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Research article

Efficacy of the antagonistic fungus *Talaromyces tratensis* KUFA 0091 in controlling rice blast and brown leaf spot diseases in field trials

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Abstract

Importance of the work: Rice blast and brown leaf spot are the most devastating foliar diseases in rice production in Thailand and many countries.
Objectives: To evaluate the efficacy of *Talaromyces tratensis* KUFA 0091 to control rice diseases.
Materials & Methods: The efficacy was evaluated of a spore suspension (1×10^6 spores/mL) and a crude extract of KUFA 0091 at 1 g/L, 5 g/L and 10 g/L at the time of application, with one or two applications to control rice blast and brown leaf spot diseases in two susceptible rice varieties.
Results: There was no significant difference between the means of two and one applications in controlling both diseases. However, spore suspension (1×10^6 spores/mL) and the crude extract (5 g/L of *T. tratensis* KUFA 0091) were the most effective in controlling brown leaf spot, with disease reduction in the field of 52.47% and 45.04%, respectively, when applied twice. The spore suspension and the crude extract (at 1×10 g/L of *T. tratensis* KUFA 0091) were moderately effective against rice blast, reducing its incidence by 35.83% and 35.11%, respectively, when applied twice under field conditions.
Main finding: There was antagonistic activity of *T. tratensis* KUFA 0091 via antibiosis against rice pathogens, as it produced substances absorbed by the rice plant, which then moved to newly emerging leaves, protecting them from pathogen infection.

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Introduction

Rice blast, caused by *Pyricularia oryzae*, is the most devastating rice disease in many countries, causing significant yield losses (Nalley et al., 2016; Asibi et al., 2019). The spores of this fungus can infect all parts of the plant and all stages of its growth (Chen et al., 2019). Brown leaf spot disease, caused by *Bipolaris oryzae*, is also considered a major constraint on rice yield (Dethoup et al., 2018). Both diseases are important foliar diseases affecting rice production in many countries (Nalley et al., 2016; Kaewsalong et al., 2019). The spores of these diseases are widely dispersed by wind and rain and can infect host plants within 24 hr under suitable conditions (Martin-Urdiroz et al., 2016). As seed-borne pathogens, they cause poor germination and seedling rot disease, the latter serving as the source of a new inoculum that infects new crops. These pathogens can survive in the soil and debris for many years (Martin-Urdiroz et al., 2016; Kaewsalong et al., 2019). The virulence and severity of these diseases are increased by careless practices in rice culture, including excessive application of nitrogen fertilizer and extensive planting of susceptible rice varieties (Nalley et al., 2016; Balasubramanian et al., 2007).

Although fungicide applications effectively control these rice diseases, continued and repeated applications raise concerns not only about their effects on public health and the environment, but also about the development of pathogen resistance (Thind, 2012; Hu et al., 2014). Consequently, eco-friendly approaches for controlling plant diseases, including biological control agents and plant extracts, are being sought to replace synthesized fungicides (Dethoup et al., 2018; Kokkrua et al., 2020).

Biological control agents have been extensively studied because rice blast is the most severe of the rice diseases. Bacterial antagonists of *Bacillus* and *Streptomyces* species have been found to be potent biological control agents against *Magnaporthe oryzae* (Law et al., 2017; Rais et al., 2018). Recently, Xu et al. (2019) reported that rice endophyte *S. hygroscopicus* OsiSh-2 displayed remarkable antagonistic activity with various antagonistic mechanisms toward the rice blast fungus *M. oryzae*. Li et al. (2018) reported that *B. tequilensis* GYLH001 had potential as a biological control agent against *M. oryzae*. Awla et al. (2017) reported that treatment with *Streptomyces* strain UPMRS4 could reduce disease severity by 67.9% more than other control treatments and was able to increase rice growth.

Trichoderma species have been reported to be potent antagonistic fungi against brown leaf spot caused by *B. oryzae*. For example, *T. harzianum* (Th-01) and *T. asperellum* (Ta-10) effectively inhibited the mycelial growth of *B. oryzae* by 86.6–97.7% (Prabhakaran et al., 2015), whereas *T. viride* (Tv2) effected a significant reduction of 45% in the incidence of brown leaf spot (Harish et al., 2008). Abdel-Fattah et al. (2007) reported that *T. harzianum* could overgrow *B. oryzae* colonies and that spraying of *T. harzianum* spore suspension significantly reduced disease severity and disease incidence on plant leaves, while significantly increasing the grain yield under field conditions.

Talaromyces tratensis KUFA 0091 has proved to be a potential antagonist against dirty panicle disease in rice (Dethoup et al., 2018). Dirty panicle disease is caused by various fungi, namely, *Alternaria padwickii*, *Curvularia lunata*, *Fusarium moniliforme*, and *Bipolaris oryzae* (Dethoup et al., 2018). Recently, Suasa-ard et al. (2019) reported the potential of *T. tratensis* KUFA 0091 for controlling stem end rot on mango fruit, caused by *Lasiodiplodia theobromae*. This fungus has been classified based on its metabolites, namely: wortmin, meso-1,4-bis(4-methoxybenzyl)-2,3-butanediol and tratenopyrone (Buttachon et al., 2016). *Talaromyces* species have been recorded as effective antagonists against many plant diseases, namely: scape and umbel blights in onions, caused by *Botrytis cinerea* (Abdel-Rahim and Abo-Elyousr, 2018); vascular wilt diseases, caused by *Verticillium* and *Fusarium* in potatoes and tomatoes (Naraghi et al., 2012; Bahramian et al., 2016); damping-off disease, caused by *Rhizoctonia solani* in sugar beet (Kakvan et al., 2013); rice bakanae disease, caused by *Gibberella fujikuroi* (Kato et al., 2012); and *Pythium* damping-off in cucumbers and tomatoes (Halo et al., 2019). *Talaromyces* species act against plant pathogens via many antagonistic mechanisms, such as mycoparasitism, antibiosis, and inducing resistance and competition for space and nutrients (Suasa-ard et al., 2019).

Ongoing research by the current authors has investigated eco-friendly approaches to rice disease control (Dethoup et al., 2018, 2019; Kaewsalong et al., 2019) and found that *T. tratensis* KUFA 0091 showed potent antagonistic activity by reducing the mycelial growth of *P. oryzae* and *B. oryzae* by 40–46%, forming an inhibition zone 10–15 mm in width in dual culture and suppressing the incidence of brown spot disease by 56.74% under greenhouse conditions (Dethoup et al., 2018). The objective in the current study was to determine the antagonistic activities of the fungus *T. tratensis* KUFA 0091 and its crude extract, in controlling brown leaf spot and rice blast diseases under field trials.

Materials and Methods

Fungal material

The fungal antagonist strain KUFA 0091, *Talaromyces tratensis*, used in study was found on a marine sponge, *Mycale* sp., collected in the Gulf of Thailand from a coral reef of Samaesan Island, Chonburi province. Its identification was determined using morphology and molecular methods, as previously described by Buttachon et al. (2016). Its gene sequence was deposited in GenBank with the accession number, KT728350.

Preparation of spore suspension and metabolite extract of T. tratensis KUFA 0091

The preparation of a spore suspension and a crude extract of the fungus was carried out, as previously described by Dethoup et al. (2018). Briefly, mycelium plugs of *T. tratensis* KUFA 0091 were inoculated in 200 mL of potato dextrose broth and placed on a rotary shaker at 120 rpm for 1 wk at room temperature to obtain a mycelial suspension. Then, 10 Erlenmeyer flasks (1 L), each containing 300 g cooked rice, were sterilized using standard autoclaving at 121 °C for 15 min. After this, 20 mL of the prepared fungal mycelial suspension were added to each flask and incubated at room temperature for 30 d. Then, 500 mL of ethyl acetate was poured into each flask containing the rice mold, macerated for 7 d and filtered to obtain the organic solutions. These solutions were concentrated using a rotary evaporator to give the crude ethyl acetate extract of *T. tratensis* KUFA 0091.

To obtain the spore suspension, 200 mL of sterile water were poured into a culture flask that had been prepared as above and incubated for 30 days, with a sterile glass rod used to gently scrape spores from the rice mold. Mycelial fragments were removed from the spore suspension by filtering through sterile cheesecloth. The spore suspension was diluted and adjusted with sterile water using a hemocytometer to obtain a spore suspension of 1×10^6 spores/mL.

Effects of T. tratensis KUFA 0091 against brown leaf spot disease under field conditions

Field trials were performed using natural infection in Bang Bua Thong district, Nonthaburi province. The plot size was 4 m × 3 m with 30 cm spacing within the row and between rows and arranged as a completely randomized design under a lowland cropping system. Each row consisted of 13 hills with five seedlings aged 7 d of rice var. RD57 per hill and each plot contained 13 rows per plot with a 1 m distance between plots. A standard conventional cultivation practice was applied, in accordance with the recommendation of the Thai Rice Department: fertilizer (15-15-15 of N-P-K) and insecticide (dinotefuran 10% wettable powder (WP) at 0.75 g/L) were applied the first time 1 mth after planting and then applied every 30 d. The watering was kept 10 15 cm above the soil in the experimental field trials. When disease symptoms first appeared and were observed at the tillering stage, the following were applied to the rice plants for the first time: 1) a spore suspension at 1×10^6 spores/mL; 2) a crude extract concentrations of 1 g/L, 5 g/L or 10 g/L; 3) 0.01% dimethyl sulfoxide (DMSO) + 0.02% Tween-20 (negative control); and 4) carbendazim 50% WP at 1.5 g/L (positive control). In experiments with two applications, the rice leaves received a second application 7 d after the first application. Rice plants were treated with 3 L of each treatment per plot with separate portable sprayers; each treatment consisted of five plots (replicates).

At 7 d after each application, the second or third leaf was randomly collected from the top part of each rice plant (giving a total of 100 rice leaves from 100 plants per plot) for evaluation of disease incidence. The number of brown leaf spots on each leaf was counted and the average in each treatment was calculated. The percentage of disease reduction was calculated using the formula: % disease reduction = (A - B/ A) × 100 where A is the average number of brown leaf spots in the control and Bis the average number of brown leaf spots in the *Talaromyces*/fungicide treatment. The experiments were conducted during June–July 2018 and repeated during February–March 2019.

Effects of T. tratensis KUFA 0091 in controlling rice blast disease under field conditions

Field experiments were performed at Khok Klang sub-district, Lue Amnat district, Amnat Charoen province in north-eastern Thailand under a lowland cropping system using natural infection. The plot size was the same as that described above, with a total of 13 rows per plot and the distance between plots was 1 m, arranged as a completely randomized design. Each row contained 13 hills with five seedlings aged 7 d of rice cv. KDML105 per hill. The same conventional cultivation practice was applied as described above. At the tillering stage

(40 d after planting), by which time rice blast symptoms had appeared in about 5–10% of the plants due to natural infection, the rice plants were sprayed for the first time. The following treatments were applied: 1) a spore suspension at 1×10^6 spores/mL; 2) a crude extract at 1 g/L, 5 g/L or 10 g/L; 3) 0.01% DMSO + 0.02% Tween-20 (negative control); and 4) carbendazim 50% WP at 1.5 g/L (positive control). In experiments with two applications, rice leaves were sprayed again 7 d after the first application. Rice plants were sprayed with 3 L (158 L/Acre) of each sample per plot with separate portable sprayers; each treatment consisted of five plots (replicates).

To determine disease incidence, a total of 100 second leaves of treated rice plants from each plot were hand harvested 7 d after each application. The disease incidence was measured in accordance with the scoring scale (International Rice Research Institute, 2013). The disease incidence was determined using Equation 1:

$$[\Sigma(r \times nr)/9 \times Nr] \times 100 \quad (1)$$

where r is the rating value (0–9), nr is the number of infected leaves with a rating of r and Nr is the total number of leaves tested in each replication (Madden et al., 2007).

The percentage of disease reduction was calculated using the formula: % disease reduction = (A - B/A) × 100, where A is the average disease incidence in the control and B is the average disease incidence in the *Talaromyces*/fungicide treatment. The experiments were conducted during July–August 2017 and repeated during July–August 2018.

Statistical analysis

All experiments were carried out twice. Data were analyzed using one-way analysis of variance and the means were compared for significance using Duncan's multiple range test ($p < 0.05$), using the statistical program SPSS v.19 (IBM Corporation, Somers, NY, USA).

Results

Effects of T. tratensis KUFA 0091 against brown leaf spot disease under field conditions

The effects of the spore suspension and the crude extract of *T. tratensis* KUFA 0091 in controlling brown leaf spot disease are shown in Figs. 1–2. Two applications of the spore suspension at 1×10^6 spores/mL demonstrated remarkable antagonistic activity, causing a 52.47% reduction in the disease. Two applications of the crude extract at 5 g/L and 1 g/L showed potent antifungal activity against the disease, reducing the disease incidence by 45.04% and 25.37%, respectively. However, there was no significant difference between the means of two and one applications in reducing the disease. The *T. tratensis* KUFA 0091 crude extract at 10 g/L, the highest dose, was phytotoxic to rice plants, resulting in yellowing of the leaves and leaf blight at leaf margins. The fungicide, carbendazim, displayed the greatest fungicidal activity, reducing the disease incidence by 48.27% and 60.11% when applied once and twice, respectively (Fig. 3).

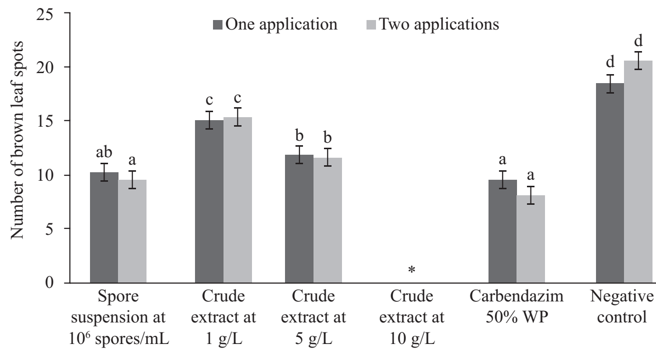

Fig. 1 Effects of spore suspension and crude extract at different concentrations of *Talaromyces tratensis* KUFA 0091 against brown leaf spot disease, with one and two applications under field conditions with control treatments, where * = phytotoxic; Histograms represent mean ± SD, above which different lowercase superscripts denote significantly different ($p < 0.05$).

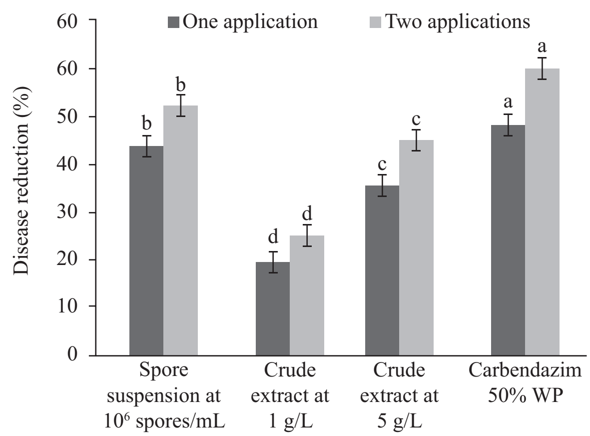

Fig. 2 Disease reduction of brown leaf spot disease under field conditions after applying spore suspension and crude extract of *Talaromyces tratensis* KUFA 0091, with one and two applications compared to control treatments; Histograms represent mean ± SD, above which different lowercase superscripts denote significantly different ($p < 0.05$).

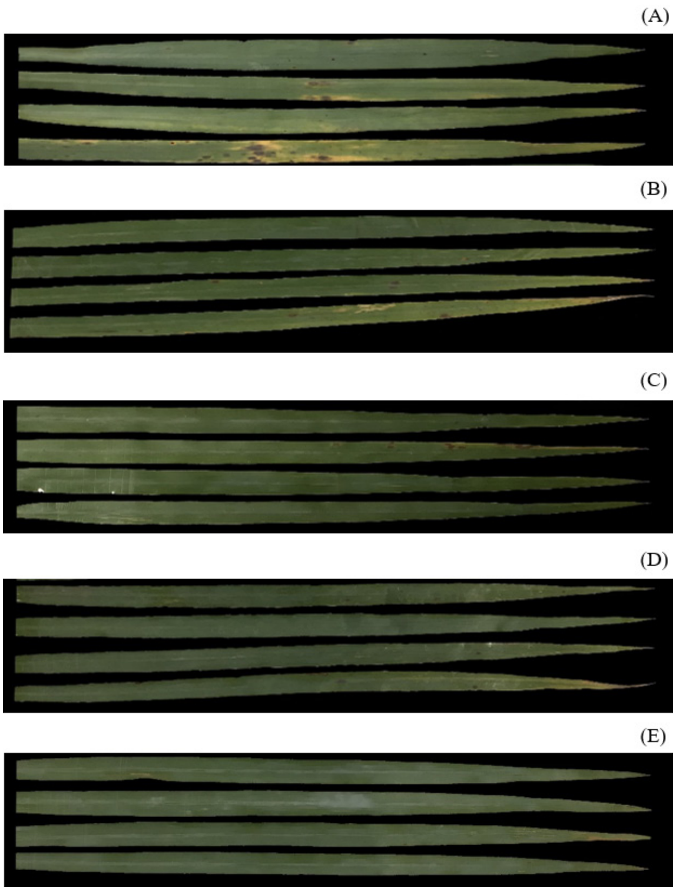

Fig. 3 Effects of spore suspension and crude extract of *Talaromyces tratensis* KUFA 0091 against rice brown leaf spot disease when applied twice in field trials: (A) water; (B) spore suspension at 1×10^6 spores/mL; (C) crude extract at 1 g/L; (D) crude extract at 5 g/L; (E) carbendazim 50% water pressure

One application to the tested samples showed lower activity in suppressing disease incidence than did two applications, but there was no significant difference with two applications. One application of the spore suspension at 1×10^6 spores/mL displayed antagonistic activity against the disease, causing a 44.05% reduction in disease. In addition, one application of the crude extract at 5 g/L or 1 g/L demonstrated fungicidal activity, reducing the disease by 35.51% and 19.67%, respectively.

Effects of T. tratensis KUFA 0091 against rice blast disease under field conditions

The results showed that two applications of either the spore suspension at 1×10^6 spores/mL or the crude extract of *T. tratensis* KUFA 0091 showed higher antifungal activity against rice blast disease than did one application, but there was no significant difference with two applications. Two applications of the crude extract at 10 g/L and the spore suspension at 1×10^6 spores/mL exhibited potent antifungal activity against the severity of rice blast disease causing a reduction in disease of 35.83% and 35.11%, respectively. Furthermore, two applications of the crude extract at 5 g/L and 1 g/L reduced the severity of rice blast disease by 27.7% and 19.08%, respectively (Figs. 4–5). However, the fungicide

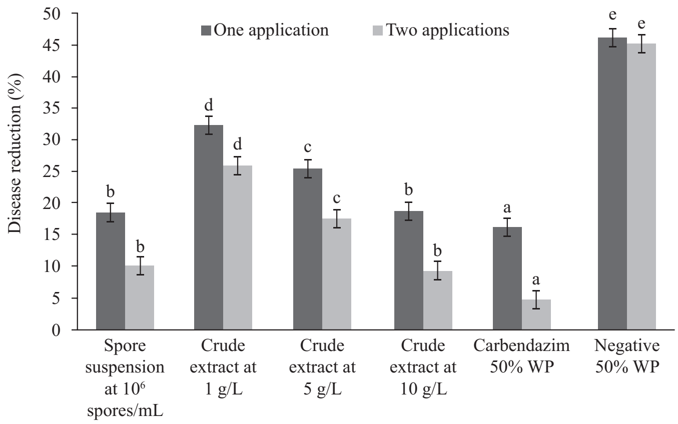

Fig. 4 Effects of spore suspension and crude extract at different concentrations of *Talaromyces tratensis* KUFA 0091 against rice blast disease, with one and two applications under field conditions with control treatments; Histograms represent mean ± SD, above which different lowercase superscripts denote significantly different ($p < 0.05$).

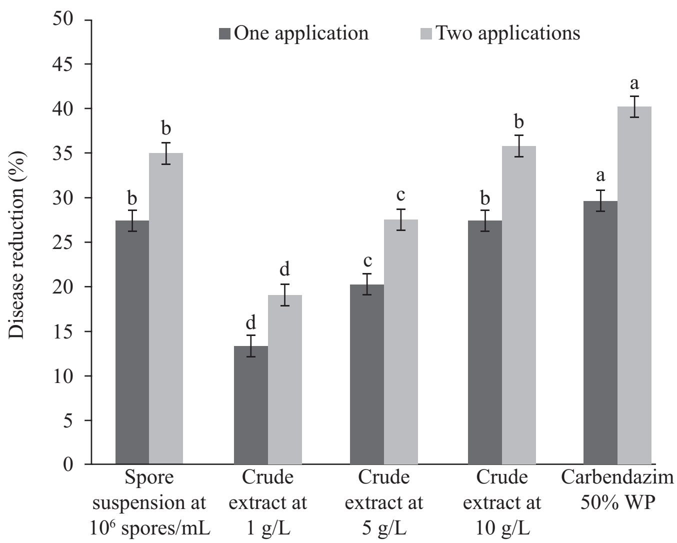

Fig. 5 Disease reduction of rice blast disease under field conditions after applying spore suspension and crude extract of *Talaromyces tratensis* KUFA 0091, with one and two applications compared with control treatments; Histograms represent mean ± SD, above which different lowercase superscripts denote significantly different ($p < 0.05$).

carbendazim demonstrated the best fungicidal activity in controlling the severity of rice blast disease when applied either once or twice, reducing disease severity by 29.73% and 40.32%, respectively (Fig. 6). One application of the crude extract at 10 g/L and the spore suspension suppressed the severity of rice blast disease by 27.19% and 27.41%, respectively, whereas the application of the crude extract at 5 g/L and 1 g/L reduced the severity by 20.44% and 13.52%, respectively.

Fig. 6 Effects of spore suspension and crude extract of *Talaromyces tratensis* KUFA 0091 against rice blast disease when applied twice in field trials: (A) water; (B) spore suspension at 1×10^6 spores/mL; (C) crude extract at 1 g/L; (D) crude extract at 5 g/L; (E) crude extract at 10 g/L; (F) carbendazim 50% water pressure

Discussion

The study results showed the efficacy of the antagonist fungus *T. tratensis* KUFA 0091 in controlling the important rice foliar diseases, brown leaf spot and rice blast, under field conditions with natural infection in endemic areas. The spore suspension and the crude extract of *T. tratensis* KUFA 0091 showed better antagonistic activity against brown leaf spot disease caused by *B. oryzae* than against rice blast caused by *P. oryzae*. The spore suspension and the crude extract at 5 g/L of *T. tratensis* KUFA 0091 reduced the disease severity of brown leaf spot up to 52.47% when applied twice. The results showed that the application of the spore suspension of *T. tratensis* KUFA 0091 had a greater effect than the application of *Callistemon citrinus* and *Cymbopogon citratus* extracts, which had been reported to reduce the disease severity of brown leaf spot in rice by 36–42% by Nguefack et al. (2013). Furthermore, it showed greater effectiveness than the application of silicon, which reduced the number of lesions per square centimeter of leaf area by 47% (Rezende et al., 2009). However, the effects of *T. tratensis* KUFA 0091 against brown leaf spot were less than those of a neem cake extract and an *N. oleander* leaf extract, reported by Harish et al. (2008), which reduced the incidence of brown leaf spot in rice by 70% and 53%, respectively. The fungal spore suspension application showed better biocontrol activity than the crude extract application in the current study perhaps because the fungal spores could germinate and grow on leaf surfaces which continually displayed antagonistic activity against pathogen infection; however, its crude extract may have been reduced in concentration due to rain or environmental factors.

Although two applications of spore suspension and the crude extract of *Talaromyces* seemed to control both rice diseases better than one application, there was no significant difference between the means of the two and one applications in controlling both diseases. This effect may have resulted from environmental effects because previous studies found that when applied to lead surfaces under field conditions, biocontrol agents may suffer a loss of population or of activity due to the effects of rain and sunshine (Galletti et al., 2020, Tyśkiewicz et al., 2022).

Limited reports are available on the control of brown leaf spot in rice under field conditions using biological control agents. This contrasts with the number of studies on their control of rice blast disease, with many studies reporting the control of rice blast with various kinds of agents and even with fungicides. In the current study, the application of the crude extract at 10 g/L and the spore suspension exhibited the greatest antifungal activity, suppressing rice blast severity by 35%. However, antagonistic bacteria were found to be even more effective agents against rice blast. For example, Rais et al. (2018) reported that *Bacillus* spp. KFP-5, KFP-7 and KFP-17 significantly reduced rice blast disease severity by 40–52%. Xu et al. (2017) reported that the spraying of *S. endus* OsiSh-2 spore solution (1×10^7 spores/mL) could reduce the severity of rice blast disease by 59.64% under field conditions. Law et al. (2017) reported the results from greenhouse trials in treating infected rice seedlings with *Streptomyces* producing up to 88.3% reduction in rice blast disease. Tokpah et al. (2016) reported that *B. cereus* strain (H-S in 24) and *B. subtilis* strain

(D-L in 76) showed remarkable biocontrol efficacy, reducing rice blast disease by 77.5% and 75%, respectively in field trials.

A few reports are available on the control of rice blast under field conditions by antagonistic fungi. Wang et al. (2013) found that the rhizosphere fungal strain *Chaetomium aureum* possessed potent antagonistic activity, reducing the disease index of rice blast by 66.07% in a field study. Jambhulkar et al. (2018) reported that the combined application of *T. harzianum* Th3 and *P. fluorescens* RRb11 displayed a synergistic reduction of rice blast severity by 69.5%. Kaewsalong et al. (2019) reported that the applications of combinations of *T. harzianum* KUFA 1051 and *T. asperellum* KUFA 1067 with *C. fenestratum* extract at 1% showed the highest level of disease reduction, achieving a significant reduction of 37.5% in rice dirty panicle disease caused by *B. oryzae*.

Dethoup et al. (2018) reported that *T. tratensis* KUFA 0091 significantly inhibited the radial growth of *B. oryzae* and *P. oryzae* in dual cultures by 46% and formed an inhibition zone of 13–15 mm in width. The crude extract of this antagonistic fungus completely inhibited the radial growth of all tested rice pathogens at a 10 g/L concentration. The crude extract of the fungus displayed a potent reduction in the incidence of dirty panicle disease of up to 48.98% under field conditions. Suasa-ard et al. (2019) found that *T. tratensis* KUFA 0091 had significant (64–67%) mycelial growth inhibition of *L. theobromae*, compared to growth on the control plates, forming clear inhibition zones against *L. theobromae* that were 15–20 mm in width. The results from the current and other studies indicated that *T. tratensis* KUFA 0091 has potent antagonistic activity against plant pathogenic fungi via remarkable antibiotic production.

Overall, the results showed the efficacy of the spore suspension and the crude extract of *T. tratensis* KUFA 0091 in controlling important foliar diseases in rice (brown leaf spot and rice blast) under field conditions. Their antagonistic activity via antibiosis may have been due to the production of substances absorbed by rice leaves that then moved to newly emerging leaves, and prevented fungal infections, resulting in the decreased incidence of both brown leaf spot and rice blast diseases. Although the effects of the crude extract of *T. tratensis* KUFA 0091 had substantial effects against brown leaf spot and rice blast diseases in a dose-dependent manner, it was noted to be toxic to rice var. KD57 when applied at the high concentration of 10 g/L. Hence, this phenomenon should be further evaluated for its toxicity.

The results obtained showed the efficacy of the spore suspension and the crude extract of *T. tratensis* KUFA 0091 in controlling the rice foliar diseases of brown leaf spot and rice blast under field conditions in the two susceptible rice varieties, KD57 and KDML105. *Talaromyces tratensis* KUFA 0091 displayed effective fungicidal activity against brown leaf spot, whereas it had a moderate effect against rice blast. The antagonist fungus *T. tratensis* KUFA 0091 could potentially be applied as an alternative to fungicides to control rice foliar diseases in the field.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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