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In vitro and *in vivo* Evaluation of Microbial Agents for Management of Rice Blast Disease in Tanzania

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Abstract: Evaluation of two microbial agents, *Trichoderma asperellum* and *Bacillus subtilis* and fungicide Linkimil 72 WP against rice blast disease, *Pyricularia oryzae* were done in a laboratory and pot experiment. Dual inoculation of *T. asperellum*, *B. subtilis* and Linkimil 72 WP caused significant inhibition of radial growth of *P. oryzae*. Both *T. asperellum* and *B. subtilis* showed over 75% inhibition of radial growth (PIRG) compared to Linkimil 72 WP with PIRG range of 21-23 % and control with 0 %. In a pot experiment, 70 % reduction in disease incidence was in plants treated with *T. asperellum* followed by *B. subtilis* (51.5 %) and Linkimil 72 WP (26.5 %). There was a 44.5 % decrease in disease severity in plants treated with Linkimil 72 WP compared to plants treated with *T. asperellum* (35.6 %) and *B. subtilis* (29.1 %). The number of lesions per leaf was low on rice plants treated with *T. asperellum* followed by Linkimil 72 WP and *B. subtilis*. *T. asperellum* and *B. subtilis* used in this study had shown a high antagonistic capacity against *P. oryzae* and they can be recommended as part of integrated management of rice blast disease.

Key words: *Bacillus subtilis* · Microbial Agent · *Pyricularia oryzae* · Rice Blast Disease · *Trichoderma asperellum*

INTRODUCTION

Rice blast disease a fungal disease caused by *Pyricularia oryzae* Cavara, a devastating disease of rice reducing yields worldwide [1, 2]. The Rice blast disease is found everywhere in the world where rice is grown [3]. The pathogen belongs to the Kingdom Fungi, Phylum Ascomycota and the Genus *Pyricularia* and infects all growth stages of rice plants [4]. It survives on infected rice crop residues, weeds and rice seeds [1, 5, 6]. Reports show that in the field the fungus dispersed through airborne spores, infected crop residues and infected rice seeds [1, 5, 7].

Initial symptoms of rice blast disease appears as white to grey-green lesions or spots, with dark green borders. Mature lesions appear cottony in the centre with a dark bluish surface due to production of conidia. On leaves, rice blast lesions are elliptical or spindle-shaped and whitish to gray centers, with red to brownish or necrotic borders [5, 8].

Several studies [9-11] indicate that management of rice blast disease is by resistant genotypes, fungicides and appropriate cultural practices. However, none of these methods can permanently control rice blast disease. The longevity of resistance of many resistant genotypes is shortened by the high pathogenic variability of *P. oryzae* [12]. The fungicide, such as Linkimil 72 WP, has a negative effect to the environment and human healthy. Sustainable and effective rice blast control can be through a combination of resistant genotypes and the use of control strategies that are environmentally friendly such as microbial agents.

In recent years, there have been an increasing number of studies on antagonistic microbial agents for control of rice blast disease [11-14]. *Trichoderma harzianum, T. viride* and *B. subtilis* have been reported to reduce rice blast disease incidence by 70% in India [11, 15]. In some parts of Africa, *T. asperellum* and *B. subtilis* are used for controlling soil borne and foliar diseases of ornamental plants and

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vegetables [16, 17]. However, there is no report on the use of *T. asperellum* and *B. subtilis* for controlling of rice blast disease in Africa. Therefore, there was a need to determine the efficacy of *T. asperellum* and *B. subtilis* against *P. oryzae*. Therefore, the current study reports on the efficacy of using *T. asperellum* and *B. subtilis* in inhibiting growth *P. oryzae* and reducing disease incidence and severity.

MATERIALS AND METHODS

Source of Microbial Agent, Linkimil 72 WP and Rice Seeds

Microbial Agents: Commercial Real *Trichoderma* (*Trichoderma asperellum*) and Real *Bacillus* (*Bacillus subtilis*) were obtained from Real IPM, Nairobi, Kenya. Linkimil 72 WP (*Mancozeb 64% + Metalaxyl 8%*) was purchased in Morogoro town. Rice genotypes such as Kihogo and Lunyuki used were collected from farmers in Morogoro district while Supa and Usiguse genotypes were from the International Rice Research Institute (IRRI) germplasm collection at DakawaAgricultural Research Institute, Morogoro, Tanzania.

Inocula Collection and Preparation: Leaf samples showing symptoms of rice blast disease were collected from rice fields in Morogoro and Tanga region, Tanzania. Samples were packed in paper envelopes and transported to the African Seed Health Centre Laboratories, Sokoine University of Agriculture (SUA), Tanzania, for isolation of *P. oryzae*. Small sections (1-1.5 cm) taken from infected parts of leaves and placed on three layers of wet filter papers lined in (90 mm) Petri dishes. The Petri dishes were incubated at 25°C. After 24 - 48-hours of incubation, the lesions were examined under a dissecting microscope to check for sporulation. Sterilized inoculating loop with small piece of Potato Dextrose Agar (PDA) were used to pick conidia that emerged and transferred it to Petri dishes containing PDA. The Petri dishes were sealed using sealing tape and incubated upside down for five days at 25°C.The pure cultures of the fungal isolates were grown on oatmeal agar for 10 -14 days to induce sporulation.

Pathogenicity Test: Different isolates were inoculated on rice seedlings (variety Supa as a susceptible check) planted in 4-litre plastic pots. Inoculation was done following procedures described by [18]. The conidia of *P. oryzae* were suspended in two drops of Tween 20 adjusted at 1 x 10^5 spores/ml and sprayed on 14 days old rice seedlings. Inoculated seedlings were covered using translucent plastic sheets and placed under screen house conditions (26 - 28) for 24 h. After 7 – 10 days of infection, assessment of rice blast disease severity was done using a 0 - 9 scales [19]. Pathogenicity of the suspected isolates of *P. oryzae* were confirmed following procedures of Koch's postulates. The fungal isolates with disease severity score value > 4 were selected for further tests.

***In vitro* Test of *Trichoderma asperellum* and *Bacillus* Sub Tilis Against Growth of *Pyricularia oryzae*:** The dual culturing technique was used to test the antagonistic effect of *T. asperellum* and *B. subtilis* against *P. oryzae* [20]. Inoculum was prepared as described by [2]. A sterile cork borer (5 mm in diameter) was used to make holes diametrically opposite to a 5 mm disc of the test pathogen. Three concentrations (0.5, 1.0 and 2.0 ml/L) of *T. asperellum*, *B. subtilis* and Linkimil 72 WP were placed singly into the media holes. Petri plates inoculated with *P. oryzae* alone were used as negative controls and Petri plates inoculated with *P. oryzae* and Linkimil 72 WP were used as positive controls. The inoculated Petri dishes were sealed using sealing tape and incubated at 20°C, 25°C and 28°C in alternating cycles of 12-hour light and darkness.

The layout of the experiment was 4 x 3 x 3 factorial in a completely randomized design (CRD) with four replications where; factor (i) was *P. oryzae* inhibition treatments: (i - 1) = *T. asperellum*, (i - 2) *B. subtilis*, (i - 3) = Linkimil 72 WP and (i - 4) = negative control. Factor (ii) consisted of concentrations of *T. asperellum*, *B. subtilis* and Linkimil 72 WP*:* (ii – 1) = 0.5 ml/L (ii - 2) = 1.0 ml/L and (ii - 3) =2.0 ml/L. Temperature was used as factor (iii) where: (i - 1) = 20°C, (ii - 2)= 25°C and (iii - 3) = 28°C. Data on colony diameter (ø) growth of *P. oryzae* was recorded for each plate after every 28 hours for up to 14 days, following procedures of [2] with modifications. Fungal colony radii towards the antagonistic colony were measured using 300 mm ruler. Percentage growth inhibition of *P. oryzae* by microbial agents was calculated using the formula described by [21] as shown below:

$$N = \frac{(Lc - Lp)}{Lc}\% \quad (1)$$

where, N is the percentage inhibition; Lc is the radius of *P. oryzae* in the negative control and the Lp is the radius of *P. oryzae* in treated dishes. Determination of antagonistic activity was done using a scale developed by Soytong, (1988) as cited by

[22]. The percentage inhibition radial growth (PIRG) was described as; > 75 % =very high antagonistic activity, 61 – 75 % = high antagonistic activity, 51 – 60 % = moderate antagonistic activity, < 50 % = low antagonistic activity and 0 = no antagonistic activity.

***In vivo* Evaluation of *Trichoderma asperellum* and *Bacillussubtilis* Against *Pyricularia oryzae*:** Rice seeds of four upland rice genotypes were planted in 4-litre-plastic pots (4 seeds/pot). Thereafter, seedlings were thinned to two plants per pot. Inoculation with *P.oryzae* was done when rice seedlings were at the age of 14 days after emergence [18]. At seven and fourteen days after inoculation with *P. oryzae,* 1 ml/L of water suspension formulation of *T. asperellum, B. subtilis* and Linkimil 72 WP (a broad-spectrum protectant and curative fungicide) were sprayed on rice seedlings. Non-sprayed rice plants were treated as a negative control, while positive control plants were sprayed with Linkimil 72 WP (4g/L). The experiment was laid out in a 4 x 4 factorial in a CRD where; factor (i) was blast disease control treatments: (i - 1) = *T. asperellum*, (ii - 2) = *B. subtilis*, (iii - 3) = Linkimil 72 WP and (i - 4) = No spray. Factor (ii) was rice genotypes where: (ii - 1) = Supa, (ii - 2) = Kihogo, (ii - 3) =Usiguse and (ii - 4) = Lunyuki. The assessment of rice blast disease incidence and severity was done at 2 and 3 weeks after inoculation for leaf blast disease and at 3 weeks after heading for the neck and panicle blast disease [23]. Plants were visually evaluated by using a scale of 0-9 developed by [19]. Rice blast disease severity scores were converted into percent disease by using the formula described by [24]. Disease incidence was calculated using the formula (ii) below:

$$\%\text{Disease sevenrity}=\frac{\text{Sum of scores X 100\%}}{\text{Number of observations x highest number on the rating scale}} \quad (2)$$

$$\text{Disease incidence}=\frac{\text{Number of diseased leaves}}{\text{Total number of inspected leaves}}\text{x}100\% \quad (3)$$

Data Analysis: Data on rice blast disease incidence and percentage disease severity were ArcSine transformed before analysis. Before transformation all 0% values were replaced by (1/4*n*) and all 100% values by (100 – 1/4*n*), where *n* is the number of units upon which the percentage data were based [25]. Logarithmic transformation was performed on the number of lesions per leaf, number of tillers per plant, number of panicles per plant. Log *(X+1)* was used for all values instead of *Log X*, where *X* represented original data [25]. All data were subjected to analysis of variance and the means were compared using Tukey's test at P ≤ 0.05. All statistics were performed using the Genstat Computer Software, 15th Edition.

RESULTS

***In vitro* Evaluation of *Trichoderma asperellum* and *Bacillussubtilis* Against Growth of *Pyricularia oryzae*:** The effect of various treatments used in the study on inhibition of mycelia radial growth are shown in Table 1. Significant differences (P≤ 0.05) were observed between *P. oryzae* growth inhibition treatments and temperature (Table 1, Figures 1 and 2, Plate 1). *Trichoderma asperellum* and *Bacillus subtilis* showed similar growth inhibition of *P. oryzae* (Figure 1). The interaction between growth inhibition treatments and temperature was significantly different (P ≤ 0.05) (Table 1). Different concentrations of *T. asperellum* and *B. subtilis* showed similar inhibition of growth of *P. oryzae* (Figure 3 and Plate 2).

Effect of Mycelia Growth Inhibition Treatments and Temperature on Radial Growth of *Pyricularia oryzae*: *Trichoderma asperellum* and *B. subtilis* at 20°C, 25°C and 28°C showed the similar percentage radial growth inhibition (PIRG) of *P. oryzae* ranging 82 - 88% (Figure 4). The highest PIRG was 88.1% by *B. subtilis* at 20°C and 86% by *T. asperellum* at 25°C. Results also showed that Linkimil 72 WP had significantly lower PIRG compared to *B. subtilis* and *T. asperellum* at 20°C, 25°C and 28°C.

***In vivo* Evaluation of Microbial Agents for Control of Rice Blast Disease**

Rice Blast Disease Incidence and Severity: Results indicate significant differences (P ≤ 0.05) on incidence of rice blast disease between microbial agents and Linkimil 72 WP (Figure 5). Rice blast disease incidence was low on rice plants treated with *T. asperellum* (18.70%) followed by *B. subtilis* (37.3 %) and Linkimil 72 WP (62.3%) compared to control (No spray) (88.8 %). Results also indicated significant differences between blast management treatments (P ≤ 0.05) on disease severity (Figure 6). Rice blast disease severity on rice plants treated with *T. asperellum* (24 %) and *B. subtilis* (30.5 %) did not differ significantly (P ≤ 0.05).

Table 1: Analysis of variance of the effect of different treatments, concentrations and temperature on mycelia radial growth inhibition of *Pyricularia oryzae*

		Mycelia radial growth (mm)											
		96 h		144 h		192 h		240 h		288h		336 h	
Treatments	df	F	P	F	P	F	P	F	P	F	P	F	P
M	3	17.3	<.001	199.9	<.001	526.3	<.001	596	<.001	464.99	<.001	650.64	<.001
C	2	3.97	0.022	0.01	0.994	2.22	0.11	2.03	0.137	2.5	0.087	0.84	0.437
T	2	102	<.001	69.37	<.001	85.99	<.001	87.37	<.001	64.68	<.001	73.31	<.001
M x C	6	2.88	0.012	1.03	0.408	2.9	0.01	2.07	0.062	2.35	0.036	1.03	0.407
M x T	6	10	<.001	6.82	<.001	6.74	<.001	8.97	<.001	10.66	<.001	10.85	<.001
C x T	4	6.25	<.001	1.17	0.327	1.1	0.36	0.48	0.748	0.34	0.849	1.31	0.271
M x C x T	12	2.19	0.017	1.17	0.313	2.05	0.03	2.19	0.017	1.26	0.251	1.47	0.148

M= Growth inhibition treatments, C= Concentration, T= Temperature, F= F, value, P= Probability

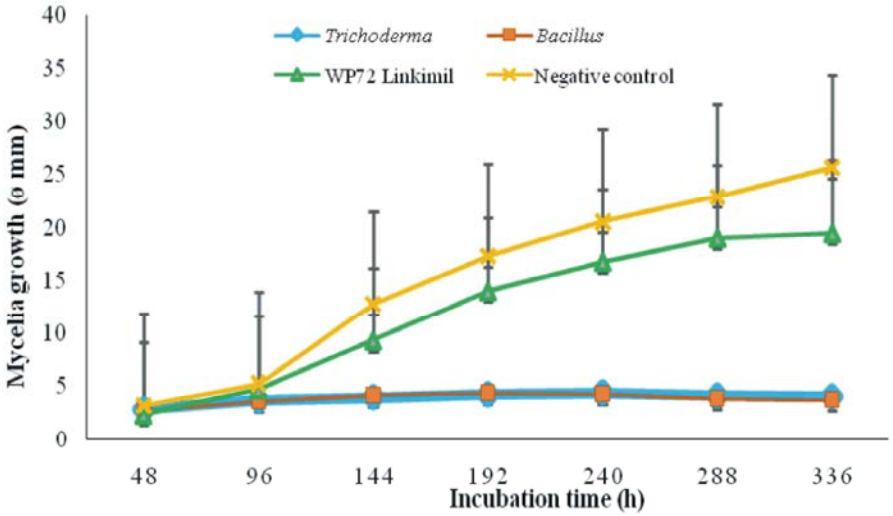

Fig. 1: The effect of *Trichoderma asperellum*, *Bacillus subtilis*, Linkimil 72WP and negative control on radial growth of *Pyricularia oryzae* after incubation for 336 hours

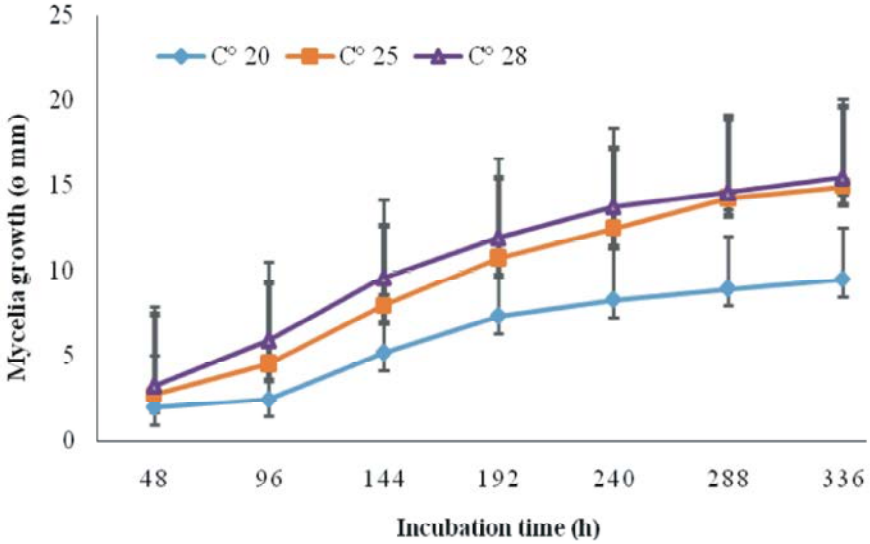

Fig. 2: The effect of different exposure temperature on radial growth of *Pyricularia oryzae* after incubation for 336 hours

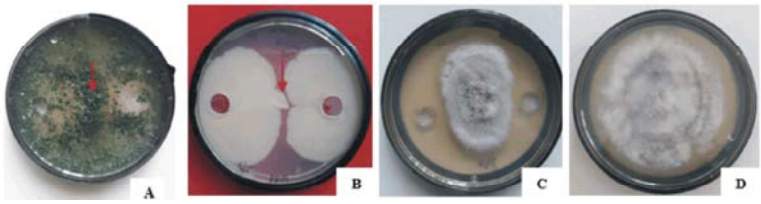

Plate 1: Effect of A= *Trichoderma asperellum*, B= *Bacillus subtilis*, C = Linkimil 72WP and D = negative control) on inhibition of growth of *Pyricularia oryzae* after incubation for 14 days

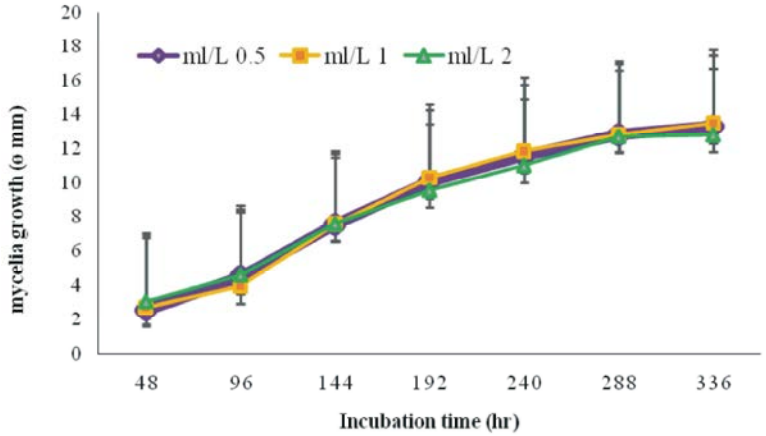

Fig. 3: The effect of different concentrations of *Trichoderma asperellum, Bacillus subtilis* and Linkimil 72 WP on radial growth of *Pyricularia oryzae* after incubation for 336 hours

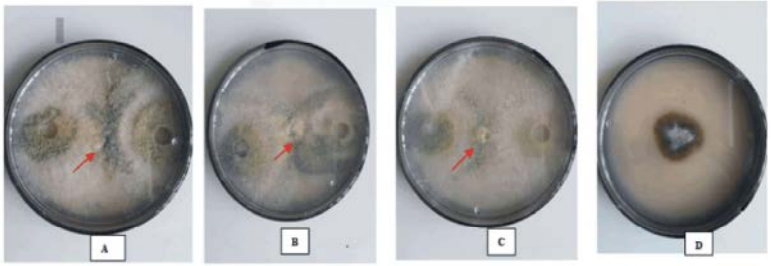

Plate 2: The effect of different concentrations of *Trichoderma asperellum* (A= 2.0 ml/L, B = 1.0 ml/L, C= 0.5 ml/L and D = negative control) on inhibition of growth of *Pyricularia oryzae* (at red arrow)

Significantly, low disease severity ($P \leq 0.05$) was observed on plants treated with Linkimil 72 WP (12.1 %) compared to negative control (59.6 %) (Figure 6). Significant differences ($P \leq 0.05$) on blast disease severity were observed on the interaction between blast management treatments and four rice genotypes (Table 4). Rice blast disease severity was significantly low on Supa (12.9 %) and Kihogo (12.9 %) rice genotypes when treated with Linkimil 72WP (Table 2).

Number of Lesions per Leaf and Size of the Lesions: The average number of lesions per leaf differed significantly ($P \leq 0.05$) between different rice blast disease management treatments. The number of lesions per leaf were low on rice plants treated with *T. asperellum* (8) followed by Linkimil 72 WP (11) and *B. subtilis* (25) compared to negative control (58) (Table 3).

The lesion size on plants treated with *T. asperellum*, *B. subtilis* and Linkimil 72 WP did not differ significantly ($P \leq 0.05$), while the large stlesion size was on the plants treated with negative control (46.3 mm) (Table 3). The lesion size on the interaction between blast management treatments and rice genotypes was not significantly different ($P \leq 0.05$). However, the lesion size (11.0 mm) on the interaction between negative control and Lunyuki rice genotype was significantly smaller ($P \leq 0.05$) than those on rice genotype Supa (54.0 mm), Kihogo (58.4 mm) and Usiguse (61.6 mm) (Table 2).

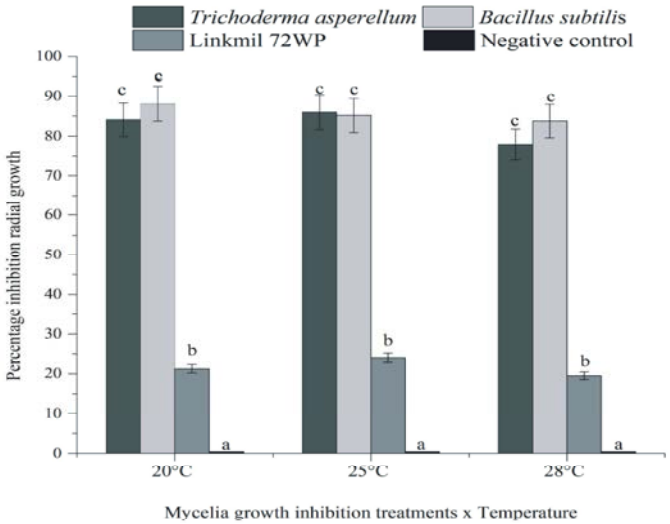

Fig. 4: Percentage inhibition of radial growth of *Pyricularia oryzae* by *Trichoderma asperellum*, *Bacillus subtilis* and Linkimil 72 WP in dual cultures

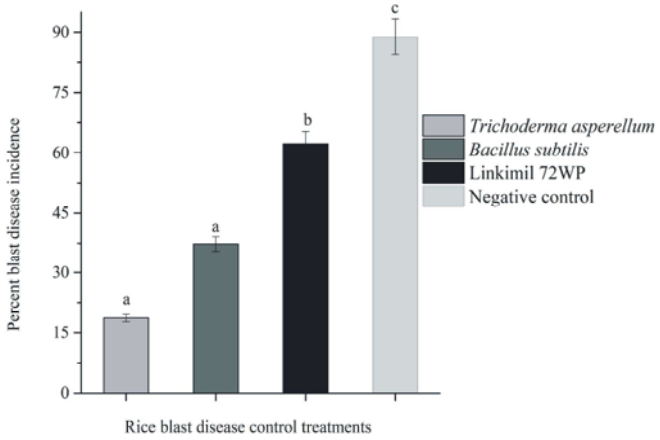

Fig. 5: The effect of different treatments on incidence of rice blast disease

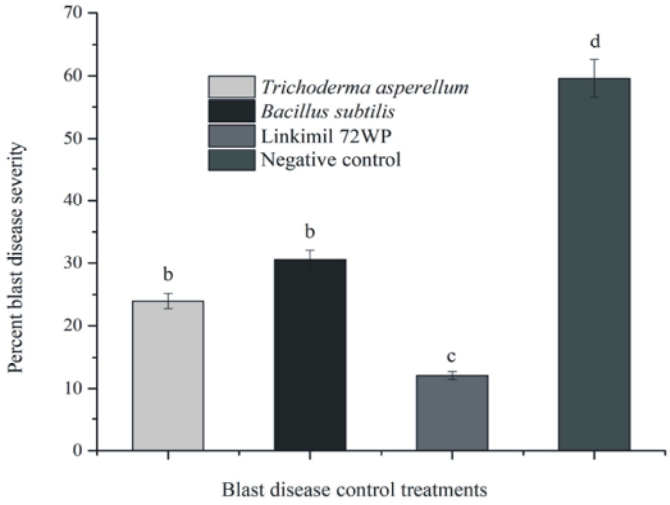

Fig. 6: The effect of different treatments on severity of rice blast disease

Table 2: The effect of different treatments on incidence and severity of rice blast disease and lesion size in four rice genotypes

	BDI (%)				BDS (%)				LS (mm)			
Treatments	V1	V2	V3	V4	V1	V2	V3	V4	V1	V2	V3	V4
T1	15.2 a	18.7 a	31.2 ab	15.9 a	25.4 abcd	36.7 bcd	29.5 abcd	14.4 ab	4.4 a	5.4 a	4.9 a	2.5 a
T2	18.7 a	52.9 abc	39.4 abc	42.2 abc	38.4 cd	38.6 cde	43.4 def	17.0 abc	7.1 a	11.9 a	6.6 a	2.1 a
T3	36.2 abc	84.1 abcd	77.3 abcd	51.6 abc	12.9 a	12.9 a	12.9 a	11.5 a	5.2 a	9.7 a	6.5 a	1.3 a
T4	85.5 d	99.5 bcd	99.9 cd	85.7 abcd	68.8 ef	70.5 f	68.6 ef	46.0 d	54.0 b	58.4 b	61.6 b	11.0 a

For each column, means followed by a common letter are not significantly different at $P \leq 0.05$ according to Tukey's test

T1 = *Trichoderma asperellum*, T2 = *Bacillus subtilis*, T3 = Linkimil 72 WP, T4 = Negative control, V1 = Supa, V2 = Kihogo, V3 = Usiguse, V4 = Lunyuki, BDI = Blast disease incidence, BDS = Blast disease severity and LS = Lesion size

Table 3: The effect of different treatments on percent filled grains, unfilled grains, panicle weight, grain weight and dry shoot weight

Source of variation	Number of lesions/leaf	Lesion size (mm)	Number of tillers/plant	Filled grains (%)	Unfilled grains (%)	Panicle weight (g)	Grain weight (g)	Dry shoot weight (g)
Trichoderma	8.0 a	4.3 a	3.0 a	71.6 b	29.0 a	9.6 b	8.7 b	23.6 b
Bacillus	25.0 b	6.9 a	3.0 a	73.8 b	27.5 a	11.0 b	10.2 b	26.3 b
Linkimil 72WP	11.0 a	5.7 a	3.0 a	73.3 b	27.6 a	10.5 b	10.1 b	23.3 b
Negative control	58.0 c	46.3 b	2.0 a	57.4 a	43.2 b	5.8 a	5.4 a	17.5 a
Mean	19.0	15.8	2.51	69.0	31.8	9.2	8.6	22.7
S.E.	2.2	12.8	0.13	0.1	12.6	2.5	2.5	4.1
CV%	26.9	81.3	32.4	14.3	39.7	27.5	28.5	18.1

For each column, means followed by a common letter are not significantly different at P = 0.05 according to Tukey's test

Number of Effective Tillers per Rice Plant: The number of tillers per plant did not differ significantly between blast management treatments ($P \leq 0.05$). The lowest number of tillers per plant (2 tillers) was observed on rice plants treated with the negative control (Table 3).

Percentage Filled and Unfilled Grains: Significant difference between blast management treatments ($P \leq 0.05$) were observed on percentage filled grains. However, the percentage filled grains on rice plants treated with *T. asperellum* (71.6 %), *B. subtilis* (73.8 %) and Linkimil 72 WP (73.3 %) were similar compared to negative control (57.4 %) (Table 2). Significant differences ($P \leq 0.05$) between blast management treatments were observed on the percentage of unfilled grains (Table 3).

Panicle and 1000 Grains Weight: The average weight of panicles did not differ significantly ($P \leq 0.05$) for rice plants treated with *T. asperellum*, *B. subtilis* and Linkimil 72 WP and the lowest panicle weight was observed on rice plants treated with the negative control (5.8 g) (Table 3). The same trend was observed on 1000 grains weight where rice plants treated with *T. asperellum* (8.7 g), *B. subtilis* (10.2 g) and Linkimil 72 WP (10.1g) did not differ significantly ($P \leq 0.05$) (Table 3).

Dry Shoot Weight: Results indicate a significant difference in dry shoot weight among blast management treatments ($P \leq 0.05$). However, there was no significant difference between *T. asperellum* (23.6 g), *B. subtilis* (26.3 g) and Linkimil 72 WP (23.3 g) compared to the negative control (Table 3).

DISCUSSION

Biological control using antagonistic fungi have been widely used to control a number of plant disease causing pathogens. Fungi belonging to the genus *Trichoderma* and bacteria such as *Bacillus subtilis* have been reported as the most effective bio-control agents against a wide range of plant pathogens [26]. In this study, different concentrations of *T. asperellum* showed similar inhibition of radial growth of *P. oryzae* within five days of incubation. Such findings are in agreement with the study by [12] which indicated that, the dual culture assays of *Trichoderma* had the highest degree of inhibition of *P. oryzae* in *in vitro* within four days. *Trichoderma* spp. uses more than one mechanism in the biocontrol activity. Such mechanisms include competition for nutrients, predation against pathogens (mycoparastism), stimulation of plant growth and immune response to induce resistance to diseases and the release of volatile antibiotics and hydrolytic enzymes such as chitinase and β-1, 3-glucanase [20, 27]. Hydrolytic enzymes have been reported to degrade the pathogen cell wall that aid to mycoparasitism [20]. The inhibition of mycelia growth could be due to competition for nutrient, antibiosis or mycoparasitism. In this study, Linkimil 72

WP had the lowest percentage radial growth inhibition of *P. oryzae* (Figure 4). According to [21] the percentage radial growth inhibition of *P. oryzae* by *T. asperellum* and *B. subtilis* in the current study indicated high antagonist activity. This result is in agreement with [28]. This indicates that both *T. asperellum* and *B. subtilis* were the best in inhibition of radial growth of *P. oryzae* in dual inoculation.

This study indicated that the incidence of rice blast disease was reduced on plants treated with *T. asperellum* followed by *B. subtilis* and Linkimil 72 WP as compared to the negative control. On disease severity, the percentage decrease in disease severity on *T. asperellum* and *B. subtilis* treated rice plants were lower than Linkimil 72 WP treated plants. These results are in agreement with previous studies which showed that reduction in blast disease incidence was significantly lower in rice plants treated with fungicide (Benlate) than those treated with bio-agent (*Trichoderma viride*) [9, 20]. In this study, significant differences in disease incidence were not observed in the interactions between *T. asperellum*, *B. subtilis,* Linkimil 72WP and four rice genotypes, indicating that *T. asperellum* and *B. subtilis* were able to reduce rice blast disease incidence regardless of rice genotypes grown.

The number of lesions per leaf did not differ significantly on rice plants treated with *T. asperellum* and Linkimil 72 WP ($P \leq 0.05$). However, the number of lesions per leaf on rice plants treated with *B. subtilis* was higher than those on *T. asperellum* and Linkimil 72 WP treated plants. The increased number of lesions per leaf reduces the photosynthetic rate by reducing the leaf are [29]. The enlargement of the lesion on rice leaf due to blast disease has been reported to reduce photosynthesis through a reduction in the green leaf area and green leaf tissues surrounding the lesions [30]. The results further showed that, lesion size decreased on rice plants treated with *T. asperellum*, followed by Linkimil 72 WP and *B. subtilis* as compared to rice plants treated with the negative control. The reduction of rice blast disease lesion size has also been achieved by application of *T. harzianum* and *T. viride* [20].

The effect of different blast management treatments on the number of tillers per plant did not differ significantly ($P \leq 0.05$). The smallest number of tillers per plant was observed on rice plants treated with the negative control. The small differences on a number of tillers per plant could be due to low range of genetic characteristics of upland rice genotypes [31].

Results indicated significant differences ($P \leq 0.05$) between blast management treatments on percentage of filled grains. However, there were no statistically significant differences on percentage of filled grains observed between *T. asperellum, B. subtilis* and Linkimil 72 WP, indicating that the two microbial agents were similar with Linkimil 72 WP in reducing the effects of rice blast disease on grain filling. The same trend was observed on percentage unfilled grains and the lowest percentage of filled and unfilled grains were observed on rice plants treated with the negative control plants (Table 2). Rice blast disease affected the photosynthesis rateand contributed to the high percentage of unfilled grain due to reduced carbohydrate supply during grain filling [32, 33].

This study showed that the average panicle weight did not differ significantly ($P \leq 0.05$) when rice plants were treated with *T. asperellum*, *B. subtilis* and Linkimil 72 WP, while a significantly low panicle weight was observed on rice plants treated with the negative control (Table 2). The same trend was observed on grains weight (Table 2). This indicates that both panicle and grain weights were greatly affected by rice blast disease on leaves. This affected the rate of photosynthesis and contributed to reduced grain filling. [32] have also reported the same finding.

Significant differences in dry shoot weight were not observed between rice plants treated with *T. asperellum*, *B. subtilis* and Linkimil 72 WP. However, high, dry shoot weight was observed on rice plants treated with *B. subtilis*. *Bacillus subtilis* have been reported to have a bio fertilizing effect that enhances the capacity of roots to mobilize and take up nutrients and substance that improve plant growth and accumulation of biomass [34]. Dry shoot weight was significantly lower ($P \leq 0.05$) on rice plants treated with the negative control. Such findings may be attributed by high rice blast disease severity observed in this study and accelerated senesces on infected leaf tissues that affected the accumulation of dry matters [33].

CONCLUSION

In this study *T. asperellum* and *B. subtilis* showed high antagonistic activity against *P. oryzae* in dual culture inoculation. The effective concentrations of the two microbial agents were ranging 0.5 - 2.0 ml/L. In the screen house experiment *T. asperellum* and *B. subtilis* were effective in reducing rice blast disease incidence, severity, number of lesions per leaf, and size of the lesion.

The two microbial agents were also effective in increasing the panicle weight, percentage of filled grains and grains weight. Therefore, these commercial *T. asperellum* and *B. subtilis* may be recommended for the integrated management of rice blast disease in Tanzania.

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