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Role of bio-agents and plant extracts in resistance induction against *Sclerotium rolfsii* under *in vitro* condition

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Abstract
Cluster bean (*Cyanosis tetragonoloba* L.) Taub is a major leguminous crop in India's dry and semi-arid regions during the Kharif season. Cluster bean stem rot, caused by *Sclerotium rolfsii* Sacc., has become a severe concern in recent years, resulting in yield losses of 50-70 percent, depending on the severity of the disease. A wide range of chemical pesticides currently available and the continuous use of these pesticides effect the food substance of crop as well as their texture and the performance of soil in order to control all these vulnerabilities. In this study, evaluated the effects of bio-agents and plant extracts against the pathogen. In the case of bioagents, maximum growth inhibition (70.78%) was recorded in *T. harzianum* and *P. fluorescens* (50.78%) among fungal and bacterial bioagents respectively. In comparison to 5, 10, and 15%, the 20 percent concentration of plant extracts was most effective in preventing fungus development. *Citrullus colocynthis*, *Azadirachta indica*, and *Datura stramonium* extracts were considerably superior in inhibiting fungal growth at 20% concentration, with 60.50 per cent, 53.77 per cent, and 52.29 per cent, respectively. Using bioagents and plant extracts to prevent cluster bean stem rot disease and its catastrophic consequences is both ecologically benign and effective. The bioagents not only inhibits the disease, but it also promotes healthy crop growth.

Keywords: Cluster bean, bio agents, plant extracts, stem rot

Introduction
Cluster bean (*Cyanosis tetragonoloba* L.) Taub also known as Guar, a major leguminous crop in India's dry and semi-arid regions during the *Kharif* season. It belongs to the family *Leguminosae* (*Fabaceae*). It is a self-pollinated, short-duration legume crop grown in marginal and sub-marginal areas (Kumar, 2005) [11]. It has deep root system and is drought tolerant (Kumar and Rodge, 2012) [10]. Rainfed crops benefit from its deep penetrating root system. Cluster bean pods are green, long, thin vegetable pods. Calcium, phosphorus, iron, and vitamins A and C abound. Cluster beans are grown as a vegetable, green fodder, green manure, and seed. It is also used as an animal concentrate, and the gum extracted from the seed endosperm is appropriate for a range of industrial purposes (Joshi and Arora, 1993). Cluster bean seeds contain 28-33% gum. In addition to textiles and paper, cluster bean gum is used in numerous other industries including as mining and oil drilling. It also enhances soil fertility by fixing nitrogen from the environment. Guar gum and its derivatives are highly prized globally.
Cluster bean are majorly growing in India, Pakistan, Indonesia, America, Italy, Mexico, Brazil, and South Africa. Cluster bean cultivation covers 4.26 million hectares in India, with a yield of 2.42 million tonnes and a productivity of 567 kg/ha (Anonymous, 2020) [1]. Rajasthan is the country's largest cluster bean producer, accounting for over 80% of total cluster bean production. The cluster bean crop is grown on 35.30 lakh hectares in Rajasthan, with a production of 14.04 lakh tonnes and a productivity of 398 kg/ha (Anonymous, 2020) [1].
The production and productivity of cluster bean in terms of grain and fodder is highly affected by several phytopathogenic fungal and bacterial diseases *viz*., Bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*), Alternaria leaf spot (*Alternaria cyamopsidis*), Anthracnose (*Colletotrichum capsici* f.sp. *cyamopsicola*), *Curvularia* leaf spot (*Curvularia lunata*), Charcoal rot/Damping-off (*Macrophomina phaseolina*), Dry root rot/Leaf blight *(Fusarium solani* and *Rhizoctonia solani*), Myrothecium leaf spot (*Myrothecium roridum*), powdery mildew (*Oidiopsis taurica*), wilt (*Fusarium caeruleum*). Cluster bean stem rot, caused by *Sclerotium rolfsii* Sacc., has become a severe concern in

recent years, resulting in yield losses of 50-70 percent, depending on the severity of the disease. It has become a limiting factor for cluster bean crop cultivation due to its severity and destructive nature (Le *et al*., 2011). As a result, the current study focuses on using various bio-agents and plant extracts to combat this damaging disease

Material and Method

Efficacy of bio-agents and plant extracts against *Sclerotium rolfsii in vitro*

Isolation of fungal antagonists

TSM and Martin's Rose Bengal Agar were employed to isolate fungal antagonists. Ten g soil was added to 90 mL sterile water and gently stirred for 4-5 minutes. To isolate *Trichoderma spp.,* 0.2ml stock soil suspension was serially diluted up to 10-7, and added to the surface of Trichoderma selective medium (Elad and Chet, 1983) and Martin's Rose Bengal Agar media in Petri dishes. The dirt suspension was spread uniformly with a glass spreader. To identify and further use the fungi, the fungal colonies were sub cultured on potato dextrose agar media and examined under a microscope for 7 days at 28°C in a BOD incubator. Appendix I specifies the media components used.

Isolation of *Pseudomonas fluorescens*

P. fluorescens was isolated using *P. fluorescens* agar selective media (HiMedia) (King *et. al.,* 1954). The stock soil solution was made by gently shaking 10 g dirt in 90 ml sterile distilled water for 2–4 minutes. The stock soil suspension was diluted up to 10^{-7}. A 0.2 ml soil suspension was dispersed evenly over the media in Petri plates using a glass spreader. After 48 hours at 28°C, the colonies were subcultured on PAF medium for identification and future use.

Isolation of *Bacillus subtilis*

Stock solution was prepared by mixing 10 g soil in 90 ml sterile nutritious broth for 2–4 minutes. Then they were put in a 55°C water bath for 3-5 minutes. A 48-hour room-temperature incubation of the soil suspension followed. The initial soil suspension was serially diluted up to 10^{-7} in sterile distilled water. A 0.2 ml soil suspension was placed equally over the nutritional agar (NA) media in Petri plates. After 48 hours at 28 ± 1^{o}C, the colonies were subcultured on nutrient agar media for identification and future usage.

Evaluation of antagonistic potential of fungal antagonists

The antagonistic ability of *Trichoderma spp.* and other fungal antagonists was determined using a dual culture approach. In Petri plates spaced 5 cm apart, one mycelial disc (5 mm diameter) of each pathogen and antagonist was kept on the surface of potato dextrose agar medium. For 7 days, the inoculated Petri dishes were incubated at 28 ± 1^{0}C. For each fungal antagonist, three replications were retained. In the case of the control, the Petri dishes were simply injected with the test pathogen's mycelial disc. After 4 days of inoculation, the test pathogen's mycelial growth was assessed. The percentage of growth inhibition was estimated using the formula

Formula

$$\text{Per cent growth inhibition} = \frac{\text{C}-\text{T}}{\text{C}} \times 100$$

C = Mycelial growth of *S. rolfsii* in control (mm)
T= Mycelial growth of *S. rolfsii* in presence of antagonist (mm)

Evaluation of antagonistic potential of bacterial antagonists

The paper disc inoculation method was used to investigate bacterial antagonists' antagonistic ability. Agar slants with *P. fluorescens* and *B. subtilis* stock cultures were streaked for 8 hours at 28 ± 1^{0}C. Each slant carrying a new colony of corresponding bacterial antagonists was suspended by scraping the bacterial growth with a sterilised inoculating needle. The suspension was then transferred to sterile Petri dishes. Sterilised filter paper discs (5 mm diameter) were dipped in the bacterial suspension. Four infected discs were placed in opposite directions on top of potato-dextrose-agar mixture in Petri dishes. Mycelial discs (5 mm diameter) were collected from the periphery of an actively developing *S. rolfsii* culture grown on potato dextrose agar medium. As a control, Petri dishes were only inoculated with test pathogen mycelial discs. Three copies of each bacterial antagonist were kept. They were cultured at $28+1^{0}$C in a BOD incubator. *S. rolfsii* mycelial growth was assessed after 7 days. The following formula was used to calculate bacterial antagonists' inhibition of mycelium growth.

Formula

$$\text{Per cent growth inhibition} = \frac{\text{C}-\text{T}}{\text{C}} \times 100$$

C = Mycelial growth of *S. rolfsii* in control (mm)
T= Mycelial growth of *S. rolfsii* in presence of antagonist (mm)

Preparation of plant extract

The plant parts were collected, washed with water and air dried. 100 g plant material was pulverised in a pestle and mortar with 100 ml sterilised distilled water (1: 1 w/v). In this case, the crushed material was squeezed through cheese cloth, and the extracts were centrifuged for 5-10 minutes at 10,000 rpm. The antifungal activities of plant extracts were studied using poisoned food. Various botanical stock solutions (5, 10, 15, and 20%) were sterilised and mixed with 95, 90, 85, and 80 ml of PDA medium, respectively. 20 mL of this medium was poured in sterile Petri plates and hardened aseptically. With a sterile corkborer, 5 mm mycelial discs were cut from an active *S. rolfsii* culture and placed onto each Petri plate. Each treatment replicated thrice. To maintain control, PDA plates was not amended with plant extracts. After 7 days at 28+2°C, the radial development of the control plates was measured.

Table 1: List of different plant extracts used against *S. rolfsii in vitro*

S. No.	Botanical Name	Common Name	Plant part used
1.	*Datura stramonium* L.	Datura	Stem, flower, leaf
2.	*Calotropis procera Aiton.*	Aak	Leaves & flower
3.	*Allium sativum* L.	Garlic	Bulb
4.	*Allium cepa* L.	Onion	Bulb
5.	*Eucalyptus globules Labil*	Eucalyptus	Leaves
6.	*Azadirachta indica*	Neem	Leaves

7.	*Azadirachta indica*	Neem (NSKE)	Seed kernel
8.	*Citrullus colocynthis*	Tumba, bitter apple	Fruit
9.	*Tinospora cordifolia*	Giloe	Stem
10.	*Curcuma longa*	Turmeric powder	Rhizome

Result and discussion

Effect of bio agents and plant extracts against *Sclerotium rolfsii in vitro*

Efficacy of bio agents

The efficacy of fungal and bacterial bioagents were evaluated against *S. rolfsii* by using dual inoculation and paper disc inoculation methods respectively. The bioagents performed better against control in inhibiting the growth of the test fungus. *T. harzianum* (70.78%). inhibited the growth followed by *T. viride* (57.67), *P. fluorescens* (50.78%) and *B. subtilis* (45.52%).

Table 2: Efficacy of different bio-agents on mycelial growth of *Sclerotium rolfsii in vitro*

Treatments	Mycelial growth (mm)	Mycelial growth inhibition (%)
T_1 -*T. harzianum*	26.30 (30.84)	70.78
T_2 -*T. viride*	38.10(38.10)	57.67
T_3 - *P. fluorescens*	44.30 (41.71)	50.78
T_4 - *B. subtilis*	49.30 (44.59)	45.52
T_5 – Control	90.00 (71.59)	-
S.Em±	0.59	
CD (P = 0.05)	1.89	

Figures in parenthesis are angular transformed values

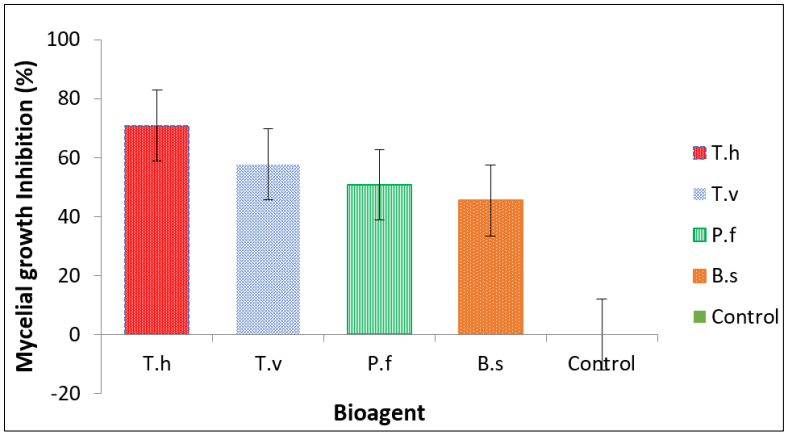

Fig 1: Efficacy of different bio-agents on mycelial growth of *Sclerotium rolfsii in vitro*

All bioagent isolates significantly slowed the growth of the test fungus compared to the control. Different microbial bio-agents such as *Trichoderma spp*., *P. fluorescens*, *B. subtilis*, and others have suppressed *S. rolfsii* pathogenic to diverse hosts as reported by others (Mukhopadhyay *et al.*, 1987, Khan and Hussain, 1990, Dev and Dawande 2010, Wahyudi *et al.*, 2011, and Reetha *et al.*, 2014) [15, 8, 5, 19]

The antagonists bind to the pathogen's active mycelial tip and impede *S. rolfsii* mycelial growth. Madhavi and Bhattiprolu (2011) [12] used dual culture to test two *Trichoderma sp.* against *S. rolfsii* and found growth inhibition of 55.8% and 57.5%respectively. *Trichoderma viride* inhibited the maximal mycelial expansion of *S. rolfsii* by 63.33 per cent (Nagamma & Nagaraja, 2015) [16]. Kapadiya and Moradiya (2017) [7] evaluated the efficiency of three bioagents against *S. rolfsii* growth. Only *T. viride* hindered *S. rolfsii* mycelial development and sclerotial germination. *In vitro*, *Trichoderma viride*, *Aspergillus niger*, and *Penicillium species* were particularly efficient against *S. rolfsii*. Ekundayo *et al*. (2015) found similar results with *Aspergillus flavus*, *A. niger*, *T. viride*, and *Penicillium italicum* against *S. rolfsii* mycelium expansion. *Trichoderma viride* and *T. harzianum* metabolites reduced *S. rolfsii* collar rot disease incidence in sunflower and increased crop yields (Maji and Nath, 2016) [13].

Plant extracts

Ten plant extracts were evaluated against *S. rolfsii*. There was a significant reduction in fungal growth in all plant extracts tested at dosages of 5, 10, 15, and 20%. At a 20% concentration of plant extract, *Citrullus colocynthis* extract (60.50%) performed better in preventing fungal growth followed by *Azadirachta indica* (53.77%), *Datura stramonium* extracts (52.29%), and NSKE (43.88%). The results showed that *Citrullus colocynthis* and *Azadirachta indica* extracts were effective against *S. rolfii* by inhibiting the maximum growth.

The results were in accordance with Magar *et al*. (2011) [14] who reported *Allium sativum* bulb extract were effective against *Macrophomina phaseolina* followed by *Zingiber officinalis* and *Allium cepa* bulb extracts. The findings coincided with Butt *et al.* (2016) [3] who found that increasing the concentration of Neem extract (0, 1, 2, 5%) inhibited the growth of harmful fungus mycelia. The results are also matched with Suryawanshi *et al.* (2015) [18] who examined the efficacy of plant extract at 10% and 20% concentrations on *S. rolfsii.*

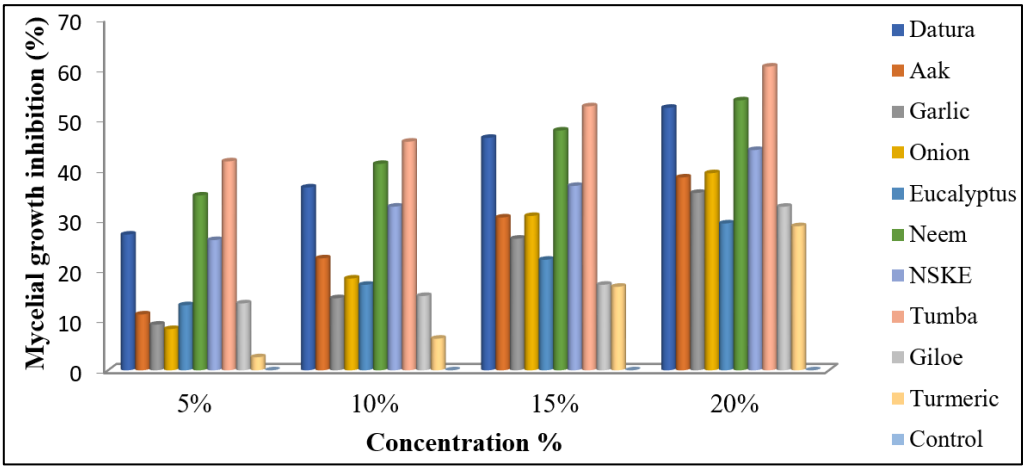

Fig 2: Effect of different plant extracts on mycelial growth inhibition of *S. rolfsii in vitro*

Table 3: Effect of different plant extracts on mycelial growth of *S. rolfsii in vitro*

Treatments	Mycelial growth (mm)				Mycelial growth inhibition (%)			
	5 %	10 %	15 %	20 %	5 %	10 %	15 %	20 %
T_1- *Datura stramonium* L. (Datura)	65.66 (54.11)	57.22 (49.13)	48.33 (44.02)	42.34 (40.57)	27.04	36.42	46.30	52.29
T_2- *Calotropis procera Aiton.* (Aak)	79.98 (63.74)	69.93 (56.73)	62.60 (52.28)	55.43 (48.10)	11.13	22.30	30.44	38.41
T_3- *Allium sativum* L. (Garlic)	81.81 (64.74)	77.07 (61.33)	66.41 (54.56)	58.19 (49.69)	9.10	14.36	26.21	35.34
T_4- *Allium cepa* L. (Onion)	82.60 (65.36)	73.54 (59.03)	66.57 (54.66)	62.33 (52.59)	8.22	18.28	30.74	39.26
T_5- *Eucalyptus globules Labil* (Eucalyptus)	78.32 (62.25)	74.67 (59.76)	70.17 (56.87)	63.67 (52.92)	12.97	17.03	22.03	29.25
T_6- *Azadirachta indica* (Neem leaf)	58.66 (49.97)	53.01 (46.70)	47.01 (43.26)	41.60 (40.15)	34.82	41.11	47.76	53.77
T_7- NSKE	66.65 (54.71)	60.66 (51.13)	56.04 (48.45)	50.50 (45.27)	25.94	32.60	36.73	43.88
T_8- *Citrullus colocynthis* (Tumba/Bitter Apple)	52.54 (46.43)	49.01 (44.41)	42.67 (40.76)	35.55 (36.58)	41.62	45.54	52.58	60.50
T_9-*Tinospora cordifolia* (Giloe)	78.00 (62.01)	76.68 (61.13)	74.34 (59.56)	60.67 (51.14)	13.33	14.80	17.04	32.58
T_{10}- *Curcuma longa* (Turmeric Powder)	87.66 (69.47)	84.33 (66.67)	75.01 (59.99)	64.17 (53.21)	2.60	6.30	16.64	28.70
T_{11}- Control	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	90.00 (71.54)	-	-	-	-
S.Em±	1.29	1.11	1.19	1.01				
CD (P = 0.05)	3.83	3.03	3.52	3.00				

Figures in parenthesis are angular transformed values

Future scope

Testing the efficacy of plant extracts and bioagents can also help prevent plant disease. Using a high concentration of a plant extract helps cut cultivation costs. This research helps farmers choose an efficient herbicide for cluster bean stem rot. This can include long-term effects on soil and human health. Cluster bean growers can enhance yields by reducing *Sclerotium rolfsii* infection, which causes stem rot.

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Conflict of interest

The authors state that they have no conflicting interests and have reported no potential conflicts of interest

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