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In vitro efficacy of seed extracts of Allum sativumagainst stem rot pathogen of and its effect on proximate composition of Groundnut (Arachis hypogea L.) in Adamawa State, Nigeria

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ABSTRACT
Groundnut fungal stem rot disease caused by Sclerotium rolfsii a destructive soil-borne fungal pathogen is one of the most importance disease of groundnut in Nigeria. The survey and control of stem rot disease of groundnut in Adamawa State was conducted from 2017 to 2023. The research focused on proximate compositionand in vitromanagementof the disease pathogen using seed extracts of garlic (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 composition of the infected groundnut seeds showed a decrease in lipid (37.20 %), protein (22.74 %), ash (3.36 %), fibre (1.02 %) and carbohydrates content (8.51%) while there was an increase in moisture content (8.31 %) as a result of the activities of the pathogen. Plant extract materials were effective in inhibiting the growth of Sclerotium rolfsiiin vitro ($P \leq 0.05$). The level of inhibition increased with increase in concentrations but was not significantly different. 80% concentration had least radial mycelial growth (1.06 cm) while the non treated control had the highest radial mycelial (2.91 cm).It was recommended that further research should be conducted to evaluate the level of toxin contaminations caused by the isolate on infected groundnut seeds.

Keywords: Arachis hypogea, Sclerotium rolfsii, Allium sativum, Stem rot and Phytochemistry

I. 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). 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 (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

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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).

Groundnut is considered as a valuable legume crop cultivated over an area of 994 hectares in Pakistan with a production of about 1019 kg/hectare or 101 tons during 2001 – 2002 (Anon, 2002). Groundnut seed contains 50 % edible oil (Verma et al., 2003). Seeds are rich in fats, proteins, vitamin B_1, B_2, B_6, nicotinic acid other vitamins (Paramasivam, 2005). Recently the consumption of groundnut has been associated with metabolic dysfunctions which lead to obesity and metabolic syndrome (Coates and Howe, 2007). It is a major seed crop with a great global economic importance (Coates and Howe, 2007). It is found in a wide range of grocery products, its shells are used in the manufacture of plastic, wallboard, abrasives and fuel.

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

II. 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 32 ^{o}C 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 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. 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 waslabeled according to the location. Three (3) farms were selected at random from each L.G.A at different locations fromwhere samples were collected.

Medium for isolation and identification

The medium used for the isolation and in vitro control trials was Potato Dextrose Agar (PDA) (Zakawa et al. 2018). 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, 10 Pa were allowed to cool.

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 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

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the infected part wasthen 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 waslabeled 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 (Watanabe, 2010). Morphological structures such as septation of mycelia and nature of spores was also observed under the microscope and was compared with the structures in Alexopouluset al. (2002).

Pathogenicity test

Pathogenicity test was carried out using techniques of Okigboet al. (2009). Certified groundnut seeds form Adamawa Agricultural Development Program, Yola (AADP)were sown in container containing sterilized soil. After germination, the 2 ml 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 stem 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 Ijatoet al. (2011) was used to prepare the ethanol extract. Fresh root, bulbs, leaves, seed and flower 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 parts (stem, root, Bulb, flower and leaf) 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 1000 ml of distilled water and shaken vigorously to give 80 % concentration, likewise 60 g, 40 g and 20 g were dissolved into 1000 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.

Effect of extracts on fungal mycelia growth

The approach of Ijatoet al. (2011) 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 (Madari and Singh, 2005).

About 2ml of extracts of root, bulbs, leaves, seed and flower 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 (Nene and Thalpiyal, 2000).

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

Where; DC – Average diameter for fungi growth in the control

DT- Average diameter of fungal growth with the treatment.

Proximate composition of healthy and infected groundnut

The methods described by Association of Analytical Chemist (AOAC, 2007) and Aniet al.(2012) were used to determine the crude fibre, crude protein, carbohydrates, per centage lipids/oil, moisture content and the ash content of both infected groundnut seeds and healthy groundnut seed to determine the effect of S. rolfsii.

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Data Analysis

All the data wasanalyzed 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 analyze the result was Statistical Analysis Software (SAS) version 7.

III. RESULTS

Table 1: Effect of Stem Rot Disease on the Proximate Composition of Groundnut

Status of Groundnut	Proximate composition (%)					
	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 (P≤0.05)	0.36	1.05	0.40	0.75	0.33	0.95

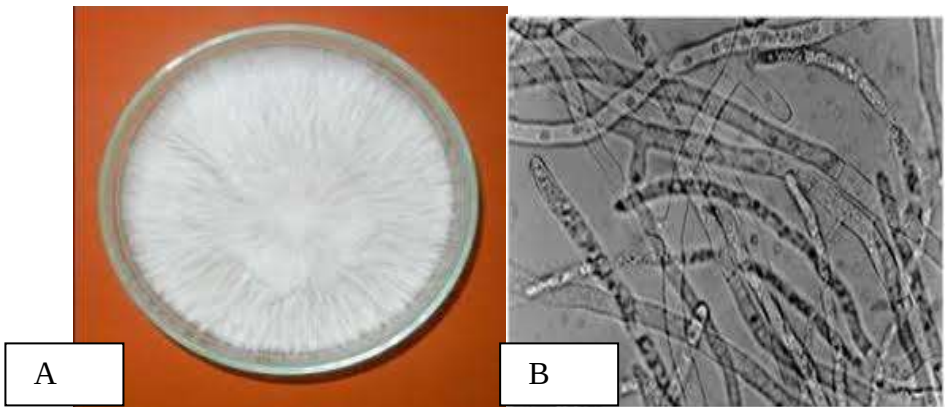

Plate I: (A) Four-day old pure culture Sclerotium rolfsii(B): Mircograph of Four Day Old Sclerotium rolfsii

Table 2: Mean In vitro Effect of A. sativumSeed Extracts on Radial Mycelial Growth of S. rolfsii after 7 Days

Treatments	Radial Mycelial Growth (cm)
Seed	1.39
Control	2.80
LSD (P≤0.05)	0.27

Table 3: In vitro Effect ofA. sativumSeed Extracts and Concentrations on Radial Mycelial Growth of S. rolfsii After 7 Days

Concentration (%)	Radial Mycelial Growth (cm)
Control	2.91
20	0.90
40	1.05
60	1.05
80	1.06
LSD (P≤0.05)	0.14

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IV. DISCUSSION

Sclerotium rolfsii is the pathogen responsible for stem rot disease of groundnut in Adamawa State. 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. Genesanet al. (2007), Doley and Jite (2013) as wellas 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. Tarafdar et al. (2018) reported Sclerotium rolfsiias the major pathogen that reduces groundnut production by nearly 30 % asa 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 (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 patternand 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.

Result from the comparative proximate analysis of the infected and healthy seeds of groundnut shows that there was a decrease in the percentage of protein, fats/oil, ash, crude fibre and carbohydrates in the infected groundnut seeds while there was an increase in moisture content. 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 Aspergillusspp 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. (2003) and Amusa et al. (2006) in which African star apple fruits and guava fruits infected by fungi had a significant lowerpercentagenutrient composition in the infected fruits. Waliyaret 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 Makeriet 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).

The result from the management of stem rot disease of groundnut showed that Allium sativum seed extracts were able to inhibit the radial mycelial growth of groundnut stem rot pathogen Sclerotium rolfsii in vitro at different degree. This agrees with Arifa et al. (2020) who reported that the bark, bulb, leaves, flowers and stem of Alliumsativum 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 (Arifa et al., 2020). The findings of this research agree with that of Kiran et al. (2006) who reported Allium sativumbulb asone of the botanicals that effectively inhibits mycelialgrowth of Sclerotium rolfsii in vitro. The genus Allium are known to inhibit the growth of microorganism such as bacteria, fungi, viruses and parasites (Kyung, 2012). Garlic is an antibacterial as well as antifungal agent (Rivlin, 2001; Block, 2010). It contains several hydrophobic antimicrobial compounds, such allicin, vinyldithiins, ajoenes and diallylpolysulfides (Jabbeset al. 2012; Kopec et al.,2013). Similarly, Abel-Salam et al. (2014) reported that the essential oil of Allium sativum bulb was found to have the highest antimicrobial activity at a concentration of 60 mg/ml against the tested bacterium (Staphylococcus aureus) and fungi (Fusariumoxysporum) with an inhibition zone diameter of 19mm and 15 mm respectively. The ethanolic and aqueous extracts were also found to be effective against the tested organisms but at a lower inhibition zone diameter (Abed-Salam et al., 2014). The inhibitory effect of plant extracts against pathogenic fungi has been studied (Alkhail, 2005; Kongkaew and Phichai, 2010). The garlic (Allium sativum L.) is a plant reported to possess various biological activities including antifungal activity (Mahmoudabadi and Nasery, 2009; Garcia, 2011). Sittisartet al. (2017) found that garlic extracts exhibited potential inhibition on the mycelia growth of Colletotrichum gloeosporioides which was isolated from leaves of the para rubber tree. Noengpaet al. (2004) reported that water extract of garlic showed inhibitory effects on Colletotrichum gloeosporioidesand 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 (Gunda et al. 2018). The antifungal activity of the extracts can be linked to the presence of secondary metabolites which have been shown to possess bioactive properties (Thamaraiselvi et al., 2012).

Recommendations

Research should be carried out to isolate the active component within the plant material that is responsible for the control of Sclerotium rolfsii. Further research should be conducted to evaluate the level of toxin contaminations caused by the isolate on infected groundnut seeds.

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