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A Mixture of Piper Leaves Extracts and Rhizobacteria for Sustainable Plant Growth Promotion and Bio-Control of Blast Pathogen of Organic Bali Rice

Ni Luh Suriani [1,*], Dewa Ngurah Suprapta [2], Novizar Nazir [3], Ni Made Susun Parwanayoni [1], Anak Agung Ketut Darmadi [1], Desy Andya Dewi [2], Ni Wayan Sudatri [1], Ahmad Fudholi [4], R. Z. Sayyed [5], Asad Syed [6], Abdallah M. Elgorban [6], Ali H. Bahkali [6], Hesham Ali El Enshasy [7,8,9] and Daniel Joe Dailin [7,8]

1 Biology Department, Faculty of Mathematics and Natural Science, Udayana University, Bali 80361, Indonesia; parwanayoni@unud.ac.id (N.M.S.P.); darmadi@unud.ac.id (A.A.K.D.); wayan_sudatri@unud.ac.id (N.W.S.)
2 Biopesticide Laboratory, Faculty of Agricultural, Udayana University, Bali 80361, Indonesia; ngurahsuprapta@unud.ac.id (D.N.S.); desy_andya@student.unud.ac.id (D.A.D.)
3 Faculty of Agricultural Technology, Andalas University, Padang 25163, Indonesia; Nazir_novizar@yahoo.com
4 Solar Energy Research Institute, Universiti Kebangsaan Malaysia, Bangi 43650, Malaysia; a.fudholi@gmail.com
5 Department of Microbiology, PSGVP Mandal's Arts, Science, and Commerce College, Shahada 425409, Maharashtra, India; sayyedrz@gmail.com
6 Department of Botany and Microbiology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia; asadsayyed@gmail.com (A.S.); aelgorban@ksu.edu.sa (A.M.E.); abahkali@ksu.edu.sa (A.H.B.)
7 Institute of Bioproduct Development (IBD), Universiti Teknologi Malaysia (UTM), Johor Bahru, Johor 81310, Malaysia; henshasy@ibd.utm.my (H.A.E.E.); jddaniel@utm.my (D.J.D.)
8 School of Chemical and Energy Engineering, Faculty of Engineering, Universiti Teknologi Malaysia (UTM), Johor Bahru, Johor 81310, Malaysia
9 City of Scientific Research and Technology Applications (SRTA), New Burg Al Arab, Alexandria 21934, Egypt
* Correspondence: niluhsuriani@unud.ac.id; Tel.: +62-816-578-266

Received: 20 August 2020; Accepted: 23 September 2020; Published: 14 October 2020

Abstract: Rice is a crop that is consumed as a staple food by the majority of the people in the world and therefore failure in rice crops, due to any reason, poses a severe threat of starvation. Rice blast, caused by a fungus *Pyricularia oryzae*, has been ranked among the most threatening plant diseases of rice and it is found wherever rice is grown. All of the rice blast disease management strategies employed so far have had limited success and rice blast has never been eliminated from rice fields. Hence, there is a need to look for the best remedy in terms of effectiveness, sustainability, and organic nature of the method. This study was aimed at determining the plant growth-promoting and fungicidal effects of a mixture of *Piper caninum* and *Piper betle* var. Nigra leaves extracts and rhizobacteria. Gas chromatography–mass spectrophotometry (GC-MS) analysis of a mixture of leaves extracts of these plants revealed the presence of new bioactive compounds such as alpha.-gurjunene, gamma.-terpinene, and ethyl 5-formyl 3-(2-ethoxycarbonyl) in a mixture of leaves extracts of *P. caninum* and *P. betle* var. Nigra. The mixture of these extracts reduced the intensity of blast disease, inhibited *P. oryzae*, and improved the growth, yield, and quality of Bali rice. All treatments comprising of different concentrations of a mixture of leaves extracts of *P. caninum* and *P. betle* var. Nigra plus rhizobacteria exhibited biocontrol and bioefficacy. However, a 2% concentration of a mixture of these leaves extracts with plant growth-promoting rhizobacteria (PGPR) exhibited potent inhibition of growth of *P. oryzae*, a significant reduction in the intensity of blast disease, and a maximum increase in growth, yield, and quality of Bali rice. In the 15th week, the intensity of blast disease decreased from 80.18% to 7.90%. The mixture of leaves extract + PGPR also improved the

height of the plant, the number of tillers, number of leaves, number of grains per panicle, number of heads per panicle, and the full-grain weight per clump. Applications of various concentrations of a mixture of leaves extracts + PGPR resulted in improvement in the potential yield of rice, however, the application of 2% extracts + PGPR gave the highest potential yield of 5.61 tha^{-1} compared to the low yields in the control and other treatments. The high grain yield observed with the treatment was caused by the low intensity of blast disease. This treatment also strengthened the stem and prevented the drooping of the plant and improved the quality of rice grain.

Keywords: bioactive substances; botanical fungicides; biocontrol; *Pyricularia oryzae*; PGPR

1. Introduction

Rice is the principal food for the majority of the population in the world, and in Asia, it is consumed by 2.7 billion people. It is grown in almost every part of the world [1] and about 90% of the world's rice comes from the Asian continent [2]. Rice farming is one of the principal sources of income and employment for the majority of households across the world [3,4]. It is an important cereal cultivated for food security and income by marginal farmers [5]. The productivity of such an important, most widely and commonly used crop is, however, affected by various biotic stresses. Rice blast caused by *Pyricularia oryzae* or *Magnaporthe oryzae* is among the major threats to rice productivity besides brown spot sheath blight, bacterial leaf blight, and sheath rot of rice. Rice blast has been regarded as one of the most damaging diseases of rice worldwide because of its high magnitude of destructiveness [2] and danger to global food security [6]. Rice blast epidemics in various parts of the world have resulted in about 50% to 90% and under extreme cases up to 100% losses in crop yield [3] compared to 20%–50% losses caused due to sheath blight, 50%–70% yield loss caused due to brown spot disease, 25% crop losses caused by bacterial leaf blight, and 20%–80% yield losses caused due to sheath rot disease [3]. Pre-harvest and post-harvest pest infestations of rice are yet another major yield-limiting factor that causes about 33% production loss [7]. Grain discoloration also accounts for the heavy yield losses of about 18.9% [2]. Although rice productivity has improved substantially, it is not insufficient to meet the present-day global demand [8], which is estimated to be 140 million tons, i.e., almost a 50% more requirement compared to the requirement in the year 2009 [9,10]. On the other hand, the challenges to produce high quality, nutritive, and organic rice at lower costs continue all while in the presence of unforgiving and unrelenting pathogens [11].

Rice blast is a fungal disease caused by *Pyricularia oryzae* or *Magnaporthe oryzae*. The causative agent exists in diverse pathogenic forms, each carrying different virulence genes, and which frequently mutates to form new pathogen races [12]. It attacks the leaf part causing symptoms of leaf blast; it also affects the neck of the rice and causes neck blast [3]. The attack begins with the formation of spores that fall on leaves and stems and germinate to produce blast symptoms. Spores from infected plants are blown away by the wind and seed through water splash. The rainy season or the presence of water splashes increases the spread of blast spores. Low temperature (22 to 28 °C) at night, high relative humidity (>95%), extended leaf wetness period (>10 h), cloudy and drizzling weather, and high nitrogen content are other predisposing factors [13]. The progression of the disease to the leaf and stem causes empty rice grains leading to a reduction in crop yield [14,15]. Healthy plant leaves account for more plant productivity as they carry out photosynthesis that provides the pool of nutrients required for the growth of plants. If leaf growth is affected, then plant productivity is also reduced [13,15]. Red Balinese rice plants have a higher average height compared with hybrid rice and are hence more vulnerable to drooping due to infestation on the stem part and will droop before the yellowing of rice. Thus, the effect on the quality of rice will reduce rice production [15].

Different approaches have been proposed to manage the blast disease. This includes the use of disease-resistant varieties, burning of blast affected straw and stubbles, use of disease-free seeds,

split application of nitrogen, prevention of stagnating water in the field, and use of systemic chemical fungicides [13]. Although the use of chemicals to control blast fungus gave appreciable results, however, it imposed numerous demerits such as the development of resistance in the pathogen, increased production cost, and hazardous to the environment, agro-ecosystem, and humans [16]. This warrants an urgent need to search a sustainable approach for improvement in rice crops along with eco-friendly management of rice blast disease. Among the various strategies applied to manage rice blast so far, a combination of leaves extracts of piper and plant growth-promoting rhizobacteria (PGPR), such as *Azotobacter*, *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, *Allorhizobium*, *Azospirillum*, *Pseudomonas*, *Alcaligenes*, *Enterobacter*, *Stenotrophmonas*, *Acetobacter*, *Klebsiella*, *Xanthomonas*, *Erwinia*, *Caulobacter*, *Serratia*, *Arthrobacter*, *Micrococcus*, *Burkholderia*, *Flavobacterium*, *Chromobacterium*, *Agrobacterium*, *Hyphomicrobium*, and *Bacillus* sp., etc., appears as a cost-effective, sustainable, and organic method [17]. It offers numerous advantages over chemical fungicides [18–22] as they are eco-friendly, biodegradable in nature, and safe to the environment, human health, and useful soil rhizobia [19].

Piper is a member of the Piperaceae, the largest angiosperm family having about 1050 species [23]. *Piper caninum* and *Piper betle* var. Nigra of this family are known as the best source of botanical pesticides [18]. *P. caninum* is a semi-woody dioecious creeping plant attached to parent plants, such as jackfruit, coconut, etc. Its stem is blackish-red while the leaves are blackish-green. It is distributed in various parts of the world, especially in Indonesia [24,25]. It grows in places with an altitude of 600 m above sea level [12,13,20]. Leaves of the plant are rich in flavonoids, alkaloids, phenols, and steroids. *Piper betle* var. Nigra is an endemic Indonesian plant that grows in bushes; it crawls and attaches to shady plants and survives on all trees [19]. It grows in places with an altitude of 500–700 m above sea level. The leaves contain volatile oil known as betle oil and a wide variety of bioactive compounds, such as alkaloids, terpenoids, steroids, flavonoids, polyphenols, tannins, saponins, hydroxychavicol, chavicol, piperbetol, chavibetol, piperol A, methylpiperbetol, and piperol [26–30]. The metabolites of piper plants serve as organic fertilizers that support plant nutrition and their antioxidant, insecticidal, and fungicidal properties help in plant disease management [20]. The secondary metabolites of piper inhibit the growth of plant pathogens by lysis of the cell wall, breaking of the peptide, glycosidic, etc. bonds, altering their metabolism through competition for nutrients and niches [21,22,31–35].

A combination of leaf extracts of *P. caninum* and compost biofertilizer have been reported to effectively suppress the rice blast fungus *P. oryzae* Cav. and promote the growth and yield of Bali rice [1,36,37]. *P. caninum* Blume leaf extracts have been reported to exhibit antifungal activity against rice blast pathogen [38]. A mixture of leaf extracts of *P. caninum* Blume and *P. betle* var. Nigra is known to inhibit the blast fungus [39]. Although the piper leaves extracts exhibit antioxidant and antifungal properties alone or in combination, there are no reports on the plant growth promotion and biocontrol of rice blast due to the application of a mixture of leaves extracts of *P. caninum* and *Piper betle* var. Nigra and PGPR. A combination of the mixture of these two extracts and PGPR may exert more pronounced effects than the effect of a single preparation [18,37,40]. Hence the present research was aimed to evaluate the effects of a mixture of leaves extracts of *P. caninum* and *Piper betle* var. Nigra and PGPR on plant growth promotion and yield improvement in rice and a decrease in disease intensity and an increase in the degree of inhibition of *P. oryzae*.

2. Materials and Methods

2.1. Plant Source and Extraction

Piper caninum and *Piper betle* var. Nigra leaves were collected from the village of Senganan, Tabanan Regency, Bali Indonesia (longitude 115.0, latitude 8.45, and altitude 249 m above sea level). For the extraction purpose, 1 kg leaves each of *P. caninum* and *P. betle* var. Nigra were collected, air-dried for 4 days, cut it into small pieces, and blended to form a powder. A 300 g powder of each plant's leaves was separately dissolved in 3 L of 70% methanol and kept for 2 days at 8 °C. After 2 days,

each methanolic extract was filtered and methanol was evaporated within a rotary evaporator at 40 °C. A 100 g powder of crude extract of leaves of each plantwas mixed in the equal ratio (1:1) to make a composite extract [18,19].

2.2. Source of Rice Seeds, P. oryzae, and Biofertilizers

Balinese rice seeds were obtained from the local market of Bali, Indonesia. The rice blast pathogen *P. oryzae* earlier identified based on the homology of 18S rRNA gene sequences [40] was obtained from the culture repository of the Biopesticide Laboratory of Udayana University, Bali, Indonesia [11]. Liquid organic fertilizer (Suria green) was procured from the local market in Bali, Indonesia. It contained a mixture of three PGPR strains, namely, *Enterobacter cloacae*, *Bacillus subtilis*, and *Stenotrophomonas maltophilia*.

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

GC-MS analysis is a method of choice performed for the characterization or identification of active ingredients in piper [41]. Leaves extracts of these plants contain steroids, phytosterols, phenols, and saponins that exhibit antioxidant and antifungal activity [35]. The GC-MS analysis of leaves extracts of *P. caninum* and *P. betle* var. Nigra and a mixture of leaf extracts of these two plants was performed on GC-MS (Wakosil ODS/5, Germany) equipped with a C18–200 column (4.6 × 200 mm) and mass spectrophotometer. One microliter of the sample was injected with a split ratio of 10:1 and allowed to run with the flow of carrier gas (nitrogen) at1 mL/min. The oven temperature of the column was initially set to 130 °C for 2 min, increased at the rate of 30 °C/min to 250 °C, and held at this temperature for 10 min. The temperature of the injector and detector was 230 and 250 °C, respectively. The mass spectra generated from this analysis were compared with the mass spectra of standard compounds available in the mass spectra library of the National Institute of Standards and Technology (NIST), USA [37]. The peaks of the spectra were initially identified based on their matching of relative retention time (RT) with respect to the standard sample.

2.4. Research Design

This research was carried out during the rainy season (October–April) 2019 in the rice fields of the village of Senganan, Penebel, Tabanan Bali, Indonesia with randomized block design (RBD), as shown in Figure 1, with five treatments and six replicates [37]. Each treatment was provided with a buffer distance. The experimental plots were kept at a distance of 2 feet from each other. There were 30 experimental units, and each unit consisted of six plants, i.e., a total of 180 plants [37]. Rice seeds were treated as follows: F0 = control; F1 = treatment with 0.5% leaves extract + PGPR; F2 = treatment with 1% extracts + PGPR; F3 = treatment with 1.5% leaves extracts + PGPR; F4 = treatment with 2% leaves extracts + PGPR.

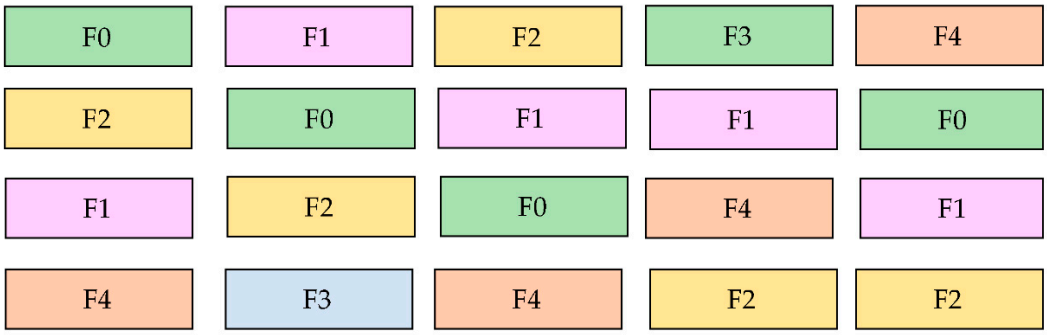

Figure 1. Experimental design to evaluate the effects of a mixture (1:1) of leaves extracts of *Piper caninum* and *Piper betle* var. Nigra in combination with plant growth-promoting rhizobacteria (PGPR) strains.

All the treatments (F1–F4) except control (F0) were sprayed with Suria liquid biofertilizer (at 1% for 5 L/ha). This liquid biofertilizer contained 6×10^{-6} cells/mL of *E. cloacae*, *B. subtilis*, and *S. maltophilia* [19].

2.5. Seeding and Transplanting

Seeds of Bali rice were soaked in water overnight and then drained, and germinated on a tray before seeding in the soil. The seedlings were watered every day for 15 days. The rice field was mixed with compost (30 kg/ha). The soil was mixed with compost when ploughing, left for a week, and ploughed again and planted with red rice plants as per the treatments mentioned above. Healthy seedlings of homogeneous size were planted in the field as per the experimental design. The field was irrigated to maintain the water level in the field at 5 cm from the soil level [19].

2.6. Application of PGPR, P. oryzae (Pathogen), and Leaves Extracts of Piper

A 1% liquid biofertilizer of PGPR containing 6×10^{-6} cells/mL of each PGPR strain, namely, *Enterobacter cloacae*, *Bacillus subtilis*, and *Stenotrophomonas maltophilia*, was sprayed evenly on the surface of rice seedlings (at 5 L/ha). After 30 days of seedling growth, 20 mL of spore suspension (25×10^{-4} spores/mL) of rice blast pathogen *P. oryzae* was sprayed. The application of pathogen was repeated four times with an interval of one week. Spraying of the pathogen was followed by the application of a composite mixture (1:1) of leaves extracts of *P. caninum* leaves and *P. betle* var. Nigra and PGPR. The desired concentration of the composite extract was made by dissolving 100 gof the crude extract of *P. caninum* leaves and *P. betle* var. Nigra leaves (ratio 1:1) in 1.0 L of sterile water and from this stock solution a concentration of 0.5%, 1%, 1.5%, and 2% were prepared and added with 1% (*w*/*v*) agister as adhesive and 5% (*v*/*v*) tween−80 [9].The control plants were sprayed with sterile distilled water.

2.7. Measurement of Disease Intensity and Plant Growth and Yield Parameters

Rice plants from test and control plots were harvested after 8, 12, and 15 weeks. Measurements were taken of disease intensity, the inhibitory activity of treatments, and plant growth parameters, such as plant height, the number of leaves, and the number of tillers, and yield components like the number of productive tillers and the number of pithed rice per panicle. The weight of pithed rice per clump, percentage of empty grain, and yield achieved were measured by weighing the rice produced, obtaining the total production, and converting the values in tha^{-1}. The leaf spot disease intensity was determined using the following formula [37]:

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

The observations were recorded as: 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).

2.8. Data Analysis

All the experiments were replicated 6 times and an average of six replicates was subjected to the analysis of variance, and Duncan's multiple range tests at a 5% level [18].

3. Results

3.1. Gas Chromatography–Mass Spectrometry (GC-MS) of Leaf Extracts

GC-MS analysis of leaf extracts of *P. caninum*, and *P. betle* var. Nigra, and a mixture of leaves extracts of *P. caninum* and *P. betle* var. Nigra yielded nine peaks of bioactive compounds, as shown in Figure 2. The extract of *P. caninum* contained benzene, xylene, tetradecane, dodecanoic acid, heptadecane, hexadecanoic acid, octadeca methyl cyclononasiloxane, phthalic acid, and docosatrienoic acid, and 1,2-benzenedicarboxylic acid.The extract of leaves of *P. betle* var. Nigra revealed the presence of alpha.-pinene, benzene, dl-Limonene, copaene, benzaldehyde, acetamide, benzoic acid,

alpha-gurjunene, and tetrasiloxane, while the mixture of these two extracts showed the presence of alpha.-pinene benzene, gamma.-terpinene, copaene, benzenemethanol, acetamide, alpha.-gurjunene, 5,8-epoxy-15-nor-labdane, and ethyl 5-formyl-3-(2-ethoxycarbonyl). The mixture contained three new compounds that were not found in the extract of either plant, as shown in Table 1. The new compounds that caused different reactions were gamma.-terpinene, alpha.-gurjunene, and ethyl 5-formyl-3(2-ethoxycarbonyl), as shown in Table 1.

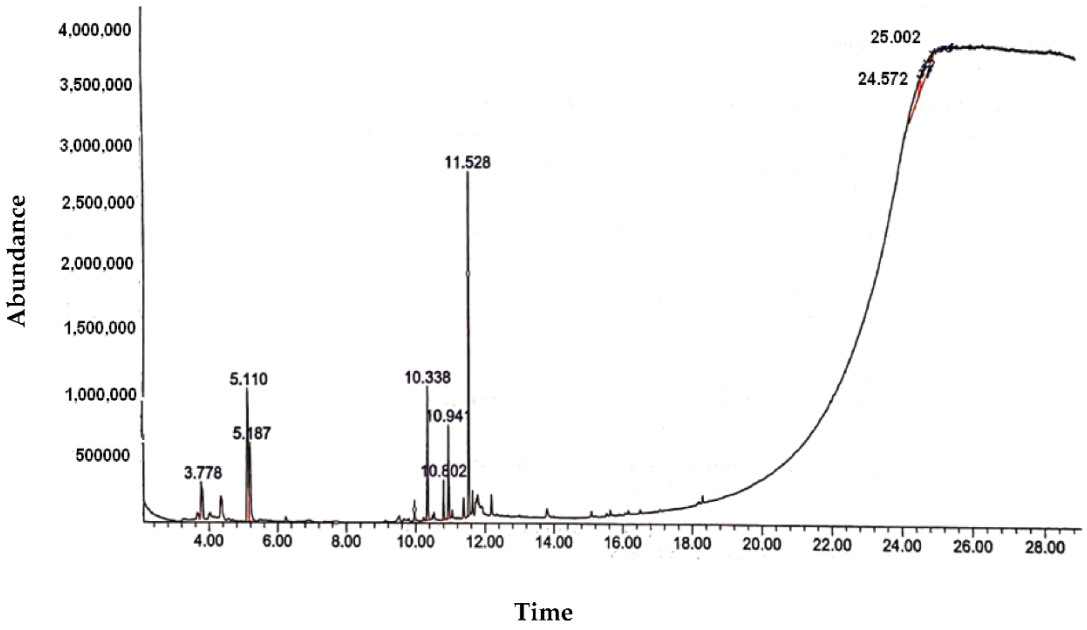

Figure 2. GC-MS spectra of a mixture of leaves extracts of piper plants indicating the presence of nine bioactive compounds. The GC-MS analysis of leaf extracts was performed on GC equipped with a C18–200 column and detector. The peaks were identified based on their retention time (RT) and comparison of mass spectra with the standards of mass spectra from the National Institute of Standards and Technology (NIST) library.

Table 1. GC-MS analysis of leaves extracts of *P. caninum* and *P. betle* var. Nigra and a mixture of leaves extracts of these two plants, indicating the number of peaks, retention time, area occupied by each peak in the chromatogram, and bioactive compounds present in each extract and a mixture of leaves extract of two plants.

Peak No.	RT (min) of Peaks			Area (%) Occupied by Peaks			Bioactive Compounds Present in the Piper Leaves Extracts		
	Pc	Pb	Mix.	Pc	Pb	Mix.	Pc	Pb	Mixture
1	3300	3791	3778	47,688	5.67	1.45	Benzene	Alpha.-pinene	Alpha.-pinene
2	3457	5115	5110	40,974	16.21	10.07	Xylene	Benzene	Benzene
3	1132	5185	5187	111,881	10.55	12.04	Tetradecane	dl-Limonene	Gamma.-terpinene
4	12,909	10.339	10.338	454,456	8.65	10.19	Dodecanoic acid	Copaene	Copaene
5	13,851	10.803	10.802	24,038	2.63	2.99	Heptadecane	Benzaldehyde	Benzenemethanol
6	17,420	10.942	10.941	36,460	5.78	7.18	Hexadecanoic acid	Acetamide	Acetamide
7	17,963	11.485	11.528	12,973	14.41	25.59	Octadecamethyl cyclononasiloxane	Benzoic acid	Alpha.-Gurjunene
8	18,426	11.529	24.572	34,013	28.91	9.27	Phthalic acid	Alpha-gurjunene	5,8-Epoxy-15nor-labdane
9	19,136	24.975	24.629	8641	4.48	2.38	8,11, 14-Docosatrienoic acid	Tetrasiloxane	Ethyl 5-formyl-3-(2-ethoxycarbonyl)
10	22,935	–	–	82,531	–	–	1,2-Benzenedicarboxylic acid	–	–

Pc = leaves extract of *P. caninum*, Pb = leaves extract of *P. betle* var. Nigra, Mix = 1:1 mixture of leaves extracts of *P. caninum* and *P. betle* var. Nigra; leaves extracts of *P. caninum*, Pb = leaves extract of *P. betle* var. Nigra were chromatogrammed on GC-MS separately and in a mixture; a 1 µL of leaf extract of each plant and mixture of both were individually injected into the C18 column of GC-MS and allowed to run with nitrogen (carrier gas) at 1 mL/min at 250 °C for 10 min. The compound present in each sample was identified based on their relative RT and comparison with mass spectra of standards available in the mass spectra library of NIST.

3.2. The Intensity of Blast Disease

Significant differences in the intensities of blast disease were observed in different treatment groups after 8, 12, and 15 weeks of applications of different preparations. Control plants that did not receive a mixture of leaf extracts and PGPR showed higher disease intensities (69.98%) and no inhibition of growth of pathogen treatments. The F4 treatment, i.e., application of 2% of composite leaves extract of two piper plants under study and PGPR significantly reduced disease intensity to 9.19% from 69.98% of the control and showed maximum inhibition of fungal growth (86.87%) compared to the control after 8 weeks, as shown in Table 2. The disease intensity further decreased from 76.43% to 8.98% and 80.18% to 7.99%, respectively, after 12 and 15 weeks of application of F4 treatment. The disease intensity and inhibitory activity of leaves extracts + PGPR were found to increase with the increasing incubation period, 88.25%, and 90.15% inhibition of fungal growth was evident on the 12th and 15th weeks, respectively. Minimum disease intensity, 8.98% and 7.90%, was evident on 12th and 15th weeks, respectively, and was after 12 and 15 weeks of application of F4 treatment, as shown in Table 2. Thus, the F4 treatment came out as an effective treatment in checking the disease intensity and inhibiting the fungal growth throughout the incubation period.

Table 2. The effect of leaves of *P. caninum* and *P. betle* var. Nigra and mixture of leaves extracts of these two plants and PGPR on percent reduction in the intensity of rice blast disease and inhibition of *Pyricularia oryzae* after 8, 12, and 15 weeks of applications of these preparations.

Treatment	% Reduction in the Intensity of Rice Blast Disease after			% Inhibitory Activity of Various Preparation against *P. oryzae* after		
	8 Weeks	12 Weeks	15 Weeks	8 Weeks	12 Weeks	15 Weeks
F0 (Control)	69.98 a*	76.43 a*	80.18 a*	0.0	0.0	0.0
F1 (0.5% extract + PGPR)	53.88 b	40.12 b	37.29 b	23.01	47.51	53.49
F2 (1% extract + PGPR)	34.29 c	23.14 c	21.20 c	51.00	69.72	73.60
F3 (1.5% extract + PGPR)	19.37 d	15.01 d	13.09 d	72.32	80.36	83.67
F4 (2.0% extract + PGPR)	9.19 e	8.98 e	7.90 e	86.87	88.25	90.15

F0 (Control) = no application of leaves extracts + PGPR; Extract = leaves of *P. caninum*, and *P. betle* var. Nigra mixed in equal ratio (1:1); values are the average of six replicates; * values followed by the same letters at the same column show no significant difference based on Duncan's multiple range test at the P = 5%; Nd = not detected. a = not significant difference compared to the control, b = significant difference compared to the control, c = significant difference with the control and with F1, d = significant difference compared to the control, F1 and F2, e = significant difference with the control, F1, F2 and F3.

3.3. Plant Growth and Yield Parameters

3.3.1. Number of Tillers

Application of a mixture of leaves extracts of *P. caninum* and *P. betle* var. Nigra and PGPR positively affected the growth and the number of tillers in Balinese rice. The number of tillers increased in all treatments compared to the control. The highest numbers of tillers (13.72) were recorded in the F4 treatment, i.e., 2% mixture of leaves extract + PGPR on week 4 compared to 8.56 tillers in the control. In the 8th and 12th week, this treatment showed further increase in the number of tillers to 13.85 and 14.11 compared to 8.99 and 9.09 of the control treatment, respectively; this treatment resulted in 4.12 more tillers compared to the control, as shown in Table 3. Applications of 0.5%, 1.0%, and 1.5% mixture of leaves extract + PGPR resulted in a less significant increase in the number of tillers on the 4th, 8th, and 12th week. The comparison of increase in the number of tillers between the control and treatment after 12 weeks was 34.48%, 48.93%, 45.83%, and 45.83% between F1 to F4, as shown in Table 3.

Table 3. The effect of inoculation of leaves extracts of *P. caninum*, and *P. betle* var. Nigra (1:1 ratio) and PGPR on plant growth and yield parameters, such as the average number of tillers, leaves, and stem height of Bali rice. Plant growth and yield parameters were measured after 4, 8, and 12 weeks of applications of these preparations.

Treatments	Number of Tillers			Number of Leaves			Stem Height (cm)		
	4 Weeks	8 Weeks	12 Weeks	4 Weeks	8 Weeks	12 Weeks	4 Weeks	8 Weeks	12 Weeks
F0 (Control)	8.56 a*	8.99 a*	9.09 a*	29.09 a*	44.90 a*	41.39 a*	28.35 a*	118.11 a*	178.97 a*
F1 (0.5% Extract + PGPR)	10.12 b	12.15 b	12.09 b	29.50 a	46.77 b	39.45 a	38.92 b	121.29 b	175.98 b
F2 (1% Extract + PGPR)	10.23 b	12.59 b	12.49 bc	33.23 b	49.86 c	43.22 b	39.02 b	122.50 b	175.67 b
F3 (1.5% Extract + PGPR)	10.51 b	13.01 b	13.00 c	38.33 c	69.80 e	62.76 c	40.59 b	122.45 b	174.87 b
F4 (2.0% Extract + PGPR)	13.72 c	13.85 b	14.11 c	42.98 d	71.51 e	65.91 d	40.61 b	121.98 b	172.99 c

F0 (Control) = no application of leaves extract + PGPR; Extract = leaf extract of *P. caninum*, and *P. betle* var. Nigra mixed in equal ratio (1:1); values are the average of six replicates; * values followed by the same letters at the same column show no significant difference based on Duncan's multiple range test at the P = 5%. a = not significant difference compared to the control, b = significant difference compared to the control, c = significant difference with the control and with F1, d = significant difference compared to the control, F1 and F2, e = significant difference compared with the control, F1, F2 and F3.

3.3.2. Number of Leaves

All the treatments produced more leaves compared to the control. An increase in the number of leaves was evident in the fourth and eighth weeks of application of a mixture of leaves extracts + PGPR. On the 12th week, the numbers of leaves were less compared to the 4th and 8th weeks. More numbers of leaves in treated plants compared to the untreated (control) plants indicated the plant growth-promoting potential of a mixture of leaves extracts + PGPR. The numbers of leaves were found to increase with an increase in the concentration of a mixture of leaves extract. The highest numbers of leaves were observed in 2% extract + PGPR treatment compared to the control. The average number of leaves increased from week 4 to week 8 and then decreased on the 12th week. The highest number of leaves (71.51) was observed in the 2% leaves extract treatment group compared to 44.90 leaves in the control on the 8th week and 42.98, 42.98, and 65.91 after the 4th and 12th weeks. The percentage comparison of the number of leaves between the control and treatment, F1, F2, F3, and F4 treatment after 12 weeks of growth was 0.15%, 4.42%, 51.63%, and 59.24%, respectively.

3.3.3. Plant Height

Red Balinese rice plants have a higher average height compared to hybrid rice and hence it is more sensitive to drooping once the grain becomes heavy and the plant may droop before the yellowing of rice. This affects the quality and hence the productivity of rice. The height of the stem was found to increase with an increase in the concentration of the mixture of leaves and growth of the crop from the 4th to 12th week. All the concentrations, except 2% leaf extracts, promoted the height of the stem compared to the control up to 8 weeks. After 12 weeks of applications of 2% mixture of extract + PGPR (F4 treatment), a short stem (172.99 cm) of rice plant was evident over the long stem (178.97 cm) obtained in the control, 0.5% extract + PGPR treatment (175.98 cm), 1.0% extract + PGPR treatment (175.67 cm), and 1.5% extract + PGPR treatment (174.87 cm), as shown in Table 3. The percentage comparison between control and F1, F2, F3, and F4 treatments after 12 weeks was 1.70%, 1.88%, 2.35%, and 3.46%, respectively.

3.3.4. Yield

The rice plants in the treated group, as shown in Figure 3a, showed a strong stalk and a bigger grain size compared to the control plant, as shown in Figure 3b. The strong stalk improved the stem strength and prevented the falling down of the plant until its full growth. The control treatment produced a tall stem with a weak stalk that fell at the age of 4 months. The highest numbers of productive tillers, i.e., 13, was observed in both F3 (1.5% extract + PGPR) and F4 treatments (2.0% extract + PGPR), compared to 8.7 tillers obtained in the control plants, as shown in Table 4. The number of productive tillers was found to increase with an increase in the concentration of leaves extract + PGPR; however, 2% of leaf extract + PGPR yielded more tillers. The application of leaf extract + PGPR also yielded more numbers of grains per panicle. Plants treated with 0.5% and 1.0% leaf extracts + PGPR, i.e., F1 and F2 treatments, produced 16.01 and 19.34 more grains per panicle, respectively, compared to the control plants. Meanwhile, the application of 1.5% leaves extract + PGPR (F3) and 2% leaf extract + PGPR (F4) treatments yielded the highest numbers of grains per panicle compared to the control and other treatments. These treatments yielded 23.10 and 23.13 more numbers of grains per panicle compared to the control. The highest full grain weight per clump (81.20 g) was observed in the F4 treatment (2% extract + PGPR) compared to the lowest full grain weight (41.56 g) obtained in the control, F1 (53.98 g), F2 (61.76 g), and F3 treatments (75.98 g). Thus, the higher concentration of leaves extracts of piper with PGPR resulted in full-grain weight per clump. Application of 1.5% (F3 treatment) and 2.0% extract + PGPR (F4 treatment) helped in reducing the percentage of empty grains per clump from 12.03% (control) to 2.45% and 2.41%, respectively, compared to more numbers of empty grains per clump in the control and other treatments. Thus, the application of higher concentration (1.5% and

2%) of a mixture of leaves extract of two piper plants and PGPR gave a minimum number of empty grains per clump, as shown in Table 4, and also increased the yield components.

(**a**) (**b**)

Figure 3. (**a**) Inoculation of a 1:1 mixture of leaves extracts of piper and PGPR produced strong stems and plants' stalks remained strong and did not fall even after 4 months; (**b**) the stem of control plants (no application of a mixture of leaves extracts of piper and PGPR) remained weak and fell before maturation, i.e., 4 months.

Increase in Yield and Quality of Rice

All the treatments resulted in more yield compared to the control, however, the F4 treatment (2% extract + PGPR) resulted in the highest potential yield among the other treatments, as it produced 5.61 t/ha of rice compared to 3.23 t/ha, 3.81 t/ha, 4.2 t/ha, and 5.49 t/ha obtained from the control, F1, F2, and F3 treatments, respectively. The potential yield of rice was found to increase with an increase in the concentration of leaves extracts. Application of higher (2%) concentration of leaves extracts + PGPR produced 70% more rice yield compared to the untreated plant (control).

Application of a mixture of leaves extracts of piper and PGPR also promoted the quality of rice grains in terms of the size. The plants treated with leaf extracts + PGPR produced bigger grains with more width and more diameters compared to the control. The increase in the concentration of leaves extracts + PGPR increased the grain size. Application of higher (2%) concentration of leaves extract + PGPR produced the grain of maximum length (0.79 mm), maximum width (0.31 mm), and larger diameter (0.43 mm) compared to the control (0.51 × 0.20 × 0.30 mm), as shown in Table 4. Applications of 0.5%, 1.0%, and 1.5% leaves extract + PGPR resulted in a grain size of 0.59 × 0.23 × 0.35 mm, 0.61 × 0.25 × 0.35 mm, and 0.68 × 0.29 × 0.38 mm size, as shown in Table 4. Thus, the application of 2% leaves extracts of *P. caninum* and *P. betle* var. Nigra (1:1) and PGPR not only increased the yield and yield parameters of rice but also improved the quality of the rice grain. Rice grains with improved quality attract the consumers and may give more profit to the growers.

Table 4. The effect of inoculation of various contractions of a mixture of leaves extracts of *P. caninum* and *P. betle* var. Nigra (1:1 ratio) and PGPR on yield components, such as number of productive tillers, number of grains per panicle, full-grain weight per clump reduction in the number of empty grains per clump, yield of rice, and quality parameters such as length, width, and diameter of rice grain. Plants were harvested after 4 months, dried for 3 days, and subjected to the measurements of yield parameters, yield, and quality of treated rice compared to the control rice plants.

Treatment	No. of Productive Tillers	No. of Grain/Panicle	Full-Grain Weight/Clump (g)	Empty Grain/Clump (%)	Potential Yield (t/ha)	% Yield Increase vis-a-vis Control	Rice Quality (mm)		
							Length	Wide	Diameter
F0 (Control)	8.7 a*	219.09 a*	41.56 a*	12.03 a*	3.23 a*	-	0.51 a*	0.20 a*	0.30 a*
F1 (0.5% extract + PGPR)	11.04 b	235.10 b	53.98 b	9.01 b	3.81 a	17.97	0.59 b	0.23 b	0.35 b
F2 (1% extract + PGPR)	12.87 c	238.43 c	61.76 c	6.91 c	4.24 b	31.27	0.61 b	0.25 b	0.35 b
F3 (1.5% extract + PGPR)	13.00 c	242.19 d	75.98 d	2.45 d	5.49 c	69.97	0.68 c	0.29 c	0.38 c
F4 (2.0% extract + PGPR)	13.00 c	242.22 d	81.20 e	2.41 d	5.61 c	70	0.79 d	0.31 c	0.43 d

F0 (Control) = no application of leaf extract + PGPR; Extract = leaves extract of *P. caninum*, and *P. betle* var. Nigra mixed in equal ratio (1:1); values are the average of six replicates; * values followed by the same letters at the same column show no significant difference based on Duncan's multiple range test at the P = 5%. a = not significant difference compared to the control, b = significant difference compared to the control, c = significant difference with the control and with F1, d = significant difference with the control, F1 and F2, e = significant difference compared to the control, F1, F2 and F3.

4. Discussion

GC-MS analysis of a mixture of *P. caninum* and *P. betle* var. Nigra revealed the presence of new bioactive compounds such as alpha.-Gurjunene, gamma.-terpinene, and ethyl 5-formyl3-(2-ethoxycarbonyl) [42]. The formation of these new compounds could be due to the mixing of various components of both the extracts. The presence of characteristic functional groups, retention time, and the properties of these bioactive compounds resembled those of standard compounds [43].

The inhibition of rice blast pathogen *P. oryzae* following the application of leaves extracts of *P. caninum* and *P. betle* var. Nigra and PGPR is due to the presence of antifungal components in the mixture of leaf extracts and antifungal activity of PGPR strains. Potent antifungal activity of a mixture of leaves extracts of piper plants is due to the combined effects of antifungal compounds present in piper and antifungal metabolites produced by PGPR strains. Mixing of extracts of two piper plants resulted in the formation of a new compound that might have exerted more antifungal activity than the antifungal activity of compounds present in the single extract of either plant.

The active antifungal metabolite, such as terpinene, present in the leaves extracts of piper has been reported to inhibit the growth of many pathogens including *Enterococcus faecalis*, *Rhizomucor miehei*, and *Candida glabrata* [44–48]. The essential oils present in *P. caninum* are known for their antioxidant and antimicrobial properties [49–51]. The leafextract of *P. caninum* is known for its antifungal potential against rice spotting disease caused by *Curvularia verruculosa* [38,52]. However, a mixture of leaf extracts of *P. caninum* and *P. betle* var. Nigra leaf extracts, i.e., a mixture of compounds, exhibited a more inhibitory effect against rice blast pathogens.

A mixture of leaves extracts of *Mansoa alliacea* L. and *Allamanda cathartica* L. has been reported to exhibit greater inhibitory effects than a single extract against the peanut sprout fungus *Athelia rolfsii* [8]. New compounds, i.e., hexadecanoic acid, formed after mixing leaf extracts of *P. caninum* and is known for its antifungal activity [39,53]. Formulation of a mixture of biopesticide preparation of *Beauveria bassiana* and *Azadirachta indica* has been found as a more potent biocontrol agent than a single preparation [38]. A decrease in the attack of blast disease results in the healthy growth of rice plants. Inhibition of growth of *P. oryzae*, as well as growth promotion in rice, is due to antifungal phytochemicals that can suppress blast disease and the presence of hormones that stimulate plant growth [38,54]. The mixture of *P. caninum* and *P. betle* extracts contains phytochemicals that can inhibit *Pyricularia oryzae* by damaging its cell wall, causing the cell fluid to leak out, and resulting in the damage and cell lysis.

P. caninum leaf extract is known to suppress blast disease and increase the number of tillers of certain rice hybrids [55]. The inhibition of the fungal pathogen may also be due to the antagonistic activity of *B. subtilis* present in liquid biofertilizer. Members of the *Bacillus* sp. exert biocontrol activity against a wide range of plant pathogens [9,38]. Chen et al. [20] reported effective control of *Bacillus subtilis 5*, *B. cereus 3S5*, and other PGPR against rice blast in rice cultivar UPLRi-5. They found 31% more inhibition of intensity of *M. oryzae* infestation compared to the control. Kumar [56,57] reported the efficacy of *B. subtilis* MBI 600 in the effective management of sheath blight of rice. *E. cloacae* has been reported to produce various antifungal metabolites, such as antibiotics [36,58], hydrogen cyanide [59–62], siderophore [63–65], and many hydrolytic enzymes [66–69]. Sayyed et al. [70] reported plant growth-promoting effects of exopolysaccharide producing *Enterobacter* sp. RZS5. Suprapta et al. [71] reported the potential of various species of *Enterobacter agglomerans* and other rhizobacteria in reducing the rice blast intensity. They claimed more than 50% reduction in the intensity of rice blast in plants treated with *Enterobacter agglomerans* and other rhizobacteria. They further claimed *E. agglomerans* Gg14D as one of the promising biocontrol agents (BCAs) to manage rice blast disease.

Significant increase in the number of tillers following the application of a mixture of leaves extracts of *P. caninum* and *P. betle* var. Nigra is due to the reason that this mixture can eliminate *P. oryzae* that causes rice blast disease and make the rice crop healthy [71]. Reduction in the numbers of empty grains is because of the inhibition of blast pathogen following the applications of a mixture of leaves extract

of piper plant. The high grain yield obtained with the treatments was caused by the low intensity of blast disease, which causes empty grains [40].

One of the problems in rice agriculture is fungal diseases, such as the blast disease caused by *P. oryzae*, which results in up to 90% loss in rice yield [42]. Rice blast caused by *P. oryzae* results in empty grains and the severity of the disease leads to a higher percentage of empty grains. A decrease in the number of empty grains following the application of a mixture of the two extracts is due to the inhibition of the growth of pathogen that otherwise produces more empty grains [1,11,14].

A decrease in the number of leaves at 2% concentration of leaf extracts may be due to the toxic effects of this high concentration of the preparation that caused endurance of the plant and increased the intensity of blast disease [1]. Suriani et al. [38] observed that the use of the leaves extract of *P. caninum* above optimal concentration exhibits toxic effects on both the growth and crop yield and they correlated this effect with the toxic nature of many active components present in the extract. A decrease in the number of leaves may also result from the aging that causes shading of leaves.

The grain yield (t/ha) mostly depends on the intensity of blast disease. The higher the intensity of the disease, the more will be the formation of empty grains that fail the harvest. Since rice quality depends considerably on grain size [38], the application of a mixture of *P. caninum* and *P. betle* can be an effective method to improve the quality of Bali rice. In earlier studies [38], the application of a single extract of *P. caninum* was found to decrease blast disease intensity (41.22%–8.10%) and improved grain yield (3.95–5.0 t/ha), whereas the application of *P. betle* var. Nigra leaves extract resulted in a decrease in disease intensity (42.40%–9.65%) and more improvement in grain yield (3.81–4.90 t/ ha) [71].

An increase in the yield of rice due to various treatments is likely due to the addition of liquid biofertilizer that contained different strains of PGPR, such as *Enterobacter cloacae*, *Bacillus subtilis*, and *Stenotrophomonas maltophilia*. This microbial consortium stimulates plant growth, as well as exhibits biocontrol activity, protecting plants from disease, and thus improving crop yield [55]. Kumar [56,57] reported the increase in rice yield following the inoculation of Integral®, a commercial biofertilizer preparation that contained *B. subtilis* MBI 600. Pooja and Katoch [58] claimed the effective management of blast disease. Sagar et al. [60] reported stimulation of seed germination and an improvement in growth parameters of rice var. Sahbhagi due to the inoculation of *Enterobacter cloacae*. Sagar et al. [61,62] found ACC deaminase positive *Enterobacter* sp. PR14 promotes the growth of rice. Multifarious PGPR has been reported to promote plant growth and inhibit fungal phytopathogens [63–71]. Spence et al. [72] reported biocontrol activity of rhizospheric bacteria against the foliar rice fungal pathogen, *M. oryzae* pathovar 70–15. This BCA exhibited antibiosis through the production of hydrogen cyanide and the induction of systemic resistance in rice. Suriani et al. [38,73] observed effective suppression (57.17%) of blast disease and improvement in crop yield of rice following the application of a mixture of 2% leaves extract of *P. caninum* Bl. leaves extract and 40% compost.

5. Conclusions

Plant extracts are one of the eco-friendly approaches for an organic (chemical-free) management of plant diseases. They also serve as the best source for plant nutrition for plants and hence support organic agriculture. Although each botanical fungicide possesses plant growth-promoting potential and antifungal activities, a combination of two extracts may increase their antifungal potential by many folds. The mixing of extracts of two plants results in the formation of newer compounds that otherwise are absent in a single extract. These new compounds promote more plant growth and more antifungal activity. The significant reduction of blast disease due to the application of a mixture of piper leaves extracts and PGPR improved the growth and yield parameters. The addition of PGPR in these extracts increases their bioefficacy in many folds. However, more and continuous field studies under different cropping seasons and in different agro-climatic zones are needed to establish the bioefficacy and biocontrol potential of a combination of piper leaves extracts and PGPR.

Author Contributions: Conceptualization and writing original draft, N.L.S.; Data Analysis, N.W.S.; Review and editing, N.N.; Supervision, D.N.S.; Methodology, N.M.S.P. and A.A.K.D.; Analysis and Project Administration, D.A.D.; Reviewing and editing, A.F., R.Z.S., H.A.E.E., D.J.D. and A.S.; Fund acquisition; A.M.E., A.H.B. and H.A.E.E. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by LPPM Udayana University, Bali, Indonesia through UNUD INNOVATION grants number: B/1588-58/UN14.4.A/PT.01.03/2020, the Researchers Supporting Project number RSP-2020/15, King Saud University, Riyadh, Saudi Arabia, the Research Project number, Allcosmos Industries Sdn. Bhd. through research project No. R.J130000.7344.4B200 and UTM-TNCPI research fund for partial support of the APC.

Acknowledgments: The authors extend their appreciation to LPPM UNUD through UNUD INNOVATION grants. The authors extend their appreciation to the Researchers supporting project number (RSP-2020/15) King Saud University, Riyadh, Saudi Arabia. Laboratory of Biopesticide and Department of Biology, Udayana University, Bali, Indonesia, and Allcosmos Industries Sdn. Bhd. Johor Bahru, Johor, Malaysia.

Conflicts of Interest: The authors declare no conflict of interest. All the authors have read and approved the manuscript for submission in *Sustainability*.

References

1. Suriani, N.L. Bioactive substance use of leaf extract of *Piper caninum* blume pressing for blast disease and increase production in rice. *Int. J. Life Sci.* **2018**, *2*, 42–50. [CrossRef]
2. Fahad, S.; Adnan, M.; Noor, M.; Arif, M.; Alam, M.; Khan, I.A.; Ullah, H.; Wahid, F.; Mian, I.A.; Jamal, Y.; et al. Major Constraints for Global Rice Production. In *Advances in Rice Research for Abiotic Stress Tolerance*; Hasanuzzaman, M., Fujita, M., Nahar, K., Biswas, J.K., Eds.; Woodhead Publishing: Cambridge, UK, 2019; pp. 1–22. [CrossRef]
3. Baite, M.S.; Raghu, S.; Prabhukarthikeyan, S.R.; Keerthana, U.; Jambhulkar, N.N.; Rat, P.C. Disease incidence, and yield loss in rice due to grain discoloration. *J. Plant Dis. Prot.* **2020**, *127*, 9–13. [CrossRef]
4. Darsono, N.; Yoon, D.H.; Raju, K. Effects of the sintering conditions on the structural phase evolution and T C of Bi1.6 Pb0.4 Sr2 Ca_2 Cu_3 O_7 prepared using the citrate sol-gel method. *J. Supercond. Nov. Magn.* **2016**, *29*, 1491–1497. [CrossRef]
5. McCouch, S.R.; Wright, M.H.; Tung, C.W.; Maron, L.G.; McNally, K.L.; Fitzgerald, M.; Singh, N.; DeClerck, G.; Agosto-Perez, F.; Korniliev, P. Open access resources for genome-wide association mapping in rice. *Nat. Commun.* **2016**, *7*, 10–532. [CrossRef] [PubMed]
6. Nalley, L.; Tsiboe, F.; Durand-Morat, A.; Shew, A.; Thoma, G. Economic and environmental impact of rice blast pathogen (*Magnaporthe oryzae*) alleviation in the United States. *PLoS ONE* **2016**, *11*, e0167295. [CrossRef]
7. DWR. *Vision 2050*; Directorate of Weed Research, Indian Council of Agricultural Research: New Delhi, India, 2015; pp. 1–2.
8. Thirze, H. Modelling Grain Surplus and Deficit in Cameroon for 2030. Master's Thesis, Lund University, Lund, Sweden, 2016; p. 59.
9. Aziiba, E.A.; Qiang, C.; Jeffrey, A.C. Rice blast: A disease with implications for global food security. *Agronomy* **2019**, *9*, 451. [CrossRef]
10. Prasad, R.; Shivay, Y.S.; Kumar, D. Current status, challenges, and opportunities in rice production. In *Rice Production Worldwide*; Chauhan, B., Jabran, K., Mahajan, G., Eds.; Springer: Cham, Switzerland, 2017; pp. 1–32. [CrossRef]
11. Matuzahroh, J.N.; Zuraidassanaaz, N.I.; Sulistyorini, L. Antifungal and antibacterial activity of black betel (*Piper betle* L. var Nigra) extract. *Biosci. Res.* **2017**, *4*, 750–755.
12. Kuyek, D.B. Biotech, and Big Business: Implications of Corporate Strategies in Rice Research in Asia. Agricultural, R. & D. Available online: https://www.grain.org/article/entries/36-blast-biotech-andbigbusiness-implications-of-corporate-strategies-on-rice-research-in-asia (accessed on 1 March 2019).
13. Leung, H. Disease- and Pest-Resistant Rice. Available online: http://irri.org/ourwork/research/betterrice-varieties/disease-and-pest-resistant-rice (accessed on 1 March 2019).
14. Kihorol, J.; Boscol, N.; Murage, H.; Ateka, E.; Makihara, D. Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. *Springer Plus* **2013**, *2*, 308. [CrossRef]
15. Management of Rice Blast. Available online: http://agropedia.iitk.ac.in/content/management-rice-blast (accessed on 20 September 2020).

16. Kunova, A.; Pizzatti, C.; Bonaldi, M.; Cortesi, P. Sensitivity of nonexposed and exposed populations of (*Magnaporthe oryzae*) from rice to tricyclazole and azoxystrobin. *Plant Dis.* **2014**, *98*, 512–518. [CrossRef]
17. Ribeiro, A.F.A.; de Morais, S.M.; Nogueira, S.A.C.; da Silva, I.N.G.; Galvão, M.C.; de Sousa, S.A.A.; dos Santos, F.R.O. Chemical composition, antioxidant and antifungal activities of essential oils and extracts from *Plectranthus* sp. against dermatophytes fungi. *Bras. Saúde Prod. Anim. Salvador* **2018**, *19*, 105–115.
18. Ngurah, D.S.; Khamdan, K. The research report. In *Development of Bio-Agent to Control the Blast Disease, Promote Growth, and Increase the Yield of Rice*; Faculty of Agriculture, Udayana University: Bali, Indonesia, 2012; p. 50, (In Indonesia Language).
19. Suriani, N.L.; Dewa, N.S.; Khamdan, K.; Anak, A.K.D.; Ni, M.S.P.; Novizar, N.; Ahmad, Z.B.Z.; Bohari, M.Y. Antagonism *Trichoderma* sp. for pressing blast disease on red Bali rice plants (*Oryza sativa*). *J. Adv. Res. Dyn. Control Syst.* **2019**, *11*, 70–76. [CrossRef]
20. Chen, W.E.; Chiou, T.Y.; Aileen, L.D.; Chien-Sen, L. The control of rice blast disease by the novel biofungicide formulations. *Sustainability* **2019**, *11*, 3449. [CrossRef]
21. Sayyed, R.Z.; Ilyas, N.; Tabassum, B.; Hashem, A.; Abd_Allah, E.F.; Jadhav, H.P. Plausible role of plant growth-promoting rhizobacteria in future climatic scenario. In *Environmental Biotechnology: For Sustainable Future*; Sobti, R.C., Arora, N.K., Kothari, R., Eds.; Springer Nature: Singapore, 2019; pp. 175–197. [CrossRef]
22. Zope, V.P.; Enshasy, H.E.; Sayyed, R.Z. Plant growth promoting rhizobacteria: An overview in agricultural perspectives. In *Plant Growth Promoting Rhizobacteria for Sustainable Stress Management, Rhizobacteria in Biotic Stress Management*; Sayyed, R.Z., Ed.; Springer: Singapore, 2019; Volume II, pp. 345–362. [CrossRef]
23. Suwanphakdee, C.; Simpson, D.A.; Hodkinson, T.; Chantaranothai, P. Taxonomic notes on the genus Piper (*Piperaceae*). *Nordic J. Bot.* **2016**, 1–14. [CrossRef]
24. Moeljanto, R.D.; Mulyono, S. *Khasiatdan Manfaat Daun Sirih Obat Mujarabdari Masake Masa*, 1st ed.; Agromedia Pustaka: Jakarta, Indonesia, 2004.
25. Kumar, N.; Misra, P.; Dube, A.; Bhattacharya, S.; Dikshit, M.; Ranade, S. *Piper betle* L. a maligned pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery. *Curr. Sci.* **2009**, *99*, 922–932.
26. Gundala, S.R.; Aneja, R. Piper betel leaf: A reservoir of potential xenohormetic nutraceuticals with cancer-fighting properties. *Cancer Prev. Res.* **2014**, *7*, 477–486. [CrossRef]
27. Jaiswal, S.G.; Patel, M.; Saxena, D.K.; Naik, S.N. Antioxidant properties of *Piper betle* (L.) leaf extracts from six different geographical domain of India. *J. Biores. Eng. Technol.* **2014**, *2*, 12–20.
28. Nouri, L.; Nafchi, A.M. Antibacterial, mechanical, and barrier properties of sago starch film incorporated with betel leaves extract. *Int. J. Biol. Macromol.* **2014**, *66*, 254–259. [CrossRef]
29. Kumari, O.S.; Rao, N.B. Phyto-chemical analysis of *Piper betle* leaf extract. *World J. Pharm. Pharm. Sci.* **2015**, *4*, 699–703.
30. Widawati, M.; Randi, M.U. Study of herbal topical repellent made of betel leaves (*Piper betle*) and patchouli oil (*Pogostem oncablin*) mixture against yellow fever mosquito (*Aedes aegypti*). *Biotrop* **2015**, *22*, 45–51.
31. Shaikh, S.S.; Sayyed, R.Z. Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. In *Plant Microbes Symbiosis: Applied Facets*; Arora, N.K., Ed.; Springer: Singapore, 2015; pp. 337–351. [CrossRef]
32. Shaikh, S.S.; Reddy, M.S.; Sayyed, R.Z. Plant growth-promoting rhizobacteria: An eco-friendly approach for sustainable agroecosystem. In *Plant Soil-Microbes*; Hakeem, K., Akhtar, M., Abdullah, S., Eds.; Springer: Cham, Switzerland, 2016; pp. 182–201. [CrossRef]
33. Jadhav, H.P.; Shaikh, S.S.; Sayyed, R.Z. Role of hydrolytic enzymes of rhizoflora in biocontrol of fungal phytopathogens: An Overview. In *Rhizotrophs: Plant Growth Promotion to Bioremediation*; Mehnaz, S., Ed.; Springer-Nature: Singapore, 2017; pp. 183–203. [CrossRef]
34. Ahmed, K.Z.; Sayyed, R.Z.; Hesham, A.E. Biosynthesis of antibiotics by PGPR and their roles in biocontrol of plant diseases. In *Plant Growth Promoting Rhizobacteria for Sustainable Stress Management. Rhizobacteria in Biotic Stress Management*; Sayyed, R.Z., Ed.; Springer: Singapore, 2019; Volume 2, pp. 1–36. [CrossRef]
35. Chayanika, S.; Prasad, R.; Sawinder, K.; Jyoti, S.; Joginder, S.; Yogesh, G.; Umar, G.; Damanpreet, K.; Kajal, D. Antioxidant and antimicrobial potential of selected varieties of *Piper betle* L. (Betel leaf). *An. Acad. Bras. Cienc.* **2018**, *90*, 3871–3878. [CrossRef]
36. Ni, L.S.; Dewa, N.S.I.; Made, S.; Rai, M.T. Antifungal activity of *Piper caninum* against *Pyricularia oryzae* cav. the cause of rice blast disease on rice. *J. Biol. Agri. Healthcare* **2015**, *5*, 72–79.

37. Suriani, N.L. *Piper caninum* blume leaf extract and compost to suppress blast disease and increase the production of Bali red rice (*Oryza sativa*) in green-house. *Int. Res. J. Eng. IT Sci. Res.* **2019**, *5*, 46–54. [CrossRef]
38. Suriani, N.L.; Darmadi, A.A.K.; Parwayoni, N.M.S.; Bohari, M.Y. A combination of *Piper caninum* Blume leaf extracts and compost fertilizer for pressing blast disease and improving the growth of Bali red rice (*Oryza sativa* Linn) in the field. *Int. J. Adv. Sci. Eng. Info. Technol.* **2020**, *9*, 518–525. [CrossRef]
39. Suriani, N.L.; Suprapta, D.N.; Khalimi, K.; Darmadi, A.A.K.; Parwanayoni, N.M.S. The combination of *Piper caninum* Blume and *Piper Betle* Var. Nigra leaf extract to press blast disease on *Oryza sativa* linn in vitro. *J. Res. Lepidoptera* **2019**, *50*, 196–204. [CrossRef]
40. Parwanayoni, N.M.S.; Suprapta, D.N.; Temaja, I.G.R.M.; Swantara, I.M.D.; Khalimi, K. Synergistic activity of leaves extracts of *Mansoaalliacea,* L. and *Allamanda cathartica* L. to inhibit *Athelia rolfsii*, the cause of stem rot disease in peanut plants. *J. Biol. Agric. Healthcare* **2018**, *8*, 29–35.
41. Kumar, B.K.; Nayak, C.; Mehta, B.K. Gas chromatography massspectrometry GC-MS analysis of the hexane and benzene extracts of the *Piper betle* leaf stalk Family *Piperaceae* from India. *J. Med. Plant. Res.* **2010**, *4*, 2252–2255. [CrossRef]
42. Suliansyah, I.; Yusniwati, D.; Dwipa, I. Genetic diversity, and association amongst west Sumatra brown rice genotype based on morphological and molecular markers. *IJASEIT* **2018**, *8*, 610–615. [CrossRef]
43. Appuaka, A.; Ekwenchi, M.M.; Adashak, D.A.D. Biological activities of characterized isolates of H-Hexane extract of *Azadirachta indica* A. Jus (Neem) leaves. *J. Nat. Sci.* **2013**, *11*, 171–178.
44. Rehman, A.U.; Gul, S.; Abbasi, M.A.; Nafeesa, K.; Siddiqa, A.; Khan, K.M.; Shahid, M.; Subhani, Z.N. Substituted derivatives of 5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl-2-sulfanyl acetamide as valuable bioactive compounds. *J. Chem. Soc. Pak.* **2014**, *36*, 503–511.
45. Simionatto, E.; Vanderlea, F.L.B.; Peres, M.T.L.P.; Hess, C.S.; Candido, A.C.S.; Diraimo, D.L.; Poppi, N.R.; Matos, M.D.C.F.; Santos, E.C.S.; Oguma, P.M.; et al. Bioactivity and chemical composition of the essential oils of *Croton urucurana* Baill on (*Euphorbiaceae*). *JEOBP* **2009**, *12*, 250–261. [CrossRef]
46. Ubaid, J.M.; Husein, H.M.; Hameed, I.H. Determination of bioactive chemical composition of *Callosobruchus maculutus* and investigation of its anti-fungal activity. *Int. J. Pharmacog. Phytochem. Res.* **2016**, *8*, 1293–1299.
47. Helen, M.P.A.; Vargheese, T.A.; Kumari, J.J.J.; Abiramy, M.R.; Sajina, N.; Sree, J.S. Phytochemical analysis and anticancer activity of essential oil from *Myristica fragrans*. *Int. J. Curr. Pharma. Rev. Res.* **2011**, *2*, 188–198.
48. Salleh, W.M.N.H.W.; Ahmad, F.; Yen, K.H. Chemical constituents from *Piper caninum* and antibacterial activity. *J. Appl. Pharma. Sci.* **2015**, *5*, 20–25. [CrossRef]
49. Kumar, S.; Lal, A.A.; Kumar, N.; Jaiswal, S.; Kumar, H.; Kumar, A.; Kumar, M. Effect of biocontrol agents and botanicals against blast of paddy caused by *Pyricularia oryzae*. *Int. J. Chem. Stud.* **2017**, *5*, 314–318.
50. Salleh, W.M.N.H.W.; Ahmad, F.; Yen, K.H.; Sirat, H.M. Chemical compositions, antioxidant and antimicrobial activities of essential oils of *Piper caninum* Blume. *Int. J. Mol. Sci.* **2011**, *12*, 7720–7731. [CrossRef] [PubMed]
51. Wang, J.; Guo, X.; Ling, L.; Qiu, H.; Zhang, Z.; Wang, Y.; Sun, G. Application of the fluorescent dye bodypy in the study of lipid dynamics of the rice blast fungus *Magnaporthe oryzae*. *Molecules* **2018**, *23*, 1594. [CrossRef]
52. Suriani, N.L.; Suprapta, D.N.; Nazir, N.; Darmadi, A.A.K.; Parwayoni, N.M.S.; Sudatri, N.W.; Yamin, M.B. *Caninum* leaf extracts against *Curvularia* spotting disease on rice plants. *Indian J. Agric. Res.* **2020**, *54*, 411–419.
53. Junairiah, J.; Zuraidassanaaz, N.I.; Sulistyorini, L. Isolation and identification of secondary metabolites of black betel (*Piper betle* L. varNigra). *J. Kimia Riset.* **2019**, *3*, 131–138. [CrossRef]
54. Ratnasari, A.M.T. The formulation of biopesticides combination (Splt MNPV Virus, *Beauveria bassiana*, and neem seed (*Azadirachta indica* A. Juss.) as biocontrol agents against soybean pests. *Adv. Sci. Lett.* **2017**, *23*, 11962–11966. [CrossRef]
55. Chandrasekaran, M.; Chun, C.; Wook, J.; Paramasivan, M.; Saini, R.K.; Joseph, J.S. *Bacillus subtilis* CBR05 for tomato (*Solanum lycopersicum*) fruits in South Korea as a novel plant probiotic bacterium (PPB): Implications from total phenolics, flavonoids, and carotenoids content for fruit quality. *Agronomy* **2019**, *9*, 838. [CrossRef]
56. Vijay, K.K.K.; Yellareddygari, S.K.R.; Reddy, M.S.; Kloepper, J.W.; Lawrence, K.S.; Zhou, X.G.; Sudini, H.; Groth, D.E.; Raju, S.K.; Miller, M.E. Efficacy of *Bacillus subtilis* MBI 600 against sheath blight caused by *Rhizoctonia solani* and on growth and yield of rice. *Rice Sci.* **2012**, *19*, 55–63. [CrossRef]

57. Kumar, K.V.K.; Sudhakar, P.; Yellareddygari, S.K.R.; Kloepper, J.W.; Sudini, H.E.; Surendranatha, C.K.S.R.; Lawrence, K.S.; Podile, A.R.; Suresh, K.; Sruthi, V.S.; et al. Efficacy of Integral® (*Bacillus subtilis* MBI 600) in sheath blight management and yield enhancement of rice. In Proceedings of the 3rd Asian PGPR Conference, Manila, Philippines, 21–24 April 2013; Reddy, M.S., Sayyed, R.Z., Ilao, R.O., Sudini, H.K., Faylon, P.S., Kumar, K.V.K., Dar, W.D., Armanda, A.B., Batchelor, W.D., Gopalkrishnan, S., Eds.; Cambridge Scholars Press: London, UK, 2014; pp. 431–436.
58. Pooja, K.; Katoch, A. Past, present, and future of rice blast management. *Plant Sci. Today* **2014**, *13*, 1651–1673. [CrossRef]
59. Shaikh, S.S.; Patel, P.R.; Patel, S.S.; Nikam, S.D.; Rane, T.U.; Sayyed, R.Z. Production of biocontrol traits by banana field fluorescent pseudomonads and comparison with chemical fungicide. *Indian J. Exp. Biol.* **2014**, *52*, 917–920. [PubMed]
60. Sagar, A.; Shukla, P.K.; Sayyed, R.Z.; Ramteke, P.W. Stimulation of seed germination and growth parameters of rice var. Sahbhagi by *Enterobacter cloacae* in presence of ammonia sulphate as substitute of ACC. In *PGPR: Prospects for Sustainable Agriculture*; Springer-Nature: Singapore, 2019; pp. 117–124. [CrossRef]
61. Sagar, A.; Riyazuddin, R.; Shukla, P.K.; Ramteke, P.W.; Sayyed, R.Z. Heavy metal stresses tolerance in *Enterobacter* sp. PR14 is mediated by plasmid. *Indian J. Exp. Biol.* **2020**, *58*, 115–121.
62. Sagar, A.; Sayyed, R.Z.; Ramteke, P.W.; Sharma, S.; Marraiki, N.; Elgorban, A.M.; Syed, A. ACC deaminase and antioxidant enzymes producing halophilic *Enterobacter* sp. PR14 promotes the growth of rice and millets under salinity stress. *Physiol. Mol. Biol. Plants* **2020**. [CrossRef]
63. Sayyed, R.Z.; Seifi, S.; Patel, P.R.; Shaikh, S.S.; Jadhav, H.P.; Enshasy, H.A.E. Siderophore production in groundnut rhizosphere isolate, *Achromobacter* sp. RZS2 influenced by physicochemical factors and metal ions. *Environ. Sustain.* **2019**, *1*, 295–301. [CrossRef]
64. Wani, S.J.; Shaikh, S.S.; Sayyed, R.Z. Statistical-based optimization and scale-up of the siderophore production process on laboratory bioreactor. *3 Biotech* **2016**, *6*. [CrossRef]
65. Shaikh, S.S.; Wani, S.J.; Sayyed, R.Z. Impact of interactions between rhizosphere and rhizobacteria: A review. *Bacteriol. Mycol.* **2018**, *5*, 1058.
66. Shaikh, S.S.; Wani, S.J.; Sayyed, R.Z.; Gulati, T.A. Production, purification, and kinetics of chitinase of *Stenotrophomonas maltophilia* isolated from rhizospheric soil. *Indian J. Exp. Biol.* **2018**, *56*, 274–278.
67. Jadhav, H.P.; Sayyed, R.Z. Role of hydrolytic enzymes of PGPR in crop protection. *MOJ Cell Sci. Rep.* **2016**, *3*, 135–136.
68. Jadhav, H.P.; Sonawane, M.S.; Khairnar, M.H.; Sayyed, R.Z. Production of alkaline protease by rhizospheric *Bacillus cereus* HP_RZ17 & *Paenibacillus xylanilyticus* HP_RZ19. *Environ. Sustain.* **2020**, *3*, 5–13. [CrossRef]
69. Jadhav, H.P.; Sayyed, R.Z.; Shaikh, S.S.; Bhamare, H.M.; Kumari, S.; Enshasy, H.A.E. Statistically designed bioprocess for enhanced production of alkaline protease in *Bacillus cereus* HP_RZ17. *J. Sci. Ind. Res.* **2020**, *79*, 491–498.
70. Sayyed, R.Z.; Patel, P.R.; Shaikh, S.S. Plant growth promotion and root colonization by EPS producing *Enterobacter* sp. RZS5 under heavy metal contaminated soil. *Indian J. Exp. Biol.* **2015**, *53*, 116–123.
71. Suprapta, D.N. *Pestisida Nabati: Potensidan Prospek Pengembangan*; Penerbit Pelawa Sari: Denpasar, Indonesia, 2014.
72. Spence, C.; Alff, E.; Johnson, C.; Ramos, C.; Donofrio, N.; Sundaresan, V.; Bais, H. Natural rice rhizospheric microbes suppress rice blast infections. *BMC Plant Biol.* **2014**, *14*, 130. [CrossRef] [PubMed]
73. Suriani, N.L.; Suprapta, D.N. *Innovation Research Report*; Udayana University: Denpasar, Indonesia, 2019.