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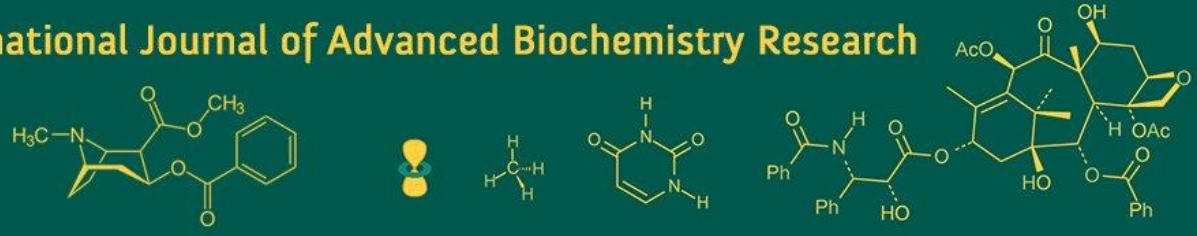

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In vitro evaluation of fungicides and bio-agents against *Rhizoctonia solani* causing sheath blight disease of rice

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Abstract
Sheath blight of rice caused by *Rhizoctonia solani* is one of the threat limiting to rice cultivation in India. Considering economic importance of the crop as well as destructive nature of the disease, present *in vitro* studies were undertaken to evaluate the efficacy of seven fungicides and six bio-agents, to assess their potential against *R. solani.* Results indicated that at a lower concentration, mycelial growth inhibition was numerically highest and cent per cent (100%) with Hexaconazole 5% EC, Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 11% + Tebuconazole 18.3% SC. Whereas, at a higher concentration, mycelial growth inhibition was numerically highest and cent per cent (100%) with Hexaconazole 5% EC, Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin 11% + Tebuconazole 18.3% SC and Azoxystrobin 23% SC. Among the six bio-agents tesed *Trichoderma harzianum* recorded significantly highest mycelial growth inhibition (63.89%) of the test pathogen, followed by *Pseudomonas fluorescens* (53.34%), *T. longibrachiatum* (34.45%), *T. konigii* (32.78%) and *Bacillus subtilis* (30.56%).

Keywords: Sheath blight, *Rhizoctonia solani*, fungicides, bio-agents, inhibition

1. Introduction
Rice (*Oryza sativa* L.) belongs to family Poaceae, serves as the primary diet for approximately 67% of the world population. In the Asian region, the demand for rice production is the highest in the world, due to the increased preference for rice among the population (Mohanty, 2013) [7]. Rice plays a vital role in sustaining life and boosting the global economy. The crop tenants a significant position in the culture and heritage of many Asian countries. In India, particularly in the eastern states, it is apart of almost every ritual. The crop has been referred in the Vedas, Ramayana, Mahabharata, Buddhist and other ancient literature (Pathak *et al.*, 2018) [12].

Rice grown in different agro ecological conditions, such as deep water, waterlogged lands, hilly terrains, areas experiencing high humidity or temperatures, saline and alkaline soils and flood-susceptible regions in India. The cropping intensity differs from one environment to the other with a maximum of three rice growing seasons in a year in the fertile deltaic regions due to availability of continuous irrigation.

Rice is a highly nutritious, easily digestible and palatable cereal, making it an excellent staple for daily meals. It's packed with 80% carbohydrates, 7-8% protein, 3% fat, and 3% fiber, along with essential minerals like iron, zinc, potassium, manganese and copper. It also provides crucial essential amino acids such as tryptophan, histidine, methionine, cysteine and arginine. Beyond its direct consumption, rice has diverse applications. It can be processed into various food products like ice cream, gel, bread, snacks, cookies and biscuits. Industrially, it's used to produce edible oil, cosmetics, synthetic fibers, detergents, emulsifiers, soap and fatty acids.

The crop is vulnerable to a variety of fungal, bacterial, viral and nematode diseases. The major rice diseases that often cause great economic losses are Rice blast (*Magnaporthe grisea*), Brown leaf spot (*Helminthosporium oryzae*), Sheath blight (*Rhizoctonia solani*), Sheath rot (*Sarocladium oryzae*), False smut (*Ustilaginoidea virens*), Bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae*), Bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzicola*) and Rice tungro disease (Rice Tungro Baciliform and Spherical Viruses) negatively impact

both the quality and quantity of rice yield, affecting the crop from the nursery stage all the way to harvest (Narasimhamurthy *et al*., 2021) [9].

Among these diseases Sheath blight caused by *Rhizoctonia solani* is one of the threat limiting to rice cultivation in India. The yield losses upto 50% have been reported depending on the crop stage at the time of infection, severity of the disease and environmental conditions (Singh *et al*., 2004; Zheng *et al.*, 2013; Bhunkal *et al*., 2015) [19, 23, 2]. Considering economic importance of the crop as well as destructive nature of the disease, present *in vitro* studies were undertaken to evaluate the efficacy of fungicides and bio-agents, to assess their potential against *R.solani.*

2. Materials and Methods

The present study was conducted at the Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli during the year 2023-24.

2.1. *In vitro* evaluation of fungicides

Various standard commercial formulation of fungicides were evaluated *in-vitro* against *Rhizoctonia solani* by applying standard poisoned food technique (Nene and Thapliyal, 1993) [10] and using potato dextrose agar as basal culture medium.

Based on active ingredient, the required quantity of fungicide was calculated and mixed thoroughly with sterilized potato dextrose agar (PDA) medium in conical flasks to obtained desired concentration of fungicides. PDA medium without fungicides was served as untreated control. Fungicides amended PDA poured in Petri plates and allow to solidify at room temperature. After solidification of the medium, all the plated were inoculated with 5 mm culture disc of the test fungus obtained from a week old growing pure culture *R. solani*. The disc was placed on PDA in inverted position in the centre of the Petri plate and plates were incubated at 27 ± 2°C temperature. The present *in vitro* experiment was conducted using a completely randomized design, with three replications maintained for each treatment.

Observation on radial mycelial growth/colony diameter were recorded when untreated control plate was fully covered with mycelial growth of test fungus. Per cent mycelial growth inhibition of the test fungus by the test fungicides over untreated control was calculated by formula given by Vincent, (1947) [22].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent Inhibition

C = Growth (mm) of the test fungus in untreated control plate

T = Growth (mm) of the test fungus in treated plates.

2.2. *In vitro* evaluation of bioagents

A total of six bio-agents were evaluated against *R. solani* by applying Dual culture technique (Dennis and Webster, 1971) [4] and using PDA as a basal medium. Seven days old culture of test fungus and test bio-agents were used for the study. Disc of PDA along with culture growth of test fungus and test bio-agents were cut out with cork borer and placed on Petri plates containing PDA at equidistance and exactly opposite to each other and the plates were incubated at 27 ± 2 °C. PDA plates inoculated with only culture disc of test fungus were maintained as untreated control. The present *in vitro* experiment was conducted using a completely randomized design, with three replications maintained for each treatment.

Observations on radial mycelial growth or diameter of colony of the test pathogen were taken after seven days of inoculation. Per cent mycelial growth inhibition of the pathogen by the bio-agents over untreated control was calculated by using the formula given by Vincent (1947) [22].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Percent Growth Inhibition

C = Growth (mm) of test fungus in control plate.

T = Growth (mm) of test fungus in treated / intersecting plate.

3. Results and Discussion

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

The efficacy of seven fungicides *viz*., Azoxystrobin 23% SC, Propiconazole 25% EC, Hexaconazole 5% EC, Thifluzamide 24% SC, Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin 11% + Tebuconazole 18.3% SC and Carbendazim12% + Mancozeb 63% WP were evaluated against *R. solani* at two different concentration. It was observed from the result that, as the concentration of the fungicides increased, there was a drastic decrease in mycelial growth and an increase in inhibition of mycelial growth (Table 1, Plate I and Fig.1).

3.1.1. Radial mycelial growth

Result (Table 1, Plate I and Fig.1) revealed that at a lower concentration, the radial mycelial growth of *R. solani* ranged from 0.00 mm to 33.50 mm. However, Hexaconazole 5% EC, Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 11% + Tebuconazole 18.3% SC arrested the mycelial growth of the test pathogen (0.00 mm). These were followed by Azoxystrobin 23% SC (13.50 mm), Carbendazim 12% + Mancozeb 63% WP (18.50 mm), Propiconazole 25% EC (23.50 mm) and Thifluzamide 24% SC (33.50 mm), as against complete mycelial growth (90.00 mm) in untreated control.

At a higher concentration, the radial mycelial growth of *R. solani* ranged from 0.00 mm to 25.5 mm. However, Hexaconazole 5% EC, Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 11% + Tebuconazole 18.3% SC and Azoxystrobin 23% SC did not showed mycelial growth of the test pathogen. These were followed by Carbendazim 12% + Mancozeb 63% WP (15.00 mm), Propiconazole 25% EC (19.00 mm) and Thifluzamide 24% SC (25.00 mm), as against maximum mycelial growth (90.00 mm) in untreated control.

Table 1: *In vitro* efficacy of fungicides against *R. solani*

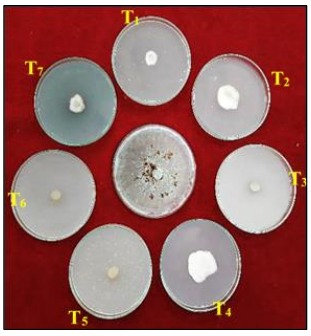
Tr. No.	Treatments	Conc. (%) used		Mean colony diameter (mm)		Per cent inhibition	
		Lower Conc. (%)	Higher Conc. (%)	Lower Conc. (mm)	Higher Conc. (mm)	Lower Conc. (%)	Higher Conc. (%)
T_1	Azoxystrobin 23% SC	0.1	0.15	13.5	00.00	85.00 (67.21)**	100.0 (90.00)
T_2	Propiconazole 25% EC	0.1	0.15	23.5	19.00	73.89 (59.27)	78.89 (62.65)
T_3	Hexaconazole 5% EC	0.1	0.15	00.00	00.0	100.0 (90.00)	100.0 (90.00)
T_4	Thifluzamide 24% SC	0.1	0.15	33.5	25.5	62.78 (52.40)	71.67 (57.84)
T_5	Tebuconazole 50% + Trifloxystrobin 25% WG	0.1	0.15	00.00	00.0	100.0 (90.00)	100.0 (90.00)
T_6	Azoxystrobin 11% + Tebuconazole 18.3% SC	0.1	0.15	00.00	00.0	100.0 (90.00)	100.0 (90.00)
T_7	Carbendazim12% + Mancozeb 63% WP	0.2	0.25	18.5	15.00	79.44 (63.04)	83.33 (65.90)
T_8	Control	----	----	90.00	90.00	----	----
SE±				0.44		0.42	
CD at 1%				1.35		1.28	

*: Mean of three replications ** Values in parentheses are arc-sine transformed values

3.1.2. Mycelial growth inhibition (%)

Results showed in Table 1, Plate I and Fig. 1 stated that, at a lower concentration, mycelial growth inhibition of *R. solani* varied from 62.78 to 100 per cent. However, it was numerically highest and cent per cent (100%) with Hexaconazole 5% EC, Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 11% + Tebuconazole 18.3% SC. These were followed by Azoxystrobin 23% SC (85.00%), Carbendazim 12% + Mancozeb 63% WP (79.44%), Propiconazole 25% EC (73.89%) and Thifluzamide 24% SC (62.78%).

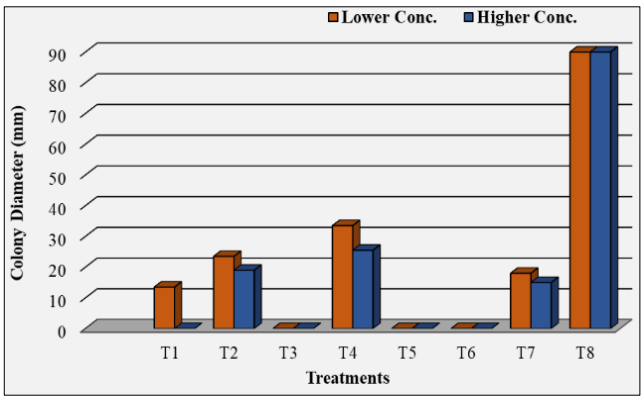
At a higher concentration, mycelial growth inhibition of *R. solani* varied from 71.67 to 100 per cent compared to untreated control (0.00%). However, it was numerically highest and cent per cent (100%) with Hexaconazole 5% EC, Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin 11% + Tebuconazole 18.3% SC and Azoxystrobin 23% SC, which was followed by Carbendazim 12% + Mancozeb 63% WP (83.33%), Propiconazole 25% EC (78.89%) and Thifluzamide 24% SC (71.67%).



Lower Conc.

Higher Conc

Plate I: *In vitro* efficacy of fungicides against *Rhizoctonia solani*



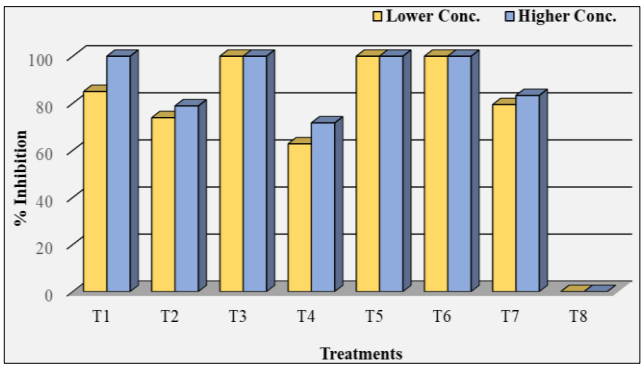


Fig 1: *In vitro* efficacy of fungicides against *R. solani*

These results are in conformity to the findings of several earlier workers. Pal and Mandal (2015) [11] evaluate *in vitro* efficacy of seven fungicides (each @ 100, 200 and 500 ppm) against *R. solani*, causing rice sheath blight and reported maximum mycelial growth inhibition of test pathogen with Azoxystrobin 18.2% + Difenoconazole 11.4% SC (78.9%, 100.00% and 100.00%), followed by Tebuconazole 50% + Trifloxystrobin 25% WG (71.8%, 96.3% and 100.00%). Mohanty *et al*. (2020) [8] reported that Tebuconazole 50% + Trifloxystrobin 25% WG and Hexaconazole 5% SC recorded cent per cent (100%) mycelial growth inhibition of *R. solani*, (each @ 200 ppm), followed by Propiconazole 25% EC (93.10%), Azoxystrobin 25% EC (90.60%). Madhavi *et al*. (2021) [6] reported complete mycelial growth inhibition of the *R. solani*, was observed due to Propiconazole 25% EC, Tebuconazole 25.9% EC, Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin 23% EC and Carbendazim 50% WP (each @ 100, 500 and 1000 ppm), followed by Carbendazim 12% + Mancozeb 63% WP.

The results of present investigation are also in close consonance with earlier reports of Roy *et al*., 2022 [16]; Chauhan and Singh, 2022 [3]; Pawar *et al*., 2024 [14] and Sahu *et al*., 2025 [17].

3.2. *In vitro* efficacy of bioagents against *R. solani*

Four fungal bioagents *viz*., *Trichoderma viride*, *T. harzianum*, *T. longibrachiatum*, *T. konigii* and two bacterial bioagents *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* were tested against *R. solani in vitro.*

Among bioagents tested, *T. harzianum* was found most effective with least mycelial growth (32.50 mm) and highest mycelial growth inhibition (63.89%) followed by *Pseudomonas fluorescens* (42.00 mm and 53.34%), *T. longibrachiatum* (59.00 mm and 34.45%), *T. konigii* (60.50 mm and 32.78%). *Bacillus subtilis* exhibited highest mycelial growth (62.50 mm) and least mycelial growth inhibition (30.56%) of *R. solani* (Table 2, Plate II and Fig.2).

Table 2: *In vitro* efficacy of bioagents against *R. solani*

Tr. No.	Bioagents	Colony diameter of test pathogen (mm)*	Per cent Inhibition
T_1	*Trichoderma viride*	61.50	31.67 (34.25)**
T_2	*T. harzianum*	32.50	63.89 (53.06)
T_3	*T. longibrachiatum*	59.00	34.45 (35.94)
T_4	*T. koningii*	60.50	32.78 (34.93)
T_5	*Pseudomonas fluorescens*	42.00	53.34 (46.92)
T_6	*Bacillus subtilis*	62.50	30.56 (33.56)
T_7	Control	90.00	----
SE±		0.64	
CD at 1%		1.98	

* Mean of three replications ** Values in parentheses are arc-sine transformed values

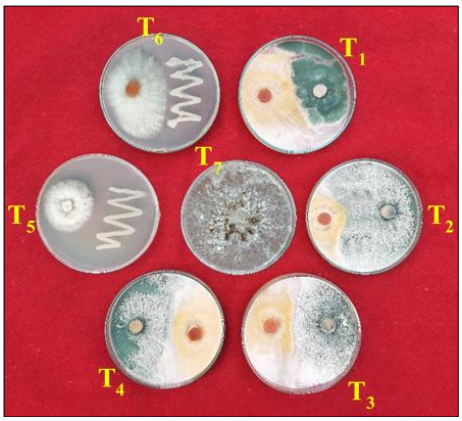


Plate II: *In vitro* efficacy of bioagents against *Rhizoctonia solani*

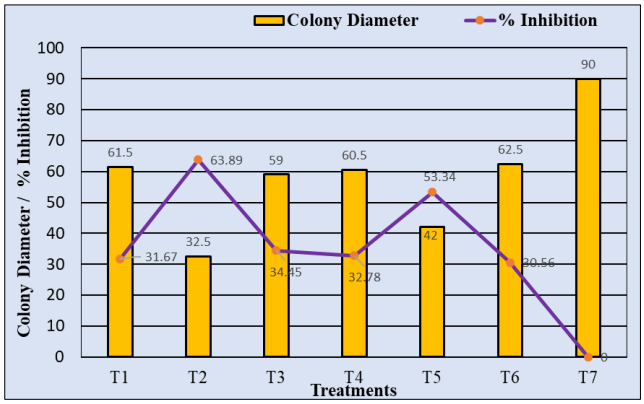


Fig 2: *In vitro* efficacy of bioagents against *R. solani*

These results of the present study are in consonance with the reports of several earlier scientists. Singh *et al*. (2008) [20] evaluated *in vitro* efficacy of four bioagents against *R. solani* and reported that highest mycelial growth inhibition with *Trichoderma harzianum* (75.55%), followed by *T. viride* (65.93%), *Gliocladium virens* (57.77%) and least inhibition was found with *Aspergillus* sp. (45.74%). Similarly, Patole and Narute (2012) [13] reported that *T. harzianum* resulted with highest mycelial growth inhibition (82.15%) of *R. solani*, followed by *T. viride* (70.22%), *T. hamatum* (67.97%) and *B. subtilis* (55.98%). Hussain *et al*. (2014) [5] reported that highest mycelial growth inhibition with *T. harzianum* resulted with significantly, maximum mycelial growth inhibition of *R. solani*. Rajput and Zacharia (2017) [15] reported that *Trichoderma harzianum recorded* highest mycelial growth inhibition (63.37%) of *R. solani* causing sheath blight of paddy, followed by *T. asperellum* (58.16%). Sharma *et al.* (2019) [18] reported that *T. viride* recorded highest mycelial growth inhibition (74.44%) of *R. solani*, followed by *T. harzianum* (68.14%), *T. longibrachiatum* (67.41%), *Pseudomonas fluorescens* I (56.66%), *P. fluorscens* II (55.74%) and *Bacillus subtilis* (47.77%). Soundarya *et al*. (2021) [21] reported that *P. fluorescens* Pf 3 resulted with significantly highest mycelial growth inhibition (76.40%), followed by, *P. fluorescens* Pf 1(73.64%) and *P. fluorescens* Pf 4 (70.63%). Banne and Suryawanshi (2023) [1] reported that *T. asperellum* resulted with highest mycelial growth inhibition (87.42%), which was on par with *T. harzianum* (86.17%), followed by *T. virens* (66.36%), *Aspergillus niger* (65.16%), *Metarhizhium anisopliae* (59.75%), *Pseudomonas fluorescens* (38.55%) and *Bacillus subtilis* (33.95%).

4. Conclusion

From the results, it is concluded that Hexaconazole 5% EC, Tebuconazole 50% + Trifloxystrobin 25% WG and Azoxystrobin 11% + Tebuconazole 18.3% SC (each @ 0.1 and 0.15% concentration) found most effective with cent per cent mycelial growth inhibition of *R. solani*, causing sheath blight disease of rice. Amongst the six bioagents evaluated *in vitro* against *R. solani*, *Trichoderma harzianum* and *Pseudomonas fluorescens* were found most effective. Thus, judicious use of these fungicides and bio-agents can be recommended to combat sheath blight disease of rice.

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