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Original article

Survey and control fungal stem rot disease of groundnut (*Arachis hypogaea* L.) using garlic leaf extracts (*Allium sativum* L.) in Adamawa State

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ABSTRACT

Groundnut fungal stem rot is one of the most important limiting factors to groundnut production and one of the most important diseases of groundnut caused by *Sclerotium rolfsii* which is a destructive soil-borne fungal pathogen. The survey and control of stem rot disease of groundnut in Adamawa State was conducted from 2017 to 2023. The research focused on groundnut incidence and severity of stem rot pathogen, phytochemical screening of the various garlic parts used and *in vitro* management of the pathogen using Leaf extracts of *Allium sativum*. Samples collected from nine local government areas of Adamawa State were taken to Plant Science laboratory of Modibbo Adama University, Yola in a dry sterile polythene bag. Laboratory work was carried out in the Department of Plant Science and Biotechnology. Potato Dextrose Agar (PDA) was used for the isolation and in vitro control trials. The result for incidence of stem rot disease of groundnut from the nine Local Government Areas of Adamawa State showed Mubi North had the highest incidence of 22.34 %, while Guyuk had the least incidence of 6.75 %. The level of stem rot disease severity revealed that Ganye recorded the highest severity of 4.60 and Guyuk had the least with 2.40. Qualitative phytochemical analysis reveals the presence of alkaloids, flavonoids, phenols, steroids and terpenoids in the aqueous garlic leaf extracts. Quantitatively, alkaloids were recorded as the highest occurring phytochemical with 6.71% while tannins were the lowest with 1.40%. Plant extract materials were effective in inhibiting the growth of *Sclerotium rolfsii* in vitro. The level of inhibition increased with increase in concentrations. It is therefore recommended that the use of *A. sativum* leaf extracts for the management of groundnut stem rot should be encourage among local farmers at a concentration between 40-60 % because of its effectiveness, affordability and environmental friendliness.

Keywords: *Arachis hypogaea, Sclerotium rolfsii, Allium sativum,* stem rot and phytochemical

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is also known as peanuts, earthnuts, gobbers, pinders, manila nuts (1). It is a member of the genus *Arachis* in the family Leguminosae which has replaced the traditional Bambara groundnut (*Vigna subterranean*) in most countries of the world. It is an annual, self-pollinated, wet season growing plant found in many tropical, subtropical and temperate countries of the world (2). Groundnut kernels are consumed directly as raw, roasted or boiled kernels or oil extracted from the kernels is used as culinary oil. It is also used as animal feed (oil pressing, seeds, green materials and straw) and industrial raw material (oil cakes and fertilizer). The uses of groundnut plant make it an excellent cash crop for domestic markets as well as for foreign trade in several developing and developed countries (3). It is now grown in about 108 countries of the world (4). Annual world Bambara groundnut production is estimated at approximately 0.3 million tons, of which 0.2 million tons were produced in Africa (5). The largest producers of Bambara groundnut in Africa are Burkina Faso, Niger, Cameroon and Nigeria (5). Nigeria leads gross Bambara groundnut production with 100,000 metric tons per annum while Burkina Faso leads with highest production yield (5). Yields in developing countries are very low ranging from 0.3 to 0.9 tons per hectares compared (due to poor soil nutrients and microbial diseases) to very high yields of 2.8 tons per hectare in the United States of Amierica (6).

Groundnut is considered as a valuable legume crop cultivated over an area of 994 hectares in Pakistan with a production of about 1019kg/hectare or 101 tons during 2001 – 2002 (7). Groundnut seed contains 50% edible oil. Seeds are rich in fats, proteins, vitamin B_1, B_2, B_6, nicotinic acid other vitamins (8). Recently the consumption of groundnut has been associated with metabolic dysfunctions which lead to obesity and metabolic syndrome (9). It is a major seed crop with a great global economic importance (9). It is found in a wide range of grocery products, its shells are used in the manufacture of plastic, wallboard, abrasives and fuel (10).

Southern blight, also known as stem rot, is caused by a soilborne fungus. The disease is widespread on peanuts and other crops (11). The fungus primarily attacks the base of stems near the soil line, but any plant part in contact with soil may be damaged. Infected plants are generally killed prior to maturity. Peg and pod infections are common and result in pod loss at harvest. Populations of *S. rolfsii* increase in infested fields cropped to peanut unless control measures are taken (11). High populations of the pathogen combined with favorable conditions for southern blight can result in yield losses of 25 percent or more.

MATERIALS AND METHODS

Study Area

The study was carried out in the Botanical Garden and Laboratory of Department of Plant Science, Modibbo Adama University, Yola. Base on GPS coordinates, Adamawa State is located on Latitude 9^o 19’ 60.00 “N

and Longitude 12° 29' 59.99" E (12). It shares boundaries with Taraba State in the south and West, Gombe in its Northern Guinea Savanna ecological zone. The climate of the area is tropical with average temperature of 32°C and a relative humidity ranging from 15% to 68% (13). The mean annual rainfall of Adamawa State ranges from 700mm in the North Western part to 1600mm in the Southern part; the length of the rainy season ranges from 120 – 210 days mostly distributed from May to October (14). The state relative humidity peak is usually in the months of August and September (15).

Sources of Groundnut Samples and Sample Size

Groundnut crop (whole plant) with stem rot symptoms was randomly collected from the three different farms of each Local Government Area (L.G.A.) selected among 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 on Figure 1. 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 systematic sampling technique and was labeled according to the location. Three (3) farms were selected at random from each L.G.A at different locations from where samples were collected.

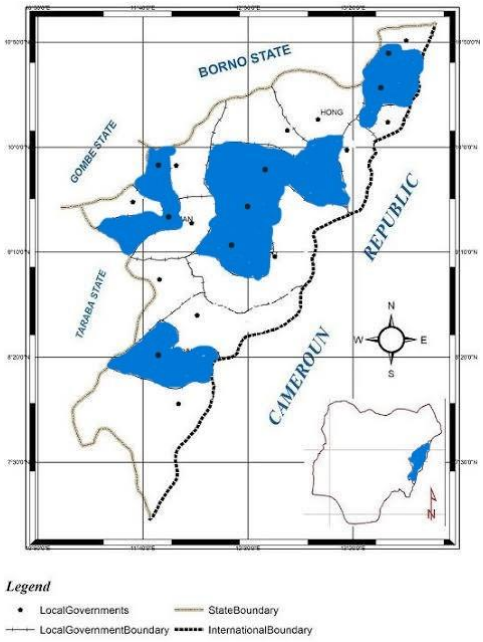


Figure 1: Map of Adamawa State Showing Study Areas (Blue)

Collection of Disease Plant Specimen

Incidence of groundnut stem rot on farm was determined. A quadrant of 3X3m 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 (16)

$$\frac{\text{Number of infected groundnut plants}}{\text{Total number of groundnut plants simpled}} \times 100\ \%$$

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 infected.

The symptoms on the stem based on the 1-5 visual scales were grouped in the following categories based on the (17) 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 average of local government area were used to estimate that of the state.

Medium for Isolation and Identification

The medium used for the isolation and in vitro control trials was Potato Dextrose Agar (PDA) (18). Thirty-nine (39) grams of PDA was dissolved into 1 litter of distilled water. The PDA was poured into conical flask, then covered with cotton wool and wrapped with aluminum foil before autoclaving it at 121^0 C for 15 minutes at 10 lbs pressure, and 200 hundred milligrams of chlorophenicol was added to the sterilized media, just before pouring into Petri-dishes to prevent bacterial growth and allowed to cool and solidify. The prepared media was autoclaved for 15minutes, 10Ib pressure and allowed to cool.

Isolation of the Pathogen

The method of (19) was used. The diseased tissues (DT) from the periphery of the rotten groundnut stem were sectioned into $5mm^2$ pieces using sterilized scalpel after sterilizing the seeds in 0.1% mercuric chloride solution for 30 seconds and was rinsed in three changes of sterile distilled water. Sterilized pieces were picked with sterilized hot-flamed forceps, allowed to cool for a minute and were dried between sterile filter papers. With cold sterilized forceps, a sterilized piece of the infected part was then plated out on sterile solidified potato dextrose agar (PDA) and incubated at 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 labeled according to organisms. 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 on to 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 (20). Morphological structures such as septation of mycelia and nature of spores was also observed under the microscope and will be compared with the structures in (21).

Pathogenicity Test

Pathogenicity test was carried out using techniques of (22). Certified groundnut seeds form Adamawa Agricultural Development Program, Yola (AADP)were sown in container containing sterilized soil. After germination, the 2ml of dissolved isolate was sprinkled to the crop and was observed for any symptom of the disease. The diseased crop was removed and the portion to be surface sterilized with 0.1% mercuric chloride solution for thirty seconds to remove surface contaminant and was rinsed in three changes of sterile distilled water and then dried using Whatman No. 1 filter paper. On establishment of disease symptoms, inocula from the infected seeds were taken for each isolate and cultured. The symptom of the infected crop and the isolated organism was compared with the first symptoms observed.

Collection and Preparation of Plant Extracts

The method of (23) was used to prepare the aqueous extract. Fresh leaves of garlic plant were collected from Girei main market, Girei Local Government Area, Adamawa State. These were taken to the Plant Science Department of Modibbo Adama University, Yola.

The collected garlic leaves were rinsed thoroughly under running tap water and were allowed to air dry under shade for 7 days. These were ground separately, 80 g each of the plant material was dissolved in 100 ml of distilled water and shaken vigorously to give 80% concentration, likewise 60 g, 40 g and 20 g were dissolved into 100ml of distilled water each to give 60 %, 40 % and 20 % concentration respectively in separate conical flasks and were kept for 24 hours. The sample was filtered with three layers' cheese cloth. The aqueous filtrate was used for control trials.

Effect of Extracts on Fungal Mycelia Growth

The approach of (23) was used to evaluate the effect of the extracts on fungal growth by creating four equal sections on each plate by drawing two perpendicular lines at the bottom of the plates. The point of interception indicates the centre of the plates. This was done before dispensing PDA into each of the plates. The extracts were then poured into the flask plug with cotton wool and were kept at room temperature (24).

About 2ml of extracts of leaf extracts of *Allium sativum* were separately introduced into the Petri-dish containing the media and pure isolates (poisoned food method). Control experiment was without addition of any plant extract but sterile distilled water. Fungus growth inhibition was determined in terms of percentage growth (25).

$$\text{Inhibition percentage (\%)} = \frac{DC-DT}{DT} \text{ X } 100$$

Where; DC – Average diameter for fungi growth in control
DT- Average diameter of fungal growth with treatment.

Qualitative and Quantitative Phytochemical Analysis

The qualitative phytochemical screening of samples was carried out as described by (26) and (27). The plant extract materials were screened for alkaloids, flavonoids, steroid, phenols, tannins, saponin, glycosides, anthraquinine and terpenoids.

Data Analysis

All the data was analyzed using one-way and two-way analysis of variance (ANOVA) according to (28). Least Significant Difference (LSD) was used to separate the means where there is a significant difference. The statistical package used to analyze the result will be Statistical Analysis Software (SAS) version 7.

RESULTS

Incidence and Severity of Stem Rot Disease of Groundnut

Survey on the incidence and severity of stem rot disease of groundnut in Adamawa State is presented on Table 1 below. The level of disease incidence shows that the state had high level of stem rot disease of groundnut with high variations among locations. Result revealed that there was a high significant difference among all the locations. Mubi North recorded significantly higher incidence of 22.34 % which is statistically the same with that recorded in all other locations; this was followed by Ganye and Girei which had incidence of 18.20 % and 17.32 % respectively. This is followed by Yola South and Numan which had incidence of 14.56 % and 12.23 % respectively, Song had an incidence of 9.10 %, Michika had 8.43 % and Mubi South had 7.54 %. The least incidence of 6.75 % was recorded in Guyuk. Base on the geopolitical zones, there was a statistically significant difference at $P \leq 0.05$ between the Central Senatorial Zone and the other two zones. Central Senatorial Zone had an incidence of 13.67 % followed by Northern Senatorial Zone with 12.77 % and then Southern Senatorial Zone with 12.39 % as shown in Table 2.

The analysis of variance on severity of stem rot disease of groundnut showed significant difference among all the Local Governments except between Michika, Mubi South and Numan at $p \leq 0.05$. Ganye had the highest level of stem rot disease with 4.60, followed by Mubi North with 4.40, Yola South with 4.00, Girei had 3.20, Michika, Mubi South and Numan had 3.00, Song had 2.60 while Guyuk had the least disease severity with 2.40 (Table 1). Analysis of variance (ANOVA) on severity shows no significance difference between the three geopolitical zones of Adamawa State (Table 2).

Table 1: Incidence and Disease Severity of Groundnut Stem rot in Adamawa State

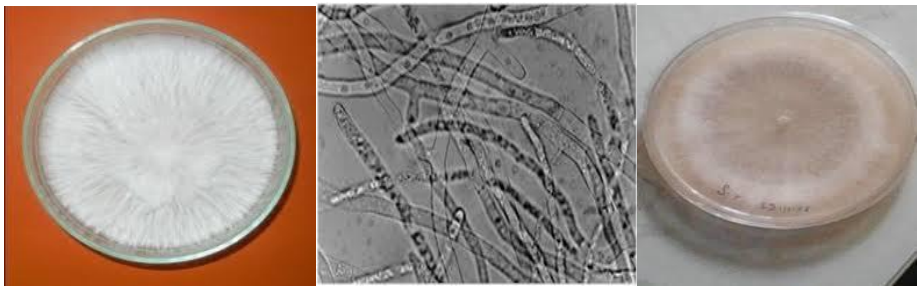
Locations	Disease incidence (%)	Severity level
Mubi North	22.34	4.40
Girei	17.32	3.20
Numan	12.23	3.00
Michika	8.43	3.00
Yola South	14.56	4.00
Song	9.10	2.60
Mubi south	7.54	3.00
Ganye	18.20	4.60
Guyuk	6.75	2.40
LSD	1.23	1.10

Table 2: Incidence and Disease Severity of Groundnut Stem Rot in Geopolitical Zones of Adamawa State

Geopolitical Zones	Incidence (%)	Severity Level
Northen Senatorial Zone	12.77	3.47
Central Senatorial Zone	13.67	3.27
Southern Senatorial Zone	12.39	3.33
LSD	1.23	1.10

Description and Identification of *Sclerotium rolfsii*

Sclerotium rolfsii produces abundant white mycelium on infected plants and in culture (Plate I a). Advancing mycelium and colonies often grow in a distinctive fan-shaped pattern and the coarse hyphal strands may have a somewhat ropy appearance. In culture, mycelium appears smooth at first, but some cultures may develop aerial mycelial that cover all or part of the culture after a few days (Plate Ic). The fungus produces at least two types of hypae, large diameter (5 to 9 µm) main branch hyphae and smaller diameter (2 to 4 µm) branch hyphae (Plate Ib). Cells are hyaline with thin cell walls and sparse cross walls. Main branch hyphae may have clamp connections on each side of the septum. In agar plate culture, sclerotia are not formed until the mycelium covers the plate. In vitro or in vivo, sclerotia begin as small tufts of white mycelium that form spherical sclerotia 0.5 to 1.5 mm in diameter. Sclerotia darken as they mature, becoming tan to dark brown in color. Young sclerotia often exude droplets of clear to pale yellowish fluids. Mature sclerotia are hard, slightly pitted, and have a distinct rind. Although most sclerotia are spherical, some are slightly flattened or coalesce with others to form an irregular sclerotium. *S. rolfsii* does not form asexual fruiting structures or spores.



A **B** **C**

Plate I: (A) Four-day old pure culture *Sclerotium rolfsii* (B): Mircograph of Four Day Old *Sclerotium rolfsii* (C): Seven-day Old Pure Culture of *Sclerotium rolfsii*

Qualitative Phytochemical Analysis of Garlic Extracts

Result for the qualitative phytochemical analysis of the garlic leaf used as extracts in the management of stem rot disease of groundnut caused by *S. rolfsii* are

presented on Table 3. Analysis was carried out for alkaloids, anthraquinne, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids. The analysis revealed that alkaloids, flavonoids, phenols, steroids and terpenoids were in the aqueous leaf extract of garlic. Glucosides were not detected in the leaf.

Quantitative Phytochemical Analysis of Garlic Extracts

Result for the quantitative phytochemical analysis of all the garlic leaf used as extracts in the management of stem rot disease of groundnut caused by *S. rolfsii* are presented on Table 4. The analysis shows that alkaloids is the highest occurring phytochemical in aqueous leaf extract of garlic with 6.71, followed by phenols with 5.02, flavonoids with 4.40, steroids (4.06), anthraquinne (2.90), saponins (2.07) while tannins is the least occurring phytochemical.

Table 3: Qualitative Phytochemistry of *A. sativum* Leaf Extract

Phytochemicals	Leaf	
Alkaloids		+
Anthraquinne		+
Flavonoids		+
Glycosides		-
Phenols	+	
Saponins	+	
Steroid		+
Tannins	+	
Terpenoid		+

Key:
+ = Present
- = Absent

Table 4: Quantitative Phytochemistry of *A. sativum* Extracts

Phytochemicals	Leaf (%)	
Alkaloids	6.71	
Anthraquinne		2.90
Flavonoids		4.40
Glycosides		-
Phenols	5.02	
Saponins	2.07	
Steroid		4.06
Tannins	1.40	
Terpenoid		2.55

Effect of *A. satium* Leaf Extracts Concentration on Radial Mycelial Growth of *S. rolsii*

Leaf Extracts of *A. sativum* were able to inhibit the radial mycelial growth of groundnut stem rot pathogen (*S. rolfsii*) *in vitro* at different concentration levels. From the analysis of variance (ANOVA), there was a significant difference between the treatments and the control at P≤ 0.005. From Table 5, 20 % concentration had the highest level of inhibition of radial mycelial growth of the pathogen after 7 days of incubation with *A. sativum* leaf extract (1.05 cm) while 80 % concentration had the least inhibition level with leaf extract (1.16 cm). there was a decrease in level of inhibition with increase in concentrations of the plant materials as shown in Table 5 below.

Table 5: Effect of *A. satium* Leaf Extract Concentration on Radial Mycelial Growth

Concentration (%)	Radial Mycelial Growth (cm)
0	2.75
20	0.99
40	1.04
60	1.06
80	1.13
LSD	0.14

DISCUSSION

Sclerotium rolfsii is the pathogen responsible for stem rot disease of groundnut in Adamawa State. The pathogen was also reported by (29) to be the causative agent of stem rot disease of groundnut in Wuhan, Hubei, China. (30) as well as (31) all reported this same pathogen (*S. rolfsii*) as the organism responsible for the stem rot disease of groundnut in their separate research conducted in India. (31) reported *Sclerotium rolfsii* as the major pathogen that reduces groundnut production by nearly 30 % as a result of stem rot disease caused by the pathogen. *Sclerotium rolfsii* is a destructive soil-borne fungal pathogen, it affects more than 600 plant species especially economically important agricultural and horticultural crops to include groundnut, soybeans, wheat, cotton, tomato, potato, cucurbit and onions (29). *Sclerotium rolfsii* can infect stems, root, pegs and pods of groundnut and cause branch wilting and even whole plant wilting. The pathogen produces white mycelium on infected plants and in culture, advancing mycelium and colonies often grow in a distinctive fan-shaped pattern and coarse hyphal strands may have a somewhat ropy appearance. In agar plate culture, sclerotia are not form until the mycelium covers the plate. Sclerotia darken as they mature, becoming tan to dark brown in colour. Stem rot disease was recorded in all the local government areas visited during the survey and the virulence exhibited by the pathogen on groundnut seedling/plants were rated high.

From the result of the phytochemical constituents present in the leaf garlic extracts used for this research, it shows

that alkaloids, anthraquinne, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids are present in the plant materials at different levels. The results agree with (32) who reported the presence of all nine phytochemicals in both aqueous and ethanolic extracts of garlic bulb. The extract of *A. sativum* bulb showed the presence of alkaloid compounds, glycosides, saponins, flavonoids, steroids, proteins, carbohydrates, oils, reducing sugar and acid compounds (33). (34) stated that garlic is highly rich in vitamins, antioxidants, flavonoids and minerals. It also contains flavonoids, saponins and sapogenin, phenolic compounds (35). The active compounds of garlic that are reported to have antimicrobial activities include Allin, Ajoenes, Allyisulfides and 1,2-vinyldthiin (36; 37; 38; 39; 40). (41) reported the presences of flavonoids, terpenoids and glycosides in chloroform, methanol, DMSO and aqueous extracts of garlic. Phytochemicals give plants their colour, flavour, smell and are part of a plant`s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites (42). It is believed that the antimicrobial activity of garlic is attributed to a sulfoxide compound isolated from fresh ground garlic pulp known as Allicin.

Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties (43). Steroids are importance in pharmacy as they possess compounds like sex hormones and can be used for drug production. Tannin and saponin were present in the extract. Saponins protect against hypercholesterolemia and antibiotics properties. In addition, it has been found that saponins have antitumor, antioxidant and anti-mutagenic activities and can lower the risk of human cancers by inhibiting the growth of cancer cells (42). The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins (44). The finding of this study correlate with the finding of (45) which found that clove extract possessed a broad spectrum of antimicrobial activity exhibited for both bacteria and fungi due to presence saponin, tannin, flavonoid and terpenoid. The result of this study on Phytochemistry of Garlic supported the study conducted by (46) who found that garlic extracts showed activity against both gram negative (*E. coli*, *Salmonella* spp, and *Citrobacteren terobacter*, *Pseudomonas Klebsiella*) and gram positive (*S. aureus*, *S. pneumonia, streptococcus* and *Bacillus anthrax*) due to presence of some phytochemicals such saponin and tannin.

The result from the management of stem rot disease of groundnut showed that *Allium sativum* leaf extracts were all able to inhibit the radial mycelium growth of groundnut stem rot pathogen *Sclerotium rolfsii* in vitro at different degree. This agrees with (33) who reported that the bark, bulb, leaves, flowers and stem of *Allium sativum* showed antimicrobial activity. The potential of antimicrobial activity of each part of the plant depends on the solvent used, the concentration and level of secondary metabolites contain there in (33). The findings of this research agree with that of (47) who reported *Allium sativum* bulb as one of the botanicals that effectively inhibits mycelial growth of *Sclerotium rolfsii in vitro*. The genus *Allium* are known to inhibit the growth of microorganism such as bacteria, fungi, viruses and parasites (48). Garlic is an antibacterial as well as antifungal agent (49; 50). It contains several hydrophobic antimicrobial compounds, such allicin, vinyldithiins, ajoenes and

diallylpolysulfides (51;39). The garlic (*Allium sativum* L.) is a plant reported to possess various biological activities including antifungal activity (52; 53). Garlic extracts exhibited potential inhibition on the mycelial growth of *Colletotrichum gloeosporioides* which was isolated from leaves of the para rubber tree. (54) reported that water extract of garlic showed inhibitory effects on *Colletotrichum gloeosporioides* and *Fusarium* spp spore growth. *A. sativum* leaves produced the largest diameter of inhibition zone at a concentration of 100 ul against *Bacillus subtilis* and *Aspergillus niger* (55). The antifungal activity of the extracts can be linked to the presence of secondary metabolites which have been shown to possess bioactive properties (56).

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