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Efficacy of indigenous *Trichoderma* isolates of West Timor, Indonesia, as biocontrol agents of brown spot (*Drechslera oryzae*) on two upland rice varieties

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Abstract

Background: Brown spot disease is one of the most destructive foliar diseases of rice. Biological control of this disease is considered more environmentally safe, but the biological control agents have limited availability. The fungus *Trichoderma* can be used as a biocontrol agent for various plant diseases, including brown spots. Indigenous *Trichoderma* species from West Timor were evaluated in this study to reveal their effectiveness in reducing brown spot disease of upland rice. The study was conducted under a screen house condition using a factorial treatment design laid out in a completely randomized design. The two factors were the brown spot disease control method (*T. viride, T. harzianum, T. hamatum*, fungicide Trivia 73 WP, and a control treatment) and upland rice genotype (Inpago 7 and Breun Senaren 2).

Results: The results showed that *Trichoderma* spp. and the fungicide Trivia 73 WP caused significantly lower AUDPC and apparent infection rate than the control treatment. The total phenol content and agronomic characters (number of reproductive tillers per plant, number of filled grains per panicle, grain yield per plant) were higher in the *Trichoderma* and fungicide treatments than the control treatment. Meanwhile, tannin and saponin levels were non-significantly different among treatments. *Trichoderma* and the fungicide Trivia treatments effectively suppressed brown spot disease of upland rice, and the efficacy was dependent on the rice variety, with a mean efficacy range over the two varieties of 27.05–36.42%. The *Trichoderma* and the fungicide Trivia treatments increased the grain yield per plant differentially in the 2 rice varieties, with a mean grain yield increase over the 2 varieties of 13.0% (*T. viride*), 23.5% (*T. harzianum*), 35.0% (*T. hamatum*), and 19.9% (Trivia 73 WP).

Conclusion: Efficacy of local *Trichoderma* of West Timor and the fungicide Trivia treatments were varietal dependent with *T. hamatum* being the most effective in reducing the brown spot disease and producing the highest grain yield increase over the 2 rice varieties. The local *Trichoderma* species of West Timor have a future potential as biocontrol agents of upland rice brown spot disease, but it is necessary to further evaluate their effectiveness against the disease in replicated field trials.

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Keywords: Rice disease, Brown spot disease, *Trichoderma*, Local species, Biological control, Effectiveness

Background

Leaf brown spot caused by the fungus *Drechslera oryzae* (Breda de Haan), Subram & Jain [syn. *Helminthosporium oryzae, Bipolaris oryzae* (Breda de Haan) Shoemaker], is one of the primary diseases of upland rice that causes high yield losses (IRRI 2009).

In East Nusa Tenggara (ENT) Province, Indonesia, leaf brown spot has long been associated with upland rice farming. The traditional cultivation practices and the poor dry land soil nutrition are the factors that cause the disease to occur more frequently. Consequently, most of the local upland rice cultivars in the region are susceptible and moderately susceptible to leaf brown spot disease, and the yield loss caused by the disease ranged from 10.46 to 56.15% (Mau et al. 2020). Leaf brown spot is, therefore, considered one of the factors contributing to the low upland rice productivity (2 t/ha) in ENT Province, while under controlled and optimum field conditions, the local cultivars can produce a grain yield of 5–6 t/ha (Ndiwa and Mau 2019). Therefore, seeking effective control measures for leaf brown spot disease is necessary to prevent high yield losses in upland rice and hence increase rice productivity.

Brown spot disease can be controlled using various methods (Asghar et al. 2019), including chemical spray, disease-free seeds, use of resistant varieties, biological control, etc. Resistant varieties are the most economical and environmentally sound control measure (Magar 2015). However, the brown spot resistant varieties are limited, and the resistance is frequently short-lived due to the frequent emergence of new virulent races (Arshad et al. 2008). Thus, an integrated control strategy that involves a more environmentally friendly method, such as biological control agents, also needs to be implemented to tackle the disease effectively. For example, the fungus *Trichoderma* sp. is a biological control agent proven to control various plant diseases effectively.

Trichoderma is a highly active fungus in the rhizosphere, soil, and the plant canopy environment, and it can also be a parasite to other fungi (Harman et al. 2004). *Trichoderma* produces several types of extracellular proteins and compounds that are fungi toxic and fungi-static to several fungal pathogens and also can stimulate plant growth, so this fungus has received serious attention as a biological control agent (Puyam 2016).

The ability of *Trichoderma* to suppress pathogen and disease development occurs through various mechanisms such as competition (Khalili et al. 2012), mycoparasitism (Dix and Webster 1995), antibiosis (Khalili et al. 2012) and also by inducing the host plant's resistance (Levy et al. 2015) and promoting the host plant growth (Hassan et al. 2017). Induction of host plant resistance can occur through the production of secondary metabolites such as phenol, saponin, and tannin (Yanti et al. 2018), while plant growth promotion may occur through the production of plant growth hormones, etc. (Alison and Robert 2014).

In an in vitro preliminary study, that several indigenous *Trichoderma* species of West Timor were found inhibiting the aerial growth of the pathogenic fungus *D. oryzae* with an efficacy of 35–50% (Mau et al. 2021). The results indicated the potential of local *Trichoderma* isolates as biological control agents of rice brown spot disease in rice. Evaluating these local *Trichoderma* isolates along with a fungicide spray on different upland rice varieties may lead to finding more effective ways of controlling brown spot disease in upland rice. The objectives of this study were to elucidate the effectiveness of indigenous *Trichoderma* species of West Timor against the brown spot disease on two upland rice genotypes and observe their effects on the agronomic characters on the tested upland rice genotypes.

Methods

Study locations and materials

The study was conducted at the Plant Disease Laboratory, Faculty of Agriculture, Universitas Nusa Cendana, and in a screen house in Lasiana Village, Kupang District, East Nusa Tenggara Province, Indonesia. The study was conducted from April to November 2021. Research materials used in the study included *T. viride, T. harzianum, T hamatum* (obtained from a collection of Plant Disease Laboratory, Universitas Nusa Cendana), *D. oryzae,* the fungicide Trivia 73 WP, and 2 Indonesian upland rice varieties, i.e., Inpago 7 and Breun Senaren 2.

Experimental design

The experiment employed a completely randomized design consisting of a factorial treatment design of 2 factors, i.e., upland rice genotype (G) and disease control method (C). The upland rice genotype consisted of 2 varieties (Inpago 7 and Breun Senaren 2), while the disease control method consisted of 5 treatments, i.e., *T. viride, T. harzianum, T. hamatum*, the fungicide Trivia 73 WP (active ingredients: Fluopicolide 6% and Propineb 66.7%), and a control treatment (without disease control). A total of 15 treatment combinations were evaluated, consisting of 3 replicates.

Procedures

Plant cultivation

Rice plants were grown in polybags of 40 cm × 30 cm (height × width) containing soil taken from the surrounding uncultivated field. The soil was taken from 1 – 20 cm depth, cleaned from the debris, and air-dried for 3 days before being finely ground and filtered to obtain a uniform soil particle. Next, 10 kg air-dried filtered soil was placed into a polybag and then watered to a field capacity level. Four upland rice seeds were planted in each polybag, and only one plant was retained from 2 weeks of age until harvest. A standard upland rice agronomic practice was applied throughout the plant growing cycle. All the plants were maintained in a screen house made from a transparent para net to allow maximum sunlight intensity.

Preparation of Trichoderma solution and foliar application of the treatment

Before being used in the experiment, the *Trichoderma* culture was purified by a single spore method on a potato dextrose agar (PDA) medium for a week at room temperature. Then, the *Trichoderma* was re-identified to confirm their identity by comparing their colony appearance and microscopic characters with the manual description provided by Kubicek dan Harman (2002) and Watanabe (2002). The treatments were applied to the rice plants in a liquid formulation of *Trichoderma* and fungicide through foliar spraying. The *Trichoderma* isolate was grown on a potato dextrose agar (PDA) growth medium for 7 days. Then, 5 disks ($\Theta = 0.6$ cm) of the *Trichoderma* were taken and placed into a 250-ml flask containing 100 ml PD broth medium. The flasks containing the PD broth culture were then continuously shaken at room temperature for 7 days at 150 rpm. The colonized PD broth medium was filtered using a 0.45 µm mesh membrane paper. The filtered filtrate was adjusted to a final concentration of 10^8 conidia/mL and used as a working solution. The fungicide Trivia 73 WP working solution was prepared by dissolving one gram of the fungicide powder into 1 L distilled water and shaken thoroughly to obtain a homogenous working solution with a final concentration of 1 g/L.

Trichoderma and the fungicide Trivia 73 WP were administrated by foliar spray 19 days after planting (2 days before plant inoculation using *D. oryzae*). The working solutions of *Trichoderma* and fungicide Trivia were sprayed onto the rice plant's leaves until runoff occurred. The foliar application was carried out at around 8 to 9 am. The treatment applications were conducted 4 times on a two-weekly basis. Plants that received no *Trichoderma* and fungicide sprays served as the control (no disease control treatment).

Preparation of D. oryzae suspension and plant inoculation

The fungus *D. oryzae* was obtained from infected rice fields. The diseased leaf disks were cultured on a PDA medium for 7 days, and a single spore was isolated and further grown on a PDA medium for another 7 days under room temperature for purification and identification of *D. oryzae*. Identification was done by comparing the colony appearance and microscopic characters with the authentic description provided by Ellis (1971) and Agarwal et al. (1989). A PDA medium was used to propagate the *D. oryzae* isolate for plant inoculation. After 14 days, the fungus mycelia mats were gently scraped with a spatula and added with 10 ml sterile water containing 0.02% Tween 20 to obtain the conidial suspension. The conidial concentration of the stock solution was determined using a hemocytometer and then adjusted to 1×10^6 conidia/ml for plant inoculation. Plant inoculation was conducted 21 days after planting (2 days after foliar sprays of *Trichoderma* sp. and the fungicide). All tested plants were inoculated by foliar spraying until runoff occurred. Inoculation was done from 8 to 9 am.

Observation

Observation was done on individual plants, including disease severity, phenol content, tannin and saponin levels, the number of reproductive tillers per plant, the number of filled grains per panicle, and grain yield per plant. Disease severity was assessed every week from 7 days after inoculation until the early flowering stage. The disease was assessed based on a disease scale according to the standard evaluation system of IRRI (2013) and was then used to calculate the disease severity level using the formula:

$$\mathrm{DS} = \frac{\sum (n \times v)}{Z \times N} \times 100\%$$

where DS = disease severity, n = number of leaves with a certain disease score, v = disease score, Z = the highest observed disease score, and N = the total number of leaves observed in each plant. The mean disease severity of each plant was averaged from all leaves in the plant.

All disease severity recordings were used to calculate the area under the disease progress curve (AUDPC) based on the formula of Campbell and Madden (1990).

$$\mathrm{AUDPC} = \sum_{i}^{n-1} \frac{Y_i + Y_{i+1}}{2}(t_{i+1} - t_i)$$

where Y_i = disease severity at the ith observation, n = the last disease assessment (number of assessment), and t_i = time period of assessment at the ith observation.

Table 1 Mean square values of epidemiological, phenol content, yield, and yield-attributing parameters of two rice genotypes treated with different brown spot disease control methods

Source of variation	df	Epidemiological parameters		Total phenol content	Yield and yield-contributing parameter		
		AUDPC	*rt*		NRTP	NFGP	GYP
Genotype (G)	1	208,484.98**	0.00021*	0.14784**	607.500**	1128.53**	54.68**
Control method (C)	4	224,706.62**	0.00006**	0.12559**	11.283**	579.46**	27.75**
G*C	10	4024.40.ns	0.00042*	0.52327**	1.250.ns	366.24**	22.05**
Error	20	13,046.33	0.00004	0.00012	1.667	49.02	5.55
CV (%)	–	11.31	19.73	0.60	6.47	6.01	11.93

df = degree of freedom, AUDPC = urea under the disease progress curve, *rt* = apparent infection rate, NRTP = number of reproductive tiller per plant, NFGP = number of filled grain per panicle, GYP = grain yield per plant, CV = coefficient of variation, **highly significant ($P < 0.01$). *Significant ($P < 0.05$), ^{ns}not significant ($P > 0.05$)

The mean apparent infection rate was calculated based on the time-varying apparent rate (Madden et al. 2007) as follows:

$$r_{t+1} = \left[\ln\left(\frac{y_{t+1}}{1-y_{t+1}}\right) - \ln\left(\frac{y_t}{1-y_t}\right)\right]/(t_{t+1} - t_t)$$

where y_t = disease severity at the tth observation, and t_t = time period of assessment at the tth observation.

Total phenol content and tannin and saponin levels were determined on leaves of the tested rice plants 3 days before the final disease assessment. Five grams of fresh leaf sample of each plant was taken and used for the analysis in the Laboratory of Biosains, Universitas Nusa Cendana. Total phenol content was determined based on the method of McDonald et al. (2001), while saponin and tannin levels were qualitatively assessed based on the method of Syahadat and Azis (2012).

Agronomic data were recorded at harvest. The number of reproductive tillers was obtained by counting the reproductive tiller per plant, while the number of grains per panicle was averaged from the number of grains of all panicles within a plant. The grain yield was measured as grain weight (± 14% moisture content) per plant.

Data analysis

Quantitative data such as AUDPC, apparent infection rate, total phenol content, and agronomical data were subjected to ANOVA followed by an LSD post hoc test, while saponin and tannin levels were descriptively analyzed. Correlation analysis was performed to see the associations among variables. The ANOVA was performed using GenStat 12th (VSN International 2009), while the correlation analysis was conducted using PAST ver. 3 (Hammer et al. 2001).

Results

Brown disease severity and efficacy of the treatments

Ten recordings of brown spot disease severity were made during the study. The disease symptoms started to appear from the first week after inoculation. At the last assessment, the disease severity ranged on the 2 tested varieties which were 19.1–26.6 and 19.6–26.1%, respectively.

Data in Table 1 summarize the results of factorial ANOVA of epidemiological and agronomical parameters. The single factor genotype and disease control method significantly or highly significantly affected the AUDPC and apparent infection rate. In contrast, the interaction between the 2 factors caused non-significant effect on AUDPC but significantly affected the apparent infection rate.

Data (*) in Table 2 show significant AUDPC among treatments within every single factor of rice genotype (G) and disease control method (C). The overall mean AUDPC over the 2 varieties (mean G) was highest in the control treatment (1345.03%.day), while the lowest was in *T. hamatum* (855.20%.day), but did not differ significantly from that of *T. harzianum* (908.59%.day). Consistent with the mean genotype, the highest AUDPC within each genotype was observed on the control treatment, and the lowest was on *T. hamatum* and *T. harzianum* applications. The overall mean AUDPC of the disease control method factor was higher in Breun Senaren 2 (1093.41%.day) than Inpago 7 (926.68%.day). Within each disease control method, Inpago 7 consistently showed lower AUDPC than Breun Senaren 2, indicating that the former had a higher brown spot resistance than the latter.

Apparent infection rates also varied significantly among treatments. The treatment combination of Breun Senaren 2 × *T. viride* showed the lowest apparent infection rate (0.0077 disease/unit/day), while the highest was on Inpago 7 × control treatment (0.0343 disease/unit/day). On average, the apparent infection rate in Inpago 7 (0.019 disease/unit/day) was higher than Breun Senaren 2 (0.016 disease/unit/day). The mean apparent infection rate of the disease control method over the 2 rice genotypes was lowest in *T. harzianum* (0.0093 disease/unit/day), and the highest was on the control treatment (0.0297 disease/unit/day).

Table 2 Means of AUDPC, apparent infection rate, and efficacy of leaf brown spot disease under combined treatments of disease control method and upland rice genotype

Control method (C)	AUDPC (%.day)			Apparent infection rate (disease/unit/day)			Efficacy (%)		
	Rice genotype (G)			Rice genotype (G)			Rice genotype (G)		
	Inpago 7	Breun Senaren 2	Mean (G)	Inpago 7	Breun Senaren 2	Mean (G)	Inpago 7	Breun Senaren 2	Mean (G)
T. viride	866.83 a	1095.69 b	981.26 b	0.0126 a	0.0077 a	0.0101 bc	31.71 a	22.88 a	27.05 a
	A	B		A	B		B	A	
T. harzianum	806.28 a	1010.91 ab	908.59 ab	0.0103 a	0.0084 a	0.0093 a	36.48 a	28.85 a	32.45 a
	A	B		A	A		B	A	
T. hamatum	807.21 a	903.19 a	855.20 a	0.0171 b	0.0207 b	0.0189 c	36.40 a	36.43 b	36.42 b
	A	B		A	A		A	A	
Trivia 73 WP	883.83 a	1036.46 ab	960.15 b	0.0209 b	0.0181 b	0.0195 c	30.37 a	27.05 a	28.62 a
	A	B		A	A		A	A	
No control	1269.26 b	1420.80 c	1345.03 c	0.0343 c	0.0252 c	0.0297 d			
	A	B		A	B				
Mean (C)	926.68	1093.41		0.0190	0.0160				
	A	B		A	B				

Lowercase denotes comparison within the same column, and uppercase indicates comparison within the same row. Values within the same column/row with the identical lowercase or uppercase are not significantly different based on the LSD (0.05) post hoc test

*Determined as the percentage of decrease in AUDPC from that of the control treatment

Data in Table 2 also show that the disease control method using *Trichoderma* sp. and fungicide sprays effectively reduced the disease AUDPC than the control treatment. The efficacy of the *Trichoderma* and the fungicide Trivia varied significantly between the 2 rice varieties. Over the 2 varieties, the highest efficacy (36.42%) was obtained from *T. hamatum* spray. In comparison, the effectiveness of the other 3 treatments was 27.05% (*T. viride*), 28.62% (fungicide Trivia 73 WP), and 32.45% (*T. harzianum*), which did not significantly differ from each other. Efficacy of *Trichoderma* and the fungicide Trivia was statistically similar on Inpago 7 but was significantly different on Breun Senaren 2 with *T. hamatum* the most effective.

Phenol, tannin, and saponin levels

Total phenol content was highly significantly affected by the interaction of disease control method and rice genotype (Table 1). Similarly, the single factor treatments showed a highly significant effect on the total phenol content. Data in Table 3 show that the treatment combination of *T. hamatum* × Inpago 7 produced the highest phenol content (2.40 mg GAE/g), followed by *T. harzianum* × Inpago 7 (2.07 mg GAE/g) and the fungicide Trivia 73 WP × Breun Senaren 2 (2.00 mg GAE/g) in the second place. Meanwhile, the lowest phenol content was recorded on the control × Inpago 7 treatment combination (1.42 mg GAE/g). On the overall mean of the 2 varieties, *T. harzianum* spray produced the highest phenol content (2.01 mg GAE/g), which was significantly higher than other treatments, while the lowest was observed on the control treatment (1.61 mg GAE/g). The mean phenol content of the the five disease control treatments over the two varieties differed significatly from each other. The overall mean phenol content of disease control method treatment was higher in Inpago 7 (1.91 mg GAE/g) than Breun Senaren 2 (1.76 mg GAE/g). In contrast to total phenol content, tannin was equally produced in all treatment combinations at a high level in both varieties (Table 3). Meanwhile, saponin was produced in low to medium levels in both varieties. The control treatment had a low level of saponin in both varieties, which was also similar to the treatment combinations of *T. hamatum* × Inpago 7, *T. viride* × Breun Senaren 2, and *T. harzianum* × Breun Senaren 2, while the rest treatment combinations produced medium levels of saponin.

Number of reproductive tiller per plant and number of filled grain per panicle

Interaction of rice variety (G) and disease control method (C) caused non-significant effect on the number of reproductive tillers per plant. In contrast, both the single factor treatments caused a highly significant effect on the trait (Table 1). Mean reproductive tillers per plant over the 2 genotypes were highest in the fungicide Trivia 73 WP (21.33 tillers), but it was non-significantly different from *Trichoderma* sp. applications (Table 4). In comparison,

Table 3 Means of leaf phenol, tannin, and saponin levels under combined treatments of disease control method and upland rice genotype

Control method (C)	Total phenol (mg GAE/g)						Tannin level*		Saponin level*	
	Rice genotype (G)						Rice genotype		Rice genotype	
	Inpago 7		Breun Senaren 2		Mean (G)		Inpago 7	Breun Senaren 2	Inpago 7	Breun Senaren 2
T. viride	1.83	c	1.81	b	1.82	b	+++	+++	++	+
	A		B							
T. harzianum	2.07	d	1.94	c	2.01	e	+++	+++	++	+
	A		B							
T. hamatum	2.40	e	1.27	a	1.84	c	+++	+++	+	++
	A		B							
Trivia 73 WP	1.80	b	2.00	d	1.90	d	+++	+++	++	++
	A		B							
No disease control	1.42	a	1.80	b	1.61	a	+++	+++	+	+
	A		B							
Mean (C)**	1.91		1.76							
	A		B							

Lowercase denotes comparison within the same column, and uppercase denotes comparison within the same row. Values within the same column/row with the same lowercase or uppercase are not significantly different based on the LSD (0.05) post hoc test. *Qualitatively assessed: $^{+}$low level, $^{++}$medium level, $^{+++}$high level

Table 4 Means of number of reproductive tillers per plant and number of grain per panicle under combined treatments of disease control method and upland rice variety

Control method (C)	Reproductive tiller per plant			Number of grain per panicle/tiller			Grain yield per plant (g)		
	Rice Genotype (G)			Rice Genotype (G)			Rice Genotype (G)		
	Inpago 7	Breun Senaren 2	Mean (G)	Inpago 7	Breun Senaren 2	Mean (G)	Inpago 7	Breun Senaren 2	Mean (G)
T. viride	23.00 a	14.00 b	18.50 a	118.33 b	121.22 bc	119.78 c	18.50 a	19.00 b	18.75 b
	A	B		A	A		A	B	
T. harzianum	26.00 b	16.00 b	21.00 b	130.44 c	93.78 a	112.11 b	25.50 c	16.33 a	20.92 bc
	A	B		A	B		B	A	
T. hamatum	25.33 b	15.67 ab	20.50 b	114.78 ab	112.78 b	113.78 b	22.50 b	22.33 c	22.42 c
	A	B		A	A		A	A	
Trivia 73 WP	25.67 b	17.00 bc	21.33 b	140.33 d	122.78 c	131.56 d	21.17 b	18.67 b	19.92 b
	A	B		A	B		B	A	
No disease Control	22.33 a	14.67 a	18.50 a	109.44 a	101.44 a	105.44 a	17.83 a	15.67 a	16.75 a
	A	B		A	A		B	A	
Mean (C)**	24.47	15.47		122.67	110.40		21.10	18.40	
	A	B		A	B		B	A	

Lowercase denotes comparison within the same column, and uppercase indicates comparison within the same row. Values within the same column/row with the identical lowercase or uppercase are not significantly different based on the LSD (0.05) post hoc test

the lowest number of reproductive tillers was recorded on the control treatment (18.50 tillers).

On average, the number of reproductive tillers per plant of Inpago 7 (24.47) was higher than Breun Senaren 2 (15.47 tillers). A similar situation did happen within each disease control method (Table 4).

Interaction of disease control method and rice variety highly affected ($P<0.01$) the number of filled grains per panicle (Table 1). The highest number of grain per panicle was observed on the treatment combination of Trivia 73 WP × Inpago 7 (140.33 grains), followed by *T. harzianum* × Inpago 7 (130.44 grains) and Trivia 73 WP × Breun Senaren 2 (121.22 grains) in the second and

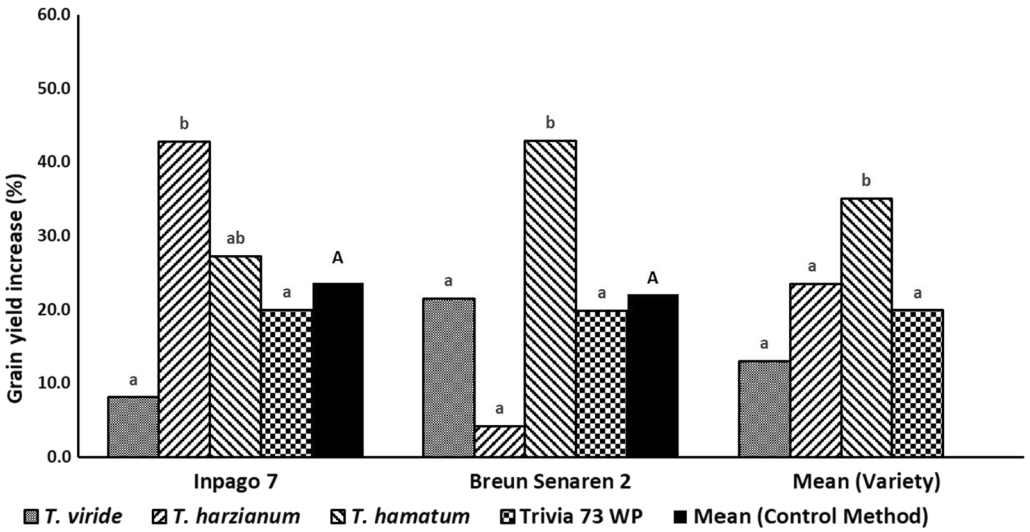

Fig. 1 Mean percentage of grain yield increase under combined treatments of disease control method and upland rice variety. Lowercase denotes comparison among disease control methods within the same variety or mean variety; uppercase denotes comparison between Inpago 7 and Breun Senaren 2. Values/bars within the same group with the identical lowercase or uppercase are not significantly different based on the LSD (0.05) post hoc test

the third places, respectively (Table 4). Meanwhile, the lowest number of grains per panicle was observed on the treatment combination of no disease control × Inpago 7 (109.44 grains) and no disease control × Breun Senaren 2 (101.44 grains) (Table 4).

On the overall mean of the 2 varieties, the fungicide spray using Trivia 73 WP produced the highest number of grains per panicle (131.56 grains), followed by *T. viride* (119.78 grains) in the second, and *T. harzianum* and *T. hamatum* in the third place. On the other hand, the control treatment produced the lowest number of grains per panicle (105.44 grains). On the overall means of disease control method, Inpago 7 produced a higher number of grains per panicle (122.67 grains) than Breun Senaren 2 (110.40 grains) (Table 4).

Grain yield per plant and increase (%) of grain yield per plant

The ANOVA results revealed that the interaction between the disease control method and rice genotype significantly affected grain yield per plant (Table 1). Mean grain yield per plant is presented in Table 4.

On the Inpago 7 genotype, *T. harzianum* produced the highest mean grain yield/plant (25.50 g), followed by *T. hamatum* (22.50 g) and Trivia 73 WP (21.17 g). In contrast, the control treatment produced the lowest grain yield/plant (17.83 g). *T. hamatum* had the highest mean grain yield plant/plant on Breun Senaren 2 (22.33 g), followed by *T. viride* (19.0 g), Trivia 73 WP (18,67 g), *T. harzianum* (16.33 g), and the control treatment (15.67 g). On the overall mean of the two varieties, *T. hamatum* produced the highest grain yield/plant (22.42 g), followed by *T. harzianum* (20.92 g), Trivia 73 WP (19.92 g), and *T. viride* (18.75 g). The control treatment produced the lowest grain/plant (16.75 g).

The results of grain yield per plant indicate that foliar applications of *Trichoderma* species and fungicide Trivia 73 WP could increase the grain yield of tested upland rice varieties compared to no disease control treatment. The percentage of grain yield increase is presented in Fig. 1, where the percentage of grain increase caused by *Trichoderma* and Trivia fungicide applications was dependent on the variety. On Inpago 7, *T. hamatum* caused the highest increase of grain yield (42.8%), while on Breun Senaren 2, *T. hamatum* produced the highest increase of grain yield per plat (42.9%). Over the 2 varieties, the percentage of grain yield increase was highest on *T. hamatum,* while *T. viride*, *T. harzianum*, and Trivia 73 WP caused a statistically similar percentage of grain yield per plant.

Correlation analysis

Correlation analysis was performed to see the association among the studied variables, employing the replicated variable's data of the treatment combinations (Table 5). AUDPC had a positive correlation with apparent infection rate ($r=0.47$), and it had a negative correlation with the reproductive tiller ($r=-0.49$), the number of filled grain ($r=-0.49$), and grain yield ($r=-0.55$), but it had non-significant correlation with phenol content

Table 5 Correlation analysis of the observed variables

	AUDPC	AIR	TPC	NRTP	NFGP	GYP
AUDPC	1	0.47*	− 0.33	− 0.49*	− 0.46*	− 0.55*
AIR		1	− 0.49*	− 0.09	− 0.10	− 0.08
TPC			1	0.35	0.13	0.17
NRTP				1	0.53*	0.49*
NFGP					1	0.54*
GYP						1

AUDPC = Area under disease progress curve, AIR = apparent infection rate, TPC = total phenol content, NRTP = number of the reproductive tiller per plant, NFGP = number of filled grain per panicle, GYP = grain yield per plant. Values in the table are correlation coefficients. * = significant based on *t* test ($P < 0.05$)

($r = -0.33$). The apparent infection rate was negatively correlated with phenol content ($r = -0.49$) and AUDPC, but it did not correlate with other variables. Total phenol had no association with other variables except with apparent infection rate (a negative correlation).

The number of reproductive tillers correlated positively with both the number of filled grain ($r = 0.53$) and grain yield ($r = 0.49$), but it negatively correlated with AUDPC. Grain yield per plant correlated positively with both the number of filled grains per panicle ($r = 0.49$) and the number of filled grain per panicle ($r = 0.541$), but it was negatively correlated with AUDPC (Table 5).

Discussion

In this study, foliar applications of local *Trichoderma* species and the fungicide Trivia 73 WP were investigated for their effectiveness in suppressing the brown leaf spot in two genotypes of upland rice. The disease control treatments using *Trichoderma* sp. and fungicide treatments were able to suppress the leaf brown spot disease progress on two upland rice varieties as indicated by their lower AUDPC than in the control treatment. In addition, the lower AUDPC in the *Trichoderma* sp. and the fungicide applications was consistent with the lower mean apparent infection rate of these treatments than the control treatment. The efficacy of *Trichoderma* sp. and the fungicide treatments ranged from 27.05 to 36.42%. Foliar application of *T. hamatum* was the most effective against the disease, while *T. viride* and *T. harzianum* were as effective as fungicide spray using Trivia 73 WP. The ability of *Trichoderma sp*. to suppress leaf brown spot in the current study is consistent with previous study results by Khalili et al. (2012). The results also confirmed the effectiveness of *Trichoderma* application against blast diseases of rice (Chou et al. 2020) and various crop diseases (Soesanto et al. 2019).

Results of the present study revealed that total phenol content was higher in plants receiving *Trichoderma* applications than in the control treatment. The results also showed that *Trichoderma* application reduced the AUDPC and apparent infection rate, supported by the positive correlation between the 2 parameters ($r = 0.47$). Meanwhile, there was a significantly negative correlation between phenol content and the apparent infection rate ($r = -0.49$), indicating the involvement of phenol in suppressing the disease progression rate. Although previous studies had reported phenol involvement in induced resistance against *D. oryzae* (Hassan et al. (2017), the novelty of the present study results is that phenol production of local *Trichoderma* of West Timor and Trivia applications was varietal dependent. This suggests that phenol involvement in suppressing leaf brown spots depends on the rice variety and the *Trichoderma* species, implying different mechanisms of host–pathogen interaction, resulting in various resistance levels.

All treatment combinations produced similarly high-level tannin content, indicating that tannin may not involve in the host plant response against *D. oryzae* and, presumably, the high-level tannin content was produced constitutively, which is in line with Dixon (2001). In contrast, Yanti et al. (2018) reported the involvement of tannin in resistance against dieback disease (*Botryodiplodia theobromae*) of cocoa seedlings. Unlike tannin, saponin was produced in low to medium levels with no apparent pattern among rice genotype and disease control treatments, which renders its involvement in resistance to brown spot disease inconclusive. Nevertheless, previous studies demonstrated that saponin is involved in biochemical resistance against various plant diseases (Yanti et al. 2018).

The number of productive tillers per plant was negatively correlated ($r = -0.49$) with AUDPC, indicating that an increase in AUDPC will be followed by a decrease in productive tiller number and vice versa. Presumably, the increase in AUDPC caused a decline in the plant's photosynthetic capacity since the brown spot symptoms mainly occurred on rice leaves, which reduces the photosynthetic area of the plant. This will, in turn, cause a decline in the number of reproductive tillers (Dariush et al. 2020). The ability of *Trichoderma* and fungicide

applications to produce a higher number of tillers than the control treatment was presumably related to the lower AUDPC, and hence a higher leaf photosynthetic capacity they possessed. The genetic factor may contribute to the significant difference between varieties in the reproductive tiller number as the ability to produce reproductive tiller is varietal dependent (Ndiwa and Mau 2019).

The treatment combinations highly affected the number of filled grains, implying that this trait is highly variable among the disease control treatments and the rice variety. The differences in disease severity may cause different photosynthetic capacities of the rice plants, which resulted in a significant grain number per panicle among treatments. A previous study by Hassan et al. (2017) showed that *Trichoderma* foliar application reduced the brown spot infection and caused an increase in agronomic characters, including the number of discolored (filled) grain.

The grain yield was affected significantly by the disease control method and rice genotype interaction, which may reflect the genetic potential of the tested rice genotypes. The expression of this genetic potential is further influenced by environmental factors, i.e., disease control treatments using *Trichoderma* and the fungicide. The grain yield of the 2 varieties in the *Trichoderma* and fungicide treatments was significantly higher than the control treatment. The lower grain yield in the control treatment may be caused by the decrease in both reproductive tiller number and number of grains per panicle as the direct effect of leaf infection by the disease, which reduced the photosynthetic capacity of rice plants (Pantha et al. 2017). This presumption is supported by the negatively significant correlation between AUDPC with, respectively, grain yield ($r=-0.55$), the number of the reproductive tiller ($r=-0.49$), the number of filled grains per panicle ($r=-0.46$), and the positive correlation between grain yield with, respectively, the number of the reproductive tiller ($r=0.49$) and the number of filled grains ($r=0.54$).

A previous study by Hassan et al. (2017) showed that *Trichoderma* foliar application reduced the brown spot infection but increased the agronomic characters (leaf chlorophyll content, plant height, panicle length, the number of filled grain, and grain yield) as compared to the non-treated plants. The ability of *Trichoderma* to improve the agronomic characters is consistent with the previous findings that *Trichoderma* can stimulate the host plant growth (Hassan et al. 2017).

The present study results revealed that the efficacy of *Trichoderma* and fungicide treatments against brown spot ranged from 27.05 to 36.42%, while the grain yield of the plants receiving the treatments was about 13–35% higher than the control. This discrepancy indicated that the grain yield observed in this study may reflect the genetic potential of the tested rice genotypes, which may or may not be directly related to leaf brown spot severity. The higher grain yield in the *Trichoderma*-treated plants than the control might be occurred through both the reduced leaf brown spot infection and the mechanism of plant growth stimulation. These included an increased number of reproductive tillers and filled grain. As a comparison, previous study (Dariush et al. 2020) confirmed that brown spot infection reduced the grain yield by reducing the reproductive tiller number and the number of filled grains. However, the host plant's growth stimulation was not examined physiologically in the present study, which is the limitation of the current study that needs to be considered in future works.

Conclusions

The indigenous *Trichoderma* species isolated from West Timor and the fungicide Trivia 73 WP effectively reduced brown spot disease of upland rice, and the efficacy was dependent on the rice variety, with a mean efficacy range over the 2 varieties from 27.05 to 36.42%. The *Trichoderma* and the fungicide Trivia treatments increased the grain yield per plant differentially in the 2 rice varieties, with a mean grain yield increase over the 2 varieties of 13.0% (*T. viride*), 23.5% (*T. harzianum*), 35.0% (*T. hamatum*), and 19.9% (Trivia 73 WP). The local *Trichoderma* species of West Timor have a future potential as biocontrol agents of brown spot disease of upland rice as they were able to suppress the disease and increase the grain yield of upland rice under a screen house condition. However, before being further employed as potential biological agents for brown spot disease, future works are needed to evaluate the effectiveness of this local *Trichoderma* against the disease in replicated field trials.

Abbreviations

ENT: East Nusa Tenggara; WP: Wettable powder; DS: Disease severity; AUDPC: Area under the disease progress curve; ANOVA: Analysis of variance; AIR: Apparent infection rate; TPC: Total phenol content; NRTP: Number of the reproductive tiller per plant; NFGP: Number of filled grain per panicle; GYP: Grain yield per plant; LSD: Least significant difference.

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Author contributions

YSM organized the research team, provided the research supplies, and contributed to all experiment stages. RSP, KDN, and HK contributed to all

stages of the experiment. JBDH and SW assisted in designing the experiment, performed laboratory analysis, and performed data analysis and interpretation. MVH assisted in data analysis and interpretation and assisted in drafting and finalizing the manuscript, and YRYG assisted in designing the experiment and assisted in data analysis and interpretation. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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Not applicable.

Consent for publication
The manuscript has not been published completely or in part elsewhere.

Competing interests
The authors declare no conflicts of interest.

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