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Comparative Testing of Multibioagent Inoculants to Control *Bipolaris spicifera* R15 on Rice Plant

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ABSTRACT

The present research deals with greenhouse studies on the efficacy of *Cladosporium halotolerans* CIR 18_ITS and *Meyerozyma guilliermondii* MIR 15_ITS compared with a compatible *Trichoderma* isolate T.4679 to control the phytopathogenic *Bipolaris spicifera* R15 fungus. An experiment was carried out under controlled conditions in a greenhouse with sterilised soil, and 13 parameters were evaluated. The greenhouse results triggered significant differences [$p<0.05$] on rice plants after two-month post planting in all treatments compared with the untreated control due to pre-inoculation with three multibiocontrol agents. In addition, results showed the significant interaction amongst three multibiocontrol agents on the growth parameters of the rice plant, fresh weight of shoot and root, dry weight of shoot, root, shoot and root length and greater efficiency of reducing disease severity when treated with *the Trichoderma* isolate T.4679, *M. guilliermondii* MIR 15_ITS *and C. halotolerans* CIR 18_ITS individually or in combination with each other. The greenhouse experiment exhibited that *C. halotolerans* CIR 18_ITS alone, *M. guilliermondii* MIR 15_ITS alone, *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS and *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS have greater efficiency of reducing disease infection and severity by approximately 11.11% and 6.67%, respectively, amongst all treatments mentioned.

Keywords: *Cladosporium halotolerans*, *Meyerozyma guilliermondii*, multibiocontrol agents

INTRODUCTION

Most microorganisms used as biocontrol agents against various phytopathogenic fungi have inconsistent performance in their activity to inhibit pathogens completely compared with synthetic fungicides. Some of them display unstable performance when applied under certain cases of agro-ecosystem [Hamdia, 2014].

The inconsistent performance of the biocontrol agents is due to the use of single biocontrol agents or old isolates. Moreover, some researchers do not combine more than one biocontrol agent to suppress a plant pathogen under any environmental condition. Therefore, alternatives, such as the application of biocontrol agents including fungi and bacteria, require continuous screening for effective biocontrol agents to reduce the damage to the environment and public health. Hence, isolation and screening of new fungal and bacterial isolates as biocontrol agents from different soil environments are important. Currently, several factors have shifted the disease management strategies towards the increased dependence on biological agents and cultural controls and reduced pesticide application [Hamdia et al., 2016c; Hamdia et al., 2018; Chaibub et al., 2020a].

Over the past decades, several researchers have documented the control of plant pathogens through the use of *Trichoderma* spp., which is considered a strong candidate in biocontrol agents due to its high potential to affect plant pathogens by attacking and linking the causal agents by sugar linkage as well as releasing cell

wall degradation enzymes (CDWEs), such as chitinase, glucanase, proteinases and lipases [Coley-Smith et al., 1974; Leylaie and Zafari, 2018; Nur and Noor, 2020]. *M. guilliermondii* previously known as *Pichia guilliermondii* is considered an important species in the biological control of soil borne pathogens [Choińska et al., 2020], and used for the biological control of *Botrytis cinerea* and *Penicillium expansum* [Kurtzman and Suzuki, 2010; Aban, 2017b]. Ncediwe [2016] was the first who observed the biocontrol ability of *M. guilliermondii* against the *Alternaria solani* fungus when isolated from tomato leaf surface. *M. guilliermondii* is considered the best candidate for agricultural application [Elena, 2018] because it shows high indole-3-acetic acid (IAA) production and phosphate solubilisation activity [Aban, 2017 a]. It produces a hormone gibberellic acid (GA) that acts throughout the life cycle of plants. GA is affected primarily by significantly increasing the extension stem, seed germination and biomass of paclobutrazol-treated rice seedlings and wheat seeds [Coda et al., 2013; Aban, 2017a]. The *M. guilliermondii* strains can promote the growth of plants by vitamins and soluble nitrogen compounds [Cheng et al., 2018].

Several investigators have reported that the combination of *M. guilliermondii* and *Trichoderma* spp. can control plant pathogens and obtain significant differences to decrease mycelial growth and conidial production [Shahbazi et al., 2014; Kasfi et al., 2018]. The effect of combined treatment of *Trichoderma* spp. and *M. guilliermondii* was shown through the subsequent secretion of extracellular cell wall, degrading enzymes (CDWEs), such as β-1, 3-glucanases, chitinase [endochitinases], proteinases and lipases, to bind pathogen lectins for first contact [Alizadeh et al., 2020]. *M. guilliermondii* is widely distributed in soil and in association with plants [Zajc et al., 2019]. Rahbi et al., [2021] used *M. guilliermondii* as a bio-fungicide to control the *Alternaria alternata* causal agent of fruit rot on strawberry.

The Cladosporium species have been used widely as biocontrol agents against plant pathogens [Köhl et al., 2015; Chaibub et al., 2016; Chaibub et al., 2019; Ercan, 2019; Gámez-Guzmán et al., 2019]. Kohl et al., [2015] showed the biocontrol ability of *C. cladosporioides* against the pathogenic *Venturia inaequalis* fungus when isolated from apple orchards. Chaibub et al., [2016] reported that *Cladosporium* sp. has high efficiency to control rice leaf blast. Moreover, Chaibub et al., [2019 and 2020b] considered that the *C. cladosporioides* isolate C24G as a non-pathogenic fungus. They concluded that this fungus induced plant parameter growth of soybeans, beans, corn as well as millet, and they recommended using this fungus as green fertiliser. Amongst the six fungi, *Metarhizium* sp. E369, *Beauveria bassiana* E1041, *Metarhizium robertsii* E652 and *Metarhizium anisopliae* alone, *Trichoderma gamsii* strains (1032, 1064) and *C. halotolerans* E126 showed the highest effectiveness against the fungal growth and *Fusarium graminearum*, *Aspergillus parasiticus* and *Penicillium chrysogenum* on stored grain of wheat seed [Segers et al., 2016; Ercan, 2019].

Thus, this research aimed to compare the *Trichoderma* isolate T.4679, *M. guilliermondii* MIR 15_ITS and a new candidate of biocontrol agent, i.e. *C. halotolerans* CIR 18_ITS that was used for the first time in this study to inhibit the *B. spicifera* R15 rice pathogen under controlled conditions.

MATERIALS AND METODS

Laboratory experiment

Three fungal organisms were used, namely, *C. halotolerans* CIR 18_ITS (Accession Number MT415956), *M. guilliermondii* MIR 15_ITS (Accession No. MT420256) and *Trichoderma* isolate T.4679, including compatible *Trichoderma* isolates. T.4 + T.7, T.4 + T.9 and T.6 + T.7 (*Trichoderma* sp. isolate T.4), (*Trichoderma harzianum* isolate T.6 Iraq, Accession No. MT649477), (*Trichoderma asperellum* isolate T.7 Iraq, Accession No. MT648463), and *Trichoderma* sp. isolate T.9) were selected by pre-screening [Hamdia et al., 2018; Hamdia et al., 2020] to determine the compatible activity with *C. halotolerans* CIR 18_ITS and *M. guilliermondii* MIR 15_ITS against the *B. spicifera* R15 pathogen that was isolated from the rice plants in previous studies [Hamdia et al., 2016a and b].

Antagonistic study between multibiocontrol agents against the *B. spicifera* R15 pathogen

Antagonistic activity was conducted using dual culture technique. Three multibiocontrol agents; *Trichoderma* isolate T.4679, *M. guilliermondii*

MIR 15_ITS and *C. halotolerans* CIR 18_ITS were selected for lab assay. In this study, one week of fresh fungal culture of the *Trichoderma* isolate T.4679 and *C. halotolerans* CIR 18_ITS were placed individually on one half of the plates that were divided into two equal portions and the other side of the plate had *B. spicifera* R15, all the plates were incubated for 4 days at 28°C according to the scale by Alfredo and Aleli [2011] that involves four degrees; 1- (+++ degree), the antagonistic fungus was able to grow over the pathogen and the pathogen growth was completely inhibited. 2- (++ degree), the pathogen growth was completely inhibited, but the antagonist was not able to grow over the pathogen. 3- (+degree), mutual inhibition initially, but the antagonist was overgrown by the pathogen.

4- (-degree), the pathogen growth was not inhibited and the antagonist was overgrown by the pathogen. *M. guilliermondii* MIR 15_ITS was prepared following the protocol mentioned by Titiya et al., [2007]. Four (4) days post-incubation, the antagonistic ability of *M. guilliermondii* MIR 15_ITS against *B. spicifera* R15 was evaluated on nutrient agar (NA) plate by scoring the inhibition zone according to the formula by Mojica-Marin et al., [2008].

$$Inhibition = \frac{Control\ growth - Fungal\ growth\ (cm)}{Control\ growth} \times 100\% \quad (1)$$

Greenhouse experiment

The antagonistic activity between the multibiocontrol agents and *B. spicifera* R15 under laboratory conditions was confirmed through the greenhouse experiment that was conducted to compare *Trichoderma* spp., *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS as biocontrol agents to inhibit the *B. spicifera* R15 rice pathogen.

Evaluation of the effectiveness of multibiocontrol agents against *B. spicifera* R15

Preparation of fungal organisms

In the study, 5 mm mycelial discs of the *Trichoderm*a isolate T.4679, *C. halotolerans* CIR 18_ITS and *B. spicifera* R15 were individually cultured from a subculture of PDA plates on a medium containing wheat bran with corn cob and water (3: 7: 3 – weight/weight/size), incubated at (28 ± 2°C), and assessed visually for five days, depending on the method mentioned by Hamdia et al. [2018]. The *M. guilliermondii* MIR 15_ITS yeast was cultured inside 250 mL conical flasks, which contain nutrient broth (NB) medium, and incubated overnight at 28°C for 24 hours.

Inoculum concentrations of fungal organisms

The inoculum size of whole fungal organisms was determined using haemocytometers. The inoculum size of the *Trichoderma* isolate, *C. halotolerans* CIR 18_ITS and the *B. spicifera* R15 fungal pathogen used in this experiment for approximately 3 g/kg soil approximately included 29×10^{10}, 17×10^{6} and 12×10^{6} spore/mL, respectively. The inoculum size of *M. guilliermondii* MIR 15_ITS was approximately 3 mL/kg, which included 1×10^{7} cell/mL.

The pots (25 cm diameter) were filled by using autoclaved soil at 121°C/1.5 kg/cm^2 for 1.5 hours (3 kg clay loam soil clay/pot), inoculated separately with fresh cultures from *B. spicifera* R15 *and C. halotolerans* CIR 18_ITS (tested separately) and stored for one week. Subsequently, the same process was repeated with the following treatments: the *Trichoderma* isolate T.4679, *C. halotolerans* CIR 18_ITS, *M. guilliermondii* MIR 15_ITS and control treatment as natural control alone (all tested separately without pathogen as control). In addition to the interaction treatments, the first group was conducted with *B. spicifera* R15, that is, the *Trichoderma* isolate T.4679 + *B. spicifera* R15 co-inoculations, *C. halotolerans* CIR 18_ITS + *B. spicifera* R15 co-inoculations, *M. guilliermondii* MIR 15_ITS + *B. spicifera* R15 co-inoculations and the *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *M. guilliermondii* MIR 15_ITS + *B. spicifera* R15 combined inoculations (tested separately).

The second group was conducted with *C. halotolerans* CIR 18_ITS as follows: the *Trichoderma* isolates T.4679 + *C. halotolerans* CIR 18_ITS co-inoculations, *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS co-inoculations, *M. guilliermondii* MIR 15_ITS + *C. halotolerans* CIR 18_ITS co-inoculations and the *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *M. guilliermondii* MIR 15_ITS + *B. spicifera* R15 combined inoculations (tested separately). The experiment was carried out in triplicate. The pots were mixed thoroughly and watered daily. Local rice seeds AL-Baraka

obtained from Agriculture Research Directorate were used, and six rice seeds were sown into preinfested pots. The inhibitory effect of the *Trichoderma* isolate T.4679,*C. halotolerans* CIR 18_ITS, *M. guilliermondii* MIR 15_ITS on disease infection and severity was scored according to Woltz and Arthur [1973] based on the following formulas:

$$Disease\ infected = \frac{No.\ infected\ plants}{Total\ of\ observed} \times 100\% \quad (2)$$

$$Disease\ severity = \frac{Total\ (number\ of\ plants\ in\ class\ \{(0 \times 0) + \ldots + (number\ of\ plants\ in\ class\ (5 \times 5)\}}{Total\ plants \times 5} \times 100\% \quad (3)$$

Plant growth parameters

Fresh and dry weights of shoot and root were evaluated for each replicate, and the length of each root and shoot was measured. Then, the results were analysed statistically using a randomised complete design according to the method described in Duncan's Multiple Rating Test.

RESULTS AND DISCUSSION

Laboratory experiment

Antagonistic study between multibiocontrol agents against the B. spicifera R15 pathogen

As seen with multibiocontrol agents; the *Trichoderma* isolate T.4679, *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS were varied in their ability to reduce the radial growth of the *B. spicifera* R15 pathogen four days post-inoculation (Figure 1). The *Trichoderma* isolate T.4679 scored (++ degree) according to Alfredo and Aleli [2011], and inhibited the growth of *B. spicifera* R15 through the coverage /over growth of the 9 cm petri dishes (Figure 1a). *C. halotolerans* CIR 18_ITS stopped the growth of the *B. spicifera* R15 pathogen prior to contact by approximately (++ degree). The interaction between *C. halotolerans* CIR 18_ITS and *B. spicifera* R15 is the best example of the influence of metabolites produced by both the organisms on each other as seen in (Figure 1b). Moreover, this experiment exhibited that *M. guilliermondii* MIR 15_ITS prevented the mycelium growth of *B. spicifera* R15, and the inhibition zone was roughly 75.5%, as in comparison with control (Figure 1c).

Greenhouse experiment

Effect of multibiocontrol agent on the plant growth parameters to control B. spicifera R15

The data in Table 1 shows the effect of the *Trichoderm*a isolate T.4679, *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS as the biological control agents added to the autoclaved soil on the plant growth parameters, namely, fresh weight of shoot and root, dry weight of shoot and root and height of the shoot and root of the rice plant at the end of the season.

The result showed that the fresh weight of the shoot increased significantly ($p<0.05$) compared with the natural control in *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS, *M. guilliermondii* MIR 15_ITS alone, *M. guilliermondii* MIR 15_ITS + *Trichoderm*a isolate T.4679 + *C. halotolerans* CIR 18_ITS + *B. spicifera* R15 and *M. guilliermondii* MIR 15_ITS + *Trichoderm*a isolate T.4679 + *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS (Figure 3a and b), which reached 42.67, 34.11, 33.87 and 33.34 g, respectively (Figure 2). However, *B. spicifera* R15 alone obtained the lowest value of approximately 8.55 g (Table 1 and Figure 2e). The treatment *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS (Figure 3b), which yielded higher rate (42.67 g) than *C. halotolerans* CIR 18_ITS alone without preinoculation with *C. halotolerans* CIR 18_ITS, obtained a value of 32.68 g. The fresh root weight showed the highest value in *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS, *M. guilliermondii* MIR 15_ITS alone and *M. guilliermondii* MIR 15_ITS + *Trichoderm*a isolate T.4679 + *C. halotolerans* CIR 18_ITS + *B. spicifera* R15 which at 13.18, 12.45 and 8.42 g, respectively, compared with *B. spicifera* R15 alone, which obtained the lowest value of 1.91 g.

The dry weight of the shoot of the rice plant inoculated with the *Trichoderm*a isolate T.4679, *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS increased in *C. halotolerans* CIR18_ITS + *C. halotolerans* CIR 18_ITS, *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *B. spicifera* R15 and *M. guilliermondii* MIR 15_ITS alone by 13.59, 12.79 and 12.77 g, respectively.

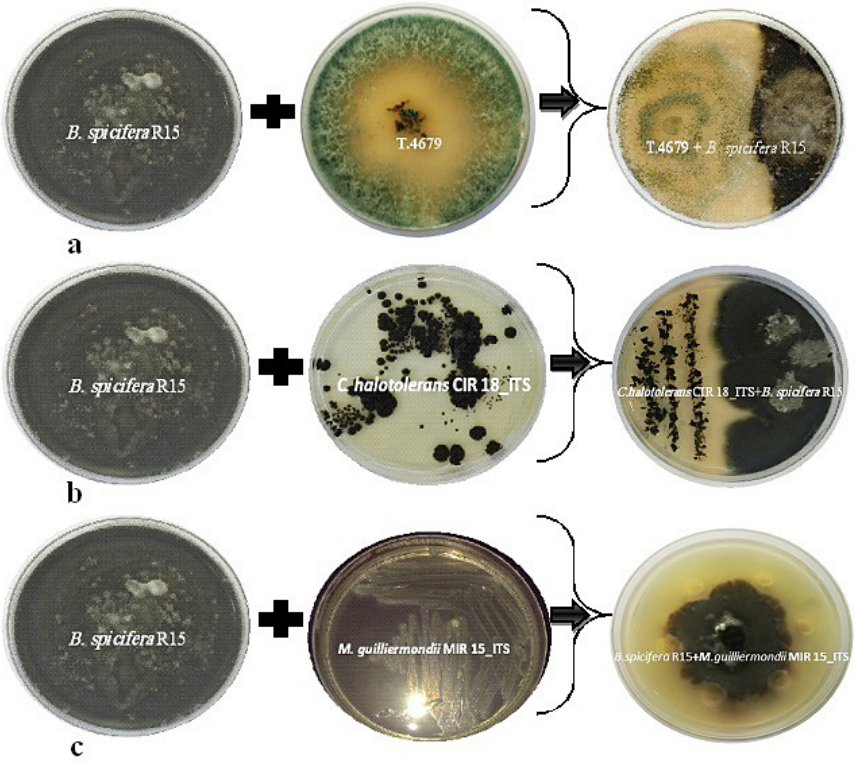

Figure 1. Antagonistic activity between multibiocontrol agents and rice pathogens *B. spicifera* R15: (a) Antagonistic activity between *Trichoderma* isolate T.4679 (left side) and *B. spicifera* R15 (right side), (b) Antagonistic activity between *C. halotolerans* CIR 18_ITS (left side) and *B. spicifera* R15 (right side), (c) Antagonistic activity between *B. spicifera* R15 and *M. guilliermondii* MIR 15_ITS surrounding the *B. spicifera* R15 plug that placed in the middle of 9 cm petri dish.

However, *B. spicifera* R15 alone revealed the lowest value (4.24 g). The data in Table 1 exhibited that *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS, *M. guilliermondii* MIR 15_ITS alone and *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *B. spicifera* R15 significantly ($p<0.05$) increased dry weight of root (4.24, 4.13 and 3.77 g, respectively) compared with the pathogen treatment of *B. spicifera* R15 alone, which had the lowest value of 0.89 g. For the data on shoot height indicated that *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS, *M. guilliermondii* MIR15_ITS + *B. spicifera* R15 and *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *B. spicifera* R15 obtained the best score by approximately (82.33, 76.67 and 76.33 cm, respectively) compared with *B. spicifera* R15 alone, which obtained 56.33 cm. The results of the root height showed that *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS, *M. guilliermondii* MIR15_ITS alone and *C. halotolerans* CIR 18_ITS alone exhibited the highest value (29, 28.67 and 27.67 cm, respectively), whereas *B. spicifera* R15 obtained the lowest value by approximately 16.67 cm, as denoted in Table 1.

The research evaluated fungus efficiency of each *Trichoderma* isolate T.4679 and *C. halotolerans* CIR 18_ITS, besides yeast *M. guilliermondii* MIR 15_ITS via dual culture assay on plates and then in the soil. The greenhouse studies determined the effectiveness of the *Trichoderma* isolate T.4679, *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS as biological control agents against a fungal pathogen of rice plant, *B. spicifera* R15.

The data in Table 1 shows that the interaction and compatibility of the *Trichoderma* isolate T.4679, *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS are very important to plant activity and growth. The compatibility of these fungi as biological control agents led to a regular increase in the growth parameters of the rice plant compared with the *B. spicifera* R15 fungal pathogen after three months. High increases in fresh weight of shoot and root at 42.67 and 13.18 g, respectively, in the treatment with *C. halotolerans* CIR 18_ITS + *C. halotolerans* was also indicated (Figure 3b). *B. spicifera* R15 alone led to low degree in fresh weight of shoot and root

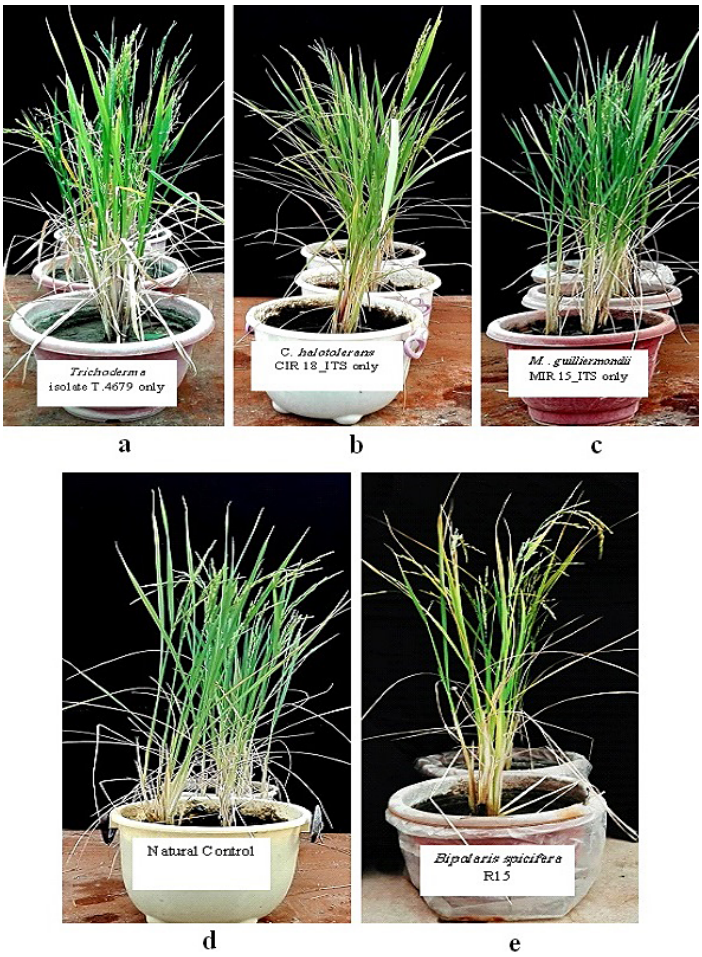

Figure 2. Evaluation of multibioagents effectiveness of *Trichoderma* isolate T.4679, M. guilliermondii MIR 15_ITS and *C. halotolerans* CIR 18_ITS as biocontrol agents to control rice fungal pathogen B. spicifera R15 under greenhouse conditions by using autoclave soil: (a) *Trichoderma* isolate T.4679 alone, (b) *C. halotolerans* CIR 18_ITS, (c) Plants inoculated with *M. guilliermondii* MIR 15_ITS, (d) Control untreated (natural control), (e) Plants inoculated with pathogen *B. spicifera* R15 alone. All labels started from left side to the right.

treatment at approximately 8.55 and 1.91 g, respectively. These results were in agreement with those of several researchers [Segers et al., 2016; Ercan, 2019; Chaibub et al., 2019 and 2020a; Al-Rahbi et al., 2021], who pointed out that the *Trichoderm*a spp. isolates, *M. guilliermondii* and *C. halotolerans* as biocontrol agents had excellent potential to suppress pathogens. This finding indicated high compatibility of these fungi, thereby suggesting high growth of parameters.

The results indicated that the multibiocontrol agent activities significantly influenced plant growth. For example, dry weight of shoot and root showed a significant increase in *treatment C. halotolerans* CIR18_ITS + *C. halotolerans* CIR 18_ITS with higher values of 13.59 and 4.24 g, respectively, than the *B. spicifera* R15 pathogen alone, which recorded the lowest values (4.24, 0.89 and 0.78 g, respectively) as indicated in (Table 1 and 2).

Effect of multibiocontrol agent on the disease infection and severity under greenhouse conditions

Table 2 shows the results of disease infection and severity parameters of a local rice variety (cv.) Al-Baraka against the *B. spicifera* R15 causal agent. The results showed significant difference ($p<0.05$) levels of stimulation and increasing rice plant growth when treated with the *Trichoderm*a isolate T.4679, *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS. The greenhouse experiment showed that *C. halotolerans* CIR 18_ITS alone, *M. guilliermondii* MIR 15_ITS alone (Figure 2b and c), *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS and *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS (Figure 3b) were effective in inducing

Table 1. The effect of *Trichoderma* isolate T.4679, *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS as biological control agents on plant growth parameters under greenhouse conditions

Treatments	Plant Growth Parameters					
	*Fresh weight of shoot [g]	*Fresh weight of root [g]	*Dry weight of shoot [g]	*Dry weight of root [g]	*Plant shoot length [cm]	*Plant root length [cm]
Natural control	14.35 g	3.51f	8.27 de	2.62 bcd	65 c	22.33 f
B. spicifera R15 alone	8.55 i	1.91 g	4.24 f	0.89 e	56.33 d	16.67 g
C. halotoler. CIR 18_ITS alone	32.68 c	6.54 c	10.20 b	3.05 bc	72.33 bc	27.67 ab
Trichoderma isolate T.4679 alone	24.99 e	5.29 d	9.96 bc	2.58 bcd	70 bc	25.67 cd
M. guillier. MIR 15_ITS alone	34.11 b	12.45 a	12.77 a	4.13 a	73 bc	28.67 a
Trichoderma isolate T.4679 + *B. spicifera* R15	13.28 h	4.03 ef	7.98 e	2.62 bcd	67.67 c	23 ef
*C.halotoler.*CIR18_ITS+ *B.spicifera* R15	14.68 g	4.14 ef	9.83 b	2.54 cd	67.33 c	23 ef
*M.guillier.*MIR15_ITS+ *B.spicifera* R15	13.94 gh	4.60 de	8.36 cde	2.45 d	76.67 ab	23.33 ef
*M.guillier.*MIR15_ITS+ *Trichoderma* isolate T.4679 + *C.halotoler.* CIR18_ITS + *B. spicifera* R15	33.87 b	8.42 b	12.79 a	3.77 a	76.33 ab	26.67 bc
Trichoderma isolate T.4679 + *C.halotoler.* CIR18_ITS	31.67 d	4.64 de	10.02 b	2.50 cd	70.67 bc	23.33 ef
C.halotoler. CIR18_ITS+ *C.halotoler.* CIR 18_ITS	42.67 a	13.18 a	13.59 a	4.24 a	82.33 a	29 a
M. guillier. MIR 15_ITS+ *C. halotoler.* CIR 18_ITS	22.34 f	6.89 c	10.07 bcd	3.11 b	69.33 bc	24.33 de
*M.guillier.*MIR15_ITS+ *Trichoderma* isolate T.4679 + *C.halotoler.*CIR 18_ITS + *C.halotoler.*CIR18_ITS	33.34 b	5.45 d	10.19 b	3.09 b	69 bc	25.33 cd

Note: * Mean of three replicate of each treatment. Numbers in each column that have same letter do not differ significantly from each other at $p<0.05$ according to Duncan's multiple range test.

Table 2. The effect of *Trichoderma* isolate T.4679, *M. guilliermondii* MIR 15_ITS and *C. halotolerans* CIR 18_ITS as biological control agents on disease infection and disease severity under greenhouse conditions

Treatments	*Disease infection [percentage, %]	**Disease severity [percentage, %]
Natural control	44.44 bc	20 c
B. spicifera R15 alone	100 a	71.11a
C. halotoler. CIR 18_ITS alone	11.11 c	6.67 d
Trichoderma isolate T.4679 alone	22.22 bc	13.33 cd
M. guillier. MIR 15_ITS alone	11.11 c	6.67 d
Trichoderma isolate T.4679+ *B. spicifera* R15	55.56 b	33.33 b
*C.halotoler.*CIR18_ITS+ *B.spicifera* R15	44.44 bc	30 b
*M.guillier.*MIR15_ITS+ *B.spicifera* R15	44.44 bc	20 c
*M.guillier.*MIR15_ITS+*Trichoderma* isolate T.4679 + *C.halotoler.* CIR18_ITS + *B. spicifera* R15	33.33 bc	20 c
Trichoderma isolate T.4679+ *C.halotoler.* CIR18_ITS	22.22 bc	13.33 cd
C.halotoler. CIR18_ITS+ *C.halotoler.*CIR 18_ITS	11.11 c	6.67 d
M. guillier. MIR 15_ITS+*C. halotoler.* CIR 18_ITS	22.22 bc	13.33 cd
*M.guillier.*MIR15_ITS+ *Trichoderma* isolate T.4679+ *C.halotoler.*CIR 18_ITS+ *C.halotoler.*CIR18_ITS	11.11 c	6.67 d

Note: Numbers in each column that have same letter do not differ significantly from each other at $p<0.05$ according to Duncan's multiple range tests.*Disease infection and **Severity of rice plants, the experiments were conducted in triplicate for each pathogen. *Disease infection and **Severity were scored after 4 months from sowing according to **[**Woltz and Arthur, 1973**]**.

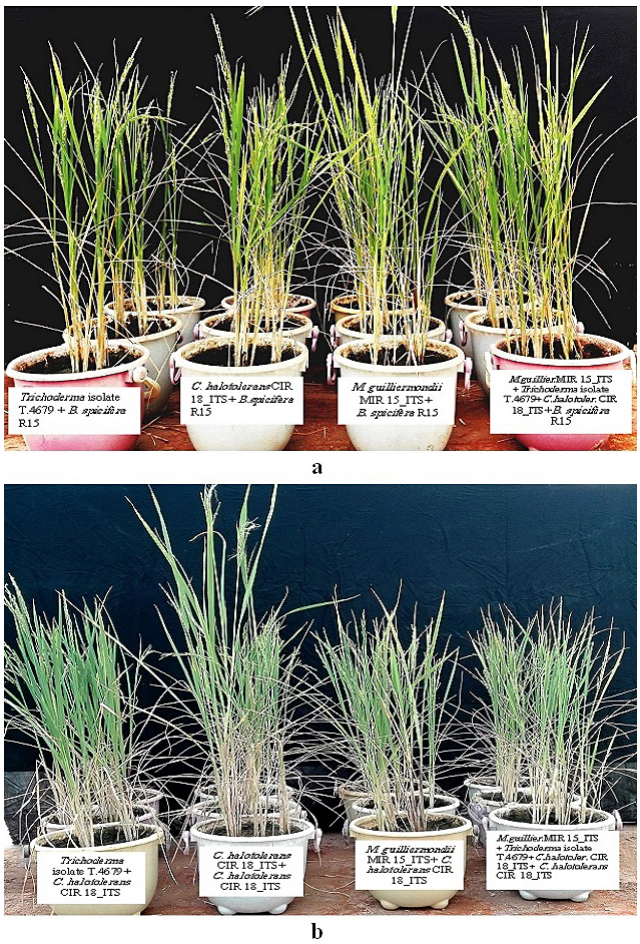

Figure 3. Comparison between soils inoculated with *B. spicifera* R15 (as pathogen) and *C. halotolerans* CIR 18_ITS (as biocontrol agent) under greenhouse conditions by using autoclave soil. (a) Pots inoculated with; B. spicifera R15 + *Trichoderma* isolate T.4679, B. spicifera R15 + *C. halotolerans* CIR 18_ITS, *B. spicifera* R15 + *M. guilliermondii* MIR 15_ITS, *B. spicifera* R15 + *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS. (b) Pots inoculated with; *C. halotolerans* CIR 18_ITS+ *Trichoderma* isolate T.4679, *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS, *C. halotolerans* CIR 18_ITS + *M. guilliermondii* MIR 15_ITS, *C. halotolerans* CIR 18_IT + *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS. All labels started from left side to the right.

significant decrease ($p<0.05$) in disease infection and obtained the lowest value of 11.11% on all treatments (Table 2). However, the *B. spicifera* R15 causal agent had the highest level in disease infection at approximately 100% (Table 2). Visual ratings of disease severity parameter in the greenhouse experiment decreased significantly in *C. halotolerans* CIR 18_ITS alone, *M. guilliermondii* MIR 15_ITS alone, *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS and *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS; they exhibited greater efficiency of reducing disease severity 6.67% than the pathogen with significant increase ($p<0.05$) of 71.11%.

The greenhouse experiment results of disease infection and severity parameters reduced significantly, and the best multibiocontrol agent potential was displayed by *C. halotolerans* CIR 18_ITS alone, *M. guilliermondii* MIR 15_ITS alone, *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS and *M. guilliermondii* MIR 15_ITS + *Trichoderma* isolate T.4679 + *C. halotolerans* CIR 18_ITS + *C. halotolerans* CIR 18_ITS. They were effective and provided clear evidence of significant reduction ($p<0.05$) in disease infection and severity with the lowest values 11.11% and 6.67%, respectively, on all treatments. However, the disease infection and severity obtained high values when the *B. spicifera* R15 pathogen was applied, showing significant increase ($p<0.05$) of approximately 100% and 71.11%, respectively (Table 2), at the end of season.

Ultimately, *C. halotolerans* CIR 18_ITS was the most compatible and exhibited the greatest

efficiency in reducing disease severity when combined with the biocontrol agents or applied individually, as shown in Tables 1 and 2. Moreover, these results were more evident and in accordance with the previous findings that certain *Cladosporium* species can improve plant growth and act as biocontrol agents [Köhl et al., 2015; Chaibub et al., 2016; Chaibub et al., 2019; Ercan, 2019; Gámez-Guzmán et al., 2019]. In summary, Chaibub et al., [2020b] concluded that the *Cladosporium* genus, such as *C. cladosporioides*, *C. tenuissimum, C. subuliforme* and *C. miyabeanus*, can be utilised successfully as biocontrol agents and protect rice plant effectively when infected by fungal pathogens, including *Cochliobolus miyabeanus*, *Magnaporthe oryzae*, *Monographella albescens* and *Sarocladium oryzae*. Moreover, the researcher found that these species have the potential of producing several enzymes, such as β-1,3-glucanase, chitinase and protease. The *C. halotolerans* CIR 18_ITS results show that the increase in the plant growth parameters through excellent compatibility with the *Trichoderma* isolate T.4679 and *M. guilliermondii* MIR 15_ITS provided a strong evidence that *C. halotolerans* CIR 18_ITS is not a pathogenic fungus to the rice plant, especially in the entire period of plant growth.

CONCLUSIONS

This study determined the effectiveness of *C. halotolerans* CIR 18_ITS as a biocontrol agent compared with the *Trichoderma* isolate T.4679 and *M. guilliermondii* MIR 15_ITS to control the pathogenic *B. spicifera* R15 fungus under greenhouse conditions.

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