Effectiveness of plant growth-promoting Rhizobacteria (PGPR) to control gall rust of *Falcataria moluccana* under field conditions

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Abstract. Gall rust caused by *Uromycladium* spp. is a most devastating disease of *Falcataria moluccana* plantations in Indonesia since a decade. There is no effective control measures to suppress rust gall disease. The objectives of the research was to investigate the effectiveness of PGPR on seedlings growth, gall rust severity and growth of young *Falcataria moluccana* trees. Talc-based powder formulation of PGPR mixtures of *Pseudomonas fluorescens*, *Bacillus polymixa* and *Rhizobium* spp. was used. First experiment consisted of testing as a seed treatment by PGPR using four concentrations of PGPR i.e. 0 (untreated), 2.5 g/L, 5 g/L and 10 g/L equal to 1.25 x 10⁶ cfu/L, 2.5 10⁶ cfu/L and 5 x 10⁶ cfu/L. Emergence rate and seedlings growth were evaluated. Second experiment was a field trial using young three months age-seedlings trees under pathogen natural infestation, designed as a complete randomized block design with six replications. Treatments were PGPR 2.5 10⁶ cfu/kg and 5 x 10⁶ cfu/kg, untreated and mancozeb fungicide. Powder formulation of PGPR was mixed with granule NPK fertilizer and applied as soil incorporation at planting date and 42 days after transplanting. Disease severity, plant height and stem diameter were observed weekly at 1 to 11 week after transplanting. Seed treatment with all concentration of PGPR did not affect germination rate, but increased seedlings growth. Moreover, field application of PGPR at the rate of 5 x 10⁶ cfu/kg in PGPR-NPK fertilizer mixture was the best treatment to control gall rust of young plants under field conditions with an effectiveness rate of 42.28%, compared to fungicide treatments which has effectiveness rate of 34.39%. The treatments also enhanced the growth of young plants.

Keywords: biocontrol, fertilizer, seedlings, *Uromycladium*, young plants

1. Introduction

*Falcataria* is a multipurpose trees cultivated by farmers in Indonesia because of its rapid growth and it has high economic value. The main problem faced by farmers in falcataria tree cultivation is pest and disease attacks. The problem that has been faced by farmers for several years is gall rust on the trees caused by *Uromycladium* sp.. In Indonesia, gall rust was first reported in 1996 on Seram Island, Maluku. Then in 2002 it was reported that rust also attacked Falcataria, as a coffee tree cover, in East Timor with a percentage of attacks 57-90%. In 2006, the Forestry Research and Development Center received a report from the Lumajang District Forestry Office that the Falcataria plant in the location of the GN-RHL / GERHAN 2003 planting area was 300 ha, 2004 was 1350 ha, and in 2005 775 ha was affected by gall rust [1]. Nurseries or young plants is the most susceptible to rust disease because it causes death.
The incidence of rust disease in nursery plants can reach 90-100%, while in plants aged 2 months, rust can cause 82% of plant death [2].

Disease control was done by farmers is conventional control that focuses on chemical control. However, the current control is not effective and expensive, aside from potential harming the environment. For this reason, control is currently being developed in an integrated manner. Currently, pest and disease control is being promoted by utilizing microorganisms known as biocontrol agents. One potential biocontrol agent is PGPR (*plant growth promoting rhizobacteria*). Based on previous research, PGPR has been proven effective in controlling white rust caused by *Puccinia horiana* in chrysanthemums [3]. The effectiveness of PGPR against gall rust of falcataaria has not been investigated yet. This study aimed to examine the effect of PGPR on the growth of Falcataaria seedlings, and gall rust disease and growth in the field.

2. Material and Methods

2.1. Seeds and Nursery Seedlings

Seeds dipping with bacterial suspensions: *e.* 0 (untreated), 2.5 g/L, 5 g/L and 10 g/L equal to 1.25 x 10^6 cfu/L, 2.5 10^6 cfu/L and 5 x 10^6 cfu/L. Five replications, one replication 10 seeds. Polybag diameter 5 cm x 10 cm. Nursery media. Variable measured were emergence rate, plant height, stem diameter and root length. Data was tabulated used Microsoft Excel program version 2010. Data were analyzed statistically using analysis of variance according to SAS version 9.0. The differences of means were identified by Duncan Multiple Range Test at 0.05 levels.

2.2. Experiment on young plants in the fields

Seedlings at the age of six months and without PGPR treatment were used in this study. The experiment was used Randomized Complete Block Design with four replications. Each replication consisted of ten plants. Experiment was carried out in plantation owned by “Sengon Lestari” Community Forest Farmer Group (KTHR), Karang Tengah Village, Cilongok District, Banyumas Regency, Central Java Indonesia. Talc based formulation of PGPR mixed with NPK fertilizer: 1:50 (5 x 10^6 cfu/kg) and 1: 100 (2.5 x 10^6 g/kg), applied at rate of  20 g/plants at transplanting date, and 6 weeks after transplanting. As control untreated plants was used. Other comparison was mancozeb-based fungicide applications. In untreated and mancozeb treatment, same dose of NPK fertilizer was applied. Variables measured was plant growth (height and stem diameter), diseases incidence and disease severity. Observation of variables was done every week started from 1 week after transplanting up to 11 weeks after transplanting.

\[
\text{Diseases Incidence (DI)} = \frac{n}{N}
\]

with (DI) is diseases incidence of gall rust, (n) is the number of plants infected with *Uromycladium tepperianum*, (N) is the number of plants that were tested in each treatment.

Each plant from each place was given a score against the severity of the gall rust disease that occurred and its severity was calculated. Determination of scoring is determined based on the many gall rust that exist in plants.

The gall rust severity score is:

0 = Free of disease  
1 = branch and leaves <10% infected  
2 = leave and branch 11 – 20% infected  
3 = leave and branch 21 – 50% infected  
4 = >50% or branch infected

\[
\text{Diseases Severity (DS)} = \frac{\sum n_i \times v_i}{N \times V}
\]
with (DS) is diseases severity of gall rust, \((n_i)\) is the number of plants with a score to \(i\), \((v_i)\) is the disease scores (from 0 to 5), \((N)\) is the Number of plant samples observed, \((V)\) is the highest score.

Data was tabulated used Microsoft Excel program version 2010. Data were analyzed statistically using analysis of variance according using SAS program package. The differences of means were identified by Duncan Multiple Range Test at 0.05 levels.

3. Result

3.1. Effect of PGPR on Plant Growth Seedling

In this study, growth was observed and measured every week up to five weeks after sowing. The growth variables were plant height, stem diameter and root length. The application of PGPR at all tested concentrations affected seedlings height of \(F.\ moluccana\). The significant effect on plant height can be seen in all PGPR concentration treatment at 5 weeks after sowing (Table 1).

| PGPR concentration \((x 10^6\ \text{cfu/L})\) | Seedlings Height (cm) at age of…… |
|-------------------------------------------------|-----------------------------------|
|                                                 | 2 WAS   | 3 WAS   | 4 WAS   | 5 WAS   |
| 0                                               | 2.80 a  | 3.50 a  | 5.32 a  | 6.16 a  |
| 1.25                                            | 3.22 a  | 3.89 a  | 5.48 a  | 6.47 b  |
| 2.5                                             | 3.10 a  | 3.83 a  | 6.14 a  | 6.93 b  |
| 5                                               | 2.72 a  | 3.33 a  | 5.28 a  | 6.54 b  |

Note: Number in same column with same symbols are not significantly different in DMRT test at \(P <0.05\)

Besides having a significant effect on plant height, in this study PGPR at all tested concentration have a significant effect on the stem diameter. The application of PGPR affects diameter seedlings of \(F.\ moluccana\). The significant effect on plant diameter can be seen in all PGPR treatment at 5 weeks after sowing (Table 2).

| PGPR concentration \((x 10^6\ \text{cfu/L})\) | Stem diameter (cm) at age of…… |
|-------------------------------------------------|--------------------------------|
|                                                 | 2 WAS  | 3 WAS  | 4 WAS  | 5 WAS  |
| 0                                               | 0.78 a | 0.90 a | 0.91 a | 0.88 a |
| 1.25                                            | 0.81 a | 0.93 a | 0.91 a | 0.98 b |
| 2.5                                             | 0.76 a | 0.90 a | 0.94 a | 0.98 b |
| 5                                               | 0.8 a  | 0.91 a | 0.91 a | 0.97 b |

Note: Number in same column with same symbols are not significantly different in DMRT test at \(P <0.05\)

The application of PGPR affected root length of seedlings of \(F.\ moluccana\). The significant effect on root length can be seen in all tested concentration and highest effect was on \(5 \times 10^6\ \text{cfu g/L}\) concentration (Table 3). PGPR did not affect the emergence rate (data not sown), but affected the plant growth (plant height, plant diameter, and root length of plants) at all tested concentrations.
Table 3. Root length of seedlings of *F. moluccana* with PGPR treatments

| PGPR concentration (x 10^6 cfu/L) | Root length (cm) |
|----------------------------------|-----------------|
| 0                                | 7.58a           |
| 1.25                             | 8.51b           |
| 2.5                              | 8.63b           |
| 5                                | 9.62c           |

Note: measured at 5 weeks after transplanting, number in same column with same symbols are not significantly different in DMRT test at P <0.05

3.2. Effect of PGPR on Gall Rust Disease and Plant Growth in Field Experiment

In general, PGPR has a significant effect in suppressing the incidence and severity of gall rust disease. This was indicated by the fact that there are significant differences in disease incidence, disease severity. Interestingly, PGPR treatments at the rate of 5 x 10^6 cfu/kg had the same effectiveness with synthetic fungicide (mancozeb) treatment in controlling gall rust. Significant effect of PGPR from the incidence of rust disease occured at the age of 2nd, 6th, 8th weeks after transplanting (Table 4).

Table 4. Disease incidence of gall rust of Falcataria with PGPR applications under field conditions

| PGPR concentration (10^6 cfu/kg) | Age of plants (week after transplanting) |
|----------------------------------|-----------------------------------------|
| 0                                | 1        2 3 4 5 6 7 8 9 10 11          |
| 0                                | 0a 20.00b 23.33a 33.33a 38.67a 35.33b 32.00a 41.67b 4067a 50.00a 46.67a |
| 2.5                              | 0a 3.33a 13.33a 23.33a 20.00a 20.00ab 20.00a 13.33a 33.33a 40.00a 43.33a |
| 5                                | 0a 6.67a 6.67a 20.00a 20.00a 13.33a 16.67a 20.07a 28.00a 31.33a 36.00a |
| Mancozeb                         | 0a 6.67a 16.67a 33.33a 36.67a 30.00a 17.33a 16.67a 30.67a 37.00a 37.33a |

Note: Number in same column with same symbols are not significantly different in DMRT test at P <0.05

At the first week observation, severity of gall rust disease in all plants tested showed a value of 0, this means that no plants were affected by gall rust disease. Starting at the second week of observations, the results of the observations showed the severity of the rust of the rust varied. In observing the severity of gall rust disease, it can be seen that in general the severity of gall rust disease is almost always increasing every week. Significant influence on the severity of gall rust disease occurred in higher concentration of PGPR (5 x 10^6 cfu/kg) and fungicide treatments, which occurred at the 2nd, 10th, and 11th week of observation (Table 5).

Table 5. Disease severity of gall rust with PGPR applications under field conditions

| PGPR concentration (10^6 cfu/g) | Age of plants (week after transplanting) |
|----------------------------------|-----------------------------------------|
| 0                                | 1 2 3 4 5 6 7 8 9 10 11                |
| 0                                | 0a 12.5b 15.83a 20.83a 26.57a 26.48a 23.98a 28.01b 29.51a 34.61b 51.60b |
| 2.5                              | 0a 3.33a 5.00a 11.67a 13.33a 12.5a 7.50a 12.51ab 25.83a 33.33b 34.17ab |
| 5                                | 0a 3.33a 7.50a 17.50a 14.17a 8.33a 9.10a 20.74ab 22.69a 28.80a 28.70a |
| Mancozeb                         | 0a 5.83a 12.52a 19.17a 20a 17.5a 14.91a 8.33a 19.07a 19.54a 25.74a |

Note: Number in same column with same symbols are not significantly different in DMRT test at P <0.05

In this study, growth was observed and measured every week for eleven observations. The indicators used in the measurement of plant growth are plant height and stem diameter. In general, PGPR concentration of 5 x 10^6 cfu/kg had a significant effect on plant height, especially at 2 WAT, 3 WAT and 11 WAT.
Table 6. Height of young Falcataria with PGPR applications under field conditions

| PGPR concentration (10^6 cfu/kg) | Age of plants (week after transplanting) |
|---------------------------------|------------------------------------------|
| 0                               | 0 1 2 3 4 5 6 7 8 9 10 11               |
| 0                               | 74.73a 82.72a 93.53a 97.44a 105.32a 107.94a 112.38a 118.54a 126.68a 131.80a 135.28a 138.47a |
| 2.5                             | 75.25a 93.05a 121.00b 123.98ab 132.71a 135.94a 146.92a 151.31a 158.85a 152.28a 170.42a 173.85ab |
| 5                               | 74.10a 100.5a 120.92b 126.38b 135.94a 136.77a 140.68a 154.31a 160.24a 164.58a 169.39a 183.90b |
| Mancozeb                        | 73.90a 81.90a 99.72ab 109.69ab 117.60a 119.46a 128.36a 128.85a 135.32a 141.36a 145.32a 157.57ab |

Note: Number in same column with same symbols are not significantly different in DMRT test at P <0.05

Table 7. Stem diameter of young Falcataria with PGPR applications under field conditions

| PGPR concentration (10^6 cfu/kg) | Age of plants (week after transplanting) |
|---------------------------------|------------------------------------------|
| 0                               | 0 1 2 3 4 5 6 7 8 9 10 11               |
| 0                               | 0.62a 0.67a 0.94a 0.98a 1.04a 1.07a 1.10a 1.16a 1.21a 1.24a 1.27a 1.29a |
| 2.5                             | 0.65a 0.77a 1.15ab 1.19a 1.23a 1.26a 1.34a 1.37a 1.43a 1.57a 1.50a 1.56ab |
| 5                               | 0.62a 0.73a 1.21b 1.24a 1.27a 1.28a 1.41a 1.45a 1.48a 1.62a 1.71b |
| Mancozeb                        | 0.59a 0.63a 1.00ab 1.07a 1.14a 1.18a 1.24a 1.29a 1.30a 1.35a 1.40a 1.45ab |

Note: Number in same column with same symbols are not significantly different in DMRT test at P <0.05
In general, the average stem diameter, PGPR treatment especially at high concentration tested i.e. 5 \( \times 10^6 \) cfu/kg, only 2nd and 11th week of observation whose results are significantly different (Table 7), but based on Table 7 it can be seen that plants with the PGPR 5 \( \times 10^6 \) cfu/kg treatment had the largest diameter in almost all weeks while the diameter of the control plant stem was almost always the lowest diameter.

4. Discussion

PGPR formulation used in this study, which contain *Bacillus polimyxa*, *Pseudomonas fluorescens* and *Rhizobium* sp. was proven effective stimulate seedling growth, even though had no any effect on emergence rate. Seedlings growth is an important component of Falcataria sylviculture, to obtain optimum growth of trees under field condition, therefore PGPR treatment can be used as important technology for Falcataria nursery.

The PGPR at concentration of 5 \( \times 10^6 \) cfu/kg also promoted growth young plants and control effectively gall rust diseases under field condition. The effectiveness of PGPR in controlling gall rust disease under field condition is 42.28\%, higher than the use of synthetic fungicide (mancoeb) (effectiveness of 34.39\%). For our knowledge this is the first report on the effectiveness of PGPR treatment on Falcataria trees under field condition.

Based on the benefits and functions, PGPR can produce IAA hormones, cytokines and gibberellins and can bind N2 from the air and dissolve phosphate (P) in the soil, PGPR is able to stimulate and increase plant growth. Rhizobacteria *Bacillus* sp. produce IAA with a concentration range between 25.99-34.97 mg / ml filtrate, while the *Pseudomonas* sp. between 28.51-100.56 mg / ml filtrate, and *Serratia* sp. between 24.16-27.98 mg / ml filtrate. There are many types of PGPR that can fix N2 so as to increase plant growth. PGPR colonizes plant roots and has beneficial effects on plant growth and development with a variety of mechanisms [5]. To be effective in PGPR, bacteria must be able to colonize roots because bacteria need sufficient populations to produce beneficial effects [6].

Many factors affect PGPR application in nature, environmental factors greatly affect the application of PGPR, micro fertilizers, a combination of micro and PGPR fertilizers, as well as fungicides on plants; such as climate, high levels of pathogen virulence, and many pathogenic inoculums. The use of PGPR has become a common practice in many regions of the world. Although significant effects on controlling plant pathogens have been demonstrated by PGPR in laboratory and greenhouse research, results in the field are inconsistent [7]. However, this research show the effectiveness of PGPR in controlling gall rust - a most destructing diseases of Falcataria. The result of this research may contribute for development of effective and economic management of gall rust disease under field condition. In controlling gall rust disease of Falcataria tree, the use of synthetic fungicide had limitation due to difficulty in application and require high cost.

The success of PGPR depends on the formation of effective population densities of active cells in the plant rhizosphere. Because this is a simple principle, it has proven difficult to determine the effect of response to doses at which the rate of increase in plant growth or disease suppression can be directly correlated with the size of the PGPR population [8]. In this experiment population density of 5 \( \times 10^6 \) cfu/L was enough for providing promoting effect of Falcataria seedlings. Under field conditions, concentration of PGPR in PGPR+ NPK mixture in concentration 5 \( \times 10^6 \) cfu/kg was proven to be effective in controlling gall rust disease and promoting plant growth. Application of PGPR by mixture with granule NPK fertilizer also give advantage for easiness of applications and saving labour work.

This PGPR can suppress disease by inducing resistance to disease. Rhizobacteria induce resistance in plants through a induced systemic resistance (ISR) [9]. The mechanism of PGPR in inducing plant resistance is to increase phytohormone production, siderophore production and dissolving phosphate minerals and other nutrients / minerals [10]. Induction of systemic resistance due to PGPR treatment is broad-spectrum. With the application of PGPR, plants will be more resistant to diseases caused by fungi, bacteria and viruses. *Bacillus* spp as PGPR can produce 2.3 volatile butanediol which can induce the
resistance [11]. Whereas Pseudomonas fluorescens can produce chitinolytic enzymes as anti-fungus [12].

Based on the study, the use of PGPR is a key technique for Falcataria silviculture. PGPR can be developed to be a beneficial technology for nursery management of Falcataria. In addition, PGPR treatments on young plants is an effective technique for gall rust control, with beneficial effect, promoting plant growth.

References

[1] Anggraeni I 2009 Penyakit karat tumor pada sengon (Paraserianthes falcataria (L) Nielsen) di Perkebunan Glenmore Banyuwangi, Jawa Timur Jurnal Penelitian Hutan Tanaman 6 311-21

[2] Rahayu S 2010 Biology and management gall rust disease caused by Uromycladium tepperianum on Falcataria moluccana in Indonesia nursery Proceeding of the Seventh Meeting of IUFRO Working Party, ed C M Michelle (Hawaii: United States Departement Agriculture) pp 109-12

[3] Munawaroh R 2010 Pengendalian penyakit karat putih (Puccinia horiana Henn.) pada krisan dengan menggunakan filtrat guano, bakteri perakaran pemacu pertumbuhan tanaman (PGPR), dan khamir antagonis di lapangan (Bogor: Bachelor thesis of Institut Pertanian Bogor)

[4] Sutariati G A K, Widodo, Sudadarso and Satriyas I 2006 Pengaruh perlakuan rizobakteri pemacu pertumbuhan tanaman terhadap viabilitas benih serta pertumbuhan bibit tanaman cabai Bull. Agron 34 46-54

[5] Cumming S P 2009 The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops; potential and problems. Environmental Biotecnology 5 43-50.

[6] Ashrafuzzaman M, Hossen F A, Ismail M R, Hoque M A, Islam M Z, Shahidullah S M and Meon S 2009 Efficiency of plant growth promoting rhizobacteria(PGPR) for the enhancement of rice growth African Journal of Biotechnology 8 1247-1252.

[7] Saharan B S and Nehra V 2011 Plant growth promoting rhizobacteria: a critical review Life Sciences and Medicine Research Vol 2011 LSMR-21.

[8] Viveros O M, Jorquera M A, Crowley D E, Gajardo G and Mora M L. 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J. Soil Sci. Plant Nutr. 10 293 – 319.

[9] Taufik M, Rahman A, Wahab A and Hidayat S H 2010 Mekanisme ketahanan oleh Plant Growth Promoting Rhizobacteria (PGPR) pada tanaman cabai terinfeksi Cucumber Mosaic Virus (CMV). J. Hort. 20 274-83.

[10] Gholami A, Shahsavani S and Nezarat S 2009 The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize Journal World Academy of Science, Engineering and Technology 49 19-24.

[11] Bakker P A H M, Pieterse C M J, V and Loon L C 2007 Induced systemic resistance by fluorescent Pseudomonas spp. The Nature and Application of Biocontrol Microbes III: Pseudomonas spp. 97 239-243

[12] Hariprasad P, Divakara ST and Niranjana S R 2011 Isolation and characterization of chitinolytic rhizobacteria for the management of Fusarium wilt in tomato Crop Protection 30 1606-12