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Research Article

EFFECTIVENESS OF SOME NOVEL FUNGICIDES AGAINST THE SHEATH BLIGHT PATHOGEN *RHIZOCTONIA SOLANI* IN RICE UNDER *IN VITRO* AND *IN VIVO* CONDITIONS

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ABSTRACT

Sheath blight of rice, caused by *Rhizoctonia solani*, is one of the most aggressive and damaging diseases in rice cultivation, leading to significant crop losses. Consequently, chemical fungicides are the most effective method to inhibit *R. solani* infection and control this disease. In the present study, *R. solani* isolate was obtained from infected rice plants, and its pathogenicity was confirmed using Koch's postulates on 20-day-old rice seedlings at three different inoculum levels. The lowest percent disease index and disease incidence were recorded with the 1 ml pathogen inoculum suspension, while the 2 ml and 3 ml suspensions resulted in 100% disease incidence, significantly affecting plant parameters. Furthermore, five different fungicides, Duphetar Plus, Evito, Nativo, Ridomil Gold, and Shincar, were evaluated at three concentrations (10, 100, and 1000 ppm) against *R. solani* using poisoned food method. The results revealed that two fungicides, Shincar and Nativo, resulted in maximum mycelial growth inhibition of *R. solani*, whereas the other fungicides failed to inhibit the mycelial growth of the pathogen at all concentrations. Shincar and Nativo were further tested in a greenhouse setting to control pathogen infection in rice plants. These fungicides showed excellent results, reducing *R. solani* infection and minimizing disease incidence in treated rice plants compared to the control. The highest root length, shoot length, and plant weight were observed with Shincar, followed by Nativo. It is suggested that the use of these fungicides may reduce sheath blight disease incidence, effectively control pathogen infection, and improve plant growth parameters in field conditions.

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INTRODUCTION

Rice (*Oryza sativa* L.) is a globally consumed cereal crop and ranks as the second most widely cultivated cereal after wheat, feeding approximately 50% of the world's population (Chen et al., 2014). It is grown in around 110 countries, with an estimated global production of 645

million tons. Asia contributes 90% of this output, with China and India being the top producers (IRRI, 2013). In Pakistan, Sindh and Punjab are the major rice-producing provinces, accounting for 53% and 26% of the country's total production, respectively. Rice is Pakistan's second-largest crop, cultivated on 2.899 million hectares (Bashir et al., 2019).

Rice crops face numerous biotic and abiotic stresses, with fungal diseases being a significant challenge to maintaining production levels (Singh et al., 2019). Fungal diseases alone are responsible for 70-80% of the losses in rice crops (Nayak et al., 2021). Among these, sheath blight and rice blast, caused by *Rhizoctonia solani* and *Magnaporthe oryzae*, are particularly damaging, significantly reducing yields and posing a threat to global food security (Khaskheli et al., 2020; He et al., 2022). *R. solani*, the pathogen responsible for sheath blight, is especially aggressive and damaging (Neha et al., 2016). This soil-borne pathogen causes lesions on the sheath, leading to yield reductions and a decline in rice grain quality (Peng et al., 2014; Singh et al., 2019).

First discovered in Japan in 1910, it has since spread globally, particularly in regions where rice is extensively cultivated (Kabdwal et al., 2023). Yield losses due to this pathogen range from 4-50% worldwide, depending on environmental conditions, disease severity, and the crop stage at the time of infection (Singh et al., 2003; Richa et al., 2016). The pathogen can survive for many years in soil debris, generating infections at any crop stage. It also behaves as a saprophyte, thriving on dead and decaying organic matter when the host plant is absent (Ghosh et al., 2018).

Currently, no naturally resistant rice cultivars against *R. solani* have been identified (Das et al., 2022; Iqbal et al., 2023a). As a result, chemical control remains one of the most effective methods for managing this disease (Hirooka and Ishii, 2013). Both systemic and non-systemic fungicides are used to control the disease, but systemic fungicides have been reported to be more effective (Gullino et al., 2000). Fungicide spraying remains the primary method of disease control, as crops either lack immunity or have high levels of susceptibility. However, prolonged use of a single fungicide can harm natural ecosystems and increase the risk of resistance (Corkley et al., 2022). Dual fungicides in controlled-release formulations with different modes of action may provide new solutions to these challenges (Li et al., 2021a).

In light of these issues, the present study aimed to evaluate the efficacy of different fungicides against *R. solani* under *in vitro* and greenhouse conditions.

MATERIAL AND METHODS

Sample collection

Rice plants in the Larkana district of Sindh province were severely infected with sheath blight disease. The plants exhibited grayish-white, water-soaked lesions with dark brown margins, typically more than one inch in size, located near the waterline in the fields. Infected plants were collected in paper bags with location tags and transported to the Disease Diagnostic Laboratory at Sindh Agriculture University, Tando Jam, for pathogen isolation.

Isolation and Identification of Pathogen

The infected samples were washed three times with tap water, cut into small pieces of 3-5 cm, and surface-sterilized with a 5% solution of commercial bleach (sodium hypochlorite) for 30 seconds. The pieces were then dried on sterilized filter paper. Subsequently, they were placed on potato dextrose agar (PDA) medium plates. Antibiotics (streptomycin and penicillin) at a concentration of 1 ml/L were added to the PDA medium to prevent bacterial contamination (Shi et al., 2019). Five pieces were placed in each Petri plate, sealed with parafilm tape, and incubated at 25°C ± 2°C for 4 days. After incubation, whitish-brown to pale brown colonies were observed and carefully transferred to fresh PDA plates for purification using the hyphal tip method. The morphological characteristics and taxonomy of *R. solani* were confirmed by comparison with previously reported literature (Barnett et al., 1999).

In vitro efficacy of different fungicides against *R. solani*

Five different fungicides, Duphetar Plus, Evito, Nativo, Ridomil Gold, and Shincar, were tested against *R. solani* using the poisoned food method as described by Dhingra and Sinclair (1985). These fungicides were applied at three concentrations: 10, 100, and 1000 ppm. Detailed information about each fungicide, including brand name, active ingredients, formulation, and manufacturer, has been provided in Table 1. To assess the efficacy of each fungicide, the appropriate amount was added to conical flasks containing PDA medium, which was then sterilized in an autoclave at 121°C for 20 min.

Following sterilization, the medium was poured into Petri dishes (90 mm diameter) under aseptic conditions at a volume of 15 ml per Petri plate. Additionally, 2 ml of

streptomycin and penicillin antibiotics were added in PDA medium for avoid bacterial contamination. PDA medium without fungicide served as the control. After the medium solidified, each plate was inoculated with a 0.5 mm disk of 7-day-old pure culture of the pathogen. The plates were sealed with parafilm tape and incubated at 25°C ± 2°C for 7 days. The plates were observed daily, and the average colony growth diameter was measured using a scale from the backside of the Petri dish every 24 h until the fungal growth filled each plate.

Table 1. Fungicides, active ingredients, brand names and recommended dosages of fungicides used against sheath blight of rice.

Brand name	Active ingredient	Concentration/formulation	Manufacture
Shincar	Carbendazim	52 WG	FMC
Nativo	Teboconazil + Trifloxistrobin	75 WG	Bayer
Evito	Fluoxastrobin + Tebuconazole	80 WG	Arysta
Ridomil Gold	Metalaxyl-M + Mancozeb	40g + 640g WG	Syegenta
Duphetar plus	Flumorph + Fosetyl. Aluminium	50 WG	Kanzo

Pathogenicity test

The rice seeds were purchased from a nearby shop and brought to the laboratory. The seeds were surface-sterilized with 70% ethanol and washed three times with distilled sterilized water (ddH_2O). They were then placed on 90 mm Petri dishes containing two layers of sterilized filter paper soaked in water and incubated at room temperature. After 7 days, the seedlings were transferred into 80 cm pots containing sterilized soil (1.5 kg per pot). Rice plants that were 20 days old were used to confirm pathogen risk.

To prepare the inoculum suspension of the isolated fungus, *R. solani* was cultured on a PDA medium until mature mycelium and sclerotia were produced. Fifty milliliters of distilled sterilized water were poured onto the culture plate, and the mycelia were gently scraped with a sterile applicator to create a fungal suspension. The concentration of the pathogen suspension was adjusted to 1×10^5 spores/ml using a hemocytometer.

Fifteen days after transplanting, 1 ml, 2 ml, and 3 ml of the pathogen suspension were inoculated into the rice roots, while 3 ml of ddH_2O were applied to the control plants. The plants were observed daily. After 15 days of inoculation, disease symptoms were observed, and the plants were taken to the laboratory for re-isolation of the pathogen and confirmation of pathogenicity. The incidence of sheath blight disease was recorded using the IRRI (0 to 9) rating scale (Table 2) as described by Sriraj et al. (2014). The percentage of disease index (PDI) was calculated using the formula provided by Qin and Zhang (2005).

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\text{Total No. of plant observed}} + \frac{100}{\text{Max. grade}}$$

Table 2. Sheath blight disease rating scale for rice (0-9).

Grade	Description (% leaf area affected)
0	No lesion
1	> 1
3	1-5
5	6-25
7	26-50
9	51-100

Pot experiment

To evaluate the effectiveness of different fungicides against sheath blight in rice, a pot experiment was conducted. The rice seeds were grown, transferred, and inoculated following the method described in the pathogenicity test. Each pot was inoculated with 3 ml of the pathogen suspension. Two days after inoculation, 1000 ppm of two fungicides, Shincar and Nativo, were sprayed onto the rice plants using a hand-operated atomizer, while the control plants were inoculated with only the pathogen suspension. Fifty milliliters of sterilized water were added daily, and the plants were observed.

After ten days, disease symptoms developed, and the incidence and percentage of disease index were recorded using the previously mentioned scale and formula. Plant parameters, including shoot length, root length, and plant weight, were also measured.

Statistical Analysis

The experiment was conducted using a randomized block design with six replicates for each treatment. The data obtained were subjected to ANOVA and LSD multiple comparison tests using Statistix software version 8.1. Graphs were created with GraphPad Prism (version 8.1) and further refined using Adobe Illustrator (2019).

RESULTS

Plant material

During the field survey in the Larkana district, infected rice plants exhibited small, water-soaked spots or irregularly shaped brown to dark brown lesions with irregular margins. In severe cases, infected plants showed symptoms of wilting, and leaves became necrotic and lifeless.

Isolation, identification, and purification of the pathogen

The fungal colonies that developed on PDA agar plates exhibited rapid mycelial growth. Initially, the mycelium was whitish-brown and fluffy, gradually becoming denser and more cottony over time. As the culture aged, the color shifted to tan or brown. The colonies of *R. solani* were circular or irregular in shape, with well-defined, distinct margins that appeared irregularly lobed. Over time, the culture produced sclerotia, which were compact, dark brown to black, and irregularly shaped structures. Each colony was carefully isolated using the hyphal tip method, transferred to a fresh PDA medium plate, and purified for further study.

Microscopic characteristics

A 7-day-old pure culture plate was used for microscopic study. The examination revealed the presence of septate hyphae, which were often darkly pigmented. In addition to the septate hyphae, non-septate (aseptate) hyphae were also observed. Sclerotia were carefully examined under the microscope and were found to consist of densely packed hyphae with a dark outer layer. The hyphae frequently branched at 90-degree angles.

In vitro efficacy of different fungicides on the colony growth of *R. solani*

The effect of five different fungicides *viz*. Nativo, Shincar, Duphetar Plus, Evito, and Ridomil Gold was evaluated against the colony growth of *R. solani* using the poisoned food method. The fungicides were tested at three different concentrations (10, 100, and 1000 ppm). All fungicides inhibited the mycelial growth of the pathogen to varying degrees. Among them, Nativo and Shincar were the most effective, showing maximum inhibition of mycelial growth at all concentrations. Duphetar Plus also demonstrated moderate effectiveness, with mycelial growth ranging from 18 to 20 mm across all concentrations. In contrast, Ridomil Gold and Evito were less effective, with mycelial growth ranging from 43 to 90 mm, respectively (Figure 1).

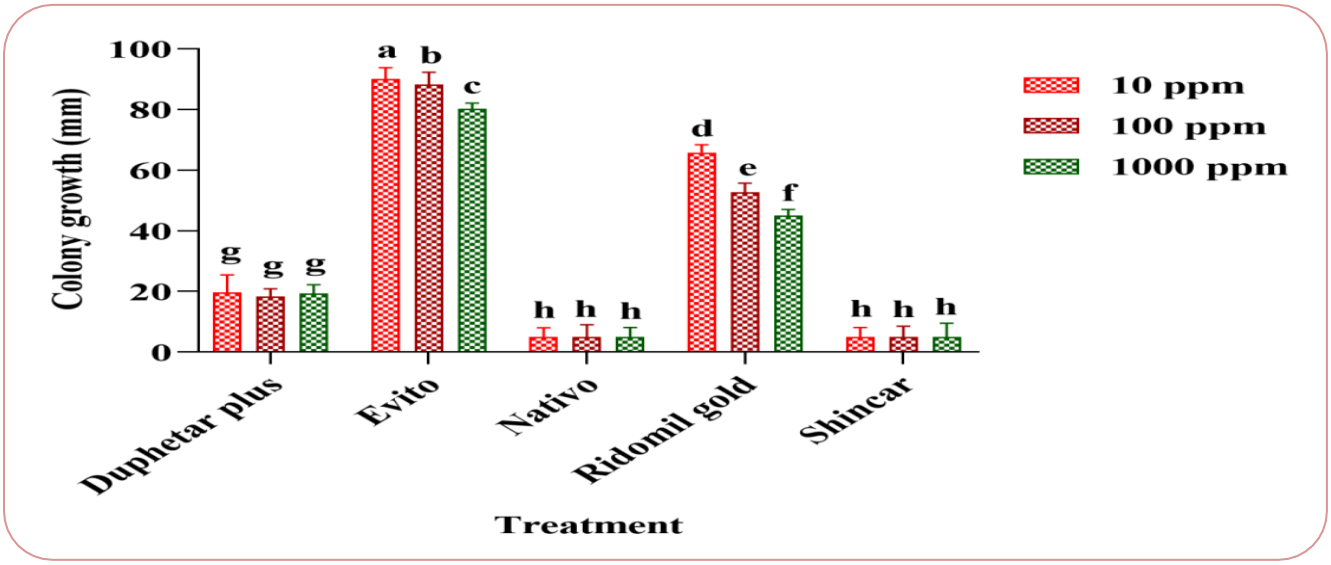

Figure 1. Response of *R. solani* to different fungicides. Each bar represents the mean ± SD from six replications, with letters indicating significant differences between treatments.

Pathogenicity test

The risk of pathogen infection was confirmed by inoculating a conidial suspension of the pathogen into 20-day-old rice seedlings. Three different volumes of inoculum suspension (1, 2, and 3 ml) were used for inoculation. Fifteen days after inoculation, rice plants treated with 2 and 3 ml suspensions exhibited typical symptoms of sheath blight disease, including water-soaked lesions on the leaf sheaths, elongated lesions with irregular margins, and white, cottony mycelial

growth at the lesion edges, compared to those treated with 1 ml and the control group. To confirm *R. solani* infection, the pathogen was successfully recovered from the diseased parts of the plants on PDA medium plates.

Disease incidence and percent disease index

The severity of the disease was dependent on the volume of the pathogen inoculum suspension applied to the rice plants. The minimum disease incidence (40%) and a (4%) percent disease index was found in 1 ml inoculum suspension inoculated plants. In contrast, the 2 and 3 ml suspensions resulted in 100% DI, with PDI of 27% and 33%, respectively (Figure 2a). Thus, the DI and PDI significantly increased with the amount of inoculum. The results indicated a strong positive correlation (0.97) between the *R. solani* inoculum level and disease incidence/PDI in rice (Figure 2b).

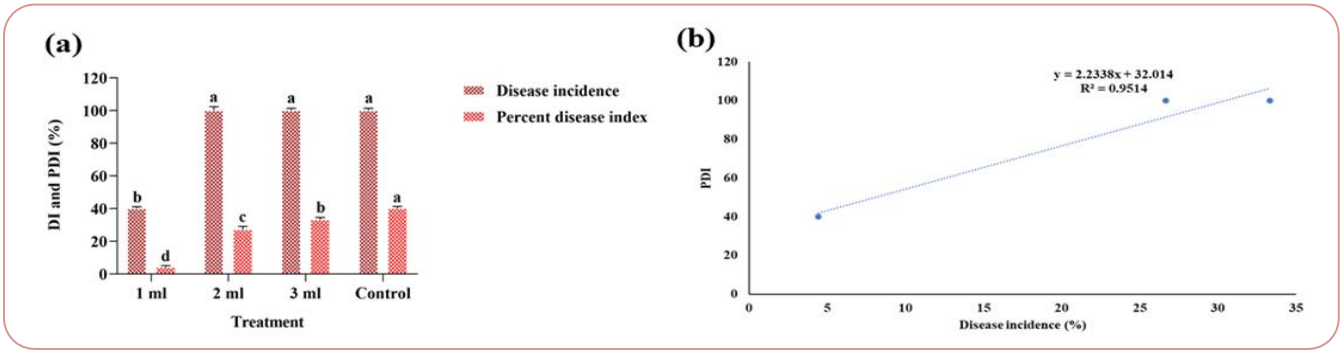

Figure 2. Disease incidence (DI) and percent disease index (PDI) of rice plants inoculated with different levels of *Rhizoctonia solani* inoculum. (a) Percentage of DI and PDI; (b) Correlation between *R. solani* inoculum level and DI and PDI. Error bars and letters indicate significant differences ($p < 0.05$).

Root and shoot length

All inoculum suspensions of the pathogen significantly reduced the root and shoot lengths of rice plants compared to the control. The shortest root and shoot lengths (7.26 cm and 14.6 cm, respectively) were observed in plants inoculated with 3 ml of *R. solani* suspension, followed by those inoculated with 2 ml (8 cm and 15.6 cm, respectively), as compared to the uninoculated control plants (Table 3).

Plant weight

Similarly, the lowest plant weight (0.10 g) was recorded in plants inoculated with 3 ml of suspension, followed by those inoculated with 2 ml (0.12 g). Plants inoculated with 1 ml of suspension showed a weight of 0.29 g, compared to the control (Table 3).

Table 3. Effect of inoculation of *Rhizoctonia solani* on root length, shoot length, and plant weight

Treatment	Root length (cm)	Shoot length (cm)	Plant weight (g)
Un-inoculated	13.6 ± 1.095 a	25.06 ± 2.167 a	0.36 ± 0.061 a
1 ml Inoculum	11.8 ± 1.923 b	23.4 ± 3.209 b	0.29 ± 0.073 b
2 ml inoculum	8.00 ± 1.303 c	15.6 ± 0.894 c	0.12 ± 0.016 c
3 ml inoculum	7.26 ± 0.54 d	14.6 ± 1.923 d	0.10 ± 0.018 d

Effect of different fungicides on disease development

To assess the effect of fungicides on the development of sheath blight disease in rice, a pot experiment was conducted. Two fungicides, Nativo and Shincar, were applied both alone and in combination with a conidial suspension of *R. solani* to rice plants. A control group was inoculated only with the pathogen's conidial suspension. Both fungicides significantly reduced disease incidence compared to the control. Shincar was found to be the most effective, resulting in the lowest disease incidence of 22.22% after 28 days of inoculation. In contrast, the control plants exhibited the highest disease incidence of 55.55%, followed by 40% in Nativo-treated plants after the same period (Figure 3). These results demonstrate that the fungicides were effective in reducing the incidence of sheath blight disease in rice plants.

Both fungicides positively impacted the prevention or mitigation of *R. solani* effects on rice plant roots

compared to the control. Shincar-treated plants had the maximum root length of 15.94 cm, followed by 14.4 cm in Nativo-treated plants, while control plants had the minimum root length of 13.32 cm (Figure 4a). Similar trends were observed for shoot length, with Shincar-treated plants showing the highest shoot length of 20.50 cm, compared to 19.02 cm for Nativo-treated plants and 16.24 cm for the control (Figure 4b). Additionally, the maximum plant weight of 0.68 g was recorded in Shincar-treated plants, followed by 0.41 g in Nativo-treated plants, with the minimum plant weight of 0.27 g in control plants (Figure 4c).

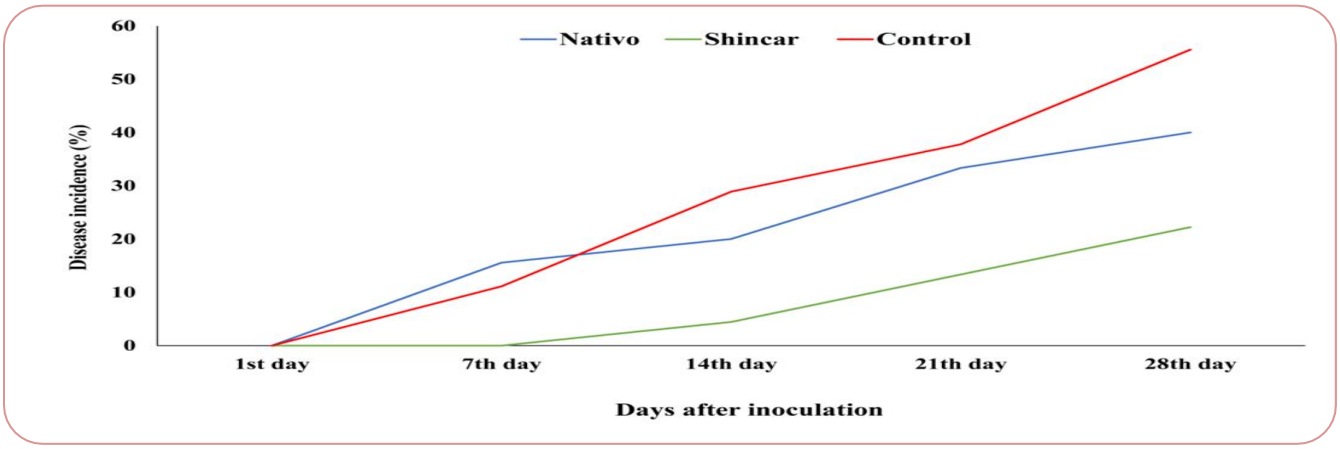

Figure 3. Comparison of disease incidence in rice plants treated with fungicides (Nativo and Shincar) vs. untreated plants inoculated with *R. solani*. Bars represent the mean ± SD of six replicates, with letters indicating significant differences among groups.

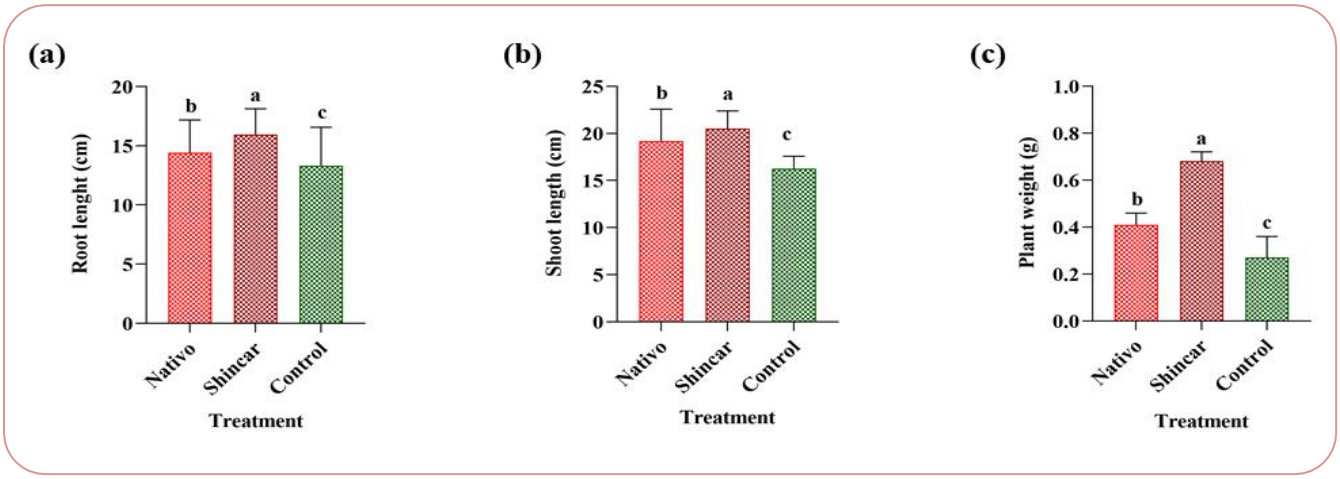

Figure 4. Effect of Nativo and Shincar fungicides on various rice growth parameters inoculated with *R. solani.* (a) Root length, (b) Shoot length, and (c) Plant weight. Each bar represents the mean ± SD of six replications. Letters indicate significant differences among treatments.

DISCUSSION

Rice is one of the most important food crops globally, feeding about 50% of the population. It serves as a model plant for researchers in genetics and agroecosystems (Wing et al., 2018) and is the second most consumed crop worldwide after wheat (Esmaeilzade-Moridani et al., 2013). Recent research has confirmed that rice faces numerous issues, including problems caused by fungi, bacteria, viruses, and nematodes. Among these, fungal diseases caused by various genera of fungi are particularly aggressive and destructive (Mahmood et al., 2019; Iqbal et al., 2023b). *Rhizoctonia solani*, which causes sheath blight disease in rice, is a major pathogen responsible for significant

annual losses in this crop (Singh et al., 2019).

Historically, various approaches have been used to control this pathogen (Mukhtar et al., 2023), but they have proven less effective and have limitations. Chemical fungicides remain one of the most important methods for controlling sheath blight disease in rice (Iqbal et al., 2014; Bag et al., 2016; Iqbal and Mukhtar, 2020; Shahbaz et al., 2023; Yaseen et al., 2024). Fungicides such as carbendazim, copper oxychloride, metalaxyl, mancozeb, and propiconazole have been used against various fungal pathogens. Of these, only carbendazim and mancozeb have been found highly effective (Hao et al., 2020; Akhtar et al., 2024).

In the present study, a survey was conducted in rice fields in the Larkana district of Pakistan to identify the causal agents of sheath blight disease. *R. solani* was isolated from infected samples using isolation methods. The pathogenicity of *R. solani* was confirmed using Koch's postulates, establishing that this pathogen is responsible for sheath blight disease in the region. Microscopic and morphological characterization further confirmed *R. solani* as the pathogen causing sheath blight, consistent with previous studies (Li et al., 2021b). According to Neha et al. (2017), *R. solani*-infected plants exhibited a high percent disease index and disease incidence compared to control plants, with disease severity depending on the inoculum level of the pathogen. In this study, five different fungicides, Duphetar Plus (flumorph + fosetyl aluminium), Evito (fluoxastrobin + tebuconazole), Nativo (tebuconazole + trifloxystrobin), Ridomil Gold (metalaxyl-M + mancozeb), and Shincar (carbendazim), were tested at three concentrations (10, 100, and 1000 ppm) using the poisoned food technique to assess their effects on the colony growth of *R. solani*.

Nativo and Shincar were found to be the most effective, completely inhibiting the mycelial growth of the pathogen at all concentrations. Duphetar Plus was moderately effective, while Evito and Ridomil Gold were the least effective against the pathogen at all concentrations. Similarly, carbendazim completely inhibited the mycelial growth of *R. solani* at all doses, as described in the literature (Boukaew et al., 2013). Dhami and Maharjan (2023) reported that Nativo, Bavistin, and Saaf fungicides completely inhibited the mycelial growth of *R. solani* at higher concentrations. Another study found that three high concentrations of two different fungicides completely inhibited the mycelial growth of *R. solani* under *in vitro* conditions (Nagaraj et al., 2005). Acharya et al. (1997) also reported that Ziram, Tolclofos methyl, Carbendazim, and Edifenphos fungicides showed complete inhibition of mycelial growth of *R. solani* at high concentrations using the poisoned food technique. Rajendraprasad et al. (2017) found that four fungicides, including Carbendazim, Captan + Hexaconazole, Propiconazole, and Tebuconazole + Trifloxystrobin, were highly effective in reducing 100% colony growth of *R. solani* in tomato. The present study also found that the minimum concentration of fungicides affected the mycelial growth of the pathogen.

In a pot experiment, two fungicides, Nativo and Shincar, were evaluated for their effectiveness against the sheath blight pathogen *R. solani*. Both fungicides demonstrated high efficacy in inhibiting pathogen infection and reducing sheath blight disease in rice seedlings. The treated plants showed significantly lower disease incidence compared to the uninoculated control plants. Among the five fungicides tested, carbendazim was found to be the most effective under field conditions. Gao et al. (2022) reported that Epoxiconazole, Hexaconazole, and Tebuconazole are likely viable substitutes for Jinggangmycin in managing rice sheath blight. Upmanyu et al. (2002) observed that foliar applications of Carbendazim at 0.1% and Tebuconazole at 0.05% effectively reduced sheath blight severity in rice plants. Similarly, Nativo fungicides reduced disease incidence by controlling *Rhizoctonia bataticola* infections in cotton (Khan et al., 2021). Khaliq et al. (2020) also found that Nativo fungicides decreased disease incidence and controlled root rot pathogens in chickpea crops under field conditions.

The application of Nativo and Shincar showed a positive effect on mitigating the impact of *R. solani* on rice plant parameters compared to the control. Shincar-treated plants exhibited the longest root length, shoot length, and weight, followed by plants treated with Nativo. Carbendazim treatment also resulted in the maximum root length, shoot length, and both fresh and dry weight of rice plants (Kabdwal et al., 2023). Neha et al. (2016) reported that carbendazim-treated rice plants had significantly greater root and shoot lengths compared to the control. Under *in vivo* conditions, both Shincar and Nativo were effective in inhibiting the development of sheath blight disease in sugarcane. Some fungicides have been found to be moderately to highly effective against sheath blight disease in rice (Meena et al., 2018).

CONCLUSION

The study successfully isolated and identified *Rhizoctonia solani*, the pathogen responsible for sheath blight disease in rice. The symptoms and pathogen identification confirmed the presence of sheath blight. The effectiveness of five different fungicides against the colony growth of *R. solani* was evaluated. Among these, Nativo and Shincar fungicides proved to be the most effective, reducing pathogen growth at both lower and higher doses *in vitro* and at maximum concentrations *in vivo*. Therefore, it is recommended that both fungicides be used to control sheath blight disease in rice, as they effectively reduce *R. solani* infection and positively impact plant growth parameters under field conditions.

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AUTHORS' CONTRIBUTIONS

BAM, SZ and GYD designed the study and did experimental works; NK and TH collected data, MAR and SHS contributed in statistical analysis and paper writing; OI did monitoring, evaluation, and edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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