]. Furthermore, the
disease spreads out rapidly to other clove production areas throughout Indonesia, such as in South Sumatera, Bengkulu, Jambi, West Java, and East Java [3].

Symptoms of XLB disease are wilting, falling leaves, and dead twigs. Falling leaves and dead twigs generally start from the top of the infected plant that often followed by the lower parts of the plant. The infected plant dies within 6-18 mo. On the surface of the heavily infected plants (wooden tissue), brownish to grayish lines symptoms appear. If the stems and roots are cut, the milky bacterial exudate can be observed. By the time, the diseased plant shows symptoms at the top of the tree, the bacterial exudates have clogged the vascular tissues of the root and the base of the stem. Symptoms of falling leaves and dead twig on the top of the plant is as a result of the malfunctioning of the roots and the presence of blockage of vascular vessel tissues [4].

XLB disease is caused by Pseudomonas (Ralstonia) syzygii [4, 5]. The bacteria are very closely related to P. solanacearum, however, its colonies size is smaller than P. solanacearum [6]. Morphological characteristic of R. syzygii are cylindrical shape, 0.5-0.6 × 1.0-1.5 μm in size, aerobic, has no flagella and does not move, Gram negative, does not form spores, grows slowly in nutrient agar medium, optimum temperature for its growth is 28 °C [5, 7].

XLB disease is spread air by insect vector of Hindola striata (in Java) and H. fulva (in West Sumatera) [6, 8, 9]. Therefore, the pattern of disease spread in the garden is irregular. In highland areas, the disease intensity is generally higher and spread more rapid than in lowland areas [4].

The presence of XLB diseases is very detrimental for farmers, therefore they were not interested to grow cloves. Recently, it was reported that a similar disease of XLB was found infecting clove plants in some growing areas. Observation made in some clove growing areas in Wonogiri and Karanganyar Districts, Central Java Province indicated that the symptom of the disease was similar to XLB. So far, the causal agent of the disease has not been confirmed yet. However, the insect vector H. striata were found in those areas. The disease spread rapidly and become more severe. Therefore, it is necessary for immediate efforts to control both the pathogen and its insect vector.

Control techniques of the disease are still limited, therefore, there is a need of integrated research to produce technological components that effectively prevent and control this disease. Although chemical control, such as using antibiotics infusion and synthetic pesticide treatment can suppress disease development and its insect vector (Hindola spp.), the continuous use of synthetic chemical pesticide intensively can cause negative effects, such as resistance, resurgence, and also polluted environmental and agricultural products. In addition, synthetic chemical pesticides are also expensive and currently difficult to obtain in farmers scale. Therefore, recently the disease control program is being focused to the use of biological agents and cultivation technique that are environmental friendly.

Isolation of endophytic and rhizobacteria from healthy clove and its rhizosphere, followed by molecular assay were able to identify Bacillus subtilis and B. cereus. They could produce indole acetic acid (IAA), dissolve phosphate, and generate the biggest inhibition zones against Ralstonia syzygii subsp. syzygii [10].

Disease control using biological agents is expected to prevent and suppress the incidence of XLB disease and increase the productivity of cloves. In this case, the exploration of biological control agents, testing their activity in the laboratory, glass house, and field are needed. The most effective biological control agents hopefully could be used as a control recommendation in field. The aim of this research was to evaluate the effectiveness of biological control agents against XLB disease of clove and its insect vector under field condition.

2. Methods
Experiment was carried out from Apr to Dec 2018 in a disease-endemic area at a farmer clove plantation in Wonogiri District, Central Java. Treatments consisted of biocontrol agents, such as a mixed of bacterial isolates (Bacillus sp.+ Pseudomonas) for the control of R. syzygii, (the pathogen of XLB disease), a formula of fungal isolate (B. bassiana), as well as a botanical insecticide (Bioprotector) for controlling of H. striata (an insect vector of R. syzygii). An antibiotic (oxytetracycline) and a chemical insecticide (fipronil) were also used as comparison. Infected clove
plant with disease intensity less than 50% were treated with each of biocontrol agents (bacterial or fungal isolates), antibiotic, and chemical insecticide. All treated clove plants were fertilized with organic (manure) and anorganic fertilizers in accordance with GAP for clove plant. Research was arranged in a completely randomized block design consisted of 12 treatments and 4 replicates (Table 1). Clove plants were sprayed with each of biocontrol agents (BH), synthetic insecticide (IF), botanical insecticide (BB), and infused with antibiotic (AB) or biocontrol agents (BR) according to the treatment (Table 1). Parameters observed were: number of *H. striata* (insect vector), disease development (percentage of plant damage), as well as, clove plant growth as reflected by young leaf shoot formation. Observation carried out before and at 1, 2, and 3 mo after treatment. Population of *H. striata* (insect vector) was observed by counting all stages (eggs, nymphs, and adults).

**Table 1.** Treatment for controlling XLB and its vector *H. striata*.

| Treatments                          | Application method          |
|-------------------------------------|-----------------------------|
| Antibiotic (Oxytetracycline) (AB)   | Stem infusion (2ml/L, 500 ml/plant) |
| Biocontrol agents (*Bacillus*-*Pseudomonas*) for *R. syzygii* (BR) | Stem infusion (10³ cfu, 500 ml/plant) |
| Synthetic Insecticide (Fipronil) for *H. striata* (IF) | Canopy spraying (2 ml/L, 7 l/plant) |
| Biocontrol agents (*Beauveria* sp.) for *H. striata* (BH) | Canopy spraying (10 g/L, 7 l/plant) |
| Botanical Insecticide (Bioprotector) for *H. striata* (BB) | Canopy spraying (5 ml/L, 7 l/plant) |
| AB + IF                             | Stem infusion + Canopy spraying |
| AB + BH                             | Stem infusion + Canopy spraying |
| AB + BB                             | Stem infusion + Canopy spraying |
| BR + IF                             | Stem infusion + Canopy spraying |
| BR + BH                             | Stem infusion + Canopy spraying |
| BR + BB                             | Stem infusion + Canopy spraying |
| Control                             | Untreated                   |

Clove plant was selected of those ±1.5 m from the ground level (as high as the observer could reach). The young leaf shoot formation was scored as follow: 0 = no shoot, 1 = a little shoot, 2 = medium shoot, 3 = many shoot. All treatments were re-applied after 1 mo from the first application. Effectiveness of tested biocontrol agents were evaluated by comparing the number of *H. striata*, disease development, and young leaf shoot formation on treated and untreated clove plants.

Effectiveness of each treatment on *H. striata* population was analysed using the following formula:

\[ EP = \frac{Ca-Ta}{Ca} \times 100\% \]

With, \( EP \) = effectiveness of treatment on population (%)  
\( Ca \) = population of *H. striata* in control  
\( Ta \) = population of *H. striata* in treatment.

A formula was considered effective if the value of the level of efficacy (EI) was ≥ 50% [11]. Effectiveness of each treatment on plant damage was determined using the following formula:

\[ EPD = \frac{Ca-Ta}{Ca} \times 100\% \]

With, \( EPD \) = Effectiveness of treatment on plant damage (%)  
\( Ca \) = Average of plants damage in control treatment (%)  
\( Ta \) = Average of plants damage in treatment.
A pesticide was considered effective if the value of the level of efficacy (EI) was ≥ 50% [11]. Data were analyzed with ANOVA (analysis of variance) and Duncan multiple range test (DMRT) using SAS at α = 0.05.

3. Results and discussion

*Hindola striata* population decreased up to zero (0) at the first observation (one mo after treatment). Both in treated and untreated clove plants, the eggs, nymphs, and adults of *H. striata* were not found. This might be due to the effect of the treatments or other causes, such as the insects died naturally. The population of *H. striata* (eggs and nymphs) increased in the next observation (2 mo after treatment) (Table 2).

| Treatments                  | The average of *H. striata* population (individu/plant)* | EP (%)d |
|-----------------------------|----------------------------------------------------------|---------|
|                             | Before treatment** | 1 matb | 2 mat* | 3 mat** |         |
| *B. bassiana*               | 2.75 b           | 0 a    | 0 a    | 1.75 ab | 30.00   |
| Oxytetracycline             | 8.00 a           | 0 a    | 0 a    | 0 c     | 100.00  |
| Fipronil                    | 6.50 ab          | 0 a    | 0 a    | 0 c     | 100.00  |
| Bioprotector                | 4.25 ab          | 0 a    | 0 a    | 0.5 bc  | 80.00   |
| *Bacillus*-Pseudomonas + fipronil | 6.50 ab     | 0 a    | 0 a    | 0.75 bc | 70.00   |
| Oxytetracycline + fipronil  | 5.00 ab          | 0 a    | 1.5 a  | 0 c     | 100.00  |
| Control                     | 5.00 ab          | 0 a    | 0.5 a  | 2.50 a  |         |
| Oxytetracycline + bioprotector | 5.00 ab      | 0 a    | 0 a    | 1.00 abc| 60.00   |
| *Bacillus*-Pseudomonas      | 7.50 a           | 0 a    | 0 a    | 0 c     | 100.00  |
| Oxytetracycline + *B. bassiana* | 6.75 ab      | 0 a    | 0 a    | 0 c     | 100.00  |
| *Bacillus*-Pseudomonas + *B. bassiana* | 7.50 a | 0 a    | 0 a    | 0 c     | 100.00  |
| *Bacillus*-Pseudomonas + bioprotector | 7.25 ab   | 0 a    | 0 a    | 0.25 bc | 90.00   |
| CV                          | 18.61           | 0      | 23.92  | 16.58   |

*a* Numbers followed by the same letters in the same column were not significantly different at DMRT 5%, where mat = mo after treatments.

b Note: \(\sqrt{x + 1}\)

c Note: \(\sqrt{x + 2}\)

d EP = effective population percentage.

The population of *H. striata* found in clove plants might be also affected by the biology of the insect. The duration of its egg was 12-18 d (the average 14 d), while the nymph was 32-90 d (the average 44 d), and the adult insect could live 2-35 d (the average 10 d) [12]. Egg and nymph were the inactive stages of *H. striata*, while the adult insect was the active stage since this stage could move and suck the nutrient from young clove leaves and then transmitted the bacterial pathogen from diseased plants to healthy plants. Based on the data, the population of *H. striata* was detected again at 2 and 3 mat (mo after treatment). This might be due to rainy season during observation period. Wet weather caused new leaf shoots grew that might attract adult insects came to lay eggs on the new young leaf shoots. Moreover, rainfall, temperature, and humidity might give the environment to support *H. striata* to live.
Population of *H. striata* in the treatments of oxytetracycline, fipronil, oxytetracycline + fipronil, *Bacillus* - *Pseudomonas*, oxytetracycline + *B. bassiana*, *Bacillus* - *Pseudomonas* + *B. bassiana* were the lowest. This indicated that those treatments were highly effective in controlling *H. striata* (100%).

The insect vector (*Hindola* spp.) was controlled with synthetic insecticides, such as pyrethroid cyhalothrin, deltamethrin, and organophosphate monocrotophos. Spraying with those insecticides had an influence on the population of *Hindola* spp. [13]. Two pyrethroids, fenpropatrin and cypermethrin were as effective as cyhalothrin in killing *H. striata* nymphs. BPMC and buprofezin gave significant decrease in nymph population [14]. Granule form of insecticides, such as aldicarb was also effective [15].

The plants’ damage declined especially in plants treated with *Bacillus-Pseudomonas+fipronil*, oxytetracycline + fipronil, *Bacillus-Pseudomonas*, and *Bacillus-Pseudomonas+B. bassiana* (Table 3). Thus, it indicated that those treatments were the most effective in suppressing plants’ damage (82.26%-91.94%).

Table 3. The effect of treatments to percentage of plants’ damage.

| Treatments | Before treatment | 1 mat | 2 mat | 3 mat | EPD (%)<sup>c</sup> |
|------------|-----------------|-------|-------|-------|-----------------|
| *B. bassiana* | 26.25 a | 30.00 bcd | 32.50 cde | 42.50 abc | 45.16 |
| Oxytetracycline | 30.00 a | 26.25 c | 25.00 e | 67.50 ab | 12.90 |
| Fipronil | 35.00 a | 36.25 abc | 27.50 de | 27.50 cde | 64.52 |
| Bioprotector | 25.00 a | 31.25 ab | 52.50 abc | 45.00 abc | 41.94 |
| *Bacillus-Pseudomonas + fipronil* | 28.57 a | 32.50 bc | 27.50 de | 8.75 de | 88.71 |
| Oxytetracycline + fipronil | 32.50 a | 33.75 bcd | 36.25 bcde | 13.75 cde | 82.26 |
| Control | 30.00 a | 46.25 a | 66.25 a | 77.50 a | 50.00 |
| Oxytetracycline + bioprotector | 31.25 a | 35.00 ab | 52.50 abc | 38.75 abcd | 56.45 |
| *Bacillus - Pseudomonas* | 28.75 a | 41.25 bcd | 42.50 abcd | 12.50 cde | 83.87 |
| Oxytetracycline + *B. bassiana* | 35.00 a | 33.75 bcd | 38.75 bcde | 33.75 bcde | 64.52 |
| *Bacillus-Pseudomonas + B. bassiana* | 31.25 a | 38.75 bc | 46.25 abcd | 6.25 e | 91.94 |
| *Bacillus-Pseudomonas + bioprotector* | 32.50 a | 46.25 ab | 52.50 ab | 31.25 bcde | 59.68 |
| CV | 21.44 | 18.89 | 16.74 | 27.06 | |

<sup>a</sup>Numbers followed by the same letters in the same column were not significantly different at DMRT 5%, where mat = no after treatments.

<sup>b</sup>Note: √x + √y

<sup>c</sup>EPD = effective plant’s damage (%).

The ability of biocontrol agents in suppressing disease progression could be associated with inhibitory mechanisms by antibacterial compounds, such as antibiotics produced by the strain of the biological agent. Furthermore, strains of the biological agents, such as *P. fluorescens* and *Bacillus* spp. also has a high ability in colonizing plant roots, therefore the biological agent strain is able to compete in nutrients with pathogenic bacteria [16]. In this case, especially biological agents, the effectiveness of *P. fluorescens* and *Bacillus* spp. in suppressing pathogens determined by their ability to produce antibiotics, induce resistance, nutrient competition, and colonizing the root system over a long period of time, as well as environmental factors, and the spread of bacteria in the soil [17].

One of the control methods of XLB disease was infusing clove plants with antibiotics into the stem or roots. Application of those antibiotics could delay the onset of symptoms, slow the progression of
the disease, reduce the number of blocked vascular tissues, extend the productive life of plants [18]. Many controlling measurements including resistant host plants [19], grafting of clove to resistant myrtenaceous plant [20], antibiotic [21] and insecticides for insect vectors [14] had been tried. However, only antibiotic and insecticidal treatment become promising alternative in reducing disease development [22].

The abundant young leaf shoot formation was on Bacillus-Pseudomonas+fipronil, oxytetracycline+fipronil and Bacillus-Pseudomonas+B. bassiana treatments (Table 4). Thus, the best treatments for young leaf shoots formation (plant growth) that reflect the reduction of plant damage were those treatments. They showed the most effective in stimulating young leaf shoot growth.

Table 4. The effect of treatments to average score of shoots growth.

| Treatments                        | Average of shoots growth<sup>a</sup> | 1 mat<sup>b</sup> | 2 mat<sup>b</sup> | 3 mat<sup>b</sup> |
|-----------------------------------|-------------------------------------|-------------------|-------------------|-------------------|
| B. bassiana                       | 1.00 a                               | 1.25 ab           | 2.25 ab           |
| Oxytetracycline                   | 0.75 a                               | 0.25 b            | 1.00 bc           |
| Fipronil                          | 1.00 a                               | 1.00 ab           | 2.25 ab           |
| Bioprotector                      | 1.00 a                               | 1.00 ab           | 1.50 abc          |
| Bacillus-Pseudomonas + fipronil   | 1.00 a                               | 1.50 a            | 3.00 a            |
| Oxytetracycline + fipronil        | 1.00 a                               | 1.25 a            | 3.00 a            |
| Control                           | 0.00 b                               | 0.75 ab           | 0.25 c            |
| Oxytetracycline + bioprotector    | 0.75 a                               | 1.75 a            | 1.50 abc          |
| Bacillus-Pseudomonas              | 0.75 a                               | 0.75 ab           | 2.25 ab           |
| Oxytetracycline + B. bassiana     | 1.00 a                               | 1.25 ab           | 1.50 abc          |
| Bacillus-Pseudomonas + B. bassiana| 1.00 a                               | 1.00 ab           | 3.00 a            |
| Bacillus-Pseudomonas + bioprotector| 1.00 a                               | 0.50 ab           | 1.75 ab           |
| CV                                | 7.57                                | 19,10             | 18.05             |

<sup>a</sup>Numbers followed by the same letters in the same column were not significantly different at DMRT 5%, where mat = mo after treatments.

<sup>b</sup>shoots: 1= a little, 2= any and 3 = very much

<sup>c</sup>Note: √ x + T

The plant growth, especially that reflected by the formation of young leaf shoots on plants that treated with biocontrol agents might be associated with the reduction of disease attacks, as a result of the high ability of indigenous endophytic bacterial strains (Bacillus spp. and P. fluorescens) in suppressing disease progression. In addition, it might be associated as well with the indirect effects of Pseudomonas activity to produce growth hormones that can stimulate root growth [16, 23].

Clove plants treated with oxytetracycline and fipronil produced abundant young leaf shoots as well. This might due to fipronil is a systemic insecticide that friendly and could stimulate the growth of roots [24]. Based on Table 2-4 it could be concluded that the best treatment for controlling XLB disease was (Bacillus-Pseudomonas+B. bassiana). The population of H. striata and the percentage of plant damage in plants treated with (Bacillus-Pseudomonas+B. bassiana) was the lowest. Moreover, the score of young leaf shoot formation production was the highest than in plants treated with other treatments.

Many control techniques have been developed and used for controlling XLB disease of clove. However, there was no effective control measures that could completely control the disease. Since, the disease develops and spreads out rapidly, as well as the complexity of the bacterial pathogen in nature. Therefore, preventive control of the disease would be more valuable and effective than the curative ones.
4. Conclusion

Application of mixed biocontrol agents of (Bacillus-Pseudomonas+B. bassiana) on diseased clove plants could reduce the population of H. striata and percentage of plant damage, as well as stimulate the production of young leaf shoots. The effectiveness of this mixed biocontrol agents was comparable with synthetic pesticides (oxytetracycline+fipronil). Therefore, this mixed of (Bacillus-Pseudomonas+B. bassiana) could be recommended for the control of XLB disease on clove plants. The use of biocontrol agents hopefully could reduce and substitute the use of synthetic pesticide in the control of XLB disease of clove.

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References

[1] Pusdatin 2014 Outlook Komoditi Cengkeh (Jakarta: Pusat Data dan Informasi Pertanian Sekretariat Jenderal Kementerian Pertanian) pp 83
[2] Waller J M and Situpu D 1975 PANS 21 141-7
[3] Hartati S Y 2013 Sirkuler Informasi Teknologi Tanaman Rempah dan Obat 16
[4] Bennet C P A, Hunt P and Asman A 1985 Plant Pathol. 34 487-94
[5] Robert S J, Eden-Green S J, Jones P and Ambler D J 1990 Systematic and Appl. Microbiol. 13 34-43
[6] Eden-Green S J and Machmud M 1988 ACIAR Bacterial Wilt Newsletter 3 5
[7] Bennet C P A, Jones P and Hunt P 1987 Plant Pathol. 36 45-52
[8] Eden-Green S J and Adhi E M 1986 ACIAR Bacterial Wilt Newsletter 1 2-3
[9] Balfas R and Eden-Green S J 1988 Pertemuan Teknis Penanggulangan Penyakit Bakteri Pembuluh Kayu Tanaman Cengkeh 4 38-46
[10] Dwimartina F, Arwiyanto T and Joko T 2017 Asian J. of Plant Pathol. 11 191-8
[11] Ditjen BSP 2004 Standar Pengujuan Efikasi Insektisida (Jakarta: Ministry of Agriculture) pp 136
[12] Balfas R, Eden-Green SJ and Sutajar T 1986 Pemberitaan Penelitian Tanaman Industri 11 51-5
[13] Nurmansyah, Stride A B and Lomer C J 1989 Indus. Crops Res. J. 1 1-6
[14] Mardiningsih TL, Balfas R, Sutarjo T and Lomer CJ 1990 Indus. Crops. Res. J. 2 13-7
[15] Lomer CJ, Stride AB, Nurmansyah, Siswanto, Sutarjo T and Mardiningsih TL 1990 Suppl. to Annals of Appl. Biol. 116 10-1
[16] Campbell R 1989 Biological Control of Microbial Plant Pathogen (Cambridge: Cambridge University Press)
[17] Janisiewicz W J, Tworkoski T J and Sharer C 2000 Phytopath. 90 191-200
[18] Hunt P, Bennet C P A and Asman A 1985 Plant Pathol. 34 495-501
[19] Pool P A, Eden-Green S J and Muhammad M T 1986 Euphytica 35 149-59
[20] Jarvis J K, Koerniati S and Pool P A 1986 Pemberitaan Penelitian Tanaman Industri 11 56-9
[21] Hunt P, Bennet C P A, Syamsu H and Nurwenda E 1987 Plant Pathol. 36 154-63
[22] Hartati S Y, Boa E R, Supriadi, Adhi E M and Karyani N 1991 Crops Res. J. 4 1-4
[23] Nasrun and Burhanuddin 2016 Bul. Littiri. 27 67-76
[24] BASF 2019 Regent & 50 SC RED Insektisida BASF Perlindungan Tanaman.www.petani-sejahtera.basf.co.id/insektisida/regent-50-SC [March 13th 2019]