# POTENTIAL ACTIVITIES OF ALLIUM SATIVUM *in vitro* FLOWER EXTRACT AGAINST FUNGAL STEM ROT DISEASE OF *Arachis hypogeae* IN ADAMAWA STATE, NIGERIA

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## ABSTRACT

*Using synthetic chemicals to control fungi is cost-effective, non environmentally friendly, toxic to humans, and causes pollution to the water bodies. The research for biodegradable, non-toxic to humans, and economically viable for controlling fungi infection led to using traditional plants. Allium sativum has fungicidal activities against various organisms. The aim of this research is to determine the efficacy of the aqueous extract of Allium sativum flower against groundnut stem rot pathogen. The infected groundnut was obtained from various farms; the fungus was isolated and identified. The in vitro antifungal activity and its pathogenicity were also determined. The fungal Sclerotium rolfsii was isolated from the infected ground nut. The certified ground nut was planted on the sterile soil. The germinated seedlings were sprinkled with the pure isolate of Sclerotium rolfsii and the symptoms of the infection were observed. The organism was isolated from the infected leaves and stems. The anti-fungal activity of the extract of 20, 40, 60, and 80 % of Allium sativum flower aqueous extract shows significant inhibition on the mycelial growth of Sclerotium rolfsii. The Allium sativum flower aqueous extract has shown promising inhibitory effects against Sclerotium rolfsii.*

**Keywords**: *Groundnut, Stem Rot Disease, Aqueous, Sclerotium rolfsii, Allium sativum,*

## INTRODUCTION

Agricultural activities are the source of employment in Nigeria, especially in the northeastern part, which accounts for about one-third of the gross domestic product (Adejoorin et al., 2024). In Adamawa state, legumes, cereals, and root crops are farming activities while other cash crops such as sugar cane and cotton groundnut are also cultivated. Farming food and cash crops within Adamawa state depends on the socio-economic and culture of the people, coupled with the area's climate and other environmental conditions (Gelaye & Luo, 2024). Groundnuts are used in national and international markets as cash crops in developed and developing nations, with 108 countries, for industrial use, such as oil, cakes, fertilisers, animal feeds, etc. Nigeria produced about 500, 000 metric tons of ground in 1960 due to infectious diseases, the ground nut output dropped to about 2.6 million tons of groundnuts, while Kano, Kaduna, Taraba, Bauchi, Borno, and Adamawa states account for about 83 – 88%, as reported by Oni & Grace (2021).


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Many fungi that cause severe damage to pre- and post-harvest crops were generally managed by synthetic chemicals and considered efficient and effective. The synthetic chemicals are non-biodegradable, affect the environment and water bodies, and cause severe toxicity to human health (Parven et al., 2024). Searching for a biodegradable, environmentally friendly, and economically viable substance that can control the fungi in post and pre-harvest groundnut led to using traditional plants (Nxumalo et al., 2021). Hence, the investigation of Allium sativum aqueous extract against the *Sclerotium rolfsii*

## MATERIALS AND METHODS

### Study Area

The study was conducted in the Botanical Garden and Laboratory of the Department of Plant Science, Modibbo Adama University, Yola. Based on GPS coordinates, Adamawa State is located on Latitude 9 $^{0}$ 19' 60.00 "N and Longitude 12 $^{0}$ 29' 59.99" E (Google Maps, 2023), as shown in Figure 1. It shares boundaries with Taraba State in the South and West and Gombe in its Northern Guinea Savanna ecological zone. The area's climate is tropical, with an average temperature of 32 C and a relative humidity ranging from 15 % to 68 % (Chimatemps.com, 2015). The mean annual rainfall of Adamawa State ranges from 700 mm in the northwestern part to 1600mm in the Southern part; the length of the rainy season ranges from 120 – 210 days, mostly distributed from May to October (Adebayo, 2004). The state relative humidity peak is usually in the months of August and September (Chama *et al*., 2007).

### Sources of Groundnut Samples and Sample Size

Groundnut crop (whole plant) with stem rot symptoms was randomly collected from the three different selected farms in each Local Government Area (L.G.A.) in each of the geographical zones of Adamawa State (Mubi South, Mubi North, Michika from the Northern Senatorial zone, Song, Girei, Yola South from the central Senatorial zone and Ganye, Guyuk, Numan from the Southern Senatorial zone) as shown in Figure 1. The diseased groundnut crop was collected in a sterilized dry polythene bag and conveyed to the laboratory for laboratory analysis. A total of 270 samples were collected from nine (9) different Local Government Areas, with 30 samples from each L.G.A (10 samples from each farm) using a systematic sampling technique and labelled according to the location. Three (3) farms were randomly selected from each L.G.A at different locations where samples were collected.


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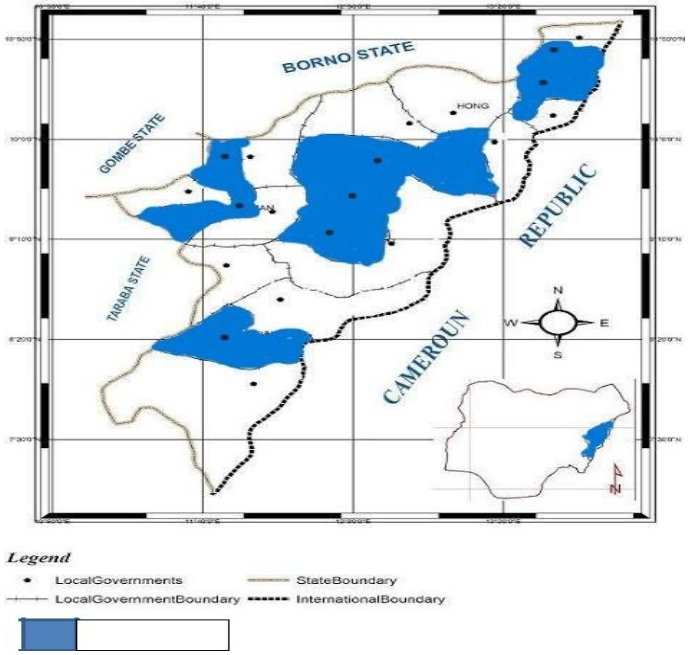
Figure 1: Map of Adamawa State Showing Study Areas




***Isolation of the pathogen***

The method of Burgess *et al.* (2008) was used. The diseased tissues (DT) from the periphery of the rotten groundnut stem were sectioned into 5 $mm^2$ pieces using a sterilised scalpel after sterilising the seeds in 0.1 % mercuric chloride solution for 30 seconds. They were rinsed in three changes of sterile distilled water. Sterilised pieces were picked with sterilised hot-flamed forceps, allowed to cool for a minute and dried between sterile filter papers. With cold sterilised forceps, a sterilised piece of the infected part was then plated out on sterile solidified potato dextrose agar (PDA) and incubated at a temperature of 30 ± 2 $^0C$ for 5 – 7 days and constant observation for any growth for sub-culturing. Pure isolates of fungal species were obtained by repeated sub-culturing on solidified sterile media, and pure cultures were preserved in McCartney bottles containing solidified PDA in a slant position. This was labelled accordingly.

The slants were corked loosely initially to enable the content fungus to grow and were then tightly corked and stored at a minimum temperature in a refrigerator to serve as stock cultures.

***Identification of isolated fungus***

The isolate was stained with Lactophenol cotton blue and observed at X 40 objective lens of microscope for fungi structure as described by Watanabe (2010) and compared with those in Alexosoulus *et al.* (2002)

***Pathogenicity test***

The pathogenicity test was carried out using the techniques of Okigbo *et al.* (2009). Certified groundnut seeds from the Adamawa Agricultural Development Program, Yola (AADP), were sown in a container containing sterilised soil. After germination, the 2 ml of dissolved isolate was sprinkled on the crop and observed for any disease symptoms. The diseased crop was removed, and the portion was to be surface sterilised with 0.1 % mercuric chloride solution for thirty seconds to remove the surface contaminant was rinsed in three changes of sterile distilled water and then dried using Whatman No. 1 filter paper. Inoculum from the infected stem was taken and cultured on the establishment of disease symptoms. The symptom of the infected crop and the isolated organism was compared with the first symptoms observed.

***Collection and preparation of plant extracts***

Ijato et al. (2011) used the method for ethanol extract of Allium sativum flower. The flower of Allium sativum (garlic) was obtained from Girei market, was rinsed under running water, and shade dried. The ground flowers weighing 80, 60, 40, and 20 g were soaked in 1000 mL of water to give 80, 60, 40, and 20 % concentrations, respectively and were kept for 24 hours with constant shaking. The sample was filtered with three layers of cheesecloth, and the filtrate was used in this study.

**In vitro anti-fungal of the extract**

The extract obtained was tested for its inhibitory activity against the groundnut stem rot pathogen *Sclerotium rolfsii* as described by Ijato *et al*. (2011). In the laboratory, fungal mycelium was grown on a PDA plate to observe the growth. The flower extract of 2.0 mL was applied to the centre of the PDA petri dish, and a small piece of mycelium was added to the PDA Medium. In the control petri dish, sterile water was added instead of the extract. The Petri dishes were incubated for 7 days at room temperature, and the growth inhibition was determined as described by Nene and Thalpiyal (2000).

**Data Analysis**

All the data was analysed using one-way and two-way analysis of variance (ANOVA), according to Gomez and Gomez (1984). Least Significant Difference (LSD) was used to separate the means where there was a significant difference. The statistical package used to analyse the result was Statistical Analysis Software (SAS) version 7.

**Result and discussion**

Groundnut stem rot is associated with various pathogens in the field and post-harvest period, leading to a grossly short supply of groundnuts. Abubakar et al. (2024), Yan et al. (2021), Leona et al. (2020) and Tarafdar et al. (2018) reported that the major pathogen that reduces the output of the groundnut by 30 % or more is *Sclerotium rolfsii.* The organism also affects other agricultural produce, such as cotton, soybeans, potatoes, onions, etc (Haveri, 2017). The treatment with 20, 40, 60 and 80 % of the garlic extract inhibits the growth of mycelium pathogen *Sclerotium rolfsii* in vitro and shows dose-dependent.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The activity of this extract may be due to the presence of some phytochemical components like terpenoids, flavonoids, alkaloids, and saponins, which have been reported to have anti-fungal activity. The presence of these antifungal substances in the plant at low concentrations is called fungistatic, but at high concentrations, they become fungicidal (Maksimov et al., 2021).

The pathogen is widely reported in America, Asia, Europe, and Africa (Willbur et al., 2019). The *Sclerotium rolfsii* can survive harsh conditions by hibernating in soil or within the environment until favourable conditions (Zheng et al., 2020). *Sclerotium rolfsii* is protected against chemical and biological degradation by the melanised cuticles (Greenhill, 2016). In conclusion, the garlic flower extract inhibits the Radial Mycelial Growth. Therefore, it can be used in the management of *Sclerotium rolfsii*.


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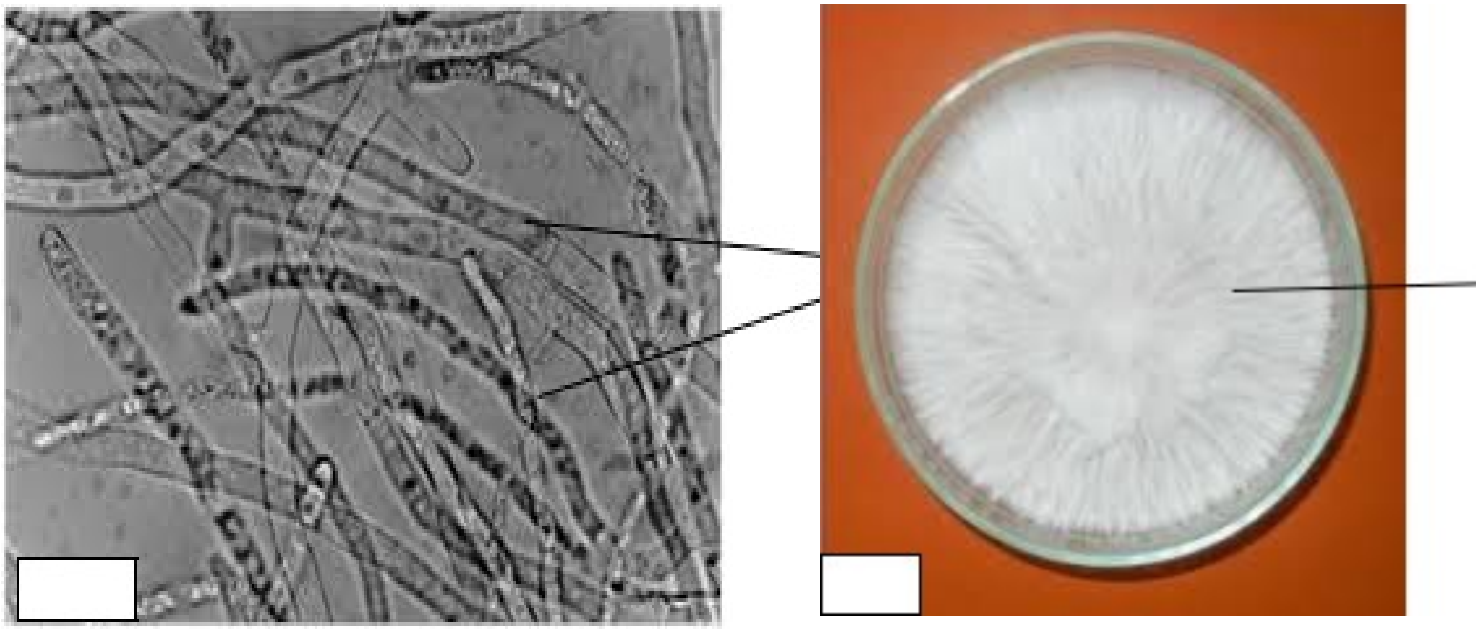

Figure 1: Pure culture of *A. sativum*

Table 1: Mean In vitro Effect of *A. sativum* Extracts on Radial Mycelial Growth of *S. rolfsii* after 7 Days

| Treatments | Radial Mycelial Growth (cm) |
|---|---|
| Flower | 1.39 ± 0.02 |
| Control | 2.80 ± 0.51 |
| LSD (P≤0.05) | 0.27 ± 0.01 |

Values are mean ± SEM of three determinants

Table 2: In vitro Mean Interaction Between *A. sativum* Extracts and Concentrations on Radial Mycelial Growth of *S. rolfsii* After 7 Days

| Treatment | Concentration (%) | Radial Mycelial Growth (cm) |
|---|---|---|
| Flower | 0 | 2.96 ± 0.32 |
| | 20 | 1.04 ± 0.21 |
| | 40 | 1.00 ± 0.13 |
| | 60 | 1.00 ± 0.17 |
| | 80 | 0.98 ± 0.01 |
| LSD (P≤0.05) | | 0.14 |

Values are mean ± SEM of three determinants


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