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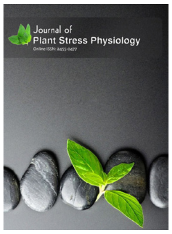
Research Article



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Biochar soil application induces stress tolerance in *Arachis hypogaea* L. Varieties inoculated with stem rot fungus (*Athelia rolfsii* (Sacc.) C.C. Tu & Kim.)

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ABSTRACT

This study aims to investigate the extent to which soil amendment with biochar can promote growth and induce biotic stress tolerance in *Arachis hypogaea* accessions (TAH-183, TAH-142, TAH-164, TAH-124 and TAH-134) inoculated with stem rot fungus (*Athelia rolfsii*). The experiment was set up in a complete block design. 5 seeds of *A. hypogaea* accessions were planted in 10 kg of sterilized soil in triplicates (control(C), *A. rolfsii* alone (T) and *A. rolfsii*+Biochar (A+T). Characterized pathogen *A. rolfsii* causing vine rot and wilt disease was obtained from Botany pathology laboratory and spawned with 300 g of millet seeds. 5 g of *A. rolfsii* colonized seeds were used to infect *A. hypogaea* accessions seedlings. Growth parameters and disease severity index (DSI) were taken using standard methods, while the total photosynthetic pigments (TPP) were determined using the atLeaf chlorophyll meter. All *A. hypogaea* accessions showed 100% germination across all treatments after 11 days except TAH-183 (80%) and TAH-134 (60%). TAH-142 and TAH-183 accessions inoculated with *A. rolfsii* were the most susceptible with disease severity index of 50% and 33.30% respectively. However, biochar application treatments recorded 0% DSI for all accessions. At 6 weeks after planting (WAP), it was observed that inoculation of *A. hypogaea* accessions with *A. rolfsii* significantly ($p<0.001$) reduced the growth parameters such as shoot length for TAH-142 and TAH-124 which recorded the lowest values; T=3.40±181 cm, C=15.50±1.45 cm, A+T=20.00±1.70 cm; and T=8.33±2.33 cm, C=15.20±1.20 cm, A+B=21.12±1.90 cm while TAH-183 and TAH-164 had the highest shoot growth (T=19.33±1.36 cm, C=20.00±1.54 cm, A+T=24.77±1.67 cm; T=16.83±1.76 cm, 17.00±1.13 cm, A+T=28.30±2.00 cm) when compared to their controls and biochar treatments. TAH-142 (T=30.00 mg/kg, C=37.90 mg/kg, A+B=40.00 mg/kg) recorded the least TPP contents, while TGM-183 (T=36.00 mg/kg, C=40.10 mg/kg, A+T=44.50 mg/kg) recorded the highest TPP. For postharvest parameters; TAH-142 (5.77±0.09 g) had the lowest fresh biomass yield while TAH-164 (58.70±1.56 g) had a better tolerance to *A. rolfsii* infection. However, TAH-183 showed greater fresh biomass yield when biochar was applied (89.67±4.53 g) while TAH-142 recorded the lowest (78.90±2.21 g). Amelioration of soil with biochar significantly (p=0.001) stimulated growth above the treatment and control. Similar trend was observed for leaf area, petiole length, leaf number, stem girth and internode length. This study has shown that *A. rolfsii* infection had a negative effect on the growth and biomass yield of *A. hypogaea* and soil amended with biochar conferred disease tolerance in all *A. hypogaea* accessions thus eliminating the need for the use of fungicides and additional fertilizer application.

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INTRODUCTION

Groundnut or peanut, (Arachis hypogaea L.) is an important oilseed crop that is cultivated in many countries of the world under irrigation system or rainfed agriculture (Ibrahim *et al.*, 2021). It is a member of the family Fabaceae, the third-largest family of flowering plants (Purohit *et al.*, 2023) and the second most widely grown legumes after soybean (FAO, 2019). In terms of world production index, Nigeria is the third largest producer of groundnut and the highest producer in West Africa (Ajeigbe *et al.*, 2015). Groundnut production in Nigeria is mainly in the Savannah regions (Vabi *et al.*, 2019), however, the essentiality of this crop as a rich source of vegetable oil, dietary mineral nutrients, feedstuffs to animals, income to small-scale farmers, and its ability to improve the fertility status in soil through the fixation of soil nitrogen (Ajeigbe *et al.*, 2015) makes it an important component in rotational and mixed cropping systems practiced in Southern Nigeria.

Groundnut production is impeded by biotic and abiotic stress that undermines the yield and productivity of the crop. Notably, microbial pathogens represent the major living components that threatened groundnut production in many countries of West Africa (Subrahmanyam *et al.*, 1991; Ajeigbe *et al.*, 2015). Approximately 10% of annual yield loss of agricultural production is attributed to fungal infection and this figure is likely to increase due to incessant change in climatic conditions (Lo Presti *et al.*, 2015).

Stem rot disease of groundnut is caused by *Athelia rolfsii*; a necrotrophic fungal pathogen that reduces the yield and quality of groundnuts (Bosamia *et al.*, 2020). It is an important soilborne disease that is predominant in the tropics and subtropical regions. A. *rolfsii* is an emerging fungal pathogen of many important crops including vegetables and weeds in some temperate countries of the world (Pethybridge *et al.*, 2019). It is highly destructive in regions with favourable temperature range (24-40 °C) and high soil moisture level that supports its germination and pathogenicity (Garcia-Gonzalez *et al.*, 2022). The pathogen is the causal agent for southern blight of tomato and sweet potato (Liamngee *et al.*, 2015; Garcia-Gonzalez *et al.*, 2022), corm rot in banana (Acabal Jr *et al.*, 2019), bulb rot in garlic (Cavalcanti *et al.*, 2018) and vine rot of fluted pumpkin (Ismaila *et al.*, 2024). According to Mullen (2001), over 500 plant species are susceptible to A. *rolfsii* disease and yield loss greater than 60 percent have been reported on peanut (Ambika *et al.*, 2023), tomato (Liamngee *et al.*, 2015) and fluted pumpkin (Ismaila *et al.*, 2024).

A. *rolfsii* pathogen is difficult to control due to its broad host range, ability to relay on decaying plant parts and form sclerotia (resting stage) in soils (Karthikeyan *et al.*, 2006). The mycelium is whitish in colour, grow profusely, and can colonize host parts within 3-5 days after infection, resulting in rot and rapid dead of the plants (Garcia-Gonzalez *et al.*, 2022; Ismaila *et al.*, 2024). Early symptoms of infection on the plants include dark-brown lesions on the stems, whitish mycelia mat on lower stems and other infected areas. Infected plants become pale and wilt spontaneously (Termorshuizen, 2007; Ismaila *et al.*, 2024). Several methods of plant diseases control have been evaluated on A. *rolfsii* fungus. For instance, the effectiveness of chemical fungicides, cultural practices and biological agents have been reported on A. *rolfsii* infected tomato (Keinath & DuBose, 2017), garlic (Cavalcanti *et al.*, 2018) sweet potato (Garcia-Gonzalez *et al.*, 2022) and peanuts (Garren, 1958; Garren & Duke, 1958; Garren & Bailey, 1963). However chemical fumigants are expensive, toxic to non-host and may form sources of chemical residues in foods (Garcia-Gonzalez *et al.*, 2022).

An integrated approach that involves screening of peanut cultivars for host resistance and soil amendment with carbon-rich materials (biochar) may be a better alternative in the control of A. *rolsii* pathogen. Although, peanuts cultivars that are resistant to A. *rolfsii* disease are scares, however there are reports of remarkable level of host resistance in some cultivars of peanuts (Edmunds *et al.*, 2003; Hagan *et al.*, 2015) and the mechanism of response to the pathogen in some varieties of groundnut have been studied (Bosamia *et al.*, 2020). In Nigeria, different cultivars of groundnut that are high yielding, early maturing with significant level of host resistant to varying stress conditions are grown. However, there are no studies to evaluate the level of response of these cultivars to A. *rolfsii* disease in Southern Nigeria.

Cultural practices such as soil amendments with biochar promote plant growth and productivity and enhance plant resistance to biotic stress in diverse ways. Biochar-soil amendment is a cheap component of integrated disease management strategies, it is readily available, zero-toxic to farmers and the mode of actions of biochar prepared from different organic sources on plants biotic stress factors have been extensively studied (Iacomino *et al.*, 2022). The study was conducted to evaluate the effect of biochar-soil amendments on the growth and host resistance of five varieties of groundnut infected with A. *rolfsii* pathogen.

MATERIALS AND MATERIALS

Description of Study Area

The experiment was conducted at the Department of Botany, Faculty of Biological Science, Akwa Ibom State University, Nigeria. The study area is located on coordinates 4.6214N, 7.7639 E and altitude of 185 metre above sea level. The rainfall pattern in this region is bimodal, with the first rain around March/April and peaks in July followed by a short break in August. The second rain peak is usually in October.

Source and Preparation of Planting Materials; Seeds, Biochar and *Athelial rolfsii*

Five improved cultivars of groundnut; TAH-124, 134, 142, 164 and 183 were gotten from the International Institute of Tropical Agriculture (IITA) Ibadan and were used in the study. Wood-derived biochar and a characterized isolate of A. *rolfsii* were gotten from the Department of Botany, Faculty of Biological Science, Akwa Ibom State University, Nigeria. A pure culture of 5 days old A. *rolfsii* pathogen was used to colonised sterilised millet and the A. *rolfsii*-spawned millet was used to infect the groundnut seedlings.

Sowing of Groundnut Seeds and Inoculation with Pathogen (*Athelia rolfsii*)

Fifteen seeds of *Arachis hypogaea* from each variety were sown at five seeds/plastic pots containing 10kg sterilized loamy soil and labelled thus: C=control , T=treatment and A+T=amendment and treatment . Biochar-amended pots contained 10% finely grind biochar. Percentage composition of biochar was calculated: $\frac{1kg\,biochar}{10kg\,Soil} \times 100 = 10\%$

The experiment was arranged in a complete block design in three replicates. Upon germination and establishment of seedlings, 5 g A. *rolfsii*-spawned millets were used to infect the seedlings in each pot at 21 days after sowing (DAS) in the T and A + T labelled pots respectively. The control plots (C) were inoculated with sterile distilled water. All agronomic practices were adequately maintained throughout the study period. The plants were irrigated with distilled water based on field requirement.

Evaluation of Growth Parameters

Upon germination, the following growth parameters were assessed.

Germination percentage

Number of established seedlings/varieties was counted and rate of germination was expressed in percentage as thus:

$$\%\ \text{germination} = \frac{\textit{number of germinated seeds}}{\textit{total number of seed sown}} \times 100$$

Shoot/petiole length

i. Shoot length and petiole length were measured using transparent plastic ruler calibrated in centimetre scale at 5 days interval and recorded.
ii. Number of leaves/established seedlings were counted and recorded at 5 days interval in all the varieties.
iii. Leaf area was determined by measuring the length and breadth of three randomly selected leaves and expressed as area and the average value was recorded for each variety in each treatment.
iv. Stem girths was measured using micro-meter screw gauge.
v. Total Photosynthetic Content (TPC) was assessed using atLeaf chlorophyll meter at 5 days interval throughout the study period.
vi. Root length: The plants were uprooted and the roots length were measured using meter rule (cm) in all the treatments.

Total fresh/dry weight

The total fresh and dry weight was taken using electronic weighing balance and recorded in grammes (g).

Assessment of disease incidence and severity

Upon inoculation with A. *rolfsii*, disease incidence was assessed using disease scoring scale described in our previous study (Ismaila *et al.*, 2024) in the pathogen infected plants and biochar-pretreated plants. The disease severity index for each variety were converted and presented in chats (Table 1).

Analysis of Data

Data collected on all the growth parameters and disease severity were analysed using Statistics for pure and social science package (SPSS) and the results presented in chats.

RESULTS

Growth and Seedling Development of A. *hypogaea* Varieties Before A. *rolfsii* Inoculation

There were statistically significant variations in the rate of germination and seedling growth performance of the groundnut varieties. The highest growth rate was observed in the TAH-124, 142 and 164 plants. TAH-134 variety had the least germination percentage followed by TAH-183 (Figure 1).

Growth Response of A. *hypogaea* Varieties Infected with A. *rolfsii* Alone and in Biochar-amended Soil

A. *rolfsii* infection caused statistically significant effect on all the agronomic characteristics; shoot and petiole length, number of leaves leaf area and stem girth in all the varieties when compared to the control and the biochar-soil amended plants. The highest affected varieties were TAH-142 and 124 with severe stunted growth and number of wilted branches (Figure 2a & b). TAH-142 was among the fastest growing variety before inoculation with the pathogen (Figure 1), but its growth rate was inhibited upon inoculation with the pathogen. In the biochar-amended soil, all the varieties of groundnut had higher growth parameters than the control and plants infected with A. *rolfsii* alone. Although there were statistically significant variabilities in the growth parameters in all the varieties, TAH-142 had the least rate of response in the growth parameters in biochar-soil amended treatment. TAH-183 variety had longer petiole length in all the treatments and in the control, TAH-142 and 164 was statistically similar in the petiole length in both the control and biochar-amended soil. Other varieties had varying length of petiole across the treatments and in the control (Figure 2d).

Biochar-soil amendment enhanced the size of the stem (stem girth) and number of leaves in all the varieties, and the rate of response in both parameters was statistically similar, variety TAH-164 had larger stem girth (Figure 2d), but less number of leaves (Figure 2b). A. *rolfsii* inoculation caused severe wilting of branches and reduced stem girth. There were no statistically significant variations in the number of leaves in varieties TAH- 164 and 124 in the A. *rolfsii* infected plants and control plants (Figure 2b).

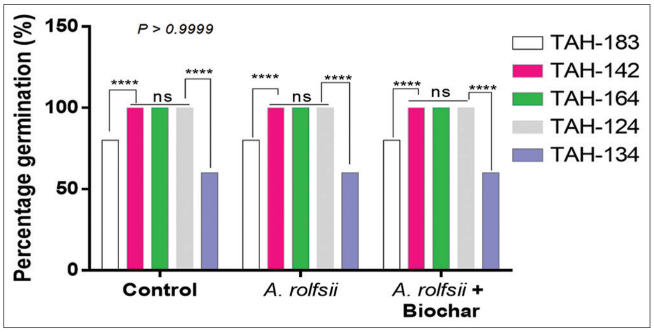

Figure 1: Percentage germination of *A. hypogaea* varieties before inoculation with the pathogen; (****- highly significant, ns- not significant)

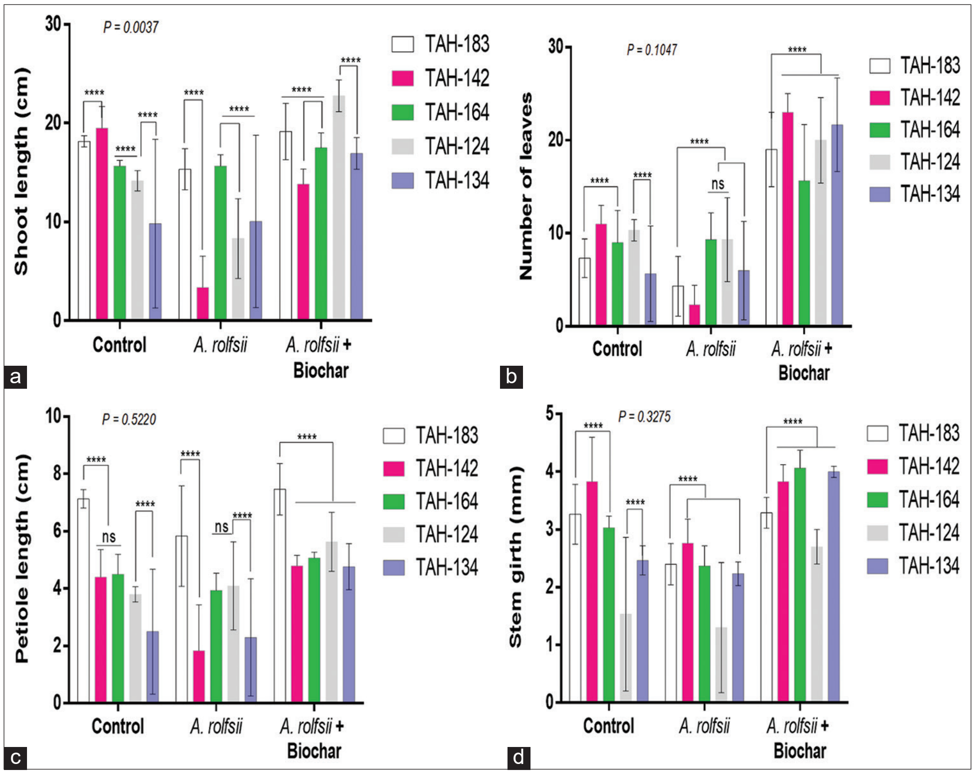

Figure 2: (a-d) Growth response of *A. hypogaea* varieties grown in biochar-amended soil and infected with *A. rolfsii*

Effect of *A. rolfsii* Pathogen and Biochar-soil Treatment on Shoot Biomass, Root Length and Total Photosynthetic Content (TPC) of the Groundnut Varieties

The Total Photosynthetic Content (TPC) was significantly enhanced by biochar-soil amendment amidst pathogen infection in all the varieties of groundnut. Varieties TAH-124 and 164 had a higher TPC, while TAH 134 and 183 had the least value for TPC, and were statistically higher and different from the control and non-biochar treated plants. A similar trend was observed in the total fresh and dry weight parameters. A. *rolfsii* alone infected plants had the least values followed by the non-infected plants (control) for all the varieties. The variabilities in the TPC in all the varieties infected with A. *rolfsii* alone were not statistically different (Figure 3a).

A Biochar-soil amended plant had higher total fresh and dry weight and was statistically different from the control and plants infected with A. *rolfsii* alone (Figure 3b & c). However, there were similarities in the root length in all the varieties in the control, A. *rolfsii* infected plants and biochar-soil amended plants. In all the treatments, the root length for variety TAH-134 was not statistically significant. TAH-164 and 183 had the longest root length in the control, but shared statistical similarity in the biochar-amended and A. *rolfsii* alone infected plants. The least root length was recorded on TAH-142 variety infected with A. *rolfsii* alone (Figure 3d).

Evaluation of *A. rolfsii* Disease Progression: Symptoms and Severity

Figure 4a shows the growth characteristics of the varieties to biochar-soil amendment, A. *rolfsii* pathogen and no pathogen inoculation. The most susceptible variety had severe symptoms of A. *rolfsii* infection (mycelia mat and sclerotia), stem rot and wilting of plants within 7-11 days post inoculation (Figure 4b & c). Varieties TAH-134 and 142 were the most susceptible to the pathogen (Figure 4d), but were not showing any symptoms in the biochar-amended soil. Generally, there were no symptoms of disease in the control and biochar-soil amended plants (Figure 5).

DISCUSSION

The Groundnut varieties evaluated in this study show varying level of responses to A. *rolfsii* disease and biochar-soil amendment significantly enhanced the growth performance and host resistance to the disease in all the varieties. Within 11 days after sowing (DAS), all the varieties germinated and were fully established. The rate of growth was higher in TAH-164, 142 and 124 varieties. Other growth parameters evaluated were also variety-dependent. The variations in the growth parameters observed in this study may be attributed to their genotypes (Essilfie *et al.*, 2020). There are reports of variations in the growth pattern and growth responses among peanuts cultivars evaluated in different regions of the world (Konlan *et al.*, 2013; Onat *et al.*, 2016).

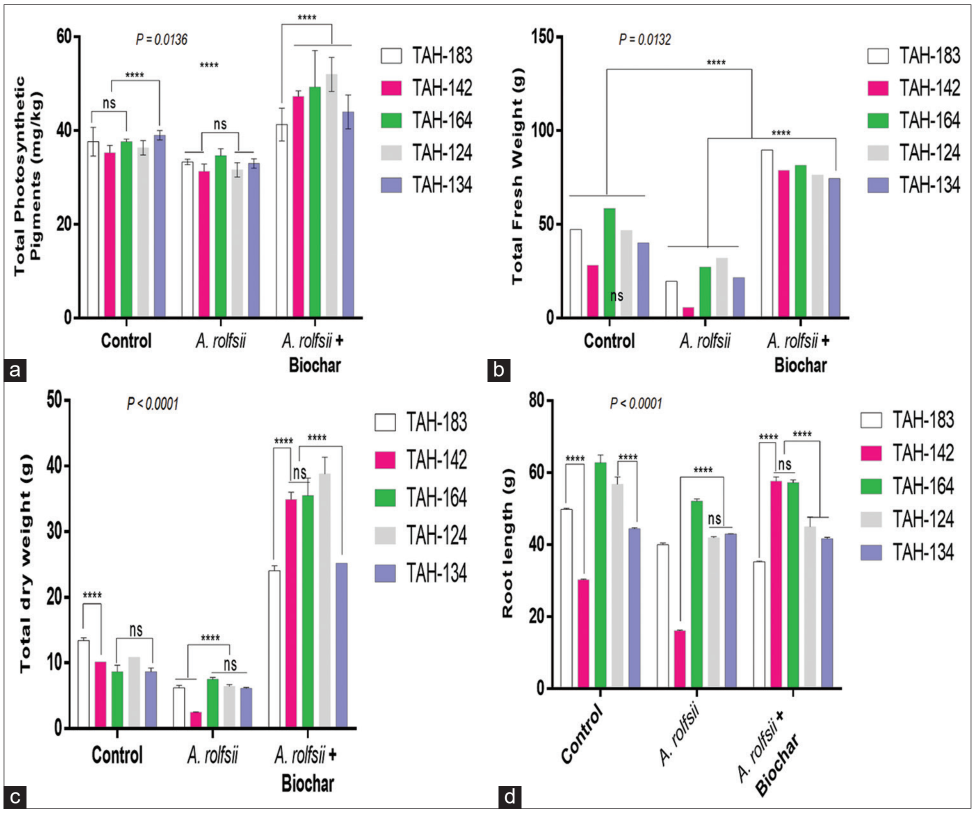

Figure 3: (a-d) Effect of *A. rolfsii* and biochar-soil amendment on root growth, and shoot biomass of groundnut varieties.

Figure 4: (a-d) Groundnut varieties infected with A. rolfsii pathogen and the control showing symptoms of infection (B+T=biochar+*A. rolfsii*; T=*A. rolfsii* alone; C=control; no pathogen/biochar)

A. *rolfsii* inoculation caused stem rot, wilted branches and stunted growth in the non-biochar pre-treated plants. The severity of infection and disease progression were incomparable to the control and biochar-soil amended plants. Although TAH 124, 164 and 134 varieties had the least effect of the pathogen, TAH 142 and 183 were the most affected. However, while the non-biochar pre-treated plants had severe symptoms of infection with wilted branches, the biochar-soil amended plants continue to grow with no symptoms of infection by the pathogen. The varying level of responses to A. *rolfsii* infection in the varieties of groundnut may likely be induced by their genetically improved traits. There are numerous reports of genetically improved cultivars of peanuts that showed high level of resistance to stem rot disease caused by A. *rolfsii* (Edmunds *et al*., 2003; Hagan *et al*., 2015) and the mechanism of response in A. *rolfsii*-resistant variety has been evaluated (Bosamia *et al*., 2020).

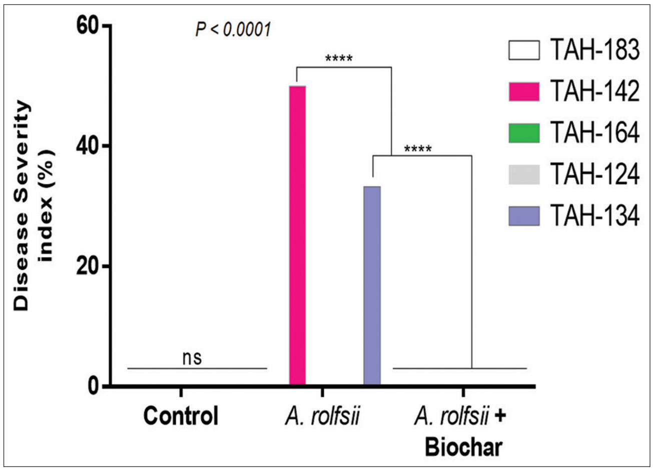

Figure 5: A. *rolfsii* disease incidence; symptoms and severity on the five varieties of groundnut

The groundnut varieties used in this study are genetically improved cultivars, thus, there were no records of completely

Table 1: Disease rating scale used in scoring stem rot disease on *A. hypogaea* varieties

Rating scale	Description
0	No symptoms of disease
1	<25 percent of the plant's parts showing symptoms
2	25 to 50 percent of the plant's parts showing symptoms
3	50 to 75 percent of the plant's parts showing symptoms
4	>75 percent of the plant's parts showing symptoms/dead plants

wilted plants due to A. *rolfsii* infection in the non-biochar pre-treated plants. Moreover, some of the infected plants were able to slowly recover from the disease. This resulted in a slow growth rate and stunting in those severely infected plants; TAH-142 and 183. A. *rolfsii* is a necrotrophic fungal pathogen that infects vast diversity of plants species using a complex mechanism. Consequently, completely resistant plants varieties are scarce. Garcia-Gonzalez *et al.* (2022) reported low-medium level of resistance in some cultivars of sweet potato and similar results was seen in some peanut cultivars (Edmunds *et al.*, 2003).

The result of this study has demonstrated the possibility of combining genetically improved cultivars of groundnut and biochar-soil amendment in the management of stem rot disease caused by A. *rolfsii* and improving the growth performance of groundnuts in southern Nigeria. The biochar pre-treated plants had higher growth parameters, shoot biomass, total photosynthetic content, roots length and were highly resistant to the disease. At 5 days after inoculation with the pathogen, there were visible symptoms (mycelia mat) around the plant base in all the varieties, however, each variety continued to grow with no wilted branches nor stem rot incidence. This result was in line with the reports by (Hua *et al.*, 2012; Upadhyay *et al.*, 2014; Mohawesh *et al.*, 2021; Lévesque *et al.*, 2022) on the role of biochar in plant growth performance and chlorophyl content. Although the rate of biochar application that is optimum for crop growth performance has not been established. There are adverse effects of biochar application on plants growth performance when applied in quantities above 30 t/ha (Upadhyay *et al.*, 2014). The quantity of biochar used in this study (10%) was within the range earlier reported by Zwart and Kim (2012), and must have been sufficient for the growth and protection of the groundnut varieties from stem rot pathogen, but further studies will be necessary to evaluate the efficacy of biochar-soil amendment in field studies.

The mechanism of action of biochar-soil amendment in terms of enhancing the resistance or tolerance to the disease and promoting the growth response in the groundnut genotypes was not analysed in this study. However, essential mineral elements that are present in biochar and their roles in soil fertility improvement and stress response in plants have been studied (Poveda *et al.*, 2021). In terms of amelioration of biotic stress, biochar application increases the population of soil antagonistic microbes such as arbuscular mycorrhizal fungi (AMFs) or Pseudomonas that induces systemic resistance in plants (Poveda *et al.*, 2020). Application of biochar and greenhouse waste reduced disease incidence in cucumber and bean plants (Jaiswal *et al.*, 2014, 2015) and activated systemic defense against foliar diseases and necrotic disease caused by *Botrytis cinera* ((Poveda *et al.*, 2020). Similarly, biochar-mediated defense has been effective in the management of Fusarium wilt diseases (Eo *et al.*, 2018) and seedling disease caused by *Rhizoctonia solani* (Verwaaijen *et al.*, 2017).

Organic chemicals identified in biochar that are inhibitory to soil borne pathogens are numerous and pathogenesis-related (PR) genes associated with biochar-mediated defense have been characterized (Iacomino *et al.*, 2022). In this study, biochar-soil amendment acts as biological elicitors of systemic resistance to the pathogen and improved shoot growth and root establishment in all the genotypes of groundnut. This was contrasting to earlier reports, where soil pre-treated with inorganic chemical fungicides caused slow vegetative growth rate and delayed fruiting in F1-Resistant Tomato Hybrid (Lindo-F1), but enhanced systemic defense response against indigenous strains of *Fusarium oxysporum* (Borisade & Uwaidem, 2017). The results in this study presented data on the interaction of groundnut genotypes with stem rot pathogen (A. *rolfsii*) and soil amendment with wood-based biochar.

CONCLUSION

This study revealed that biochar-soil amendment improved the growth performance, total photosynthetic content and enhanced host resistance in groundnut varieties infected with A. *rolfsii*. There were statistically significant variabilities in the groundnut varieties in terms of the rate of germination, growth characteristic and overall responses to the pathogen. The most susceptible varieties to the pathogen (TAH-142 and 134) however, had no visible symptoms of infection in the biochar-soil pretreatment. The overall growth performance of the plants in the biochar-amended soil was statistically significant and non-comparable to the control. This result indicated the potentials of biochar-soil amendment as organic elicitor of host systemic defense response against A. *rolfsii* disease.

AUTHORS CONTRIBUTION

O. G. Okon and Y. I. Uwaidem: Design the experiment, analyzed the data and write the manuscript. U. E. Antia and B. F. Archibong: Did the literatures search and read the final copy of the manuscript. J. E. Okon: Prepared the experimental materials and methodology. All authors read and approved the final copy of the manuscript.

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