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Rice blast management in Cambodian rice fields using *Trichoderma harzianum* and a resistant variety

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ABSTRACT

Rice blast (*Pyricularia oryzae* Cavara) is one of the most devastating diseases affecting the rice crop in Cambodia and other countries in the world. The fungus *Trichoderma* spp. is known as one of biological control agents applied as a soil treatment, seed treatment and foliar application, that is used for suppression of various diseases caused by fungal pathogens. *Trichoderma harzianum* strain BTB 022 is one of the commercial biological control products available in Cambodian markets. The combined use of *T. harzianum* and a resistant variety, to manage blast disease, are considered as sustainable approaches to reduce yield losses and to cope with recent restrictions on fungicide use. A series of consecutive experiments was conducted to examine the effectiveness of *T. harzianum* on suppression of rice blast incidence in Koktrap and Polors agricultural research stations during wet and dry seasons in 2016 and 2017. In both years, the treatments consisted of the use of *Trichoderma* on susceptible and resistant rice varieties. In 2017 the two treatments were combined with conventional practice treatments representing the average farmers' practice. The experiments were arranged in randomized complete block design with three replications in 2016 and four replications in 2017. Leaf blast incidence was assessed at five and four growth stages in 2016 and 2017, respectively, and the area under the leaf blast progress curve (AULBPC) was computed for each year and location. Neck blast (NB) incidence was assessed at dough stage and grain yield (GY) was measured at ripening stage. *T. harzianum* reduced the incidence of leaf blast and neck blast on IR504 (susceptible strain), but its efficacy was not consistent. The magnitude of disease suppression by *T. harzianum* was higher for neck blast than for leaf blast. GY variation was correlated with AULBPC and NB incidence, which suggests that disease reduction corresponded to an increase in yield (AULBPC: $r = -0.877$, $P < 0.001$; NB incidence: $r = -0.567$, $P < 0.001$). *T. harzianum* effectively reduced neck blast at high disease pressure. Growing a resistant variety, e.g. CAR14, effectively reduced AULBPC and NB incidence compared to *T. harzianum* and farmers' practice of fungicidal use but the association of *T. harzianum* and resistant variety did not increase the effect in the control of disease.

1. Introduction

Rice (*Oryza sativa*) is the staple food in Cambodia. It constitutes over 80% of total crop production area (Beecher et al., 2014) and is the main source of income for 85% of rural households (Tong et al., 2013; Dary et al., 2017). Rice production in the country has increased rapidly during the recent decade. Cultivated rice area increased from 2,585,905 ha in 2007 (Ministry of Agriculture, Forestry and Fisheries, MAFF, 2011) to 3,118,143 ha in 2016 (MAFF, 2017). However, rice production in Cambodia is still constrained by several insect pests and diseases. One of the most devastating diseases is rice blast which is caused by the fungal pathogen *Pyricularia oryzae* Cavara (syn. *Magnaporthe oryzae* B. C. Couch). Leaf blast causes elliptical lesions on the leaves during the vegetative and reproductive phase (Bastiaans, 1991) and neck blast causes grain sterility and reduces grain size, yield and quality traits of seeds (Khan et al., 2014). The annual yield loss is about 25% of total

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production in Japan (Ribot et al., 2008) and at around US$ 55 million in South and Southeast Asia (Kuyek, 2000). However, rice blast can be managed by the use of resistant cultivars, chemical pesticides, agronomic practices and biotechnological methods (Ribot et al., 2008).

Deployment of blast resistant genes is one of the alternative management strategies (Sharma et al., 2012). CAR 14 (IR06L164), a rice variety with resistance to rice blast and high grain yield, was released in Cambodia in 2015 (Zhao et al., 2016; Cambodian Agricultural Research and Development Institute, CARDI, 2017). Although the use of resistant varieties is considered as the most practical and economical approach to manage blast, Cambodian farmers who export rice to Vietnam prefer to grow susceptible varieties e.g. "IR50401-77-2-1-3 (IR504) due to its demand in the market (CARDI, 2017). Farmers usually control blast on this variety through prophylactic and calendar-based application of fungicide. The widespread availability and ease to use of chemical fungicide further increase the popularity of this disease management option (Flor et al., 2018).

However, widespread and long-term use of fungicides, particularly those with the same mode of action, cause resistant fungal populations (Deising et al., 2002), and also have an adverse effect on the soil and water quality of rice ecosystems (Phong et al., 2009; Wandscheer et al., 2017), grain quality (Dors et al., 2011; Zhang et al., 2015; Telo et al., 2015), and human health (Pingali, 1995). Recently, the European Union, which accounts for 63% of rice exported by Cambodia, has tightened regulations for the use of the fungicide, tricyclazole in rice, by reducing the maximum threshold limit from 1 to 0.01 mg per kg (Arora et al., 2014; Nader et al., 2014). An alternative approach to sustainably manage blast is necessary to reduce the reliance of farmers on fungicides. These strategies should include the use of biological control agents (BCAs) and their integration with the use of resistant varieties. BCAs can suppress the incidence or severity of agricultural pests and diseases without leaving harmful residues (Elad, 2000; Gupta and Dikshit, 2010; Kawalekar, 2013; Katti, 2013; Kamble et al., 2016). The fungus, *Trichoderma* spp., is being used as a BCA against various plant pathogens (Harman, 2006; Schuster and Schmoll, 2010). In Cambodia, a powder formulation of *T. harzianum* BTB 022 is commercially available, and some programs promote its use as bio-fungicide to control fungal diseases and improve the yield of vegetables and other major crops. Although *Trichoderma* spp. showed 100% growth inhibition of *P. oryzae* under laboratory conditions (Prabhakaran et al., 2013), it is necessary to conduct field testing of *Trichoderma* spp. as a biocontrol agent of rice blast.

In this study, a series of on-station experiments was conducted to investigate the effectiveness of the rice variety CAR14 with resistance against rice blast, and a commercially available *T. harzianum* formulation as a biological control agent to suppress rice blast incidence under field conditions during wet and dry seasons. Experiments were conducted in Prey Veng and Svay Rieng provinces of Cambodia in 2016 and 2017.

2. Materials and methods

2.1. Experimental location

Field experiments were conducted at two agricultural research stations; Koktrap and Polors. The Koktrap Agriculture Research Station is located in Svay Rieng province (Latitude: Longitude - 11.12245: 105.67060), with Koktrap soil occurring on the old alluvial terraces, which has a dark gray to very dark brown topsoil with a clayey or loamy texture; the Polors Agriculture Research Station is located in Prey Veng Province (11.16326: 105.42856) with Prateah Lang soil occurring on the old alluvial terraces or the colluvial-alluvial plains, which has a sandy topsoil less than 40 cm thick over a subsoil with a loamy or clayey texture (White et al., 1997, 2000; Ngin et al., 2017). Svey Rieng and Prey Veng provinces are located in southeastern Cambodia and are considered as two of the major rice-growing areas of the country; the monthly temperature and rainfall in the two provinces were shown in Fig. 1 (MAFF, 2017). As in most intensively cropped areas, many of the rice farmers use high seeding rates and high amounts of chemical pesticides to control pests. There is a perception among farmers that these practices are associated with higher yields (Ngin et al., 2017).

2.2. Experimental design and treatments

The experiments were conducted in the wet season (WS) and dry season (DS) of 2016 and 2017 using two rice varieties, IR504 as a susceptible variety and CAR14 as a resistant variety. CAR14 (IR06L164) was grown in the dry and wet season, and it is characterized as resistant against rice blast (CARDI, 2017; Zhao et al., 2016). IR504 (IR50401-77-2-1-3) is widely grown in Cambodia, and it is susceptible to rice blast (Luu and Bui, 1999). The seeds of IR504 were acquired from AQIP Seed Company and local seed distributors. The CAR14 seeds were acquired from CARDI. The treatments and number of replications vary with the season and are summarized in Table 1. Each sample was collected from 10 randomly selected quadrats (10 cm × 10 cm) within 10 m × 10 m plots in size.

Formulated *T. harzianum* BTB 022 (Td) has been commercialized in Cambodia by GMF4 Company (Td biological control agent, 1×10^7 CFU). In the treatments involving Td (Table 1), two hundred and fifty grams of powdered form Td was used to treat 20 kg of rice seeds. Additionally, Td was also foliarly applied four times a season at the rate of 20 g/20 L of water. The timing of applications followed the manufacturer's recommendation: at 20 days after sowing (DAS) coinciding with early tillering stage, at 30 DAS, at booting stage and, finally, at the dough stage. Plastic sheets were used around the fields during foliar application of Td and other treatments to avoid drift across treatments.

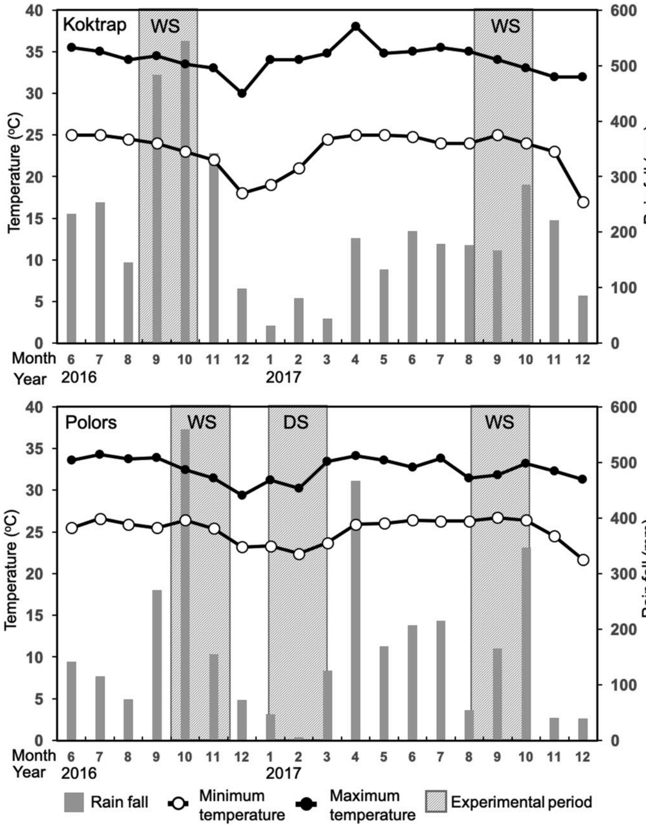

Fig. 1. Monthly temperature and rainfall in Svay Rieng province where Koktrap is located (upper panel) and in Prey Veng Province where Polors is located (lower panel). WS, wet season; DS, dry season. Closed circles, open circles and gray columns indicate the mean value of each month.

Table 1
Description of experimental treatments.

Year	Season	Location	Number of quadrats[a]	Number of replications	Total sample size	Treatments
2016	Wet	Koktrap and Polors	10	3	30	BIPM[b]+CAR14 + Trichoderma
			10	3	30	BIPM[b]+IR504 + Trichoderma
			10	3	30	BIPM[b]+CAR 14+ (No Trichoderma)
			10	3	30	BIPM[b]+IR 504+ (No Trichoderma)
2017	Dry	Polors	10	4	40	Same as above with Conventional practice
	Wet	Koktrap and Polors	10	4	40	Same as above with Conventional practice

[a] One quadrat was constructed from 10 cm × 10 cm. Total sample size in each treatment was composed of 10 quadrats multiplied by replication number.

[b] BIPM: Basic Integrated Pest Management which stands for treatment with fertilizer and twice weed management without pesticide rodenticide nor molluscicide, as described in text.

Moreover, dikes were constructed around each replicated plot to manage the water level within the plots.

In the $T_{1\text{-}4}$ (Table 1), approximately 100 kg/ha of certified seeds were row-seeded using a drum seeder with a 20 cm row spacing. Fertilizer was applied according to soil type as based on the technical recommendation by CARDI (White et al., 1997). For weed management, the herbicide Sofit 300 EC (Pretilachlor: 300 g/L + Fenclorim: 100 g/L) was applied at two DAS and followed by the post emergence herbicide Nominee 10SC (Bispyribac-Sodium: 100 g/L) at 15 DAS. No insecticide, rodenticide nor molluscicide was applied in $T_{1\text{-}4}$ because pest pressure was minimal.

The conventional practice treatment (T_5) was added in 2017. The input in this treatment was defined as based on the information gathered in a survey of 40 farmers in Prey Veang Province. Based on the survey, T_5 consists of a chemical fungicide application (Trade name of Hanovil with Hexaconazol as the active ingredient) at 15 DAS and 30 DAS. IR504 was used in T_5, established by hand-broadcasting using a very high seed rate (335 kg/ha).

2.3. Data collection

Across all treatments, leaf blast was assessed in ten randomly selected quadrats (10 cm × 10 cm) per plot by counting the total number of leaves and diseased leaves at the tillering, panicle initiation, booting and dough stage. Neck blast was assessed by counting the number of tillers and diseased panicles in the sampled quadrats at the dough stage. The incidence of leaf blast was computed as the percentage of leaves with lesions, whereas neck blast was computed as the percentage of panicles with lesions on the neck.

In all trials, the area under the leaf blast progress curve (AULBPC), was computed using the data obtained from all of the growth stages. AULBPC was computed to take into account variation in time of the incidence, the rate of increase of disease incidence, and final disease incidence (Shaner and Finney, 1977; Campbell and Madden, 1990).

Grain yield of each plot was measured from two randomly designated crop cut areas, each measuring 2 m × 2.5 m. Sampled grains from each crop cut area were separately harvested, threshed and cleaned. The grains were dried to approximately 14–20% moisture content. The moisture content of the grains from three separate portions of total bulk grain was measured using a grain moisture tester (Riceter f521, Belt and Bearings, Japan) and grain yield estimation at 14% moisture content was calculated. The reported yield data is the average of the two crop cut areas for each plot.

2.4. Statistical analysis

For each year/season-location combination, the incidences of leaf blast and neck blast as well as yield data were compared across the treatments using an analysis of variance (ANOVA) followed by a multiple comparison test (Tukey-Kramer test). In the multiple comparison test, the 2016 and 2017 data were analyzed separately due to the difference in the number of treatments. No experiment was conducted in Koktrap in dry season 2017, thus the data for dry and wet season of 2017 were analyzed separately. All statistical tests were performed using the Statistical Package for Social Sciences (IBM SPSS Statistics, ver. 22.0).

3. Results

3.1. Variation of the AULBPC, blast incidence and yield

In the 2016 and 2017 wet season, analysis of variance showed a significant effect of the treatment on the blast disease AULBPC with P value < 0.0001 (Table 2); there was no significant difference in AULBPC between the field location (ANOVA, $P = 0.9247$ and 0.8502). Two-way interaction among "Location x Treatment" significantly affected AULBPC ($P < 0.001$). A similar tendency was obtained in the NB incidence and yield. In the dry season, however, all of AULBPC, NB incidence and yield indicated no significant difference. Overall variation in the AULBPC, NB incidence and Yield are mainly governed by the treatment.

Table 2
Analyses of variance for the area under leaf blast progress curve (AULBPC), NB incidence and yield.

Variable	Source of variation	DF	Mean square	F value	p value
2016 Wet season					
AULBPC	Location	1	622	0.01	0.9247
	Treatment	3	1199861	26.78	<0.0001
	Location x Treatment	7	1236042	10.76	<0.0001
NB incidence	Location	1	237	1.35	0.2580
	Treatment	3	1097	26.80	<0.0001
	Location x Treatment	7	578	145.15	<0.0001
Yield	Location	1	191531	0.48	0.4943
	Treatment	3	2745184	80.96	<0.0001
	Location x Treatment	7	1217479	49.78	<0.0001
2017 Dry season					
AULBPC	Treatment	4	86235	3.12	0.0472
NB incidence	Treatment	4	2	3.69	0.0276
Yield	Treatment	4	139538	3.19	0.0438
2017 Wet season					
AULBPC	Location	1	1124	0.04	0.8502
	Treatment	4	273729	110.07	<0.0001
	Location x Treatment	9	1139878	90.30	<0.0001
NB incidence	Location	1	663	6.12	0.179
	Treatment	4	825	19.58	<0.0001
	Location x Treatment	9	472	26.61	<0.0001
Yield	Location	1	67506	0.79	0.3701
	Treatment	4	322951	5.57	0.0014
	Location x Treatment	9	171926	2.91	0.0134

DF, degree for freedom; AULBPC, area under leaf blast progress curve; NB, neck blast.

3.2. AULBPC

At both stations, during wet season 2017, the application of *T. harzianum* reduced AULBPC on IR504 but not on CAR14. No effect of Td treatment on AULBPC was detected on CAR14 across locations, seasons and year (Table 3). In WS of 2017, Td-treated susceptible variety (IR504) had significantly lower AULBPCs. Our data indicates that the use of Td does not affect AULBPC on a resistant variety but it can significantly reduce AULBPC on a susceptible variety (e.g. Wet season data for 2017 for both locations). Plots with conventional practice (i.e. use of susceptible variety with fungicide application) consistently had significantly higher AULBPCs compared to the plots using a resistant variety. Td-treated susceptible variety had lower or similar level of AULBPC compared to conventional practice, but a statistically significant difference can be detected between these two treatments.

3.3. Leaf blast incidence

In Koktrap station (KT) during wet season 2016, Td treatments did not affect leaf blast incidence in both rice varieties (Fig. 2A). However, higher incidence of blast disease was observed on the susceptible variety during PI and dough stages compared to the resistant variety. No significantly different rates of LB incidence were observed between IR504 and CAR14 in the seedling and booting stages (Fig. 2A).

In Polors station during wet season 2016 (Fig. 2A), significantly higher LB incidences were observed on IR504 compared to CAR14 during tillering, booting and dough stages. Td treatments had no significant effect on LB incidences on both varieties. However, numerically lower LB incidences were observed on Td-treated plots of IR504 compared to those without Td treatments.

In dry season of 2017, the leaf blast incidence was very low compared to the wet seasons (Fig. 2B). No significant difference was observed in leaf blast incidence across all treatments at tillering stage. During panicle initiation and booting stages, the only significant difference was observed between conventional practices and Td-treated resistant variety. Leaf blast incidence in wet season of 2017 at Koktrap was the highest on IR504 without Td at tillering and panicle initiation stages (Fig. 2B). At all observation periods, use of a resistant variety reduced leaf blast incidence compared to the susceptible variety and conventional practice. Application of Td significantly reduced leaf blast incidence on the susceptible variety at tillering, panicle initiation and booting stages but not on the resistant variety. Conventional practice plots showed significantly lower blast incidence compared to the susceptible variety without Td. However, when Td was used on the susceptible variety, there was no significant difference in leaf blast incidence compared to conventional practice.

In wet season of 2017 at Polors, we observed higher leaf blast incidences on IR504 compared to CAR14, especially in the tillering and panicle initiation stages (Fig. 2B). During the same observation periods, we did not observe significant differences in leaf blast incidence between Td treated and no Td treated plots in both varieties. Plots with conventional practices showed no significant difference in leaf blast incidence compared to IR504 plots. However, plots using CAR14 had significantly lower blast incidence compared to conventional practice plots (Fig. 2B). At booting, the only significantly different observation in leaf blast incidence occurred between IR504 without Td and CAR14 with Td. There was no significant difference in leaf blast incidence across all treatments at the dough stage.

3.4. Neck blast incidence

Wet season data from both years and locations showed that NB incidence on CAR14 was significantly lower than on IR504 (Table 4). In the wet season of 2016, Td treatments significantly reduced NB incidence on IR504 in both stations (Table 4), but in 2017 there was no difference in NB incidences between Td-treated and non Td-treated plots. Overall NB incidence was lower in wet season 2017 compared to wet season 2016. In dry season 2017 at Polors, the NB incidence was generally very low (Table 4). The NB incidence in conventional practice during the wet season of 2017 was not significantly different compared to IR504 and was significantly higher compared to CAR14.

3.5. Grain yield

Grain yield was highly correlated with AULBPC ($r = -0.877$, $P < 0.001$) and moderately correlated with neck blast incidence ($r = -0.567$, $P < 0.001$). *T. harzianum* effectively reduced neck blast at high disease pressure.

In the wet season of 2016, at both locations, the yields of CAR14 were significantly higher compared to IR504 regardless of the Td treatments (Table 5). In 2016, Td treatments did not affect the yield of both varieties in all locations.

In dry season of 2017, there was no significant difference among all treatments. In wet season of 2017, there was no significant difference among all treatments in Polors. In Koktrap, the lowest yield was recorded in plots using IR504 without Td treatments while the highest yield was recorded in plots using CAR14 with Td treatments. Td treatment did not significantly affect yield on CAR14, but Td-treated IR504 yield was significantly higher compared to IR504 without Td treatment.

4. Discussion

Our data showed that deployment of a resistant variety clearly reduced leaf blast incidence, AULBPC and neck blast compared to a susceptible variety and conventional practice (i.e. use fungicide as practiced by farmers). Yield data also showed that in seasons with high blast incidence (e.g. 2016), use of the resistant variety yielded significantly higher compared to the susceptible variety.

The use of *Trichoderma* did not significantly reduce leaf blast and neck blast incidence on the resistant variety. In a susceptible variety, *Trichoderma* application was shown to reduce neck blast incidence in one year (e.g. at 2016 on both locations) and leaf blast incidence in some selected year and location (e.g. AULBPC data at 2017 at both locations). The yield data showed that in a select location and year combination (Koktrap, wet season at 2017), the use of *Trichoderma* on a resistant variety significantly improved yield compared to other treatments including the conventional practice. Although *Trichoderma* application on a susceptible variety produced a similar yield as the conventional

Table 3

Comparison of area under leaf blast progress curve (AULBPC) at two different sites and different seasons with or without *Trichoderma harzianum* treatment (Td).

Location	Season	Year	Total Sample size	CAR14 no Td	CAR14 with Td	IR504 no Td	IR504 with Td	Conventional practice
Koktrap	wet	2016	30	664.71 ± 151.05a	502.83 ± 235.71a	1058.26 ± 152.42a	863.81 ± 143.82a	
Polors	wet	2016	30	548.27 ± 10.79 b	493.49 ± 37.14 b	1042.23 ± 85.24 a	964.89 ± 13.45 a	
Polors	dry	2017	40	238.77 ± 10.35 a	233.05 ± 130.96 a	352.00 ± 97.50 a	372.28 ± 69.41 a	387.21 ± 54.87 a
Koktrap	wet	2017	40	169.34 ± 23.82 c	99.65 ± 11.55 c	517.58 ± 42.04 a	339.97 ± 46.29 b	477.72 ± 61.57 a
Polors	wet	2017	40	184.73 ± 45.48 c	113.64 ± 30.75 c	607.31 ± 21.39 a	383.78 ± 42.91 b	367.82 ± 17.61b

Data are mean ± SD. Different alphabetical small letters in the same line indicate a significant difference ($p < 0.01$, Tukey-Kramer multiple comparison test). Sample size and number of replications are described in Table 1. Average 43.29 leaves per sample were assessed.

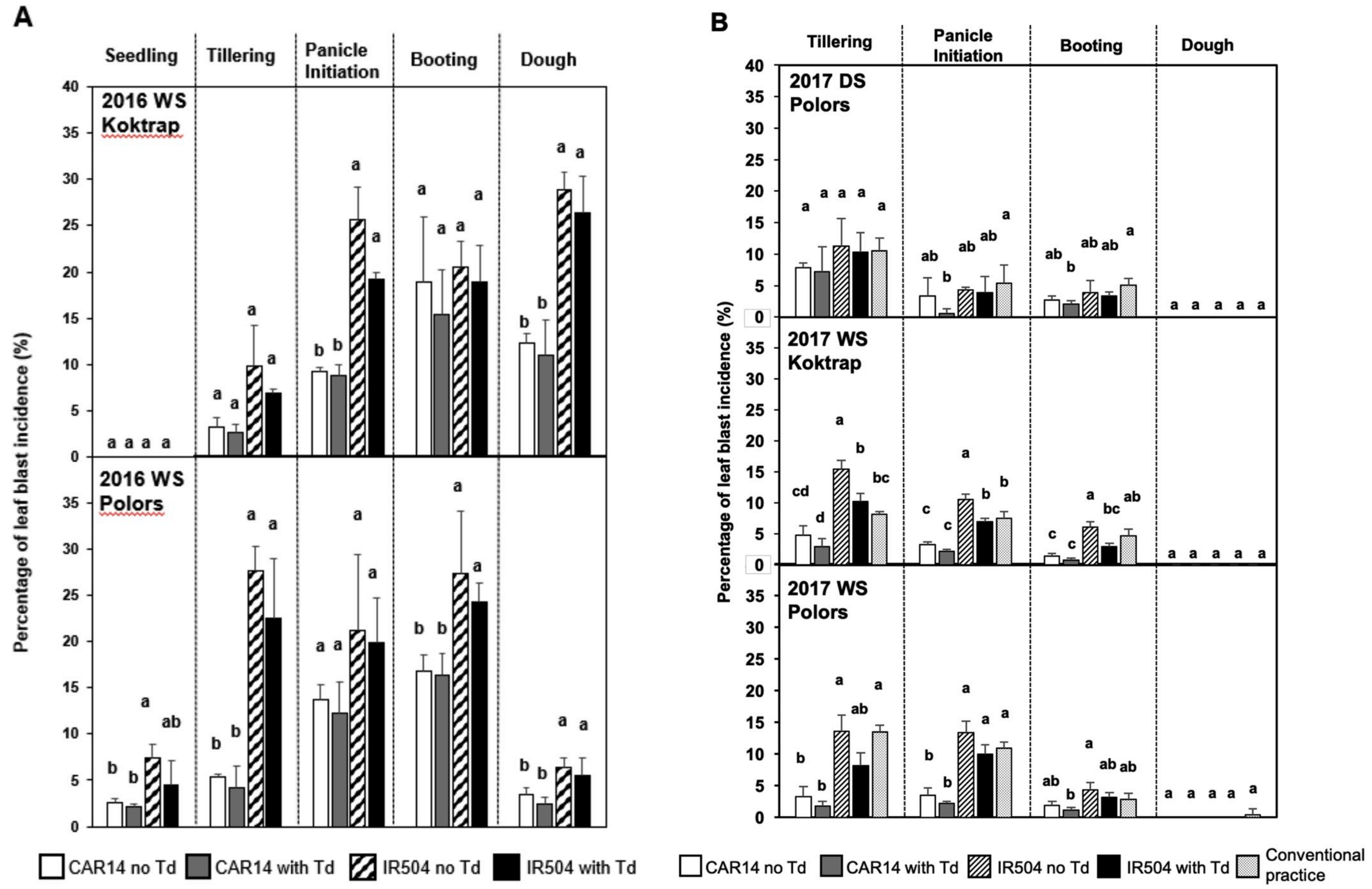

Fig. 2. Incidence of rice leaf blast on each treatment and growing stage. (A) 2016 wet season in Koktrap and Polors. (B) 2017 dry and wet season in Koktrap and Polors.

Experimental year, season and location are indicated in the upper left on each panel; WS, wet season; DS, dry season. Crop growth stages are indicated at the top of panels. Bars represent means ± SD; Means marked with different letters are significantly different from each other within that crop growth stage (Tukey-Kramer multiple comparison test; $P < 0.01$). Sample size and number of replications are described in Table 1. Average 43.29 leaves per sample were assessed.

Table 4

Incidence of neck blast on two rice varieties, with or without *Trichoderma harzianum* treatment, at two different sites.

Place	Season	Year	Total Sample size	CAR14 no Td[1]	CAR14 with Td	IR504 no Td	IR504 with Td	Conventional practice
Koktrap	wet	2016	30	7.61 ± 1.12 c[2]	4.33 ± 1.12 c	22.70 ± 1.25 a	15.52 ± 2.15 b	
Polors	wet	2016	30	4.59 ± 0.88 c	1.89 ± 0.56 c	41.12 ± 1.94 a	27.73 ± 4.29 b	
Polors	dry	2017	40	0 a	0 a	1.68 ± 1.68 a	0 a	0.44 ± 0.54 a
Koktrap	wet	2017	40	1.40 ± 0.92 b	0.51 ± 1.01 b	16.43 ± 4.01 a	9.13 ± 2.12 ab	15.73 ± 6.94 a
Polors	wet	2017	40	4.43 ± 1.68 b	1.26 ± 1.20 b	28.59 ± 5.62 a	23.47 ± 7.04 a	26.17 ± 4.61 a

Data are means ± SD. [1] "no Td" means each variety treated without *T. harzianum*; with Td, each variety treated with *T. harzianum.* [2]Different alphabetical small letters in the same line indicate a significant difference ($p < 0.01$, Tukey-Kramer multiple comparison test). Sample size and number of replications are described in Table 1. Average 11.13 tillers per sample were assessed.

Table 5

Comparison of rice yield produced by four different treatments at two different sites and years.

Location	Season	Year	Total Sample size	CAR14 no Td[1]	CAR14 with Td	IR504 no Td	IR504 with Td	Conventional practice
Koktrap	wet	2016	30	3399.96 ± 67.86 a[2]	3515.91 ± 256.03 a	2031.87 ± 112.91 b	2340.22 ± 237.36 b	
Polors	wet	2016	30	3513.73 ± 54.59 a	3607.26 ± 145.42 a	2347.97 ± 163.35 b	2620.02 ± 85.63 b	
Polors	dry	2017	40	4147.53 ± 192.08 a	4214.39 ± 306.40 a	3925.85 ± 252.98 a	4021.44 ± 119.08 a	3913.99 ± 317.39 a
Koktrap	wet	2017	40	3909.61 ± 81.57 abc	4054.76 ± 70.35 a	3718.64 ± 82.61 c	3947.3 ± 96.85 ab	3807.99 ± 71.73 c
Polors	wet	2017	40	4072.54 ± 463.63 a	4293.82 ± 394.21 a	3550 ± 186.74 a	4042.02 ± 237.96 a	3890.73 ± 310.17 a

Data are mean ± SD. [1] "no Td" means each variety treated without *T. harzianum:* with Td, each variety treated with *T. harzianum.* [2] Different alphabetical small letters in the same line indicate a significant difference ($p < 0.01$, Tukey-Kramer multiple comparison test). Each sample size and number of replications are described in Table 1. Average number of panicle per sample was assessed as 10.57.

practice, yield in a selected location and year combination, was higher than a susceptible variety without *Trichoderma* application.

Our data indicate that the use of a resistant variety is highly effective in reducing leaf blast and neck blast incidence on rice and to reduce yield loss due to blast infection, especially in seasons with a high blast incidence rate. Over 100 quantitative blast resistance genes have been documented on rice globally (Sharma et al., 2012) and a score of resistant varieties has been developed by deploying some of these genes (Srivstana et al., 2017). However, some of these varieties may lose their tolerance to blast disease due to a quick shift of field blast races (Singh et al., 2018). The longevity of rice varieties with single resistance genes (before they are broken down by a pathogenic fungal race) is estimated to be less than 3 years in Japan (Kiyosawa, 1982). To protect against resistance breakdown by the rice blast pathogen, varietal rotation (i.e. resistance gene(s) rotation) and varietal mixtures have been deployed (Leung et al., 2003). CAR14 was newly released in 2015 and no information currently exists on the specific resistance genes deployed in the variety.

While *Trichoderma* application did not show blast reducing effects on the tested resistant variety, it reduced neck blast and leaf blast incidence in the susceptible variety, albeit inconsistently. The variability in the efficacy of *Trichoderma* in reducing leaf and neck blast incidence on susceptible varieties may be associated with the disease pressure for a particular season. Seasons with high humidity, high concentration of nitrogen in the soil, poor sunshine duration and cold weather have been associated with high blast pressure (Suzuki, 1975).

Gohel and Chauhan (2015) reported that *Trichoderma* foliar application treatment proved effective and reduced rice leaf blast and neck blast incidence and increased the yield parameters. In a protected environment, application of *T. harzianum* strain CEPA A-34 showed a decrease of up to 67.5% of AULBPC (Pérez-Torres et al., 2018). In this study, we tested a locally-produced and commercially-available *Trichoderma* biocontrol product in Cambodia. It is possible that other strains of *T. harzianum* more effectively suppress rice blast disease. Thus, further field studies, examining other *Trichoderma* products legally imported into Cambodia, are necessary.

5. Conclusion

The use of a resistant variety is shown to be effective in reducing leaf and neck blast incidence and in protecting the yield due to blast infection, especially in seasons with high blast pressure, at field scale. The deployment of host plant resistance should indeed be used as the first line of defense against blast disease.

The *Trichoderma harzianum* treatment could reduce rice blast incidence in susceptible variety (IR504). The yield associated with Trichoderma application on a susceptible variety is comparable to that of conventional practice on the same variety. When a susceptible variety such as IR504 constitutes the main option to farmers, due to high demands of the market for example, *Trichoderma* spp. as a biological control agent should be used to reduce neck blast and leaf blast incidence.

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