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ORIGINAL ARTICLE

Effect of indigenous microbes on growth and blister blight disease of tea plant

Fani Fauziah[1]*, Mieke Rochimi Setiawati[2], Eko Pranoto[1], Dwi Ningsih Susilowati[3], Yati Rachmiati[1]

[1] Pre-Harvest, Research Institute for Tea and Cinchona, Gambung, Indonesia

[2] Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Indonesia

[3] Microbiology Molecular, Indonesian Center for Agricultural Biotechnology and Genetic Resource Research and Development, Bogor, Indonesia

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*Corresponding address:
fani_fauziah@ymail.com

Abstract

The role of the tea commodity in the economy of Indonesia is quite strategic. Various types of microorganisms in nature have been known to increase the benefit of the root function, suppress disease, and accelerate plant growth. This study aimed to determine the potential of indigenous bacteria (Azoto II-1, *Acinetobacter* sp., bacteria Endo-5, bacteria Endo-65 and Endo-76) on the growth of tea plants and their potential in increasing resistance to blister blight disease. The test of microbes' potential effect on growth and blister blight was conducted in Gambung, West Java in an experimental field using a randomized block design (RBD) with six treatments and each treatment was replicated four times. The composition of the treatments was: A) Endo-5; B) Endo-65; C) Endo-76; D) Azoto II-1; E) *Acinetobacter* sp.; and F) control (without microbes). Bacterial suspension was applied directly to the soil at a dose of 2 l · ha^{-1}. The bacterial suspension was applied six times at 1 week intervals. The results of field observations indicated that the intensity of blister blight decreased in all treatments but did not significantly differ from the control. Meanwhile, the results of *Acinetobacter* sp. treatment in tea shoots was 17.26% higher than the control.

Keywords: fertilizer, indigenous microbes, plant growth promoting rhizobacteria (PGPR), tea

Introduction

The role of tea commodities in the economy of Indonesia is quite strategic. However, the area of tea plantations has declined over the years. Tea production is often constrained by many factors such as weather disturbances, pests and diseases. To improve the quality and quantity of tea plants, balanced macro and micro nutrients and effective pest management strategies are needed. The supply of nutrients and control of pests with chemicals still dominate in tea plantations. Currently inorganic fertilizers and chemical pesticides create some concerns. The excessive use of inorganic fertilizers and chemical pesticides can cause environmental pollution. Therefore, for fertilization and disease control to be efficient and environmentally friendly, soil microorganisms and endophytic bacteria can be utilized.

The ability of the soil as an ecosystem component depends on the diversity of soil microbial communities. Almost 90% of the important processes occurring in the soil involve soil microbes (Nannipieri *et al*. 2003; Sengupta and Dick 2015). Various types of microorganisms in nature have been known to play a role as biological agents, plant growth promoting rhizobacteria (PGPR), suppressing disease, and accelerating plant growth (Sturz *at al.* 2000; Saharan and Nehra 2011). Besides soil bacteria, there are endophytic bacteria with several benefits such as N_2 air-inhibition, the production of phytohormones such as indole-3 acid (IAA), cytokinin, and growth stimulation (Setiawati *et al*. 2009).

In 2015, one soil bacteria (Azoto II-1) and three endophytic (*Acinetobacter* sp., Endo-5, Endo-65, and Endo-76) bacteria were isolated from tea plantation in

Gambung, West Java. The result of molecular characterization showed that the bacterial isolates have potential as bio-fertilizers and bio-control agents (Rachmiati, unpublished). The bacterial isolates have survival capabilities under both biotic and abiotic stress conditions by producing ACC deaminase. Hypersensitivity test results showed that the bacterial isolates did not cause necrosis in tobacco plants. This means that the four isolates were not pathogenic when applied to the plant. The detection of the presence of IAA-coded genes also indicated that the isolates were 148 bp (Rachmiati, unpublished).

The mechanism of PGPR in suppressing plant diseases can occur directly or indirectly. The mechanism of disease suppression can indirectly occur if the disease that attacks the plant does not interact directly with biological agents (Saharan and Nehra 2011). The procedure of inducing plant resistance can be done by splashing bacterial suspensions, mixing with sterile soil, soaking the roots of seeds when germinating in bacterial suspension, coating the seeds with media containing bacteria or by soaking the seeds in a bacterial suspension (Kuc 1987; Kloepper *et al*. 1992).

This study aimed to combine soil bacteria and endophytic bacteria to increase tea plant growth and induce plant resistance. We found that the *Acinetobacter* sp. could improve tea yield by 17.26% more than the control and all combinations of bacteria were synergist.

Materials and Methods

Microbial isolates tested were used in the synergism test. This study aimed to determine the effect of each microbial application against blister blight. The experiment was conducted at Gambung Experimental Garden, Research Institute for Tea and Cinchona on TRI 2024 clone. The study used Randomized Block Design (RBD) with six treatments and four time replications. The treatment arrangement was as follows: A) Endo-5; B) Endo-65; C) Endo-76; D) Azoto II-1; E) *Acinetobacter* sp.; and F) control (without microbes).

Bacterial suspension was applied directly to the soil at a dose of 2 l · ha^{-1}. The bacterial suspension was applied six times at 1 week intervals. Observations were made once a week at the time of plucking. There were three preliminary observations, and six observations after the treatment application. The main observation parameter was the intensity of blister blight. As supporting data, tea shoots per plot, rain rate and humidity were also observed.

The intensity of blister blight was determined by counting the number of healthy and infected Pecco + 3 leaves (P + 3) shoots from 200–500 g of fresh shoot samples taken randomly from each plots. Disease precent intensity was calculated by the formula (Rayati 2011):

$$DPI = \frac{\sum \frac{n_1 v_1}{N_1 Z_1} + \sum \frac{n_2 v_2}{N_2 Z_2} + \sum \frac{n_3 v_3}{N_3 Z_3}}{3} \times 100\ [\%],$$

where: DPI – disease percent index; v_1 – value scale type of reaction; n_1 – the number of leaf samples for each reaction type scale value; Z_1 – the highest reaction type scale value; N_1 – the number of leaf samples observed for the reaction type; v_2 – the scale value of the density of spots on leaves; n_2 – the number of leaf samples for each spot density on the leaves; Z_2 – the scale value of the density of spots on the highest leaves; N_2 – the number of leaf samples observed for the density of spots on the leaves; v_3 – the scale value of the density of spots on the shoots (p + 3); n_3 – the number of leaf samples for each density of spots on top (p + 3); Z_3 – the scale value of the density of spots on the shoots (p + 3); N_3 – the number of leaf samples was observed for the density of spots on the shoots (p + 3).

The scale value type of reaction of blister blight disease was presented in the Table 1. The scale value of density of blister blight spots on leaves and shoots presents the Table 2.

Results and Disscusion

Three preliminary observations (PO) showed that at the beginning of the trial prior to treatment application, the condition of blister blight was homogeneous throughout the experimental area. The mean intensity of disease (±72.67%) in the third preliminary observation was the initial condition before the treatment application (Table 3).

The results showed that all treatments decreased blister blight intensity after the first application. The results of statistical analysis on blister blight intensity showed no difference between treatments or the control (Table 4). This could have been caused by an insufficient number of applications of microbes, so the effectiveness did not significantly differ from the controls. A similar study was conducted by Saravanakumar *et al*. (2007). In their study the application of *Pseudomonas fluorescens* with 7 day application intervals consistently reduced the intensity of blister blight for two seasons, which was equivalent to the application of chemical fungicides and significantly increased tea plant production when compared to the controls.

Table 1. Value scale type of reaction of blister blight disease in the table and visible on tea leaves

Value scale	Description of the type of reaction
0	No spots
1	The diameter of translucent spots <1 mm
2	The diameter of translucent spots surrounded by dark green rings, 1–2 mm (flat spots)
3	The diameter of translucent spots surrounded by dark green rings, 3–6 mm (already curved to the bottom of the leaf)
4	Sporadic, part or all of its surface
5	Partial or whole spot has turned brown, dry, and often released to produce holes

Table 2. Scale value of density of blister blight spots on tea leaves and shoots

Value scale	The number of the spots on leaves	Value scale	The number of the spots on shoots
0	0	0	0
1	1–5	1	1–5
2	6–10	2	6–10
3	11–20	3	11–20
4	>20	4	21–40
		5	>40

Table 3. Disease percent intensity of blister blight at the preliminary observation on tea plant [%]

Treatment	PO 1	PO 2	PO 3
Endo-5	81.93	67.04	69.87
Endo-65	85.47	68.77	72.59
Endo-76	71.71	68.80	71.96
Azoto II-1	78.50	63.52	74.07
Acinetobacter sp.	74.32	62.64	73.13
Control	66.24	69.05	74.41
Significance	NS	NS	NS

*NS = not significant; PO = preliminary observation

The mechanism of antagonism by an indirect microbe is called induced resistance (Hasanuddin 2003). Induced resistance is a form of defense generated by plants as a reaction to certain stimulation (Van Loon *et al.* 1998). Induction of plant resistance to various diseases can be done with inducer agents in the form of pathogens, manure extracts, and plant leaf extracts (Semangun 2000; Hersanti 2005). The inducing agent will activate the plant's defense system regulated by the defense gene (Kuc 1987; Suganda 1999).

Application of *P. fluorescens*, *Bacillus amyloliquofaciens*, *Serratia marcenes*, and *B. pumilus* may promote growth and induce tea plant resistance against tea leaf disease (Saravanakumar *et al.* 2007; Chakraborty *et al.* 2013). Induced resistance leads to the activation of the plant resistance system or stimulates a plant-resistant mechanism. Several factors that can trigger induced systemic resistance include siderofor chemical compounds, antibiotics, Fe ions, and bacterial cell components such as microbial cell wall, flagella, filli, lipopolysaccharide membrane (LPS) which act as elicitors in inducing systemic resistance (Ton *et al.* 2002).

The clones in this study are susceptible to blister blight, the TRI 2024. In general, the application of the inducer agent causes the plant to become sensitive so it can respond to pathogen infection quickly. Decreased pathological effects may be regarded as evidence of

Table 4. Blister blight intensity [%] observed on tea plant after microbial application

Treatment	Weeks after microbial application					
	1	2	3	4	5	6
Endo-5	69.96 a	70.99 a	65.41	55.68	80.43	48.95
Endo-65	76.05 ab	85.13 ab	57.60	43.06	72.11	50.42
Endo-76	84.82 ab	78.18 ab	70.80	46.77	71.69	54.63
Azoto II-1	90.00 b	90.00 b	57.82	34.45	78.71	59.11
Acinetobacter sp.	73.40 ab	84.82 ab	55.72	54.63	75.41	55.08
Control	72.24 a	72.48 ab	58.66	49.29	81.76	48.06
Significance			NS	NS	NS	NS

*the values in the column followed by the same letter are not significantly different according to Duncan's Multiple Range Test at 5%
NS = not significant

induced resistance, but in susceptible plants the latent resistance may be rapidly expressed to control pathogens (Kloepper *et al*. 1992).

The occurrence of a plant disease can be influenced by three important factors, namely, susceptible host plants, virulent pathogens and appropriate environmental conditions (Semangun 2000). If these three factors are present, plant diseases will emerge. Environmental factors that influence the development of diseases such as temperature, humidity and high rainfall tend to increase the intensity of the disease. This certainly indicates that environmental factors are important in supporting the occurrence of plant diseases.

The average humidity during the experiment was 80% (Fig. 1). Humidity greatly affects the increase and decrease in disease intensity. Based on observations, decreased disease intensity corresponds with decreased humidity, and vice versa. The highest humidity reached 82% during the fifth observation, causing disease intensity to increase. Humidity over 80% is required for germination, establishment, and release of spores (Departemen Pertanian 2002). Spores that fall on the surface of leaves with sufficient moisture will germinate and penetrate into the leaf tissue (Astuti 2013).

Rainfall is another factor that influences the development of blister blight. Although during the experiment there was no rain, the relative humidity still supported the development of blister blight. The average intensity of blister blight disease remained high until the last observation was made. This may have been affected by vulnerable clones and environmental conditions favorable to the development of blister blight.

Statistical analysis of tea yield per plot cumulatively from six observations showed that the tested microbial treatments did not significantly differ (Table 5). However, the cumulative tea yield on the *Acinetobacter* sp. was 17.26% higher than the control treatment. The results of this study are in accordance with Phukan *et al.* (2012) who found that the application of PGPR in tea plants can increase tea production by 13–30%.

The decrease in the intensity of blister blight was not accompanied by increased yield of fresh shoots. The rate of blister blight infection does not always result in differences in production. Loss of yield caused by blister blight is not quantitatively related to disease control (Van der Knaap 1955; De Silva *et al*. 1974).

The production of tea shoots has a very high variability and is influenced by many factors such as shoot

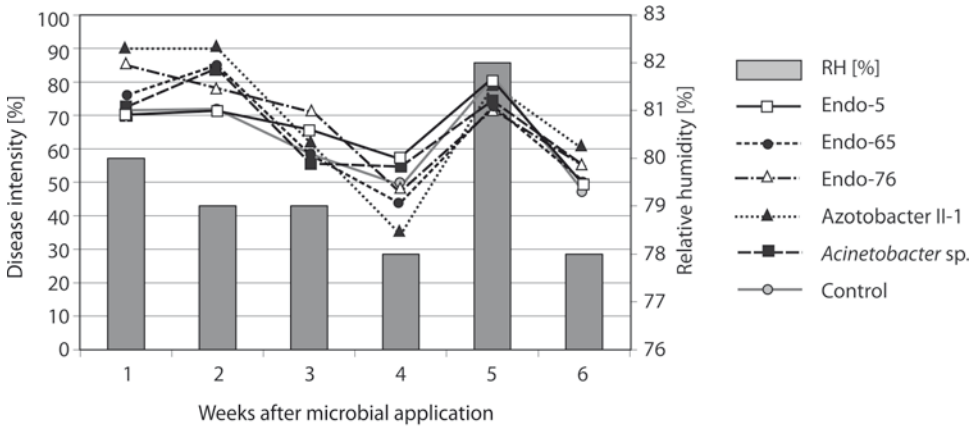

Fig. 1. Relative air humidity during the experiment

Table 5. Results of cumulative of fresh shoot on various microbial treatments

Treatment	Cumulative of fresh shoot [kg · plot^{-1}]*	% Yield increase compared to control
Endo-5	2,014	–1.09
Endo-65	1,907	–6.33
Endo-76	2,145	5.35
Azoto II-1	2,132	4.68
Acinetobacter sp.	2,388	17.26
Control	2,036	0
Significance	NS	

*cumulative from six times application
NS = not significant

production in a relatively short period of time. In addition, the relationship between shoot production and the intensity of blister blight attacks can only be seen in cases of high rates of blister blight. In clonal plants with high yield potential and rapid growth, yield loss on each plucking can be rapidly compensated for, resulting in cumulative production that is not significantly different over a relatively long time (Van der Knaap 1955; De Silva *et al*. 1974).

Several species of the genus *Azospirillum, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium* and *Serratia* may be associated with the plant rhizosphere and may affect plant growth (Saharan and Nehra 2011). Endophytic bacteria have several benefits, such as, N_2 air-inhibition, the production of phytohormones such as indole-3 acetic acid (IAA), cytokinins, as well as the spurring of growth and others. Increased concentrations of endogenous N_2 stimulating endemic bacteria tend to significantly increase plant N uptake (Setiawati *et al*. 2009).

Conclusions

The results showed that the intensity of blister blight decreased in all treatments but did not significantly differ from the control. Microbial applications must be added to enhance the effects of tea plant resistance against blister blight. However, the results of *Acinetobacter* sp. treatment in tea shoots was 17.26% higher than the control. Therefore, the microbes have the potential to increase shoot production.

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