



## Use of emulsifiers for control of rice blast and sheath blight in organic rice cultivation

Se Ji Jang <sup>1†</sup>, Ha-il Jung <sup>2†</sup>, Young Beom Yun <sup>1</sup>, Kyu Hwan Hyun <sup>1</sup>, Do-Ik Kim <sup>3</sup>, Carol Mallory-Smith <sup>4</sup>  
and Yong In Kuk <sup>1\*</sup>

<sup>1</sup> Department of Development in Oriental Medicine Resources, Suncheon National University, Suncheon 540-742, Republic of Korea. <sup>2</sup> Division of Soil and Fertilizer, National Academy of Agricultural Science, 166 Nongsaengmyeong-ro, Iseo-myeon, Wanju-gun 565-851, Republic of Korea. <sup>3</sup> Jeonnam Agricultural Research and Extension Service, Naju 520-715, Republic of Korea. <sup>4</sup> Department of Crop and Soil Science, Oregon State University, Corvallis, Oregon 97331, USA. \*e-mail: yikuk@suncheon.ac.kr

Received 14 December 2014, accepted 30 March 2015.

### Abstract

Currently, practices for controlling disease in organically produced rice (*Oryza sativa* L.) crops are not as effective as conventional ones. The objective of this research was to determine the effectiveness of various organic emulsifiers (natural emulsifier A and B, loess sulphur, brown rice vinegar, and insecticidal soap) on the suppression of fungal pathogens, rice blast (*Pyricularia oryzae* Cavara) and sheath blight (*Rhizoctonia solani* Kühn), and rice growth. Suppression activity of emulsifiers on mycelia growth of rice blast was proportional to dosage, and the order of effectiveness was: loess sulphur > natural emulsifier B > insecticidal soap > brown rice vinegar > natural emulsifier A. Rice sheath blight suppression of mycelia growth was also proportional to emulsifier concentration. Rice sheath blight was totally suppressed by 0.5% loess sulphur, 3% brown rice vinegar or insecticidal soap. The order of effectiveness of these emulsifiers was: loess sulphur > insecticidal soap > brown rice vinegar > natural emulsifier B > natural emulsifier A. Rice injuries due to blast infection were significantly reduced by the organic emulsifiers (0.5-10%), compared to untreated control. Therefore, the results suggest that organic emulsifiers could effectively control rice blast and sheath blight without contributing to adverse in rice growth.

**Key words:** Rice growth, control, organic emulsifiers, *Pyricularia oryzae*, *Rhizoctonia solani*.

### Introduction

Rice (*Oryza sativa* L.) is not only the most widely cultivated crop in Asia but also the principal food source for more than half of the world's population <sup>1,2</sup>. Two major fungal pathogens of rice, blast (*Pyricularia oryzae* Cavara) and sheath blight (*Rhizoctonia solani* Kühn) cause great yield losses in rice-growing countries <sup>3,4</sup> and decrease food supply <sup>5</sup>. Synthetic chemical fungicides have been routinely applied for controlling rice blast and sheath blight in the field <sup>3,6</sup>. In contrast, synthetic chemical fungicides are generally considered harmful to the human health and detrimental to the environment <sup>5</sup>. Additionally, host plant resistance to synthetic chemical fungicides may be impermanent in the field <sup>7</sup> and continued use of synthetic chemical fungicides may result in strains of fungal pathogens that are resistant to the applied fungicides <sup>5,7</sup>. Public demand for safe, robust, and sustainable food production has increased interest in investigating crop management practices that do not heavily rely on the broad application of synthetic chemical fungicides, insecticides, and herbicides <sup>8</sup>. Therefore, the demand and development of natural products for disease control have become increasingly promising, particularly for organic agriculture production systems <sup>8,9</sup>.

Recently, many studies on the practical approaches of plant extracts and essential oils against plant pathogenic fungi have been conducted in organically produced rice crops <sup>10</sup>. Natural products have been of interest, especially in organic farming, as

the application of vinegars from aerobic bacteria oxidation of ethanol in fermented grains, fruit juice, or nearly any other liquid containing alcohols, has been demonstrated to enhance plant growth of pea and sweet corn and to control some diseases <sup>11</sup>. In addition, oils play a key role in integrated programs against pests and diseases on roses and potatoes in the USA and Australia <sup>12</sup>. Insecticidal soaps have been demonstrated to work on contact and cause insect death by disturbing the cuticle permeability <sup>13,14</sup>. Nevertheless, limited research on the practical approaches of various emulsifiers in pest management in organically produced rice and other crop cultivation has shown that the products can be effective alternatives to synthetic pesticides. Therefore, the objectives of this research were to evaluate the effectiveness of organic emulsifiers (natural emulsifier A and B, loess sulphur, brown rice vinegar, and insecticidal soap) against the pathogenic growth of rice blast and sheath blight using *in vitro* bioassays and *in planta* greenhouse experiments.

### Materials and Methods

**Organic materials:** The emulsifiers used in this study are approved for use in organic farming by the Rural Development Administration Guideline in Korea. Loess sulphur and natural emulsifier A were formulated and provided by Jeonnam Agricultural Research & Extension Services. The loess sulphur was composed of 250 g sulphur, 5 g red clay powder, 15 g bay salt, 5 g phyllite

† These authors contributed equally to the work.

powder, 5 g calcium powder, 200 g NaOH, and 500 ml distilled water per 1 litre of product. The final concentration of sulphur in the final product was 37%. Natural emulsifier A was composed of 32 g KOH, 180 ml canola oil and 788 ml distilled water per 1 litre of product. Brown rice vinegar was purchased from Natural Food Inc. (Seoul, Korea). The brown rice vinegar was made from aerobic bacterial oxidation of ethanol in fermented brown rice (80 g per L) and contains 7% acetic acid. The insecticidal soap, purchased from EMbio Inc. (Seoul, Korea), was composed of natural vegetable fats and oils, NaOH and EM (effective microorganisms) fermented liquor in a ratio of 77:17:6. Natural emulsifier B was purchased from Sugar Bubble Inc. (Seoul, Korea).

**Determination of organic emulsifiers effect on suppression activities of rice blast and sheath blight using in vitro bioassays:**

Ten ml of 0, 0.05, 0.1, 0.3, 0.5, 1, 5, and 10% of the emulsifiers were added to potato dextrose agar (PDA) media in Petri dishes (90 mm). After solidification, a mycelia plug (10 mm diameter) of test the fungus (*Pyricularia oryzae* KACC-40441 or *Rhizoctonia solani* AG-1 KACC-40101) was placed in the centre of the Petri dishes and incubated at 26°C in darkness. Three-day old cultures of *Pyricularia oryzae* KACC-40441 and *Rhizoctonia solani* AG-1 KACC-40101 grown on PDA medium were used for bioassays. Radial mycelia growth of the test fungus was recorded at 3 days after treatment. The suppression activity was calculated using colony diameter growth of treated plates compared to control plates (PDA medium without emulsifiers) according to the following equation:

$$\text{Suppression activity (\%)} = [(\text{Colony diam. on untreated} - \text{Colony diam. on treated}) / \text{Colony diameter on untreated}] \times 100\%$$

**Determination of organic emulsifiers effect on suppression of blast in infected rice seedlings:**

A susceptible rice cultivar, cv. Ilmibyeo, was used for greenhouse blast trials. Ilmibyeo plants were sown at a high density (30 seeds) in pots (200 ml) filled with commercial potting soil (Pungnong NPKO, Seoul, Korea). Blast was induced under low temperature and high humidity conditions as followed. The plants were maintained with day/night mean temperatures of 28/22°C, relative humidity of 70/90%, 500 μmol m<sup>-2</sup>s<sup>-1</sup> photosynthetically active radiation, and a 14/10 h day/night period. Twenty day-old seedlings that were 20% infected with blast were sprayed with 0, 0.5, 1, 3, 5, and 10% of emulsifiers. The rice injury from the blast was evaluated visually (0-100; 0 = no damage, 100 = complete death) at 3, 7, 11, and 15 days after treatment and plant height and shoot fresh weight were measured at 15 days after treatment. Pots were arranged as a randomized complete block with three replicates per treatment.

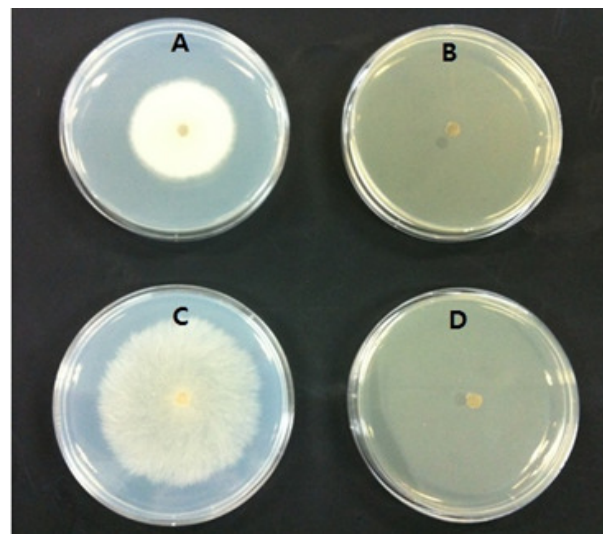
**Determination of organic emulsifiers effect on growth of rice seedlings:**

Two cultivars, the blast-susceptible cv. Ilmibyeo and blast-resistant cv. Dongjinbyeo were used to monitor the effect of organic emulsifiers on rice growth. Twenty-day-old seedlings were sprayed with 0, 0.5, 1, 3, 5, and 10% of emulsifiers. Rice injury, plant height, and shoot fresh weight were recorded at 7 days after treatment. Pots were arranged as a randomized complete block with three replicates per treatment.

**Statistical analysis:** Data were analysed using analysis of variance (ANOVA) procedure in the Statistical Analysis Systems (SAS) software<sup>15</sup>. Means were separated using Duncan's multiple range test ( $P=0.05$ ).

**Results and Discussion**

**Effect of organic emulsifiers on suppression of rice blast and sheath blight:** *In vitro* bioassays of emulsifier suppression were determined by measuring the diameter of mycelia growth of rice blast (*Pyricularia oryzae* KACC-40441) or sheath blight (*Rhizoctonia solani* AG-1 KACC-40101) on solid potato dextrose agar (PDA) plates compared to mycelia growth on control PDA plates with no added emulsifier (Fig. 1). 0.05% loess sulphur resulted in 48.1% suppression activity of mycelia growth on PDA, and this suppression activity increased with increased dosage. Furthermore, 5% loess sulphur totally suppressed the mycelia growth (Table 1). On the other hand, 0.05% insecticidal soap suppressed the mycelia growth by 17.0%, and this suppression activity was proportionally increased with the increase in concentrations of insecticidal soap; 10% insecticidal soap suppressed mycelia growth by 100%. Addition of 0.05% brown rice vinegar suppressed mycelia growth by 3.0%, and 0.5% suppressed blast growth by 48.1%. Natural emulsifier B of 0.1% suppressed mycelia growth by 54.1%, whereas 5% natural emulsifier B could suppress blast totally. Natural emulsifier A of



**Figure 1.** Mycelia growth of rice blast (*P. oryzae*) at 3 days after placement on PDA plates without (A) and with (B) 5% loess sulfur, and rice sheath blight (*R. solani*) without (C) and with 0.5% loess sulphur (D).

**Table 1.** Suppression activities of organic emulsifiers against the growth of rice blast (*P. oryzae*) at 3 days after placement on PDA medium *in vitro* bioassay.

Emulsifiers	Suppression activity* (%)						
	0.05%	0.1%	0.3%	0.5%	1%	5%	10%
Control	0.0 <sup>c</sup>	0.0 <sup>c</sup>	0.0 <sup>d</sup>	0.0 <sup>e</sup>	0.0 <sup>d</sup>	0.0 <sup>d</sup>	0.0 <sup>c</sup>
Natural emulsifier-A	0.0 <sup>c</sup>	0.0 <sup>c</sup>	10.0 <sup>cd</sup>	13.3 <sup>d</sup>	41.7 <sup>c</sup>	62.2 <sup>c</sup>	62.5 <sup>b</sup>
Natural emulsifier-B	0.0 <sup>c</sup>	54.1 <sup>a</sup>	65.2 <sup>ab</sup>	89.6 <sup>a</sup>	87.5 <sup>a</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>
Loess sulphur	48.1 <sup>a</sup>	58.5 <sup>a</sup>	72.6 <sup>a</sup>	75.0 <sup>b</sup>	83.0 <sup>a</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>
Brown rice vinegar	3.0 <sup>c</sup>	6.7 <sup>c</sup>	17.0 <sup>c</sup>	48.1 <sup>c</sup>	-	-	-
Insecticidal soap	17.0 <sup>b</sup>	23.7 <sup>b</sup>	60.0 <sup>b</sup>	66.7 <sup>b</sup>	83.0 <sup>a</sup>	87.5 <sup>b</sup>	100.0 <sup>a</sup>

\*Suppression activity was calculated relative to the colony diameter by comparison with the control.

Means within a column followed by the same letters are not significantly different at 5% level according to the Duncan's multiple range test.

0.3% suppressed mycelia growth by 10.0% and the greatest suppression (62.5%) was appeared, when 10% natural emulsifier A was added. Although loess sulphur showed suppression activity at lower concentrations (0.05, 0.1, and 0.3%), compared to natural emulsifier B, complete suppression (100%) of rice blast mycelia growth at a 5% concentration with either emulsifier suggests that loess sulphur could be as effective as natural emulsifier B did. Overall, *in vitro* bioassays revealed that suppression activity of emulsifiers on mycelia growth of rice blast was proportional to dosage, and the order of effectiveness (from greatest to least) was: loess sulphur > natural emulsifier B > insecticidal soap > brown rice vinegar > natural emulsifier A.

*In vitro* bioassays of rice sheath blight showed the same trend as for blast where suppression of mycelia growth was proportional to emulsifier concentration (Fig. 1 and Table 2). PDA plates containing 0.01% loess sulphur resulted in 45.6% suppression of mycelia growth, whereas 0.5% loess sulphur completely suppressed mycelia growth. Insecticidal soap of 0.01% suppressed mycelia growth by 26.4% but 3% insecticidal soap totally suppressed growth. A concentration of 0.1% of brown rice vinegar suppressed mycelia growth by 23.5%, while 3% brown rice vinegar resulted in 100% suppression. Natural emulsifier B added to PDA plates at 0.1% resulted in 17.7% suppression, while the same concentration of natural emulsifier A suppressed mycelia growth by 7.4%. Full suppression (100%) of rice sheath blight was not achieved using either emulsifier at the concentrations tested. Bioassays monitoring suppression of mycelia growth of rice sheath blight suggest that the order of effectiveness (from most to least effective) of these emulsifiers was: loess sulphur >

insecticidal soap > brown rice vinegar > natural emulsifier B > natural emulsifier A.

**Effect of organic emulsifiers on suppression of blast-infected rice:** *In vitro* bioassays trials suggested that the emulsifiers tested were able to suppress mycelial growth of rice blast. To confirm suppression ability, cv. Ilmibyoo, a cultivar susceptible to rice blast was grown in a greenhouse in high density and infected with blast (see Methods). Twenty-day old seedlings that were 20% infected with blast were chosen for subsequent treatment with 0.5–10% emulsifier concentration. The effectiveness of the emulsifiers to alleviate damage caused by blast was determined by observing injury in seedlings at 3, 7, 11, and 15 days after treatment, and recording shoot height, and fresh weight at 15 days after treatment.

Rice injuries due to blast infection in control plants were calculated as 15.0, 35.6, 79.0, and 90.6% at 3, 7, 11, and 15 days after treatment (DAT), consistent with the progression of disease if fungal pathogens are left untreated. In contrast, injury due to blast was significantly decreased if plants were treated with 0.5% emulsifier (Table 3). Injury in plants treated with natural emulsifier B was recorded to be 8.3, 8.3, 38.3, and 48.3% at 3, 7, 11, and 15 DAT, respectively. The same concentration of brown rice vinegar decreased blast injury to 8.3, 5.0, 16.7, and 25.0% at 3, 7, 11, and 15 DAT, respectively. In contrast, there was no significant difference in injury between untreated plants and plants treated with 0.5% loess sulphur at 3 and 7 DAT; however, injury due to blast was documented as 35.0 and 40.0% at 11 and 15 DAT, suggesting that treatment with 0.5% loess sulphur results in slower inhibition of blast injury compared to the same concentration of natural emulsifier B and brown rice vinegar. Increasing loess sulphur concentration to 1% resulted in a decrease of injury due to blast at 3.0, 14.0, 18.3, and 26.7% at 3, 7, 11, and 15 DAT, respectively, compared to controls. The same percentage of natural emulsifier B reduced injury to 4.3, 13.3, 18.3, and 28.3% at 3, 7, 11, and 15 DAT, respectively. When plants were treated with 1% natural emulsifier A, injury due to blast was 5.0, 11.0, 31.7, and 53.3% at 3, 7, 11, and 15 DAT, respectively. Plants treated with 3% natural emulsifier B resulted in 4.0, 15.0, 18.3, and 28.3% at 3, 7, 11, and 15 DAT, respectively. Loess

**Table 2.** Suppression activities of organic emulsifiers against the growth of rice sheath blight (*R. solani*) at 3 days after placement on PDA medium *in vitro* bioassay.

Emulsifiers	Suppression activity* (%)						
	0.01%	0.05%	0.1%	0.5%	1%	3%	5%
Control	0.0 <sup>c</sup>	0.0 <sup>c</sup>	0.0 <sup>e</sup>	0.0 <sup>f</sup>	0.0 <sup>e</sup>	0.0 <sup>d</sup>	0.0 <sup>d</sup>
Natural emulsifier-A	-	-	7.4 <sup>d</sup>	23.5 <sup>e</sup>	38.2 <sup>d</sup>	48.5 <sup>c</sup>	70.6 <sup>c</sup>
Natural emulsifier-B	-	-	17.7 <sup>c</sup>	53.0 <sup>c</sup>	66.1 <sup>c</sup>	70.6 <sup>b</sup>	83.8 <sup>b</sup>
Loess sulphur	45.6 <sup>a</sup>	61.7 <sup>a</sup>	88.2 <sup>a</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>
Brown rice vinegar	-	-	23.5 <sup>c</sup>	44.1 <sup>d</sup>	75.0 <sup>b</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>
Insecticidal soap	26.4 <sup>b</sup>	57.4 <sup>b</sup>	72.0 <sup>b</sup>	86.8 <sup>b</sup>	97.0 <sup>b</sup>	100.0 <sup>a</sup>	100.0 <sup>a</sup>

\*Suppression activity was calculated relative to the colony diameter by comparison with the control. Means within a column followed by the same letters are not significantly different at 5% level according to the Duncan's multiple range test.

**Table 3.** Effect of organic emulsifier application to 20-day-old rice blast (*P. oryzae*)-infected cv. Ilmibyoo seedlings. Injury due to blast infection was evaluated at 3, 7, 11, and 15 days after treatment (DAT), while shoot height and fresh weight of rice were measured at 15 days after treatment.

Emulsifier concentration (%)	Rice injury* (%)			Shoot height (cm)		Shoot FW <sup>†</sup> (g/pot)	
	3 DAT	7 DAT	11 DAT	15 DAT	15 DAT	15 DAT	
Control	0	15.0 <sup>ab</sup>	35.6 <sup>a</sup>	79.0 <sup>a</sup>	90.6 <sup>a</sup>	22.0 <sup>c</sup>	1.5 <sup>b</sup>
Natural emulsifier A	1	5.0 <sup>cd</sup>	11.0 <sup>bc</sup>	31.7 <sup>bc</sup>	53.3 <sup>b</sup>	27.0 <sup>ab</sup>	6.3 <sup>a</sup>
	5	10.0 <sup>bc</sup>	17.7 <sup>bc</sup>	38.3 <sup>b</sup>	36.7 <sup>bcd</sup>	29.7 <sup>ab</sup>	6.0 <sup>a</sup>
	10	6.7 <sup>cd</sup>	11.7 <sup>bc</sup>	21.7 <sup>bcd</sup>	28.3 <sup>cd</sup>	29.0 <sup>ab</sup>	6.7 <sup>a</sup>
Natural emulsifier B	0.5	8.3 <sup>cd</sup>	8.3 <sup>c</sup>	38.3 <sup>b</sup>	48.3 <sup>bc</sup>	27.3 <sup>ab</sup>	6.1 <sup>a</sup>
	1	4.3 <sup>cd</sup>	13.3 <sup>bc</sup>	18.3 <sup>bcd</sup>	28.3 <sup>cd</sup>	28.3 <sup>ab</sup>	7.0 <sup>a</sup>
	3	4.0 <sup>cd</sup>	15.0 <sup>bc</sup>	18.3 <sup>bcd</sup>	28.3 <sup>cd</sup>	28.3 <sup>ab</sup>	6.4 <sup>a</sup>
Loess sulphur	0.5	16.7 <sup>a</sup>	25.0 <sup>ab</sup>	35.0 <sup>b</sup>	40.0 <sup>bcd</sup>	26.7 <sup>b</sup>	5.7 <sup>a</sup>
	1	3.0 <sup>d</sup>	14.0 <sup>bc</sup>	18.3 <sup>bcd</sup>	26.7 <sup>cd</sup>	28.3 <sup>ab</sup>	6.6 <sup>a</sup>
	3	4.3 <sup>cd</sup>	11.7 <sup>bc</sup>	16.7 <sup>bcd</sup>	25.0 <sup>cd</sup>	28.3 <sup>ab</sup>	7.5 <sup>a</sup>
Brown rice vinegar	0.5	8.3 <sup>cd</sup>	5.0 <sup>c</sup>	16.7 <sup>bcd</sup>	25.0 <sup>cd</sup>	28.0 <sup>ab</sup>	7.9 <sup>a</sup>
	3	5.0 <sup>cd</sup>	5.0 <sup>c</sup>	5.0 <sup>cd</sup>	16.7 <sup>d</sup>	30.3 <sup>a</sup>	8.3 <sup>a</sup>
	5	4.3 <sup>cd</sup>	9.0 <sup>c</sup>	8.3 <sup>cd</sup>	18.3 <sup>d</sup>	28.0 <sup>ab</sup>	7.8 <sup>a</sup>

\*Rice injury from the blast was evaluated visually (0-100; 0 = no damage, 100 = complete death). <sup>†</sup>FW, fresh weight. <sup>††</sup>Means within a column followed by the same letters are not significantly different at 5% level according to the Duncan's multiple range test.

sulphur and brown rice vinegar treatment at 3% resulted in 4.3, 11.7, 16.7, and 25.0%, and 5.0, 5.0, 5.0, and 16.7% injury, respectively, at 3, 7, 11, and 15 DAT. At 5% treatment, brown rice vinegar reduced blast injury to 4.3, 9.0, 8.3, and 18.3%, while natural emulsifier A reduced blast injury to 10.0, 17.7, 38.3, and 36.7% at 3, 7, 11, and 15 DAT, respectively. Increasing natural emulsifier A treatments to 10% resulted in 6.7, 11.7, 21.7, and 28.3% injury at 3, 7, 11, and 15 DAT. Although there were no differences in shoot height before 15 days (data not shown), treatment with organic emulsifiers increased shoot height compared to controls (Table 3). Interestingly, shoot height of plants treated with natural emulsifier A or B did not respond to increases in dosage, where shoots of plants treated with the lowest concentration of emulsifier (0.5 or 1%) were the same height as plants treated with the highest concentration of emulsifier (3 or 10%). Increases in loess sulphur concentration from 0.5% to 1% resulted in increased shoot height (Table 3), but there were no differences in shoot height of plants treated with 1 or 3% loess sulphur. Brown rice vinegar increased shoot height also, where shoots of plants treated with 3% brown rice vinegar were taller than plants treated with 0.5 or 5% brown rice vinegar. Overall, shoot height data suggests that increasing concentrations of organic emulsifier to treat blast-infected rice is more effective in reducing injury than increasing shoot height or fresh weight. Indeed, like shoot height, fresh weight of plants treated with organic emulsifiers was greater than the control plants, the concentration of emulsifier treatment did not result in any changes to fresh weight (Table 3). Although blast infection was 100% reduced when we used a conventional fungicide, 0.1% tricyclazol (Beam, data not shown) the results outlined above indicate that all the organic emulsifiers used in this study can inhibit the two rice fungal pathogens blast (*P. oryzae*) and sheath blight (*R. solani*) *in vitro* and blast pathogen *in planta*.

**Effect of organic emulsifiers on rice injury and growth:** Although emulsifier treatment resulted in decreased injury and increased shoot height and fresh weight (Table 3) in blast-infected rice, it is possible that application of the emulsifier itself can inhibit growth or cause injury. Therefore, emulsifiers were applied to healthy rice plants and injury and shoot height and fresh weight were analysed

at 7 DAT. To confirm if emulsifier treatments caused hidden stress to rice, we applied the same concentration of emulsifiers used in trials testing for *in planta* blast suppression (Tables 3 and 4) to 20-day-old seedlings of two rice cultivar plants: the blast susceptible cv. Ilmibyeo, and a blast-resistant cultivar cv. Dongjinbyeo.

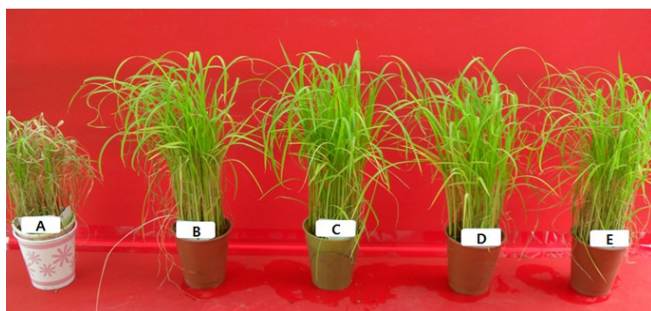
Ilmibyeo seedlings treated with natural emulsifier A, loess sulphur, and brown rice vinegar showed no injury at 7 DAT (Table 4). In contrast, treatment with 1 or 5% natural emulsifier B treatment resulted in 1.3% injury, while a concentration of 3% caused 0.7% injury. Insecticidal soap caused 5.0% injury to Ilmibyeo plants, and higher concentrations correlated with increased injury (Table 4). Insecticidal soap also caused the same percentage of injury in Dongjinbyeo seedlings, while treatment with the other emulsifiers showed no damage (Table 4). Shoot height of Ilmibyeo seedlings increased at 7 DAT in all emulsifiers used, while shoot height of plants treated with 1 and 3% loess sulphur and 5% insecticidal soap (Table 4) were not different compared to controls. Shoot height of Dongjinbyeo seedlings treated with 10% natural emulsifier A, 1% loess sulphur, and all concentrations of insecticidal soap were not significantly different compared to controls, while all other concentrations and emulsifier treatments increased shoot height (Table 4). Shoot fresh weight was enhanced in Ilmibyeo seedlings treated with 5% natural emulsifier A, 1 and 3% natural emulsifier B, 0.5 and 1% loess sulphur, and 1 and 5% brown rice vinegar. In contrast, shoot fresh weight of Dongjinbyeo plants increased only with 5% natural emulsifier A, 3 and 5% natural emulsifier B (Table 4).

Although injury appeared in both cultivars treated with insecticidal soap and in Ilmibyeo seedlings after natural emulsifier B treatment, the degree of damage was minor and did not affect shoot height and fresh weight (Table 4 and Fig. 2). Additionally, treatment using organic emulsifiers on blast-infected rice was effective in reducing injury caused by blast (Table 3) <sup>11</sup>. These data suggest that treatment with organic emulsifiers is a possible avenue of controlling fungal disease in rice <sup>8,9</sup>. Further study is also required to clarify the mechanisms underlying the enhancement of shoot height and fresh weight, and the suppression of fungal diseases in rice plants.

**Table 4.** Effect of organic emulsifier application to 20-day-old cv. Ilmibyeo and cv. Dongjinbyeo seedlings. Injury, shoot height, and shoot fresh weight were evaluated at 7 days after treatment (DAT).

	Emulsifier concentration (%)	Ilmibyeo			Dongjinbyeo		
		Rice injury* (%)	Shoot height (cm)	Shoot FW <sup>†</sup> (g/3plants)	Rice injury (%)	Shoot height (cm)	Shoot FW (g/3plants)
Control	0	0.0 <sup>e</sup>	36.7 <sup>f</sup>	0.70 <sup>b</sup>	0.0 <sup>d</sup>	32.7 <sup>fg</sup>	0.57 <sup>cd</sup>
Natural emulsifier A	1	0.0 <sup>e</sup>	40.0 <sup>bcd</sup>	0.83 <sup>ab</sup>	0.0 <sup>d</sup>	38.3 <sup>abc</sup>	0.69 <sup>abc</sup>
	5	0.0 <sup>e</sup>	42.7 <sup>ab</sup>	0.86 <sup>a</sup>	0.0 <sup>d</sup>	38.7 <sup>ab</sup>	0.71 <sup>ab</sup>
	10	0.0 <sup>e</sup>	40.0 <sup>bcd</sup>	0.81 <sup>ab</sup>	0.0 <sup>d</sup>	35.3 <sup>cdef</sup>	0.66 <sup>abcd</sup>
Natural emulsifier B	1	1.3 <sup>d</sup>	40.0 <sup>bcd</sup>	0.87 <sup>a</sup>	0.7 <sup>d</sup>	37.7 <sup>abcd</sup>	0.70 <sup>abc</sup>
	3	0.7 <sup>de</sup>	43.3 <sup>a</sup>	0.86 <sup>a</sup>	0.0 <sup>d</sup>	37.0 <sup>abcd</sup>	0.72 <sup>a</sup>
	5	1.3 <sup>d</sup>	40.3 <sup>bcd</sup>	0.79 <sup>ab</sup>	0.7 <sup>d</sup>	38.0 <sup>abcd</sup>	0.71 <sup>ab</sup>
Loess sulphur	0.5	0.0 <sup>e</sup>	39.3 <sup>cde</sup>	0.89 <sup>a</sup>	0.0 <sup>d</sup>	36.3 <sup>bcd</sup>	0.54 <sup>d</sup>
	1	0.0 <sup>e</sup>	39.0 <sup>def</sup>	0.85 <sup>a</sup>	0.0 <sup>d</sup>	35.0 <sup>efg</sup>	0.59 <sup>bcd</sup>
	3	0.7 <sup>e</sup>	37.3 <sup>ef</sup>	0.79 <sup>ab</sup>	0.7 <sup>d</sup>	37.7 <sup>abcd</sup>	0.59 <sup>abcd</sup>
Brown rice vinegar	1	0.0 <sup>e</sup>	40.0 <sup>bcd</sup>	0.88 <sup>a</sup>	0.0 <sup>d</sup>	37.3 <sup>abcd</sup>	0.64 <sup>abcd</sup>
	3	0.0 <sup>e</sup>	41.3 <sup>abcd</sup>	0.83 <sup>ab</sup>	0.0 <sup>d</sup>	38.7 <sup>ab</sup>	0.64 <sup>abcd</sup>
	5	0.0 <sup>e</sup>	42.0 <sup>abc</sup>	0.90 <sup>a</sup>	0.0 <sup>d</sup>	39.7 <sup>a</sup>	0.68 <sup>abc</sup>
Insecticidal soap	1	5.0 <sup>c</sup>	39.3 <sup>cde</sup>	0.77 <sup>ab</sup>	5.0 <sup>c</sup>	33.7 <sup>efg</sup>	0.62 <sup>abcd</sup>
	3	10.0 <sup>b</sup>	39.7 <sup>cde</sup>	0.80 <sup>ab</sup>	10.0 <sup>b</sup>	32.3 <sup>g</sup>	0.59 <sup>bcd</sup>
	5	15.0 <sup>a</sup>	38.7 <sup>def</sup>	0.71 <sup>b</sup>	15.0 <sup>a</sup>	33.3 <sup>fg</sup>	0.58 <sup>cd</sup>

\*Rice injury under the emulsifier treatment was evaluated visually (0-100; 0 = no damage, 100 = complete death). <sup>†</sup>FW, fresh weight. <sup>‡</sup>Means within a column followed by the same letters are not significantly different at 5% level according to the Duncan's multiple range test.



**Figure 2.** Effect of organic emulsifiers in suppressing injury in blast (*P. oryzae*)-infected seedlings (cv. Ilmibyeo).

Emulsifier treatment was applied to 20-day-old plants harbouring 20% infection by rice blast. Photographs were taken at 15 days after treatment. Shown are untreated plants (A), and plants treated with 10% natural emulsifier A (B), 10% natural emulsifier B (C), 3% loess sulfur (D), and 3% brown rice vinegar (E).

### Conclusions

The data clearly show that the emulsifiers are strongly effective to control the rice blast pathogen *P. oryzae* and sheath blight pathogen *R. solani* *in vitro* bioassays, and *P. oryzae* *in planta*. Furthermore, it is highly considerable that synergistic effectiveness between controlling these diseases and enhancing rice growth would be detected. Therefore, these results indicate that the emulsifier application would lead to reduce fungicide usage in rice and support sustainable and robust agriculture.

### Acknowledgements

This work was carried out with the support of “Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ008423)” Rural Development Administration, Republic of Korea. The authors would like to thank Dr. Sheena Gayomba for helpful discussion concerning the writing of the manuscript.

### References

- <sup>1</sup>Yang, D., Wang, B., Wang, J., Chen, Y. and Zhou, M. 2009. Activity and efficacy of *Bacillus subtilis* strain NJ-18 against rice sheath blight and *Sclerotinia* stem rot of rape. *Biol. Control* **51**:61–65.
- <sup>2</sup>Li, B., Liu, B. P., Yu, R. R., Lou, M. M., Wang, Y. L., Xie, G. L., Li, H. Y. and Sun, G. C. 2011. Phenotypic and molecular characterization of rhizobacterium *Burkholderia* sp. strain R456 antagonistic to *Rhizoctonia solani*, sheath blight of rice. *World J. Microb. Biot.* **27**:2305-2313.
- <sup>3</sup>Skamnioti, P. and Gurr, S. J. 2009. Against the grain: Safeguarding rice from rice blast disease. *Trends Biotechnol.* **27**:141–150.
- <sup>4</sup>Saikia, R., Gogoi, D. K., Mazumder, S., Yadav, A., Sarma, R. K., Bora, T. C. and Gogoi, B. K. 2011. *Brevibacillus laterosporus* strain BPM3, a potential biocontrol agent isolated from a natural hot water spring of Assam, India. *Microbiol. Res.* **166**:216-225.
- <sup>5</sup>Paster, N. and Bullerman, L. B. 1988. Mould spoilage and mycotoxin formation in grains as controlled by physical means. *Int. J. Food Microbiol.* **7**:257–265.
- <sup>6</sup>Padaria, J. C. and Singh, A. 2009. Molecular characterization of soil bacteria antagonistic to *Rhizoctonia solani*, sheath blight of rice. *J. Environ. Sci. Heal. B.* **44**:397-402.
- <sup>7</sup>Avis, T. J. 2007. Antifungal compounds that target fungal membranes: Applications in plant disease control. *Can. J. Plant Pathol.* **23**:323-329.
- <sup>8</sup>Gomiero, T., Pimentel, D. and Paoletti, M. 2011. Environmental impact of different agri-cultural management practices: conventional vs. organic agriculture. *Crit. Rev. Plant Sci.* **30**:95–124.
- <sup>9</sup>Dayan, F. E., Cantrell, C.L. and Duke, S.O. 2009. Natural products in

crop protection. *Bioorganic Medicin. Chem.* **17**:4022-4034.

- <sup>10</sup>Choi, G. J., Kim, J. C., Jang, K. S., Lim, H. K., Park, I. K., Shin, S. C. and Cho, K. Y. 2006. *In vivo* antifungal activities of 67 plant fruit extracts against six plant pathogenic fungi. *J. Microbiol. Biotechnol.* **16**:491-495.
- <sup>11</sup>Daly, M. J. and Stewart, D. P. C. 1999. Influence of “Effective Microorganism” (EM) on vegetable production and carbon mineralization a preliminary investigation. *J. Sustain. Agric.* **14**:15–25.
- <sup>12</sup>Nicetic, O., Watson, D. M. and Beattie, G. A. C. 2002. A horticultural mineral oil-based program for control of two spotted mite and powdery mildew on roses in greenhouses. In Beattie, G. A. C., Watson, D. M., Stevens M. L., Rae, D. J. and Spooner-Hart, R. N. (eds). *Spray Oils Beyond 2000*. University of Western Sydney, pp. 387–395.
- <sup>13</sup>Henn, T. and Weinzierl, R. 1989. Botanical insecticides and insecticidal soaps. Alternatives in insect management. Office of Agricultural Entomology, University of Illinois at Urbana-Champaign, College of Agriculture, Cooperative Extension Service, in cooperation with the Illinois Natural History Survey. Circular 1296. 18 p.
- <sup>14</sup>Jansen, J. P., Defrance, T. and Warnier, A. M. 2010. Effects of organic-farming-compatible insecticides on four aphid natural enemy species. *Pest Manag. Sci.* **66**:650-656.
- <sup>15</sup>Statistical Analysis System [SAS] 2000. *SA/STAT User’s Guide, Version 7*, Statistical Analysis System Institute, Electronic Version, Cary, NC.