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Rhizo-Microbiome for the Sustenance of Agro-Ecosystems in the Changing Climate Scenario






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## Article

# The Synergistic Action of Three Piper Plant Extracts and Biofertilizer for Growth Promotion and Biocontrol of Blast Disease in Red Rice

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**Abstract:** Bali is a world tourist destination and has many natural resources that need to be developed to support the tourism sector. One of the local Bali resources that has the potential to be developed to support tourism and food is the local red Bali rice. This local Balinese rice is a characteristic of the ecotourism area of the Jatiluwih village of Tabanan, Bali. Balinese rice is grown with inorganic pesticides and there is an urgent need to develop organic pesticides as a sustainable approach to rice farming. In this regard, extracts of piper plants can serve as the best and greenest biopesticides as plant growth-promoting rhizobacteria (PGPR), and compost functions as organic fertilizer. The present research aimed to evaluate PGPR, compost, and the synergistic biopesticidal effects of extracts of three piper plants, namely *Piper caninum*, *Piper betle* var. *Nigra*, and *Piper betle*, against blast disease in Bali red rice plants. The results showed that the synergistic action of PGPR, compost, and crude extract of piper plant provided an inhibitory activity against blast disease in rice plants where the greatest inhibition was found in a mixture of the three extracts with an inhibition of 50 cm. This shows that the mixed compounds of the three piper extracts work synergistically in suppressing blast disease; in addition, PGPR also exhibited a positive impact on the growth of red rice because PGPR produce growth hormones and various antifungal metabolites that help the plant growth and induce systemic resistance against phytopathogens. The active principles were identified as citronella, trans-geraniol, and 4.6-dipropyl-nonan-5-one. A combination of these extracts with compost and PGPR showed potential antifungal activity against blast disease at a concentration of 2%. This application also promoted the growth of Bali red rice. There is a significant increase in the number of leaves and the number of tillers, where the height is inversely proportional to the higher the extract up to 2%, as the height of the red Bali rice plant decreases. This is good because it reduces the red Bali rice stalks' possibility of falling during small production. The piper extract mixture at a concentration of 2% had the highest effect on grain production/tonne (6.59 tonne/ha) compared to the control at only 3.21–3.41 tonnes/ha. The 2% concentration of the extracts from the mixture of the three pipers

has the highest effect on growth and red Bali rice production, and provides the greatest obstacle to the intensity of blast disease in red Bali rice.

**Keywords:** biopesticide; compost; PGPR; piper plant; red Bali rice

## 1. Introduction

Bali is a world tourist destination, very well known throughout the world for its culture, natural beauty, and biodiversity. One of Bali's local biodiversities that is used for ecotourism is Bali red rice, which is only found in the Jati Luwih village, Penebel, Tabanan, Bali [1]. This red Bali rice is rich in fibers, antioxidants, vitamins, and minerals. It is suitable to be developed as a healthy food [2]. However, this important crop is affected by a large number of plant pathogens and pests. Traditional methods of controlling the pathogens and pests through the use of chemical pesticides in Bali rice have badly impacted the environment, farmers' health, and community health, and, so far, a comprehensive organic method for red Bali rice has not yet been found [3,4]. To overcome the adverse effects of chemical pesticides, the use of plant-based biopesticides has been suggested as one of the best approaches to suppress diseases and pests in Bali rice [5]. A combination of botanical pesticides and plant growth-promoting rhizobacteria (PGPR) serves the dual purpose of biocontrol and plant growth promotion. This approach is expected to reduce the chemical inputs in agriculture [6,7].

The use of biopesticides, such as plant extracts, has begun to develop in the world of agriculture, to replace chemical biopesticides [8]. The use of *Piper caninum* extract to suppress blast disease caused by *Pyricularia oryzae* and *Curvularia oryzae* increases the growth and yield of dry grain in Ciherang rice and Bali red rice [9–11]. The use of a combined extract of *P. caninum* and *P. betle* var. *Nigra* provides better results on the inhibition of blast disease in rice and has a better effect on the growth and yield of dry unhulled rice [12]. The inhibitory activity of the mixed extract of three piper plants is stronger compared to a single extract because the compounds in the mixture work in synergy, exerting more inhibitory effects on blast disease pathogens [12]. When the extract treatment is combined with PGPR, it gives maximum results in decreasing blast disease intensity and growth, and increasing rice yield. PGPR provides systemic resistance and produces growth hormones to be used as a growth stimulant, while acting as a biocontrol against diseases in rice plants and as a biological fertilizer to increase rice yields [12]. The present study aims to develop an effective formulation by offering a combination of three plant extracts, namely *Piper caninum*, *Piper betle* var. *Nigra* and *Piper betle*, along with PGPR and compost for the integrated management of blast pathogens and to improve the yield of Bali rice.

## 2. Materials and Methods

### 2.1. Research Time and Location

This research was conducted in 2 locations (longitude 115.0; latitude –8.45, and altitude 249 m above sea level), namely in the laboratory and the field. Research in the laboratory was carried out in the Analytical Laboratory of F. MIPA UNUD and in the Biopesticide Laboratory, and field research was carried out in Senganan village, Penebel, Tabanan Bali. The first phase of research (2020) was conducted from February–October 2020.

### 2.2. Source of Microbial Cultures

The fungal pathogens, *Pyricularia oryzae* Cav., the cause of blast disease used in the present study, were obtained from the Biopesticide Laboratory, Faculty of Agriculture, Udayana University, Udayana, Bali, Indonesia. The PGPR cultures came from Suria green liquid organic fertilizer that contained a mixture of *Bacillus subtilis*, *Enterobacter cloacae*, and

*Stenothropomonas maltophilia*, and were procured from Suria Green Pvt Ltd., Bali, Indonesia, from the local market.

### 2.3. Extraction of Biopesticides from Piper Plants

The leaves of *P. caninum* Blume, *P. betle* var. *Nigra* and *P. betle* were collected from Munduk Paku, Senganan village, Tabanan Regency, Bali, Indonesia. A 10 kg volume of mature leaves were cut into small pieces, dried in the wind for 3 days until the leaves were dry, and mashed using a blender. After drying, maceration was carried out using a 90% methanol solution with a weight/volume ratio of 1:10 for 2 days and 2 nights. Then the mixture was filtered using gauze and Whatman filter paper No. 1. The filtrate solution was evaporated at 40 °C and this crude extract was used for further study [10].

### 2.4. Compost Preparation

A compost preparation of 200 kg was prepared by mixing 180 kg cow dung, 20 kg agricultural waste, 0.5 kg liquid starter (Suria green), and *Trichoderma sp.*, followed by the addition of water until a little crumb appeared, then it was stirred and covered with tarpaulin. After 12 days the mixture was turned and closed for 1 month. After 1 month, the compost was ready for use [10].

### 2.5. Making Botanical Biopesticides

The biopesticide extract of piper was made from leaves of *P. caninum*, *P. betle* var. *Nigra*, and *Piper betle*. A 5 kg volume of the mature leaves was dried in the wind, cut into thin strips, dried, blended, and macerated with methanol for 48 h in a dark place. Then it was filtered and evaporated in a rotary vacuum evaporator at 40 °C. This crude extract was then ready for application [13].

### 2.6. Seeding/Seeds

Good quality local Balinese red rice seeds, obtained from a local market, were initially soaked for 48 h in clean water then mixed in the rice field media comprising paddy soil (90%) and compost (10%). The excess water was drained and coated seeds were air dried [9].

### 2.7. Preparation of Planting Media

Wet paddy soil media was added into a bucket (30 cm diameter) to the level of about  $\frac{3}{4}$  of the bucket, mashed, mixed, watered, and stirred to form mud. Each bucket except the control had 50 g of compost added.

### 2.8. Greenhouse Trials

The greenhouse trials were conducted in a randomized block design (RBD) with 4 groups as follows:

- Group 1—*P. caninum* leaf extract;
- Group 2—*P. betle* var. *Nigra* leaf extract;
- Group 3—*P. betle* leaf extract; and
- Group 4—Mixture of the three extracts.

Each group had 4 treatments and five replications so that there were 20 experimental units, each unit consisting of 10 clumps. There were 200 clumps for the field for each group. The treatment for each group consisted of:

- F0 = Control (without extract);
- F1 = 0.5% extract;
- F2 = 1% extract;
- F3 = 1.5% extract; and
- F4 = 2.0% extract.

The implementation of the experiment includes sowing seeds, preparing planting media, planting seeds, maintaining plants, fertilizing, and harvesting [10].

The seeds were 15-day-old, healthy, disease-free rice seeds of uniform height ( $\pm 15$  cm). The extraction of seeds and planting were performed in the morning. Each pot was planted with 2 rice seeds. Planting was carried out perpendicular to a depth of  $\pm 3$  cm.

The addition of the fertilizer was done with 2 mL/pot of liquid organic fertilizer (Suria green), which contained *Enterobacter cloacae*, *Bacillus subtilis*, and *Stenotrophomonas maltophilia*. diluted with 500 mL of water, fertilizing at the age of 2 and 6 weeks after planting.

Inoculation of the fungus *P. oryzae*, which causes blast disease in rice plants, was carried out after 30 days of red Bali rice planting. The spore suspension of the pathogen was spread at the rate of 20 mL per clump, then covered with plastic for 12 h to retain moisture [14].

The extract was applied after 1 day of inoculation of the fungus to the rice plants (according to treatment). Each rice plant was sprayed with as much as 20 mL of piper extract and repeated 4 times at 1 week intervals [15]. Rice plant maintenance included embroidery, watering, weeding, and fertilization. Embroidering was carried out on those plants that did not grow or had abnormal growth with plants that had been prepared in advance in a tray so that their growth was uniform. Watering was done every day (morning or evening) from planting. Weeding was done to keep the plants from being disturbed by weeds and to prevent competition for nutrients between weeds and rice plants.

Harvesting was done after all the rice had turned yellow within 4.5 months. The rice was dried in the sun, then milled in oozing grain to produce organic rice [12]. Measurement of plant growth parameters: growth parameters (plant height, number of tillers, and number of leaves), yield components (number of grain/panicle, full-grain weight/clump (g), and empty grain/clump (%)), and estimated yield/ha (calculated by weighing the rice produced, totaling production, and converting into Tonne/ha) [16].

### 2.9. Measurement of Blast Disease Intensity

Measurement of the intensity of leaf spot disease was carried out using the following formula:

$$DI = \frac{\text{No. of infected plants}}{\text{No. of plants observed}} \times 100 \quad (1)$$

Observations were recorded as follows:

0 = No attack;

1 = Very mild attack (0–10% damage to leaf surface);

2 = Mild attack (10–30% damage to leaf surface);

3 = Moderate attack (30–50% damage to leaf surface);

4 = Severe attack (50–75% damage to the leaf surface); and

5 = Heavy attack (75–100% damage to the leaf surface).

### 2.10. Measurement of Antifungal Activity Test with Diffusion Well Method

The antifungal activity test was carried out by testing the crude extract of *P. caninum*, *P. betle var. Nigra*, and *P. betle*, and a mixture of the three extracts with a ratio of 1:1:1 to the growth of *P. oryzae* fungus that causes blast disease in rice. A 200  $\mu$ L of *P. oryzae* spore suspension was mixed with 15 mL molten PDA and poured into the Petri plate. After solidification, 2 diffusion wells of 5 mm diameter were made in each plate. Each well was filled with 20  $\mu$ L of crude extract and plates were then incubated at 30 °C for 48 h and observed for the inhibition of fungal growth [17].

### 2.11. Determination of Antifungal Activity Test on Fungal Colony Growth

The concentration of *P. caninum* leaf crude extract, *P. betle var. Nigra*, and *P. betle*, and a mixture of the three extracts with a ratio of 1:1:1, respectively, namely 0% as a control, 0.5%, 1%, 1.5%, and 2%, were tested for inhibition against the growth of *P. oryzae* fungal colonies on PDA media. A 0.5 mL amount of 10% extract was mixed with 9.5 mL of PDA in a Petri plate, mixed, and left for solidification. One piece of *P. oryzae* fungal mycelia taken from the edge of the 5-day-old fungal colony on a PDA was placed at the center of a Petri plate. A

total of 5 Petri plates were prepared for each extract concentration tested. The control plate was prepared without an extract. These cultures were incubated at 30 °C in a dark place for 7 days. Observations were made every day by measuring the diameter of the fungus in each treatment. The percentage of inhibition was calculated by comparing the growth of fungus in the test plates with the control plate. The inhibition of extract treatment on colony growth was calculated using the following formula [18]:

$$\text{Inhibition activity (\%)} = \frac{\text{Diameter of control colonies} - \text{Diameter of treated colonies}}{\text{Diameter of control colonies}} \times 100 \quad (2)$$

#### 2.12. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined on PDA previously seeded with *P. oryzae* by the well diffusion method with various concentrations of the extract in the range of 0.1% to 5%. A preparation without extracts served as a control. Each well was added with 20 µL of individual concentration of crude extract. Plates were incubated at 30 °C for 48 h and observed for the inhibition of the fungal pathogen [19].

#### 2.13. Gas Chromatography–Mass Spectrophotometry (GC-MS) Analysis

To determine the content of phytochemicals in the extracts of *P. caninum*, *P. betle* var. *Nigra* and *P. betle*, they were tested for GC-MS [20,21]. The samples were analyzed in the form of plant leaf extracts. The most active and relatively pure fraction samples were analyzed by GC-MS. The molecular weight, fragmentation pattern, and the structure of the isolated compound were compared with the compound in the library in the GC-MS system [12,15,19].

#### 2.14. Statistical Analysis

The data presented in this study are the mean of five replications and were analyzed quantitatively by using analysis of variance (ANOVA). If the treatment caused a difference to the observed variable, it was followed by a Duncan's Multiple Range Test (DMRT) at the 95% significance level.

### 3. Results

#### 3.1. Plant Growth Parameters

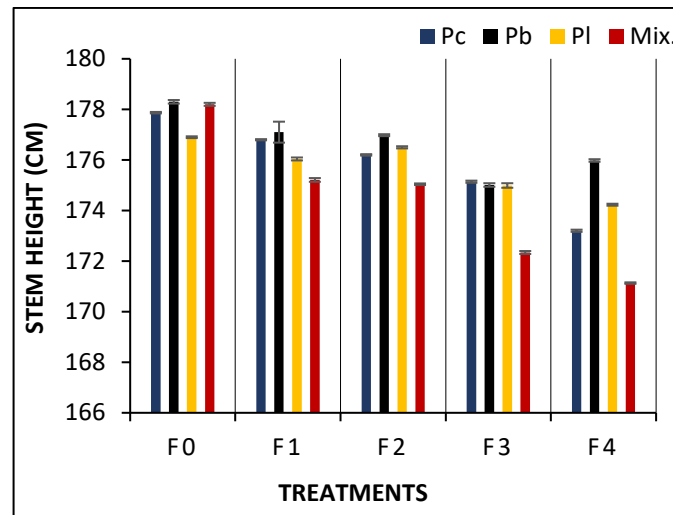
The treatment of piper leaf extract, compost, and PGPR on Balinese red rice plants had a significant effect (Figures 1–3) on the data parameters for growth in terms of the number of tillers and the number of leaves. Rice stalks became shorter if the extract concentration was added; this is beneficial because the stems of Balinese rice with an average height of more than 2 m typically fall during production. If the stems are shorter, it can stop them from falling. During the treatment of single extracts, the highest number of tillers was found in the *P. caninum* leaf extract treatment (12.90). The leaf number parameter was also observed in the *P. caninum* extract treatment (57.12) at a concentration of 2%. For the combined extracts (*P. caninum*, *P. betle* var. *Nigra*, and *P. betle*): for the number of tillers (15.98), the number of leaves (67.98) showed the best data and the height parameter (171.13 cm).

#### 3.2. Yield Components

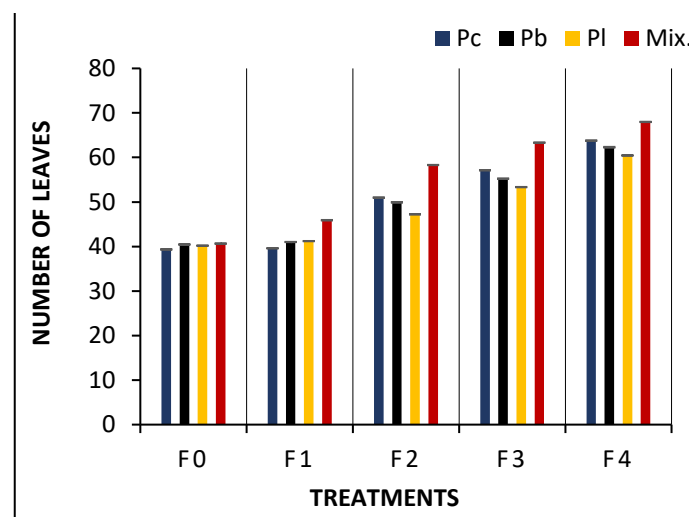
The effects of single extracts (Table 1, Figures 4 and 5) combined with PGPR and compost showed significantly different yield components from controls. The higher the treatment, the higher the yield component. The *P. caninum* extract had the greatest effect on the yield, followed by the *P. betle* var. *Nigra* extract and the *P. betle* extract. When compared with the mixed extract, the mixture of three extracts showed a superior effect on the yield component. This shows that the mixture of three extracts works synergistically to produce new compounds that are more toxic to rice blast disease, and can increase rice yields. Likewise, the weight treatment of empty grain (Figure 6) was significantly different from the control. The higher the treatment, the smaller the empty grain weight; likewise, the mixture extract showed the empty grain weight was smaller than the single extract. In



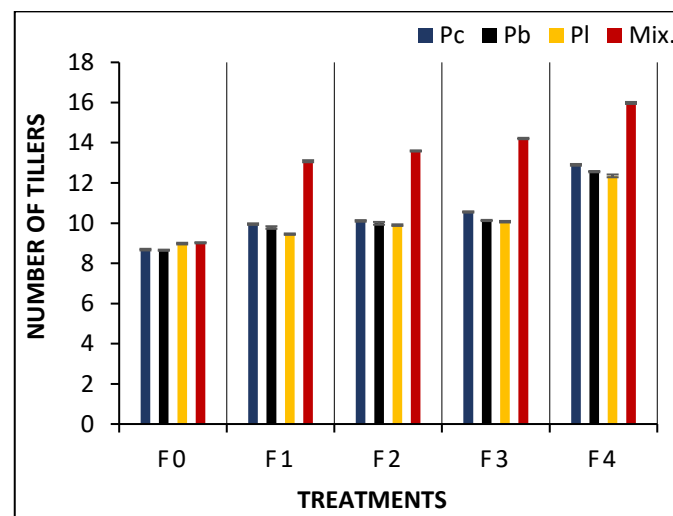
addition to extracts functioning to suppress blast disease, PGPR also functions to suppress disease and can produce growth hormones that can increase growth so that it can induce plant systemic defenses [22].



**Figure 1.** Effect of piper leaf extract, PGPR, and compost on stem height of Bali red rice at 12 weeks. Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mix extract Pc, Pb, and Pl. F0 (control), F1 (0.5% extract + PGPR + compost), F2 (1% extract + PGPR + compost), F3 (1.5% extract + PGPR + compost), and F4 (2.0% extract + PGPR + compost). Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.



**Figure 2.** Effect of piper leaf extract, PGPR, and compost on number of leaves in Bali red rice at 12 weeks. Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mix extract Pc, Pb, and Pl. F0 (control), F1 (0.5% extract + PGPR + compost), F2 (1% extract + PGPR + compost), F3 (1.5% extract + PGPR + compost), and F4 (2.0% extract + PGPR + compost). Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.



**Figure 3.** Effect of piper leaf extract, PGPR, and compost on number of tillers of Bali red rice at 12 weeks. Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mix extract Pc, Pb, and Pl. F0 (control), F1 (0.5% extract + PGPR + compost), F2 (1% extract + PGPR + compost), F3 (1.5% extract + PGPR + compost), and F4 (2.0% extract + PGPR + compost). Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.

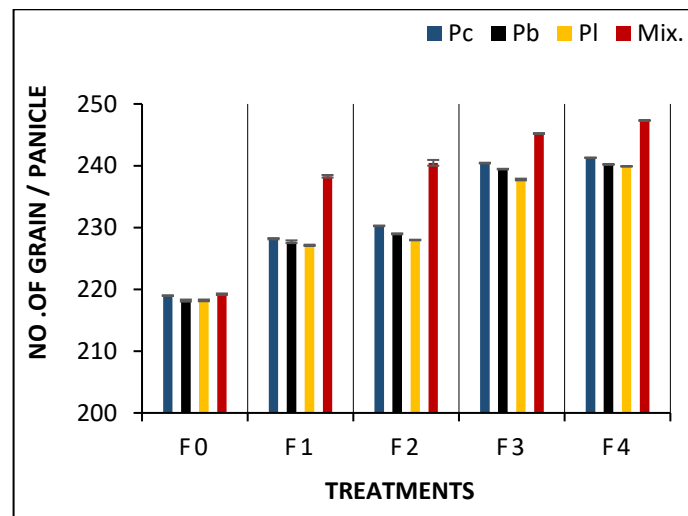
**Table 1.** Effect of leaf extract treatment, PGPR, and compost on yield components.

Treatment	Potential Yield (tonne/ha)				% Yield Increase Vis-a-Vis Control			
	Pc	Pb	Pl	Mix	Pc	Pb	Pl	Mix
F0 (Control)	3.21 a *	3.15 a *	3.10 a *	3.40 a *	-	-	-	-
F1 (0.5% extract + PGPR + compost)	3.50 a	3.48 a	3.41 a	4.20 b	10.48	10.48	10	23.53
F2 (1% extract + PGPR + compost)	3.91 ab	3.87 a	3.56 a	4.91 b	21.81	13.02	14.84	44.41
F3 (1.5% extract + PGPR + compost)	5.42 c	5.23 b	5.12 b	6.21 c	68.85	62.54	65.16	82.65
F4 (2.0% extract + PGPR + compost)	5.45 c	5.27 b	5.15 b	6.59 c	69.78	67.30	66.13	93.83

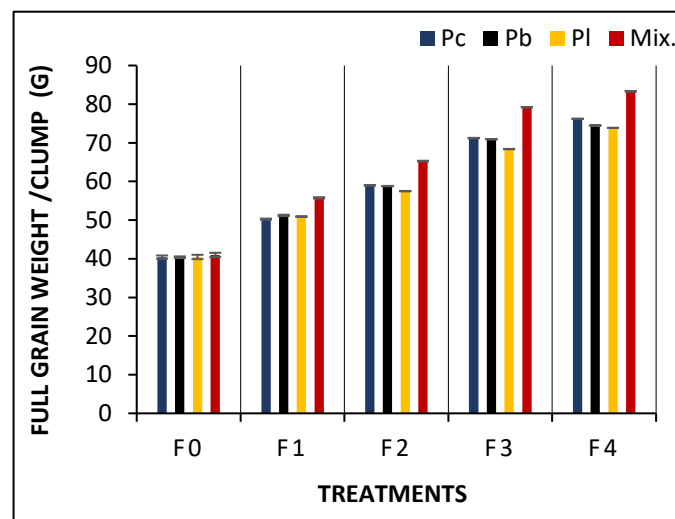
Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mixture of extract Pc, Pb, and Pl.  
 \* the same letter is not significantly different between treatments. Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.

The content of alkaloid compounds, flavonoids, phenolics, and terpenoids in extracts can damage the cell walls of pathogens, which then causes the pathogens to be developmentally interrupted [23]. Likewise, the permeability of pathogenic cell membranes is inhibited by phytochemical compounds in plant extracts [24]; in addition, it can inhibit pathogen germination [25,26]. Phytochemical compounds in extracts can also influence polysaccharides and proteins in pathogens [27], and activate systemic resistance in plants [28]. A mixture of *P. caninum* leaf extract and *P. betle var. Nigra* leaf extract combined with PGPR, which has a higher inhibitory against disease in red Bali rice, can increase yields compared to the effects of single extracts [12].

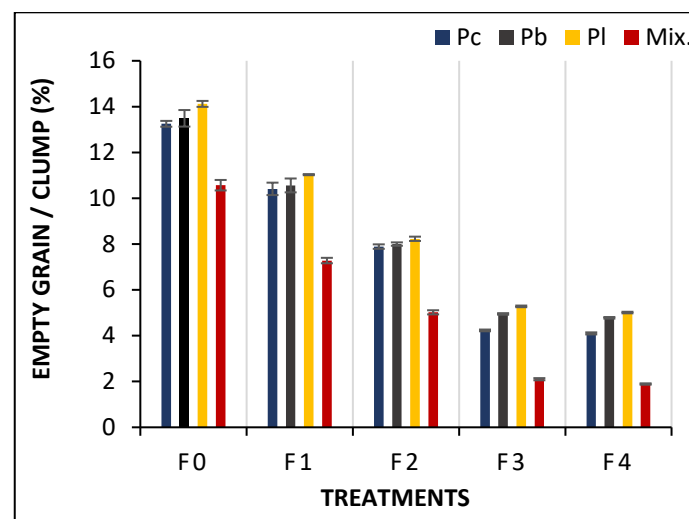




**Figure 4.** Effect of piper leaf extract, PGPR, and compost on number of grains/panicle. Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mix extract Pc, Pb, and Pl. F0 (control), F1 (0.5% extract + PGPR + compost), F2 (1% extract + PGPR + compost), F3 (1.5% extract + PGPR + compost), and F4 (2.0% extract + PGPR + compost). Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.



**Figure 5.** Effect of piper leaf extract, PGPR, and compost on full grain weight/clump. Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mix extract Pc, Pb, and Pl. F0 (control), F1 (0.5% extract + PGPR + compost), F2 (1% extract + PGPR + compost), F3 (1.5% extract + PGPR + compost), and F4 (2.0% extract + PGPR + compost). Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.



**Figure 6.** Effect of piper leaf extract, PGPR, and compost on empty grain/clump. Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mix extract Pc, Pb, and Pl. F0 (control), F1 (0.5% extract + PGPR + compost), F2 (1% extract + PGPR + compost), F3 (1.5% extract + PGPR + compost), and F4 (2.0% extract + PGPR + compost). Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.

### 3.3. Blast Disease Intensity

The intensity of blast disease in Bali red rice at week 12 after planting showed a significant difference between control and treatment. The smallest disease intensity of all treatments was found in the 2% extract treatment. The higher the extract to a concentration of 2%, the less the disease intensity (Table 2). The smallest blast disease intensity for a single extract was found in the *P. caninum* treatment, which was 8.10%, followed by the *P. betle var. Nigra* extract treatment at 9.65%, then the *P. betle* extract treatment at 9.95%. The lowest was the mixed extract. The three extracts were *P. caninum*, *P. betle var. Nigra* and *P. betle*. A mixture of the three extracts can reduce the intensity of blast disease in Bali red rice. The extract that was most effective in reducing the intensity of blast disease was the mixed extract with the smallest disease intensity (5.97%) at a concentration of 2% and 92.58% inhibition. The higher the extract concentration, the smaller the blast disease intensity. Piper extract has the potential to be developed as a biopesticide to suppress blast disease in rice. When compared with the use of a mixture of two extracts, namely a mixture of *P. caninum* with *P. betle var. Nigra* at week 12, the intensity of blast disease in Bali red rice was 8.98% [12]. The use of three piper extracts (*P. caninum*, *P. betle var. Nigra*, and *P. betle*) was shown to be more effective than a mixture of two (*P. caninum* and *P. betle var. Nigra*) to reduce the intensity of blast disease in Bali red rice.

In addition to extracts that have the potential to reduce the intensity of blast disease in rice plants, there is also the use of PGPR and compost. PGPR (*Enterobacter cloacae*, *Bacillus subtilis*, and *Stenothropomonas maltophilia*) can produce antifungals that can suppress blast disease in rice, thereby increasing systemic resistance. Likewise, the use of compost using *Trichoderma* sp. as a starter can suppress blast disease in rice. Compost also contains nutrients which are a source of nutrition for rice plants [10]. *Rhizoctonia solani* is a disease in wheat plants that can be suppressed by Rhizobacteria isolates and increases the growth of wheat germ and the biomass of wheat roots [29]. PGPR isolated from tea plantations can increase the growth of tea plants and can also suppress pathogenic fungi such as *Nigrospora sphaerica*, *Pestalotiopsis there*, *Curvularia eragostidis*, *Glomerella cingulata*, *Rhizoctonia Solani*, and *Fusarium oxysporum* [30].

**Table 2.** The impact of piper extracts, compost, and PGPR on the intensity of blast disease in Bali rice plants at week 12.

Treatment	The Intensity of Blast Disease (%)				Inhibitory Activity (%)			
	Pc	Pb	Pl	Mix	Pc	Pb	Pl	Mix
F0 (Control)	80.21 a *	80.40 a *	80.64 a *	80.43 a *	-	-	-	-
F1 (0.5% extract + PGPR + compost)	41.22 b	42.40 b	42.97 b	35.10 b	48.61 b	47.38 b	46.71 b	56.36 b
F2 (1% extract + PGPR + compost)	31.89 bc	33.19 bc	35.12 b c	19.19 c	60.24 c	58.72 c	56.45 c	76.14 c
F3 (1.5.% extract + PGPR + compost)	20.21 c	25.11 c	12.89 c	11.80 cd	74.80 cd	68.77 c	84.02 d	85.33 d
F4 (2.0% extract + PGPR + compost)	8.10 cd	9.65 d	9.95 c	5.97 d	89.90 d	88 d	87.66 d	92.58 e

Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mixture of extract Pc, Pb, and Pl. \* the same letter is not significantly different between treatments. Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.

### 3.4. Inhibition of Piper Crude Extract on the Growth of *P. oryzae* Colonies

The inhibition activity of piper extract against *P. oryzae* is presented in Table 3. The highest inhibition activity for a single extract was found in *P. caninum* at a concentration of 2%, with inhibitory activity of 34.98% on the seventh day. This was followed by *P. betle var. Nigra* with an inhibitory activity of 30.01% at a concentration of 2%. The least inhibitory activity was found in the *P. betle* extract treatment at 27.02%. The greatest inhibitory activity was found in the mixture of the three extracts of 40.13% at a concentration of 2%. Inhibition of each extract and mixed extract against *P. oryzae* fungus that causes blast disease in Bali red rice was significantly different compared to the control. On day 5 and day 7 the inhibitory activity against fungal colonies for each extract was the highest at 2% extract concentration and the best overall inhibition was in the mixed extract (40.13%) at 2% extract concentration. The higher the extract concentration, the higher the inhibitory activity of *P. oryzae* fungal colonies [31].

**Table 3.** Inhibitory activity of crude extract and MIC to *P. oryzae* at 7 days.

Diameter of Crude Extract (mm)				Mic (%)			
Pc	Pb	Pl	Mix.	Pc	Pb	Pl	Mixture
40	35	37	50	0.4	0.5	0.5	0.3

Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mix = mixture of three extracts. Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.

The function of the phytochemicals in the extract can lyse *P. oryzae* fungal cells by damaging the fungal cell walls, then changing the osmotic pressure on the cell membranes so that the cell fluid leaves the cells and the fungal cells experience lysis [32]. Garlic extract containing allicin at a concentration of 80–125 µg/mL can inhibit the fungi *Drechslera tritici-repensis*, *Bipolaris sorokiniana*, and *Septoria tritici* by inhibiting colony growth, which causes disease in wheat. This garlic extract has the potential to be developed as a biopesticide in wheat farming [33]. Leaf extracts of *Nerium oleander* and *Pithecolobium dulce* can inhibit mycelia growth (77.4 and 75.1%) and spore germination (80.3 and 80%) of the *Bipolaris oryzae* fungus that causes brown spots in rice [34].

The highest inhibition against blast disease was found in the mixed extract (*P. Caninum*, *P. betle var. Nigra*, and *P. betle*). Two new compounds were found that were analyzed by GC-MS, namely citronella, trans-geraniol, and 4,6-dipropyl-nonan-5-one compounds, which have the potential to increase blast disease resistance. The mixture of three extracts combined with PGPR and compost was more effective in suppressing blast disease in rice plants caused by *P. oryzae*, and more effective at increasing growth, red Bali rice production, and yields (by 93.83%) compared to the control. Extract, PGPR, and compost are integrated measures in sustainable agriculture, which are ways to carry out sustainable development.

### 3.5. Crude Extract and MIC Inhibition

The inhibition of the three extracts and the mixed extract is presented in Table 4. The mixed extract shows the highest inhibition activity compared to the single extract. This shows that the three extracts work in a synergy resulting in greater inhibition of *P. oryzae*, which causes blast disease in rice plants. The GC-MS results showed that the mixed extract contained three new compounds, namely citronella, trans-geraniol, and 4,6-dipropyl-nonan-5-one. These three new compounds are more effective at suppressing blast disease in rice plants. Similar studies show combined *P. caninum* and *P. betle var. Nigra* extracts also have a better effect on suppressing blast disease in rice than single extracts [12]. Likewise, the mixed extract of *Mansoa alliacea L.* and *Allamanda cathartica L.* leaves has a better effect than a single extract on suppressing the disease of the peanut sprout fungus *Athelia rolfsii* [14]. Leaf extracts of *M. olerifera*, *A. indica*, and *E. globules* were very effective against *D. noxia* and surge compared to single extracts [31].

**Table 4.** Inhibitory activity of piper extracts against colonies of *P. oryzae* at 5 days and 7 days.

Treatment	Inhibitory Activity of Piper against <i>P. oryzae</i> (mm) at 5 Days (%)				Inhibitory Activity of Piper against <i>P. oryzae</i> (mm) at 7 Days (%)			
	Pc	Pb	Pl	Mixture	Pc	Pb	Pl	Mixture
F0 (Control)	-	-	-	-	-	-	-	-
F1 (0.5% extract)	15.34 a *	13.16 a *	12.01 a *	15.90 a *	15.22 a *	12.39 a *	11.92 a *	15.02 a *
F2 (1% extract)	25.76 b	20.19 b	18.33 b	26.45 b	24.98 b	20.12 b	18.11 b	26.09 b
F3 (1.5% extract)	31.12 c	24.75 c	22.56 c	33.17 c	30.10 c	23.97 c	21.99 c	32.98 c
F4 (2.0% extract)	35.45 d	30.22 d	27.90 d	40.82 d	34.98 d	30.01 d	27.02 d	40.13 d

Pc = extract of *P. caninum* leaf; Pb = extract of *P. betle var. Nigra* leaf; Pl = extract of *P. betle* leaf; and Mixture = mix extract of Pc, Pb, and Pl. \* the same letter is not significantly different between treatments. Values are the mean of five replications, analyzed by ANOVA followed by the DMRT at the 95% significance level.

MIC data at day 7 (Table 3) showed that the MIC of the *P. caninum* extract was 0.4%, while *P. betle var. Nigra* and *P. betle* were the same, namely 0.5%, and the MIC for the mixture of the three extracts was 0.3%. The mixed extract, as seen from the MIC value, has the smallest inhibition activity at a concentration of 0.3%. The smaller the MIC, the more effective the extract is at inhibiting blast disease in Bali red rice plants. *Rosmarinus officinalis L.* leaf extract has MICs of 25 µg/mL and 10 µg/mL for *Cynara scolymus L.* leaf extract against *Alternaria sp.*; the same effect shown by conventional Captan fungicide (2.5 µg/mL) [35]. *Rhizopus stolonifer* can inhibit pathogenic fungi such as *A. niger*, *A. oryzae*, *C. albican*, *P. digitatum*, and *F. Oxysporum* with a MIC value of 0.25 mg/mL, containing the phytochemical substances mycotoxin aflatoxin [36].

### 3.6. GC-MS Analysis Results

Bioactive substances from the *P. Caninum* extract were found in eight compounds: benzene, xylene, Tetradecane, dodecanoic acid, heptadecane, hexadecanoic acid, octadecamethyl cyclononasiloxane, Phthaic acid, and 8,11, 14-dococatric acid. The *P. betle var. Nigra* extract found nine compounds, such as Alpha-pinene, benzene, dl-Limonene, copaene, benzaldehyde, acetamide, benzoic acid, alpha-gurjunene, and tetrasiloxane. The *P. betle* extract contained 12 compounds, namely acetamide, 2,4 dinitrophenyl, trimethylformamide, 2-hydroxy-2-cyclopenten, 2-heptanamine, 2-propanamine, Pentanal, benzene, cyclohexene, 2-n-propoxyamphetamine, copaene, and 5,8-epoxy-15-nor-labdane. If the three extracts are mixed with an extract ratio of 1:1, 10 compounds are obtained (Figure 7), namely: citronella, trans-geraniol, dodecanoic acid, hexadecanoic acid, methyl ester, octadecanoic acid, di-n-octyl-phthalate, 4,6-dipropyl-nonan-5-one, tetradecane, 6,9-dimethyl (CAS), and 6,9. dimei. The three types of pipers, *P. Caninum*, *P. Betle var. Nigra*, and *P. Betle*, and the combination of the three types can be seen in Table 5.

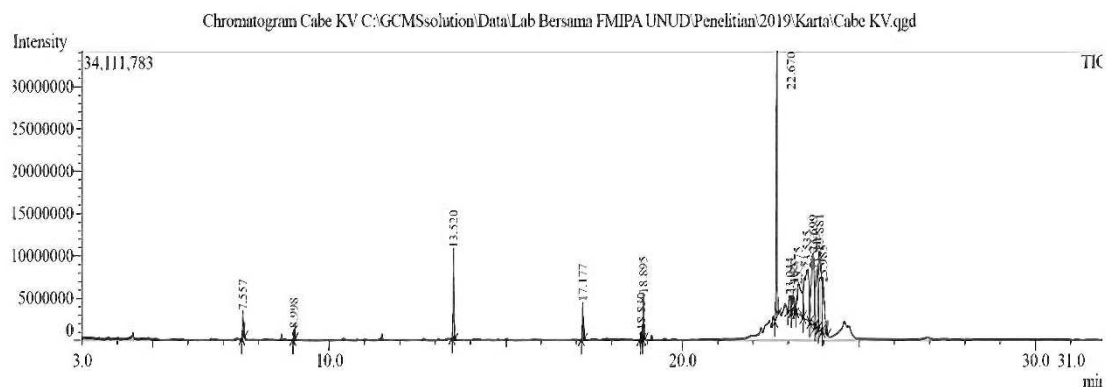


Figure 7. Chromatogram of mixture of three piper leaf extracts.

Table 5. Bioactive compounds of three piper and mixture of leaf extracts.

Peak	Retention Time (Min)				Area (%)				Bioactive Compound			
	PC	Pb	PI	Mix.	PC	Pb	PI	Mix.	PC	Pb	PI	Mixture
1	3300	3.791	2.123	7.558	47.68	5.67	2.52	13.74	Benzene	Alpha-pinene	Acetamide	Citronella
2	3457	5.115	3.145	8.999	40.97	16.21	0.71	55.54	Xylene	Benzene	2,4 Dinitrophenyl	Trans-geraniol
3	11.32	5.185	3.506	13.252	11.18	10.55	4.65	25.77	Tetradecane	dl-Limonene	Trimethylformamide	Dodecanoic acid
4	12.909	10.339	3.621	17.178	45.44	8.65	9.49	13.86	Dodecanoic acid	Copaene	2-hydroxy-2-cyclopenten	Hexadecanoic acid, methyl ester
5	13.851	10.803	3.786	18.895	24.03	2.63	4.21	16.16	Heptadecane	Benzaldehyde	2-heptanamine	Octadecanoic acid
6	17.420	10.942	4.153	22.669	36.46	5.78	4.42	10.93	Hexadecanoic acid	Acetamide	2-propanamine	Di-n-octyl-phthalate
7	17.963	11.485	4.541	23.815	12.97	14.41	1.74	34.37	Octadecamethyl cyclononasiloxane	Benzoic acid	Pentanal	4,6-dipropyl-nonan-5-one
8	18.426	11.529	5.114	23.985	34.01	28.91	7.44	33.62	Phthalic acid	Alphagurjunene	Benzene	Tetradecane, 6,9-dimethyl (CAS) 6,9-dimei
9	19.136	24.975	5.186		86.41	4.48	4.02		8,11,14-Docosatrienoic acid	Tetrasiloxane	Cyclohexene	
10	22.935	-	9.436		82.53	-	2.12		1,2-Benzenedicarboxylic acid	-	2-n-propoxyamphetamine	
11			10.339				4.54				Copaene	
12			10.941				3.33				Piperidine	
13			11.484				11.02				Benzoic acid	
14			11.528				15.39				Alpha-guaiene	
15			24.794				10.89				5,8-epoxy-15-nor-labdane	

PC = leaf extract of *P. caninum*; Pb = leaf extract of *P. betle var. Nigra*; PI = *P. betle*; and Mix = 1:1:1 mixture of leaf extracts of *P. caninum*, *P. betle var. Nigra*, and *P. betle*. Leaf extracts of *P. caninum* and leaf extracts of *P. betle var. Nigra* were chromatographed on GC-MS separately.

Table 5 shows that after the three extracts are mixed, new compounds are formed that are not found in the single extract, such as citronella, trans-geraniol, and 4,6-dipropyl-nonan-5-one. This compound works synergistically to suppress blast disease in rice caused by the fungus *Pyricularia oryzae*. Citronella compounds have antimicrobial properties, such as being antifungal, antibacterial, and antiparasitic, and are often found in citrus fruits and fragrant lemongrass [37]. Trans-geraniol is a chemical compound found in plants that functions as an anti-insecticide, antimicrobial, and an antioxidant, and is also anticancer [38]. Other phytochemical compounds, such as Alpha-gurjunene, benzene, copaene, and benzoic acid, have an antifungal function [39–41]. In the extract *P. caninum* the largest number of compounds is 1,2-Benzenedicarboxylic acid (82.53%). The *P. betle var. Nigra* extract is a compound phthalic acid (28.91%); in *P. betle* extract, the highest compounds were Alpha-guaiene (10.89%). In the mixed extract, the highest compound was trans-geraniol (55.54%).

#### 4. Discussion

The higher the extraction concentration, the higher the yield component. The highest number of grain/panicle components found in the treatment of the mixture of three extracts (247.32) with an extract ratio of 1:1:1, for the highest single extract, was found in *P. caninum*

(241.29), then *P. betle* var. *Nigra* (240.21), and *P. betle* (239.90). The full grain weight/clump between control and treatment showed a significant difference at 2% extract concentration for all treatments. In all treatments the best effect was the mixed extract treatment with a concentration of 2% (83.31 g). The highest single extract full grain weight/clump parameter was found in *P. caninum* (76.22 g). For the empty grain/clump parameter the smallest amount was in the mixed extract treatment (1.89%) at a 2% extract concentration. For the mixed extract treatment the smallest amount was in the *P. caninum* extract treatment at a 2% concentration. The yield potential per ha between the control and treatments showed a significant difference. The largest yield potential was found in the mixed extract treatment with a concentration of 2% (6.59 ton/ha) and could increase the yield by 93.83% compared to the control, followed by the single extract of *P. caninum* (5.45 ton/ha) which could increase the yield by 69.78%.

Comparing single extracts with mixed extracts in terms of yield component parameters, the mixed extract was more effective. The formation of new phytochemical compounds in the mixed extract is more effective in suppressing blast disease, at increasing the yield component, and at reducing the amount of empty grain. The mixture of two extracts (*P. caninum* and *P. betle* var. *Nigra*) showed better yield components (5.61 tonne/ha) than the single extract treatment [12]. The use of *Ficus septica* leaf extract can suppress coletotricum disease in chilies, both in vitro and in the field [19]. Likewise, cinnamon leaf extract can suppress Fusarium disease in tomato plants [15] and chilies. *P. caninum* extract can suppress the fungus *Curvullaria* sp. in rice plants and can increase dry grain yields [10]. The bark extract of *Michelia alba* barks can suppress *Curvullaria* sp. disease in rice and can also increase yields [18,42]. *Equisetum arvense* L 0.5% and *Urtica dioica* 0.1% leaves act as stimulants to cabbage seeds, increasing plant weight by 113% and 112% [43]. The treatment of young corn extract in combination with coconut water can increase the growth and viability of potato plants by 86.67–100%, in comparison to the control at 74.44% [44]. Ulva seaweed extract can increase the growth of *Arabidopsis thaliana* plants at a concentration of 1% [45].

In addition to the effects of the extracts, the use of PGPR is influential in increasing yields on red Bali plants [10]. Piper extract can increase the growth of Bali red rice plants because it contains growth hormones. Piper extract can also increase root growth and multiply and lengthen roots [12]. PGPR and compost also function to increase plant growth because PGPR can produce growth hormones such as IAA [20,46] and compost contains nutrients that can improve plant nutrition. PGPR strains such as *Bacillus* sp., *Pseudomonas* sp., and *Enterobacter* sp. are known to improve growth and suppress pathogens in avocado plants [21,22]. *Streptomyces* has the potential as biocontrol to suppress blast disease in rice [47,48]. The use of PGPR on green beans can increase seed weight (41%) and biomass (54%). The use of compost from cow dung and husk ash (7.5 ton/ha) can increase the biomass of maize in Indonesia [49]. PGPR can be used in plants as biocontrol and can function as a growth enhancer and to increase yields, and is also sustainable agriculture [50]. PGPR is an alternative to improve soil fertility due to pressure from chemical fertilizers and pesticides [51]. The addition of *B. Japonicum* and *P. Putida* and 3% biochar can increase soybean yields and soil fertility by searching for soil nutrients and enzymes in the soil, and is an alternative in sustainable agriculture [51]. PGPR isolates from plants are antifungal against several pathogenic fungi, such as the fungus *nigrospora* sp., because these isolates produce siderophores, chitinases, and cellulases, and also produce IAA hormones, phosphate solvents, and ammonia [52].

Compost, which is a source of plant nutrition, has the potential to increase crop yields in red rice plants, and also functions as a source of decomposing microbes and microbes that can suppress plant pathogens. The use of compost at 600 kg/ha can increase yield and biomass in soybean plants [53]. Likewise, the compost treatment of 20 ton/ha can increase the efficiency of using NPK fertilizer by 50% on *Phaseolus vulgaris* plants on saline soil media [54]. Compost is an alternative to turning manure into value and can support agricultural sustainability and help farmers [55,56]. However, the ability of PGPR to ferment three piper extracts is not yet known, and further research is needed that can show



how to increase the shelf life of the extract as a biopesticide and, at the same time, as an organic fertilizer.

## 5. Conclusions

The conclusion of this study is a single extract of *P. caninum*, *P. betle* var. *Nigra*, and *P. Betle* as well as a mixture of all three can inhibit blast disease in rice plants starting from the extract concentration of 0.5% and increasing to the extract treatment of 2%. The synergistic action of three extracts (2% concentration), compost (0.5%), and PGPR preparations has the potential to improve the growth parameters in red rice and exert potential antifungal activity against rice blast phytopathogens. This preparation has the potential to be developed as the best organic fertilizer-cum-biopesticide for sustainable growth of Balinese red rice and effective control of rice blast pathogen.

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