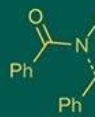


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(2): 196-201
www.biochemjournal.com
Received: 17-11-2025
Accepted: 19-12-2025

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Biocontrol of *Agroathelia rolfsii*: Harnessing plant extracts and beneficial microbes to induce host resistance *in vitro*

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i2Sc.7476>

Abstract

Cluster bean (*Cyamopsis tetragonoloba* L. Taub) is a significant leguminous crop cultivated in the dry and semi-arid regions of India during the Kharif season. In recent years, stem rot caused by the soil-borne pathogen *Agroathelia rolfsii* has emerged as a severe disease, leading to substantial yield losses ranging from 50 to 70%. The reliance on broad-spectrum chemical pesticides for disease management raises concerns regarding food safety, soil health, and environmental sustainability. Due to concerns over the environmental and health impacts of continuous chemical pesticide use, this study investigated alternative management strategies. An *in vitro* assay was conducted to evaluate the efficacy of biological control agents and plant extracts against the pathogen. Among the bioagents, *Trichoderma harzianum* and *Pseudomonas fluorescens* demonstrated the highest mycelial growth inhibition, at 70.78% and 50.78%, respectively. Evaluating plant extracts at various concentrations (5%, 10%, 15%, and 20%) revealed the 20% concentration to be most effective. At this concentration, extracts of *Citrullus colocynthis* (60.50% inhibition), *Azadirachta indica* (53.77%), and *Datura stramonium* (52.29%) were the most effective in suppressing fungal growth. The results indicate that these biocontrol agents and plant extracts represent a potent and eco-friendly strategy for the integrated management of stem rot in cluster bean, offering an effective alternative to chemical pesticides.

Keywords: Cluster bean, *Agroathelia rolfsii*, *Trichoderma*, *Pseudomonas*, *Citrullus colocynthis*, biological control

1. Introduction

Cyamopsis tetragonoloba (L.) Taub., commonly known as cluster bean or guar, is a short-duration, self-pollinating leguminous crop predominantly cultivated in the arid and semi-arid regions of India during the Kharif season (Kumar, 2005) [18]. Taxonomically, it belongs to the tribe Indigoferae within the family Fabaceae, with a diploid chromosome number of $2n = 14$. The crop is highly valued for its drought tolerance, a trait facilitated by a deep-penetrating root system that enables efficient soil moisture utilization, making it well-suited for rainfed agriculture (Kumar and Rodge, 2012) [17]. It also exhibits resilience to mild salinity and alkalinity. While its immature, slender green pods are consumed as a nutrient-rich vegetable, being a good source of vitamins A and C and minerals like calcium, phosphorus, and iron, the crop has diverse applications. These include use as green fodder, green manure, and for seed production. The seeds are of particular industrial importance due to their high (28-33%) galactomannan gum content, located primarily in the endosperm. This guar gum is a versatile hydrocolloid with critical applications in numerous industries, including food processing, pharmaceuticals, textiles, paper, cosmetics, mining, and oil drilling. Furthermore, as a legume, it contributes to soil fertility by fixing atmospheric nitrogen.

Cluster bean is cultivated in several countries, including India, Pakistan, the United States, Brazil, and South Africa. India is the global leader in its cultivation. National cultivation covers approximately 4.26 million hectares, yielding 2.42 million tonnes with an average productivity of 567 kg/ha (Anonymous, 2020) [1]. The state of Rajasthan is the dominant producer within India, contributing nearly 80% of the national output from an area of 3.53 million hectares, with a production of 1.404 million tonnes and a productivity of 398 kg/ha (Anon, 2020) [1]. Of the global producers, India is the predominant cultivator of cluster bean (*Cyamopsis tetragonoloba* L.), accounting for an estimated 75-80% of the world's total

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production, with an annual output exceeding 600,000 tonnes.

Cluster bean production is constrained by a range of phytopathogenic diseases that significantly impact both grain and fodder yield. Historically documented pathogens include *Xanthomonas axonopodis* pv. *cyamopsidis* (bacterial blight), *Alternaria cyamopsidis* (Alternaria leaf spot), *Colletotrichum capsici* f.sp. *cyamopsicola* (anthracnose), and *Macrophomina phaseolina* (charcoal rot), among others. In recent years, stem rot caused by *Agroathelia rolfsii* has emerged as a particularly severe disease, leading to yield losses of 50-70%, depending on the severity of the infection. Its aggressive and destructive nature has made it a major limiting factor for cluster bean cultivation. Consequently, there is a pressing need to develop sustainable management strategies. The utilization of biocontrol agents is garnering significant attention for their dual capacity to promote plant growth and suppress soil-borne diseases (Nagamma and Nagaraja, 2015; Dwivedi and Prasad, 2016; Dhivya, 2020) [27, 11, 9]. The efficacy of this approach is supported by numerous studies demonstrating the successful antagonism of *A. rolfsii* by specific microorganisms and plant extracts across various host crops (Muthukumar and Venkatesh *et al.*, 2014; Ekundayo *et al.*, 2015; Pacheco *et al.*, 2016) [26, 12, 28]. In light of this empirical background, the present investigation was formulated with the primary objectives of evaluating the *in vitro* antagonistic potential of native bio-control agents and plant extracts against *A. rolfsii*.

2. Materials and Methods

2.1 Efficacy of Bio-Agents and Plant Extracts against *A. rolfsii* Under Laboratory Conditions

2.1.1 Isolation of Fungal Antagonists

Fungal antagonists, primarily *Trichoderma* spp., were isolated from soil samples using selective media: Trichoderma Selective Media (TSM) and Martin's Rose Bengal Agar. A soil suspension was prepared by adding 10 g of soil to 90 mL of sterile distilled water and agitating for 5 minutes. Serial dilutions up to 10^{-7} were made, and a 0.2 mL aliquot from an appropriate dilution was spread onto the media. The inoculated plates were incubated at 28 ± 1 °C for 7 days. Emerging fungal colonies were sub-cultured on Potato Dextrose Agar (PDA) and identified microscopically for subsequent use. The composition of all media is listed in Appendix I.

2.1.2. Isolation of Bacterial Antagonists

Pseudomonas fluorescens was isolated on selective Pseudomonas Agar Fluorescens (PAF) medium (King *et al.*, 1954). A soil suspension was prepared, and serial dilutions up to 10^{-7} were spread onto PAF plates. After incubation at 28 ± 1 °C for 48 hours, colonies were sub-cultured for purification.

Bacillus subtilis was isolated by adding 10 g of soil to 90 mL of sterile nutrient broth. The suspension was heat-shocked at 55 °C for 5 minutes to select for spore-forming bacteria and then incubated at room temperature for 48 hours. Serial dilutions were prepared, spread onto Nutrient Agar (NA) plates, and incubated at 28 ± 1 °C for 48 hours. Colonies were purified on NA for further use.

2.2 *In vitro* Evaluation of Antagonistic Potential

2.2.1 Evaluation of Fungal Antagonists

The antagonistic potential of fungal isolates was evaluated using a dual culture technique on PDA. A 5-mm mycelial

disc of the antagonist and *A. rolfsii* was placed 5 cm apart. Plates inoculated only with the pathogen served as the control. All treatments were replicated three times and incubated at 28 ± 1 °C. The radial growth of the pathogen was measured after 4 days, and the percentage inhibition of mycelial growth (PIG) was calculated as:

$$\text{PIG (\%)} = (C-T)/C \times 100,$$

where C is the radial growth in control (mm) and T is the radial growth in dual culture (mm).

2.2.2 Evaluation of Bacterial Antagonists

The antagonistic activity of bacterial isolates was assessed using a paper disc diffusion assay. Bacterial suspensions were prepared from 8-hour-old cultures. Sterile filter paper discs (5 mm diameter) were impregnated with the suspension and placed on PDA plates. A 5-mm mycelial disc of *A. rolfsii* was placed in the centre. Control plates contained pathogen discs alongside sterile water-impregnated discs. After incubation at 28 ± 1 °C for 7 days, the radial growth was measured, and the percentage inhibition was calculated using the formula above.

2.3 Efficacy of Plant Extracts

2.3.1 Preparation of Plant Extracts

Aqueous extracts were prepared from ten selected plant species. The plants used were: *Datura stramonium* L. (stem, flower, leaf), *Calotropis procera* Aiton. (leaves & flower), *Allium sativum* L. (bulb), *Allium cepa* L. (bulb), *Eucalyptus globulus* Labill. (leaves), *Azadirachta indica* A. Juss. (leaves and seed kernel, denoted as NSKE), *Citrullus colocynthis* (L.) Schrad. (fruit), *Tinospora cordifolia* (Willd.) Miers (stem), and *Curcuma longa* L. (rhizome powder). Fresh plant materials were collected, washed, and air-dried. One hundred grams of each plant part were macerated in 100 mL of sterile distilled water (1:1 w/v). The homogenate was filtered and centrifuged at 10,000 rpm for 10 minutes, and the supernatant was used as a stock solution.

2.3.2 *In vitro* Antifungal Assay

The antifungal activity was evaluated using the poisoned food technique. The stock solution was incorporated into PDA to achieve final concentrations of 5%, 10%, 15%, and 20%. A 5-mm mycelial disc of *A. rolfsii* was inoculated onto the amended medium. PDA plates without extract served as the control. All treatments were replicated three times and incubated at 28 ± 2 °C for 7 days. The radial growth was measured, and the percentage inhibition was calculated as previously described.

3. Results

3.2 Efficacy of Bio-Agents, Plant Extracts, and Fungicides against *A. rolfsii* *in vitro*

3.2.1 Efficacy of Bio-Agents

The *in vitro* efficacy of two fungal (*T. harzianum*, *T. viride*) and two bacterial (*P. fluorescens*, *B. subtilis*) bio-agents was evaluated against *A. rolfsii* using dual culture and paper disc assays. All tested bio-agents significantly suppressed the mycelial growth of the pathogen compared to the control. Among the fungal antagonists, *T. harzianum* demonstrated the highest inhibition at 70.78%, followed by *T. viride* at 57.57%, both producing well-defined zones of inhibition

(Fig. 1&2). Of the bacterial antagonists, *P. fluorescens* was more effective with a 50.78% inhibition, compared to *B. subtilis* at 45.52% (Fig. 1&2).

These findings are consistent with previous research documenting the suppression of *A. rolfsii* by microbial antagonists. Madhavi and Bhattiprolu (2011) ^[19] reported mycelial inhibition of 57.5% and 55.8% by *T. harzianum* and *T. viride*, respectively. Similarly, Nagamma and Nagaraja (2015) ^[27] observed a 63.33% reduction in pathogen growth by *T. viride*. Further supporting this, Kapadiya and Moradiya (2017) ^[14] found *T. viride* to be the most effective among tested isolates, reducing mycelial growth by 69.40% and sclerotial germination by 62.50%. The efficacy of these agents is often attributed to their ability to inhibit the actively growing mycelial tips of the pathogen. The broader applicability of biocontrol is reinforced by studies on other crops, such as the suppression of *A. rolfsii* in groundnut by *T. viride* and *Aspergillus niger* (Ekundayo *et al.*, 2015) ^[12], the management of tomato crown and root rot (Curtis *et al.*, 2010) ^[7], and the reduction of collar rot in sunflower using metabolites from *Trichoderma spp.* (Maji and Nath, 2016) ^[20].

4.2.2 Efficacy of Plant Extracts

All extracts significantly inhibited fungal growth in a concentration-dependent manner, with the 20% concentration proving most effective (Fig. 3). At this concentration, *Citrullus colocynthis* extract was the most potent, achieving 60.50% mycelial growth inhibition (Fig.

3&4). This was followed by *Azadirachta indica* leaf extract (53.77%), *Datura stramonium* (52.29%), and NSKE (43.88%). *Curcuma longa* rhizome powder extract was comparatively less effective, showing 28.70% inhibition (Fig. 3&4).

These results align with existing literature on the antifungal properties of plant extracts (Chavan *et al.*, 2024; Mahadevaswamy *et al.*, 2025; and M.T. *et al.*, 2025) ^[6, 22, 25]. The antifungal activity of plant extracts against *A. rolfsii* is well-established *in vitro*. Previous studies have documented the efficacy of extracts from species such as *Lupinus rotundiflorus* and *L. exaltatus* (Bernal *et al.*, 2005), *Ricinus communis*, *Ocimum basilicum*, *Lepidium virginicum*, and *Allium sativum* (Marcano *et al.*, 2005) ^[24], *Coronopus didymus* (Drakshan and Javaid, 2011) ^[10], *A. sativum* and *Zingiber officinale* (Yasmin, 2016) ^[31], as well as garlic and onion. Similarly, the strong inhibition by *Citrullus colocynthis* corroborates the work of Hadizadeh *et al.* (2009) ^[13], who identified colocynth as a potent inhibitor of soil-borne fungi. The concentration-dependent suppression of mycelial growth, as seen with neem extract in this study, has also been documented by Butt *et al.* (2016) ^[5]. While the application of botanical extracts represents a promising alternative to synthetic fungicides (Zapata *et al.*, 2020) ^[33], their efficacy under field conditions requires further validation. Future research should focus on evaluating the impact of these extracts on the pathogen's sclerotial inoculum in the soil to develop reliable, preventive control strategies for *A. rolfsii* and other soil-borne fungi.

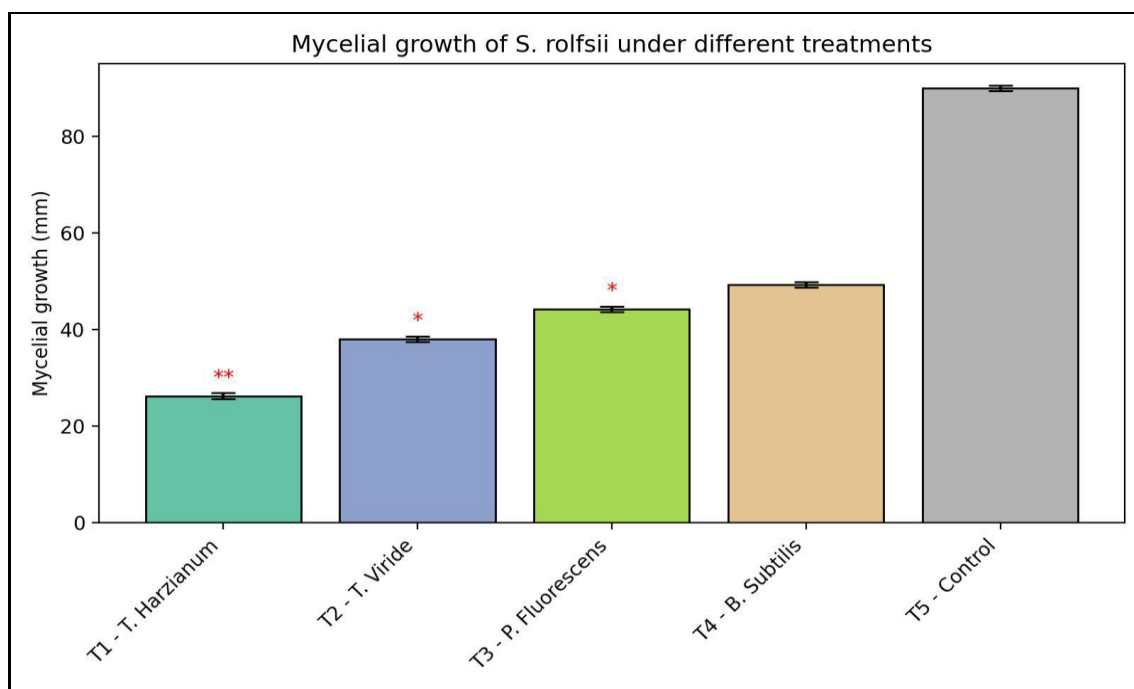


Fig 1: Efficacy of different bio-agents on mycelial growth of *Sclerotium rolfsii* *in vitro*



Fig 2: *in vitro* antagonistic effect of *T. harzianum*, *T. viride*, *P. fluorescens*, and *B. subtilis* on the mycelial growth of *A. rolfsii*, compared with the untreated control.

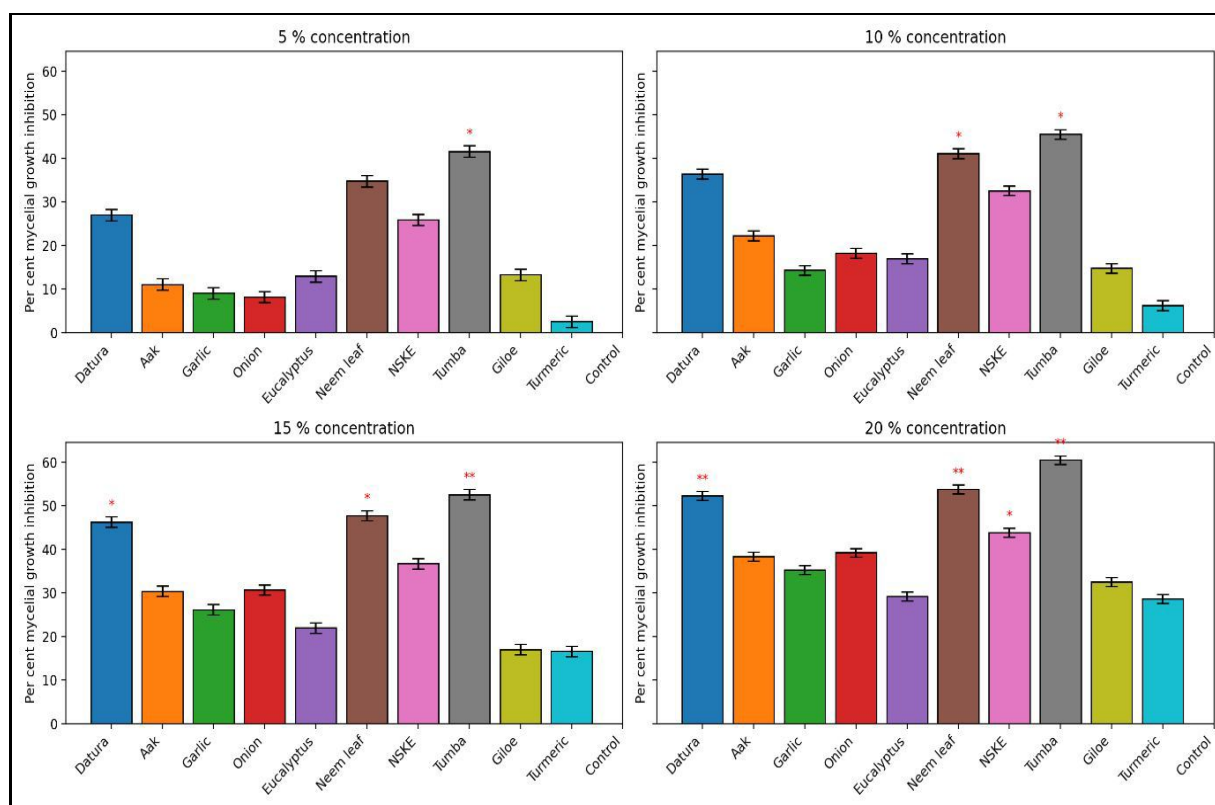


Fig 3: Effect of different doses of plant extracts on the Mycelial growth of *A. rolfsii* *in vitro*

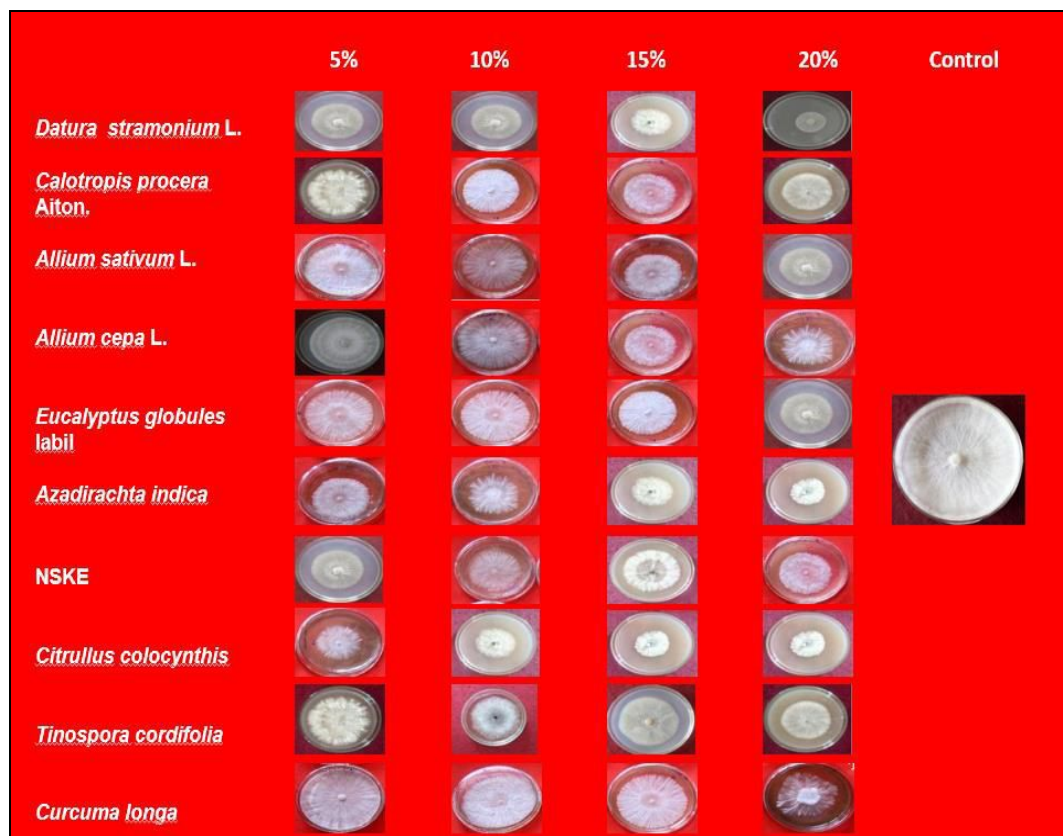


Fig 4: *in vitro* effect of different concentrations (5%, 10%, 15%, and 20%) of various plant extracts on the mycelial growth of *A. rolfsii*, compared with the untreated control.

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