Nano-milled Biochar Improves Water Use Efficiency and Stress Defence Mechanism of Okra (*Abelmoschus esculentus*) under Drought Condition

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Abstract
Climate change poses serious challenges to agriculture in the tropics and sub-tropics, having implications on food security, livelihoods, and ecosystem stability. One critical concern is the extreme weather events, including more intense droughts, floods, and storms which disrupt traditional agricultural practices leading to crop failures. The existing agronomic and soil management practices have not been able to deal with the rising problems of climate change. Over the years, nanotechnology has been employed to tackle problem in agriculture. This research studies the effects of nano-milled biochars on the performance of Okra (*Abelmoschus esculentus*) when subjected to drought stress. Okra (*Abelmoschus esculentus*) was planted on 20 kg of soil-poultry manure mixture treated with different rates of nano-milled biochars and subjected to water stress. Growth parameters were taken weekly starting from seven days after planting to harvest. The results show that water stress has no major effect on the Okra physiology for the first 3-4 weeks of planting, while the major effects are evident at the later stage of the plant growth. Best result was discovered from the application of 100 g of nano-milled biochar to 20 kg soil. The treatment significantly reduced water stress, which was evident in the reduced hydrogen peroxide levels and enhanced peroxidase and catalase activities. Proline and antioxidant activities show different impacts, emphasizing the need for careful nanoparticle management. It was concluded that okra's response to water stress is more pronounce at the later growth stages and nano-milled biochar can help the plant cope with water stress.
Keyword: biochar, nanoparticles, water stress, okra, antioxidant, water use efficiency

1.0 Introduction

Climate change has become a global issue in the tropics (Ezegwu, 2014). Its effect in Nigeria has led to a wide array of physical and biological transformations that adversely affect the ecosystem, agriculture, food security, human livelihoods, the environment, and socio-economic development of the country (Ezegwu, 2014, Okon *et al*., 2021). The sensitivity of Nigeria's agricultural productivity to climate change is a major constraint, as climate change has caused notable changes in Nigeria's agriculture practices and productivity (Ebele and Emodi, 2016). For centuries, agriculture has been a major source of income and livelihood for individuals and communities in Nigeria. The effects of climate change endanger this means of livelihood, food security, and poverty alleviation schemes (Ezegwu, 2014). Being an agricultural-predominant country, crop production dominates about 94% of Nigeria's agricultural sector. Within this category, the impact of climate change is particularly notable, with certain regions of the country experiencing up to a 20% reduction in the duration of their growing seasons (Ebele and Emodi, 2016). Efforts to combat the increasing effect of climate change and ensure sustainable agricultural practices and food security has brought about the need to explore different approaches to sustainable agriculture. Nanotechnology holds significant promise in addressing various agricultural challenges, with nanoparticles offering a compelling avenue to bridge the gap between bulk materials and atomic or molecular structures (Shang *et al*., 2019). Over the past two decades, extensive research has been conducted on nanotechnology, highlighting its diverse applications in the agricultural sector. The application of nanotechnology into agriculture began with the realization that traditional farming methods had reached their limits in terms of enhancing productivity and restoring ecosystems affected by current practices (Mukhopadhyay, 2014). This recognition stemmed from an increasing awareness of the long-lasting consequences associated with conventional farming techniques, which are detrimental, unsustainable, and inefficient (Mittal *et al*., 2020, Yaday *et al*., 2023). The growing human population and the consumer-driven behaviour of individuals are rapidly depleting natural resources, posing a significant challenge to sustainable development. Modern agriculture faces a multitude of issues, including excessive reliance on supplementary irrigation, declining groundwater levels, soil degradation, contamination from pesticides and fertilizers, reduced interest of younger generations in agriculture, and the inefficiency of existing agricultural practices (Rodel *et al*., 2009, Yaday *et al*., 2023).

Carbon nanomaterials have gained prominence as powerful resources, owing to their unique characteristics and versatile applications across a variety of fields, including materials science, agriculture, environmental management, and phytoremediation (Chausali *et al*., 2021). They excel in mitigating a wide spectrum of contaminants, encompassing organic compounds, inorganic substances, and heavy metals, making them invaluable assets in addressing environmental pollution challenges (Chausali *et al*., 2021). Biochar-based materials, encompassing both pure biochar and composites with other functional substances, are regarded as next-generation materials with a wide range of versatile applications, potentially entering the soil environment through deliberate or inadvertent means (Lin *et al*., 2022). Biochar is a solid, carbonized product derived from biomass feedstock, including agricultural waste and other lignocellulosic materials, through controlled thermal decomposition processes conducted in the absence of oxygen known as pyrolysis (Chausali *et al*., 2021).

Nano-milled biochars emerge as byproducts during the creation, weathering, and breakdown of bulk biochar. Due to their substantial surface area-to-volume ratio and high chemical reactivity, they can potentially exert significant influence on processes like metal cycling, contaminant transport, and the structure and functioning of microbial communities within aqueous systems (Pratiwi *et al*., 2021). Nanoparticles are colloidal materials consisting of tiny particles at the nanoscale, typically ranging from 1 to 100 nanometers in size (Mukhopdhyay, 2014). According to Alemayehu and Teshome (2021) the large surface areas and higher charges of colloids grant them a high cation exchange capacity (CEC), which is a major factor that governs soil fertility. Furthermore, studies have shown that nano-milled biochars can increase the soil water holding capacity and decrease the irrigation frequency required for plant growth (Behnam and Firouzi, 2022). Additionally, nano-milled biochars can enhance the water uptake of plants by improving the root system's hydraulic conductivity (Yang *et al*., 2022). These findings suggest that nano-milled biochars can be an effective strategy for sustainable agriculture practices, particularly in areas with water scarcity and mitigate the effect of climate change on agricultural productivity and food security. This study, therefore, focuses on assessing the physiological responses and water-stressed defence mechanisms of Okra (*Abelmoschus esculentus*) on a soil treated with nano-milled biochars under drought stress condition, while also accessing the effects of this treatment on the yield and water use efficiency.

2.0 Materials and Methods

2.1 Description of Study Areas

The study was carried out at the teaching and research farm of The Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. The teaching and research farm is located at latitude 7.3072306 and longitude 5.1218411. Being a replication of a drought scenario, this research work needed to be carried out in a controlled environment where it is easier to control the environmental conditions or limit the impact of the environment, such as rainfall, on the crops. Hence this research was carried out at the screen house located at the teaching and research farm. This research work was carried out in potting bags arranged in blocks in the screen house.

2.2 Sample Size

The soil sample used for planting was collected from agricultural land at the FUTA teaching and research farm. The collected soil sample was then mixed thoroughly with poultry manure at a ratio of 2:1 after which the potting sacks were filled with 20 kg of the mixture of soil and manure. Poultry manure was specifically used in this research because of the nature of the materials used in the production of the nano-milled biochar. The nanoparticles were produced from pyrolysis and milling of sawdust which is deficient in nitrogen. Using poultry manure, which is rich in nitrogen, seeks to balance the nitrogen deficit from the nanoparticle material.

The experiment consisted of 6 treatments replicated three times, which makes a total of 18 experimental units. The experimental treatments consist of different levels of nano-milled biochars applied to the 20 kg of soil. The treatments range from 0 g of nano-milled biochars to 400 g of nano-milled biochars applied to 20 kg of soil.

2.3 Preparation of Nano-milled biochar

The nano-milled biochar was prepared from sawdust through the process of pyrolysis and milling. About 36.5kg of sawdust collected from the sawmill was pyrolysed for 6 hours at a temperature of 389ºC when taken with a pyrometer mounted with a thermocouple. After this, the kiln was completely sealed for 12 days in order to allow for the complete cooling of the materials without oxygen. The pyrolysed material was opened on the twelfth day at a temperature of 42.8ºC when taken with the pyrometer. After the pyrolysis, the material was then milled to the nanoscale (1-100 nanometers) using a ball mill.

2.4 Experimental Design and Layout

For this experiment, a Completely Randomized Design was used. In this design the treatments are allocated at random to a group of experimental units. The primary purpose of this experimental design is to keep the variability among experimental units within a block as small as possible and to maximize differences among blocks. Each experimental unit was thenreplicated three times.

2.5 Sample Treatments

Each treatment contains 20 kg of soil mixed with poultry manure at a ratio of 2:1. The treatments vary in the amount of nano-milled biochars applied to each block. Two (2) treatments were used as control; controls – treatment 1 with 0 g of nano-milled biochar and treatment 6 which also has 0 g of nano-milled biochar but was subjected to standard cropping condition with regular watering. These two treatments differ only in the frequency of water supply. For treatment 1, the water supply follows the regular pattern for all treatments which replicates a drought scenario, that is, 2 litres of water at the beginning of the experiment and 1 litre at the seventh week after planting. While for treatment 6, there was a regular supply of two litres of water every week.

- **Treatment 1:** 20 kg of soil + 0 g of nano-milled biochars
- **Treatment 2:** 20 kg of soil mixture + 100 g of nano-milled biochars
- **Treatment 3:** 20 kg of soil mixture + 200 g of nano-milled biochars
- **Treatment 4:** 20 kg of soil mixture + 300 g of nano-milled biochars
- **Treatment 5:** 20 kg of soil mixture + 400 g of nano-milled biochars
- **Treatment 6:** 20 kg of soil mixture + 0 g of nano-milled biochars with regular supply of water

All treatments were supplied with 2 litre of water at planting, after which treatments one to five were subjected to drought stress. However, treatment six was supplied 2 litres of water weekly.

2.6 Data Collection

The crop growth parameters for this experiment were collected weekly on a specified day starting from 7 days after planting. The growth parameters collected weekly include; the plant height, number of leaves, and plant girth. The plant height data was collected using a measuring tape (cm), while the plant girth data was collected using a veneer calliper (cm). The data were collected from all plants in each experimental unit. The growth parameters collected during the growth period until the last harvest was used in calculating the water use efficiency of each experimental unit.

2.7 Yield Determination

Harvesting was done at the eighth (8^{th}) and ninth (9^{th}) weeks and necessary data were collected to determine. The fruits were weighed using an electronic weighing scale (g) and the fruit length and girth were measured using a veneer calliper (cm) after every harvest to get accurate data. These collected data were used in calculating the yield per plot and yield per harvest. Yield refers to the quantity of seeds or grains that may be gathered from a given geographical area. Yield per harvest was calculated using the formula:

$$Yield\ Per\ Harvest = \frac{Total\ Weight\ of\ Okra\ Harvested}{Total\ No.of\ Plants\ in\ the\ Field}$$

2.8 Determination of Water Use Efficiency

Water use efficiency (WUE) is commonly determined by assessing the grain yield or total biomass generated per unit of water consumed by crops. The estimation of water consumption by crops takes into account the total water utilized from both the plant and soil surfaces, in addition to the water retained within plant structures [32]. Adequate water availability is crucial for the growth and production of crops, especially in arid and semi-arid climates. The water use efficiency was calculated using the formula:

$$WUE = \frac{Crop\ Yield}{Water\ Applied} \times 100$$

2.9 Laboratory Analysis

Laboratory analysis was carried out to determine the proline, hydrogen peroxide, catalase and peroxidase content. These datasets were necessary to effectively determine how the treatments affected the biochemical properties of the plant.

2.9.1 Determination of Proline Content

Fresh leaves and fruit (0.25 g) were mixed with 5 mL of sulfosalicylic acid (3% w/v). Then, the mixture was filtered, and 2 mL of filtrate was taken. The reaction mixture consisted of 2 mL of proline extract, 2 mL of ninhydrin and the addition of 2 mL of glacial acetic acid. The mixture was boiled at 95°C for 60 min. After cooling the mixture, 4 mL of toluene were added to the mixture to generate two layers. The absorbance was recorded at 520 nm using a spectrophotometer.

2.9.2 Determination of Hydrogen Peroxide Content

Fresh samples of okra fruit (0.25 g) were ground in a mortar and pestle along with 0.1% TCA (5 mL). Then, the samples were filtered and 0.5 mL of phosphate buffer and 1 mL of potassium iodide were mixed with 0.5 mL of the supernatant. Subsequently, the mixture was vortexed, and the absorbance was recorded at 390 nm using a spectrophotometer.

2.9.3 Determination of Catalase Activity

Okra fruits were collected 10 days after inducers application and were excised, weighed (100 mg) and ground with a pestle in an ice-cold mortar with 2 mL of 0.05 M Na_2HPO_4/NaH_2PO_4 (pH 7.0) buffer. The homogenate was centrifuged at 4°C for 20 min at 15,000 ×g. The supernatant was used for the assay of catalase activity and total soluble proteins. Catalase activity was measured. Enzyme extract (20) was added to 3 mL of HP_2-PO_4 buffer (0.16 mL of HP2 to 100 mL phosphate buffer, pH 7.0) and the breakdown of HP2 was measured at 240 nm in a spectrophotometer. An equivalent amount of buffer containing H_2O_2 was used as reference.

2.9.4 Determination of Antioxidant Power

Laboratory analysis was carried out to determine the free radical scavenging ability, Fe^{2+} chelation power, Fe^{3+} reducing power. These datasets were necessary to effectively determine how the treatments affected the chemical composition of the fruits.

2.9.5 Determination of Free Radical Scavenging Ability

To determine the free radical scavenging ability, the procedure of Garcia *et al*. (2012) was followed with slight modification. A 1ml of each sample concentration was collected and added to 3 ml of ethanol and 1 ml of DPPH (2,2-diphenyl-1-picrilhydrazil) radical solution. They are then shaken until homogeneous. Sample was incubated at room temperature for 30 minutes. Sample absorbance was measured by using UV-VIS spectrophotometer at a wavelength of 513nm. The antioxidant strength of extracts and fractions in inhibiting DPPH radicals was calculated using the following equation:

$$\% \text{ Inhibition } = \frac{\text{Ac} - \text{As}}{\text{Ac}} \text{x } 100\%$$

Where:
% Inhibition = percentage of inhibition of radical DPPH
Ac = control absorbance
As = sample absorbance

2.9.6 Determination of Iron II (Fe^{2+}) Reducing Power

To determine the Fe^{2+} reducing ability of okra, a method based on the reduction of Fe^{3+} to Fe^{2+} was employed. This assay measures the capacity of okra or its extract to reduce Fe^{3+} ions to Fe^{2+} ions, indicating its chelation ability. The process begins by preparing an okra extract, either by homogenizing fresh okra samples or using a commercially available extract. A solution of iron (III) chloride ($FeCl_3$) is prepared to serve as the source of Fe^{3+} ions, while a solution of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) is used as the chelating agent. The reaction mixture, consisting of the okra extract, Fe^{3+} solution, TPTZ solution, and pH-adjusted hydrochloric acid, is then incubated to allow for the reduction of Fe^{3+} to Fe^{2+}. Spectrophotometric measurements are taken at a specific wavelength to determine the formation of the Fe^{2+}-TPTZ complex. The higher the chelation percentage in the results, the stronger the Fe^{2+} chelation ability of okra.

2.9.7 Determination of Iron III (Fe^{3+}) Chelation

To determine the Fe^{3+} chelation ability of okra, a laboratory assay is employed, focusing on the formation of stable complexes between okra and Fe^{3+} ions. This assay is a valuable method for assessing the chelation capacity of okra, indicating its ability to bind and sequester Fe^{3+} ions, which is important for potential applications as an iron chelator or antioxidant. The procedure begins with the preparation of an okra extract, which can be obtained by homogenizing fresh okra samples or using commercially available extracts. A solution of iron (III) chloride ($FeCl_3$) serves as the source of Fe^{3+} ions, and a solution of ferrozine, a chelating agent, is prepared. The reaction mixture is then created, combining the okra extract, Fe^{3+} solution, ferrozine solution, and a buffer to maintain a consistent pH. After an incubation period, spectrophotometric measurements are taken to assess the formation of Fe^{3+}-ferrozine complexes. A higher chelation percentage in the results indicates a stronger Fe^{3+} chelation ability of the okra extract, offering valuable insights into its potential role in binding iron ions and functioning as an antioxidant.

2.10 Data Analysis

The data collected from the field experimentation were subjected to Analysis of Variance (ANOVA) using appropriate statistical tools to measure the level of significant differences, while means were separated using Tukey HSD. Graphs were generated using Microsoft Excel.

3.0 Results

3.1 Effects of Varying Levels of Nano-milled biochars on Okra Growth Parameters

Tables 1 to 3 show the effect of varying level of Nano-milled biochars on okra height, stem and number of leaves from week 1 to 9. There was no significant difference in the height of Okra among all treatments from week 1 to week 3 ($p \geq 0.05$). However, at week 4, treatment 6 shows a height that is significantly different to all treatments ($p \geq 0.05$). At weeks 5 and 6, while there is no significant difference between the heights of treatments 1 to 5, the result also shows that treatments 1, 3, 5, and 6 are not significantly different. Meanwhile, significant difference existed between the mean heights of treatment 6 and all other treatments from weeks 7 to 9, while treatments 1 to 5 for these weeks exhibit no significant difference (Table 1).

At weeks 1 and 2, the girth for all treatments is about the same diameter when compared to the mean ($p \geq 0.05$). However, from week 3 to week 9, the girth for treatment 6 increased exponentially from treatments 1 to 5 ($p \geq 0.05$). Treatment 1 has a stem girth that's thicker than other treatments except treatment 6 at weeks 8 and 9. But for the most part of the experiment, treatment 2 exhibited a thinner stem girth when compared to other treatments.

Meanwhile, there is no significant difference in the girth across all treatments from week 1 to week 3. However, from week 4 to week 9, the difference in girth of treatment 6 becomes significantly different to treatments 1 to 5 while there is no significant difference among treatments 1 to 5 throughout the experiment (Table 2).

The mean number of leaves across all treatments shows no significant difference from week 1 to week 5 ($p \geq 0.05$). However, from week 6 to week 9, the number of leaves in treatments 1 to 5 reduced significantly in comparison to treatment 6 ($p \geq 0.05$).The data shows that from week 1 to week 3, the number of leaves among all treatments is about the same. At week 4, treatment 6 has more leaves, followed by treatments 5, 4, 3, 1, and 2 respectively. This trend continued to week 5. However, from week 6 to week 8, while treatment 6 still had more leaves when compared to other treatments, the number of leaves in all treatments reduced than in previous weeks (Table 3).

3.2 Yield and Water Use Efficiency of Okra

Figures 1 and 2 presents the influence of nano-milled biochar of the yield and water use efficiency of okra plant respectively. Treatment 0 g of biohar-based nanoparticles with adequate water supply has the highest yield while treatment 5 has the lowest yield. The second highest yield was obtained from treatment 2, followed by treatments 1, 3, and 4 respectively. Tukey test shows that the differences in the yields of treatments 1 to 5 are not significant ($p \geq 0.05$). However, a significant difference exists between treatments 1, 3, 4, and 5 when compared with treatment 6 ($p \geq 0.05$). Meanwhile, comparison between treatment 2 and treatment 6 shows that the difference between these two treatments is not significant ($p \geq 0.05$) (Figure 1).

The best Water Use Efficiency (WUE) of the Okra plant was recorded in treatment 2 while treatment 5 has the lowest WUE. The WUE for treatments 1 and 3 is also higher than in other treatments, safe for treatments 2. Meanwhile, analysis of variance shows that the differences in the WUE for these treatments are not significant ($p \geq 0.05$) (Figure 2).

3.3 Effects of Nano-milled biochars on Biochemical Properties of Okra (*Abelmoschus esculentus*)

Examining the effects of nano-milled biochars on biochemical properties of okra, the result shows that the application of 400 g of nano-milled biochars had the highest level of hydrogen peroxide content (313.29), while treatment 6 with 0 g of nano-milled biochars and adequate water supply recorded the lowest content of hydrogen peroxide (47.04). However, there was no significant difference between the treatments 3 and 4, with hydrogen peroxide content of (224.85 and 220.39) respectively ($P \geq 0.05$) (Figure 3).

Furthermore, it was discovered that the treatment 4 has the highest catalase activity (74.98), while treatment 6 is the lowest (17.61). Meanwhile, the catalase activities in treatments 4 and 3 are only slightly different, having 74.98 and 73.39 respectively. The same close interaction was also observed in treatments 2 and 5 with catalase content of 54.47 and 54.45 respectively (Figure 4). Investigation into the peroxidase activity shows that treatment 4 had the highest level of peroxidase content (10.85), while treatment 6 recorded the lowest content of peroxidase (0.78). Furthermore, the result shows that the peroxidase content in treatments 5 and 4 are not significant different ($P \geq 0.05$). This is also true for a comparison between the peroxidase content of treatments 1 and 6, as well as treatments 2 and 3. Meanwhile, treatments 2, 3, 4, and 5 are significantly different to treatment 6 and 1 ($P \geq 0.05$) (Figure 5). Conversely, proline activity is highest in treatment 5 and followed closely by treatment 4, while treatment 6 recorded the least proline activity. The results further showed a difference that is not significant in the proline activity for treatments 1, 2, 3, 4, and 5 ($P \geq 0.05$), however, treatment 6 is significantly different from all other treatments ($P \geq 0.05$) (Figure 6).

3.4 Effect of Nano-milled biochars on the Antioxidant Activity of Okra (*Abelmoscus esculentus*)

The Free Radical Scavenging Ability (FRSA) was discovered to be highest in treatment 6 (51.56) and lowest in treatment 1 (12.82). The result further showed that there is no significance difference between the FRSA intreatment 4 and treatment 3 ($P \geq 0.05$), with both having FRSA of 23.33 and 23.63 respectively. Meanwhile,a significant difference exists between the FRSA of treatments 2 and 5 ($P \geq 0.05$), with FRSA of 24.78 and 18.33 respectively (Table 4). The result for Fe Chelation shows that treatment 6 has the highest level of Fe chelation ability (42.71),while treatment 1 recorded the lowest (10.76). A further analysis shows that there was no significant difference between the Fe chelation ability of treatments 2 and 3 ($P \geq 0.05$) with both having Fe Chelation level of 26.65 and 26.22 respectively.Meanwhile,a comparison between treatments 4 and 5shows a significant difference ($P \geq 0.05$) in their Fe chelation ability, having a chelation level of 21.13 and 12.51 respectively (Table 4).

Further research shows the highest Fe reduction ability in treatment 6 (18.58) and lowest in treatment 1 (5.63). The result further shows a significant difference between the Fe reducing ability of the treatments when compared against each other ($P \geq 0.05$).

4.0 DISCUSSION

The physiological response to water deficit of different crops is of major importance in crop production and planning. Water deficit and the corresponding water stress affect the plant growth and development. However, soil water availability does not affect the height of plants for the first three weeks after planting. Meanwhile, from week 4 through week 9, the height of okra in response to water stress becomes more evident. This result can be compared to the finding by Nana *et al*. (2014) that plant water availability decreases at every stage of every growth stage when okra is subjected to water stress. However, the decline in water availability has no significant impact on the crop height for the first three weeks. Furthermore, this result concurs with Wakchaure *et al*. (2023) and Buriro *et al*. (2015) that water availability affects the height of a plant even though the effects are not noticeable until after 3 weeks of planting when the number of leaves has increased and the leave areas are large enough to dispel more water from the plant through respiration.

The number of leaves of the okra plants across all treatments is within the same range from the beginning of the experiment to the fifth week. However, the stem girth for treatment 6 became significantly different from the others ($p \geq 0.05$) from week 6 to week 9. Furthermore, the number of leaves in treatments 1 to 5 underwent a consistent decrease from week 6 to week 8 in response to the unavailability of water. The rapid decline in the number of leaves of the plants from week 5 through the end of the experiment indicates the response of the plant to cope with water stress by reducing the rate of transpiration and conserving water. Yakoub *et al*., (2016) described this phenomenon as an adaptation technique developed by plants to cope with water stress. He also noted that a decline in the physiological character of the plant, such as the number and surface areas of the leaves, will reflect on the stomata adjustment of the plant to avoid excess water loss under stress.

Furthermore, the stem girth or thickness of a plant's stem is an important parameter that indicates water stress in a plant. While water stress affects the stem girth, the effect is not noticeable until after 3 weeks of planting. The stem girth comparison between treatments 1 to 5 with treatment 6 in this experiment shows a level of significant difference ($p \geq 0.05$) from week 4 and the trend continues till the 9^{th} week, while there's no significant difference in the comparison between treatments 1 to 5 ($p \geq 0.05$). These findings align with Yakoub *et al*. (2016) that all agro-morphologic parameters, that is, plant height, stem diameter, number of leaves, leaf surface area, and dry weights, will decrease when a plant undergoes water stress. This research findings also agrees with Umar*et al*. (2021) that a decrease in plant biomass and other morphological parameters serves as a clear indication of the impact of drought stress on plants, signalling their sensitivity to water deficiency. According to the research, Umar*et al*. (2021) affirmed that under higher water stress conditions, there is a noticeable reduction in plant growth parameters such as plant height, fresh weight, and dry weight.

Yield holds the most importance in agronomic performance and is the sole essence of cropping. The yield of okra in this research is important to determine the success of this experiment. The results show that treatment 6 has a higher yield when compared to other treatments. However, the yields for treatments 2 and 6 are not significantly different ($p \geq 0.05$). Furthermore, while treatment 6 has a yield difference that's significant to treatments 1, 3, and 5 ($p \geq 0.05$), the yield difference in treatment 2 is not significant to treatments 1, 3, and 5 ($p \geq 0.05$). This experiment established that water availability is very crucial to achieve maximum crop yield and it agrees with Mkhabela *et al*. (2022) and Nana *et al*. (2014) that insufficient water during the flowering and fruiting stage can significantly diminish the yield of okra.

Furthermore, it was discovered that treatment 2 has the highest water use efficiency even though treatment 6 has the highest yield. Treatment 6 recorded the lowest water use efficiency compared to the other treatments. The low WUE to yield in treatment 6 could be as a result of supplying water above the field capacity (Aboyobi *et al*., 2009). The high water use efficiency recorded on treatment 2 can be associated with the nano-milled biochar which helps to retain water longer in the soil and made available for plant. Thus, comparing the physiological response and yield of treatment 2 to other treatments throughout the experiment, the finding shows that plants will prioritize fruiting over physiological growth under water stress conditions as established by Buriro *et al*. (2015).

This study further shows the varying defence mechanism in the production of hydrogen peroxide by okra plant in response to rate of nanoparticles applied when subjected to water stress. The response of plant to water stress depends on the severity of the stress (Osakabe *et al*., 2014), nanoparticles because of their large surface area can help the soil retain more water thereby increasing water availability during drought hence, reducing the severity of water stress to plant (Chen *et al*., 2021). In this study the analysis of hydrogen peroxide activity indicated nuanced responses to nanoparticle application. At the control, hydrogen peroxide content was 304.54. Interestingly, at treatments where 100 g, 200 g and 300 g of nanoparticles was added respectively, there was significant reduction in the hydrogen peroxide produced, which means that the nanoparticles helped the okra plant to cope with moisture stress leading to lesser hydrogen peroxide production which agrees with the report of (Orabi *et al*., 2018). Application of nano-milled biochars at the rate of 400 g however recorded the highest hydrogen peroxide production. This could be due to the colloidal properties of the nanoparticles to hold water molecules. At high rate of application, it could result in a counter effect which puts plant under water stress (Mohamed *et al*., 2009).

Peroxidase in plants serves as a key player in managing oxidative stress and reinforcing cell walls, contributing to the plant's ability to withstand water stress (Hussein *et al*., 2009). Analysis of peroxidase activity demonstrated distinct patterns throughout this experiment. The initial peroxidase content at the control was 2.59. Application of nano-milled biochars at the rate of 300 g and 400 g recorded the highest peroxidase production this could be due to stress induced by the higher rate of nano-milled biochars applied that possibly led to high content of peroxidase been produced to reduce oxidative stress (Nakano and Asada, 1981). At treatments where 0 g, 100 g, 200 g, 300 g and 0 g of nano-milled biochars (with regular water supply) were added respectively, there was reduction in the content of peroxidase produced which shows that the okra plant is less stressed at these nanoparticle application rate (Hossain *et al*., 2016). Under water stress, plants may experience increased production of reactive oxygen species (ROS), leading to oxidative damage. Catalase helps mitigate this by catalyzing the decomposition of hydrogen peroxide into water and oxygen, reducing oxidative stress (Aebi, 1983). The result obtained on catalase content shows interesting trends. At the control, catalase content was 39.78. Application of nano-milled biochars at the rate of 300 g recorded the highest peroxidase production with no significant difference from 400 g application rate which could be as a result the stress induced by the nanoparticles application rate thereby leading to high content of catalase produced to counter the stress (Roy *et al*., 2009). Notably, at treatments 2 where 100 g of nano-milled biochars was added, there was a significant reduction in the content of catalase produced which shows that the okra plant is less stressed as compared to treatment 3 and treatment 4 at these rates of application (Wang *et al*., 2018).

During drought conditions when water availability is limited, the accumulation of proline helps plants cope with water stress by regulating osmotic balance and reducing water loss, ultimately enhancing their ability to survive (Mittal *et al*., 2020). The proline content analysis revealed dynamic change over the rate of application nanoparticles. Initially, at the control, the proline content was 2.90. The application rate at treatment 5 where 400 g of nano-milled biochars was applied recorded the highest proline content (3.48) this could be a result of stress induced by the rate of nanoparticles application at this treatment level where the nanoparticles because of their colloidal properties held unto water without releasing it to the plant, there by inducing stress on the okra plant which led to increase in proline content (Mohamed *et al*., 2009). However, there is no significant difference between the treatments 1, 2, 3 and 4 with proline content of 2.90, 2.96, 3.46, and 3.27 respectively. While treatment 6 has the lowest content of proline probably due to less water stress incidence (Roy *et al*., 2009).

Furthermore, the results indicate a varied impact of nanoparticles on the antioxidant power of the okra. The application rate at treatment 6 recorded the highest FRSA (51.56), this could be attributed to the regular watering enjoyed by the plant while T1 recorded the lowest FRSA (12.82). At the application rate of 100 g of nanoparticle, the FSRA (24.78) was significantly higher than that of the T1, which means that the nanoparticles was able to retain water and make it available for the plant during periods of shortage. Sufficient water supply to plant has been linked to increased free radical scavenging ability of plants (Rai and Acharya, 2012, Villano *et al*., 2007). However, there is no significant difference between the FSRA recorded in treatments 3 and 4.

The experiment further analysed the effects of this treatment on the Fe Chelation properties of the okra. From the result, treatment 6 recorded the highest Fe Chelation activity (42.71), resulting from regular supply of water which helps the antioxidant power of the plant while treatment 1 recorded the lowest which could be attributed to stress arising from shortage of water (Eseyin *et al*., 2015, Olsen *et al*., 2008). At treatment 2 and treatment 3, the result shows that there was a favourable Fe Chelation activity which could be attributed to the water holding ability of the nano-milled biochar which influences Fe Chelation activity (Mohamed *et al*., 2009). Furthermore, the results shows that the highest Fe reducing activity at treatment 6 with (18.52) and lowest Fe reducing activity at treatment 1 with a significant difference between treatments 2, 3, 4, and 5, having Fe reduction ability of 12.84, 13.72, 11.18, and 10.35 respectively. The varied pattern in Fe reducing activity could be associated with the nanoparticles ability to retain water and as well make it available for the plant to use when subjected to stress (Chen *et al*., 2003).

Generally, water stress has a negative impact on growth, yield, antioxidant and biochemical properties of okra, however the use of nano-milled biochar at the rate of 100g could help the plant cope with drought stress. At an application rate of greater that 100kg could be counterproductive for the okra plant.

5.0 Conclusion

The investigation into the physiological responses of okra (*Abelmoschus esculentus)* revealed that water deficit significantly impacted okra's height, number of leaves, and stem girth, with noticeable effects becoming evident after three weeks of planting. Okra can grow effectively under little to no water for the first three weeks after planting. Further investigation into the yield and water use efficiency of okra demonstrated the important relationship between water availability, crop productivity, and efficient water utilization. Application of nano-milled biochar at the rate of 100 g, despite having a slightly lower yield in comparison to treatment 6 with proper irrigation, displayed the highest water use efficiency, indicating an optimal utilization of the limited water resources.
Furthermore, the findings in this study suggest that applying nanoparticles to the soil at the rate of 100 g will not maximize water use efficiency but also help the plant cope with stress arising from the drought condition. The study also reveals that proline levels remain relatively stable at treatments 3, 4, and 5, implying a threshold beyond which nanoparticle application may not significantly influence proline accumulation.

This research contributes to the broader understanding of sustainable agricultural practices, offering valuable insights that can inform future strategies for enhancing crop resilience and water management in the face of changing environmental conditions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

Aebi, H.E., (1983). Catalase. In: *Methods of enzymatic analysis*. Bergmeyer, H.U. (Ed.). Verlag Chemie Weinhem, 273-286.

Alemayehu, B., and Teshome, H. (2021). Soil colloids, types and their properties: A Review. *Open Journal of Bioinformatics and Biostatistics*, 008–113. [https://doi.org/10.17352/ojbb.000010]

Atoyobi, T., Ajayi, N., and Adeniran, K. (2009). Effect of water stress on growth and yield of okra (Abemoscus esculentus) under irrigation. *Journal of Applied Science and Technology*, *14*(1–2). [https://doi.org/10.4314/jast.v14i1-2.44338]

Behnam, H., and Firouzi, A. F. (2022). Effects of synthesis method, feedstock type, and pyrolysis temperature on physicochemical properties of nano-milled biochars. *Biomass Conversion and Biorefinery,* 1–11.

Buriro, M., Sanjrani, A., Chachar, Q., Ahmed, N., Chachar, S., Buriro, B., Gandahi, A.W., and Mangan, T., (2015). Effect of water stress on growth and yield of sunflower. *Journal of Agricultural Technology*. 11. 1547-1563.

Chausali, N., Saxena, J., and Prasad, R. (2021). Nanobiochar and biochar based nanocomposites: Advances and applications. *Journal of Agriculture and Food Research*, 5, 100191. [https://doi.org/10.1016/j.jafr.2021.100191]

Chen, X., Duan, M., Zhou, B., and Cui, L. (2021). Effects of nano-milled biochars as a soil amendment on the structure and hydraulic characteristics of a sandy loam soil. *Soil Use and Management*, 38, 836–849.

Chun O.K., Kim D.O. and Lee C.Y., (2003). Superoxide Radical Scavenging Activity of the Major Polyphenols in Fresh Plums. *Journal of Agricultural and Food Chemistry.* 51(27):8067-8072.

Ebele, N., and Emodi, N. (2016). Climate change and its impact in Nigerian economy. *Journal of Scientific Research and Reports*, 10(6), 1–13. [https://doi.org/10.9734/jsrr/2016/25162]

Effiong, Asanga, (2015). Evaluation of the Antioxidant Properties of Some Commonly Eaten Vegetables in Akwa Ibom State of Nigeria 5(2): 165-173

Eseyin, Olorunfemi A., Etiemmana, Goodnews Charlie, Enobong, Monday, Ebong, Aniekan, Etim, Iweh, Udobre, Sunday A., Johnson, Ekarika, Attih, Emmanuel, and Hossain, M.A., Bhattacharjee, S., Armin, S., Qian, P.,

Xin, W., Li, H., Burritt, D.V., Fujita, M. and LamSon, P., (2016). Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. *Front. Plant Sci*., 2015; 6: 420

Ezegwu, C. N. (2014). Climate change in Nigeria: The impacts and adaptation strategies. *SSRN Electronic Journal*. [https://doi.org/10.2139/ssrn.2543940]

Godarzi A., Bazrafshan F., Zare M., Faraji H., and Langeroodi A. R. S., (2017). Studying the effect of drought stress on yield and physiological characteristics in genotypes of canola (*Brassica napus* L.). *Helix*, 8: 1250-1258.

Hussein, M.M., Bakheta, M.A., and Orabi, S.A., (2009). Influence of abscisic acid and benzyl adenine on some growth characters, endogenous hormones and some chemical constituents of cotton plants grown under drought stress. *Egyptian Journal of Agronomy*.31: 253-270

Irmak, S., and Rathje, W. R. (2008). Plant Growth and Yield as Affected by Wet Soil Conditions Due to Flooding or Over-Irrigation. Neb Guide, G1904.[g1904.indd.]

Lin, Q., Tan, X., Almatrafi, E., Yang, Y., Wang, W., Luo, H., Qin, F., Zhou, C., Zeng, G., and Zhang, C. (2022). Effects of biochar-based materials on the bioavailability of soil organic pollutants and their biological impacts. *Science of the Total Environment*, *826*, 153956. [https://doi.org/10.1016/j.scitotenv.2022.153956]

Mittal, D., Kaur, G., Singh, P., Yadav, K., and Ali, S. A. (2020). Nanoparticle-based Sustainable Agriculture and Food Science: Recent Advances and Future Outlook. *Frontiers in Nanotechnology*, *2*. [https://doi.org/10.3389/fnano.2020.579954]

Mkhabela, S. S., Shimelis, H., Gerrano, A. S., and Mashilo, J. (2022). Phenotypic response of okra (*Abelmoschus esculentus* [L.] Moench) genotypes under drought-stressed and non-stressed conditions. *South African Journal of Botany*, *145*, 293-302. [https://doi.org/10.1016/j.sajb.2021.11.008]

Mohamed B., Jamie R L., Frank von der K., Thilo H. (2009). Environmental and human health impacts of nanotechnology, 109-161

Mukhopadhyay, S.S. (2014) Nanotechnology in agriculture: Prospects and constraints, *Nanotechnology, Science and Applications*, 2014(7), pp. 63–71. [https://doi.org/10.2147/nsa.s39409]

Nakano, Y. and Asada, K., (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase. *Plant Cell Physiology*., 22: 867-880.

Nana, R., Sawadogo, M., and Tamini, Z. (2014). Response of okra [*Abelmoschus esculentus* (L.) Moench] to water stress in the soil. *African Journal of Biotechnology*, 13(35), 3591–3596. [https://doi.org/10.5897/ajb2013.12853]

Okon, E. M., Falana, B. M., Solaja, S. O., Yakubu, S. O., Alabi, O. O., Okikiola, B. T., Awe, T. E., Adesina, B. T., Tokula, B. E., Kipchumba, A. K., and Edeme, A. B. (2021). Systematic Review of Climate Change Impact Research in Nigeria: Implication for Sustainable Development. *Heliyon*, *7*(9). [https://doi.org/10.1016/j.heliyon.2021.e07941]

Olsen R. A., Bennett J. H., Denise, Blume, and Brown J. C. (2008). Chemical aspects of the Fe stress response mechanism in tomatoes. 8: 905-921

Osakabe Y., Osakabe, K. Kazuo S., and Lam-Son P. (2014). Response of plants to water stress, *Front Plant Science*, 5: 86.

Orabi, Salwa A., Hussein, M.M., Zaki, Safi-naz S., and Sharara, Faida A., (2018). Influence of hydrogen peroxide on growth, yield and biochemical constituents of canola plants grown under different irrigation intervals, 7: 407-418

Pratiwi, D. C., Konhauser, K. O., and Alessi, D. S. (2021). Nano-milled biochars: Interactions with and impacts on soil and water microorganisms. *Biochar in Agriculture for Achieving Sustainable Development Goals*, 139-154. [https://doi.org/10.1016/B978-0-323-85343-9.00030-6]

Rai M. and Acharya K., (2012). Proximate Composition, Free Radical Scavenging and NOS Activation Properties of Ramaria aurea. *Research Journal of Pharmacy and Technology*. 5(11):1421-1427.

Rodell, M., Velicogna, I., and Famiglietti, J. S. (2009). Satellite-based estimates of groundwater depletion in India. *Nature*, *460*(7258), 999-1002. [https://doi.org/10.1038/nature08238]

Roy, R., Mazumder, P. B., and Sharma, G. D., (2009). Proline, catalase and root traits as indices of drought resistance in bold grained rice (Oryza sativa) genotypes 8(4): 6521-6528

Shang, Y., Hasan, Md. K., Ahammed, G. J., Li, M., Yin, H., and Zhou, J. (2019). Applications of nanotechnology in plant growth and Crop Protection: A Review. *Molecules*, *24*(14), 2558. [https://doi.org/10.3390/molecules24142558]

Umar, W., Zia ur Rehman, M., Umair, M., Ayub, M. A., Naeem, A., Rizwan, M., Zia, H., and Karri, R. R. (2021). Use of nanotechnology for wastewater treatment: Potential applications, advantages, and limitations. *Sustainable Nanotechnology for Environmental Remediation*, 223-272. [https://doi.org/10.1016/B978-0-12-824547-7.00002-3]

Villano D., Fernandez-Pachon M.S., Moya M.L., Troncoso A.M., Garcia-Parilla M.C., (2007). Radical scavenging ability of phenolic compounds towards DPPH free radical. *Talanta*;71(1):230–235

Wang, J., Kookana, R., Liu, X., and Fang, H., (2018). Nano-milled biochars: A review. *Environmental Science & Technology*, 52(21), 12662–12673.

Wakchaure, G., Minhas, P., Kumar, S., Khapte, P., Dalvi, S., Rane, J., and Reddy, K. S. (2023). Pod quality, yields responses and water productivity of okra (*Abelmoschus esculentus* L.) as affected by plant growth regulators and deficit irrigation. *Agricultural Water Management*, *282*, 108267. [https://doi.org/10.1016/j.agwat.2023.108267]

Yadav, N., Garg, V. K., Chhillar, A. K., and Rana, J. S. (2023). Recent advances in nanotechnology for the improvement of conventional agricultural systems: A Review. *Plant Nano Biology*, *4*, 100032. [https://doi.org/10.1016/j.plana.2023.100032]

Yakoub, A. R. B., Benabderrahim, M. A., and Ferchichi, A. (2016). Physiological and Agromorphological Responses of Tossa Jute (Corchorus olitorius L.) to Drought Stress. *Journal of Plant Physiology and Pathology*, *04*(03). [https://doi.org/10.4172/2329-955x.1000152]

Yang, W., Li, B., and Shang, J. (2022). Aggregation kinetics of nano-milled biochars in aqueous environment: Interplays of anion type and bovine serum albumin. *Science of the Total Environment*, 155148