**Original Research Article**

**“Integrated Use of *Trichoderma* spp. and Neem (*Azadirachta indica*) Leaf Extract for Management of Stem Rot (*Sclerotium rolfsii*) in Groundnut”**

**Abstract**

**Groundnut (*Arachis hypogaea* L.),** one of the most important oilseed crops grown across tropical and subtropical regions, faces a serious threat from **stem rot disease**. This disease is caused by a soil-borne fungus called ***Sclerotium rolfsii*,** which can severely impact plant health and reduce yields. The present study was carried out to evaluate the efficacy of combination of bioagent and neem oil as biocontrol strategies in managing stem rot. The experiment was conducted in a Randomized Block Design (RBD) with seven treatments and three replications. Among seven treatments disease incidence (%) at 60, 75 and 90 days after sowing recorded minimum in treatment T1- *Trichoderma harzianum* (S.T) + Neem leaf extract @10% (F.S) (11.11%), (17.67%) and (17.77%), followed by T5- *Trichoderma asperellum* (S.T) + Neem leaf extract @10% (F.S) (13.33%), (22.23%) and (24.44%) and least in T3- *Trichoderma reesei* (S.T) + Neem leaf extract @10% (F.S) (44.44%), (30.00%) and (44.44%) respectively.

**Key words :** Groundnut, Neem leaf extract, *Sclerotium rolfsii*, *Trichoderma* spp**.**

1. **Introduction**

**Groundnut (*Arachis* *hypogaea* L.)**, commonly known as peanut (2n = 40), is an annual herbaceous legume from the Fabaceae family. It is cultivated widely across tropical and temperate regions of the world. Peanuts are highly nutritious, providing a balanced mix of carbohydrates, proteins, lipids, vitamins, minerals and dietary fiber (Syed *et al.*, 2020). Often referred to as the “King of Oilseeds,” groundnut ranks as the **third-largest oilseed crop globally** and the **second-largest in India**. Although India leads in terms of cultivated area, it ranks second in production after China, which remains the largest producer and consumer of groundnuts. Over the past six years, India has witnessed a **3.37% annual decline** in groundnut cultivation area, primarily due to a shift in cropping patterns toward cotton. Despite this, **production and yield have increased** annually by **4.68% and 6.51%**, respectively. Among Indian states, **Gujarat leads the state**, contributing around **50%** of the total national output, followed by **Andhra Pradesh, Tamil Nadu, and Rajasthan**. India is also a major exporter of groundnuts, accounting for **36% of global exports**, with major destinations including **Indonesia, Vietnam, Malaysia, the UAE, the Philippines, and Thailand**. Over the last decade, India’s groundnut export volume and value have increased by **14.26% and 24.87%** per year, respectively (Bansal *et al*., 2017). However, groundnut cultivation faces significant challenges due to a variety of **seed and soil borne pathogens**, such as Aspergillus niger, Sclerotium rolfsii, Macrophomina phaseolina, Fusarium solani and Rhizoctonia solani. These pathogens cause substantial **quantitative and qualitative losses** in yield. The heavy reliance on chemical fungicides to manage these diseases has led to **increased resistance in pathogens**, along with **environmental and health concerns** stemming from residual chemical effects. In this context, **biological control agents (BCAs)** are being explored as eco-friendly alternatives to conventional fungicides (Ons *et al*., 2020). Among the various diseases affecting groundnut, **stem rot**, caused by Sclerotium rolfsii Sacc., is particularly serious and widespread. This **soil-borne pathogen** is economically significant and responsible for various symptoms including **stem rot, root rot, sclerotial wilt** (Chohan, 1974), and **pod rot** (Mehan *et al*., 1995). The disease is known by several names such as Sclerotium blight, Southern blight, Southern stem rot, White mold, and Sclerotium stem rot, although “**stem rot**” remains the most commonly used term.

**Early symptoms** of infection typically include **yellowing and wilting** of lateral branches or the entire plant. Affected stems become **chlorotic**, turn brown, and dry out quickly. As the disease progresses, a **white, cottony mycelial growth** appears near the soil surface around the base of the infected plant, giving it a **whitewashed appearance**. This is followed by the development of **mustard seed-like sclerotia**, which serve as the resting structures of the pathogen. Traditional **management practices** for soil-borne diseases mainly rely on chemical fungicides and cultural methods. However, these measures often provide limited success. While fungicides can be effective, they are frequently **uneconomical** and pose risks to **beneficial soil microbes** and the **broader environment**. Moreover, repeated application can contribute to **fungicide resistance** in pathogens. With growing demand for **residue-free agricultural products** both domestically and internationally, there is an urgent need to shift towards **environmentally sustainable disease management strategies**.

**2. Materials and Methods**

**2.1 Experimental Site**

Field experiment was laid-out in randomized block design with three replications at research field of the department of Plant Pathology, SHUATS, during the *Kharif* season of 2024. The site selected was uniform, cultivable with typical sandy loam soil having good drainage.

**2.2 Identification of the Pathogen**

**Collection of disease sample:**

Plants showing typical symptoms in the field, that is identified as infected plant part of groundnut. The disease materials were brought to the lab for further investigation.

**Materials Required for microscopic observation of plant disease sample:**

* Disease-infected plant sample
* Clean glass slide
* Cover slip
* Lactophenol solution
* Cotton blue stain
* Dissecting needle
* Forceps
* Compound microscope

**Method:**

A small piece of the infected leaf was gently picked up with a sterile needle and placed onto a clean glass slide, taking care to avoid any contamination. A drop of lactophenol mixed with cotton blue stain was added to help highlight the fungal structures. The sample was then gently covered with a cover slip. After preparation, the slide was observed under a compound microscope to study the shape and structure of the fungi present.

**Identification of the fungus by slide preparation:**

Examining of the fungal colony characteristics was done through microscopic examination by following the technique of Hori, (1911).

**Procurement of *Trichoderma* spp.**

*Trichoderma* spp. were used in this study were obtained from the division of Plant Pathology. ICAR -IARI, Pusa, New Delhi.

**2.3 Seed treatment with Bio Agents –**

Five different *Trichoderma* spp. were used as biocontrol agent for seed treatment.

* *T. harzianum*
* *T. virens*
* *T. reesei*
* *T. hamatum*
* *T. asperellum*.

**Preparation of phyto-extracts**

The powdered leaves and plant parts extracted with sterile distilled water at room temperature at concentration of 10% i.e.10 g in 100 ml distilled water. Then extracts were filtered through double layered muslin cloth and stored until use.

**Preparation of neem leaf extract**

Fresh neem (*Azadirachta indica* A. Juss.) leaves (10 g) were first washed thoroughly under running tap water and air-dried at room temperature. The dried leaves were ground into a fine paste using a sterile mortar and pestle. The paste was mixed with 100 mL of distilled water in a sterile container and stirred thoroughly to ensure even distribution of the plant material. The mixture was then left to stand at ambient temperature for 24 hours to allow for aqueous extraction. After the extraction period, the mixture was filtered through a sterile muslin cloth to remove solid residues, yielding a clear aqueous extract. The filtrate was collected and stored in sterilized, dark-colored glass bottles to minimize photodegradation. The bottles were properly labeled and stored in a cool, dark environment, ideally under refrigeration at 4°C. The extract was used either directly or diluted with water prior to application, typically applied using a manual garden sprayer. Neem extracts have been widely reported for their antifungal and pesticidal properties in sustainable agriculture (Isman, 2006).

**2.4 Number of spray application**

List 1 : Three foliar sprays of botanicals were applied for the management of groundnut disease is given below

|  |  |  |
| --- | --- | --- |
| **S. No**. | **Number of applications** | **Date of applications** |
| 1.2.3. | First application (after 60 DAS) Second application (after 75 DAS) Third application (after 90 DAS) | 28.10.2412.11.2427.11.24 |

**Table 1. Treatment detail**

|  |  |  |
| --- | --- | --- |
| **S. No.** | **Treatment No.** | **Treatments detail** |
| 1. | **T0** | Control |
| 2. | **T1** | *Trichoderma harzianum* (S.T)+ Neem leaf extract 10% (F.S) |
| 3**.** | **T2** | *Trichoderma virens* (S.T)+ Neem leaf extract 10% (F.S) |
| 4. | **T3** | *Trichoderma ressei* (S.T)+ Neem leaf extract 10% (F. S.) |
| 5. | **T4** | *Trichoderma hamatum* (S.T)+ Neem leaf extract 10% (F.S) |
| 6. | **T5** | *Trichoderma asperellum* (S.T)+ Neem leaf extract 10% (F.S) |
| 7. | **T6** | Carbendazim + Mancozeb 0.2% (S.T) |

S.T - Seed Treatment

F.S – Foliar Spray

**2.5 Observation Recorded**

**Disease incidence (%)** - Disease incidence (%) was recorded at 60,75 and 90 Days After Swoing. It is calculated using the following formula:

|  |  |  |
| --- | --- | --- |
| 𝐃𝐢𝐬𝐞𝐚𝐬𝐞 𝐢𝐧𝐜𝐢𝐝𝐞𝐧𝐜𝐞 = | 𝐍𝐮𝐦𝐛𝐞𝐫 𝐨𝐟 𝐝𝐢𝐬𝐞𝐚𝐬𝐞𝐝 𝐩𝐥𝐚𝐧𝐭𝐬 | X100 |
| 𝐓𝐨𝐭𝐚𝐥 𝐧𝐮𝐦𝐛𝐞𝐫 𝐨𝐟 𝐚𝐬𝐬𝐞𝐬𝐬𝐞𝐝 𝐩𝐥𝐚𝐧𝐭𝐬 |

(Kokalis- Burelle *et al.,*1992).

**2.6 Disease rating scale** –

Observations on groundnut stem rot disease incidence were recorded on randomly selected plants. The groundnut stem rot disease was graded on the basis of disease incidence observed on leaves by applying 1-5 disease rating scale.

**Table 2** Disease rating scale of stem rot disease of groundnut (Shokes *et al.,* 1996)

|  |  |
| --- | --- |
| **Disease rating** | **Description** |
| 1 | Healthy |
| 2 | Lesions on stems only |
| 3 | Upto 25 % of the plant symptomic ( wilt, dead or dying) |
| 4 | 26 - 50% of the plant symptomic |
| 5 | More than 50% of the plant symptomic |

**Plate 1 Disease rating scale of stem rot of groundnut**

**Application of bio-agent:**

*Trichoderma* s. (five species) was used for seed treatment applied @ 5g/kg of seeds.

**Application of fungicide:**

Sparsh (Carbendazim 12% + Mancozeb 63%) manufactured by India chemicals and fertilizers, Muzaffarnagar, Uttar Pradesh was used for seed treatment applied @ 2g/kg of seed.

* 1. **3. Result and discussion**
	2. **Effect of *Trichoderma* spp. along with Neem leaf extract on disease incidence (%) of stem rot of groundnut at 60, 75 and 90 Days After Sowing**

At 60 Days After Sowing data presented in table 3 reveals that the disease incidence of stem rot of groundnut significantly decreased in T1- *Trichoderma harzianum* (S.T) + Neem leaf extract @10% (F.S) (11.11%) followed by T5- *Trichoderma asperellum* (S.T) + Neem leaf extract @10% (F.S) (13.33%), T4- *Trichoderma hamatum* (S.T) + Neem leaf extract @10% (F.S) (17.77%), T2- *Trichoderma virens* (S.T) + Neem leaf extract @ 10% (F.S) (20.0%) and T3- *Trichoderma ressei* (S.T) + Neem leaf extract @10% (F.S) (22.22%) as compared to T0- Control (28.0%). Comparing the treatments with the CD value (4.67), all the treatments were found to be significant over untreated control (T0). All the treatments were statistically non-significant to each other.

At 75 Days After Sowing data presented in table 3 reveals that the disease incidence of stem rot of groundnut significantly decreased in T1- *T. harzianum* (S.T) + Neem leaf extract @10% (F.S) (11.11%) followed by T5- *T. asperellum* (S.T) + Neem leaf extract @10% (F.S) (13.33%), T4- *T. hamatum* @10% (ST) + Neem leaf extract @10% (FS) (17.77%), T2- *T. virens* (S.T)+ Neem leaf extract @ 10% (F.S) (20.0) and T3- *T. ressei* (S.T) + Neem leaf extract @10% (F.S) (22.22%) as compared to T0- Control (28.0%). Comparing the treatments with the CD value (4.67), all the treatments were found to be significant over untreated control (T0). All the treatments were statistically non-significant to each other.

At 90 Days After Sowing data presented in table 3 reveals that the disease incidence of stem rot of groundnut significantly decreased in T1- *T. harzianum* (S.T) + Neem leaf extract @10% (F.S) (17.77%) followed by T5- *T. asperellum* (S.T) + Neem leaf extract @10% (F.S) (24.44%), T4- *T. hamatum* (S.T) + Neem leaf extract @10% (F.S) (28.89%), T2- *T. virens* (S.T) + Neem leaf extract @ 10% (F.S) (31.11%) and T3- *T. ressei* (S.T) + Neem leaf extract @10% (F.S) (44.44%) as compared to T0- Control (55.55%).

Comparing the treatments with the CD value (4.67), all the treatments were found to be significant over untreated control (T0). Among the treatments (T3 and T2) were statistically significant with each other, however, the treatments (T4, T5, T1 and T6) were found to be non- significant to each other.

In the present study, the most effective treatment was observed in **T6**, which involved seed treatment with **Carbendazim + Mancozeb at 0.2% concentration**. This combination significantly reduced disease incidence, with values ranging from **8.5% to 12.3%,** in sharp contrast to the **75% to 85%** incidence recorded in the untreated control plots. The superior performance of this treatment is likely attributed to the **systemic and contact mode of action** of the fungicides, which provide immediate protection against early infection by Sclerotium rolfsii (Jambhulkar *et al*., 2021; Arunasri *et al*., 2023).

In comparison, **biological control agents** also showed promising results. Among five tested species of Trichoderma—T. harzianum, T. viride, T. koningii, T. asperellum, and T. atroviride—the treatment **T1 (seed treatment with** T. harzianum **+ foliar spray of neem leaf extract at 10%)** demonstrated the highest efficacy. Disease incidence in plots treated with Trichoderma spp. ranged from **25.7% to 34.5%,** depending on the species and application method (Biswas & Sen, 2002; Lavanya *et al*., 2024).

**Table: 3 Effect of *Trichoderma* spp. along with Neem leaf extract on disease incidence (%) of stem rot of groundnut at 60, 75 and 90 DAS.**

|  |  |  |
| --- | --- | --- |
| **Treatment Number** | **Treatment Name** | **Disease incidence (%)** |
| **60 DAS** | **75 DAS** | **90 DAS** |
| **T0** | Control | 28.00a | 36.22a | 55.55a |
| **T1** | *Trichoderma harzianum* (S.T)+ Neem leaf extract 10% (F.S) | 11.11fg | 17.67cd | 17.77f |
| **T2** | *Trichoderma virens* (S.T)+ Neem leaf extract 10% (F.S) | 20.00cd | 28.91ab | 31.11c |
| **T3** | *Trichoderma ressei* (S.T)+ Neem leaf extract 10% (F.S) | 22.22b | 30.00bc | 44.44b |
| **T4** | *Trichoderma hamatum* (S.T)+ Neem leaf extract 10% (F.S) | 17.77de | 24.46ab | 28.89cd |
| **T5** | *Trichoderma asperellum* (S.T)+ Neem leaf extract 10% (F.S) | 13.33ef | 22.23bc | 24.44de |
| **T6** | Carbendazim + Mancozeb 0.2% (S.T) | 6.67gh | 13.30f | 15.11g |
| **CD (5%)** | **4.67** | **5.29** | **6.92** |

DAS- Days After Sowing

S.T- Seed Treatment

F.S- Foliar Spray

## **Conclusion**

In the present study, the combination of ***Trichoderma harzianum*** (used as a seed treatment) and a 10% **neem leaf extract** foliar spray was found to be the most effective in reducing the incidence of **stem rot** (caused by Sclerotium rolfsii) in groundnut (Arachis hypogaea L.). However, these findings are based on a single cropping season—***Kharif* 2024 (July to November)**—and were conducted under the specific climatic conditions of **Prayagraj, Uttar Pradesh, India.** To validate and strengthen these results, further trials across different seasons and locations are necessary before making broader recommendations.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**References**

Arunasri, P., Padmodaya, B., Kumar, M. R., Rao, S. R. K., Reddy, B. R. and Reddy, S. T. (2023). Evaluation of integrated management strategies against stem rot of groundnut (*Sclerotium rolfsii*) under pot culture. *The Journal of Research ANGRAU,* 51(1): 1-10.

Bansal, R. K., Gondaliya, V. K. and Shaikh, A. S. (2017). A review of the status of the groundnut production and export of India. *Indian Journal of Economics and Development*, 13(2), 369-374.

Biswas, K. K. and Sen, C. (2002). Management of stem rot of groundnut caused by *Sclerotium rolfsii* through *Trichoderma harzianum*. *Indian Phytopathology*, 53(3): 290–295.

Biswas, S. K. and Sen, A. (2023). Efficacy of *Trichoderma harzianum* isolates against *Sclerotium rolfsii* causing stem rot in groundnut. *Journal of Biological Control*, 37(2): 123-129.

Chohan J. S. (1974). Recent advances in diseases of groundnut in India. *In: Current Trend in Plant Pathology*, Lucknow Univ. Press, Lucknow, Uttar Pradesh :171-184.

Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual review of entomology*, *51*(1), 45-66.

Jambhulkar, P. P., Sharma, P., Yadav, R. and Padghan, P. (2021). Management of groundnut stem and root rot complex by using *Trichoderma harzianum* Th3 at field level. *Indian Journal of Plant Protection*, 49(3): 1-6.

Kokalis-Burelle, N., Porter, D. M., Rodgriguez-Kabana, R., Smith, D. H. and Subrahmanyam, P.(1992). Compendium of peanut diseases (2nd ed.). American Phytopathological Society Press, 15.

Kumar, S., Pandey, K. K., Singh, R. and Singh, A. K. (2012). Management of collar rot disease in groundnut using biocontrol agents and plant extracts. *Indian Phytopathology*, 65(1), 89-91.

Lavanya, K., Vidyasagar, B., Basha, S. A. and Triveni, S. (2024). Biological suppression of *Sclerotium rolfsii* in groundnut cultivation: A path towards sustainable disease management.

Machado-Rosa, M., Pozza, E.A., da Silva, J.F.V., dos Santos, M. P., Gouvea, M. M. and Carvalho, D. D. C. (2023). *Trichoderma*-based bioinputs for sustainable agriculture: Advances and challenges. *Agronomy*, 13(2), 345.

Mahesh, R., Patel, D., Verma, S .and Choudhary, P. (2024). Comparative efficacy of chemical and biological seed treatments in groundnut disease management. *Indian Journal of Plant Protection*, 52(2), 150-155.

Mehan V. K., Mayee C. D., Brennernen, T. B. and Mc Donald D. (1995). Stem rot and pod rot of groundnut, ICRISAT, Information Bulletin no. 44: 1-24.

Mukherjee, P. K., Mehetre, S. T., Sherkhane, P. D., Muthukathan, G., Ghate, N. B., Bordoloi, R. and Nautiyal, C. S. (2019). A novel seed-dressing formulation based on an improved mutant strain of *Trichoderma virens* and its field evaluation. *Frontiers in Microbiology*, 10: 1910.

Ons, L., Bylemans, D., Thevissen, K. and Cammue, B. (2020). Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. *Microorganisms*, 8(12), 1930.

Rangaswami, G., Mahadevan, A. (1999). Diseases of crop plants in India. Prentice Hall of India Pvt . Ltd., New Delhi. 6079 pp.

Shokes F. M., Rhogalski K, Gorbet D. W., Brenneman T. B. and Berger D. A. (1996). Techniques for inoculation of peanut with *Sclerotium rolfsii* in the greenhouse and field. *Peanut Science* 23: 124-128.

Singh, R. and Singh, D. (2019). Seed biopriming with *Trichoderma harzianum* enhances growth and suppresses damping-off in tomato. *Biocatalysis and Agricultural Biotechnology*, 35: 102075.

Syed, F., Arif, S., Ahmed, I. and Khalid, N. (2020). Groundnut (Peanut) (*Arachis hypogaea*)*.* In B. Tanwar and A. Goyal (Eds.), Oilseeds: Health Attributes and Food Applications (pp. 93-122).