PENGGUNAAN FORMULASI *BACILLUS FIRMUS* E65-BERBASIS TALEK UNTUK PENGENDALIAN PENYAKIT HAWAR DAUN BAKTERI

Use of *Bacillus firmus* E65-Talc Based Formulation for The Management of Bacterial Leaf Blight Disease

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Abstrak: Penyakit hawar daun bakteri (HDB) yang disebabkan oleh *Xanthomonas oryzae* pv *oryzae* (Xoo) telah diketahui sejak lama merupakan penyakit bakteri utama terjadi di banyak negara-negara penghasil padi. Penelitian ini dilakukan untuk mengetahui pengaruh bakteri endofit yang diformulasikan berbahan dasar talek terhadap pengendalian hayati Xoo di agroekosistem padi berbasis sistem intensifikasi padi (SRI)-organik di Cianjur-Jawa Barat. Formulasi berbahan dasar talek disiapkan mengandung bakteri endofit *Bacillus firmus* (isolat E65) yang sebelumnya diisolasi dari padi sawah. Penekanan penyakit HDB berkisar 2,14% sampai 97,15%. Di antara kultivar yang diuji, rata-rata tingkat keparahan HDB di antara enam kultivar adalah 9,79%. Kultivar Inpari 10 (Sedang) menunjukkan tingkat keparahan penyakit yang paling rendah dibandingkan dengan yang tahan (cv. Sintanur, Mekongga dan Code). Hal ini menunjukkan bahwa efikasi biokontrol *B. firmus* E65 berbasis talek tidak dipengaruhi oleh kerentanan kultivar. Formulasi bakteri antagonis meningkatkan hasil hingga 9%. Hasil tertinggi untuk masing-masing perlakuan dengan formulasi bakteri berbasis talek diperoleh pada cv. Inpari 10 dan Cisantana. Untuk skala produksi yang dapat memperpanjang aktivitas biokontrol, kendala yang mungkin terjadi adalah umur simpan formulasi. Kajian ini menunjukkan keuntungan dari kultur tunggal bakteri antagonis yang

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digunakan dalam formulasi berbasis talekmenungkinkan untuk menekan penyakit HDB serta meningkatkan hasil padi pada kondisi lahan SRI.

Kata kunci: Hawar Daun Bakteri, *B. firmus* E65, Padi, Formulasi Talek.

Abstract: It has long been known that bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is major bacterial diseases which occured widespread in rice growing countries. This study was performed to determine effect of biological control using endophyte bacteria formulated in talc-based powder against Xoo in rice agroecosystem which grown under organic system of rice intensification (SRI) in Cianjur-West Java. Carriers formulation of talc-based powder was prepared to contain endophytic bacteria of *Bacillus firmus* (isolate E65) which was previously isolated from the rice field. BLB disease suppression was ranged 2.14% to 97.15%. Among cultivars tested, with the average BLB severity among six cultivars was 9.79%. The cv. Inpari 10 (Moderate) showed the lowest disease severity compared with that of resistance cv. Sintanur, Mekongga and Code. This suggests that the biocontrol efficacy of formulation *B. firmus* E65-talc-based powder was not affected by cultivar susceptibility. The antagonistic bacteria formulation yields up to 9%. The highest yields for each respective treatment treated with talc based-bacterial formulation were obtained using cv. Inpari 10 and Cisantana. The scale-up of biocontrol products that prolongs biocontrol activity may challenged with shelf life of formulation. This study showed advantages of single cultures of bacterial antagonist used in a talc-based formulation is possible to suppress BLB disease and promotes rice yield under SRI field condition.

Keywords: Bacterial Leaf Blight, *B. firmus* E65, Rice, Talc-Powder Formulation.

1. Introduction

Rice is an important cash crop in Indonesia. In an effort to supply national rice demands, sustainable rice production is faced with several problems i.e; high growth rate rice consumption, shrinkage land conversion, as well as global climate which affects the increase of pest infestation (Zahri *et al*., 2018). Rice diseases have a significant effect in terms of annual yield and farmer's incomes lost. The major bacterial diseases of rice such as bacterial leaf blight (BLB) due to Xoo is serious major concern in rice producing-countries, affected yield loss up to 30%. Bacterial leaf blight of rice has been reported in severalparts of the world with high incidence and severity. It was reported widespread damage in Asia particularly in rice tropical regions. Moreover, BLB disease is reported to infect wetlands and upland rice fields (Kadir *et al*., 2007; Naqvi, 2019).

Therefore, strategies adapted to particular environments must be developed to avoid possible epidemics. Control of pathogen mostly relied upon use of plant resistance (Khan et al, 2012; Fred et al, 2016); however, durability of plant resistance are easily broken due to variation of pathogenic races that occurs between seasons and locations. In Indonesia up to now at least known 12 Xoo virulence groups (Hifni and Kardin, 1998). The BLB disease progression depends upon the specific combination of host-pathogen. It has been observed in many countries that the occurrence of wide diversity among Xoo pathogenic groups, and shifting of race pathogen population may influenced by host genotypes in the fields (Wonni *et al*., 2016; Suryadi *et al*., 2016).

Use of chemicals control such antibiotics and copper-based compounds to manage bacterial disease is sometimes not effective due to environmental problems and pathogens resistance, hence use of beneficial organic systems is recommended to replace synthetic chemical (Stoleru *et al*., 2012; Munteanu *et al*., 2014).

However; field cultivation of organic system depends upon a series of properly applied cultural practices. i.e; crop rotation, cultivar selection, manure, and fertilizer application, and pest/disease control (Furlong *et al*., 2008; Pfiffner and Mader, 2009). In the past few years, the System of rice intensification (SRI) being used as rice intensive cultivation due to the efficiency in the management of soil, crop, and water-based system (Uphoff, 2003). In the previous experiment at Pusakanagara Expt. Station (W. Java) during the dry season (DS), use of biocontrol agent viz. the bacterial consortium which grown under integrated crop management practice (ICM) and organic SRI practices, could suppressed severity of rice diseases (Suryadi *et al*., 2013a).

Biological control has been given in evaluating the capability of various microorganisms in suppressing plant pathogens. However, the inconsistent field performance of biocontrol product has been limited for scale up applications. Velusamy *et al*., (2006) reported that use of rice-associated bacteria showed capability to suppress BLB in India. It was also reported by Kilic and Yuen (2004) that *Lysobacter* spp., a Gram-negative bacteria could suppressed *Bipolaris sorokiniana*. Moreover, the use of a single application of different antagonistic bacterial isolates could suppress BLB lesion length under a screen house test.

The biological approach using biocontrol agents may be integrated with other management practices such as plant resistance to attain more level of rice protection and sustainability. Although research on biocontrol of several rice diseases by microbial agents has been established, limited studies on the use of bacterial strains isolated locally from the various environment have been reported. To date, used of bioformulation containing bacterial antagonist is gaining more interest to replace synthetic chemical control measures (Kalyan*et al*., 2010; Suryadi *et al*., 2013b; Pustika et al, 2021).

To select, biocontrol formula, criteria of adaptation to the appropriate cultivar resistance to diseases and control method were taken into account (Mishra and Arora, 2016). Use of potential biocontrol agent which combined with plant resistance may affect BLB disease suppression, therefore should be investigated. In this study, we selected *B. firmus* E65 isolate capable of suppressing Xoo pathogen both *in-vitro* and *in-vivo*assays and further develop as bio-formulation. In thisstudy also, the use of strain *B. firmus* E65was kept to seeif any protective effect, in suppressing the diseasethrough induced resistance.If the bio-formulations (containing bacteria of *B. firmus* E65) is effective, then the bioproducts can potentially be used as components in an integrated disease management program for controlling BLB disease under field condition. This study was aimed a) to evaluate use of bio-formulation containing *B. firmus* E65 isolates using the talc-based to control BLB on rice cultivars with varying degree of resistance which grown under organic SRI practices, and b)to evaluate effect of bio-formulation against BLB of rice on cv. Sintanur under different cultural practices

2. Material and Method

Preparation *B. firmus* Isolate E65 and Talc-Based Carriers for Bio-Formulation Mass Production

The bacterial endophytic strain (*B. firmus* E65 isolate) was obtained from ICABIOGRAD culture collection. Bacterial stock cultures from ampoules were streaked onto nutrient agar (NA) which incubated for 24 h at 37°C; then bacterial colony was grew on flask and shaking (125 rpm) under a rotary shaker (Stuart Scientific, SI 50). Bacterial colony (10%, v/v) were sub-cultured into the fresh nutrient broth (NB), incubated at ambient temperature. The bacterial growth was determined by measuring the OD_{600nm}=0.5 adjusted to a bacterial concentration of 9 x 10^8 CFU/mL using a spectrophotometer (Hitachi-150-20).

Propagation of bio-formulation, was prepared following Vidhyasekaran and Muthuamilanmethod (1995). In brief, bacterial suspension (10 mL) at concentration 10^8 CFU/mL were transferred onto a 1000 mL flask containing NB media and shaking (125 rpm) and incubated at ambient temperature for 48 h. The composition and mixing process of the carrier materials was done under sterile conditions consist of 300 mL bacterial suspension, 1 kg of talc powder, carboxymethyl cellulose (CMC) 10%, and calcium carbonate (CaCO3) 15%. The formulation was air-dried and kept moisture content < 20% under moist room temperature (28 ± 2°C) and further it was packed and sealed using polypropylene bags (Fig 1).

Figure 1. Prototype of bio-formulation of *B. firmus* E 65 using talc based carrier

Exp 1. Field Evaluation of *B. firmus* E65-Talc Based Formulation on Five Rice Cultivarsto BLB

The field experiment was carried out at the SRI organic rice field farm of ‘Mandiri’ Group located in Cipeuyeum village Cianjur W-Java (287 m above sea level), (S 6^{o}48’40.3704”, E 107^{o}17’33.288”) under natural infection. This site was chosen based on endemic area, where previously dominated by Xoo pathotype VIII. Prior to the testing, the plot size was planted by susceptible cultivar (Ciherang) as border serve as inoculums. The local climate is characterized by an average annual temperature of 31.3°C, total average rainfall of 392 mm/year, and the soil is classified as regosol.

Five rice cultivars with varying degree of resistance to BLB viz. Sintanur (R), Ciherang (S), Inpari 13 (MS), Inpari 14 (MR), Inpari 15 (MR), and Inpari 20 (R) were planted in a 4 x 5 m^2 plot size following a randomized complete block design (RCBD) with three replications. Rice plants were sprays with bacterial formulation (3 g/L) four-times at 14, 28, 35, and 45 days after planting (DAP), and BLB disease severity was observed based on standard evaluation system (SES) for rice (IRRI, 2013). The disease scorewas based on scale of 1-9, where 1 = 1% of leaf areaaffected and 9 = 50% of leaf area affected by the disease.

Exp. 2. Effect of bio-formulation on 7 cultivars (treated vs untreated) to BLB severity

Seven rice cultivars i.e; Sintanur (R), Mekongga (MS), Inpari 10 (MR), Inpari 13 (MS), Cisantana (MR), Code (R), and Ciherang (S) were planted in a 4 x 5 m^2 plot size and arranged in a randomized complete block design (RCBD) with three replications. The study was carried out based on organic-SRI farmer practices which consist of: transplanting of single young seedlings (less than 12 days old-seedlings) with spacing 30 x 30 cm; organic material of animal compost used as fertilizer (4 t/ha); intermittent irrigation for watering, and no synthetic pesticides at all (except used of local microorganism and plant extract as a natural pesticide) in the pest management. Rice plots were treated with *B. firmus* (E 65) - talc-based formulation (3 g/L) containing $10^{8\text{-}9}$ CFU/mL, whilst control was done without formulation.

Rice cultivars were treated four-time sprays-applications with bio-formulation mixture using knapsack sprayer at 14, 28, 35, and 45 DAP. BLB disease severity was evaluated at 70 DAP using 0-9 scale basis of SES for rice (IRRI, 2013), and yield components were observed after harvesting time.

Exp 3. Effect of bio-formulation against BLB of rice on cv. Sintanur under different cultural practices.

The cv. Sintanur (crossing between cv.Lusi and Bengawan Solo) was used in this test based onfarmer’s preference i.e aromatic rice, with the characteristics of earlymaturing ability, and resistant to brown plant hoppers and BLB.Rice seedlings were established by different planting methods. Cultural practises were prepared based on System of Rice Intensification (SRI) ‘jarwo’ 2:1 (40 x 25 cm), SRI-transplanting (25 x 25 cm), Integrated Cropping Management (ICM)-transplanting (25 x 25 cm) and farmer-‘tegel’-transplanting (25 x 25 cm), respectively. SRI nursery was sowing in plastic boxes or baskets. Seeds were mixed with organic fertilizer with a ratio of 1: 1. Rice seedlings were planted at 7-10 days after sowing. One seedling per hole was transplanted with spacing of 25 cm x 25 cm (transplanting system) or 45 cm X 25 cm ("jarwo" system). This study was carried out using a randomized block design with 3 replications.

Each crop was established using well-balanced irrigation. Fertilizer used is organic fertilizer derived from animal manure, forage, organic wastes composted using local microorganism. The soil was fertilized using 2.5 t/ha of mature goat compost and plowed to a depth of 20-30 cm. Rice plants in each plots were treated with bacterial formulation (3 g/L).

Data analysis

Observations of BLB disease symptoms were assessed based on SES score (IRRI, 2013). The severity of BLB disease was then calculated by: DS(%) = [∑(n.x v)/(∑(N x V) x 100%], where: DS=disease severity; n=number of plants or parts of the sample plant with a scale of damage symptoms; v = damage scale value; N = total number of plants observed; V= the highest scale.

Variable data were examined i.e; plant height (cm), tillers number, and yield (t/ha), then data were analyzed using the variance analysis (ANOVA) method, and the significance of the differences was further assessed with Duncan multiple range tests (DMRT) at 95% degrees of confidence.

3. Result and Discussion

Exp 1. Field Evaluation of *B. firmus* E65-Talc Based Formulation on Five Rice Cultivars to BLB

From the field observation, infected leaves showed yellow lesions at the margins parallel along the leaf blade, which finaly joined together covering the entire leaf surface. Based on visual observations, the disease symptoms among the five rice cultivars evaluated, only one cultivar had lower BLB disease in the SRI field. According to cultivar description (BB Padi, 2015), the selected rice cultvars i.e. Sintanur (resistant (R) to BLB pathotype III); Inpari 14 (MR to BLB pathotype IIII and Susceptible (S) to pathotype IV, VIII); Inpari 15 (MRto BLB pathotype III and MS to pathotype IV, VIII); Inpari 13 (MS to BLB pathotype III, IV and VIII); andInpari 20 (R to pathotype III, and MS to pathotype IV, VIII).

Organic farming practices will help environmentally friendly agricultural production to improve sustainable farming (Yuttitham, 2019). In line with the organic study approaches, the results of the evaluation of bio-formulation (*B.firmus* E65-talc) effects on BLB severity on different rice cultivars are shown in Fig 2. BLB disease severity ranged from 11.4 to 27.8%. It was shown that cv. Ciherang was the most susceptible to the disease, whilst other cultivars almost showed a similar reaction (moderate) to BLB disease. Spraying of strain Pseudomonas MBPF-01 at 7 d interval was reported effective in suppressing (by 70 % over control) BLB intensity (Kalyan et al, 2010).

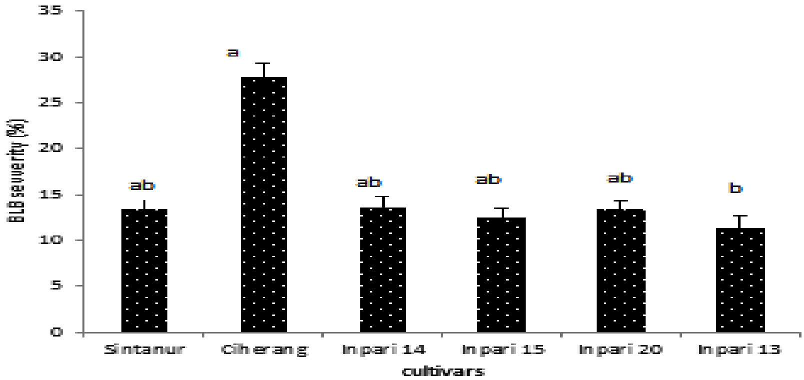

Figure 2. BLB severity of different rice cultivars under SRI. Means followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at P=0.05.

The bioformulation lead to lower BLB disease severity at Inpari 13 which is significantly different compared to the control cv. Ciherang. However, the four cultivars viz. Sintanur, Inpari 14, Inpari 15 and Inpari 20 has also not significantly differ with control cv. Ciherang.

Therelatively low severity observed in the field might bedue to different resistance response and environmental factors such as day/night temperature difference, humidity, and wind, which affect the virulence of BLB. Safni et al (2021), reported that Inpari 30 cultivar had the lowest disease severity (54.6%) when they were grown on acidic soil.

Exp. 2. Effect of Bio-formulation on 7 Cultivars (Treated vs Untreated) to BLB Severity

Table 1 summarizes the results for the tested application using E65-talc based formulation on seven different rice cultivars. Under field test conditions at Cianjur W. Java, BLB of rice occurred in the field with disease severity ranging from 0.74 to 26.01% (Figure.3).

Table 1**.** Effect of E65-talc based formulation to BLB disease severity among several rice cultivars differing resistance to BLB

Cultivar (with BLB resistance)	BLB disease severity (%)		Disease suppression (%)*
	Treated	Untreated (control)	
Sintanur (R)	10.4 [bcd**]	15.5[a]	32.9
Mekongga (MR)	14.1 [bc]	15.5[a]	9.0
Code (R)	5.2 [cd]	18.8[a]	72.3
Inpari 10 (MR)	0.7 [d]	15.7[a]	95.5
Inpari-13 (MS)	20.3 [ab]	15.9[a]	(27.7)
Cisantana (MR)	8.2 [cd]	15.0[a]	45.3
Ciherang (S)	26.0 [a]	30.0 [b]	15.4

* calculated using formula DS=(C-T)/C x 100%; where DS= disease suppression, C=BLB severity in control, T=BLB severity in treatments. R= resistant, M=moderate, S=susceptible.
**Means in column followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at P=0.05.

It was shown that spraying application using E65-talc on cv. Inpari 10 significantly reduced BLB disease severity compared with that of cv. Ciherang (control treatment). In addition, *B.*

firmus E 65 treated as a single culture with talc formulation could reduce BLB disease severity on cv. Cisantana and Code with the BLB severity of 8.2% and 5.2%; respectively; however it failed to reduce the disease on cv. Inpari 13.

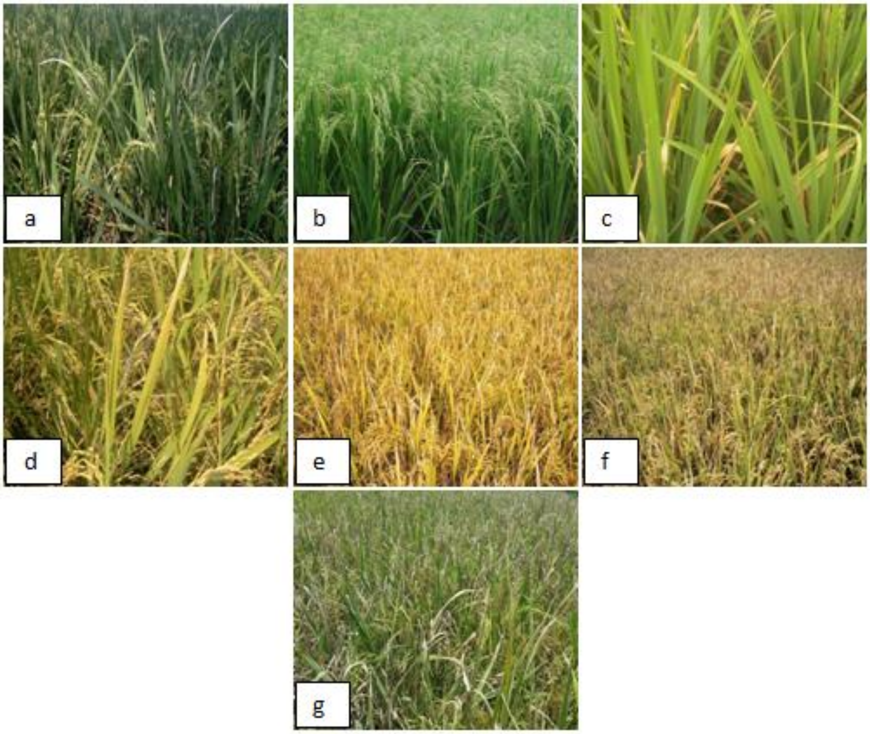

Figure 3. Example of BLB severity on diferent rice cultivars in the field. a=Sintanur, b=Mekongga, c=Code, d=Inpari 10, e=Inpari 13, f=Cisantana, g=Ciherang

Use of aromaticrice cultivars with high cooking qualities (viz. RD6 and KDML105), which widelygrown in large-scale and long-term cultivation in the North and Northeast of Thailand was reported targets for beingdamaged by the BLB pathogen (Sribunrueang and Chankaew, 2017). In this study, formulation treatment on aromatic rice i.e cv. Sintanur had 10.4% severity, which also as effective to reduced BLB severity similar to cv. Mekongga, Cisantana and Code. The cv. Code showed significantly lower disease (0.7%) than others, suggesting that *Xa7* which contain in this variety might confer a higher level of resistance.

The mean average of BLB severity ofthe six cultivars showing effective disease control was 9.79% with biocontrol efficacy (BLB suppression) ranging from 9 to 95.5%. This result suggests that biocontrol efficacy of formulation E65-talc was somewhat not affected to the susceptibility of the cultivar to BLB; for instance, Inpari 10 (MR) showed the lowest disease severity compared with that of Sintanur, Mekongga and Code (Rcultivars). Although the mechanism present of *B. firmus* E65-talc has been not determined, the result suggested the active compound of bacteria protect against BLB pathogen when applied prior to disease occurrence.

It was observed that bio-formulation containing *B. firmus* E 65 isolate improved the performance of suppressing ability in treated rice cultivars under SRI practices. Efficacy of bio-formulation varied significantly among different rice cultivars tested, with disease suppression ranged 2.14 - 97.15%. However, the *B. firmus* E 65-talc treatment was not effective in reducing BLB disease on cv. Inpari 13. The better suppression efficacy with *B. firmus* E 65-talc formulation was achieved using cv. Code (containing *Xa-4*; *Xa-7* gene for resistance to Xoo) and cv. Cisantana. In similar work, Jochum *et al.*, (2006) reported that application of biocontrol agent among different wheat cultivars showed varying degree in suppressing head blight under greenhouse study, which suggests that efficacy was not directly influenced by the resistance level. Plant morphology such as leaf surface structure (hydatodes, mesophyl) was suggested affected colonization of biocontrol agent in different rice cultivars. Under different environmental conditions, most biocontrol agents i.e; local microorganism application showed varied performance which attributed to differences in mode of action. Many Bacillus species are considered antagonistic microorganisms owing to their potential to producediverse antimicrobial compounds (Garcia et al, 2011; Li et al, 2016). *B. amyloliquefaciens* B014 has been reported aspotential to produce lipopeptide iturins and surfactins (Li et al, 2012). Asmah and Sapak (2020) pointed out that *B. subtilis* UiTMB1 is able to control the disease and enhance the growth of rice plants.

In the present study, viability of *B. firmus* E65- talc powder formulations declined in number at different storage periods. The initial population was 2.1 x 10^9 CFU/mL; whilst at final observation, population decrease to 4 x 10^7 CFU/ml, indicating that viability after 3 months of storage was gradually decreased. Hence, the development of stable formulations of bacteria is importance to support sustainable agriculture. In our study, *B. firmus* E65 based formulation was relatively effective. This could probably be due to the more diverse metabolites such as salylic acid and peroxidase produced by the bacterium (Saikia *et al.*, 2006).

Previously talc-powder, and bentonite performed well among the carriers tested as biocontrol formula (Suryadi *et al*., 2013b). The use of *B. firmus E 65* as talc powder formulation as a spray application in this study, showed that the bio-formulation suppressed BLB disease. In line with the study, El–Hassan and Gowen (2006), also reported that formulations *B. subtilis*- talc powders reduced *Fusarium oxysporum* f.sp. *lentis* wilt disease by 43.0%; while Ardakani *et al.* (2010) also reported the efficacy of talc-based formulation to control damping-off on cotton.

The present study also describes the formulation of *B firmus* E 65 in talc powder for the biocontrol of BLB under field condition. The reduction in plant symptoms indicates representative potential resources management strategies for control Xoo. Talc powder formulations resulted in a significant in BLB disease suppression (Table 1). Disease severity of untreated rice cultivars range between 15- 30% with the disease reduction of 15.4 to 95.5 % after the treatment with talc-powder formulations.

Distinctly significant differences were noted between treatments. The total production varied from 3.6 to 4.05 t/ha for treated plants compared with 3.5 to 4.1 t/ha for untreated plants. The cv. Inpari 10 and Cisantana showed positive yield differences (Table 2).

Tabel 2. Effect of E65-talc based formulation on rice yield

Cultivar	Mean Yield (t/ha)**		Yield Difference (t/ha)
	Treated	Untreated (control)	
Sintanur (R)	4.05[b]	4.1[b]	(0.05)
Mekongga (MS)	3.6[a]	3.6[a]	0
Code (R)	3.75[a]	3.8[a]	(0.05)
Inpari 10 (MR)	3.9[ab]	3.5[a]	0.4
Inpari-13 (MS)	3.75[a]	3.7[a]	0.05
Cisantana (MR)	4.0[b]	3.8[a]	0.2
Ciherang(S) (control)	3.65[a]	3.7[a]	(0.05)

*) Means in column followed by the same letter are not significantly different by DMRT (P=0.05).

**) water content 14%

Exp. 3.Effect of Bio-formulation Against BLB of Rice on cv. Sintanur Under Different Cultural Practices

Farmer *'tegel'*-transplanting is a conventional cropping pattern that is generally performed by farmers in Indonesia with a distance of 25 × 25 cm or wider which forms like tiles (Kadir *et al.*, 2006; Darmawan, 2016) (Figure.4). Nurhayati *et al*., (2015) stated that SRI '*jarwo'* 2:1: is a cropping pattern that manipulates growth where every two rows of manipulates seedlings are interspersed with a wide row spacing. Meanwhile, the Integrated crop management (ICM) system is built upon integrated pest management (IPM), utilizing a participatory 'farmer-field school' approach (Oka, 1997; Kadir *et al*., 2006).

Aromatic cultivar Sintanur used in this study showed varying degree of resistance to BLB. The severity of BLB disease was range 11.71 - 38.33%. Using SRI-‘*jarwo’*2:1 transplanting, it was shown that bioformulation resulted in lower BLB disease, whilst SRI-transplanting resulted in higher BLB severity (Table 3). The cropping patterns may affect differences in disease progression. SRI-‘*jarwo’*2:1 could decrease BLB disease by 50.3% compared with that of farmer-transplanting ‘*tegel*’ (control). Meanwhile, SRI-transplanting had higher BLB disease severity. Reduction disease severity as shown in SRI’*jarwo*’ (40 x 25 cm) might be due to less leaf canopy and more light which affected plant humidity as compare to a dense plant population in SRI-transplanting ‘*tege*l’, ICM-transplanting, and farmer transplanting-‘*tegel*’ methods (25 x 25 cm).

Treatment of bio-formulation using *B firmus* E65 in talc-based powder formulation under different rice cultural practices had a similar effect on rice yield cv. Sintanur. Based on this study the productivity of ICM-transplanting is not significantly different compared to SRI-transplanting, ICM-‘*jarwo’*2:1, and farmer-*‘tegel’*. However; based on quantitative values, ICM-transplanting showed productivity that tended to be higher.

Table 3. Effect of bio-formulation on rice cv. Sintanur against BLB and yield, under different cultural practices

Treatment	BLB severity (%)	Plant height (cm)^{NS}	Number of tiller^{NS}	Grain weight(g/m^2)^{NS}
SRI-transplanting ('SRI-*tegel*') (25 x 25 cm)	38.33 ± 4	93.6	21.8	1365
SRI '*jarwo*'2:1 (40 x 25 cm)	11.71 ± 2	92.6	23	1380
ICM- transplanting) (25 x 25 cm)	22.22± 4.5	98.6	27	1400
Farmer-transplanting'*tegel*' (25 x 25 cm)	23,6 ± 2.8	92.26	22.6	1185
CV(%)	28.52	3.47	20.87	10.27

NS=not significant

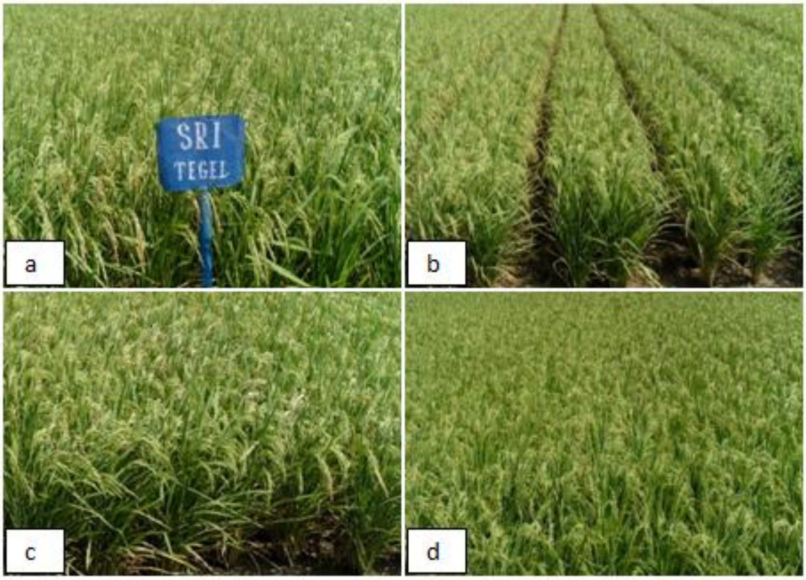

Figure 4. Performance evaluation of bioformulation on different rice cultural practices. a=SRI-transplanting (*SRI-'tegel'*); b=*SRI 'jarwo'*; c=ICM-transplanting; d=farmer-transplanting*'tegel'* (control)

ICM-transplanting also producing yields of grain weight (1400 g/1.5m^2). SRI-*'jarwo'*2:1 has more optimal plant height and tiller as well productivity reaching to 1380 grain weight/1.5m^2 compared farmer-*tegel* which only 1185 g/1.5m^2. Thus, it is very good to recommend that ICM-transplanting and SRI '*jarwo'*2:1 practices has low BLB disease severity as well as higher productivity.

The bioformulation treatment was more effective in suppressing bacterial growth under SRI- '*jarwo'*2:1, in comparison with other cultural practices. The suppressing activity is presumably due to the wider space of the plant that minimized the spread of Xoo infection between the tiller. Formulation of *B firmus* E 65 may have also facilitated increased efficacy of antimicrobial compounds present in the formula (Suryadi et al, 2013b).

Mechanisms of disease suppression talc-based powder formulations containing *B. firmus* E65 to Xoo may have been associated with chemically active compounds such as plant secondary metabolites that either affect the bacteria directly or elicit systemic resistance in host plants which results in the decrease of disease symptoms (Saikia *et al.,* 2006; Radulovic *et al*., 2013). *Bacillus* species were developed as a commercial biocontrol product to control plant disease (Schisler *et al*., 2004). The endophytes *B. subtilis* treated rice plots showed significantly lower BLB intensity compared to untreated control plots, which also produced a higher grain yield (Krishnan et al, 2013). Previous study also reported that *B. firmus* E65 was highly suppress the growth of Xoo *in-vitro* (Suryadi *et al*., 2013b).

The results highlight the potential of *B. firmus* E 65 as biopesticide to control BLB disease, also improve our understanding of the biocontrol mechanism of *Bacillus* spp. From this study, antagonistic bacteria formulation showed a good result to inhibit BLB on rice. For more effective formulation, further study should be develop formulation methods such as coating at seedlings stage as well as spraying at the generative stage. Under challenging environmental conditions such as rice organic-SRI, the presented findings support the hypothesis that the use of bacterial antagonists can serve as a tool in the BLB-control strategies to support organic crop production.

4. Conclusion

Bacillus firmus E 65 species was developed as an effective biocontrol product to control rice disease. The bioformulation lead to lower BLB disease severity at cv. Inpari 13 which is significantly different compared to the control cv. Ciherang. However, the four cultivars viz. Sintanur, Inpari 14, Inpari 15, and Inpari 20 has not significantly differ with control cv. Ciherang.

Antagonistic bacteria formulation of *B. firmus* E 65 using talc-based powder showed a good result to suppress Xoo under field condition. Among cultivar tested, application formula of E65-talc could suppresses BLB disease ranging 2.14 - 97.15%, with BLB severity of 9.79%. Cv. Inpari 10 (MR) showed the lowest disease severity compared with that of cv. Sintanur (R), Mekongga (MS) and Code (R), hence their effect toBLB should be reassessed under similar field condition in endemic areas.

Use of formula *B. firmus* E65-talc may improve yield > 9.14% as compared with untreated plot. More higher rice yield was obtained on cv. Inpari 10 and Cisantana. The cultivars treated with bio-formulation yielded up from 3.6 to 4.05 t/ha. When different cultural planting method and spray application with bio-formulation was applied on cv. Sintanur, the rice yield varied from 1185 to 1400 g/1.5m^2. Further exploitation of the bacterial isolate and suitable carrier will be beneficial in suppressing BLB disease under different agroecosystem conditions.

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Author contribution statement

YS: Conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; wrote the paper. DNS and IMS: Contributed materials analysis tools, analyzed and interpreted the data; wrote the paper.

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