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Evaluation of the efficacy of some botanicals and bioagents against the root rot pathogen of sugar beet (*Beta vulgaris* L.), *Rhizoctonia solani*

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Abstract
In vitro studies were carried out to determine whether certain botanicals and bioagents were effective against *Rhizoctonia solani*, the organism that causes root rot disease in Sugar beet (*Beta vulgaris* L.) plants. *Terminalia arjuna* and *Pithecellobium dulce* (Manila tamarind) were the botanicals, and *Trichoderma viride*, *Trichoderma harzianum*, and *T. koningii* were the bioagents used in this study. All plant extracts and antagonistic organisms significantly inhibited the growth of *Rhizoctonia solani*. However, *T. viride* had the greatest inhibitory effect of the antagonists, followed by *T. harzianum* and *T. koningii*, which have the least suppressive effect. *Pithecellobium dulce* and *Terminalia arjuna* extracts had the strongest inhibitory effects.

Keywords: Sugar beet, root rot, botanicals, Bioagents, *Rhizoctonia solani*.

Introduction
Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is a biennial plant belonging to the Amaranthaceae family. It is a crucial crop that is grown all over the world, primarily for its rich sugar content that is obtained from its fleshy storage root. Sugar beetroot is a crucial ingredient in the food and beverage industry because it is a valuable source of sucrose and contributes significantly to sugar production [1]. One of the most important sugar-producing crops in the world is the sugar beetroot (*Beta vulgaris* L.). It is crucial for supplying sugar to the global market [2].
Sugar beet, in particular, is highly susceptible to *Rhizoctonia solani* infection, leading to significant yield losses and economic impact. The pathogen infects the sugar beet roots, causing rot and decay, which ultimately hampers the plant's ability to absorb nutrients and water [3]. As a result, infected sugar beet plants become stunted and eventually die, severely impacting the sugar extraction process. This has prompted extensive research efforts to understand the pathogenicity of *Rhizoctonia solani* and develop effective control strategies to minimize its impact on sugar beet cultivation [4].
When sugar beets are infected with Rhizoctonia solani, the fungus attacks the root system, causing rotting and decay. Common symptoms of *Rhizoctonia* root rot in sugar beets include wilting, stunted growth, yellowing of leaves, and a decline in overall plant health. As the disease progresses, the roots may develop dark-coloured lesions or become soft and mushy [5]. *Rhizoctonia solani* is a ubiquitous soil-borne basidiomycete that causes disease in many economically important crops, including rice, soybean, potato, corn, and sugar beet [6].
Rhizoctonia solani can enter the sugar beet plant through wounds on the roots or at the seedling stage. It can also spread from plant to plant through soil contact or contaminated farming tools and equipment [7]. Due to its soil-borne origin, ability to persist for longer periods of time without host plants, ability to form persistent dormant structures, and soil-borne nature, management of *Rhizoctonia solani* infection is frequently a difficult task [8]. Utilizing resistant cultivars, crop rotation, synthetic fungicides, and fumigation have been the main methods used in recent decades to prevent and control soil-borne crop diseases [9]. Research areas in agricultural sciences now include the development of biological disease control and the hunt for novel, distinctive natural microbial phytopathogen antagonists [10]. However, there has been little success and little financial benefit from the use of soil fungicides. Alternative management approaches are therefore required for the environmentally friendly and long-term control of soil pathogens.

Material and Methods

Collection and authentication of test plant material

Plant materials of the selected plants for tasting *in vitro* efficacy against the isolated fungi from Sugar beet were collected from JES College Campus Jalna, Maharashtra, India. The plants with inhibitory properties were authenticated, and herbarium voucher specimens were prepared and deposited in the Herbarium, Department of Botany, JES College, Jalna Maharashtra, India for future reference.

Preparation of Plant Extracts

In this study, aqueous plant extracts were used. Plant leaf extracts of *Terminalia Arjuna* and *Pithecellobium dulce* are used for the evaluation of antifungal activity against isolated pathogen. To obtain a sample, the powdered plant material from the leaf part was taken separately and subjected to the Soxhlet extraction procedure [11]. Preliminary phytochemical analysis was carried out for each solvent extract according to the standard procedure [12-13]. The extracted samples were kept in closed glass vials and refrigerated at 4 °C when not in use [14]. Plant extracts were prepared and evaluated for their bioactivity using the agar dilution method [15].

Isolation and purification of the pathogen

The diseased plant showing the symptoms was washed thoroughly with tap water, and small pieces from infected parts 1-2 mm in dimension from the advancing margin of the spot, adjacent to healthy portions were cut with the help of a sterilized blade. These pieces were surface sterilized with 1% sodium hypochlorite solution for 30 seconds and finally washed well in three changes of sterilized distilled water to remove any trace of sodium hypochlorite. The pieces were then transferred aseptically to Petri plates containing Potato Dextrose Agar. Inoculated Petri plates were incubated at 26±1 °C for three to five days and examined at frequent intervals to see the growth of the fungus. The pure colonies were picked and inoculated in a culture tube for further research work. Pathogen was identified on the basis of their morphological and cultural characteristics with the help of available literature [16].

Isolation, purification, and identification of antagonistic microorganisms

The isolation of bioagents was done by the serial dilution technique. 10 g of rhizospheric soil was dissolved in 100 ml of sterile distilled water to get a 10^{-1} dilution. 1 ml of soil suspension was taken from stock and added to 9 ml of sterile distilled water to get a 10^{-2} dilution. This is further repeated until a final dilution of 10^{-7} is obtained. 1 ml of each soil suspension was poured into sterilised petri plates containing nutrient medium, incubated at 25±1 °C, and observed at frequent intervals for the development of colonies of bio-agents. Bioagents were identified based on cultural and mycological characters described by Barnett and Hunter [7].

Dual culture technique

Using the dual culture methods of Morton and Stroube (1955) [18] on PDA, antagonistic activities of the biocontrol agent were examined against the soil-borne plant pathogen *Rhizoctonia solani* (Sherb.). A 5 mm mycelial disc was obtained from the periphery of a 5-7 day-old culture of the pathogen on PDA, and it was positioned on a fresh PDA plate (3 cm from the bioagent centre). Next, fungal bioagents were placed 3 cm away from the inoculum of the pathogen. Three replication of each treatment were maintained with one control set without inoculating the bioinoculants. Then the plates were incubated at 26+1 °C. At the end of the incubation period, radial growth was measured. The inoculated plates with culture discs of pathogens without bioagents served as controls. After the 3rd, 5th and 7th days of incubation at 26+1 °C, radial growth of the pathogen and percent inhibition were recorded.

Determination of antifungal activity

The effectiveness of two plant extracts on the growth and inhibition of *Rhizoctonia Solani* (Sherb.) was investigated. The inoculating needle was used to take *Rhizoctonia Solani* into the extract-containing (poisoned) PDA in Petri dishes to check for its growth and PDA without extract served as the control. The extracts were then incorporated in 50 µl of different concentrations (5%, 10%, and 25%) into the agar. Each treatment was replicated 3 times. Each Petri dish was inoculated at 28±2 °C for 7 days. The radial growth of the colony was recorded on the 3rd, 5th, and 7th days of intervals, and the percent inhibition of mycelial growth was calculated over control. The tests were carried out in triplicate. Per cent inhibition of mycelial growth over control was calculated by using the formula.

$$PI = \frac{C - T}{C} \text{ X } 100$$

Where, PI: Is the percent inhibition over control, C: Is mycelial radial growth in control plate, T: Is mycelial radial growth in treatment [20].

Results and Discussion

Table 1: Preliminary Qualitative phytochemical screening of aqueous extracts of *Terminalia Arjuna* L.

Sr. No.	Phytochemicals	Test	Result
1	Flavonoids	Shibita's reaction test	++
2	Phenols	Lead test	+++
3	Steroids	Salkowski's Test	+
4	Glycosides	Borntrager's	++
5	Saponins	Froth test	+
6	Terpenoids	Salkowski test	+
7	Tannins	Ferric chloride test	++

Where (+) sign indicates presence of corresponding phytocomponents.

Preliminary Qualitative phytochemical screening of aqueous extracts of *Terminalia arjuna* (L.) results

The result of the present study is the outcomes of extracted contents of plant leaf extract (aqueous) tested for presence or absence of various phytochemicals (in qualitative form) are noted in Table 1. The results show that *Terminalia arjuna* L. plant contains a maximum ten types of phytochemical groups, such as Flavonoids, Phenols, Steroids, Glycosides, Saponins and Terpenoids. Phytochemical screening of *Terminalia arjuna* L. extracts reveals the presence of various secondary metabolites in them. Several researchers have evaluated the phytochemical properties of plant extracts by qualitative methods [20-21].

Table 2: Preliminary qualitative phytochemical screening of aqueous extracts of *Pithecellobium dulce* L.

Sr. No.	Phytochemicals	Test	Result
1	Flavonoids	Shibita's reaction test	+++
2	Phenols	Lead test	++
3	Steroids	Salkowski's Test	--
4	Glycosides	Borntrager's	++
5	Saponins	Froth test	--
6	Terpenoids	Salkowski test	++
7	Tannins	Ferric chloride test	+

Where (+) sign indicates presence of corresponding photo components.

Preliminary Qualitative phytochemical screening of aqueous extracts of *Pithecellobium dulce* L.

After the successful conventional hot-soxhlet extraction of the *Pithecellobium dulce* leaves, the preliminary phytochemical study revealed that the aqueous extract of *Pithecellobium dulce* L. contains alkaloids, flavonoids, phenols, and tannins (Table 2). Similar observations were reported for phytochemical screening of the plant, which revealed the presence of phytochemicals like tannins, flavonoids, and alkaloids [22-23]. The identification and purification of secondary metabolites found in active fractions are the next steps.

Table 3: Growth of *Rhizoctonia Solani* on PDA incorporated with three different concentrations of two plant extracts at the 3rd, 5th and 7th of incubation

Treatments	Concentration (µg/mL)	Inhibition After 3rd Day		Inhibition after 5th day		Inhibition after 7th day	
		Radial Growth of pathogen (mm)	Inhibition Ratio (%)	Radial Growth of pathogen (mm)	Inhibition Ratio (%)	Radial Growth of pathogen (mm)	Inhibition Ratio (%)
Terminalia arjuna	15	8.85±0.1	13.23	9.75±0.1	10.55	9.89±0.12	9.26
	30	5.62±0.2	44.90	6.34±0.2	40.74	7.25±0.2	33.48
	45	6.85±0.1	32.84	7.98±0.1	25.42	7.99±0.5	26.69
	60	3.22±0.11	68.43	4.42±0.17	58.69	4.98±0.1	54.31
Control		10.2±0.25	0	10.7±0.34	0	10.9±0.79	0
Pithecellobium dulce	15	9.46±0.5	22.45	9.58±0.12	25.15	9.88±0.13	23.41
	30	4.69±0.2	61.55	5.29±0.1	58.67	5.98±0.11	53.64
	45	1.38±0.11	88.68	2.48±0.11	80.77	2.78±0.15	78.44
	60	7.86±0.7	35.57	8.56±0.9	33.12	8.92±0.9	30.85
Control		12.2±0.25	0	12.8±0.29	0	12.7±0.34	0

Notes: Data in the table are mean value ± standard deviation. The different letters in the same column indicate significant differences at the 0.05 level.

Effects of plant extracts on *Rhizoctonia solani*

Different concentrations of extracts of *Terminalia arjuna* and *Pithecellobium dulce* were incorporated into agar at different volumes of 15, 30, 45 and 60 µg/mL, and the poisoned agar was inoculated with *Rhizoctonia solani*. After the third day of inoculation, the highest mycelial growth of the pathogen is reflected in amongst the concentrations of 15, 30, 45 and 60 µg/mL, of *Terminalia arjuna* extract were 8.85±0.1 mm at 15 µg/mL and lowest were observed 3.22±0.11 mm at 60 µg/mL. By the fifth day, the growth was 9.75±0.1 mm and 4.42±0.17 mm, respectively. Similarly, on the seventh day, the highest growth was 9.89±0.12 mm, at 15 µg/mL and lowest were 4.98±0.1 mm at 60 µg/mL respectively. The percent inhibition of *Rhizoctonia Solani* due to the different volumes of (15, 30, 45 and 60 µg/mL) concentrations of *Terminalia arjuna* extract incorporated into the growth media were 54.31 percent, respectively, at 7 days of incubation. This observation shows that the growth of the target pathogen on the poisoned agar plate was reduced compared with the growth on the control plates, which were not poisoned with the extract of *Terminalia arjuna* (Table 3). There was a significant difference in the growth of *Rhizoctonia Solani* in different concentrations of *Terminalia arjuna* extracts. The 60 µg/mL was observed to be the most effective followed by 15, 30, and 45 µg/mL was the least effective. Wang *et al.* reported a significant reduction in growth of *Rhizoctonia solani* by the plant extracts. At higher concentrations, mycelial growth was inhibited more than at lower concentrations. Anil Sehajpal, *et al*., evaluated crude aqueous leaf extract of *Terminalia arjuna* L. for their antifungal efficacy on the growth of *Rhizoctonia Solani* extracts in their finding revealed that higher concentrations of the extract showed greater inhibitory activity than the lower concentration.

After the third day of inoculation, the highest mycelium growth of the organism amongst the different concentrations of *Pithecellobium dulce* were 9.46±0.5 mm at 15 µg/mL, whereas on the fifth day, the growths of the target organism were seen to be 9.58±0.12 mm, respectively. Similarly, on the seventh day, the growth was 9.88±0.13 mm, respectively. The percentage inhibition of *Rhizoctonia solani* due to the 15, 30, 45 and 60 µg/mL concentrations of *Pithecellobium dulce* extract incorporated into the growth media; was 78.44% respectively, at 7 days of incubation. This shows that the highest growth of the target organism was reduced when compared with the growth on the control plate, which was not poisoned with an extract of *Pithecellobium dulce*. Here the 45 µg/mL was observed to be the most effective followed by 30 and 60 µg/mL and 15 µg/mL was the least effective.

Table 4: Influence of Trichoderma isolates on growth of *Rhizoctonia solani*

Trichoderma viride Isolates	Radial growth (mm), (7DAI)	Inhibition %	*Trichoderma harzianum* Isolates	Radial growth (mm), (7 DAI)	Inhibition %	*Trichoderma koningii* Isolates	Radial growth (mm), (7 DAI)	Inhibition %
Tv_1	14	84.44	Th_1	41.17	54.25	Tk_1	37	58.88
Tv_2	17.2	81.58	Th_2	22.39	75.12	Tk_2	51.5	42.77
Tv_3	22.3	75.22	Th_3	34.11	73.21	Tk_3	28.11	68.76
Tv_4	35.1	61	Th_4	27.67	69.25	Tk_4	32.35	64.05
Tv_5	16.95	81.16	Th_5	13	61.11	Tk_5	22.33	86.3

In vitro* bioefficacy of bioagents against *Rhizoctonia solani

Results showed that *Rhizoctonia Solani* was refractory to all tested bioagents' fungistatic and antifungal effects and that their use significantly reduced the growth of *Rhizoctonia solani* compared to the untreated control (Table 4).

At 7 days after incubation (DAI), *T. viride* isolates constrained the growth of the intended target organism, *Rhizoctonia solani* was observed maximum in Tv_1 and it was found minimum in Tp_4 respectively, which is significantly greater than the growth rates of the control plates. The mycelium of *T. viride* appeared greenish in color and fully occupied the plate, covering up the pathogen (Table 4). The mycelium of *T. harzianum* was green with fluffy white patches, and it inhibited the growth of *Rhizoctonia solani*, 7 days after incubation (DAI), maximum percent inhibition showed Th_3 and Th_2 and minimum in Th_5 there was a significant difference that revealed *Rhizoctonia solani* growth was inhibited during these times. For *T. koningii*, isolates the mycelium appeared in a ring shape, greenish at the tip and white at the center. The growth of *Rhizoctonia solani* on the 7 days after incubation the maximum percent inhibition was observed in Tk_3 and Tk_4, and minimum in Tk_2 (Table 4).

Amongst the bioagents tested, *T. viride* was found most effective, showing the least mycelial growth and its highest inhibition of the test pathogen, followed by *T. harzianum* and *T. koningii* in terms of percent mycelial inhibition. The results of the present study on the antagonistic effects of *Trichoderma* spp. against *Rhizoctonia solani* are in accordance with those reported earlier by several workers such as Mukhopadhyay reported on the effectiveness of *Trichoderma* species against *Rhizoctonia solani.* According to Papavizas and Lumsden (1980) [27] the mechanisms involved in the control of pathogens by *Trichoderma* spp. are probably: Antibiosis, lysis, competition and mycoparasitism. However, Ayers and Adams (1981) [28] indicated that interactions observed *in vitro* do not necessarily confirm their operation for the decrease in pathogen populations and reduction in diseases observed in natural conditions.

Conclusions

In contrast to synthetic fungicides, our study showed that the use of aqueous plant extracts could be a useful, secure, and affordable method for controlling soil-borne diseases *(Rhizoctonia solani*). Additionally, there are benefits to using plant extracts as fungicides in agriculture because they naturally disintegrate and don't leave a harmful residue on plants. Our study also revealed the possibility of using regional isolates of *T. harzianum*, *T. viride*, and *T. koningii* as biological control agents to shield bean plants from *Rhizoctonia solani*. Other practices that aim to enhance crop health and provide new standards of disease management where other techniques are ineffective could be incorporated into biocontrol programming.

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