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# Effects of fungicides and antagonistic marine-derived fungi on rice seedling promotion and rice sheath blight control

*Tida Dethoup*[1,2]*, *Rotchana Klaram*[1], *Arom Jantasorn*[3]

[1]*Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand*
[2]*Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima, Japan*
[3]*Bodhivijjalaya College, Srinakharinwirot University, Nakhon-Nayok, Thailand*

**Corresponding author: agrtdd@ku.ac.th*

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**Abstract:** Fourteen fungicides and three marine-derived fungi were tested for their activities in the promotion of rice seedling development and their efficacy in controlling rice sheath blight disease in two rice varieties, RD43 and KMDL105, under greenhouse conditions. The results showed that the rice seeds treated with carbendazim 50%SC and azoxystrobin 25%SC displayed the best promotion of rice growth in root length and shoot height at seven and 14 days in rice var. RD43. Rice seeds from var. KMDL105 treated with thiophanate-methyl 70%WP exhibited significant stimulation of rice seedling growth resulting in the greatest increase in root length and shoot height at seven and 14 days. In pot experiments with rice var. RD43, spraying with triforine 19%EC, propiconazole 25%EC, trifloxystrobin 50%WG, and validamycin 3%SL showed a high level of fungicidal activity in controlling sheath blight disease, with this evidenced by significant suppression of the disease severity by 71–75% and 61–73% at seven and 14 days after inoculation, respectively. The treatment of rice var. KMDL105 with validamycin 3%SL exhibited the best activity in controlling sheath blight disease by reducing disease severity by up to 62% and 64% at seven and 14 days, respectively. The antagonistic marine fungi tested in this study displayed low activity against sheath blight disease and low effects on rice seedling promotion compared to fungicides. Specifically, *Trichoderma harzianum* KUFA 0689 and *T. asperellum* KUFA 0677 reduced sheath blight disease severity by 42–46% and 27–37% at seven and 14 days, respectively.

**Keywords:** biocontrol agents; fungicide; rice growth; sheath blight disease

Rice (*Oryzae sativa* L.) is the main economic crop in Thailand. Production of 24–25 million tons was recorded in 2019–2020, with an average rice yield of 445 kilograms per rai (2 781 kg/ha) (https://www.statista.com/markets/). Rice production served local consumption and worldwide export, as rice is a staple food for the world's population, ranked second after wheat (Plodpai et al. 2013; Abbas et al. 2021). However, the continually repeated cultivation of rice on the same land has increased pest and disease infection. Sheath blight disease caused by *Rhizoctonia solani* Kühn is one important rice disease in Thailand and may cause yield losses of more than 20% (Boukaew et al. 2013). Moreover, the pathogen has a wide range of hosts of more than 100 plant species and causes many diseases such as leaf blight, stem rot, and seedling rot (Handiseni et al. 2015; Persaud et al. 2019).

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The primary symptoms of sheath blight disease are small spots that appear on rice sheaths near the water line. The spots expand to oval- to globose-shaped, pale green, water-soaked lesions, which then elongate up the rice sheath, the center appearing bleached and surrounded by irregular tan to pale brown areas (Nicolaisen et al. 2018; Singh et al. 2019). The microsclerotium quickly germinates to produce hyphae that infect the rice sheath during periods of high relative humidity and extended periods of wet weather. After infection under favorable conditions, the pathogen produces fine pale brown mycelia and then forms microsclerotia on the infected areas (Khoshkdaman et al. 2020; Xiang et al. 2021). In Thailand, sheath blight disease is severe on low land in the northeast and central regions, especially in directly seeded fields where Thai farmers like to use high seed rates resulting in dense stands of rice with inordinate tillering (Boukaew et al. 2013). The plant density leads to poor crop stands, with high competition for soil nutrients making rice stems thin and weak (Sahu et al. 2020). Differences in grain maturation and lodging can be seen during the heading stage. Moreover, farmers apply large amounts of nitrogen and other fertilizers to promote rice growth and development. This excessive use of fertilizers results in rapid rice growth causing stems to be thin and weak and highly susceptible to infection by the pathogen (Takahashi et al. 2017; Kumar et al. 2018). In addition, the dense stands of prolifically tillered rice plants reduce air circulation, and this provides favorable conditions for the dissemination of the pathogen, infection of plants, development of the disease, and severe damage to grain yield and grain quality (Sahu et al. 2020; Li et al. 2021).

These factors weaken the rice plant and make it more susceptible to infection by *R. solani*. One approach for improving the growth and vigor of the rice plant to enable it to fight the pathogen and thus prevent infection is to treat rice seeds with fungicides and antagonistic microbes (Takahashi et al. 2017; Meixia et al. 2018; Kongcharoen et al. 2020). Many studies have reported on the search for effective approaches to improve rice growth and control rice sheath blight disease caused by *R. solani* using biological control agents, chemicals, or integrated pest management (Chen et al. 2014; Uppala & Zhou 2018; Chalearmsrimuang et al. 2019; Harvianti & Kasiamdari 2021). Fungicides are applied singly or in combinations to control important rice diseases and are always effective in a short time (Eakjamnong et al. 2021). Many fungicides, both non-systemic and systemic, are used to control the disease in Thailand. The various classes of fungicides recommended for controlling the disease include triazoles, strobilurins, and antibiotics. Previous studies have shown that azoxystrobin; propiconazole; azoxystrobin + propiconazole; trifloxystrobin + propiconazole; fluxapyroxad; pyraclostrobin; flutolanil; validamycin; and kasugamcyin were highly effective in reducing the severity of sheath blight disease compared to untreated controls (Kumar et al. 2018; Uppala & Zhou 2018; Zhao et al. 2020). Moreover, integrated biological and chemical control of sheath blight disease has been reported (Boukaew et al. 2013; Peng et al. 2014).

Along with the application of fungicides, eco-friendly approaches for controlling this disease have been extensively studied, with antagonistic bacteria displaying potent activity as biological control agents. *Burkholderia* sp. strain A-7.3, when applied in paddies, significantly reduced disease incidence and increased rice yield by 7% (Nicolaisen et al. 2018). Wu et al. (2019) found that *Streptomyces* sp. N2 produced the novel antifungalmycin N2 which significantly reduced the severity of sheath blight disease. Recently, Sahu et al. (2020) reported the efficacy of endophytic bacilli from holy basil (*Ocimum tenuiflorum* L.) in promoting plant growth and inducing systemic resistance against *R. solani* in rice. Persaud et al. (2019) studied the effects of plant extracts, bioagents, and fungicides in the control of rice sheath blight in Guyana. In Thailand, Plodpai et al. (2013) reported that *Desmos chinensis* dichloromethane extract showed the best antifungal activity by damaging the membranous structures of *R. solani*. Boukaew and Prasertsan (2014) found that the culture filtrate of *Streptomyces philanthi* RM-1-138 brought about suppression of rice sheath blight of up to 65%. Moreover, Shasmita and Mukherjee (2019) reported the application of *Trichoderma erinaceum* resulted in a decrease in disease severity and improvement of rice yield. *Citrobacter freundii* and *Pseudomonas aeruginosa* inhibited the growth of *R. solani* lesions on rice leaves by 89% and 100% (without lesion), respectively, after seven days of incubation (Harvianti & Kasiamdari 2021).

Most antagonists are endophytic fungi obtained through screening soil. Marine microorganisms have also proven to be a potentially promising source

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of novel agents against plant pathogens due to their potent antagonistic properties (Dethoup et al. 2018). In a previous report, we documented our evaluation of the effects of 20 marine-derived fungi against plant pathogens, including *R. solani*, and found that *Emericella nidulans*, *Trichoderma asperellum*, and *T. harzianum* had potent antagonistic activity (of more than 60%) against the mycelial growth of *R. solani* when tested using dual cultures (Chalearmsrimuang et al. 2019). In the present study, we have further evaluated the effect of these fungi against rice sheath blight disease in laboratory and pot experiments. Moreover, while the fungicides used in this study have been promoted for controlling rice diseases in Thailand, their effects on rice seedling growth have never been reported. The objectives of this research were to evaluate the potential of fungicides and antagonistic marine-derived fungi for rice seedling promotion and for controlling rice sheath blight disease in pot experiments.

## MATERIAL AND METHODS

### Fungal material

The three marine-derived fungal antagonistic strains used in this study, namely, *Emericella nidulans* KUFA 0031, *Trichoderma asperellum* KUFA 0677, and *T. harzianum* KUFA 0689, showed antagonistic activity against *R. solani* in the laboratory and were further evaluated in the present study in pot experiments. The identification of the three marine-derived fungi was based on morphology and molecular techniques, as described previously by Chalearmsrimuang et al. (2019). Their gene sequences were deposited in GenBank with accession numbers, OQ269474 (*R. solani*), MF614160 (*E. nidulans*), KY942142 (*T. asperellum*), and MF614160 (*T. harzianum*).

### Preparation of spore suspension of marine-derived fungi

The preparation of a spore suspension of the selected marine-derived fungi was done as described previously by Dethoup et al. (2018) with slight modification. Each fungus was cultured on potato dextrose agar (PDA) for seven days, with mycelium plugs of each fungus then cut using a steel borer. To prepare a mycelial suspension, the plugs were cultured in 200 mL of potato dextrose broth in a flask and incubated on a rotary shaker at 120 rpm for seven days at room temperature. Five 100 mL Erlenmeyer flasks, each containing 200 g cooked rice, were sterilized by autoclaving at 121 °C for 15 minutes. Each flask was then inoculated with 15 mL of each mycelial suspension and incubated at room temperature for seven days. To prepare the spore suspension, 100 mL of sterile water was poured into a culture flask, prepared as above, and incubated for seven days, with a sterile glass rod used to gently scrape spores from the rice mold. To remove the mycelium fragments, the spore suspension was then filtered through the sterile chesscloth. It was then adjusted with sterile water to obtain a homogenous spore suspension of $10^6$ spores/mL using a hemocytometer.

### Effects of fungicides and antagonistic marine-derived fungi on rice growth

Rice seeds of var. RD43 and KMDL105 were surface disinfected, as described previously by Dethoup et al. (2018). Fifteen grams of rice seeds of each rice variety were coated with each fungicide at the recommended rate or a modification of the recommended rate, and each of the three antagonistic marine-derived fungi at $10^6$ spores/mL and left for seven days at room temperature. Untreated rice served as the control. The fungicides used in this study per 1 kg of rice seeds were: azoxystrobin 25%SC (1 mL); carbendazim 50%SC (3 mL); flutriafol 12.5%SC (1.25 g); mancozeb 80%WP (6 g); prochloraz 45%EC (1.5 mL); propiconazole 25%EC (3 mL); propineb 70%WP (5 g); thiophanate-methyl 70%WP (3 g); trifloxystrobin 50%WG (0.6 g); triforine 19%EC (3 mL); validamycin 3%SC (2.5 mL); difenoconazole 15% + propiconazole 15%EC (2 mL); fluopyram 20% + tebuconazole 20%SC (2 mL); and tebuconazole 25% + trifloxystrobin 50%WG (1.2 g). The rice seeds from each treatment were then placed in a plastic box (width × length × height of 8.5 × 8.5 × 9.75 cm) and 10 mL of sterile water was added. An addition of 10 mL of sterile water was made every two days. Ten rice seedlings were randomly selected from each box, with the length of their roots and the height of their shoots measured seven and 14 days after planting. Each experiment was repeated twice with five replications (boxes) each time.

### Efficacy of antagonistic marine-derived fungi against rice sheath blight disease using detached leaf method

As each fungicide used was recommended for controlling sheath blight disease in Thailand,

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only the antagonistic marine-derived fungi were tested against the disease *in vivo*. Second leaves from rice var. RD43 aged 60 days were selected and washed with tap water three times. The leaves were cut to 20 cm lengths which were soaked in a spore suspension of each antagonistic marine-derived fungus for 5 min and placed in a plastic box (width × length × height of 10 × 1 × 20 cm). Five rice leaves were inoculated with a mycelial plug of *R. solani* at the center of the leaf. The boxes containing the rice leaves were kept moist with wet paper. Four days after inoculation, the length of the disease lesions were measured and recorded. The experiment was repeated three times.

**Efficacy of fungicides and antagonistic marine-derived fungi against rice sheath blight disease under greenhouse conditions**

The rice seeds var. RD43 and KMDL105 were surface disinfecting and planted in nursery trays, grown for 14 days with watering every day, and then transplanted to pots. Four rice seedlings were transplanted into a clean plastic pot sized 25 cm in diameter and 22 cm in height, arranged in a greenhouse and watered every day to keep the water level 1 cm above soil. All treatments consisted of five pots (replications) and were set up in a completely randomized design.

At eight weeks, the rice plants were applied with 30 mL of the spore suspension of antagonistic marine-derived fungi or each fungicide with 0.01% Tween-20 per pot on all sides of the rice sheath and stem, and allowed to dry for 2 h (Tables 1 and 2). A mycelial *R. solani* disc of 1 cm in diameter was then placed in the leaf sheath at the base of the rice plant near the water line. Sixteen rice plants were used per treatment. One percent dimethyl sulfoxide (DMSO) and 0.01% Tween-20 void of either fungicides or spore suspension set as a negative control. Disease severity was recorded seven and 14 days after pathogen inoculation with this calculated using the formula = total lesion length/total length of sheath × 100.

**Statistical analysis**

One-way analysis of variance (ANOVA) followed by Duncan's multiple range test was applied to compare means in each treatment, with statistical significance set at $P < 0.05$ using SPSS (version 22), to conduct all the analyses. Data were represented as means ± standard errors with three replicates.

## RESULTS

**Efficacy of fungicides and antagonistic marine-derived fungi on rice growth var. RD43 and var. KMDL105**

The effects of the 14 fungicides and three antagonistic marine-derived fungi on the growth of rice seedlings of var. RD43 are shown in Figures 1–3. The fungicides and antagonistic marine-derived fungi stimulated, inhibited, or had no effect on rice seedling development. Seven days after planting, the rice seedlings of var. RD43 treated with carbendazim 50%SC had the greatest root length of all rice seedlings, followed by the rice seedlings treated with thiophanate-methyl 70%WP. Most of the fungicides and antagonistic marine-derived fungi had no effect on the root length of the rice seedlings; however, propiconazole 25%EC and *E. nidulans* KUFA 0031 exhibited negative activity on rice seedling root growth. Mancozeb 80%WP and propineb 70%WP displayed the greatest activity in increasing the shoot height of rice seedlings of var. RD43 seven days after planting, followed by thiophanate-methyl 70%WP and fluopyram 20% + tebuconazole 20%SC. The rest of the fungicides and all the antagonistic marine-derived fungi exhibited negative effects on the shoot development of rice seedlings, with lower shoot heights than those in the control treatment.

At 14 days after planting, rice seedlings of var. RD43 treated with azoxystrobin 25%SC exhibited the best promotion of root length growth, with carbendazim 50%SC, mancozeb 80%WP, prochloraz 45%EC, thiophanate-methyl 70%WP, trifloxystrobin 50%WG, triforine 19%EC, and difenoconazole 15% + propiconazole 15%EC all showing good promotion of root growth over the controls with significant statistical differences. The results also showed that the treatment of rice with propineb 70%WP, difenoconazole 15% + propiconazole 15%EC, and fluopyram 20% + tebuconazole 20%SC resulted in great promotion of plant growth activity in terms of rice shoot development. The antagonistic marine-derived fungi, both *T. asperellum* KUFA 0677 and *T. harzianum* KUFA 0689, also significantly promoted rice shoot growth, but *E. nidulans* KUFA 0031 showed negative effects on rice growth at both seven and 14 days after planting.

The effects of the 14 fungicides and three antagonistic marine-derived fungi on rice seedlings of var. KMDL105 are shown in Figures 1, 2 and 4.

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Table 1. Effects of fungicides and antagonistic marine-derived fungi in controlling rice sheath blight disease in rice var. RD43 and var. KMDL105 in pot experiments

| | Treatment | Chemical group/FRAC | Rate/ water (1 L) | Disease severity (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | rice var. RD43 | | rice var. KMDL105 | |
| | | | | 7 DAI | 14 DAI | 7 DAI | 14 DAI |
| Fungicides | azoxystrobin 25%SC | quinone outside inhibitors/11 | 0.5 mL | 2.79 ± 0.59$^{a–c}$ | 6.78 ± 1.26$^{c–e}$ | 3.30 ± 0.36$^{ab}$ | 5.56 ± 0.28$^{b–e}$ |
| | carbendazim 50%SC | methyl benzimidazole carbamates/1 | 1.5 mL | 3.62 ± 0.41$^{c–e}$ | 7.03 ± 1.06$^{c–e}$ | 3.24 ± 0.28$^{ab}$ | 4.63 ± 0.95$^{a–d}$ |
| | flutriafol 12.5%SC | demethylation inhibitors/3 | 0.625 g | 1.35 ± 0.18$^{a}$ | 2.35 ± 0.52$^{a}$ | 2.75 ± 0.92$^{ab}$ | 3.93 ± 0.56$^{a–c}$ |
| | mancozeb 80%WP | dithiocarbamates and relatives/M03 | 3 g | 5.15 ± 0.68$^{ef}$ | 8.25 ± 0.78$^{de}$ | 3.34 ± 0.37$^{ab}$ | 3.46 ± 0.14$^{a}$ |
| | prochloraz 45%EC | demethylation inhibitors/3 | 0.75 mL | 3.51 ± 1.28$^{b–e}$ | 3.85 ± 0.81$^{ab}$ | 3.69 ± 0.42$^{a–c}$ | 3.76 ± 0.31$^{ab}$ |
| | propiconazole 25%EC | demethylation inhibitors/3 | 1.5 mL | 1.36 ± 0.41$^{a}$ | 3.19 ± 0.94$^{a}$ | 2.31 ± 0.13$^{a}$ | 3.45 ± 0.48$^{a}$ |
| | propineb 70%WP | dithiocarbamates and relatives/M03 | 2.5 g | 2.54 ± 0.79$^{a–c}$ | 6.62 ± 0.37$^{cd}$ | 4.49 ± 0.71$^{bcd}$ | 5.72 ± 0.58$^{c–e}$ |
| | thiophanate-methyl 70%WP | methyl benzimidazole carbamates/1 | 1.5 g | 2.03 ± 0.96$^{a–c}$ | 6.80 ± 0.77$^{c–e}$ | 3.29 ± 0.25$^{ab}$ | 4.24 ± 1.06$^{a–c}$ |
| | trifloxystrobin 50%WG | quinone outside inhibitors/11 | 0.3 g | 1.5 ± 0.14$^{a}$ | 3.28 ± 0.37$^{a}$ | 3.73 ± 0.64$^{a–c}$ | 5.01 ± 1.33$^{a–d}$ |
| | triforine 19%EC | demethylation inhibitors/3 | 1.5 mL | 4.88 ± 1.59$^{d–f}$ | 7.06 ± 0.56$^{c–e}$ | 2.60 ± 0.9$^{ab}$ | 3.74 ± 1.08$^{ab}$ |
| | validamycin 3%SL | glucopyranosyl antibiotic/26 | 1.25 mL | 1.31 ± 0.26$^{a}$ | 2.21 ± 0.56$^{a}$ | 2.28 ± 0.31$^{a}$ | 3.18 ± 0.26$^{a}$ |
| | difenoconazole 15% + propiconazole 15%EC | demethylation inhibitors/3 + demethylation inhibitors/3 | 1 mL | 1.84 ± 0.36$^{ab}$ | 3.76 ± 0.41$^{ab}$ | 3.22 ± 0.97$^{ab}$ | 4.79 ± 1.48$^{a–d}$ |
| | fluopyram 20% + tebuconazole 20%SC | succinatede hydrogenase inhibitors/7 + demethylation inhibitors/3 | 1 mL | 2.88 ± 0.51$^{a–c}$ | 6.32 ± 0.57$^{c}$ | 3.16 ± 0.18$^{ab}$ | 4.12 ± 0.56$^{a–c}$ |
| | tebuconazole 25% + trifloxystrobin 50%WG | demethylation inhibitors/3 + quinone outside inhibitors/11 | 0.6 g | 2.34 ± 0.56$^{a–c}$ | 5.19 ± 0.73$^{bc}$ | 3.51 ± 0.59$^{a–c}$ | 4.25 ± 0.68$^{a–c}$ |
| Antagonistic marine-derived fungi | *Emericella nidulans* KUFA 0031 | $10^6$ spores/mL | | 5.12 ± 1.78$^{ef}$ | 6.34 ± 0.11$^{c}$ | 5.31 ± 1.78$^{cd}$ | 7.34 ± 4.11$^{ef}$ |
| | *Trichoderma asperellum* KUFA 0677 | $10^6$ spores/mL | | 3.52 ± 1.21$^{b–e}$ | 5.38 ± 0.34$^{bc}$ | 3.52 ± 1.21$^{a–c}$ | 6.38 ± 3.34$^{de}$ |
| | *T. harzianum* KUFA 0689 | $10^6$ spores/mL | | 3.27 ± 1.07$^{b–d}$ | 5.53 ± 0.18$^{bc}$ | 3.27 ± 1.07$^{ab}$ | 5.53 ± 3.18$^{b–e}$ |
| Water (control) | | | | 5.31 ± 2.58$^{f}$ | 8.47 ± 0.72$^{e}$ | 6.08 ± 1.21$^{d}$ | 8.83 ± 2.47$^{f}$ |

DAI – days after incubation; FRAC – Fungicide Resistance Action Committee

$^{a–f}$Means ± standard derivations followed by the same letter in each column do not significantly differ at $P < 0.05$, when analysed using Duncan's test of one-way ANOVA

The rice seedlings treated with thiophanate-methyl 70%WP had the greatest root length followed by the seedlings treated with triforine 19%EC, with these showing significantly greater root length than that of the controls. The azoxystrobin 25%SC, carbendazim 50%SC, trifloxystrobin 50%WG, *T. as-*

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Table 2. Efficacy of fungicides and antagonistic marine-derived fungi in controlling rice sheath blight disease in rice var. RD43 and var. KMDL105 in pot experiments

| | Treatment | Chemical group/FRAC | Rate/water (1 L) | Disease reduction (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | rice var. RD43 | | rice var. KMDL105 | |
| | | | | 7 DAI | 14 DAI | 7 DAI | 14 DAI |
| Fungicides | azoxystrobin 25%SC | quinone outside inhibitors/11 | 0.5 mL | 47.46 ± 3.68$^{c-e}$ | 19.95 ± 1.71$^{e}$ | 45.72 ± 2.31$^{ef}$ | 37.03 ± 2.67$^{i}$ |
| | carbendazim 50%SC | methyl benzimidazole carbamates/1 | 1.5 mL | 31.83 ± 2.05$^{f}$ | 17.00 ± 1.02$^{e}$ | 46.71 ± 2.07$^{d-f}$ | 47.57 ± 3.15$^{f}$ |
| | flutriafol 12.5%SC | demethylation inhibitors/3 | 0.625 g | 74.58 ± 5.83$^{a}$ | 72.26 ± 5.34$^{a}$ | 54.77 ± 3.72$^{c}$ | 55.49 ± 4.57$^{d}$ |
| | mancozeb 80%WP | dithiocarbamates and relatives/m03 | 3 g | 3.01 ± 0.28$^{g}$ | 2.60 ± 0.14$^{f}$ | 45.07 ± 3.54$^{f}$ | 43.82 ± 3.21$^{h}$ |
| | prochloraz 45%EC | demethylation inhibitors/3 | 0.75 mL | 33.89 ± 1.17$^{ef}$ | 54.54 ± 0.35$^{b}$ | 39.31 ± 2.34$^{h}$ | 57.42 ± 4.19$^{c}$ |
| | propiconazole 25%EC | demethylation inhibitors/3 | 1.5 mL | 74.39 ± 6.12$^{a}$ | 62.34 ± 4.26$^{ab}$ | 62.01 ± 4.01$^{a}$ | 60.93 ± 5.46$^{b}$ |
| | propineb 70%WP | dithiocarbamates and relatives/M03 | 2.5 g | 52.17 ± 2.58$^{b-d}$ | 21.84 ± 1.20$^{de}$ | 26.15 ± 1.46$^{i}$ | 35.22 ± 2.58$^{j}$ |
| | thiophanate-methyl 70%WP | methyl benzimidazole carbamates/1 | 1.5 g | 61.77 ± 5.11$^{b}$ | 19.72 ± 1.04$^{e}$ | 45.89 ± 3.58$^{ef}$ | 51.98 ± 4.07$^{e}$ |
| | trifloxystrobin 50%WG | quinone outside inhibitors/11 | 0.3 g | 71.75 ± 5.47$^{a}$ | 61.28 ± 4.51$^{ab}$ | 38.65 ± 2.11$^{h}$ | 43.26 ± 3.25$^{h}$ |
| | triforine 19%EC | demethylation inhibitors/3 | 1.5 mL | 8.10 ± 0.51$^{g}$ | 16.65 ± 1.03$^{e}$ | 57.24 ± 4.58$^{b}$ | 57.64 ± 4.62$^{c}$ |
| | validamycin 3%SL | glucopyranosyl antibiotic/26 | 1.25 mL | 75.33 ± 5.51$^{a}$ | 73.91 ± 5.49$^{a}$ | 62.50 ± 4.87$^{a}$ | 63.99 ± 5.45$^{a}$ |
| | difenoconazole 15% + propiconazole 15%EC | demethylation inhibitors/3 + demethylation inhibitors/3 | 1 mL | 65.35 ± 4.92$^{b}$ | 55.61 ± 3.60$^{b}$ | 47.04 ± 3.05$^{de}$ | 45.75 ± 3.13$^{g}$ |
| | fluopyram 20% + tebuconazole 20%SC | succinatede hydrogenase inhibitors/7 + demethylation inhibitors/3 | 1 mL | 45.76 ± 3.45$^{c-f}$ | 25.38 ± 1.69$^{c-e}$ | 48.03 ± 3.70$^{d}$ | 53.34 ± 4.35$^{e}$ |
| | tebuconazole 25% + trifloxystrobin 50%WG | demethylation inhibitors/3 + quinone outside inhibitors/11 | 0.6 g | 55.93 ± 3.34$^{bc}$ | 38.72 ± 2.09$^{c}$ | 42.27 ± 3.53$^{g}$ | 51.87 ± 3.91$^{e}$ |
| Antagonistic marine-derived fungi | *Emericella nidulans* KUFA 0031 | $10^6$ spores/mL | | 3.95 ± 0.16$^{g}$ | 25.15 ± 1.60$^{c-e}$ | 12.66 ± 0.81$^{j}$ | 16.87 ± 0.90$^{l}$ |
| | *Trichoderma asperellum* KUFA 0677 | $10^6$ spores/mL | | 33.71 ± 2.06$^{ef}$ | 36.48 ± 2.31$^{c}$ | 42.11 ± 2.56$^{g}$ | 27.75 ± 1.03$^{k}$ |
| | *T. harzianum* KUFA 0689 | $10^6$ spores/mL | | 38.42 ± 2.84$^{d-f}$ | 34.71 ± 2.65$^{cd}$ | 46.22 ± 3.18$^{ef}$ | 37.37 ± 2.35$^{i}$ |

DAI – days after incubation; FRAC – Fungicide Resistance Action Committee

$^{a-l}$Means ± standard derivations followed by the same letter in each column do not significantly differ at $P < 0.05$, when analysed using Duncan's test of one-way ANOVA

*perellum* KUFA 0677 and *T. harzianum* KUFA 0689 treatments had no effect on the root length of rice seedlings, with the rest of the treatments displaying negative effects on the root growth of rice seedlings at seven days after planting. Rice seedlings treated with mancozeb 80%WP showed the greatest shoot height followed by the seedlings treated with thiophanate-methyl 70%WP, triforine 19%EC, and fluopyram 20% + tebuconazole 20%SC, all of which showed significantly greater shoot height

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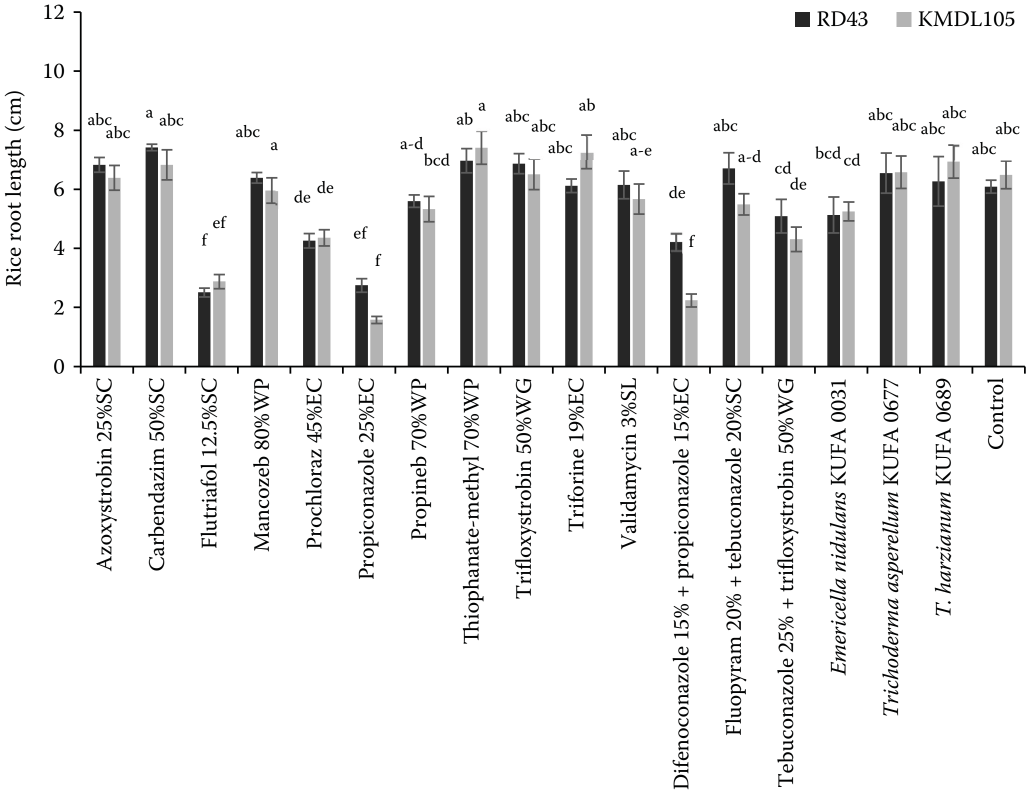
(A)




(B)

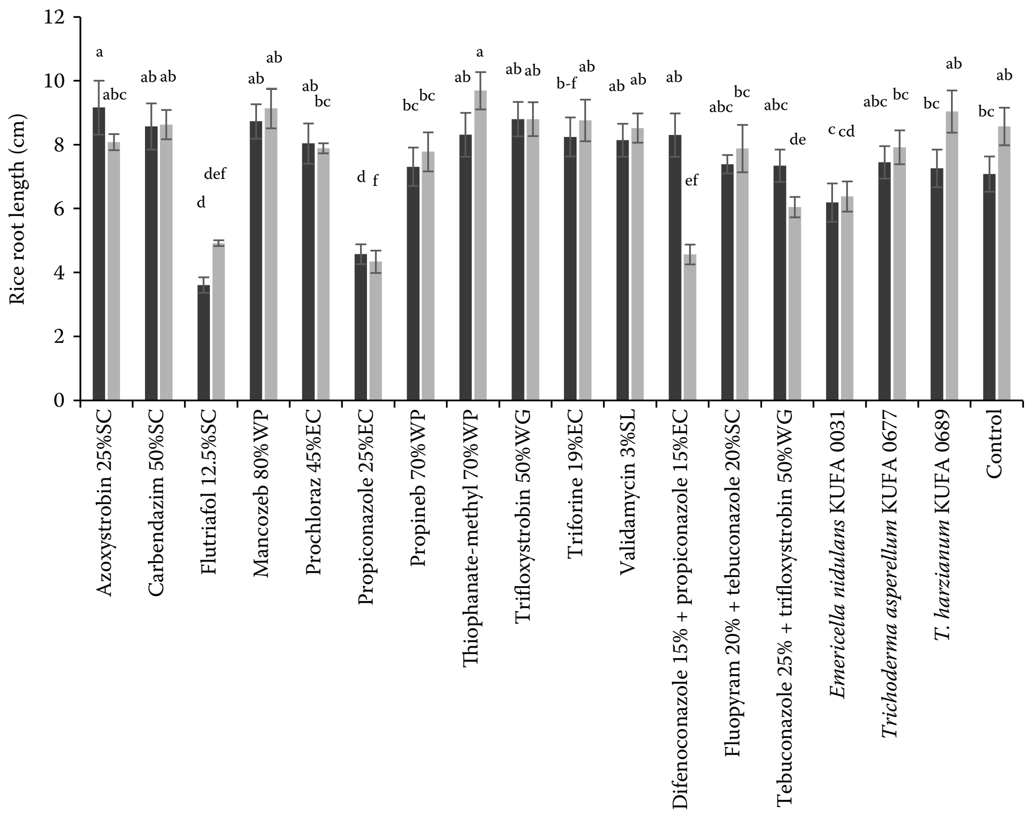



Figure 1. Effects of fungicides and biocontrol agents on rice root length in rice var. RD43 and var. KMDL 105 at seven days (A) and 14 days (B)

[a–f]Means ± standard derivations followed by the same letter in rice variety do not significantly differ at $P < 0.05$, when analyzed using Duncan's test of one-way ANOVA

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(A)

(B)

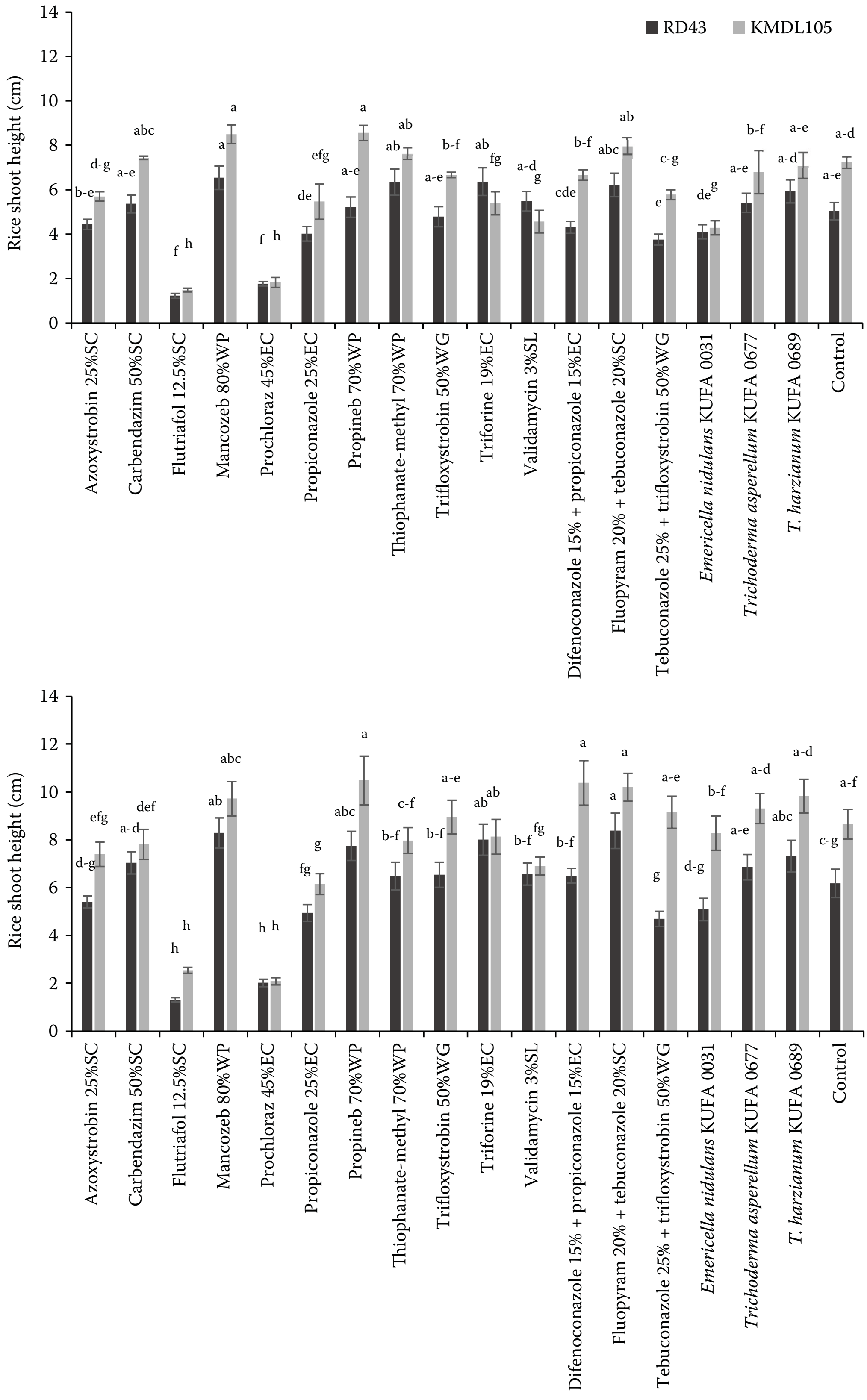


Figure 2. Effects of fungicides and biocontrol agents on rice shoot length in rice var. RD43 and var. KMDL 105 at seven days (A) and 14 days (B)

[a–h]Means ± standard derivations followed by the same letter in rice variety do not significantly differ at $P < 0.05$, when analyzed using Duncan's test of one-way ANOVA

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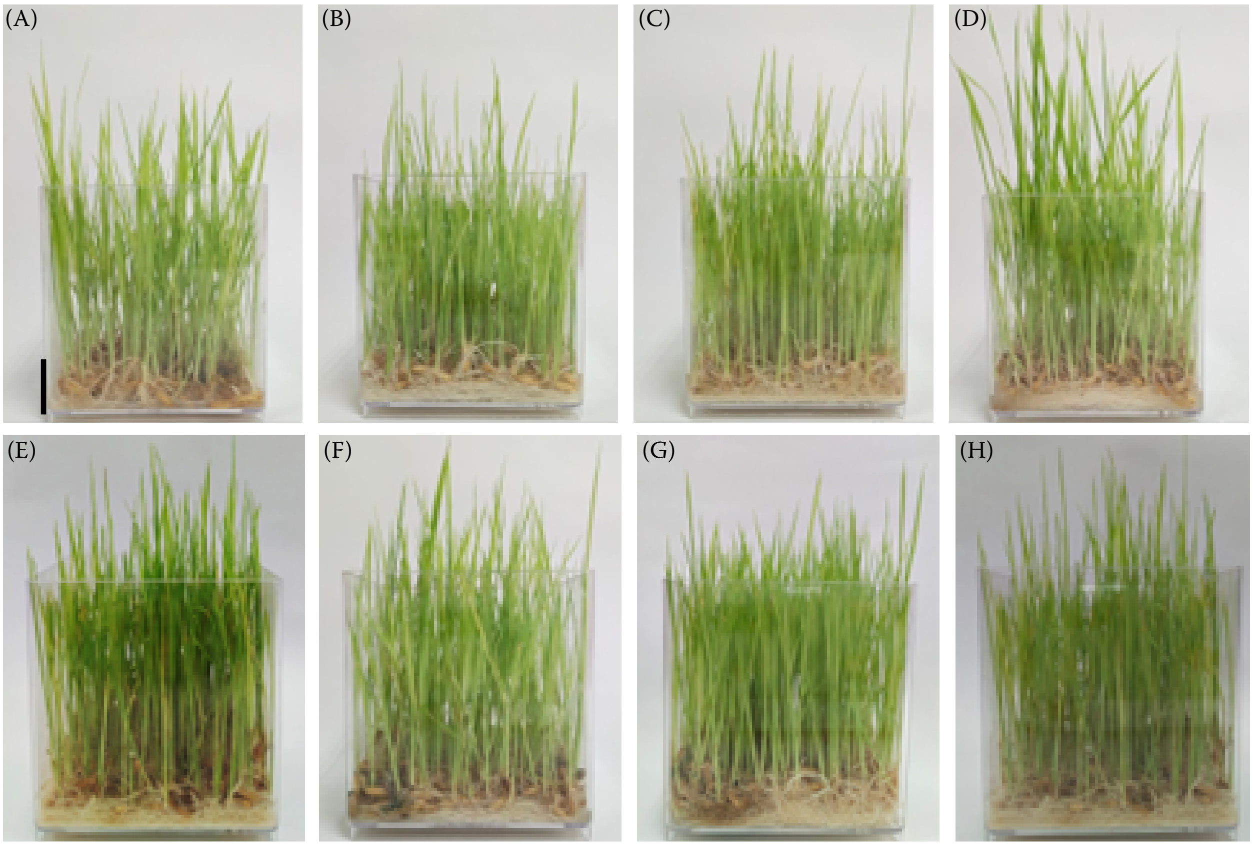


Figure 3. Effects of fungicides and biocontrol agents on rice growth promotion in rice var. RD43 at 14 days
(A) Control; (B) trifloxystrobin 50%WG; (C) fluopyram 20% + tebuconazole 20%SC; (D) propineb 70%WP; (E) difenoconazole 15% + propiconazole 15%EC; (F) mancozeb 80%WP; (G) *T. harzianum* KUFA 0689; (H) *T. asperellum* KUFA 0677
Scale bar = 2 cm

over that of the controls. The carbendazim 50%SC, propineb 70%WP, trifloxystrobin 50%WG, validamycin 3%SL, *T. asperellum* KUFA 0677 and *T. harzianum* KUFA 0689treatments had no effect on the shoot height of rice seedlings, while the other treatments displayed negative effects on rice seedling shoot height at seven days after planting.

At 14 days, rice seedlings treated with thiophanate-methyl 70%WP showed significantly improved root length, having the greatest root length among all the rice seedlings. The carbendazim 50%SC, mancozeb 80%WP, trifloxystrobin 50%WG, triforine 19%EC, validamycin 3%SL, and *T. harzianum* KUFA 0689 treatments had no effect on root length, while the rest of the treatments showed negative effects on root development with root lengths being significantly lower than those of the controls. As to the effects on shoot growth, the results showed that the rice seedlings treated with fluopyram 20% + tebuconazole 20%SC had the greatest shoot height, followed by the seedlings treated with mancozeb 80%WP, triforine 19%EC, propineb 70%WP, and *T. harzianum*. KUFA 0689, all of which had a significant promotion of rice shoot growth over the controls. The thiophanate-methyl 70%WP, trifloxystrobin 50%WG, validamycin 3%SL, and difenoconazole 15% + propiconazole 15%EC treatments also showed moderate improvement in rice shoot growth, whereas the other treatments had negative effects on the shoot height of rice seedlings at 14 days.

**Efficacy of fungicides and antagonistic marine-derived fungi against rice sheath blight disease using the detached leaf method**

The results from the detached leaf method showed that treatment of rice leaves with *T. harzianum* KUFA 0689, and *T. asperellum* KUFA 0677 displayed potent antagonistic activity in reducing the disease severity. The disease severity on rice leaves treated with *T. harzianum* KUFA 0689, *T. asperellum* KUFA 0677, and *E. nidulans* KUFA 0031 were 3.3 cm, 5.73 cm, and 5.8 cm, respective-

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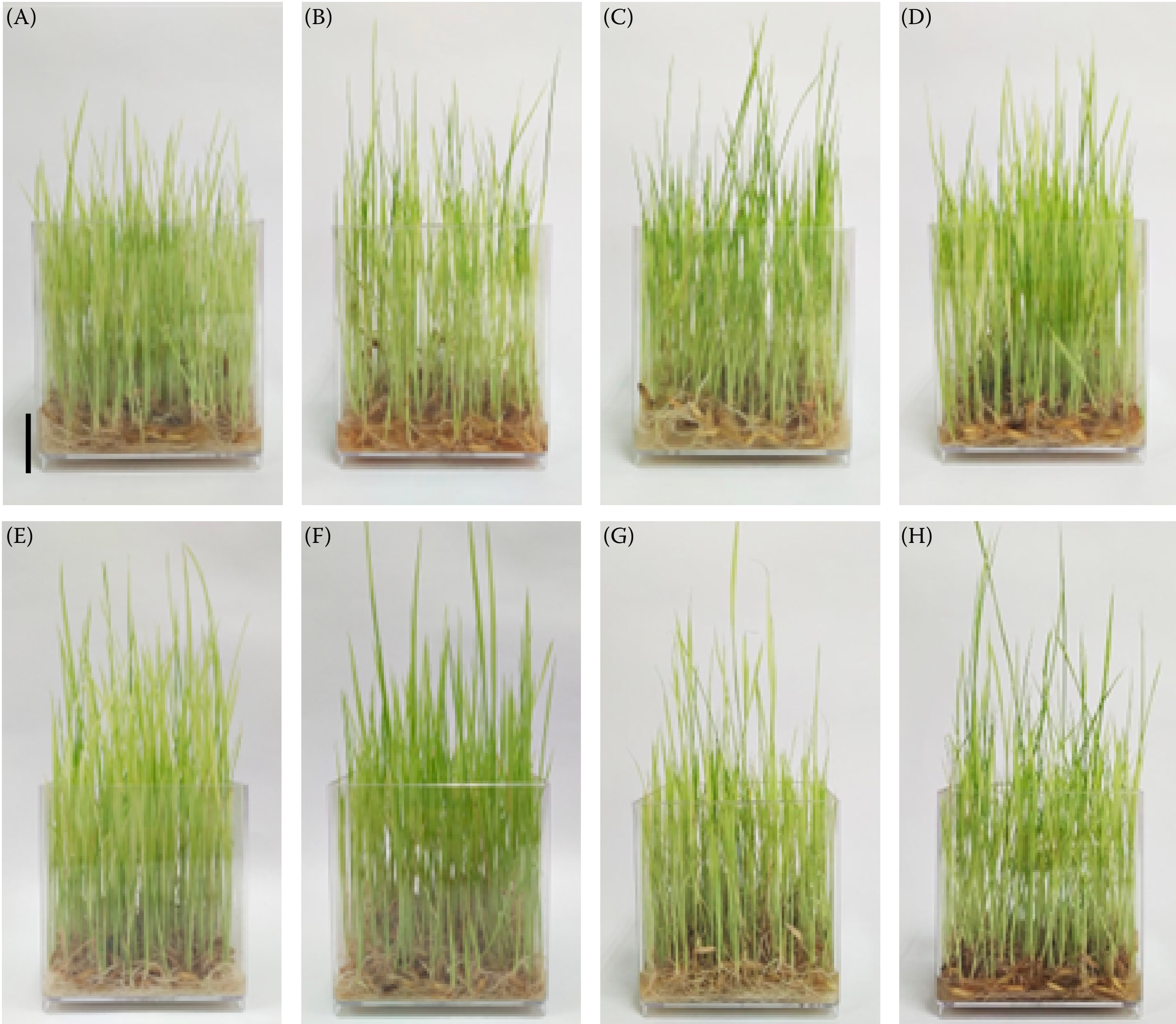


Figure 4. Effects of fungicides and biocontrol agent on rice growth promotion in rice var. KMDL105 at 14 days
(A) Control; (B) propineb 70%WP; (C) carbendazim 50%SC; (D) triforine 19%EC; (E) mancozeb 80%WP; (F) *T. harzianum* KUFA 0689; (G) *T. asperellum* KUFA 0677; (H) fluopyram 20% + tebuconazole 20%SC
Scale bar = 2 cm

ly, whereas the control treatment exhibited disease severity of 10.2 cm (Figure 5).

**Effects of fungicides and antagonistic marine-derived fungi in controlling rice sheath blight disease in rice var. RD43 and var. KMDL105 in pot experiments**

The effects of 14 fungicides and three antagonistic marine-derived fungi against sheath blight disease in rice var. RD43 at seven and 14 days after inoculation are displayed in Tables 1 and 2 and Figure 6. As shown in the results, applications of triforine 19%EC, propiconazole 25%EC, trifloxystrobin 50%WG, and validamycin 3%SL exhibited the best fungicidal activity in controlling sheath blight disease severity by significantly reducing the disease severity by 71–75% and 61–73% at seven and 14 days, respectively. Applications of the fungicide mixtures difenoconazole 15% + propiconazole 15%EC, tebuconazole 25% + trifloxystrobin 50%WG, and thiophanate-methyl 70%WP displayed significant difference in disease reduction which could reduce the disease by 55–65% at seven days. Moreover, the fungicide mixtures, difenoconazole 15% + propiconazole 15%EC, tebuconazole 25% + trifloxystrobin 50%WG, and fluopyram 20% + tebuconazole 20%SC reduced the disease severity by 55%, 38%, and 25% at 14 days,

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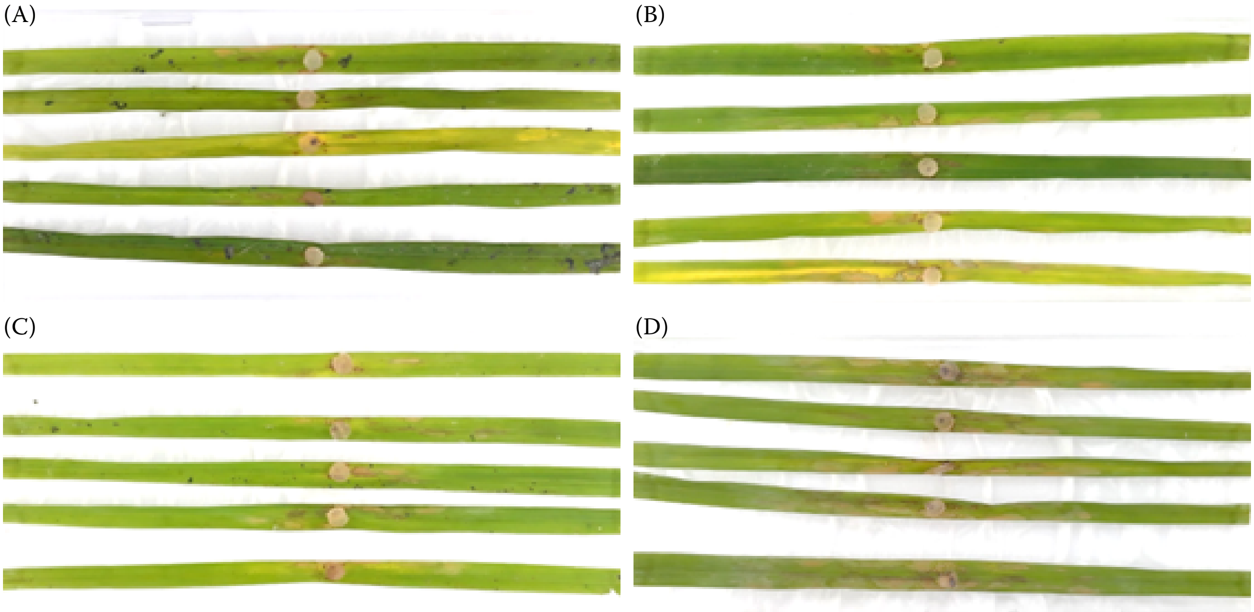


Figure 5. Effects of antagonistic marine-derived fungi against rice sheath blight using the detached leaf method at four days after inoculation
(A) *Trichoderma harzianum* KUFA 0689; (B) *T. asperellum* KUFA 0677; (C) *Emericella nidulans* KUFA 0031; (D) control

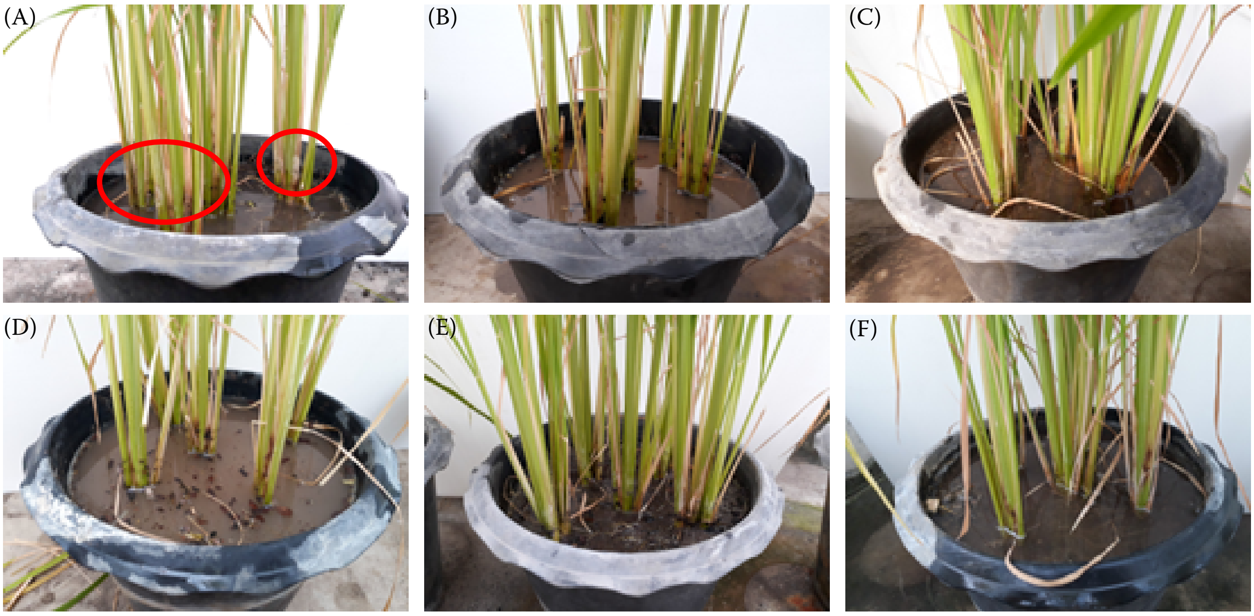


Figure 6. Effects of fungicides in controlling sheath blight in rice var. RD43 in pot experiments
(A) Control; (B) validamycin 3%SL; (C) flutriafol 12.5%SC; (D) prochloraz 45%EC; (E) trifloxystrobin 50%WG; (F) difenoconazole 15% + propiconazole 15%EC

respectively. The fungicides azoxystrobin 25%SC, carbendazim 50%SC, thiophanate-methyl 70%WP, and flutriafol 12.5%SC showed no significant fungicidal activity against the disease at 14 days after inoculation, while mancozeb 80%WP and propineb 70%WP displayed low activity against the disease. The antagonistic marine fungi, *T. asperellum* KUFA 0677 and *T. harzianum* KUFA 0689, showed antagonistic activity in controlling sheath blight disease at 33–38% whereas *E. nidulans* KUFA 0031 had low activity by 25.15% disease reduction.

Tables 1 and 2 also show the fungicidal activity of the 14 fungicides and three antagonistic marine fungi against sheath blight disease in rice

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var. KMDL105 at seven and 14 days after inoculation. As shown in the results, validamycin 3%SL and prochloraz 45%EC exhibited the best activity in controlling the severity of sheath blight disease, reducing the disease by up to 62% at seven days. Triforine 19%EC, flutriafol 12.5%SC, and fluopyram 20% + tebuconazole 20%SC reduced the disease severity by 57%, 54%, and 48%, respectively at seven days after inoculation. At 14 days after inoculation, validamycin 3%SL showed the greatest effectiveness, reducing the disease severity by 64%, followed by prochloraz 45%EC, propiconazole 25%EC, flutriafol 12.5%SC, and flutriafol 12.5%SC which exhibited high fungicidal activity against the disease, reducing severity by 55–60%, with thiophanate-methyl 70%WP and tebuconazole 25% + trifloxystrobin 50%WG reducing disease severity by 51%. The lowest fungicidal activity against sheath blight disease was found in the propineb 70%WP treatment which reduced severity of the disease by less than 40% when observed at seven and 14 days (Figure 7).

The antagonistic marine fungi displayed low activity against the disease in comparison to the fungicides. *T. harzianum* KUFA 0689 and *T. asperellum* KUFA 0677 reduced severity of the disease by 42–46% and 27–37% at seven and 14 days, respectively, whereas *E. nidulans* KUFA 0031 had the lowest antagonistic activity against the disease, reducing severity of the disease by 12% and 16% at seven and 14 days after inoculation, respectively.

## DISCUSSION

Rice diseases caused by fungi have been increasing in severity in many rice-producing countries, one of which is Thailand. The seedling stage is the time when rice is most vulnerable to infections by pathogens, with approaches to improve rice seedling growth seeking methods to avoid and prevent pathogen infection during this stage. In this study, we have reported on the efficacy of 14 fungicides and three biological control agents (i.e., antagonistic marine fungi) in the promotion of rice seedling growth and effectiveness against rice sheath blight disease caused by *R. solani*. However, studies of the efficacy of fungicidal activity on rice seedling growth are still limited, and previous reports have studied different fungicides from those in this study. Takahashi et al. (2017) found that rice seedlings treated with orysastrobin had improved resistance to abiotic stress, resulting in loss of root-cutting injury, and hydrogen peroxide ($H_2O_2$) overproduction. Some reports have shown that treating rice seeds with fungicides has reduced rice disease infection. Meixia et al. (2018) revealed the applied of the mixture of phenamacril and ipconazole (2:1) at 6 g of a.i./100 kg

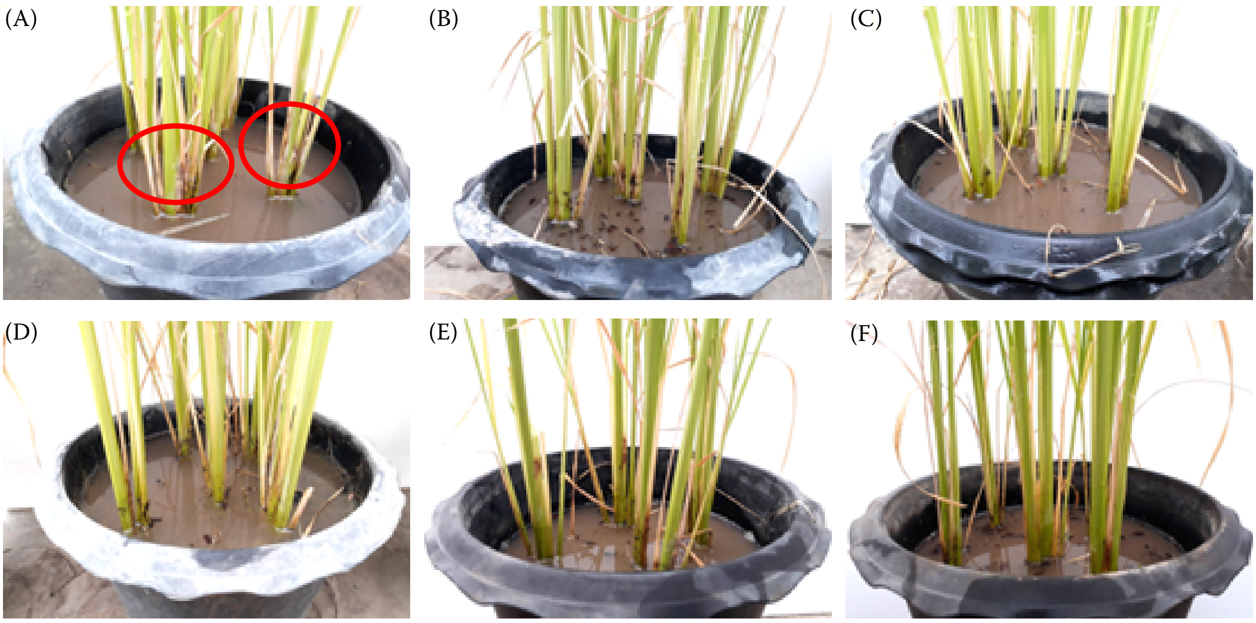


Figure 7. Effects of fungicides in controlling sheath blight in rice var. KMDL 105 in pot experiments
(A) Control; (B) validamycin 3% SL; (C) prochloraz 45%EC; (D) propiconazole 25%EC; (E) triforine 19%EC; (F) flutriafol 12.5%SC

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of rice seeds resulted in complete control of rice bakanae disease and reduced the fungicide resistance in *Fusarium fujikuroi* population.

Sheath blight disease is a devastating rice disease in Thailand due to the continuous repetition of rice planting in the same area. Many classes of fungicides have been recommended for controlling the disease; however, the efficacy of these fungicides has never been tested at the same time even under greenhouse conditions. Many reports have shown that fungicide application causes pathogen resistance. The fungicides used in the present study have also shown positive, negative, or no effects on rice seedling growth and have had different effects on rice growth in the two rice varieties used in the study. Rice var. RD43 is an insensitive variety while KMDL105 is a photoperiod sensitive variety which both of them are the popular rice varieties for production in Thailand (Kongcharoen et al. 2020). However, in plant physiology of these two rice varieties there are considerable differences such as the average of plant height in var. RD43 and KMDL105 are 103 and 140 cm, respectively (Ullah et al. 2017; Vanavichit et al. 2018). Thus, the statistically analysis of each treatment in two rice varieties was performed separately. Carbendazim 50%SC and azoxystrobin 25%SC displayed the best promotion of rice growth of root length and shoot height on rice var. RD43. For seedlings of rice var. KMDL105, treatment with thiophanate-methyl 70%WP exhibited the greatest increases in both root length and shoot height. In contrast, the antagonistic marine fungi, *T. harzianum* KUFA 0689 and *T. asperellum* KUFA 0677, showed low to moderate activity in the promotion of rice seedling growth and in sheath blight control, whereas *E. nidulans* KUFA 0031 had negative activity in rice seedling growth and sheath blight control. The latter was evidenced by rice seedling growth less than that of control treatments and the lowest effect in disease control. Previous studies found that a triazole fungicides displayed growth regulating property resulted in increasing the physiological trait of crops via stimulate photosynthesis (Petit et al. 2012; Desta & Amare 2021).

The present study's results correspond to previous reports that found that *T. asperellum* had low to moderate percentage of disease reduction against sheath blight disease. Santos de França et al. (2015) reported that the antagonistic *T. asperellum* was effective against rice sheath blight, reducing disease severity by 19%. However, reports have indicated that some antagonistic fungi demonstrated potent antagonistic activity against this rice disease. Kumar and Gouda (2018) found that the nematode-trapping fungi, *Arthobotrys conoides*, *A. eudermata*, *Dactylellina gephyropaga* and *D. phymatopaga* formed coils around the hyphae of *R. solani*, resulting in strong cell wall proliferation and subsequent disintegration of the cytoplasm of *Rhizoctonia* cells. Moreover, treatment of plants exposed to *R. solani* with these fungi suppressed sheath blight invasion in rice tillers by 43.39–50.74%. Abbas et al. (2021) reported that *Talaromyces* sp. isolate TF-04 obtained from the paddy soil displayed effective antagonistic activity by mycoparasite the hyphae and destroyed the sclerotia even coupled with enhanced rice growth, suppressed the rice sheath blight incidence and improved the rice yield.

The present study's results indicated that spraying with triforine 19%EC, prochloraz 45%EC, trifloxystrobin 50%WG, and validamycin 3%SL displayed great fungicidal activity in controlling sheath blight disease, significantly suppressing the severity of disease by 61–75%. This corresponds with a report by Boukaew et al. (2013) in which they found that chemical fungicides, including carbendazim®, validamycin®, propiconazole®, and mancozeb®, showed increased protection of rice against sheath blight disease of 47–60%. Kumar et al. (2018) reported that the application of azoxystrobin 18.2% + difenoconazole 11.4%SC showed the greatest activity in controlling sheath blight infection and enhancing the grain yield of rice, under field conditions. Applications of azoxystrobin at the label rate (0.16 kg a.i./ha) or half the label rate (0.08 kg a.i./ha) effectively suppressed the severity of sheath blight disease and increased rice yield (Uppala & Zhou 2018).

Many fungicides have proved effective in controlling sheath blight disease (Li et al. 2014). Chen et al. (2014) reported that a succinate dehydrogenase inhibitor fungicide, fluxapyroxad, interfered with complex II in the electron transport chain of *R. solani* and exhibited excellent fungicidal activity against rice sheath blight up to 94.2% control efficacy, while the old fungicide, boscalid, by up 57.0% control efficacy after two applications. Zhao et al. (2020) studied the effects of the pyrazole carboxamide compounds and found it was equivalent to the commercial fungicide thifluzamide against *R. solani* by inhibiting protein expression in physiological and biochemical pathways in the mitochondria, endoplasmic reticulum, ribosome, and other related gene ontology and biological pathways.

Furthermore, novel and advanced approaches have been studied for controlling rice sheath blight disease. Persaud et al. (2019) evaluated the effectiveness of plant extracts, bioagents, and fungicides against sheath blight disease in Guyana and found that the extracts of lemongrass, thick leaf thyme at 15% and *B. cereus* OG2L at 2 g/L suppressed disease infection. Moreover, new-generation fungicides, such as Antracol, Nativo and Serenade, recorded low percentages of disease severity of 7.62–8.51% compared to those of the untreated control of 26.25% under greenhouse conditions.

The present study's results suggested that each fungicide and antagonistic marine fungus showed different effects on rice seedling growth–either positive, negative, or nil–and different levels of efficacy in controlling rice sheath blight in two rice varieties, RD43 and KMDL105. Carbendazim 50%SC, azoxystrobin 25%SC, and thiophanate-methyl 70%WP significantly improved rice growth in both root length and shoot height when applied as a seed treatment. Applications of triforine 19%EC, propiconazole 25%EC, trifloxystrobin 50%WG, and validamycin 3%SL demonstrated effective fungicidal activity in controlling sheath blight disease. The antagonistic marine-derived fungi tested in this study displayed low antagonistic activity against rice sheath blight disease and low to moderate effects on the promotion of rice seedling growth when compared to fungicides.

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