ANTIFUNGAL EFFECT OF *in vivo* GARLIC FLOWER EXTRACT ON GROUNDNUT STEM ROT DISEASE IN ADAMAWA STATE, NIGERIA

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ABSTRACT

Groundnut is one of the most important cash crops in Adamawa state, Nigeria. The ground nut is a good source of nutrients for humans, and the leaves and haulms are rich in nutrients for animal feed. Sclerotium rolfsii is a fungal that significantly constrains ground nut production by reducing yield and quality. This research aims to investigate garlic flower extract on the groundnut stem rot pathogen. The phytochemical analysis of the garlic flower extract, isolation of pathogen responsible for groundnut stem rot and determining the yield of the groundnut after treatment for 13 weeks. The qualitative phytochemical shows the presence of alkaloids, anthraquinones, flavonoids, glycosides, phenols, steroids, and terpenoids, while saponins and tannins were not detected. The flower contains high alkaloids with 5.04 ± 0.01, moderate terpenoids, glucosides, flavonoids, and phenols with 3.33 ± 0.03, 2.51 ± 0.04, 2.03 ± 0.01, 2.08 ± 0.02 respectively. The Sclerotium rolfsii was isolated from the stem rot of groundnuts. Treatment with the garlic flower extract significantly improved the yield of the groundnut. Therefore, the garlic flower can manage Sclerotium rolfsii.

Keywords: *Sclerotium, rolfsii, groundnut, Stem rot, Disease, garlic*

INTRODUCTION

According to the Food and Agriculture Organization, 50.46 million metric tons of groundnut was produced in 2023 (Tacán et al., 2024). Asia is the leading producer, with 74 %; Africa produces 19%; and Nigeria is the top producer of the African continent. Nigeria will produce 5 million metric tons of groundnut in 2024, an increase from 2023 with 4,8 million metric tons (Tacán et al., 2024). Ground nuts are used in industries to produce oil, chocolates, peanut butter, fertiliser, animal feeds, and other products, which play a significant role in the economics of northern Nigeria and Nigeria (Sakha et al., 2022). Kano's groundnut pyramids have declined but still play a vital role in industries and other consumers (Merem et al., 2021).

Diseases have affected ground nut production, especially fungi, during pre- and post-harvest periods. Some of the pathogenic fungi are *Aspergillus niger*, *Aspergillus ruber, Macrophomina phaseoling*, *Rhizoctonia solani*, *Fusarium solani*, F. *Oxysporum*, *Aspergillus flavus, and S. rolfsii* (Abubakar et al., 2024). Synthetic chemicals must treat these fungal infections (Reddy et al., 2022). However, the fungal become resistant to chemicals that are also non-biodegradable, influence the environment, pollute water bodies, and are toxic to humans (Aboelela & Bassyouni, 2024).

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The search for biodegradable, economically viable, and human-friendly for controlling fungi infections led to traditional methods (Gupta et al., 2023). One of the plants that is used and has antimicrobial activity is *Allium sativum* (Garlic), a member of the family *Alliaceae,* which is consumed raw or processed and contains various bioactive compounds responsible for treating diseases or physiological conditions (Rauf et al., 2022). *Allium sativum* is known as Garlic (English), Tafarnuwa (Hausa), Aayu (Yoruba), and Ayo-ishi (Igbo). Garlic has a tall, erected flowering stem, up to 1 meter. The leaf blade is flat, linear, and solid. The purple flowers grow from a bulb with a strong odour with 10 to 12 cloves (Nair & Groot, 2021). This research aims to survey and effectively in vivo groundnut stem rot disease management using *Allium sativum* flower extract.

Methodology

Area of Study

The study was carried out at the Botanical Gardens of the Plant Science Department, Modibbo Adama University, Yola, Nigeria, with coordinates of Latitude 90 19' 60.00 N and Longitude 120 29' 59.99‖ E. Adamawa state has a tropical climate with an average temperature of 32^0 C and relative humidity of 15 to 68 %.

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 study Local Government Area (L.G.A.) within the three zones. The local Governments are Mubi North, Mubi South and Michika (Northern Zone), Song, Girei and Yola South (Central Zone), and Numan, Guyuk and Ganye (Southern Zone). A total of 270 samples were collected from farms using random sampling techniques.

Collection and preparation of plant extracts

The *Allium sativum* bulb was purchased from Girei market, rinsed thoroughly under running tap water, and was allowed to air dry under shade for 7 days. The dried sample was ground using a pestle and mortar to powder and was stored in a container. The concentration of 20, 40, 60, and 80 % was prepared by dissolving 20, 40, 60, and 80 g in 1000 mL of water.

Collection of disease plant specimen

The incidence of groundnut stem rot on the farm was determined.

A quadrant of 3X3 m was plotted out in each farm, and the stands were counted (healthy and diseased) samples. The samples collected from the farms were sampled out, taking the number of diseased groundnut plants out of the total number of groundnut crops within the sample plot of each farm. The incidence of groundnut infection was expressed in percentage using the adopted formula given by Singh et al. (2012).

$$\text{Incidence} = \frac{Number\ of\ infected\ groundnut\ plant}{\text{Total number of ground nut plants simple}}\ X\ 100\ \%$$

The severity of the disease on the infected plant was determined by using the visual scale of 1-5 in which:

1 = 1- 20 % of Groundnut Plants infected,
2 = 21- 40 % of Groundnut Plants infected,
3= 41- 60 % of Groundnut Plants infected,
4 = 61- 80 % of Groundnut Plants infected,
5= More than 80 % of Groundnut Plants are infected.

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The symptoms on the stem based on the 1-5 visual scales were grouped into the following categories based on the Ratanacherdchail et al. (2010) rating scale. Both the disease incidence and severity on the groundnut farm were compared. The data obtained from each farm was used to calculate and compare the averages for each LGA, and subsequently, the average of the local government area was used to estimate that of the state.

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 5mm2 pieces using a sterilised scalpel after sterilising the seeds in 0.1% mercuric chloride solution for 30 seconds and 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 slants 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

Microscopic examination was made after examining the colony characteristics such as colony colour (front and reverse) and growth pattern and rate on media. A sterile needle was used to take a portion of the hyphae containing spores onto the glass slide, which was stained with Lactophenol cotton blue and was observed under the light microscope with power objective lens X 40 for the structures of the fungi (Watanabe, 2010). Morphological structures such as mycelia's septation and spores' nature were also observed under the microscope and will be compared with the structures in Alexopouluset al. (2002).

Field Experiments

Land preparation

The land was cleared with a cutlass, ploughed with a tractor, harrowed and divided into ridges with a hoe. A field plot of 0.5 m X 0.4 m size with 0.5 m inter plot space and 1.0 m outside border was used. Groundnut seeds (Ordaaji variety) were sown with hoe within a space of 25 cm inter-row and 25 cm intra-row with a depth of 0.02m using the adopted method of Philip et al. (2010). The treatments consist of aqueous garlic flower extracts, which consist of four sub-treatments, i.e., concentration levels (20 %, 40 %, 60 % and 80 %). The experiment was laid out in a Randomized Complete Block Design (RCBD) and replicated three times. The plots were then infected with the fungal soil pathogen isolated from the laboratory and were watered for five (5) days before sowing seeds

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Sowing

Sterilised healthy seeds of the groundnut variety (Ordaaji) were selected and soaked with the extract at four different concentration levels according to the modified method of Idowu et al. (2016) and Ahmed et al. (2023). The dressed seeds were then sown at two seeds per hole, at a spacing of 25 cm on a row and 25 cm within a row. The seedlings were later thinned to one plant per hole at two weeks after planting. Weed control was carried out at the third and sixth weeks after planting using hoe to remove unwanted weeds. Remoulding was carried out at 8-9 WAP to ensure proper weed control and a clean field during harvesting.

Data collection

Data were collected for Number of Leaves, Number of Branches, Length of Leaves, Number of pods, Number of matured pods, Number of immature pods, Number of healthy pods and Number of diseased pods. The height and number of leaves per plant were taken after two weeks, while the number of matured and immature pods per plant was taken at harvest.

Data Analysis

All the data was analysed using one-way and two-way analysis of variance (ANOVA), according to Gomez and Gomez (1984).

Result and Discussion

Using synthetic chemicals affects the management of plant diseases, which is unsafe, making plant pathologists search for alternatives with little or no environmental impact (Fortunati et al., 2019). Biological disease control was safer in plant disease management (Collinge et al., 2022).

The groundnut growing area is affected by stem rot and root rot infections. Garlic flower extract was able to increase the yield and improve the growth of ground nuts. Flower abortion and leaf defoliation have significantly improved compared to control. Tables 3 and 4 agreed with Abubakar et al. (2024), who treated the ground with different concentrations of garlic seed extract, and the result was dose dependent.

The groundnut yields have increased from 10.33 to 41.00, the number of matured pods from 3.00 to 39.00, the number of healthy pods from 2.33 to 35.33, and a significant decrease in the number of disease pods from 8.00 to 2.33 (Table 7 and 8). This increase in the yield might have resulted from the presence of alkaloids, flavonoids, and phenolic compounds.

The stem rot pathogen widely isolated from groundnuts is a Sclerotium rolfsii (*S. rolfsii*) in Adamawa state. This Sclerotium rolfsii is reported by Yan et al. (2021), Leona et al. (2020), and Abubakar et al. (2024) as the causative agent for the ground nut stem rot disease from their research. Abubakar et al. (2024) also reported that *S. rolfsii* has significantly reduced groundnut yield to about a quarter of its production. The *S. rolfsii* pathological effect affects over 500 species of 100 families of plants (Haveri, 2017). This pathogen has significant economic effects on farmers of various crops. Cereals, legumes, weeds, tomatoes, chilli,

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forage plants, and vegetables have pathogenic effects on these plants. This organism secretes cellulose enzymes to degrade the plant tissues, and oxalic acid that chelates with calcium in the plant tissues is a cell wall component and lowers the external pH (Dora et al., 2022). The presence of alkaloids inhibits the enzyme cellulose, thereby preventing the degradation of the plant tissue, and phenolic compounds prevent calcium chelation by oxalic acid (Ahlawat et al., 2024).

The phytochemical screening shows the presence of alkaloids, anthraquinones, flavonoids, glycosides, phenols, steroids, and terpenoids, while saponins and tannins were not detected. This study agrees with Ali and Ibrahim (2019) and Behbahani and Fooladi (2018).

The antimicrobial activities of *Allium sativum* have been widely known. Studies have shown that the leaves, flowers, bulbs, and roots of *Allium sativum* contain high amounts of flavonoids and phenols, and these compounds have antioxidant activities, antimicrobial and antitumour (Kurnia et al., 2021). The alkaloid concentration is 5.04 ± 0.01 in the *Allium sativum* flower, which could suppress enzyme activity, disrupt cell membranes and target DNA and RNA for protein synthesis (Yan et al., 2021). Alkaloids also prevent biofilm formation and burst the immune system (Damyanova et al., 2024). Fungal growth has been inhibited by flavonoids by induction of mitochondria dysfunction, protein and RNA synthesis, cell wall formation, and efflux pump inhibition (Al Aboody & Mickymaray, 2020). The phenolic compounds can partition the lipid membranes, causing an increase in the pH of cytosolic and vacuole, ionic haemostasis and disruption of the integrity of the cellular structure (Kim, 2024).

In conclusion, the garlic flower extract inhibited the fungal *S. rolfsii*, thereby increasing the yield of the groundnut and reducing leaf abortion and defoliation. This extract can be used to manage groundnut's fungal stem rot pathogen.

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Table 1: Qualitative Phytochemistry of *A. sativum* Flower Extract

S/No	Phytochemicals	
1	Alkaloids	+
2	Anthraquinone	+
3	Flavonoids	+
4	Glucosides	+
5	Phenols	+
6	Saponins	-
7	Steroid	+
8	Tannins	-
9	Terpenoid	+

Key: + = Present, - = Absent

Table 2: Quantitative Phytochemistry of *A. sativum* Flower Extract

S/No	Phytochemicals	Concentration
1	Alkaloids	5.04 ± 0.01
2	Anthraquinone	1.50 ± 0.02
3	Flavonoids	2.03 ± 0.01
4	Glucosides	2.51 ± 0.04
5	Phenols	2.08 ± 0.02
6	Saponins	-
7	Steroid	Trace
8	Tannins	-
9	Terpenoid	3.33 ± 0.03

Values are mean ± SEM of triplicate

Table 3: Effect of *A. sativum* Extracts on Pathological Characters of Groundnut

S/No	Plant Extracts	Leaf Defoliation	Flower Abortion
1	Flower	12.16 ± 0.02	3.12 0.04
2	Control	2.14 ± 0.03	0.81 ± 0.01
3	LSD (0.05)	1.55 ± 0.04	0.57 ± 0.02

Values are mean ± SEM of triplicate

Table 4: Effect of *A. sativum* Extracts and Concentrations on the Pathological Characters of Groundnut Infected with *Sclerotium rolfsii*

Concentration (%)	Flower Abortion	Leaf Defoliation
0	261.00 ± 0.20	0.94 ± 0.01
20	12.83 ± 0.06	3.11 ± 0.04
40	16.28 ± 0.07	4.00 ± 0.10
60	18.33 ± 0.05	4.06 ± 0.02
80	10.72 ± 0.02	2.67 ± 0.08
LSD (0.05)	1.55 ± 0.03	0.57 ± 0.01

Values are mean ± SEM of triplicate at 5% significant level

Table 5: Effect of *Allium sativum* flower extract on growth characters of groundnut infected with *S. rolfsii*

Concentration (%)	Germination Count	Number of Leaves	Number of Branches
0	0.89 ± 0.02	15.89 ± 0.21	7.58 ± 0.04
20	0.67 ± 0.05	53.28 ± 0.43	22.78 ± 0.21
40	1.00 ± 0.02	71.61 ± 0.54	31.44 ± 0.43
60	1.00 ± 0.02	70.56 ± 0.21	27.11 ± 0.53
80	0.72 ± 0.03	43.50 ± 0.34	16.78 ± 0.64
LSD (0.05)	0.07 ± 0.01	0.42 ± 0.03	0.51 ± 0.07

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Table 6: Effect of *Allium sativum* flower extract on growth characters of groundnut infected with *S. rolfsii*

Concentration (%)	Leave Length	Branch Length	Plant Height
0	2.06	1.62	2.00
20	4.55	3.52	5.54
40	6.11	4.73	8.52
60	6.77	7.73	8.38
80	3.99	5.69	6.02
LSD (0.05)	0.23	0.97	0.99

Table 7: Effect of *A. sativum* Flower Extract on Yield Characters of Groundnut Infected with *S. rolfsii*

Extract	Number of pods	Number of matured pods	Number of immature pods	Number of Healthy pods	Number of Disease pods
Flower	41.12	36.50	4.44	33.67	6.10
Control	8.37	2.20	6.17	1.84	6.53
LDS (0.05)	3.11	2.33	2.91	3.48	2.84

Table 8: Effect of *A. sativum* Flower Extract on Yield Characters of Groundnut Infected with *S. rolfsii*

Concentrations	Number of pods	Number of matured pods	Number of immature pods	Number of Healthy pods	Number of Disease pods
Flower 0	10.33	3.00	7.33	2.33	8.00
20	43.00	39.00	4.33	35.33	3.67
40	37.67	36.00	1.67	34.00	2.00
60	40.67	38.67	2.00	34.33	4.33
80	41.00	37.00	4.00	34.67	2.33
LSD (0.05)	2.51	3.23	4.73	3.43	4.29

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