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In vitro evaluation of fungicides and bioagents against *Sclerotium rolfsii* causing collar rot in elephant foot yam

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Abstract
Elephant Foot Yam (*Amorphophallus paeoniifolius*) referred as "King of tubers" belongs to the family-*Araceae* is considered as one of the main tuber crop cultivated in India and worldwide. Among various diseases that effects its yields, collar rot caused by *Sclerotium rolfsii* is considered as one of the main constraints for its cultivation in India. This disease can cause yield loss upto 100% based on the favourable conditions to the causal organism. To manage this disease effectively at field level, ten fungicides (copper oxy chloride, mancozeb, thiophanate methyl, propiconazole, azoxystrobin, flusilazole, tebuconazole, difenoconazole, carbendazim + mancozeb and carboxin + thiram) were tested @ 500, 1000, 1500, 2000 & 2500 ppm. All the tested fungicides significantly controlled the mycelial growth of *S. rolfsii* at 2500ppm except copper oxy chloride and thiophanate methyl. At 500, 1000, 1500, 2000 ppm, the fungicides *i.e.*, propiconazole, azoxystrobin, flusilazole, tebuconazole and carboxin + thiram has completely inhibited the mycelial growth of *S. rolfsii*. Among the tested bioagents, *Bacillus subtilis* shows highest inhibition percentage (83.05%) followed by *P. fluorescens* (81.11%), *T. harzianum* (75%), *T. viride* (69.72%) and *T. reesei* (63.33%). As per the results obtained, application of fungicides @ 500 ppm: propiconazole, azoxystrobin, flusilazole, tebuconazole and carboxin + thiram, @2000 ppm- carbendazim + mancozeb and difenoconazole and @ 2500 ppm mancozeb, and under bioagents *Bacillus subtilis* effectively controls the growth of *S. rolfsii*.

Keywords: *Sclerotium rolfsii*, fungicides, bioagents, Elephant foot yam, collar rot

Introduction
Elephant Foot Yam (*Amorphophallus paeoniifolius*) referred as "King of tubers" and it is one of the important commercial tuber crop grown in India for its tubers which is commonly known as Suran or Jimmikand under family *Araceae*. It is grown for vegetable purpose and also used in pharmaceutical industry for preparing medicines in curing tumours, swelling of lungs, asthma, and as a blood purifier. (Chattopadhayay *et al*., 2009) [3].

In India, elephant foot yam (EFY) is cultivated in an area of 32,000 ha with production of 8,08,000 MT in the states of Andhra Pradesh, W.B, U.P, W.B, Jharkhand, Bihar and Gujarat (NHB, 2019-2020) (www.nhb.gov.in). It thrives best at 25 ° to 35 °C with annual requirement of 1000-1500 mm rainfall on sandy loam, fertile and well-drained soils. In Andhra Pradesh, it is cultivated majorly in East and West Godavari, Krishna, Guntur and Vizianagaram districts.

Elephant foot yam (EFY) as like other horticultural crops is affected with diseases such as Collar rot (*Sclerotium rolfsii*), foot rot (*Rhizoctonia solani*), Leaf spot (*Cornyspora cassiicola* Berk and Curt), Anthracnose (*Colletotrichum gloeosporioides* Penz.), Bacterial leaf spot (*Xanthomonas campestris* pv *amorphophalli*), Mosaic (*Elephant foot yam mosaic virus*), etc. (Divya *et al*., 2019) [6].

Among the fungal diseases, collar rot caused by *S. rolfsii* is considered as the main constraint for its cultivation in India (Sivapraksam *et al*., 1982) [4]. *S. rolfsii* is being well known polyphagous, ubiquitous omnivorous and most destructive soilborne fungus (Praveen *et al*., 2012) [10]. Teleomorphic stage of *S. rolfsii* is *Athelia rolfsii* (Curzi) Tu & Kimbrough which is soilborne that is widely distributed in the tropical and warm temperate regions of the world (Mehri *et al*., 2013) [1].

The collar rot caused by *S. rolfsii* causes significant yield losses of EFY about 20 to 100% due to its wide host range such as pepper, tomato, groundnut, watermelon, potato and sweet potato and more severe in rainy season followed by warm dry weather conditions (Neetha *et al*.,

2014) [9]. On close observation of collar rot infected elephant foot yam plants, collar region has deep cracks with roots getting shredded and with full of white mycelial growth (Kalmesh and Gurjar, 2001) [2].

Materials and Methods

Isolation of *S. rolfsii*

In the present study, the infected samples were collected from different locations of East & West Godavari (Kovvur, Thogummi, Venkataramannagudem, Vemuluru, Vadapalli) of Andhra Pradesh during the 2022-23 cropping season. Using a sterile scalpel, small piece of tissue (5 mm) from infected roots or stems were separated along with healthy tissue. $HgCl_2$ of 0.1 percent was used to surface sterilize the tissues for one minute. To remove mercury ions, the tissues were rinsed in sterile distilled water for three times. The surface sterilized tissues were transferred on to Potato Dextrose Agar (PDA) and incubated at 27 ± 1 °C in BOD incubator and growth was recorded day to day upto seven days (Aneja, 2003) [8].

In vitro evaluation of fungicides against *S. rolfsii*

In vitro efficacy of ten fungicides *viz*., copper oxy chloride (50% WP), mancozeb (75% WP), thiophanate methyl (70% WP), propiconazole (25% EC), azoxystrobin (23% EC), flusilazole (40% EC), tebuconazole (250 EC), difenoconazole (25% EC), carbendazim (12%) + mancozeb (63%) WP, carboxin 37.5% + thiram 37.5% (75WS) @ 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm were evaluated against *S. rolfsii* by poisoned food technique on PDA medium. Based on the active ingredient, the necessary quantity of each fungicide was estimated and applied separately to PDA in conical flasks shortly before pouring into petri plates. The test fungus, which were taken from the seven-day-old, actively growing pure culture, were inoculated aseptically and individually into the centre of each plate after the PDA had solidified. At 27 ± 1 °C, all of the plates were incubated. The experiment was designed in CRD and all the treatments were replicated thrice. Observations on radial mycelial growth were recorded in all the treatment plates, after complete growth noticed in control plates (four days), and per cent inhibition was calculated by applying the formula given by Vincent (1947) [7].

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

Where,
I= Per cent inhibition of mycelial growth
C = Fungal growth in control (mm)
T = Fungal growth in treatment (mm)

In vitro evaluation of bioagents against *S. rolfsii*

Dual culture technique was conducted study the effect antagonistic organisms *viz*., *T. viride, T. harzianum, T. reesei, P. fluorescens* and *B. subtilis* on *S. rolfsii*. Twenty ml of sterilized and cooled PDA was added to sterilized Petri plates for the *in vitro* evaluation of antagonists. By inoculating the pathogen on one side of the Petri plates and the fungal antagonist on the exact opposite side of the same plate with a 3 to 4 cm gap, the fungal antagonists were assessed. Freshly grown cultures were used for this purpose. Two mycelial discs of the pathogen were inoculated in the case of the bacterial antagonist, and the bacterial antagonist was streaked in the centre of the Petri dish. The radial growth of the pathogen was measured after the required incubation period, or when the growth in the control plate reached 90 mm in diameter, or four days. The percent inhibition over control was worked out according to the equation given Vincent (1947) [7].

Results

In vitro evaluation of fungicides against *S. rolfsii*

The results presented in the Table 1 and Fig 1 clearly revealed that all the fungicides significantly inhibited the growth of the pathogen at 2500 ppm concentration except copper oxychloride and thiophanate methyl when compared with check. Propiconazole, azoxystrobin, flusilazole, tebuconazole and carboxin 37.5% + thiram 37.5% completely inhibited the growth at all five concentrations. Copper oxy chloride showed its highest inhibition (9.26%) at 2500 ppm concentration having a mycelial growth of 81.67 mm and no inhibition at 500 ppm concentration. Thiophanate methyl showed its highest inhibition (49.63%) at 2500 ppm concentration having a mycelial growth of 45.33 mm and no inhibition at 500ppm concentration. Difenoconazole showed 100% inhibition at 2000 and 2500 ppm concentration. Carbendazim (12%) + mancozeb (63%) shows 100 percent inhibition at 2000 and 2500 ppm concentration and low inhibition (69.63%) at 500 ppm concentration.

The results are in conformity with Pawar *et al*. (2021) [15], Shirsole (2019) [11], Rimamay *et al* (2021) [13], Divya *et al* (2019) [6] and Chandra Sekhar (2020) [14]. Pawar *et al*. (2021) [15] evaluated tebuconozole@500 and 1000ppm and got 92.97% and 94.36% of inhibition of mycelial growth. Azoxystrobin@ 500 and 1000ppm shows 8.18% and 39.19% inhibition. Thiophanate methyl@ 500 and 1000ppm shows 42.16% and 67% inhibition. Mancozeb @1500 and 2000ppm shows 94% and 94.44% inhibition. Carboxin + thiram @1500 and 2000ppm shows 94.35% and 94.44% inhibition. Carbendazim + mancozeb @1500 and 2000 ppm shows 91.33% and 93.70% inhibition. Shirsole (2019) [11] evaluated propiconazole, azoxystrobin, thiophanate methyl @500ppm and got 100%, 100% and 37.49% inhibition of mycelial growth respectively. Rimamay *et al* (2021) [13] evaluated copper oxy chloride and mancozeb@1000 ppm and 2000 ppm, got 1.88%, 3.66%, 100% and 100% inhibition of mycelial growth respectively. Thiophanate methyl and propiconazole@ 500ppm and 1000ppm got 82.22%, 89.66%, 100% and 100% inhibition of mycelial growth respectively. Divya *et al* (2019) [6] evaluated thiophanate methyl and propiconazole@ 1000ppm, got 0% and 100% inhibition of mycelial growth respectively. Mancozeb and copper oxy chloride@ 2500 ppm, got 100% and 8.5% inhibition of mycelial growth respectively. Sekhar *et al* (2020) [14] evaluated difenoconazole and mancozeb@ 500ppm and 1000 ppm and got 26.48%, 92.38%, 28.59% and 93.44% of inhibition of mycelial growth respectively.

Table 1: *In vitro* evaluation of different fungicides against *S. rolfsii*

S. No	Treatments	Mean mycelial growth(mm)					Total mean	Percent inhibition					Total mean
		500 ppm	1000 ppm	1500 ppm	2000 ppm	2500 ppm		500 ppm	1000 ppm	1500 ppm	2000 ppm	2500 ppm	
1	Mancozeb (75%WP)	58.33 (49.78)*	5.67 (13.77)	5.30 (13.30)	4.80 (12.65)	0.00 (0.00)	14.82 (22.63)	35.18 (36.37)	93.71 (75.45)	94.11 (75.93)	94.66 (76.61)	100 (89.97)	83.53 (66.03)
2	Copper oxy chloride (50%WP)	90 (71.54)	87.67 (69.42)	86.33 (68.27)	84.50 (66.79)	81.67 (64.63)	86.03 (68.03)	0 (0.00)	2.6 (9.28)	4.07 (11.63)	6.11 (14.31)	9.26 (17.71)	4.40 (12.12)
3	Thiophanate methyl (70%WP)	63.33 (52.71)	58.67 (49.97)	54.67 (47.66)	48.33 (44.03)	45.33 (42.30)	54.07 (47.32)	29.63 (32.97)	34.82 (36.15)	39.26 (38.78)	46.30 (42.86)	49.63 (44.71)	39.92 (39.18)
4	Propiconazole (25% EC)	0 (0.00)	0 (0.00)	0 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)
5	Azoxystrobin (25% EC)	0 (0.00)	0 (0.00)	0 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)
6	Flusilazole (40% EC)	0 (0.00)	0 (0.00)	0 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)
7	Tebuconazole (250 EC)	0 (0.00)	0 (0.00)	0 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)
8	Difenoconazole (25% EC)	13.33 (21.41)	9.33 (17.78)	5.00 (12.92)	0.00 (0.00)	0.00 (0.00)	5.53 (13.60)	85.18 (67.33)	89.63 (71.19)	94.44 (76.33)	100 (89.97)	100 (89.97)	93.85 (75.61)
9	Carbendazim (12%) + mancozeb (63%) WP	27.33 (31.51)	21.33 (27.50)	5.67 (13.37)	0.00 (0.00)	0.00 (0.00)	10.87 (19.24)	69.63 (56.64)	76.3 (60.84)	93.71 (75.45)	100 (89.97)	100 (89.97)	87.92 (69.64)
10	Carboxin 37.5% + thiram 37.5% WS	0 (0.00)	0 (0.00)	0 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)	100 (89.97)
11	Control	90 (71.54)	90 (71.54)	90 (71.54)	90 (71.54)	90 (71.54)	90 (71.54)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	C.D(0.01)	1.876	1.423	1.454	1.219	0.42							
	S.E(m)±	0.636	0.482	0.493	0.413	0.142							
	S.E(d)	0.899	0.682	0.697	0.584	0.201							
	C.V (%)	3.538	3.368	3.801	3.455	1.248							

*Figures in parentheses are angular transformed values

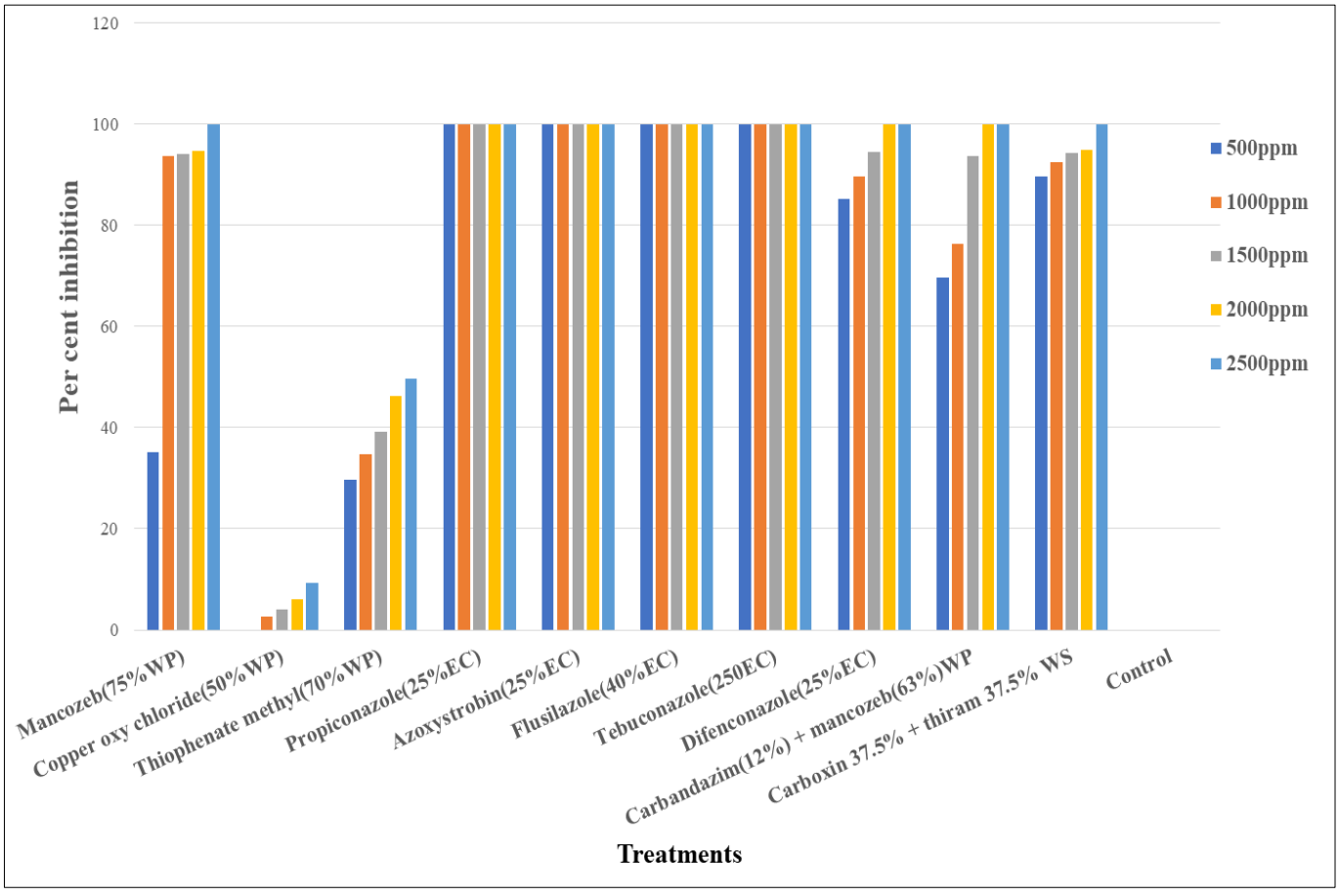

Fig 1: *In vitro* evaluation of different fungicides against *S. rolfsii*

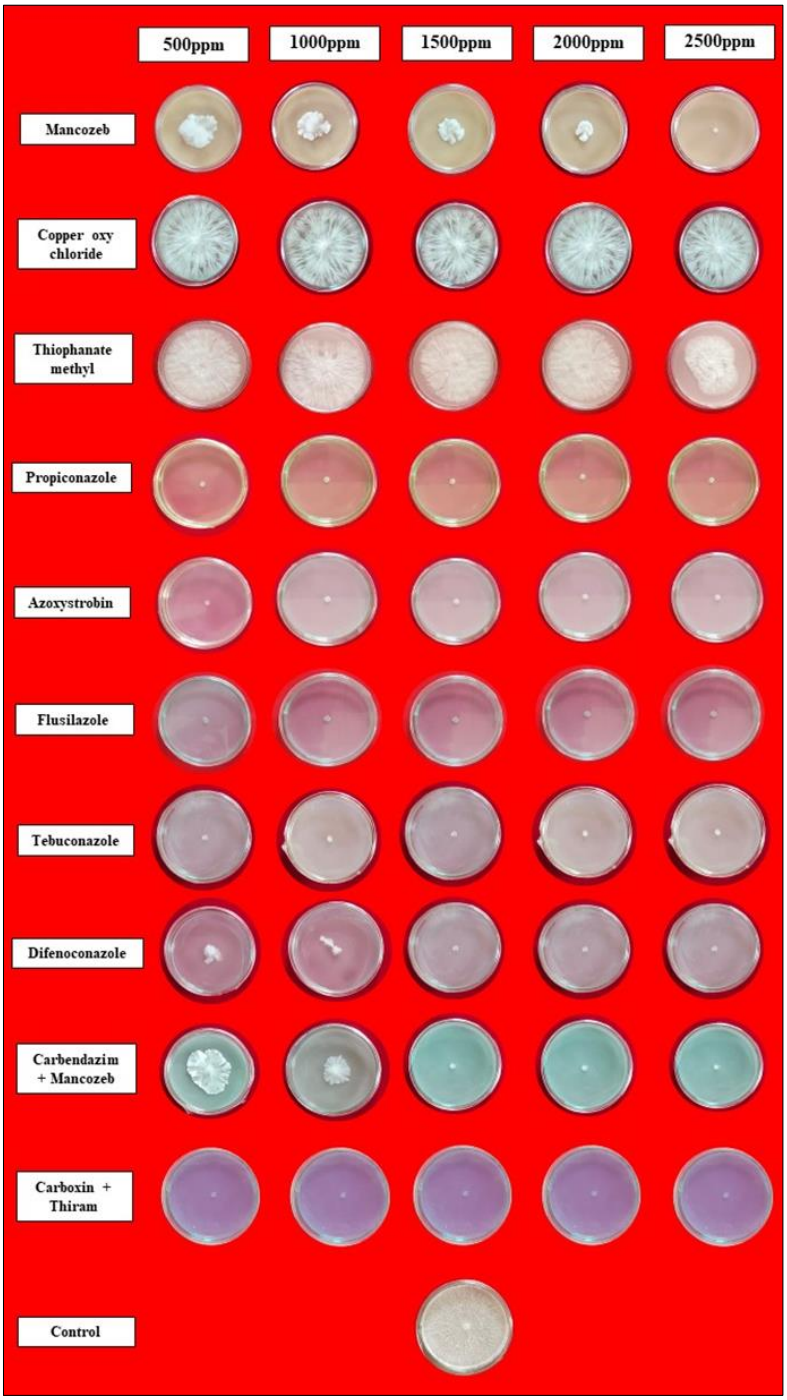

Plate 1: *In vitro* evaluation of different fungicides against *S. rolfsii*

In vitro evaluation of bioagents against *S. rolfsii*

The results presented in Table-2 and Fig.2 showed, among all the tested bioagents, *B. subtilis* was significantly superior with highest inhibition of 83.05 per cent followed by *P. fluorescens* with 81.11% inhibition which at par with each other. The other bioagents *T. harzianum* (75%), *T. viride* (69.72%) inhibited the mycelial growth which were significantly different and the least effective bioagent was found to be *T. reesei* with 63.33 per cent mycelial growth inhibition.

The results are in conformity with Mool chand *et al* (2019) who observed that *T. viride*, *T. harzianum*, *P. fluorescens* and *B. subtilis* has highest inhibition percentage of mycelial growth of *S. rolfsii* with 84.28%, 88.64%, 72.78% and 77.84% under dual culture. The results of Daunde *et al* (2018) under dual culture also found similar result with *T. viride* and *T. harzianum* with recording 73.81% and 81.33% of inhibition of mycelial growth respectively.

Table 2: *In vitro* evaluation of antagonistic organisms against *S. rolfsii*

Treatments	Mena colony growth(mm)	Percent of inhibition
T. viride	27.25 (31.46)*	69.72 (56.59)
T. harzianum	22.50 (28.31)	75.00 (59.98)
T. reesei	33.00 (35.05)	63.33 (52.71)
P. fluorescens	17.00 (24.34)	81.11 (64.21)
B.subtilis	15.25 (22.98)	83.05 (65.66)
Control	90.00 (89.97)	0 (0.00)
C.D (0.01)	2.878	
S.E(m)±	0.961	
S.E(d)	1.359	
C.V (%)	5.626	

*Figures in parentheses are angular transformed values

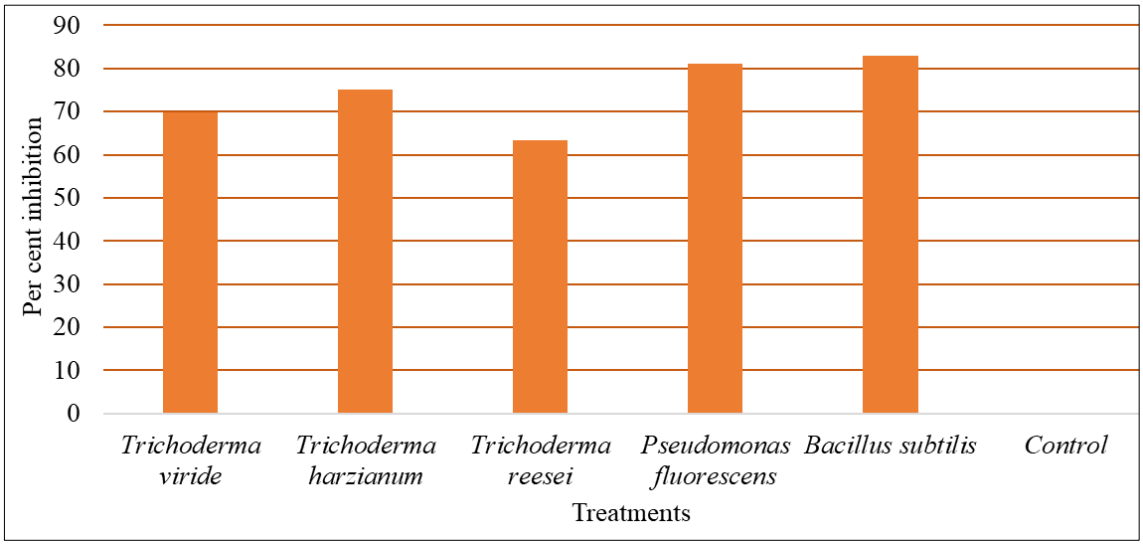

Fig 2: *In vitro* evaluation of antagonistic organisms against *S. rolfsii*

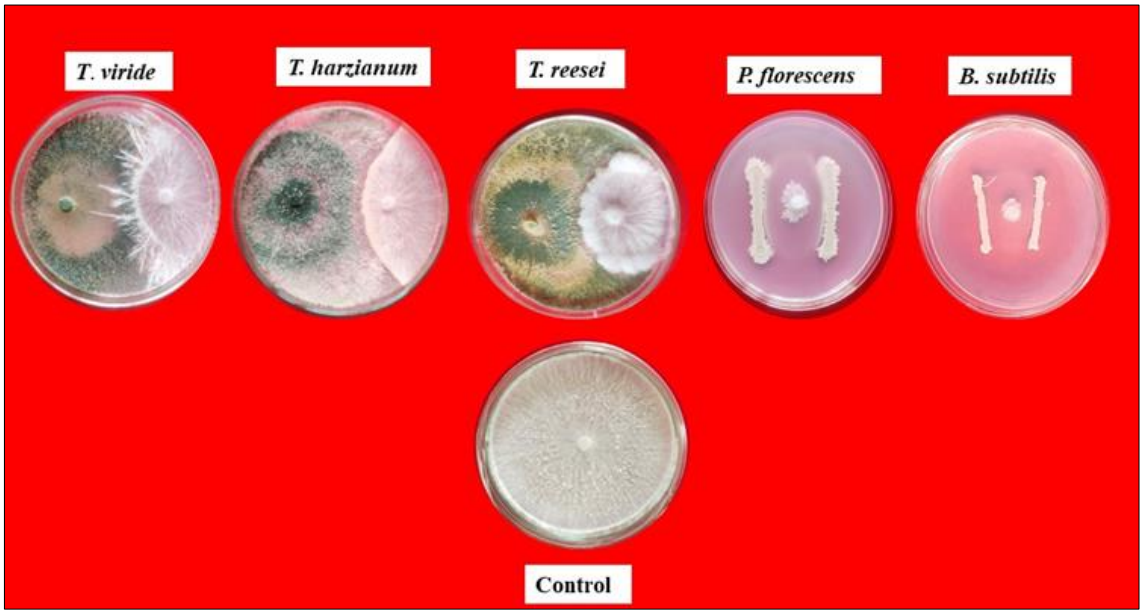

Plate 2: *In vitro* evaluation of antagonistic organisms against *S. rolfsii*

Conclusion

As per the results obtained, application of fungicides @ 500 ppm: propiconazole, azoxystrobin, flusilazole, tebuconazole and carboxin + thiram, @2000 ppm- carbendazim + mancozeb and difenconzole and @ 2500 ppm mancozeb, and under bioagents *Bacillus subtilis* effectively controls the growth of *S. rolfsii.*

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