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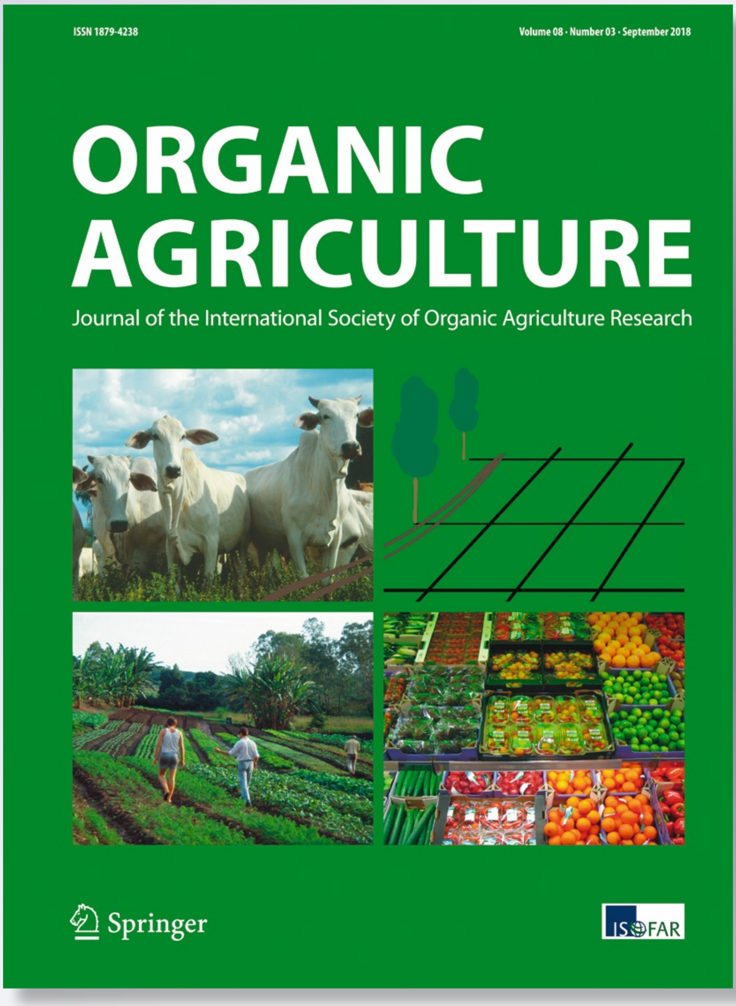

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A simple, efficient, and farmer-friendly *Trichoderma*-based biofertilizer evaluated with the SRI Rice Management System

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Abstract *Trichoderma* spp. are highly interactive fungi that live in soil, root, and foliar environments. In addition to assisting plants to resist various diseases and drought stress, *Trichoderma* has been reported to have positive effects on the growth of many crops. While *Trichoderma* inoculants have been developed for use with numerous crops, explorations of the use of *Trichoderma* inoculants in rice farming systems are still in a nascent stage. In this study, a field experiment using a randomized complete block design was conducted to determine the ability of *Trichoderma*-based biofertilizer (TBF) to enhance the growth, physiological traits, and yield of rice under System of Rice Intensification (SRI) management. The results showed significant potential for TBF to increase a rice crop's growth, physiological traits, and productivity. *Trichoderma*-inoculated rice plants exhibited significantly greater plant height, photosynthetic rate, chlorophyll *a* and *b* content, stomatal conductance, and tiller and panicle numbers. The grain yield of *Trichoderma*-inoculated rice plants was 30% more than that from the uninoculated SRI control plots, which simply due to changes in management practices produced paddy yields that were twice the current average in Malaysia. A simple, efficient, and farmer-friendly method for producing TBF, developed for SRI farmers' use to get these results, is reported here.

Keywords Biofertilizer · Symbiotic fungus · System of Rice Intensification (SRI) · Sustainable rice production · *Trichoderma*

Introduction

Trichoderma is a fungal genus with numerous functions in agricultural systems, e.g., promotion of plant growth, induction of plant defenses against pathogens, and resistance to both biotic and abiotic stresses (Hermosa

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et al. 2012; Brotman et al. 2013; Atanasova et al. 2013). As an endophytic symbiont, *Trichoderma* establishes direct links with the plant by colonizing its root system (Martinez-Medina et al. 2016a). During *Trichoderma*-plant interactions, the fungus establishes chemical communication with the plant and enhances the expression of numerous plant genes (Martínez-Medina et al. 2016b). Although *Trichoderma* becomes endophytic in plant roots, the greatest changes in gene expression are reported to occur mostly in plant shoots (Harman et al. 2012).

Species belonging to the genus *Trichoderma* are already economically important, being used in a wide range of crop plants as microbial inoculants for plant-growth promotion and in the management of different pathogens (Contreras-Cornejo et al. 2013). Consequently, the search for *Trichoderma* isolates that have significant antagonistic and biofertilizer potentials has increased in recent years (López-Bucio et al. 2015). With growing concern worldwide about the adverse effects of agricultural chemicals on the environment (Heong et al. 2015), agricultural practitioners are looking increasingly for environmentally-friendly inputs such as biofertilizers to manage their crops and cropping systems (Rivera and Fernandez 2006; Viera and Alvarez 2006; Sahoo et al. 2013).

Recently, we successfully isolated a local strain of *Trichoderma*, namely *Trichoderma asperellum* SL2, which has been proven to enhance rice plant growth, physiological traits, nutrient uptake, and yield under gnotobiotic greenhouse conditions (Doni et al. 2017, 2014a). This isolate, henceforth referred to simply as *T. asperellum*, has thus far usually been used in the form of a suspension of fungal cells applied to rice seeds or seedlings.

Trichoderma inoculants can be mass production through liquid or solid fermentation (Oancea et al. 2016; Kobori et al. 2015; Cavalcante et al. 2008). However, mass propagation of *T. asperellum* using solid agar medium in petri dishes is not an economically feasible approach for the production of large supply of this microorganism. Substrates for the production of *Trichoderma* species can come from crop residues, livestock wastes, industrial wastes, and any other natural material (Isahak et al. 2014). Our investigations looking for a carrier from among different locally available organic materials for scaled-up production of *T. asperellum* have shown that corn kernels can be a cheap and efficient carrier in the local production of this fungal agent (Doni et al. 2014b).

The System of Rice Intensification (SRI) is an agroecological approach to rice production with the following features: (i) healthy young seedlings are selected and transplanted into the rice field preferably in their two-leaf stage, which under Malaysia's tropical conditions means at an age of 5–7 days; (ii) single seedlings are planted at a spacing of 25–30 cm to encourage vigorous root growth by reducing competition for nutrients and also to support profuse tillering; (iii) mechanical weeding is carried out to eliminate weeds as well as to surface-aerate the soil; (iv) instead of continuous flooding, SRI rice paddies are flooded only intermittently to achieve better soil aeration and growth of beneficial soil microorganisms; and (v) organic fertilizers are used/preferred over chemical fertilizers (Thakur et al. 2013, 2015, 2016; Styger and Uphoff 2016; Wu and Uphoff 2015; Uphoff 2015). One factor contributing to the robust growth of SRI rice plants is the beneficial effects of symbiotic microbes such as *Trichoderma* (Doni et al. 2017; Anas et al. 2011).

One of the practices employed by SRI farmers in Malaysia is to produce home-made biofertilizers from agricultural wastes by conventional fermentation in a liquid form to be sprayed on the plants during rice cultivation (Yusoff et al. 2013). This technique is unable to produce reliable biofertilizers, however, because it combines and uses many unknown, even non-functional microorganisms in a single-culture solution. Moreover, various environmental factors such as moisture content, temperature, pH and oxygen levels affect the quality of any liquid biofertilizer produced from the fermentation of agro-wastes (Lim and Matu 2015). Given this background, we developed a standardized procedure for producing a *Trichoderma*-based biofertilizer using corn kernels as the substrate, which could meet the field requirements that are common to farmers in Malaysia. Producing and using this biofertilizer, details discussed in a supplement to this paper, could free farmers from their current dependence, or at least reduce this reliance, on purchased chemical fertilizers which are commonly used in rice farming systems.

The objective of this study was to refine and assess the effectiveness of a *Trichoderma*-based biofertilizer (TBF) formulated using corn kernels as the carrier, monitoring key changes in growth parameters, physiological traits and yield. This work involved also developing a simple, efficient and farmer-friendly procedure for producing reliable, good-quality, practical biofertilizer for application in SRI rice fields.

Materials and methods

Fungus culture

Trichoderma asperellum SL2 (UPMC 1021) was grown in petri dishes containing potato dextrose agar (PDA) medium and incubated for 7 days at 30 °C until the fungal colony became green and produced spores. These spores were then removed from the plates by flooding the plates with sterilized distilled water and transferring the spores immediately to an Erlenmeyer flask. These spores were diluted by adding sterilized distilled water until the concentration reached 10^7 spores/ml, based on hemocytometer counts.

Carrier preparation

Corn kernels were sterilized by autoclaving them at 121 °C for 15 min and then cooling them in a laminar air flow for 10 min. This treatment made the kernels non-viable for germination, but a suitable substrate for microbial nutrition. The kernels were sprayed with a suspension of *T. asperellum* spores (1 ml spores with a concentration of 10^7 spores per ml for 50 g of kernels) and then stored in sterilized polyethylene plastic bags, incubated for 15 days at 30 °C before their use (Doni et al. 2014b).

Calculation of colony-forming units for shelf-life study

Samples of the TBF were kept in the laboratory for estimating numbers of colony-forming units (CFU). Measurement of CFU of *T. asperellum* in the corn-kernel carrier was conducted by suspending 1 g of *T. asperellum* corn formulation and serially diluting it in sterilized distilled water. The suspension was then homogenized using a vortex apparatus, and 1 ml of suspension plated on fresh potato dextrose agar under aseptic conditions using spread-plate plating method, in triplicate. These plates were incubated at 30 °C, and population counts were taken at 15-day intervals, being continued up to 120 days. During the period of storage, the formulations were stored in sealed polythene bags at 30 °C (Sriram and Savitha 2011; Singh et al. 2007). We also developed at the same time a simple, efficient and farmer-friendly procedure for producing TBF at farmers' level with their knowledge and skills. Besides this, we also looked at the costs and economics of this methodology. Our findings are reported in the Supplementary File 1.

Seedling preparation

In this experiment, the Malaysian rice variety MRQ74, a medium-duration rice variety (120–125 days), was used. Before sowing, the seeds were surface-sterilized to minimize contamination from pathogenic microbes. For this, the seeds were soaked in 70% ethanol for 30 min, followed by soaking in 5% sodium hypochlorite for 30 min, and then washing repeatedly with sterilized distilled water. The seeds were then grown in 30 cm × 50 cm seedling trays containing a mixture of equal amounts of sterilized compost, soil and sand. The young plants were watered carefully two times a day using a small sprayer. The soil was kept moist, but without standing water. The seedling trays were kept inside a 3 m × 3 m growth chamber made of wood, with bamboo leaves and plastic forming a roof. Seedlings were transplanted into the open field at 7 days after sowing.

Experimental site and soil

This research was conducted at the SRI Lovely Farm, a certified organic farm in Sik, Kedah Malaysia (N 6.047668, E 100.8414271; 104 m above sea level). The experiment was performed during the wet season (December 2015 to April 2016) on a soil classified as sandy loam, with pH 5.33; EC 2.2 dS/m; total N 0.72%; total P 0.40%; total K 0.66%.

Experimental design and land preparation

The experiments were conducted using a randomized complete block design, with two treatments and five replicates, and each replicate plot measuring 5 m × 5 m. Each plot was separated by bunds 0.5 m wide to prevent cross-contamination of treatment effects, and irrigation channels 0.5 m wide were constructed to surround the plots for the purpose of controlling water. In each plot, for transplanting with regular spacing, a wooden marker was used to trace a square grid pattern on the soil's surface with demarcated distances of 30 cm × 30 cm between the perpendicular lines.

Seedlings transplanting and crop management

Rice seedlings were carefully lifted from the seedling trays to ensure that their roots were not separated from the plants, and each seedling was transplanted singly.

Seedlings were established at a shallow depth (≤1 cm), with no standing water on the muddy field during transplanting. Mechanical weeding was performed at 10-day intervals, at 10, 20, 30, and 40 days after transplanting, using a two-row motorized weeder. Details of crop management are described in Table 1.

Application of TBF

The TBF was applied as a soil application at 20 days after transplanting as indicated in Table 1, mixed with sterilized compost (N 16.2%; P 6.7%; K 11.4%) in a ratio of 1:2. The mixture of TBF and compost was then applied to the field at the rate of 60 g/m^2. This application was carried out before weeding, so that during the weeding, the mixture could be mixed into the soil. On the control plots, compost was applied at a rate of 40 g/m^2, so all plots had the same amount of compost applied.

Physiological parameters measurement

Physiological traits of rice plants on the respective *Trichoderma* and control plots were evaluated at 50 days after transplanting. Measurements were made on the flag leaves of the respective rice plants on a clear sunny day between 09:45 a.m. and 11:30 a.m. with solar radiation of >1200 μmol m^{-2} s^{-1}. Five plants were selected randomly from each of the five plots to represent each replicate with measurements of photosynthesis rate (μmol m^{-2} s^{-1}), leaf stomatal conductance (mmol m^{-2} s^{-1}), internal CO_2 concentration (ppm), and transpiration rate (mmol m^{-2} s^{-1}). These measurements were done using a LICOR 6400 (Lincoln, Nebraska, USA) and an infrared gas analyzer (IRGA) as previously described in Doni et al. (2017).

Chlorophyll content

Chlorophyll content of the leaves was monitored at 50 days after transplanting. Five plants were selected randomly from each plot representing each replicate for measurement. Samples of 0.1 g of comminuted plant leaves (fragments ~2 mm) were placed in a test tube, and 20 ml of 80% acetone was added to the tube. The mixture was homogenized using a vortex apparatus and then incubated for 2 × 24 h under dark conditions. Concentrations of chlorophyll *a* and chlorophyll *b* were analyzed using a spectrophotometer at the respective wavelengths (λ) of 663 and 645 nm. Chlorophyll *a* and *b* content were calculated according to the equations given below and were expressed as μg/g fresh leaf weight (Shibghatallah et al. 2013).

Table 1 SRI crop management

Day after transplanting	Water management	Weed management	Nutrient management
1–8	Soils were kept moist, with no standing water allowed		
9–10	Water was added overnight, not >2 cm	1st weeding	
11–18	Water was drained out, and soil conditions were maintained slightly aerobic		
19–20	Water was added overnight, not >2 cm	2nd weeding	Application of mixture of TBF and compost, application rate of 60 g/m^2 on *Trichoderma* plots; for control plots, compost was applied at rate of 40 g/m^2
21–28	Water was drained out, and soil conditions were maintained slightly aerobic		
29–30	Water was added overnight, not >2 cm	3rd weeding	
31–38	Water was drained out, and soil conditions were maintained slightly aerobic		
39–40	Water was added overnight, not >2 cm	4th weeding	
45–100	Water was maintained at 2 cm level from the soil surface		
100–120	Soils were dried and maintained with slightly cracked top soil surface, but enough soil humidity to sustain plant growth		

$$C_{chl-a} = 12.7A_{663} - 2.69\,A_{645}$$
$$C_{chl-b} = 22.9A_{645} - 4.68\,A_{663}$$

Chlorophyll content was also determined by using a SPAD 502 Plus Chlorophyll Meter (Konica Minolta Ltd.). Five plants were selected randomly from each plot, and three different rice leaves were chosen randomly from every rice plant and clipped in a SPAD meter, with the readings recorded carefully (Doni et al. 2017).

Measurement of growth and yield components

Growth and yield parameters were evaluated at 120 days after transplanting. Five plants from each plot in each replicate were randomly chosen for the measurement of growth parameters. Plant height (cm) was measured from ground level to the tip of the highest leaf using a tape meter. To measure the fresh weight of canopy and roots, the rice plants were separated carefully from the soil, then cleaned using flowing tap water, and weighed using a digital scale (in g). For the measurement of biomass in roots and canopy, the plants were dried in an oven at 65 °C for 7 days before weighing (in g).

Yield components—tiller number, panicle number, spikelets per panicle, unproductive tillers, biomass (g), filled and unfilled grains, and 1000-grain weight (g)—were all evaluated at maturity stage (Doni et al. 2017). Yield calculation was done based on yield component calculations using the amount of panicle m^{-2}, 1000-grain weight at 14% humidity, and the number of grains per panicle. Yoshida et al. (1976) and Casanova et al. (2002) have observed that calculations based on produce component are closely related to the number of panicles, rice grains, existence of weeds, and planting uniformity. Grain yield (Y) was calculated using this formula:

$$Y = \text{PANO} \times \text{SPP} \times \text{FSP} \times \text{Wf} \times 10^{-5}, \text{where Y}$$
$$= \text{grain yield}\left(\text{t ha}^{-1}\right), \text{PANO}$$
$$= \text{panicle number m}^{-2}, \text{SPP}$$
$$= \text{spikelets per panicle}, \text{FSP}$$
$$= \text{fraction of filled spikelets}, \text{Wf}$$
$$= 1000\text{−grain weight (g)}$$

Statistical analyses

All data were statistically analyzed using analysis of variance (ANOVA). The significance of the treatment effect was determined using an F test, and calculation of least significant difference (LSD) at the 5% probability level was used to determine the significance of the differences between the means of two treatments.

Results

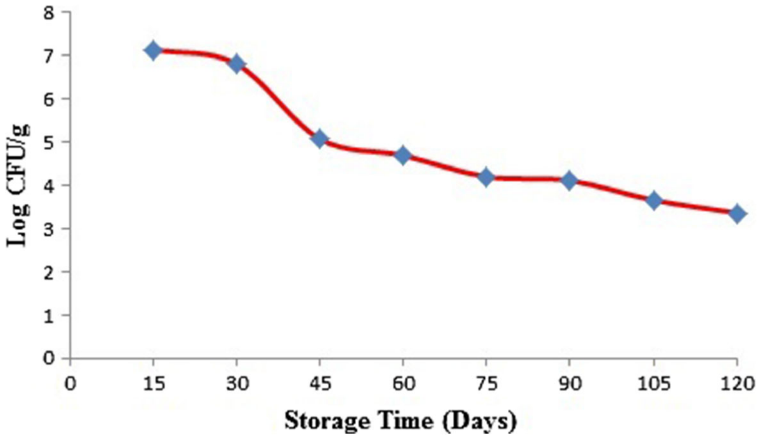
Shelf-life of TBF

For the values of measured microbial biomass in the inoculation material, the average population count (log CFU) was highest (7.14) at 15 days after the preparation of the formulation. It was reasonably stable thereafter at temperature 30 °C up to 120 days. Then a gradual loss of stability started beyond this period (Fig. 1).

Effects of TBF on rice growth

Soil application of TBF on rice fields evoked a significant increase in plant growth (Table 2). Plant height was significantly greater in the TBF-treated plots at 56.2 cm (±0.27) compared to the control plots, 45.9 cm (±1.04). Also, a positive and significant correlation ($p < 0.05$) was observed between TBF inoculation and root fresh weight (g). This was 13% higher in the TBF-treated plants compared to controls. A similar relationship was observed in canopy fresh weight measurements, with average weight of the canopies in TBF and control plants being 86.4 g (±0.72) and 66.9 g (±0.56), respectively (Table 2). There was significantly greater belowground biomass production in TBF-inoculated plants compared to control plants ($p < 0.05$). TBF-inoculated plants had an average root biomass of 33.9 g (±0.29) while control plants had 24.5 g (±0.33). Mean canopy biomass for the two treatments was also statistically different at 43.57 g (±0.38) vs. 33.22 g (±0.40), respectively (Table 2). Note that both sets of plants had been grown with SRI agronomic methods, which gave greater plant productivity than from rice plants with conventional management, as discussed below.

Fig. 1 Population density and shelf life of *T. asperellum*



Effects of TBF to rice physiological traits

TBF application significantly increased the main physiological traits of rice plants (Table 3). Net photosynthetic rate (μmol m^{-2} s^{-1}) was higher in rice plants inoculated with TBF compared to control plants. Inoculated plants had a rate of 18.2 μmol m^{-2} s^{-1} (±0.32), while control plants recorded a value of 10.7 μmol m^{-2} s^{-1} (±0.20). Significantly higher values ($p < 0.05$) of stomatal conductance were exhibited in inoculated plants. This had a mean of 863.57 mmol m^{-2} s^{-1} (±3.77), while for control plants the mean was 658.53 mmol m^{-2} s^{-1} (±3.76). Inoculation of rice plants with TBF also influenced their internal concentration of CO_2 as compared to control plants since the former had significantly lower concentrations (<30%) (Table 3). Note that a lower value on this parameter is physiologically a more favorable value for plant performance.

The rice plants inoculated with TBF had significantly greater water use efficiency compared to control plants. This was seen in their respective transpiration rates (mmol m^{-2} s^{-1}), with TBF plants recording much lower values compared to control plants (Table 3). The ratio of photosynthetic rate to transpiration rate was 2.5× greater in TBF-inoculated plants (3.05) vis-à-vis the ratio in control plants (1.19). At the same time, the TBF-treated rice plants also demonstrated higher levels of chlorophyll *a* and chlorophyll *b* in their leaves (Table 3), about 2.5 times more of both forms compared to these levels in untreated plants. This was consistent with the relative chlorophyll content (SPAD value) which was observed to be significantly higher in TBF-treated plants (>41.45%).

Effects of TBF on yield parameters of rice plants and on yield

Treatment with TBF had a significant effect on all of the yield parameters of rice (Table 4). A significant increase in the number of tillers was observed in TBF-treated rice plants as their mean number (57.3 ± 1.03) was two-thirds more than that for control plants (34.3 ± 0.65). Significantly larger numbers of panicles (30.9 ± 0.33) were also observed in TBF-treated rice plants, compared to control plants (24.2 ± 0.56). Spikelets per panicle were also higher in the TBF-treated plants compared to control plants (Table 4).

Further, the rice plants with TBF amendments showed a small enhancement in 1000-grain weight, at 24.2 g compared with 23.8 g in untreated plants (Fig. 2).

Table 2 Rice plant growth responses to *T. asperellum* inoculation

Treatments	Plant height (cm)	Root fresh weight (g)	Canopy fresh weight (g)	Root biomass (g)	Canopy biomass (g)
Trichoderma	56.20 ± 0.27	74.00 ± 0.55	86.42 ± 0.72	33.90 ± 0.29	43.57 ± 0.38
Control	45.92 ± 1.04	60.96 ± 0.43	66.92 ± 0.56	24.51 ± 0.33	33.22 ± 0.40
LSD	2.37	1.55	1.76	0.91	1.14

All means were significantly different between treatments at $p < 0.05$

Table 3 Rice plant physiological responses to *T. asperellum* inoculation

Parameters	Treatments		LSD
	Trichoderma	Control	
Photosynthetic rate (μmol m^{-2} s^{-1})	18.22 ± 0.32	10.71 ± 0.20	0.79
Stomatal conductance (mmol m^{-2} s^{-1})	863.57 ± 3.77	658.53 ± 3.76	12.46
Internal CO_2 concentration (ppm)	288.06 ± 0.67	375.58 ± 4.28	11.00
Transpiration rate (mmol m^{-2} s^{-1})	5.96 ± 0.090	9.01 ± 0.080	0.22
Chlorophyll *a* (mg/g)	2.66 ± 0.037	1.00 ± 0.028	0.11
Chlorophyll *b* (mg/g)	0.98 ± 0.42	0.39 ± 0.018	0.05
Chlorophyll relative content (SPAD)	32.79 ± 0.60	23.18 ± 0.97	2.28

All means were significantly different between treatments at $p < 0.05$

All these factors together culminated in significantly higher grain yield, 10.02 t ha^{-1} from the TBF-inoculated rice plants, compared with 7.67 t ha^{-1} from control plants (Fig. 3). The yield from TBF-treated plots was thus 30% more than the yield from control plots without TBF inoculation.

Note that all of these comparisons were made between plants, inoculated vs. uninoculated, that had been grown with SRI cultivation methods. The yield from the uninoculated SRI plots in this experiment was more double than Malaysia's national average paddy yield (2–3 tons/ha), while the yield from plots that had TBF inoculation was more than triple.

Discussion

Production and shelf-life of TBF

This study has demonstrated that *Trichoderma* biofertilizer can be successfully formulated using corn kernels as the carrier. The green coloration on the surface of the corn kernels indicated that the *Trichoderma* mycelium grew successfully and had colonized the carrier as intended (Fig. 4). The good growth of *Trichoderma* in corn kernels was due to having sufficient amounts of essential components of nutrition such as carbohydrates, proteins, minerals, and amino acids (Kim et al. 2008; El-Fattah et al. 2013). No expensive equipment is necessary as simple pressure cookers can be used to achieve sterilization at household level (Fig. 5). Material as inexpensive and accessible as corn kernels can be used effectively and safely as the delivery carrier for field application of *Trichoderma*. Possibly rice grains could be also used for this purpose if properly sterilized and treated, but this has not been investigated.

A viable microbial inoculant should give farmers microbial persistence in the soil to as to apply bioactive material to the target plants. Our study showed that a viable inoculum of *Trichoderma* produced with comminuted corn kernels can have a shelf-life of up to 120 days. However, to be widely adopted by farmers, a microbial inoculant must also be cheap and easy to apply, enabling farmers to deliver the desired microbes to host plants in an appropriate manner and form (Herrmann and Lesueur 2013). In our field trials, farmers found this technology to be operational, and our economic analysis, in the supplement, showed the technology to be quite affordable.

Table 4 Rice plant yield responses to *T. asperellum* inoculation

Treatments	Tiller number	Panicle number	Spikelets per panicle	Filled grains
Trichoderma	57.32 ± 1.03	30.88 ± 0.33	121.96 ± 0.40	120.96 ± 0.37
Control	34.32 ± 0.65	24.08 ± 0.56	119.88 ± 0.31	119.00 ± 0.23
LSD	2.38	1.45	1.05	0.93

All means were significantly different between treatments at $p < 0.05$

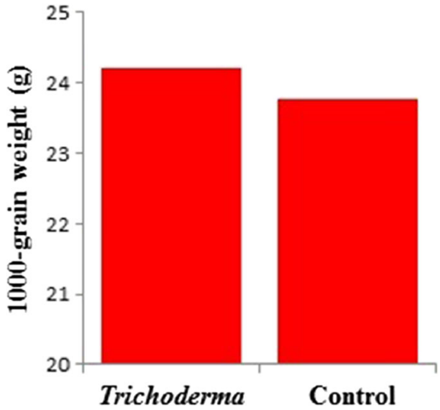


Fig. 2 1000-grain weight of rice plants upon application of TBF

TBF for increasing rice plant growth and yield

Effects on growth

As seen from the results reported, TBF soil treatment showed consistent and significant effect for increasing rice plants' performance and production. The increases in rice growth observed in this study—in plant height, root fresh weight, canopy fresh weight, root biomass, and canopy biomass—were all significantly related to the effect of *Trichoderma* as a plant growth-enhancer. Several reports from previous studies have shown that adding *Trichoderma* to soil systems can promote growth in a variety of plants, e.g., radish, tomato, pepper and cucumber (Kleifeld and Chet 1992), strawberry (Porras et al. 2007), tomato (Morsy et al. 2009), soybean (John et al. 2010), maize (Kumar et al. 2016), and grapes (Pascale et al. 2017).

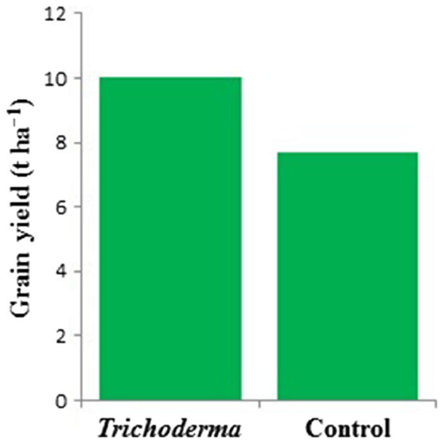


Fig. 3 Grain yield of rice plants upon application of TBF

Fig. 4 Green coloration indicates good growth of *Trichoderma*

Plant growth-promoting effects induced by *Trichoderma* are associated with several modes of action, such as production of plant growth hormones, solubilization of sparingly soluble minerals, buffering of the immediate environment, control of root and foliar pathogens, changes in the composition of micro-flora in roots, enhancing the utilization of available nutrients and water uptake, stimulating root and root hair development, increasing plant systemic resistance, and production of siderophores (Neumann and Laing 2006; Nicolás et al. 2014). Besides increasing plant growth (Fig. 6), the application of TBF can result in reduced environmental contamination, thereby contributing towards more sustainable farming systems.

Effects on yield

When the yield parameters of TBF-inoculated plants were compared to those of control plants, there were

Fig. 5 Low-cost, simple equipment for TBF production at household level

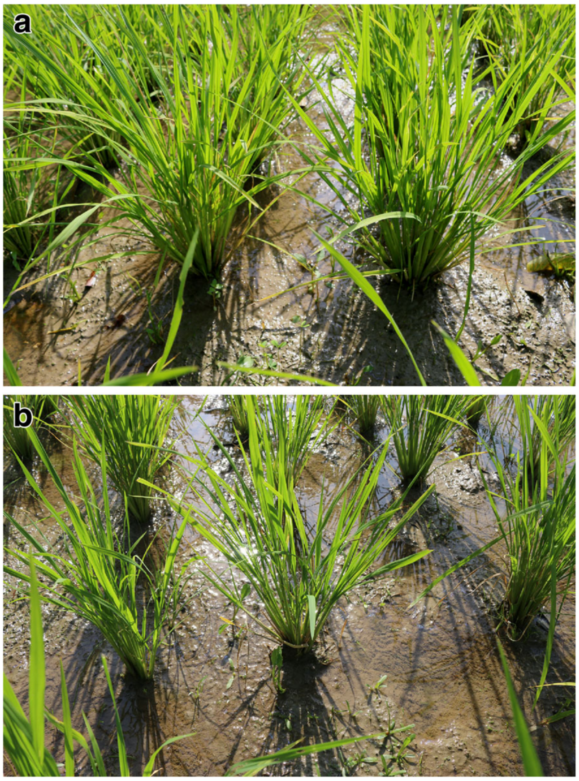


Fig. 6 Rice plants inoculated with TBF (**a**) show higher tillering and better canopy growth compared to control plants (**b**). Both pictures were taken at 30 days after sowing

significant increases in tiller number, spikelets per panicle, filled grains, 1000-grain weight, and grain yield. These increases were correlated with higher physiological capacities and greater growth of roots and canopy. Further, Shoresh and Harman (2008) have suggested a direct connection between the ability of *Trichoderma* spp. to induce energy metabolism and their ability to induce plant growth and yield. Cai et al. (2015) have reported that the contribution of endophytic *Trichoderma* maintaining stable plant yields may be due to the capacity of *Trichoderma* to enhance soil nutrient availability in root systems.

Furthermore, Colla et al. (2015) have reported that early and total yields of zucchini plants were respectively and significantly increased by 59 and 15% by the application of *Trichoderma* and *Glomus*. More recently, Buysens et al. (2016) reported that co-inoculation of *Rhizophagus* and *Trichoderma* can increase yield of potato plants under low nutrient conditions by up to 37%.

Physiological effects

Effects on photosynthesis

Benefits to rice plants from TBF inoculation include impacts on physiological traits such as photosynthetic rate, stomatal conductance, and specific and relative chlorophyll content. Other beneficial effects of TBF inoculation were significant decreases in rice plants' transpiration rate and in their internal CO_2 concentration. A higher photosynthetic rate with a lower internal CO_2 concentration and lower transpiration rate indicates that TBF-inoculated plants have a more efficient carboxylation process (Thakur et al. 2010). Carboxylation is a reaction in which Rubisco catalyzes RuBP with CO_2 to produce the carbon compounds that eventually become triose phosphates which are used by the plant for producing sugars and starches (Rezende et al. 2016).

Higher photosynthetic efficiency in TBF plants is also indicated by their higher stomatal conductance, as greater stomatal activity is linked to a higher capacity for CO_2 fixation (Valentine et al. 2006). During photosynthesis, CO_2 absorbed through the stomata is used by the plants to produce sugars. Previous studies by Augé (2000) have suggested that stomatal changes in microbe-inoculated plants result in an alteration of the plants' hormonal status. We have reported previously (Doni et al. 2017) that *Trichoderma*-inoculated rice plants exhibit higher stomatal density which is also associated with enhancement of the photosynthesis process.

The results obtained in this study showed a significant enhancement of the chlorophyll content in TBF-inoculated plants. The higher chlorophyll content with TBF application can be attributed to larger and better functioning root systems, which enable the plants to achieve greater nutrient and water up-take capacity (Thakur et al. 2010). Colonization activity of *Trichoderma* in plant roots could also activate some pathway signals that are involved in the synthesis of certain hormones, which play important roles in regulating chlorophyll content (Martinez-Medina et al. 2014; Guler et al. 2016). Furthermore, in their induction of plant resistance to biotic and abiotic stresses, *Trichoderma* strains enhance plant chloroplast pathways which have been linked to the improvement of photosynthetic efficiency. This is done by reducing the damage caused by superoxide anions and other reactive species involved in photosynthesis (Harman 2011).

Effects on water use efficiency

In addition, the lower rates of transpiration along with higher rates of photosynthesis in TBF-inoculated plants indicate that these plants use water more efficiently than do the control plants, thereby yielding "more crop per drop." These results are consistent with findings reported in Doni et al. (2014a). The increased water use efficiency afforded by *Trichoderma* can be a vital feature for rice production in future as current water scarcity becomes a more serious constraint.

Impact of *Trichoderma* on soil systems

Soil application of *T. asperellum* has been seen to enhance rice plant growth, physiological traits, and yield as effectively as the application of *Trichoderma* by seed treatment (Doni et al. 2017). Besides increasing rice plant productivity, the application of *Trichoderma* as soil treatment has been reported to increase the populations of *Trichoderma* from about 10^4 to about 10^6 CFU/g of root (Harman et al. 2008). Although *Trichoderma* spp. make up about 10 to 60% of the total culturable fungi usually isolatable from soils (Zachow et al. 2016), most *Trichoderma* strains are not endophytic, nor are they good root-colonists (Harman et al. 2008). Also, we know that not all *Trichoderma* strains are effective in increasing rice plant growth (Doni et al. 2014a). Therefore, there is a need to use a comprehensive functional approach to identify and isolate more *Trichoderma* strains that have biocontrol and growth-promoting capabilities, which can be used for improving soil health and plant productivity (Martinez-Medina et al. 2014).

Trichoderma inoculants for sustainable rice production

The effects of *Trichoderma* inoculation have been well studied and used with many crops as seen in Table 5, but not much work has been done thus far with *Trichoderma* and rice plants. As seen from our literature review summarized in Table 6, studies of *Trichoderma* inoculants for rice cultivation have not been many and have been limited in use. Some bacterial-based inoculants are becoming widely used in rice plant production, especially from the *Pseudomonas* genus. There is a need and opportunity to formulate various microbial inoculants that would have beneficial effects not only on plant growth and productivity, but that are also able to improve soil health in varied ways (Vassilev et al. 2015).

TBF and related opportunities for application in SRI agroecosystems

In recent years, there has been a growth in worldwide awareness of eco-friendly approaches to increasing the yield and production of rice plants (Charoenrak and Chamswarng 2016). Production approaches such as using crop residues as surface mulch and using various organic manures and microbial inoculants have been shown to help enhance yields and sustain soil fertility and health (Isahak et al. 2014; Doni et al. 2013; Rupela et al. 2006). At the same time, reliance on chemical fertilizers, herbicides and pesticides with their economic and environmental costs is viewed with increasing dissatisfaction in many countries (Stehle and Schulz 2015; West et al. 2014).

For increasing rice production in more environmentally-friendly and socially acceptable ways, the development and use of microbial inoculants is a promising approach for maintaining soil quality and enhancing paddy production as seen in Table 6 above. This research has shown that there are specific beneficial effects to be obtained from utilizing a particular formulation of a *Trichoderma*-based inoculation process for rice, used in conjunction with the management practices of the System of Rice Intensification, which is itself conducive to the enhancement of beneficial soil biota (Anas et al. 2011; Watanarojanaporn et al. 2013).

Conclusion

This research highlights the potential use of TBF as sustainable inoculant in rice farming system. TBF have the potential to enhance rice plant growth parameters, photosynthetic rate, stomatal conductance, chlorophyll content, and yield. Besides that, TBF was also able to decrease the internal CO_2 concentration and transpiration rate, which indicated an efficiency in converting CO_2 into carbohydrates. A simple method for producing *Trichoderma*-based biofertilizers is available for farmers to take up, thereby reducing their dependency on chemical fertilizers, giving them economic advantages, and producing wider environmental benefits.

Table 5 Reports on effects of *Trichoderma* inoculation in crop production

Species	Crops	Delivery carriers	Role in crop production	References
T. harzianum SQRT037	Cucumber	Amino acid organic fertilizer, compost of pig manure and rice straw, or of Chinese medicine production residues, and or of alcohol and vinegar production residues	Control of *Fusarium* wilt	Yang et al. (2011)
T. virens, T. hamatum, T. viride, T. harzianum	Cucumber, eggplant, and pepper seedlings	Commercially manufactured cellulose granules	Reduce damping-off caused by *Rhizoctonia solani*	Lewis et al. (1998)
T. harzianum T22	Tomato	Compost of a mix from sugar mills, poultry litter, cow dung and household/kitchen wastes	Increase plant growth, yield, and antioxidant and mineral contents	Khan et al. (2016)
T. asperellum T34	Cucumber	Agricultural waste compost	Decrease *R. solani* infection	Trillas et al. (2006)
T. harzianum T24	Tomato	Alginate	Produce hydrolytic enzymes, promote their growth	El-Katatny (2010)
T. harzianum	Chickpea	Talc, kaolin, and bentonite	Decrease *R. solani* infection	Prasad and Rangeshwaran (2000)
T. harzianum	Apple	Coconut and soybean oils	Provide protection from *Botrytis cinerea* infection	Batta (2004)
T. harzianum starin 1295-22 (ATCC 20847)	Cucumber and bean	Czapek Dox and Richard's medium with addition of V8 juice	Provide protection against attack by *Pythium ultimum*	Harman et al. (1991)
T. koningii, T. aureoviride and *T. longibrachiatum*	Sunflower	Industrial talc and milled corn kernels	Reduce disease incidence of *Sclerotinia sclerotiorum* infection	Escande et al. (2002)
T. harzianum	Eggplant	Aqueous extracts of *Azadirachtaindica, Ricinuscommunis*, *Pongamiapinnata*	Increase plant height and seedling weight	Rao et al. (1998)
Trichoderma spp.	Radish	Wheat bran	Provide biocontrol against *R. solani*	Mihuta-Grimm and Rowe (1986)
T. harzianum T22	Tomato	Kitchen wastes	Improve the yield and nutritional quality	Molla et al. (2012)
T. asperellum	Pineapple	Talc powder	Inhibitory action against *Thielaviopsis paradoxa*	Wijesinghe et al. (2011)
T. viride IF-26, *T. harzianum* ATCC 24274, *T. pseudokoningii* ATCC 26801	Pea	Apple pomace-based medium	Increase seedling growth and vigor	Zheng and Shetty (1999)
T. harzianum PDBCTH 10, *T. viride* PDBCTV 32, *T. virens* PDBCTV 12	Chickpea	Talc	Increase germination, nutrient uptake, plant height, number of branches, nodulation, yield and total biomass	Rudresh et al. (2005)
T. viride IARI P-1, *T. virens* IARI P-3, *T. harzianum* IARI P-4	Mung bean	Wheat and pulse brans	Increase growth and yield, protect against *R. bataticola* infection	Dubey et al. (2009)
T. harzianum	Pigeonpea	Talc powder		Prasad et al. (2002)

Table 5 (continued)

Species	Crops	Delivery carriers	Role in crop production	References
			Disease suppression towards *F. udum*	
T. harzianum ITEM 3636, *T. longibrachiatum* ITEM 3635	Peanut	Biodac	Increase growth and decrease disease severity of *F. solani* infection	Rojo et al. (2007)
T. harzianum	Okra	Rice straw and oil palm empty fruit bunch	Reduce disease severity of *Choanehora* wet rot caused by *Choanehora cucurbitarum*	Siddiqui et al. (2008)
T. harzianum NBRI 1055	Groundnut and chickpea	Banana pseudostem, compost, spent cob (maize), maize meal, rice husk, sawdust, sorghum grain, used tea leaves, wheat bran, bran sawdust	Reduce mortality from chickpea wilt complex and groundnut collar rot disease	Singh et al. (2007)
T. harzianum M1	Tomato	Talc, lignite, lignite + fly ash-based powder formulation, wettable powder, bentonite paste, polyethylene glycol-paste, and gelatin-glycerin-gel	Reduce incidence of damping-off disease caused by *Pythium aphanidermatum*	Jayaraj et al. (2006)
Trichoderma sp. Tri-1	Oilseed rape	Oilseed rape seedcake and straw	Increase production, and control infection of *Sclerotinia sclerotiorium*	Hu et al. (2016)
T. harzianum Th-10	Banana	Rice bran, rice chaffy grain, farmyard manure, banana pseudostem, and dried banana leaf	Control of *Fusarium* wilt	Thangavelu et al. (2004)
Trichoderma spp.	Pepper and cucumber	Mixture of vermiculite, powdered wheat bran, and dry fermenter-produced biomass	Control damping-off caused by *R. solani*	Lewis and Lumsden (2001)

Table 6 Examples of microbial inoculants being used for sustainable rice production

Microbes	Delivery carriers	Role in rice production	References
Rhodopseudomonas palustris strains TN114, PP803 and TK103	Rice straw and rice husk	Enhance rice yield, ameliorate rice seedling growth under salt stress, and reduce greenhouse gas emissions	Kantachote et al. (2016), Kantha et al. (2015)
Streptomyces corchorusii	Talcum and corn starch	Increase shoot length, plant weight, grain weight, and total grain yield	Tamreihao et al. (2016)
Consortium of phosphate-solubilizing bacteria and *Stenotrophomonas maltophilla*	Palm oil empty fruit bunches and peat soil with 48% carbon	Improve the fertility of acid sulfate soils for sustainable rice production	Panhwar et al. (2016)
Bacillus negaterium, and *T. viride*	Empty fruit bunches	Increase shoot biomass, root biomass, root length, and seedling height	Al-Taweil et al. (2009)
Rhizobium leguminosarum bv. trifolii	Peat	Increase agronomic potency and grain yield	Yanni and Dazzo (2010)
Azospirillum spp.	Charcoal powder and gum acacia	Elevate endogenous nutrient content, and improve growth and yield	Sahoo et al. (2014)
Nitrogen-fixing cyanobacteria	Wheat straw and multani mitti	Increase yield	Dhar et al. (2007)
P. fluorescens PF1, FP7 and PB2	Talc	Increase plant growth and yield, and suppress sheath blight disease.	Nandakumar et al. (2001)
P. fluorescens Pf1	Talc	Control rice blast disease, sheath blight disease, and increase grain yield	Vidhyasekaran and Muthamilan (1999), Vidhyasekaran et al. (1997)
P. fluorescens PfALR2	Peat	Control sheath blight disease and increase yield	Rabindran and Vidhyasekaran (1996)
P. fluorescens strains PF1 and FP	Mixture of talc and chitin	Control sheath blight disease and leaf-folder insect	Commare et al. (2002)
T. asperellum T12	Rice hull	Reduce sheath blight disease infection	Chen et al. (2015)

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