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Performance of a selected *Trichoderma* strain as plant pathogen inhibitor and biofertilizer

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ABSTRACT

Aims: The application of beneficial microbes is a suitable alternative to synthetic pesticides and fertilizers for agriculture. This study was aimed to evaluate the potential of a selected *Trichoderma* strain as a biocontrol agent against *Rhizoctonia* sp. and as a biofertilizer to improve paddy growth.
Methodology and results: Four *Bipolaris* strains were identified via DNA barcoding as the cause of brown spot disease, whereas two *Rhizoctonia* strains were similarly identified as the cause of sheath blight disease in Brunei Darussalam. Eight *Trichoderma* strains were initially screened using confrontation assay and were found to substantially inhibit the growth of *Rhizoctonia* sp. Hybrid rice named BDR5 was treated with *Trichoderma* sp. UBDFM01 and/or *Rhizoctonia* sp. It was found that the selected strain showed the potential as a biofertilizer by significantly increasing the vigour index I, chlorophyll a, chlorophyll b, total chlorophyll and dry shoot weight of the rice plants. The pathogen negatively affected the plants by significantly reducing the vigour index II, chlorophyll a, chlorophyll a/b ratio, total chlorophyll, and total weight of grains. *Trichoderma* strain showed the potential as a biocontrol agent by significantly diminishing the negative effects of the pathogen on the chlorophyll a, chlorophyll a/b ratio and total chlorophyll.
Conclusion, significance and impact of study: This study highlights the potential of *Trichoderma* sp. UBDFM01 as a biocontrol agent against *Rhizoctonia* sp. and also as a biofertilizer for rice plants. In addition, this study is the first to provide DNA-based evidence of *Bipolaris* sp. and *Rhizoctonia* sp. as the fungi that caused rice diseases in Brunei Darussalam.

Keywords: Biocontrol, biofertilizer, *Rhizoctonia*, rice, *Trichoderma*

INTRODUCTION

Agricultural systems require continuous improvement in order to maximize the production of safe, nutritious and high-quality food globally. The use of agrochemicals such as fertilizers and pesticides play a significant role in increasing crop yields in the shortest possible time and maintaining sufficient food supplies (Hanapi *et al.*, 2012; Meena *et al.*, 2016). However, these agrochemicals are commonly associated with many environmental and health issues. For example, the increasing excessive use of synthetic fertilizers and pesticides has resulted in resistant plant pathogens, atmospheric and groundwater pollution, soil degradation and a reduction in soil fertility (Hanapi *et al.*, 2012; Kourgialas *et al.*, 2017; Mahanty *et al.*, 2017). These issues have prompted the search for harmless, environmentally friendly, inexpensive and more sustainable solutions. In other words, practicing good agricultural practice is essential to reduce harm to human health, public goods and the environment (Bastiaans *et al.*, 2008).

An alternative to the use of synthetic pesticides and fertilizers is the utilization of biocontrol agents and biofertilizers that are derived from nature instead of synthetic chemicals. Biocontrol agents refer to microbes that are capable of suppressing disease-causing plant pathogens. In contrast, biofertilizer refers to microbes that are able to promote plant growth when applied to seeds, plant surfaces or soils by increasing the nutrients supply to the host plants. They may also inhibit pathogen diseases by producing some bioactive compounds (Hanapi *et al.*, 2012). Many *Trichoderma* species, such as *T. harzianum*, *T. atriviridae* and *T. asperellum* have previously been reported as potential biocontrol agents against various plant pathogens (Haran *et al.*, 1996). *Trichoderma* species are effective against a wide range of pathogens due to their rapid growth and diverse antagonistic mechanisms in inhibiting phytopathogens

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(Benítez *et al.*, 2004; Harman *et al.*, 2004; Vinale *et al.*, 2008; Kaewchai *et al.*, 2009; Viterbo *et al.*, 2010; Błaszczyk *et al.*, 2014). A number of *Trichoderma* species have been used commercially as biofertilizers such as *T. harzianum*, *T. koningii*, *T. polysporum*, *T. reesei* and *T. viride*.

In ensuring the security of food supply, Brunei Darussalam places much importance in agriculture, particularly rice production. As rice is mainly imported, the country aims to increase its rice production in order to be more self-sufficient. For 2020, 8.2% self-sufficiency was reported by the Department of Agriculture and Agrifood. Several challenges are associated with rice cultivation in this country, such as high soil acidity, lack of irrigation infrastructure and rice diseases. Many high-yielding hybrid rice varieties have been introduced, which is intended as one of the solutions for increasing rice production. The use of biocontrol and biofertilizer could also be considered. Therefore, this study aimed to evaluate the potential of a selected *Trichoderma* strain as a biocontrol agent and biofertilizer. This study sought to (1) isolate and identify fungal causal agents of rice diseases in Brunei Darussalam, (2) screen for potential *Trichoderma* strains with antagonistic property against a selected rice pathogen and (3) evaluate the effects of a selected *Trichoderma* strain on the growth and disease incidence of a local hybrid rice variety.

MATERIALS AND METHODS

Sample collection and *Trichoderma* strains

Symptomatic rice plant samples were collected in March 2020 from paddy fields at Wasan (4°47'16.9"N, 114°49'05.8"E) and Kandol (4°32'33.0"N, 114°34'56.8"E), Brunei Darussalam. All samples were placed in LACY'S ziploc bags during field sampling and stored in a cool Styrofoam box. After sampling, all the samples were stored in a refrigerator at 4 °C prior to isolation and identification of pathogenic fungi. Clayey soils were also collected from the paddy fields at Wasan to be used for growing paddy seeds. The rice seeds were kindly provided by the Paddy Industry Division of the Department of Agriculture and Agrifood, Ministry of Primary Resources and Tourism. The rice seeds belong to a hybrid variety called Brunei Darussalam Rice 5 (BDR5) that was developed from cross-breeding between two varieties, Laila and Pusu.

Eight *Trichoderma* strains used in the present study were provided and isolated by a previous study (Taha *et al.*, 2020). Briefly, the strains were isolated from either a mixed dipterocarp forest or a mangrove forest in Brunei Darussalam and identified by DNA barcoding. The strains used were *Trichoderma* sp. UBDFM01 (GenBank accession no. MK116428), *Trichoderma* sp. UBDFM02 (MK116429), *Trichoderma* sp. UBDFT02 (MK116418), *Trichoderma* sp. UBDFT03 (MK116419), *Trichoderma* sp. UBDFT04 (MK116420), *Trichoderma* sp. UBDFT11 (MK116421), *Trichoderma* sp. UBDFT19 (MK116424) and *Trichoderma* sp. UBDFT20 (MK116425).

Isolation and identification of pathogenic fungi

The collected plant samples were cut into tiny pieces (1 cm^2) and were washed with 1% sodium hypochlorite solution for 3 min and in sterile distilled water for 6 min. The samples were then placed on potato dextrose agar (PDA) plates and incubated at 25 °C for 4 days to allow the pathogenic fungi to grow. The fungi were sub-cultured a few times on the same growth medium by using a cork borer with a 10 mm diameter.

The pathogenic fungal isolates were identified by DNA barcoding using a previously described protocol (Taha *et al.*, 2021). Briefly, a Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, USA) was used to extract the fungal genomic DNA following the manufacturer's instructions. The extracted genomic DNA samples were exported to a service provider (Apical Scientific, Malaysia) for DNA sequencing of the rRNA-ITS sequence that was used for the DNA barcoding. A homology search on the NCBI database was conducted for the fungal isolates using the BLAST algorithm. Only the top BLAST matches were selected as two sequences with a similarity of 97% or higher is generally considered from the same species. MEGA X software (Kumar *et al.*, 2018) was used to align the DNA sequences using ClustalW and to construct a phylogenetic tree using the neighbor-joining method with the genetic distance calculated using the Kimura 2-parameter model.

Confrontation assay

A confrontation assay was used to quantify the growth inhibition of a selected pathogenic fungal isolate (*Rhizoctonia* sp. K6) by each of the eight *Trichoderma* strains. The assay was conducted following the dual culture plate method as previously described (Mayo *et al.*, 2015; Ng *et al.*, 2015). On a PDA plate, two fungal plugs (5 mm in diameter) of each *Trichoderma* strain and *Rhizoctonia* sp. were placed 5 cm apart from each other and incubated at 25 °C. For control, a PDA plate was inoculated with a fungal plug of the pathogen only. Five replicates were used for each *Trichoderma* strain. The radial growth of the pathogen was measured after 7 days, and the percentage of growth inhibition was calculated using the formula:

Inhibition = $[(r_1 - r_2)/r_1] \times 100$, where r_1 is the radial growth of the pathogen in the control plate and r_2 is the radial growth of the pathogen in test plate.

Seedling vigour and chlorophyll content

The viability of the rice seeds was tested by immersing the seeds in a beaker filled with distilled water for 30 min. Any seeds that floated were considered unviable, while the seeds that sank to the bottom of the beaker were used for testing. The seeds were surface sterilized using 1% sodium hypochlorite solution for 3 min and then washed with sterile distilled water for 6 min. The experiment consisted of four treatments – (1) treatment T:

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Seeds treated with *Trichoderma* sp. UBDFM01, (2) treatment R: Seeds treated with *Rhizoctonia* sp. K6, (3) treatment TR: Seeds treated with a mixture of *Trichoderma* sp. UBDFM01 and *Rhizoctonia* sp. K6, and (4) control: Seeds treated with sterile distilled water. To treat the seeds with fungi, the seeds were submerged in a fungal spore suspension with a concentration of 2×10^7 spores/mL. After 8 days, the seeds were placed on top of a moistened Whatman filter paper inside a square Petri dish. A total of 25 seeds were placed in each Petri dish and three replicates were made for each treatment. The seeds were left to germinate at room temperature (~25 °C).

The germination percentage was calculated on the 15th day by counting the number of seeds that germinated relative to the total number of seeds. Only the seeds that produced green shoots and radicles were considered as germinated seeds. For vigour assessment, a total of 10 seedlings were selected randomly from each replicate in each treatment. The seedling length for each replicate was measured. Subsequently, these seedlings were oven-dried at 60 ± 1 °C for 72 h and the dry weight was measured. The vigour indexes were calculated using these formulae (Swain *et al.*, 2018):

Vigour index I = Germination (%) × Seedling length (cm);
Vigour index II = Germination (%) × Seedling dry weight (mg).

Chlorophyll content of the seedlings was determined according to Porra (2002). The fresh leaf tissue (0.1 g) was chopped and transferred to a Falcon tube containing 25 mL of 80% acetone and then kept inside a refrigerator at 4 °C for 48 h. Absorbance measurements were made using a spectrophotometer at a wavelength of 663 and 645 nm. The following equations (Arnon, 1949) were used:

Chlorophyll a content = $(12.72 \times A_{663}) - (2.59 \times A_{645})$;
Chlorophyll b content = $(22.9 \times A_{645}) - (4.67 \times A_{663})$;
Total chlorophyll content = $(20.31 \times A_{645}) + (8.05 \times A_{663})$;
Chlorophyll a/b ratio = Chlorophyll a content/Chlorophyll b content.

Plant growth tests in greenhouse and NPK content

Assessment of *Trichoderma* sp. as a biological control agent against *Rhizoctonia* sp. and as biofertilizer was conducted following previous methods (Lahlali and Hijri, 2010; Swain *et al.*, 2018) with modifications. Initially, the rice seeds were surfaced and sterilized by immersing in a 1% sodium hypochlorite solution for 3 min and then in sterile distilled water for 6 min. The rice seeds were then allowed to germinate at room temperature. The clayey soils collected from the paddy fields were mixed together and autoclaved at 121 °C for 60 min by using steam under 15 psi of pressure before transferring 4 kg into each pot (12" × 12"). Four treatments were conducted – (1) treatment T: The soil was mixed with six PDA plates of *Trichoderma* sp. UBDFM01, (2) treatment R: The soil was mixed with six PDA plates of *Rhizoctonia* sp. K6, (3) treatment TR: The soil was mixed with six PDA plates of *Trichoderma* sp. UBDFM01 and six PDA plates of *Rhizoctonia* sp. K6, and (4) control: The soil was not treated with any fungi. The pots were left at room temperature for 14 days, after which one germinated seedling was transplanted into each pot. Ten pots or replicates were used for each treatment. The randomly placed seedlings were grown to adult size in a greenhouse for 120 days. The measurement of shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight were recorded per individual plant after the harvesting period. The number of tillers, grain weight and yield were also recorded per individual plant.

The total nitrogen (N) and phosphorus (P) contents of the roots and shoots of three randomly selected rice plants from each treatment were estimated using flow injection analysis (FIAstar analyzer 5000 and sampler 5027, FOSS, Sweden). The total potassium (K) content was also estimated using an atomic absorption spectrophotometer (Atomic Absorption flame emission spectrophotometer AA-6701, Shimadzu**,** Japan). The protocols for these analyses were adopted from Allen *et al*. (1974).

Statistical analysis

The Shapiro test was used to check for the normal distribution of data. For normally distributed data, One-way ANOVA and Tukey HSD test were conducted. Data that were not normally distributed were analyzed using Kruskal-Wallis test. The significance level was set at 0.05, with $p<0.05$ being statistically significant.

RESULTS AND DISCUSSION

Identification of fungi that cause rice diseases in Brunei Darussalam

The common rice diseases in Brunei Darussalam are blast disease, brown spot disease and sheath blight disease (Department of Agriculture and Agrifood, personal communication). In this study, two rice diseases were discovered during field sampling at the paddy fields in Wasan and Kandol, Brunei Darussalam: brown spot disease and sheath blight disease. However, there was no blast disease found at these selected paddy fields during field collection. Department of Agriculture and Agrifood reported that the brown spot disease in Brunei Darussalam could be caused by *Curvularia* sp., *Alternaria* sp. or *Cercospora* sp., whereas the sheath blight disease could be caused by *Rhizoctonia solani*.

This study isolated the pathogenic fungi from the rice leaf samples infected with brown spot disease. A total of four fungal isolates, namely K2, K3, K4 and K5 were identified by DNA barcoding, as species identification by morphology and biolog system is usually difficult and can also lead to misidentification. Their DNA sequences were uploaded to the GenBank database with accession

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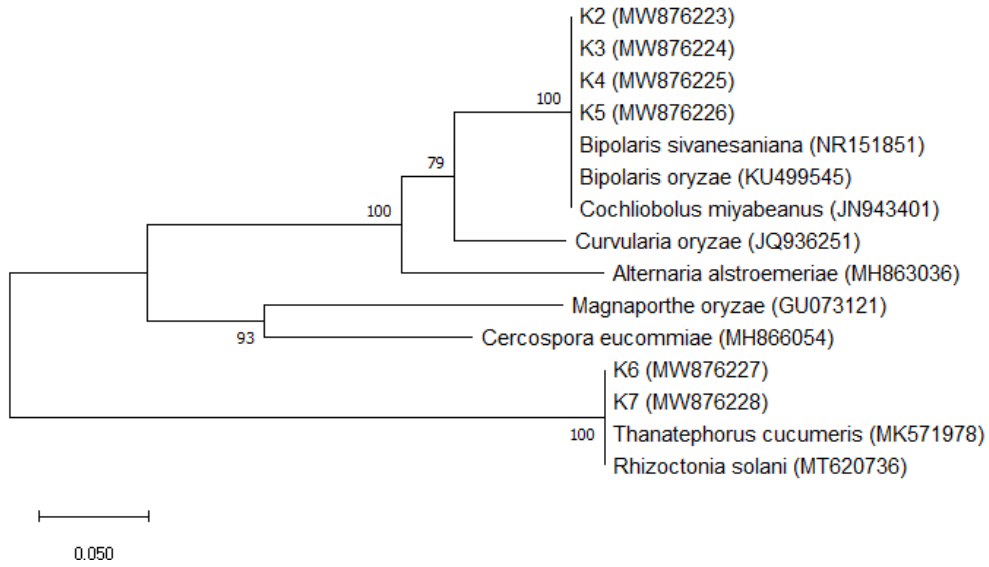

Figure 1: Phylogenetic tree of isolated pathogenic fungi infecting rice plants based on rRNA-ITS sequence. The number at the node represents the bootstrap percentage based on 1000 replicates. GenBank accession number is shown in the bracket. Scale refers to the evolutionary distance in the unit of the number of base substitutions per site.

Table 1: Pathogenic fungi infecting rice plants in Brunei Darussalam.

Isolate	Identification	GenBank accession no.	Top BLAST match	Identity (%)
K2	*Bipolaris* sp.	MW876223	*Bipolaris oryzae*	100
K3	*Bipolaris* sp.	MW876224	*Bipolaris oryzae*	100
K4	*Bipolaris* sp.	MW876225	*Bipolaris oryzae*	100
K5	*Bipolaris* sp.	MW876226	*Bipolaris oryzae*	100
K6	*Rhizoctonia* sp.	MW876227	*Rhizoctonia solani*	100
K7	*Rhizoctonia* sp.	MW876228	*Rhizoctonia solani*	100

numbers MW876223 to MW876226. When compared with the reference sequences in the GenBank database, all the four isolates showed 100% identity match with *Bipolaris oryzae* (Teleomorph: *Cochliobolus miyabeanus*), and 99% match with *Bipolaris sivanesaniana*. Therefore, these four isolates were identified as *Bipolaris* sp. (Table 1; Figure 1). *Bipolaris oryzae* has been reported as a causal agent of brown spot disease in rice plants (Debona *et al.*, 2018), which further supports this finding. As far as we are aware, this would be the first record of *B. oryzae* in Brunei Darussalam.

This study also isolated the pathogenic fungi from the rice leaf samples infected with sheath blight disease. Two fungal isolates namely K6 and K7 were also identified by DNA barcoding, which showed 100% identity match with *Rhizoctonia solani* (Teleomorph: *Thanatephorus cucumeris*). Therefore, these two isolates were identified as *Rhizoctonia* sp. (Table 1; Figure 1). Their DNA sequences were uploaded to the GenBank database with accession numbers MW876227 and MW876228, respectively. *Rhizoctonia solani* is known as a causal agent of sheath blight disease in rice plants (Li *et al.*, 2021), which further supports this finding. This study is the first to provide a DNA-based confirmation of *Rhizoctonia* sp. as the cause of the sheath blight disease in Brunei Darussalam. In this study, only *Rhizoctonia* sp. was selected for further testing.

Screening of *Trichoderma* strains

Biocontrol activity of eight *Trichoderma* strains (UBDFM01, UBDFM02, UBDFT02, UBDFT03, UBDFT04, UBDFT11, UBDFT19 and UBDFT20) against *Rhizoctonia* sp. were screened using confrontation assay. The growth of *Rhizoctonia* sp. was found to be substantially inhibited by all eight strains, with the average values ranging from 60.6 to 78.8% (Table 2). This suggests that these *Trichoderma* strains had the potential to be used as biocontrol agents. However, in this study, only *Trichoderma* sp. UBDFM01 was selected for further testing due to it showing the highest average value.

A number of biocontrol mechanisms exist in *Trichoderma,* such as mycoparasitism, antibiosis and competition. Mycoparasitism is a common mechanism by which there is an antagonistic direct contact with a pathogen. This can involve several stages, including pathogen recognition, binding to the target, enzymatic disruption of the fungus cell wall and assimilation of the cytoplasmic content (Benhamou and Chet, 1997; Vinale *et al*., 2008; Kaewchai *et al*., 2009; Rincón *et al*., 2009).

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Table 2: Biocontrol activity of *Trichoderma* strains.

Trichoderma sp.	Inhibition (%)
UBDFM01	78.8 ± 0.9[b]
UBDFM02	70.7 ± 0.8[ab]
UBDFT02	62.1 ± 0.8[a]
UBDFT03	69.0 ± 2.6[ab]
UBDFT04	70.7 ± 1.1[ab]
UBDFT11	68.7 ± 1.4[ab]
UBDFT19	60.6 ± 5.4[a]
UBDFT20	71.0 ± 1.2[ab]

Values are expressed as mean ± standard error of 5 replicates. Values with the same letter are not significantly different.

Table 3: Rice seedling vigour under different treatments.

Treatment	Vigour index I	Vigour index II
Control	317.9 ± 66.3[a]	1478.4 ± 17.9[b]
T	628.9 ± 34.9[b]	1443.0 ± 50.8[b]
R	237.2 ± 9.8[a]	1042.8 ± 17.7[a]
TR	588.8 ± 8.7[b]	1115.5 ± 2.9[a]

Values are expressed as mean ± standard error of 3 replicates. Values with the same letter are not significantly different.

Antibiosis is another mechanism by which there is an antagonistic interaction with a pathogen through the secretion of antimicrobial compounds or specific secondary metabolites that exhibit inhibitory properties and suppress pathogenic activity (Rincón *et al*., 2009). *Trichoderma* strains, for example, *T. viride*, *T. harzianum* and *T. koningii* are capable of the production and secretion of a volatile metabolite, 6-pentyl-α-pyrone (6-PAP), which is responsible for the biocontrol of several pathogenic species such as *Botrytis cinerea*, *Rhizoctonia solani* and *Fusarium oxysporum* (Błaszczyk *et al*., 2014). *Trichoderma* can also compete with a pathogen for nutrients, space and even infection sites on plant roots (Błaszczyk *et al*., 2014). The mechanisms that aid in colonizing the diverse ecological niches are exceptionally evolved and vary in *Trichoderma* species (Harman 2006; Vinale *et al*., 2008). Furthermore, the wide metabolic versatility of many *Trichoderma* species has enabled them to use different and complex carbon and nitrogen sources, which allows *Trichoderma* to limit competing pathogens from growing and spreading in the environment (Hjeljord and Tronsmo, 1998).

Evaluation of *Trichoderma* sp. UBDFM01 in affecting BDR5 rice

In order to evaluate the effects of *Trichoderma* sp. UBDFM01 on rice seedling growth, the vigour index I and vigour index II were measured (Table 3). The vigour index I assessed the growth of germinated seedlings via its length, whereas the vigour index II assessed via the accumulation of its dry matter. Increasing seedling vigour is critical for plant growth. The vigour index I appear to show a more promising result compared to the vigour index II. When treated with the *Trichoderma* strain (treatment T), the rice seedlings showed a significantly higher vigour index I compared to the control, suggesting that the strain had the potential as a biofertilizer. When treated with the pathogen (treatment R), the vigour index I of the seedlings was not significantly affected compared to the control, suggesting that *Rhizoctonia* sp. had no significant negative effect on this index. When treated with both *Trichoderma* and *Rhizoctonia* strains (treatment TR), the seedlings showed a significantly higher vigour index I. A previous study (Doni *et al.*, 2014) similarly reported that the vigour index I was higher in the rice seeds treated with *Trichoderma* compared to the untreated seeds. Similarly, inoculating rice seeds with a *Trichoderma* strain was previously found to enhance germination rate and seedling length (Doni *et al*., 2017). The biofertilizer potential might be explained by the ability of *Trichoderma* to produce and releasing various secondary metabolites and phytohormones such as auxins and gibberellins, which could help in improving seedling growth (Swain *et al.*, 2018).

The vigour index II did not provide any support to the potential use of *Trichoderma* sp. UBDFM01 as biofertilizer (Table 3). This is because when treated with the *Trichoderma* strain (treatment T), the rice seedlings did not show any significant improvement in the vigour index II. However, when treated with the pathogen (treatment R), the vigour index II was significantly reduced compared to the control, suggesting that *Rhizoctonia* sp. had a negative effect on this index. Treatment TR did not show any significant difference when compared to treatment R. This means that the *Trichoderma* strain did not have any potential as a biocontrol agent for this index since it did not manage to significantly prevent or lessen the adverse effect.

Chlorophyll in plants is essential for carrying out photosynthesis which is required for the plant carbon cycle as well as for the production of oxygen. A high chlorophyll content and optimum chlorophyll a/b ratio can result in higher photosynthetic activity. Vargas *et al.* (2009) reported the ability of *Trichoderma* to induce photosynthetic activity. Similarly, Doni *et al.* (2017) reported that *Trichoderma* inoculation positively enhanced chlorophyll content, improving plants' photosynthesis processes. In this study, the chlorophyll contents of the rice seedlings in each treatment were also measured (Table 4). When treated with the *Trichoderma* strain (treatment T), the rice seedlings showed significantly higher chlorophyll a, chlorophyll b and total chlorophyll compared to the control and the chlorophyll a/b ratio was not significantly affected. This suggests that the strain had the potential as a biofertilizer since it significantly improved the chlorophyll contents. This is in agreement with a previous study (Swain *et al.*, 2018) in which all the treatments involving six *Trichoderma* strains had significantly higher chlorophyll a, chlorophyll b and total chlorophyll compared to the control. The analysis of the chlorophyll content by a previous study (Neito-Jacobo *et al.*, 2017) supported these findings, whereby all the tested *Trichoderma* strains except one induced a significant increase in chlorophyll content.

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Table 4: Chlorophyll contents of rice seedlings under different treatments.

Treatment	Chl a (mg/g)	Chl b (mg/g)	Chl a/Chl b	Total Chl (mg/g)
Control	0.72 ± 0.00^{c}	0.36 ± 0.04^{a}	2.00 ± 0.24^{b}	1.08 ± 0.04^{c}
T	0.83 ± 0.01^{d}	0.47 ± 0.02^{b}	1.77 ± 0.09^{b}	1.29 ± 0.01^{d}
R	0.40 ± 0.01^{a}	0.34 ± 0.01^{a}	1.17 ± 0.04^{a}	0.74 ± 0.01^{a}
TR	0.61 ± 0.00^{b}	0.30 ± 0.01^{a}	2.06 ± 0.07^{b}	0.90 ± 0.01^{b}

Values are expressed as mean ± standard error of 3 replicates. Values with the same letter are not significantly different. Chl: Chlorophyll.

Table 5: Plant growth parameters under different treatments.

Treatment	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
Control	104.17 ± 2.40^{a}	44.96 ± 3.58^{a}	94.50 ± 8.45^{a}	17.50 ± 1.86^{a}	24.64 ± 3.14^{a}	2.74 ± 0.37^{a}
T	109.23 ± 2.67^{ab}	39.73 ± 2.16^{a}	126.50 ± 5.78^{ab}	28.50 ± 0.76^{b}	33.42 ± 4.20^{a}	3.13 ± 0.36^{a}
R	118.76 ± 3.06^{bc}	36.65 ± 2.25^{a}	143.00 ± 14.84^{b}	33.50 ± 3.88^{b}	46.93 ± 10.52^{a}	4.58 ± 1.50^{a}
TR	122.18 ± 3.82^{c}	41.3 ± 2.18^{a}	141.1 ± 7.76^{b}	33.30 ± 2.50^{b}	45.57 ± 7.08^{a}	4.11 ± 0.72^{a}

Values are expressed as mean ± standard error of 10 replicates except for TR that had 9 replicates. Values with the same letter are not significantly different.

Table 6: Plant yield parameters under different treatments.

Treatment	No. of tillers	No. of grains	Total weight of grains (g)
Control	6.20 ± 0.59^{a}	687.70 ± 60.51^{ab}	14.38 ± 1.37^{b}
T	7.50 ± 0.40^{a}	835.50 ± 57.78^{b}	12.34 ± 1.01^{b}
R	6.40 ± 0.58^{a}	414.90 ± 120.44^{a}	6.01 ± 1.78^{a}
TR	6.33 ± 0.24^{a}	355.56 ± 103.28^{a}	6.47 ± 1.44^{a}

Values are expressed as mean ± standard error of 10 replicates except for TR that had 9 replicates. Values with the same letter are not significantly different.

When treated with the pathogen (treatment R), the seedlings showed significantly lower chlorophyll a, chlorophyll a/b ratio and total chlorophyll compared to the control, although chlorophyll b was not significantly affected (Table 4). This seems to support the negative effects of *Rhizoctonia s*p. on rice plants. When treated with both *Trichoderma* and *Rhizoctonia* strains (treatment TR), the seedlings showed significantly higher chlorophyll a, chlorophyll a/b ratio and total chlorophyll compared to treatment R. This suggests that the *Trichoderma* strain had the potential as a biocontrol agent since it was able to significantly lessen the negative effect of the pathogen on the chlorophyll content.

To evaluate the effects of *Trichoderma* sp. UBDFM01 on agronomical rice parameters, the plant growth parameters (Table 5) and yield parameters (Table 6) were measured. Table 5 shows that there was no significant difference in the root length, fresh root weight and dry root weight among the four treatments, suggesting that these growth parameters were not significantly affected by the fungi. However, the shoot length, fresh shoot weight and dry shoot weight were significantly affected. When treated with the *Trichoderma* strain (treatment T), the rice plants produced a substantially higher dry shoot weight compared to the control, but the other growth parameters were not significantly affected. This suggests that the *Trichoderma* strain had the potential as a biofertilizer for increasing dry shoot weight. A previous study (Neito-Jacobo *et al*., 2017) reported that four *Trichoderma* strains showed a significant increase in shoot, root and total biomass but another *Trichoderma* strain had no effect. Contreras-Cornejo *et al.* (2009) reported that *Trichoderma* strains enhanced shoot biomass production through an auxin-dependent mechanism.

When treated with the pathogen (treatment R), the rice plants surprisingly produced significantly higher shoot length, fresh shoot weight and dry shoot weight compared to the control. Subsequently, when treated with both *Trichoderma* and *Rhizoctonia* strains (treatment TR), the results were not significantly different compared to treatment R. Thus, the potential of the *Trichoderma* strain as a biocontrol agent could not be judged. In this study, it was observed that the noticeable symptoms of the sheath blight disease in treatment R occurred during the flowering stage, two weeks before the rice harvesting period. A potential explanation for the positive effects observed on the shoots and roots of the infected plants could be due to the plant defense mechanism. By increasing the growth of the shoots and roots, the rice plants presumably could counteract the effects of the pathogen. According to Li *et al.* (2021), *R. solani* produces various types of compounds that can interfere with the infected plant's physiological functions, and as countermeasures, the infected plants can activate multiple layers of defenses such as enhancing cell wall lignification and producing a variety of secondary metabolites, particularly during the early stage of infection.

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Table 7: Total NPK contents of rice plants under different treatments.

Treatment	N (mg/g)		P (mg/g)		K (mg/g)	
	Root	Shoot	Root	Shoot	Root	Shoot
Control	5.58 ± 0.50[a]	5.95 ± 0.13[a]	1.45 ± 0.11[a]	1.60 ± 0.12[a]	111.54 ± 2.36[a]	107.62 ± 4.41[a]
T	5.81 ± 0.45[a]	4.93 ± 0.12[a]	1.50 ± 0.05[a]	1.42 ± 0.09[a]	108.91 ± 2.98[a]	109.02 ± 2.34[a]
R	5.43 ± 0.22[a]	7.75 ± 1.38[a]	1.16 ± 0.05[a]	2.07 ± 0.21[a]	109.99 ± 2.47[a]	111.78 ± 1.63[a]
TR	5.88 ± 0.10[a]	4.95 ± 0.45[a]	1.54 ± 0.18[a]	1.62 ± 0.14[a]	109.14 ± 2.03[a]	110.97 ± 2.23[a]

Values are expressed as mean ± standard error of 3 replicates except for T that had 2 replicates. Values with the same letter are not significantly different.

Table 6 shows that when treated with the *Trichoderma* strain (treatment T), no significant difference was observed in all the yield parameters compared to the control. Thus, it did not provide any support for the *Trichoderma* strain as a biofertilizer. When treated with the pathogen (treatment R), the rice plants produced a significantly lower total weight of grains compared to the control, although the numbers of tillers and grains were not significantly affected. This supports the negative effect of the pathogen on rice plants. As the sheath blight disease was noticeable only during the flowering stage, the fungal infection was likely the cause of this significant reduction in the total weight of grains. When treated with both *Trichoderma* and *Rhizoctonia* strains (treatment TR), the results were not significantly different compared to treatment R. Thus, under the conditions tested, it did not support the *Trichoderma* strain as a potential biocontrol agent.

The concentrations of total N, P and K of the roots and shoots of the rice plants were also measured (Table 7). The average N contents of the roots and shoots ranged from 5.43 to 5.88 mg/g and 4.93 to 7.75 mg/g, respectively. The average P contents of the roots and shoots ranged from 1.16 to 1.54 mg/g and 1.42 to 2.07 mg/g, respectively. The average K contents of the roots and shoots ranged from 108.91 to 111.54 mg/g and 107.62 to 111.78 mg/g, respectively. This study found that there was no significant difference in the concentrations of total N, P and K in the shoots and roots among the four different treatments. This suggests that the NPK content could not provide any support to the *Trichoderma* strain as a biocontrol agent or biofertilizer. In comparison, a previous study (Swain *et al.*, 2018) reported that the concentrations of total N, P and K were found to be higher in the plants treated with *Trichoderma* compared to the untreated plants. This could be due to the ability of the fungi to enhance solubilization, soil nutrients uptake such as NPK and improving root growth (Doni *et al.*, 2014; Yellareddygari *et al.*, 2014). Different strains of *Trichoderma* could potentially produce different effects on the N, P and K contents of different plants. A previous study (Ng *et al*., 2015) found that not all *Trichoderma* isolates could enhance plant growth, which might be due to the suppression effect of the metabolite produced.

CONCLUSION

In this study, *Bipolaris* sp. was identified as the cause of brown spot disease, whereas *Rhizoctonia* sp. was identified as the cause of sheath blight disease in Brunei Darussalam. Initial screening showed that all eight *Trichoderma* strains were able to substantially inhibit the growth of the pathogen, *Rhizoctonia* sp. A selected strain, *Trichoderma* sp. UBDFM01 showed the potential as a biofertilizer by significantly increasing the vigour index I, chlorophyll a, chlorophyll b, total chlorophyll and dry shoot weight of the BDR5 rice plants. The pathogen negatively affected the plants by significantly reducing the vigour index II, chlorophyll a, chlorophyll a/b ratio, total chlorophyll and total weight of grains. The *Trichoderma* strain showed the potential as a biocontrol agent by significantly diminishing the negative effects of the pathogen on the chlorophyll a, chlorophyll a/b ratio, and total chlorophyll. Future studies could look into the effects of *Trichoderma* on other rice varieties and on other phytopathogens. Other techniques for inoculating the paddy plants with *Trichoderma* could also be considered.

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