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***Bacillus megaterium* Suppresses Major Thailand Rice Diseases**

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Abstract

The fungal disease blast caused by *Pyricularia oryzae* and sheath blight disease caused by *Rhizoctonia solani* are major diseases of rice in Thailand. Recently emerging of dirty panicle disease is contributing to an additional challenge to the rice disease panorama. Dependence on fungicidal sprays for disease control adds expense to farmers and contributes to non-target damage from fungicides employed. No single rice variety has high levels of genetic resistance to all major rice diseases. Because of these challenges and gaps in the disease management strategy, integrated disease management that employs biological control measure is becoming increasingly attractive for rice producers. Biological control product formulated based upon *Bacillus megaterium* has been produced and tested in the rice field for its ability to control blast and sheath blight. During off season rice testing in Uttaradit province, Thailand, water-soluble granules showed a high level of disease efficacy for controlling sheath blight, rice blast and dirty panicle disease. Applications were made as a seed treatment once (at 1 g formulation/100 g seed), followed by 3 sprays at the seedling, tillering and flowering stages. The application rate was 200 g formulation/20 L water/rai application rate. The water-soluble granules of *B. megaterium* show potential to increase yield of rice when weed populations are kept under control thresholds and recommended amount of N-fertilizer was applied. Besides the results with the 3 aforementioned diseases, this biological control option has selected to be tested for neck blast (*P. oryzae*) efficacy. Testing results are being disseminated to local and regional farmers who are interested to produce rice organically and reduce their fungicide use.

Keywords: Strain improvement, Ultraviolet radiation, *Xenorhabdus* sp.

Introduction

Both insect pests and plant diseases have always been the limiting factors for rice production in countries where rice is cultivated extensively (Thurston, 1984). These pest problems are persisting although rice varieties with resistant characteristics have been bred and put into usage. Farmers are thus dependent upon pesticide application to combat pests although this action may cause the re-emergence of pest problems as a result of a built-up of pesticide resistance in the pest population (Dekker, 1977). A hazard to farmer's health has also become to be a major issue as numerous case of pesticide-related sickness has been reported among working youths (Calvert et al. 2003).

Pest control measure with no side effects to human health and environment has become to be a crucial component in crop production (Cook and Baker 1983). Plant extracts and microorganisms have gradually replaced chemical pesticides in pest control (Stephan et al. 2005). However, the widespread application of these natural entities is still hindered by the limited availability of the products with suitable practical characteristics (Burgess and Jones, 1998).

If microorganisms are intended to use for pest control, they must be in a form that is easily applicable by the farmers. The microorganisms must still be active, after being formulated, transported and stored, and must still be effective in controlling pests upon usage by the farmers in the real situation (Burgess and Jones, 1998). There are numerous reports of microbial formulation designed for plant disease control (Cook, 1993; Burgess and Jones, 1998). In Thailand, the focus is in the development of effective and practical bacterial formulation for controlling fungal diseases of rice (Wiwattanapatapee et al. 2007)

Formulations of the bacterium, *Bacillus megaterium*, have been developed and undergone both greenhouse and small field trial (Wiwattanapatapee et al. 2007; Chumthong et al. 2008). The formulations of this bacterium are effective in suppressing sheath blight and rice blast. This paper reports a field trial of the efficacy of the water-soluble formulation containing *B. megaterium* to control sheath blight, rice blast and dirty panicle for off season rice at Uttaradit province in the North of Thailand.

Materials and Methods

Water-soluble formulation containing B. megaterium

This formulation was produced using pharmaceutical technology. This formulation was prepared as described by Chumthong et al. (2008).

Testing site, rice paddy preparation and rice variety

Three volunteered farmers in Tambol Had-Kuat, Muang District, Uttaradit province participated in the project. Each farmer was asked to prepare a 1600 m² paddy field and they were requested to partition this field into 4 equal blocks with soil embankment to prevent water movement between blocks. The central area (approximately 100 m²) of each block was a testing site where sheath blight pathogen, *Rhizoctonia solani*, was inoculated and the additional 3-sprays at the rate of 200 g formulation/20 L water/rai were carried out.

Pitsanulok-2 was a rice variety that was used as a testing plant in this study. This variety was popular in the area but it had been reported to be susceptible to rice blast and neck blast caused by *Pyricularia oryzae*. Each farmer was instructed to employ a normal practice in planting rice as they had done in the preceding seasons.

First, this was to ensure that, if proved effective and accepted for use, the water-soluble formulation product could be integrated into the rice production system. Second, although each farmer may have followed a similar cultural practice in growing rice, they may have employed a slightly different technique (such as different in formula, frequency and rate of the fertilizer used, input in weed control and application of other plant growth promoting agents) in tending to their crop. These practices may have affected the efficacy of the formulation applied.

Pathogen inoculation

For sheath blight disease, artificially inoculation of the *Rhizoctonia solani* to rice plants was carried out 59 days after sowing. Inoculum of *R. solani* was prepared as described by Wiwattanapatapee et al. (2007). Ten rice plants in the each block were randomly inoculated with an inoculum of *R. solani*. Rice plants with sheath blight symptom were randomly sampled for assessing the incidence and severity of sheath blight disease. For rice blast and dirty panicle diseases, no artificially inoculation was conducted and these diseases were the result of infection from a natural inoculum of each disease.

Application of water-soluble formulation and Experimental design

Before sowing, seeds of Pitsanulok-2 variety were mixed with the water-soluble formulation (at 1 g formulation/100 g seed) in water and incubated overnight. Subsequently, the excessive water was drained and the seeds were then incubated in the perforated plastic bag for 2 days. The germinating rice seeds were subsequently sown into each paddy field which was partitioned into 3 blocks, each with the area of 400 m².

Control block was sown with rice seeds receiving no water-soluble formulation and the rice plants in this block received no additional sprays of water-soluble formulation.

For the additional sprays, there were 3 treatments for each farmer, including the rice plants with received either 1 spray (60 days after sowing), 2 sprays (at both 60 days and 67 days after sowing), or 3 sprays (at both 60 days, 67 days and 90 days after sowing).

Data collection and data analysis

Seven days after the first spray, three rice plants from each block (of each farmer) were randomly sampled for assessing sheath blight disease. Both a number of infected tillers and the length of the sheath blight lesion were counted and measured respectively.

For blast disease, blast lesions were counted and the severity of blast was assessed based upon the method of IRRI (2002). Twenty-one days after the second spray, another 3 rice plants from each block (of each farmer) were again randomly sampled for assessing both sheath blight and rice blast. Sixteen days after the third spray, another 3 rice plants from each block (of each farmer) were randomly sampled for assessing both rice blast and dirty panicle disease. Degree of dirty panicle disease was arbitrary established for assessing the disease severity.

At 120 days after sowing, rice plants (in the 9 m² where rice plants received additional sprays) were manually harvested and rice grains were weighted to determine the effect of water-soluble formulation in increasing yield of rice. Sheath blight disease incidence (number of infected tillers/plant), sheath blight disease severity (relative lesion height, RLH), blast disease incidence (number of lesions/leaf), disease severity (score of disease severity), dirty panicle

severity (score of disease severity) were evaluated and means of these values were compared with Duncan's Multiple Range Test between block of each farmer.

Results

The efficacy of the water-soluble formulation in suppressing sheath blight, rice blast and dirty panicle for each farmer was presented in Table 1. The water-soluble formulation of *B. megaterium* was effective in reducing %sheath blight disease incidence (in Farmer A and B case), sheath blight severity (RLH) (after the 1 spray in Farmer A, B and C case), number of lesion/leaf (after the 1 and the 2 spray in Farmer C case) and dirty panicle severity (after the 1, the 2 and the 3 spray in Farmer A and C case). The formulation, however, had no effect in suppressing %sheath blight disease incidence, number of lesion/leaf and dirty panicle severity (in Farmer B case).

Table 1 Efficacy of the water-soluble formulation containing *B. megaterium* in rice disease suppression

Treatment	%Sheath blight incidence ⁽¹⁾	RLH ⁽²⁾	Number of lesions/leaf ⁽³⁾	Number of lesions/leaf ⁽⁴⁾	Score of dirty panicle severity ⁽⁵⁾
Farmer A					
1. Control (A1)	73.9±1.0 a	26.6±3.4 a	7.3±1.2 c	4.8±0.2	0.1 b
2. Block A2	18.1±1.9 c	5.0±0.9 c	14.2±1.4 b	5.4±2.6	0.4a
3. Block A3	31.7±2.9 b	8.8±2.2 bc	16.4±1.6 b	3.3±0.1	0.1b
4. Block A4	35.0±5.0 b	9.9±0.7 b	24.8±1.1 a	4.4±1.0	0.08c
F-test	*	*	*	ns	*
CV (%)	7.8	16.7	8.3	38.2	36.6
Farmer B					
1. Control (B1)	100.0±0.0	36.8±4.1 a	10.4±2.2 ab	3.8±1.1	0.1b
2. Block B2	100.0±0.0	37.0±0.9 a	9.4±2.9 ab	3.9±0.6	0.07c
3. Block B3	100.0±0.0	29.9±1.5 b	8.9±1.8 b	5.7±3.7	0.5a
4. Block B4	100.0±0.0	11.6±2.3 c	13.5±1.4 a	3.9±0.5	0.2b
F-test	ns	*	*	ns	*
CV (%)	0.0	8.7	20.4	56.0	21.0
Farmer C					
1. Control (C1)	100.0±0.0 a	40.1±2.6 a	8.4±1.6 a	4.9±0.7 a	1.0a
2. Block C2	62.6±2.5 b	21.4±2.4 b	6.6±0.6 ab	5.1±0.8 a	0.0c
3. Block C3	12.5±1.8 d	2.2±0.4 c	5.9±1.6 b	2.7±0.3 b	0.0c
4. Block C4	56.1±1.4 c	15.9±1.6 d	4.7±0.2 c	3.5±0.0 c	0.2b
F-test	*	*	*	*	*
CV (%)	2.9	9.8	18.5	16.6	19.9

ns = Means the same column are not statistical significantly different

* Means followed by the same letter are not significantly different by Duncan's Multiple Range Test at $p < 0.05$

^{(1), (2)} Results of the water-soluble formulation in sheath blight disease suppression after the 1 spray

^{(3), (4)} Results of the water-soluble formulation in blast disease suppression after the 1 and 2 spray, respectively

⁽⁵⁾ Results of water-soluble formulation in dirty panicle disease suppression after the 3 spray

Discussion

The water-soluble formulation was reported to be effective in suppressing sheath blight and blast diseases in the greenhouse and small field trials (Chumthong et al. 2008). This field trial has re-affirmed the positive result of the efficacy of this formulation carried out in the preceding greenhouse and small field experiment.

Nevertheless, in the larger and open field trial with comparatively less-regulated and lesser-controlled environment, the efficacy of this formulation, however, was dependent upon the cultural practices of each farmer and other biotic and a-biotic factors. Rice plants in Farmer B block had been given with unknown plant hormone and had been fertilized too much (with N at 19.25 Kg/rai against the recommended rate N at 8.6-14.0 kg/rai). This action may have nullified the efficacy of the formulation to control rice diseases. Application of high N fertilizer will make rice plants susceptible to rice blast and other diseases (Thurston, 1984). Although high N fertilizer application was intended to increase rice yield, this action may have caused lodging and yield reduction (Basak et al. 1962). The average rice yield from Farmer A, B, and C block was almost the same although Farmer A and B had applied relatively 50% less N fertilizer than Farmer B had.

Yield of rice from Farmer A which received 3 sprays was 1 kh higher than that of other treatments when the rice yield was assessed from the area of 9 m² (data not shown). This may be a result of the growth promotion capacity of the formulation if rice paddy is well managed in weed control and proper use of N-fertilizer. Yield of rice from Farmer B and Farmer C did not increase when the plants were sprayed. Farmer B had high degree of rice lodging while Farmer C did not keep weed population under control. However, more research with respect to the growth- and yield-enhancing capacity mechanism of this bacterial formulation is needed (López-Bucio et al. 2007).

Conclusions

Ultraviolet radiation has shown to be beneficial for improving the efficacy of *Xenorhabdus* sp.(X1) for controlling mites, and the obtained information can be used for bacterial strain development for industrial scales. In the future, the Mutant RP58 may be further improved by other mutagens for enhancing its efficacy and consistent virulence to mushroom mite.

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