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Survey and *In vivo* Control of Stem Rot Pathogen of Groundnut (*Sclerotium rolfsii* L.) Using Garlic Leaf Extracts in Adamawa State

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ABSTRACT

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 of stem rot pathogen, proximate composition and its *in vivo* 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. Field management of *Sclerotium rolfsii* was conducted in the Departmental and Federal Polytechnic Mubi farm. 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 composition of the infected groundnut seeds shows a decrease in lipid, protein, ash, fibre and carbohydrates while there was an increase in moisture as a result of the activities of the pathogen. The level of inhibition increased with increase in concentrations. High increase in growth (NL=87 and NB=31) and yield characters (NP=54, NMP= 51 and NHP= 52) were also recorded in the treated groundnut farms compared with the non-treated control.

Key words:*Arachis hypogea, Allium sativum, Sclerotium rolfsii,* Incidence, Concentrations and Extracts

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is also known as peanuts, earthnuts, gobbers, pinders, manila nuts (Beghin *et al*., 2003). It is a member of the genus *Arachis* in the family *Leguminosae* (Fabacaea) 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 (Halima, 2000). 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 (FAO, 2006). It is now grown in about 108 countries of the world (Srivastava *et al.,* 2011). Asia with 63 – 65 % land mass produces 71.72 % of world groundnut followed by Africa with 18.6 % production and North-central America with 7.5 % (Malakar *et al.,* 2008). The Nigerian's annual production of groundnut (yield in-shells) in 1990, 1995 and 1998 were 0.992, 1.6 and 2.6 million tons while areas under cultivation were 0.7, 1.8 and 2.3 million hectares respectively (Danladi, 2000). 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 (International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), 2012).

Southern blight, also known as stem rot, is caused by a soilborne fungus. The disease is

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widespread on peanuts and other crops (Subrahmanyam *et al*., 2000). 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 (Subrahmanyam *et al*., 2000). 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 9^0 19' 60.00 "N and Longitude 12^0 29' 59.99" E (Google Map, 2023). 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 32ºC and a relative humidity ranging from 15% to 68% (Chimatemps.com, 2015). 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 (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 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. 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.

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 Singh *et al.* (2012)

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

Isolation and Identification of the Pathogen

The medium used for the isolation and in vitro control trials was Potato Dextrose Agar (PDA) (Zakawa *et al*. 2018). The method of Burgess *et al.* (2008) 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

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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^{0}C 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.

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 (Watanabe, 2010). 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 Alexosoulus *et al*. (2002).

Pathogenicity Test

Pathogenicity test was carried out using techniques of Okigbo *et al.* (2009). Certified groundnut seeds form Adamawa Agricultural Development Program, Yola (AADP)were sown in container containing sterilized soil. After germination, 2ml of dissolved isolate was sprinkled unto 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 Ijato *et al*. (2011) was used to prepare the ethanol 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 plant 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 100 ml 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.

Proximate Composition of Groundnut Caused by Fungal Pathogens

Proximate analysis was conducted on both healthy and diseased groundnuts using the methods described by Association of Analytical Chemist (AOAC, 2007) and Ani *et al.* (2012) for the following; crude fibre, crude protein, ash content, oil/lipid content, moisture content and carbohydrates.

Field Experiments

Land preparation

The land was cleared with cutlass, ploughed with tractor, harrowed and divided into ridges

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with a hoe. Field plot of 0.5 m X 0.4 m size with 0.5 m and 0.5 m inter plot space, and 1.0 m outside border was used as adopted by Ibrahim and Dadari (2000). Groundnut seeds (Ordaaji variety) were sown with hoe within a space of 0.2 m inter-row and 0.4 m intra-row with a depth of 0.02m using the adopted method of Philip *et al.* (2010). The treatments consist of aqueous leaf extracts (LE) of garlic, each treatment consists of four sub-treatments i.e., concentration levels (20 %, 40 %, 60 % and 80 %) 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 of seeds. Sterilized healthy seeds of groundnut variety (Ordaaji) were selected and soaked with each 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 seed per hole, at a spacing of 0.2 m on row and 0.4 m within row. The seedlings were later be thinned to one plant per hill at two weeks after planting.

Data collection

Data were collected on for Germination Count, Number of Leaves, Number of Branches, Leaf Length, Branch Length, Leaves Defoliation, Flower Abortion, Number of pods, Number of matured pods, Number of immature pods, Number of healthy pods and Number of unhealthy pods. Height and number of leaves per plant was taken after two weeks while numbers of matured and immature pods per plants were taken at harvest.

RESULTS AND DISCUSSION

Incidence of Stem Rots Disease of Groundnut

Survey on the incidence 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 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.

Sclerotium rolfsii is the pathogen responsible for stem rot disease of groundnut in Adamawa State (Plate 1 a, b and c). The pathogen was also reported by Yan *et al.* (2021) to be the causative agent of stem rot disease of groundnut in Wuhan, Hubei, China. Doley and Jite (2013) as well as Leona *et al.* (2020) 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. Rangarari *et al.* (2017) 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,

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cucurbit and onions (Yan *et al.,* 2021). *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.

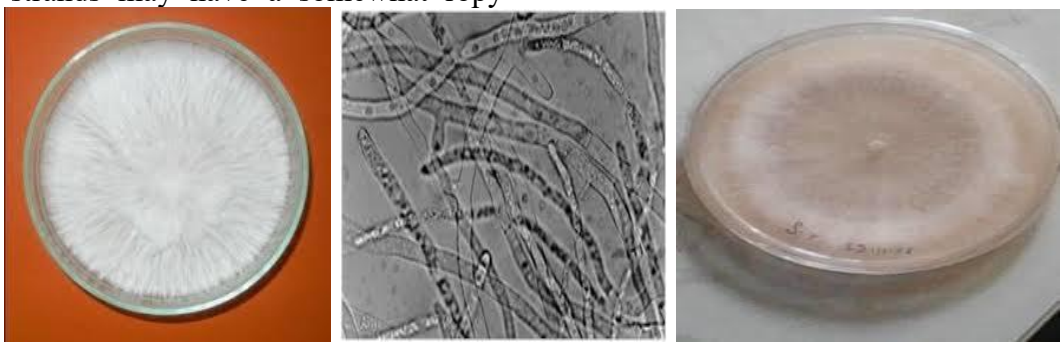

Plate 1: (a) Four days old pure culture of *S. rolsii* (b) Microgram of *S. rolsii* (c) Seven days old pure culture of *S. rolsii*

Table 1: Incidence of Groundnut Stem rots in Adamawa State

Locations	Disease incidence (%)
Mubi North	22.34
Girei	17.32
Numan	12.23
Michika	8.43
Yola South	14.56
Song	9.10
Mubi south	7.54
Ganye	18.20
Guyuk	6.75
LSD	1.23

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

Geopolitical Zones	Incidence (%)
Northen Senatorial Zone	12.77
Central Senatorial Zone	13.67
Southern Senatorial Zone	12.39
LSD	1.23

Proximate Analysis of Infected and Healthy Groundnut Seeds

The effect of stem rot disease on the proximate composition of the groundnut understudy is presented in Table 3. From the analysis of variance (ANOVA), there is a significant difference in the proximate composition of the infected and the healthy groundnut seeds at $P \leq 0.05$. There was a decrease in the percentage of protein from 26.45% in the healthy to 22.74 %, fats/oil decreases from 58.02 % to 37.20 %, Ash also decreases from3.47 % to 3.36 %, crude fibre

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decreases from 2.27 % to 1.02 % while carbohydrates decrease from 12.05 % to 8.51 %. However, moisture increases from 7.74 % to 8.31 % as a result of the action of the fungal pathogen on the groundnut seeds.This is in agreement with the works of Ekhuemelo and Abu (2018) who reported decrease in crude protein, fats/oil and crude fibre of groundnut seeds infected by *Aspergillus* spp in Makurdi, Nigeria with an increase in moisture content. Also, the reduction in the nutritional content of the infected groundnut seeds in this study agrees with the reports of Amusa *et al.* (2006) in which African star apple fruits and guava fruits infected by fungi had a significant lower percentage nutrient composition in the infected fruits. Waliyar *et al*. (2015) noted the decline of groundnut quality resulting from aflatoxin incidence. Reduction in nutrients contents is due to the utilization of the nutrients by the fungi for growth and survival (Marschner and Baumann, 2003). The lipid content of the groundnut falls between the range (41-48 %) reported by Makeri *et al*. (2011); Boli *et al*. (2013) and Ekhuemelo and Abu (2018). Also, the moisture content of groundnut falls within the range (7.48 %) of raw groundnut seeds reported by Ayoola and Adeyeye (2010) and Boli *et al*. (2013).

Table 3: Effect of Stem Rot Disease on the Proximate Composition of Groundnut

	Status of Proximate composition (%)					
Groundnut	**Protein**	**Fats/oil**	**Ash**	**Moisture**	**Fibre**	**Carbohydrates**
Infected 22.74	37.20	3.36	8.31	1.02	8.51	
Healthy 26.45	48.02	3.47	7.74	2.27	12.04	
LSD	0.36	1.05	0.40	0.75	0.33	0.95

In vivo Effect of Plant Extracts on Growth Related Characters of Groundnut Infected with *S. rolfsii*

Treatment of groundnut with *A. sativum* extracts (leaf) was able to inhibit the activities of *S. rolfsii* pathogen on growth related characters of groundnut in the field (Plate 2 a and b). The analysis of variance (ANOVA) shows a significant difference among the plant extracts and the non-treated control used on the different growth characters at P≤ 0.05. For germination count, number of leaves were highest at 40 % treatment (50) and lowest in the non-treated control, it follows the same pattern for mean number of leaves, leaf length and flower defoliation. Leaf length was highest in the 60% treatment with 5.98 while it was lowest in the non-treated control (2.03). Leaf defoliation occurred highest in the 60% treatment with 4.76 while it was lowest in the non-treated control as shown on Table 4.

Effect of *A. sativum* Leaf Extract Concentrations on the Yield Parameters of Groundnut Infected with *S. rolfsii* in Adamawa State

Effect of *A. sativum* leaf extract concentrations on the yield characters of groundnut infected with stem rot pathogen in Adamawa State is presented on Table 5. There was a statistically significant difference between *A. sativum* leaf extract concentrations and the non-treated control at P≤0.005. For mean number of pods per plant, treatment of *A. sativum* leaf with 60 % concentration had the highest pod yield with 47.00 while the non-treated control had the least mean number of pods per plant with 5.00. For mean number of matured pods per plant, treatment concentration of 80 % *A. sativum* leaf extracts had the highest mean number of pods per plant with 42.33 while the non-treated control had 1.33. For mean number of immature pods per plant, treatment concentration of 20 % *A. sativum*

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leaf extracts had the highest mean number of pods per plant with 7.33 while the non-treated control had 3.67. For mean number of healthy pods per plant, treatment concentration of 80 % *A. sativum* leaf extracts had the highest mean number of healthy pods per plant with 39.33 while the non-treated control had 1.00. For mean number of diseased pods per plant, treatment concentration of 20 % and 40 % *A. sativum* leaf extracts had the highest mean number of diseased pods per plant with 9.67 while the non-treated control had 4.00.

The plant height, number of leaves, number of branches and leaf shade of this research were better than that of the control. This agrees with Adeleke (2016) who reported that groundnut plants treated with the lower concentration of garlic extracts compared favorably with the control, while those with higher concentrations decrease in leaf area and plant height. Flower defoliation increases in treatments with garlic extract materials, this is however not in agreement with Koita *et al*. (2017) who reported that all plant extracts used controlled defoliation to a significant level compared to the negative control, which recorded the highest defoliation rate. In terms of yield, there was an increase in number of pods per plant, number of matured pods per plant and number of healthy pods per plant in all the plant treated with garlic extract materials. This agrees with Koita *et al*. (2017) that aqueous extracts from four plant species increased pod yield of infected groundnut over the negative control. Krishna and Pande (2005) reported that foliar application of *Prosopis juliflora* extract effectively reduced groundnut foliar disease severity and increased the pod yield. Another study revealed that foliar application of neem leaf extracts recorded significant improvement in pod yield and other yield characters of groundnut (Kumawat *et al*.,2009). Kongkaew and Phichai (2010) also found that dried garlic powder, which was extracted, using a maceration method in distilled (DI) water and 95 % ethanol solvent, was effective at inhibiting the growth of *Trichoderma* spp. isolated from Yanagi mushroom. Sittisart *et al*. (2017) reported that the dried leaves and fruits of garlic extracted using a Soxhlet extractor in DI water solvent where solvent capable of preventing fungal infection in groundnut crop.

Plate 2: (a) Healthy groundnut seedling from experimental farm (b) Diseased groundnut seedling from experimental far

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Table 4: Effect of *A. sativum* Leaf Extracts Concentration on the Some Growth Characters of Groundnut Infected with *Sclerotium rolfsii*

Concentration (%)	Germination Count	Number of Leaves	Number of Branches	Leaf Length (cm)	Flower Abortion	Leaf Defoliation
0	0.94	15.72	7.22	2.03	2.22	0.83
20	0.67	36.28	16.39	4.07	7.44	2.94
40	1.00	50.67	26.50	4.99	14.89	4.72
60	1.00	48.33	21.78	5.98	12.06	4.76
80	0.72	30.56	14.72	3.91	8.00	2.94
LSD (0.005)	0.07	0.42	0.51	0.99	1.55	0.57

Table 5: Effect of *A. sativum* Leaf Extract and Concentrations on the Yield of *S. rolfsii* infected Groundnut

Concentrations (%)	Number of Pods	Number of Matured Pods	Number of Immature Pods	Number of Healthy Pods	Number of Diseased Pods
0	5.00	1.33	3.67	1.00	4.00
20	41.33	34.00	7.33	31.67	9.67
40	46.00	39.33	6.67	36.33	9.67
60	41.00	37.33	3.67	33.33	7.67
80	47.00	42.33	5.00	39.33	7.67
Mean	26.67	30.86	5.27	28.33	7.74
LSD (0.005)	2.51	3.23	4.73	3.43	4.29

CONCLUSION

In conclusion, the incidence of stem rot disease was rated as high in the farms visited/surveyed. *Sclerotium rolfsii* was isolated as an agent of stem rot disease of groundnut in Adamawa State. *Sclerotium rolfsii* produces abundant white mycelium on infected plants and in culture. Advancing mycelium and colonies often grow in a distinctive fan-shaped pattern and the coarse hyphal strands may have a somewhat ropy appearance. There was a lost in nutritional composition of the groundnut due to the activities of the pathogen. Higher values in both growth and yield parameters were observed in the treatments with plant material (leaf) compared to the non-treated control especially in the number of pods per plant and number of healthy pods per plant which led to increase in yield.

Fungal contamination is a direct relationship with seeds, and this can alter the yield quantity and quality of the plant. Other factors affecting groundnut production are rainfall, temperature and other cultural practices. Improper seed management and soil treatment contributes to the spread of the pathogen responsible for stem rot disease. Seeds and soil that are not well treated will exposed to groundnut plants to various disease-causing pathogens. Hence, the use of garlic leaf extracts is a promising control alternative to fungal stem rot of groundnut.

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