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Original Article

Efficacies of wettable pellet and fresh culture of *Trichoderma asperellum* biocontrol products in growth promoting and reducing dirty panicles of rice

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ABSTRACT

Wettable pellets and fresh culture (fungus-colonized rice seeds) bioproducts of *Trichoderma asperellum* isolates 01-52 and CB-Pin-01, respectively, were evaluated for their efficacy in reducing dirty panicle or seed discoloration, and to increase the growth and yield of rice var. Chai Nat 1. Rice seeds were soaked (Sk) in a spore suspension of wettable pellets (20 g/20 L) or a fresh culture of bioproducts (100 g/20 L) of the fungus for 24 h. Soaked seeds were incubated for another 24 h before sowing. The 21-day-old seedlings were transplanted into small plots (1 m^2 × 3 m^2). Rice plants were sprayed (Sp) three times during the growing period with a spore suspension from the two bioproducts. The results indicated that both wettable pellet 01-52 (Sk + Sp) and fresh culture CB-Pin-01 (Sk + Sp) formulations significantly increased the plant height, number of tillers per hill, 1000-seed weight and total yield of rice compared to the untreated control. The incidence of dirty whole rice panicles, dirty panicle infected seed and empty seed were significantly reduced, while healthy seed were increased compared to the untreated control. Rice root colonization by *T. asperellum* isolates 01-52 and CB-Pin-01 were detected from the seedling through to the harvesting stage. The seedling vigor index and seedling health index of seedlings grown from 5 month-stored healthy and dirty panicle infected seeds treated by both wettable pellet 01-52 (Sk + Sp) and fresh culture CB-Pin-01 (Sk + Sp) bioproducts were significantly higher than the untreated control. All *Trichoderma* treatments increased whole kernels plus head rice and reduced broken rice in milled brown rice.

Introduction

In Thailand, rice (*Oryza sativa* L.) is one of the most important economic crops for both domestic consumption and for exporting and Thailand was ranked the number one rice exporter globally in 2014 when total milled rice exported from Thailand was 10.97 million t and worth USD 5439 million (Thai Rice Exporters Association, 2015). Chettanachit et al. (2009) discussed the distribution and impact of dirty panicle disease also known as seed discoloration, which is one of the most important constraints for rice production. This disease is commonly found on most rice varieties in all rice production regions of the country. The dirty panicle pathogens spread by wind and are also seed borne. If the disease is severe it not only reduces the percentage of rice seed germination, but also the quality of brown rice and milled rice.

The causal fungal pathogens of this disease include *Curvularia lunata* (Wakk) Boed., *Bipolaris* (*Helminthosporium*) *oryzae* (Breda de Haan) Shoem., *Alternaria* (*Trichoconis*) *padwickii* (Ganguly) M.B. Ellis., *Cercospora oryzae* I. Miyake., *Fusarium incarnatum* (Roberge) Sacc. (Berk & Rav.) and *Sarocladium oryzae* Sawada (Chettanachit et al., 2009; Jaisong, 2010; Bureau of Rice Research and Development, 2015). Besides using chemical fungicides, the application of antagonistic microorganisms or biocontrol agents and their products is an alternative method to control dirty panicle disease of rice.

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Trichoderma spp. are among the most successfully used biological control agents in agriculture, with more than 60% of the registered biofungicides wordwide being *Trichoderma* based (Verma et al., 2007). They are presently marketed as biopesticides, biofertilizers, growth and yield enhancers and as nutrient solubilizers and organic matter decomposers (Woo et al., 2014). *Trichoderma* spores can be produced in liquid media and in solid state fermentation on sterile rice, corn or other grains, and then the *Trichoderma*-colonized substrate as a fresh culture can be applied directly to the crops or soil, with the spores then being separated from the substrate by sifting and re-suspending in water (Woo et al., 2014). *Trichoderma asperellum* T12, used as a solid state fermentation preparation added to soil, effectively controlled sheath blight of rice (Chen et al., 2015). A mixture of four isolates of *T. asperellum* was efficient in reducing the severity of sheath blight and increasing the rice yield and grain weight in Brazil (de França et al., 2015). The mode of action of *T. asperellum* was revealed using scanning electron microscopy to involve hyphae of *Gibberella fujikuroi*, the causal agent of "Bakanae" disease of rice, being penetrated by the hyphae of *T. asperellum* SKT-1 and degradation of cell walls of *G. fujikuroi* was observed (Watanabe et al., 2007).

In Thailand since 2002, *T. asperellum* CB-Pin-01 (formerly identified as *Trichoderma harzianum*) has been distributed as a pure stock culture in a powder bioformulation used for producing a fresh culture bioproduct (semi-cooked broken milled rice colonized with the fungus) by the simple procedure developed by Chamswarng and Intanoo (2002). Spore suspensions were prepared by washing the spores from fresh culture bioproduct with clean water. This spore suspension is widely used for seed soaking, soil drenching and plant spraying against fungal diseases of various plants including cereals, fruits, vegetables, ornamentals and hydroponic lettuces (Chamswarng, 2015). *Trichoderma* fresh cultures can be stored in a refrigerator for only 1–2 mth; therefore, *T. asperellum* isolate 01-52, which effectively reduced several diseases of rice was developed as a wettable pellet bioproduct with a longer shelf life (Chamswarng et al., 2012a, 2013). In the current study, experiments were conducted to evaluate the effectiveness of the wettable pellet formulation of isolate 01-52 and the fresh culture formulation of isolate CB-Pin-01 as seed soaks and plant sprays for reducing dirty panicle disease and increasing growth and yield of rice in small plots.

Materials and methods

Trichoderma asperellum bioproducts

T. asperellum isolates CB-Pin-01 and 01-52 (= 03/7-I34) (Molecular taxonomic based; Unartngam, personal communication) used in this study were provided by Associate Professor Dr. Chiradej Chamswarng, Plant Disease Biocontrol Laboratory, Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom province, Thailand. These two isolates were formerly identified as *T. harzianum* based on fungal growth and morphology (Chamswarng and Intanoo, 2007; Inwang and Chamswarng, 1986). *T. asperellum* CB-Pin-01 was cultured on semi-cooked broken milled rice as a fresh culture bioproduct according to the procedure developed by Chamswarng and Intanoo (2002). *T. asperellum* 01-52 was prepared as a wettable pellet bioproduct (1×10^8 colony forming units/g; unpublished data).

Rice seed soaking and plant spraying with Trichoderma

A sample of 20 g of rice seed (paddy) cv. Chai Nat 1 was placed in double layers of cheesecloth and soaked in a spore suspension of *T. asperellum* fresh culture bioproduct (100 g/20 L of water) or wettable pellet bioproduct (20 g/20 L of water) for 24 h. After the spore suspension was drained off, soaked seeds were kept moist and incubated for 24 h before sowing in 288-hole trays (12 mL/hole). The 21-day-old seedlings were transplanted into small plots ($1\ m^2 \times 3\ m^2$) consisting of paddy field soil. The whole rice plants were sprayed with each *Trichoderma* isolate three times with the spore suspensions prepared from wettable pellet (625 g/500 L/ha) or fresh culture (2.5 kg/500 L/ha) bioproducts at the booting stage (60 d after sowing; DAS), 5% of panicle-forming stage (75 DAS) and milk-forming stage (95 DAS).

A randomized complete block design was used with five treatments, four replications for each treatment and 30 plants per replication (plot). The first and second treatments were comprised of rice seed soaking (Sk) and plant spraying (Sp) with each isolate of *T. asperellum* (CB-Pin-01 and 01-52). The third treatment was rice seeds soaked only with *T. asperellum* isolate 01-52. The fourth treatment was rice seeds soaked with clean water, as the negative control, while the fifth treatment was rice seeds soaked with mancozeb (80% WP) (3 g/100 mL/100 g of seed) and the plants were sprayed with a fungicide mixture (propiconazole + difenoconazole 30% weight per volume emulsifiable concentrate) and this served as the positive control. Chemical fertilizers were applied three times to each plot as recommended by the Rice Department (Bureau of Rice Research and Development, 2016). These included N-P-K formulations of 16-20-0 (156.25 kg/ha), 46-0-0 (125.00 kg/ha), and 46-0-0 (62.50 kg/ha) which were applied at 15, 45 and 55 DAS, respectively.

Dirty panicle pathogens inoculation

Pure cultures of *Bipolaris oryzae*, *C. lunata* and *Alternaria padwickii* isolated from discolored rice seeds were used for rice plant inoculation. Each pathogen was grown on autoclaved rice seeds contained in a plastic bag and incubated under near ultraviolet light for 10 d or until the spores could be observed (modified from Chamswarng and Intanoo, 2002). Spore suspensions were prepared and the spore concentration was adjusted to 1×10^4 spores/mL using a haemacytometer. Inoculation was performed by spraying the spore suspension on the whole rice plants at the early stage of panicle formation.

Sample collection and data acquisition

Rice growth, disease incidence and yield components of each treatment were recorded from 60-day-old and 120-day-old rice plants. These included the height (the length from the base of tiller to the terminal of the flag leaf) and the number of tillers per rice hill. The severity of dirty panicle disease on whole panicles of rice plants was determined twice at 2 and 4 wk after the third *Trichoderma* spray by sampling 25 whole panicles from each replication and four replications per treatment (modified form Chettanachit et al., 2009). Disease severity from 10 g of panicle-detached seed in each replication (four samples per replication) was further determined as the percentages of healthy seed, dirty panicle infected seed and empty seed (unfertile or undeveloped seeds). For the yield assessment, rice panicles were collected and the moisture content of the rice seed was reduced to 14%. All seed samples were detached from the panicles, then the total rice yield, 1000-healthy seed weight, and 1000-seed weight were recorded. The quality of milled brown rice was determined by sampling paddies from each treatment (1 kg per replication, four replications per treatment) for the brown rice milling process. The weights of healthy seed or whole kernels plus head rice and

broken rice from 10 g per randomly collected sample of milled brown rice were recorded.

Trichoderma colonization of rice roots

The percentages of rice roots colonized by *T. asperellum* were detected by collecting rice root systems from harvested plants of each replication. Roots were washed three times under running tap water, cut to a single piece, blotted dry using sterilized tissue paper and placed on Martin's medium supplemented with 100 parts per million rifampicin. After incubation under light at room temperature (25–30 °C) for 3–5 d, the numbers of rice roots colonized with *T. asperellum* were recorded.

Seedling vigor index of healthy rice seeds

A sample of 100 healthy seeds from each replication were selected from 5-month-stored seed (10 °C) and incubated for 14 d in sterilized Petri dishes placed on moist paper towels under constant lighting at room temperature (25–30 °C). The percentages of seed germination, seedling shoot height and root length of 100 seedlings were recorded. The seedling vigor index (SVI) was calculated using the formula; SVI = Germination (%) × Seedling length (Doni et al., 2014b).

Seedling health index of dirty panicle infected rice seeds

Dirty panicle infected seeds were selected from 5-month-stored rice seeds (10 °C; 50 seeds/replication) and incubated in sterilized Petri dishes on moist paper towels under constant lighting at room temperature (25–30 °C) for 7 d. The percentage of seed germination was determined and seedling health was recorded based on a scale from 0 to 4 (0 = seed germinated with dead seedling; 1 = seed germinated with brown, shortened roots; 2 = seed germinated with green shoot and dark brown, whole roots; 3 = seed germinated with green shoot and light brown at root tips; and 4 = seed germinated with green shoot and healthy roots). The seedling health index (SHI) was calculated using a formula modified from a disease severity index (Suhaida and NurAinIzzati, 2013) as: $SHI = (\Sigma(A \times n))/\Sigma B$, where A = seedling health rating scale (0–4); n = number of seedlings in a specific health rating and B = total number of seedlings.

Statistical analyses

All data were statistically analyzed using ANOVA. The significance of differences between the treatment means was determined using Duncan's Multiple Range Test in SPSS version 16.0 (SPSS Inc.; Chicago, IL, USA). The significance level was set at $p \leq 0.05$.

Results and discussion

Trichoderma-based formulations are commercialized as wettable powders, granules, liquids and solids that include substrates such as a coco mat or peat moss, cereal grain such as rice or broken corn which support the growth of *Trichoderma* culture until sporulation (Woo et al., 2014). In Costa Rica, a local strain of *Trichoderma atroviride* was grown on autoclaved rice in small rooms for direct use by growers to control diseases on different crops. These *Trichoderma*-colonized rice seeds were used as fresh material without further processing or drying (Obregón, 2002). In Venezuela, *Trichoderma* spp. were produced and used as dried-colonized rice seeds for plant disease biocontrol in agriculture. The colonized product was both ground and applied as granules or spores were extracted and used as a powder formulation (Harman et al., 2010). In Thailand, Chamswarng and Intanoo (2002) developed a simple method for producing fresh cultures of *T. asperellum* CB-Pin-01 (local strain). This strain was cultured on semi-cooked broken milled rice seeds placed in heat-tolerant plastic bags. The substrate was prepared using a common, automatic rice cooker instead of using an automatic autoclave. Moreover, rice growers can produce this fresh culture by themselves within 6–7 d without using any complicated equipment, specific conditions and or even a sterile room. Spore suspensions were prepared by washing spores from colonized rice seeds with clean water (Charoenrak and Chamswarng, 2015).

All commercialized pellets and granular bioproducts available globally are formulated as ready-to-use, and are directly applied for example to the soil by incorporation at the time of seeding, transplanting or planting and include products such as RootShield®-Granule, DRH Pellets® and Binap P® (Woo et al., 2014). In the current study, a wettable pellet bioproduct of *T. asperellum* 01-52 developed by Chamswarng and Intanoo in 2012 (unpublished data) was applied as a seed soak and plant spray for the control of rice diseases (Chamswarng and Kumchang, 2012; Chamswarng et al., 2012a). Wettable pellets were used for preparing the spore suspension by mixing the pellets with water and stirring for a few minutes in order to suspend pellet fragments with dispersing spores in the water. This wettable pellet formulation is probably similar to the water-dispersible granule formulation of the nontoxic *Aspergillus flavus* strain K-49 developed by Lyn et al. (2009).

Rice plant growth

All treatments significantly increased the heights of rice plants at the booting (60 DAS) and harvesting stages (120 DAS) by 9.22–15.55% and 5.98–9.20%, respectively, compared with the untreated control. The plant heights in treatments with seed soaking and spraying (Sk + Sp) with CB-Pin-01 fresh culture and 01-52 wettable pellet bioproducts were significantly higher than in the chemical fungicide treatment at both the booting and harvesting stages of rice growth (Table 1). All *Trichoderma* treatments increased the number of tillers per rice hill by 50.72–72.69% and 3.24–25.47% at 60 and 120 DAS, respectively, compared with the untreated control. At the harvesting stage (120 DAS), only two *Trichoderma* treatments— CB-Pin-01 fresh culture bioproduct (Sk + Sp) and 01-52 wettable pellet bioproduct (Sk + Sp)— significantly increased the number of tillers per rice hill by 25.09% and 25.47%, respectively, compared to the control (Table 1). These results were consistent with those recently reported by Doni et al. (2014a) where all seven *Trichoderma* isolates tested were able to enhance rice growth components including the plant height, leaf number, tiller number, root length and root fresh weight. Similar results were obtained by da Silva et al. (2012) who reported that *Trichoderma* isolate T-52 enhanced the shoot and root dry weight in rice (*O. sativa*) by up to 38%. The proposed mechanisms to explain plant growth promotion associated with *Trichoderma* species include synthesis of phytohormone (either by microbes or the plants), production of vitamins, enhanced solubilization and uptake of soil nutrients, enhanced root development and increases in the rate of carbohydrate metabolism, photosynthesis and plant defense mechanisms (Kleifeld and Chet, 1992; Inbar et al., 1994; Harman et al., 2004; Harman, 2006).

Rice root colonization

Roots of rice seedlings (21 DAS) from all *Trichoderma* treatments were completely colonized (100%) by the isolates CB-Pin-01 and 01-52. However, root colonization percentages at 120 DAS were reduced to 77.78%, 75.56% and 44.44% in CB-Pin-01 fresh culture

Table 1
Effect of *Trichoderma asperellum* isolate formulations on plant height, number of tillers per rice plant and root colonization percentage of rice plants (var. Chai Nat 1) at 60 and 120 d after sowing (DAS).

Treatment	Plant height (cm)		Tiller number per plant		Root colonization (%)	
	Booting stage (60 DAS)	Harvesting stage (120 DAS)	Booting stage (60 DAS)	Harvesting stage (120 DAS)	Seedling stage (21 DAS)	Harvesting stage (120 DAS)
1. *T. asperellum* CB-Pin-01 fresh culture bioproduct (Sk + Sp*)	85.76[a†] (+15.55%)[‡]	131.26[a] (+7.87%)	16.89[ab] (+72.69%)	28.97[a] (+25.09%)	100.00[a]	77.78[a]
2. *T. asperellum* 01-52 wettable pellet bioproduct (Sk + Sp)	82.94[b] (+11.75%)	132.88[a] (+9.20%)	14.74[c] (+50.72%)	29.06[a] (+25.47%)	100.00[a]	75.56[a]
3. *T. asperellum* 01-52 wettable pellet bioproduct (Sk)	81.06[bc] (+9.22%)	128.96[ab] (+5.98%)	15.41[bc] (+57.57%)	23.91[b] (+3.24%)	100.00[a]	44.44[b]
4. mancozeb (Sk)/propiconazole + difenoconazole (Sp)	80.50[c] (+8.46%)	124.62[bc] (+2.42%)	17.59[a] (+79.86%)	26.25[ab] (+13.34%)	ND[§]	0.00[c]
5. Control (untreated)	74.22[d]	121.68[c]	9.78[d]	23.16[b]	ND	0.00[c]

*Sk = seed soak; Sp = plant spray.
† Means in each column followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.
‡ Percentage of increase (+) or decrease (−) of each treatment mean compared with untreated control.
§ ND = Not determined.

(Sk + Sp), 01-52 wettable pellets (Sk + Sp) and 01-52 wettable pellet (Sk), respectively (Table 1). Many beneficial effects could be obtained from the plant roots of both dicotyledons and monocotyledons which were colonized by *Trichoderma* strains (Harman and Shoresh, 2007). The close physical association between *Trichoderma* and plant roots will contribute increases in the growth, nutrient uptake and fertilizer utilization efficiency, leaf greenness, photosynthetic rate and plant hormones (IAA, GA3 and ethylene) as a result of improvements in the plant growth development and yield (Harman, 2011; Hermosa et al., 2013; Studholme et al., 2013; Stewart and Hill, 2014). Vinale et al. (2008) reported that *Trichoderma* species which colonized plant roots could produce compounds that changed the plant's metabolism and stimulated the plant's defenses against plant pathogens. A recent report indicated that increased chitinase activity in 45-day-old leaves of rice plants resulted from seed soaks and plant sprays with spore suspension derived from *Trichoderma* dry powder, wettable pellet and fresh culture formulations (Charoenrak et al., 2012). High percentages of rice root colonization by *Trichoderma* in this study may have resulted in induced systemic resistance in rice plants against dirty panicle disease.

Rice disease incidence

All *Trichoderma* treatments significantly reduced dirty panicle incidence on whole panicles at the milk-forming stage (2 wk after the third spray) and at harvest (4 wk after the third spray) by 50.24–52.33% and 26.69–44.86%, respectively, compared with the control. The efficacy of *Trichoderma* treatments was comparable to the chemical fungicide treatment which gave 60.06% and 39.76% disease reduction. Among *Trichoderma* treatments, CB-Pin-01 fresh culture (Sk + Sp) and 01-52 wettable pellet (Sk + Sp) treatments gave significantly lower dirty panicle incidences (6.92% and 6.95%, respectively) than the 01-52 wettable pellet (Sk) treatment (9.20%) as shown in Table 2. These results suggest the important effect of the plant spray on dirty panicle disease reduction. Examination of seed detached from rice panicles revealed that all *Trichoderma* treatments provided a significant reduction in discolored seed by 22.62–39.51% compared with the untreated control. The lowest percentage of dirty panicle infected seed was obtained from the treatment CB-Pin-01 (Sk + Sp) (39.51%). Moreover, the efficacy of all *Trichoderma* treatments in reducing discolored seed was comparable to the use of chemical fungicides (Table 2). All *Trichoderma* treatments significantly increased the healthy seed percentages and reduced empty (unfertile or undeveloped) seed percentages by 15.25–20.15% and 35.15–45.47%, respectively, compared with the untreated control. Healthy seed percentages derived from all *Trichoderma* treatments (76.01–79.24%) were not significantly different from the chemical fungicide treatment (75.56%). However, empty seed percentages obtained from CB-Pin-01 fresh culture (Sk + Sp) and 01-52 wettable pellet (Sk + Sp) treatments (10.57% and 10.30%, respectively) were significantly lower than for the fungicide treatment (13.64%) as shown in Table 2.

Rice yield and seed quality

All *Trichoderma* treatments increased the 1000-healthy seed weight and 1000-seed weight by 0.32–1.84% and 0.18–1.69%, respectively. In particular, treatment 01-52 wettable pellet (Sk) significantly increased the 1000-healthy seed weight and 1000-seed weight by 1.84% and 1.65%, respectively, compared with the untreated control. The efficacy levels of *Trichoderma* treatments were comparable to the chemical fungicide treatment. The rice yields significantly increased in the *Trichoderma* treatments of CB-Pin-01 fresh culture (Sk + Sp) and 01-52 wettable pellet (Sk + Sp) by 26.35% and 35.83%, respectively. *T. asperellum* 01-52 wettable pellet (Sk + Sp) provided the highest rice yield (4.40 t/ha) but was not significantly different from the chemical fungicide treatment (4.07 t/ha) as shown in Table 3. Previous results showed that a wettable pellet formulation of *T. asperellum* isolate 01-52 increased the yield of rice vars. Pathum Thani 80 and Pin Kaset (Chamswarng and Kumchang, 2012; Chamswarng et al., 2012a). In addition, a dirty panicle reduction and a rice yield increase were obtained by using a spore suspension prepared from fresh culture bioproducts of *T. asperellum* CB-Pin-01 and 03-7/I34 by seed soaking and plant spraying (Chamswarng and Intanoo, 2007). These results support the current research findings that both fresh culture and wettable pellet bioproducts provided comparable efficacy for increasing rice yield. The reports of Doni et al. (2014a) revealed that *Trichoderma* spp. were able to increase several physiological processes in rice plants including the net photosynthetic rate, stomatal conductance, transpiration, internal CO_2 concentration and water use efficacy. Improving rice physiological characteristics could contribute to the achievement of a high rice yield (Makino, 2011; Li et al., 2012). Some *Trichoderma* strains were able to produce plant growth hormones such as cytokinin-like molecules, for example, zeatin and gibberellin GAB-related molecules, which directly affected plant growth (Howell, 2003; Benítez et al., 2004). Nevertheless, the rice yield of the 01-52 wettable pellet (Sk) treatment was significantly lower than from the CB-Pin-01 fresh culture (Sk + Sp) and 01-52

Table 2
Effect of *Trichoderma asperellum* isolate formulations on dirty panicle incidence on whole rice panicles and percentages of healthy seed, dirty panicle infected seed and empty seed of seed samples harvested from rice plants (var. Chai Nat 1).

Treatment	Dirty panicle disease incidence (%)		Paddy characteristic		
	2 wk after 3rd spray (milk-forming stage)	4 wk after 3rd spray (harvesting stage)	Healthy seed (%)	Dirty panicle infected seed (%)	Empty seed (%)
1. *T. asperellum* CB-Pin-01 fresh culture bioproduct (Sk + Sp*)	3.09^{b†} (−50.24%)‡	6.92^{c} (−44.86%)	79.24^{a} (+20.15%)	9.17^{c} (−39.51%)	10.57^{c} (−44.04%)
2. *T. asperellum* 01-52 wettable pellet bioproduct (Sk + Sp)	2.96^{b} (−52.33%)	6.95^{c} (−44.62%)	78.19^{a} (+18.56%)	10.48^{bc} (−30.87%)	10.30^{c} (−45.47%)
3. *T. asperellum* 01-52 wettable pellet bioproduct (Sk)	3.07^{b} (−50.56%)	9.20^{b} (−26.69%)	76.01^{a} (+15.25%)	11.73^{b} (−22.62%)	12.25^{bc} (−35.15%)
4. mancozeb (Sk)/propiconazole + difenoconazole (Sp)	2.48^{b} (−60.06%)	7.56^{c} (−39.76%)	75.56^{a} (+14.57%)	11.17^{bc} (−26.32%)	13.64^{b} (−27.79%)
5. Control (untreated)	6.21^{a}	12.55^{a}	65.95^{b}	27.89^{a}	18.89^{a}

*Sk = seed soak; Sp = plant spray.
† Means in each column followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test at $p \leq 0.05$.
‡ Percentage of increase (+) or decrease (−) of each treatment mean when compared with untreated control.

Table 3
Effect of *Trichoderma asperellum* isolate formulations on weights of 1000-healthy seed, 1000- seed, yield and weights of whole kernels plus head rice and broken rice of milled brown rice obtained from rice plants (var. Chai Nat 1).

Treatment	Weight (g)		Rice yield (t/ha)	Weight of milled brown rice	
	1000 healthy seed	1000 seed		Whole kernels (+ head rice) (g)*	Broken rice (g)*
1. *T. asperellum* CB-Pin-01 fresh culture bioproduct (Sk + Sp†)	28.30^{ab‡} (+0.32%)§	27.94^{ab} (+0.18%)	4.17^{a} (+26.35%)	752.80^{ab} (+4.53%)	247.20^{ab} (−11.61%)
2. *T. asperellum* 01-52 wettable pellet bioproduct (Sk + Sp)	28.62^{ab} (+1.45%)	28.36^{a} (+1.69%)	4.40^{a} (+35.83%)	800.97^{ab} (+11.21%)	199.03^{ab} (−28.83%)
3. *T. asperellum* 01-52 wettable pellet bioproduct (Sk)	28.73^{a} (+1.84%)	28.35^{a} (+1.65%)	3.50^{b} (+7.90%)	781.57^{ab} (+8.52%)	218.43^{ab} (−21.92%)
4. mancozeb (Sk)/propiconazole + difenoconazole (Sp)	28.51^{ab} (+1.06%)	28.23^{ab} (+1.22%)	4.07^{a} (+25.42%)	817.87^{a} (+13.56%)	182.13^{b} (−34.88%)
5. Control (untreated)	28.21^{b}	27.89^{b}	3.23^{b}	720.23^{b}	279.77^{a}

*Weights of whole kernels plus head rice and broken brown rice from 1 kg of milled brown rice (four replications/treatment).
† Sk = seed soak; Sp = plant spray.
‡ Means in each column followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test $p \leq 0.05$.
§ Percentage increase (+) or decrease (−) of each treatment mean when compared with untreated control.

wettable pellet (Sk + Sp) treatments. The lower yield from this treatment may have resulted from the lower plant height, fewer tiller numbers per hill (Table 1) and the higher dirty panicle disease incidence (Table 2). This result indicated the important effects of an integrated application of both fresh culture and wettable pellet bioproducts as a seed soak and plant spray on disease reduction and the growth and yield enhancement of rice.

The rice seed quality after brown rice milling revealed that whole kernels plus head rice from all *Trichoderma* treatments had increased (4.53–11.21%) but was not significantly different compared to the untreated control. The weights of broken brown rice in *Trichoderma* treatments were reduced 11.61–28.33% compared to the untreated control (Table 3). The current results agreed with previous reports of Chamswarng et al. (2012a, 2012b, 2013) which revealed the efficacies of 01-52 wettable pellet and powder for increasing both the percentage and weight of whole kernels, while reducing the percentage and weight of broken rice after the milling process for brown rice. In addition, mineral analyses of milled brown rice indicated the enhancement of phosphorus in rice seeds (Chamswarng et al., 2012b). This evidence could be explained by the reports of Shoresh et al. (2010) and Harman (2011) which demonstrated the ability of *Trichoderma* species to increase nitrogen and phosphorus uptakes in plants. Increased uptake of minerals in the rice plant may result in increased health and hardiness of the paddy. Therefore, higher brown rice milling efficiency can be obtained from paddies treated with *Trichoderma*. The above results demonstrate the potential of both *Trichoderma* bioproducts to provide not only better yield components but also increased grain quality of milled brown rice compared to the untreated control.

Rice seedling vigor index and seedling health index

The germination percentages of 5-month-stored healthy seeds derived from all *Trichoderma* treatments (78.89–88.33%) were not significantly different compared to the untreated control (85.44%). The SVI values from those germinated seeds of *Trichoderma* treatments were significantly increased by 4.54–19.65% compared with the untreated control. Moreover, the SVI values from the *Trichoderma* treatments—CB-Pin-01 fresh culture (Sk + Sp) and 01-52 wettable pellet (Sk + Sp)—significantly increased compared to both the untreated control and chemical fungicide treatments (Table 4).

The germination percentages of 5-month-stored dirty panicle infected seed among all treatments were not significantly different. However, significant increases in seed germination (3.12–12.50%) produced by the *Trichoderma* treatments were observed. Bioproducts of all *Trichoderma* treatments significantly increased the SHI by 45.00–72.50% compared with the untreated control. There were no significant differences in the SHI values among all *Trichoderma* treatments, while the 01-52 wettable pellet bioproduct (Sk + Sp) treatment produced the highest increase in SHI (72.50%) compared to the untreated control. The SHI of this *Trichoderma* treatment (2.76) was significantly higher than that of the fungicide treatment (1.86) as shown in Table 4. The results revealed the capability of *Trichoderma* bioproducts to improve and maintain the

Table 4
Effect of *Trichoderma asperellum* isolate formulations on seed germination, seedling vigor index and seedling health index of rice seedlings sown from 5-month-stored seeds harvested from rice plants (var. Chai Nat 1).

Treatment	5-mth-stored healthy seeds		5-month-stored dirty panicle infected seed	
	Seed germination (%)	Seedling vigor index	Seed germination (%)	Seedling health index
1. *T. asperellum* CB-Pin-01 fresh culture bioproduct (Sk + Sp*)	87.33[a†] (+2.21%)[‡]	1382.10[a] (+19.00%)	87.50[a] (+9.38%)	2.32[ab] (+45.00%)
2. *T. asperellum* 01-52 wettable pellet bioproduct (Sk + Sp)	88.33[a] (+3.38%)	1389.58[a] (+19.65%)	90.00[a] (+12.50%)	2.76[a] (+72.50%)
3. *T. asperellum* 01-52 wettable pellet bioproduct (Sk)	78.89[a] (−7.67%)	1214.17[ab] (+4.54%)	82.50[a] (+3.12%)	2.45[ab] (+53.12%)
4. mancozeb (Sk)/propiconazole + difenoconazole (Sp)	82.89[a]	1183.68[b] (+1.92%)	80.00[a] (+0%)	1.86[bc] (+16.25%)
5. Control (untreated)	85.44[a]	1161.39[b]	80.00[a]	1.60[c]

*Sk = seed soak; Sp = plant spray.
† Means in each column followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test (DMRT) ($p \leq 0.05$).
‡ Percentage of increase (+) or decrease (−) of each treatment mean when compared with the untreated control.

quality of both healthy and dirty panicle infected seeds during storage. The exact mechanisms whereby *Trichoderma* bioproducts induce better SVI and SHI values in rice seed than in the untreated control are not known; however the enhanced uptake of minerals such as nitrogen and phosphorus in rice plants (Shoresh et al., 2010; Harman, 2011) and the accumulation of such minerals in rice seed could be involved. Further study to confirm and strengthen this data will provide beneficial information for improving and enhancing the vigor and health of rice seed in seed production programs.

In conclusion, the current results suggested that both *T. asperellum* fresh culture and wettable pellet bioproducts applied as seed soaks (Sk) and plant sprays (Sp) not only enhanced the growth and yield of rice, but also significantly increased the number of healthy seed and reduced the percentage of dirty panicle infected seed and empty (unfertile, undeveloped) seed. Moreover, after milling of harvested paddy, the whole kernels plus head rice weights of milled brown rice derived from all *Trichoderma* treatments were increased, whereas for broken rice they were reduced. These results indicated that both fresh culture and wettable pellet bioproducts compared to fungicide use provided comparable efficacy for reducing dirty panicle diseases and increasing rice growth and yield. Therefore, these bioproducts will provide benefits for normal agricultural practices and sustainable organic rice production in the future.

Conflict of interest

There is no conflict of interest.

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