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Full Length Research

Screening for induced drought stress tolerance on some selected rice varieties (*Oryza glaberrima* l.) using silver nanoparticles (AgNPs)

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ABSTRACT: Rice is the main staple food for one-third of people worldwide providing up to 80% of these individuals' daily calories. Drought is adverse, affecting crop plants' growth, yield, quality and nutritional value. Likewise, it causes social unrest and economic loss to the farmers and governments. The current study aimed to examine the impact of environmentally friendly silver nanoparticles (AgNPs) on the physiological, yield, and growth parameters of rice plants in response to drought. The most widely manufactured engineered nanomaterials at the moment, silver nanoparticles (AgNPs) are present in a large variety of commercial products. The aqueous leaf extract of *Azadrachta indica* was used to create the plant-based AgNPs. The leaves of *Azadirachta indica* were extracted aqueously to create the plant-based AgNPs. In order to determine the metabolites of drought stress, the AgNPs were characterised using X-ray diffraction (XRD), GC-MS, furrier transform infrared (FTIR), UV-visible spectrophotometry, and scanning electron microscopy (SEM). Brown colouration was observed during synthesis, and this was confirmed by UV-visible at an absorbance peak of 300 nm. An FTIR machine also confirmed the presence of amine, alcohol and carboxylic, alkene, nitrogenous molecule, and alkyl halide at five significant peaks, spherical and with a crystalline size of 32.8 nm. GC-MS was used to identify octadecanoid acid, a metabolite associated with drought stress. Rice plants treated with different doses of AgNP in field studies became more tolerant to drought stress. It was discovered that the AgNPs significantly affected physiological, growth, and yield parameters in addition to total biomass. It was discovered that AgNPs at a concentration of around 20 mg/L were efficient in inducing metabolic alterations that led to drought stress. Furthermore, roots treated with AgNPs under drought stress showed less root aerenchyma than roots treated with AgNPs under well-watered conditions, which showed a greater number of aerenchymas. Due to the surface plasmon vibration, excitation of bio-reduction, capping or stabilising agent, and neem leaves' presence of octadecanoic acid (ethyl ester), which is essential in reducing drought stress tolerance in plants, especially rice, neem leaves were suggested for the synthesis of silver nanoparticles.

Keywords: *Azadirachta indica*, drought, metabolite, nanotechnology, rice (*Oryza glaberrima* L.), silver nanoparticle.

INTRODUCTION

According to Khush (2005) and Burlando and Cornara (2014) rice provides up to 80% of the daily calories for two-thirds of the world's population. However, due to its short root system, thin cuticular wax, and rapid stomatal closure, rice is regarded as one of the most drought-susceptible plants (Ji *et al.*, 2012). According to Serraj *et al.* (2011), over 23 million hectares of rain-fed rice are under drought stress. By screening and characterizing rice germplasm at several molecular, genetic and morphological levels while under drought stress, Swamy and Kumar (2013) were able to identify genetic variants in the rice genes important for drought resistance. Consequently, by creating drought-tolerant types, it might be able to negate drought stress in rice in the future.

Numerous factors, including humidity, winds, temperature and geographic features, regulate the changeability of the primary water supply and precipitation over an area (Mishra and Singh, 2010). It was found that drought and global warming are primarily responsible for the majority of local climatic changes. According to predictions made by Sallam *et al.* (2019), 1.8 billion people worldwide will experience absolute water scarcity and shortage in the first quarter of the twenty-first century, while 65% of people will experience partial water shortage.

Farmers can improve crop yield and quality by improving soil conditions, but they can also use sensors to automate the agricultural sector and predict local weather forecasts by using nano-fertilizers, nano-pesticides, nano-biosensors, and nano-metrological instruments (Dimetry and Hussein, 2016; Manna and Bandyopadhyay, 2019). Due to the simplicity of the reaction conditions, energy intensity, cost-effectiveness and production of biocompatible nanoparticles, green metal nanoparticle synthesis has advantages over employing dangerous chemicals (Javed *et al.,* 2020).

The most widely used designed nanomaterial at the moment is silver nanoparticles (AgNPs), which may be found in a variety of commercial goods (Davies, 2009). In agriculture, AgNPs have been linked to crop improvement. Furthermore, research advances have highlighted the agricultural application of AgNPs as alternative growth promoters, stress resistors or pesticides. As such, it is imperative to understand the phyto-complex activity of AgNPs to evaluate their environmental implications. Although this is a subject of intense investigation, most of the reported results come from environmentally irrelevant concentrations (Lemire*et al*., 2013; Tamilselvan *et al.,* 2016). An analysis of the publications using relevant concentrations demonstrates that AgNPs may provide several benefits to crop plants. (Chen *et al*., 2023). According to numerous publications, the right concentrations of AgNPs are essential for promoting seed germination and plant growth (Shelar and Chavan, 2015), increased chlorophyll content, photosynthetic quantum efficiency, and water and fertilizer usage effectiveness (Kaveh *et al.,* 2013; Rahman *et al*., 2023).

This study aimed to determine the response of two rice (Faro 60 and Faro 67) varieties to drought stress conditions using nanoparticles by synthesizing and characterizing Silver Nanoparticles AgNPs and the effect of the nanoparticles on growth parameters. The research was meant to identify metabolites for drought stress tolerance and assess the effect of physiological and yield parameters.

The present study was carried out at Gombe State University in Nigeria, and the findings will help Gombe rice farmers better manage their crops during drought conditions and provide strategies for eradicating the factors that lower expectations for the state's rice yield.

EXPERIMENTAL

Description of the study area and collection of sample materials

This research work was conducted at the Botanical Garden in Gombe State University, Gombe State, Nigeria. The Campus is situated in Tudun-Wada within Gombe State, Nigeria. It lies between latitude 10˚15'N and longitude 11˚10'E (Figure 1). The rice (Faro 60 and faro 67) seeds were obtained from Biu local market, Borno State, and the silver nanoparticles were prepared in the Chemistry Department at Gombe State University.

Synthesis of nanoparticles

Dispersions of silver nanoparticles, used in this study, were prepared at the Chemistry Department Laboratory, Gombe State University, Gombe State, Nigeria using the method of Igwe and Ekebo (2018). UV-visible spectroscopy analysis was done to confirm the formation of nanoparticles and observe the extract plasmon vibration and excitation.

The wavelengths were varied at regular wavelengths of 200, 300, 400, 500, 600, 700 and 800 nm (Javed *et al.,* 2020). FTIR analysis was done on both the leaf extracts and the synthesized silver nanoparticles to determine the functional group present in the samples. The SEM analysis was done to determine the morphology of the synthesized silver nanoparticles. Copper grids were used to mount the samples by using the drop coating method (Javed *et al*., 2020). XRD analysis was done to find out the average crystalline size.

The Debye Scherer equation was used to calculate the average crystalline size. GC-MS analysis was done by the SHIMAZDU QP2010, at oven temperature from 50 to 280°C at 4°C/min and held at this temperature for 5 min; inlet and interface temperatures were 250°C and 280°C, respectively. Carrier gas was He at a flow rate of 1.0 ml/min (constant flow). 0.2 ml of sample was injected under a split of 20:1. EIMS: electron energy, 70 eV.

Preparation of treatment materials and drought stress application

The silver nanoparticles were made in the Gombe State

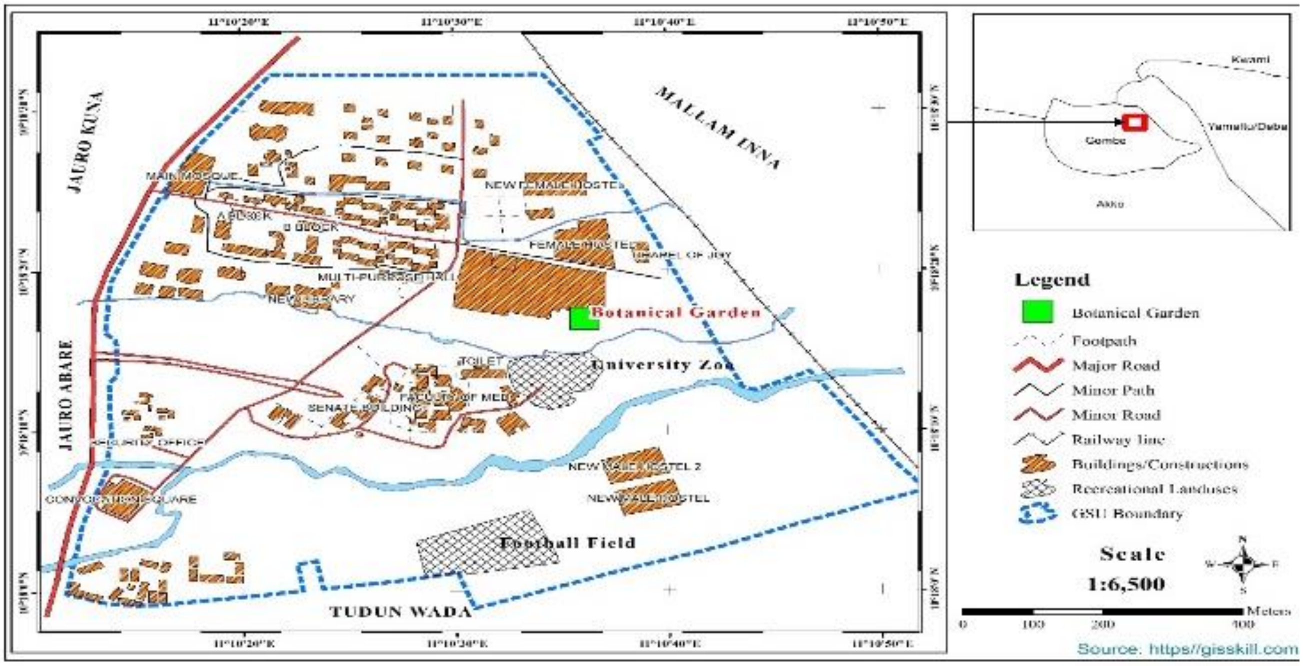

Figure 1. MAP of Gombe State University showing the location of the Botanical Garden.

University Chemistry Department, while the seeds were acquired from the Biu local market in Borno State. In this investigation, four watered level treatments and three concentrations of silver nanoparticles that were produced in a lab were used. Three applications of the AgNPs were made during the tillering, stem elongation, and reproductive stages. Under typical irrigation conditions, plants were subjected to alternating wetting and drying (one week of watering, one week of drought) and well watering (WW) daily (DS) (Figure 2). There were 72 pots in each treatment and three replicates of each cultivar, for a total of 270 pots per treatment.

Seedlings in each nanoparticle treatment were randomly assigned to one of two groups based on soil moisture stress at the end of the nanoparticle treatments (day 28), both well-watered (WW) and stressed by drought (DS) (Figure 2). The seedlings were divided into three duplicates at random following four weeks of germination.

The following treatment was applied to each cultivar of rice: a water deficit at day 28, also known as drought stress treatment (DS). Water was provided daily as part of the second treatment (WW) (Figure 2). Every week, growth metrics were noted for every cultivar. At the conclusion of the experiment, the biomass of the dry shoot and root was also ascertained.

Measurement of growth and physiological parameters

Growth parameters such as plant height, flag leaf area, tiller number and stem diameter were measured. Plant height was measured using the meter rule, flag leaf area was measured using the following formula, flag leaf area (cm^2) = flag leaf length (cm) × maximum width (cm) × 0.75 (Islam *et al.*, 2016), tiller number was counted and the outer diameter internodes were measured. The data on relative water content (RWC), chlorophyll content (CC), chlorophyll stability index (CSI), and transpiration of rice plants treated with AgNPs were recorded.

To determine the relative water content youngest fully expanded plant per pot was collected and immediately weighed to obtain the fresh weight, then rehydrated in petri dishes containing distilled water for 24 hours under dim light and room temperature to get the turgid weight. Subsequently, the leaves were oven-dried to record the dry weight. The RWC was calculated using the method devised by García-Mata and Lamattina (2001) using the following equation:

$$\mathrm{RWC}\ (\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}}\ x\ 100$$

The chlorophyll content was measured using paper chromatography where the leaves were crushed in a mortar with a pestle by adding 80% acetone, then the extracts were dropped on a 2 cm line drawn on the paper chromatography to get thick concentration and then paper was placed gently into the beaker that contains prepared running solvent (petroleum ether and acetone) to the mark of 20 ml (Reiss, 1994). The CSI was calculated by using

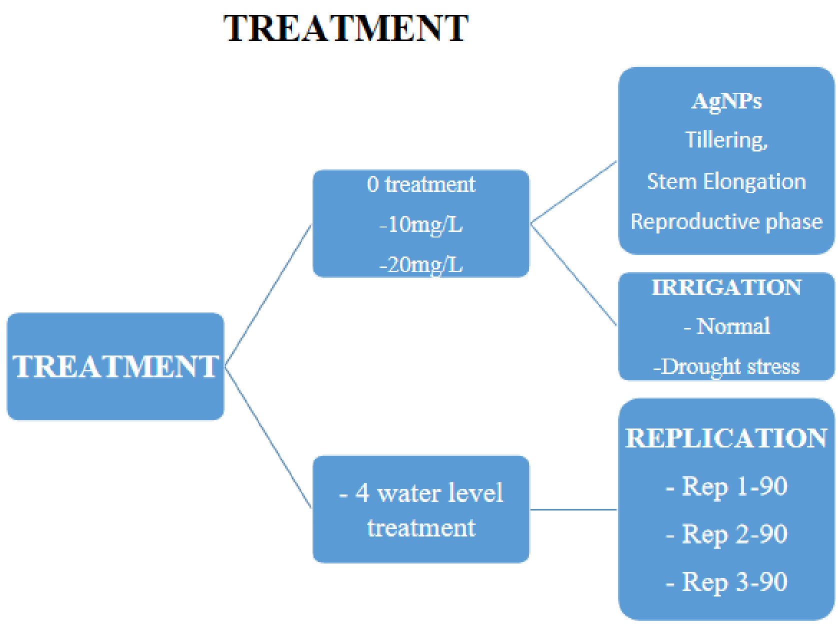

Figure 2. Experimental design and drought stress treatment application.

the following formula (Rai *et al.,* 1995):

$$\text{CSI (\%)} = \frac{\text{Total chlorophyll contents (stressed)}}{\text{Total chlorophyll contents (control)}}\ x\ 100$$

To determine the amount of water that transpired, the polythene bags were tired on shoot with five leaves inside at noon and allowed for 40 mins and the water was collected with a syringe and measured in milliliter.

Morphological/ yield parameters

A variety of morphological characteristics were assessed, including fresh root and shoot biomass (cm), dried root and shoot biomass (gm), spike length, spikelets per spike, number of grains per spike, and 100-grain weight (gm). Using the meter rule, the length of the spikes was measured.

The number of spikelets and seeds per spike was also recorded. 100 grains were weighed, and then the weight of 1000 grains was computed. Following harvest, the total biomass of the dried roots and shoots was determined.

The data collected was subjected to analysis of variance (ANOVA) using Jamovi (2021) Version 1.8 computer software. The treatment was tested at least a level of significance and differences were determined by Tukey and Pearson correlation at <0.05 level of significance.

Microscopy

The microscopic sections were obtained from one-third of the root tip and then aerenchyma formation was compared within treatments. The root-cross section was prepared from the plant sample. It was examined using an electron microscope (Cutler, 1978).

RESULTS AND DISCUSSION

UV-visible spectrophotometry analysis

Figure 3 shows the spectrum obtained from the UV-visible spectrophotometric analysis of *Azadirachta indica* extract and silver nanoparticles. This is consistent with the results of Shankar *et al.* (2003), who reported that the yellowish-brown appearance of silver nanoparticles in aqueous solution was induced by surface plasmon oscillations.

In the present study, *Azadirachta indica*'s largest absorbance peak was found at 400 nm in the UV-visible spectrum. At a wavelength of 300 nm (Figure 4), *Azadirachta indica* generated silver nanoparticles. The absorbance peak of the spectra of the silver nanoparticles produced in reaction media is located at 300 nm (Veerasamy *et al.,* 2011). This is in line with the findings of Suriyathana *et al*. (2018), where it was discovered that the absorbance peaks with the biggest magnitudes were at 350, 315 and 400 nm, respectively.

Fourier Transform Infrared (FTIR) analysis

Numerous studies have indicated that the absorption maxima of iron nanoparticles (FeNPs) should be between 280 and 420 nm. Similar investigations were conducted by

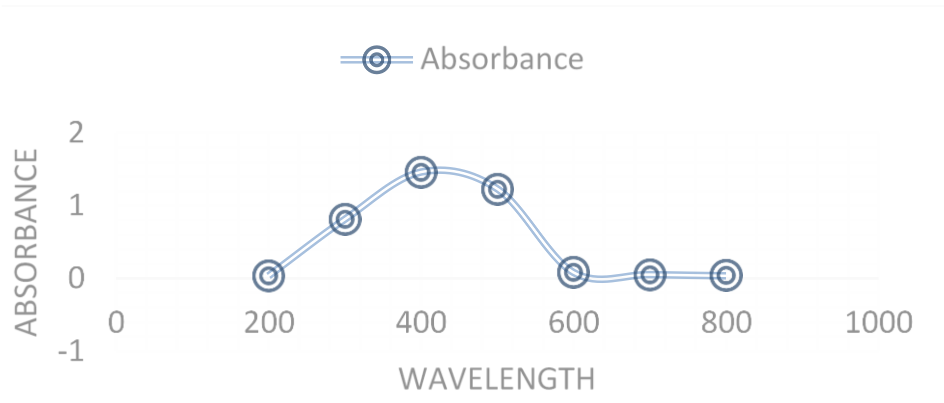

Figure 3. UV-visible for *Azadirachta indica* extract showed the highest absorbance at 400 nm.

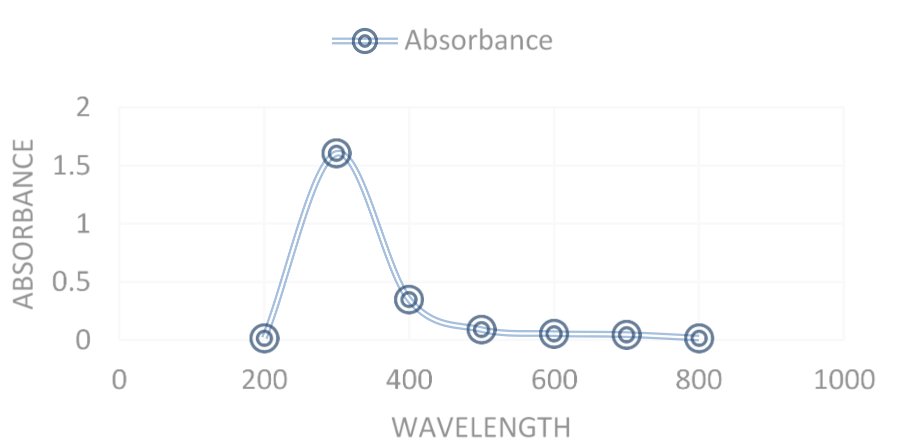

Figure 4. UV-visible spectrum for AgNPs.

Mechalingam *et al*. (2016), who discussed the potential discovery of AgNPs' plasmon band in the 400–420 nm range. The bio-reduction of Ag+ ions to silver nanoparticles is caused by a reduction in the capping material of plant extract, as confirmed by FTIR research. Proteins in the leaf extract can bind to silver nanoparticles through free amino or carboxyl groups, according to Gole *et al.* (20010). C=C (alkene), N-O (nitrogenous molecule), O-H (alcohol & carboxylic) and C-Br (alkyl halide) are present in this study's FTIR spectrum. Prasad *et al*. (2011) showed that the carboxyl (–C=O), hydroxyl (–OH) and amine (–NH) groups are the main groups of leaf extracts utilised in the production of silver nanoparticles. The acquired results are in agreement with the literature given by Preeti (2017), which suggests that the presence of polyphenols and flavonoids is indicated by the discovered FTIR bands. The leaf extract of *Azadirachta indica* yields silver nanoparticles with an average crystallographic size of 32.8 nm (Figures 5 and 6).

Scanning Electron Microscopy (SEM) analysis (hitachi.4160)

AgNPs' anisotropic, almost spherical, SEM morphology is revealed in Figure 7, which also shows the presence of certain cubical nanoparticles. The SEM investigation revealed that the spherical nanoparticles had a range of morphologies and sizes between 10 and 50 nm. Savithramma *et al.* (2011) observed that in *Shorea tumbuggaia* and *Boswellia ovalifoliolata*, comparatively spherical L-shaped silver nanoparticles developed with diameters ranging from 30 to 40 nm.

X-Ray Diffraction Analysis (XRD)

For the synthesized AgNPs, an X-ray diffraction (XRD) analysis pattern was noted. Five notable peaks, which can be indexed by the plain of (111), (110), (101) and (222),

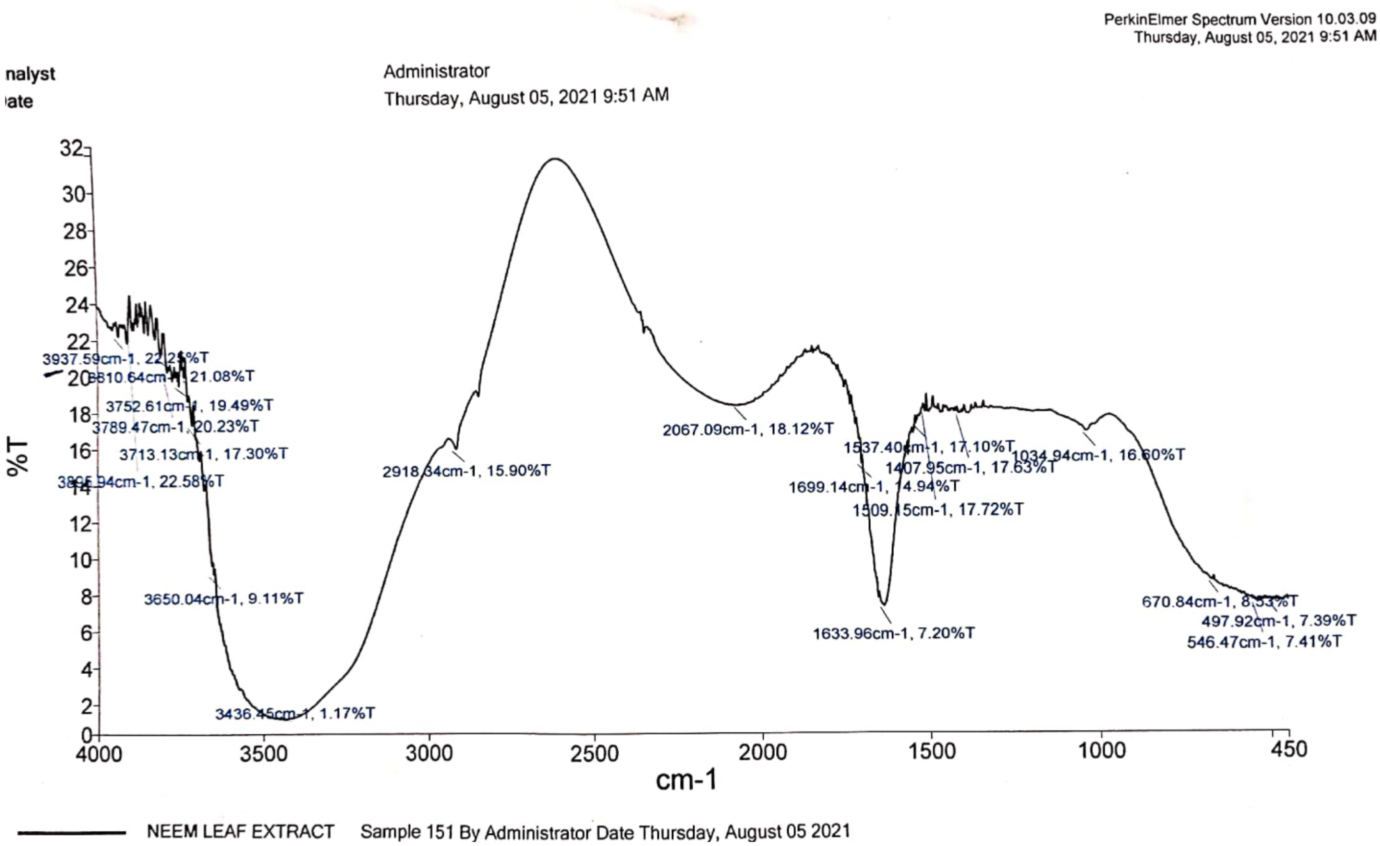

Figure 5. Showing the FTIR spectrum of the *Azadirachta indica* leaf extract.

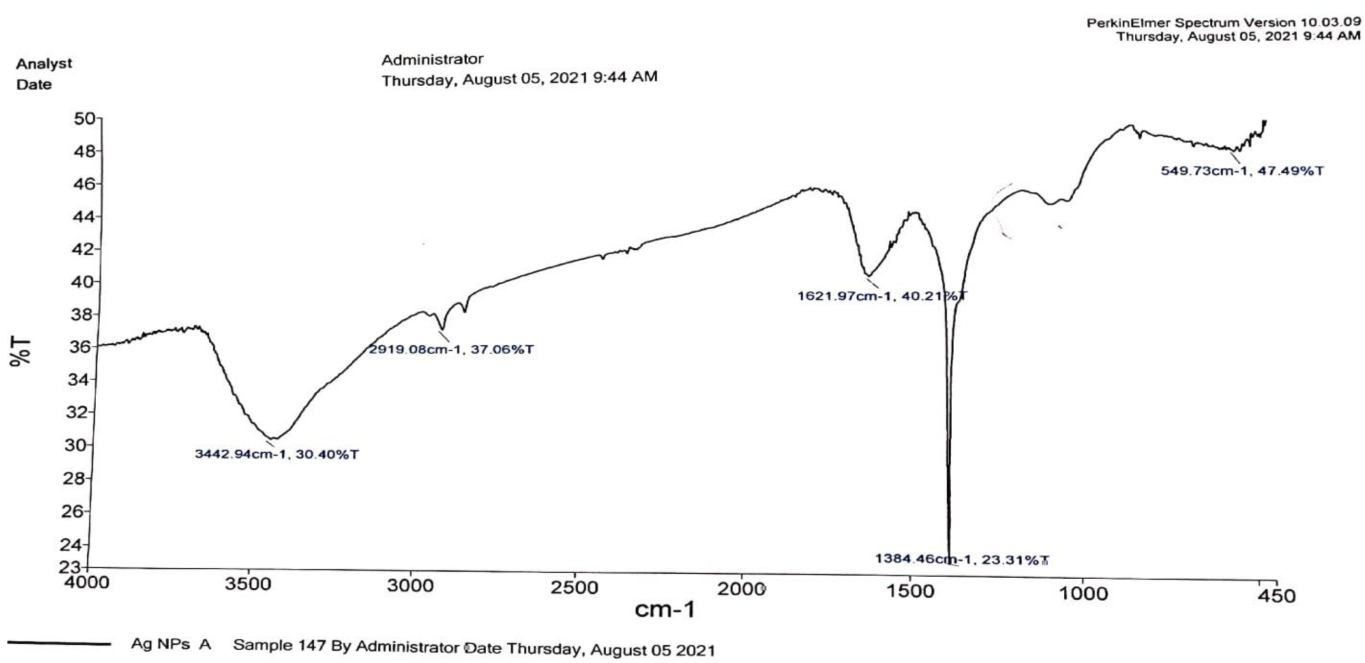

Figure 6. Showing the FTIR spectrum of the silver nanoparticles (AgNPs)

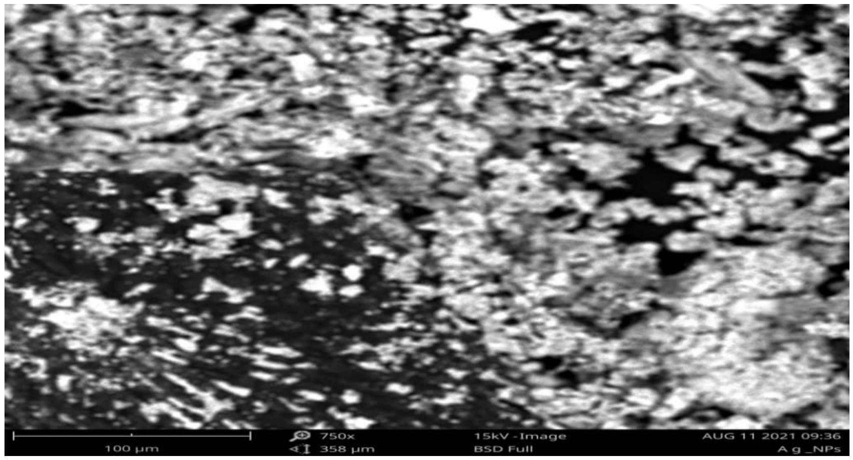

Figure 7. SEM micrograph of silver nanoparticles synthesized by using the *Azadirachta indica* leaf extract.

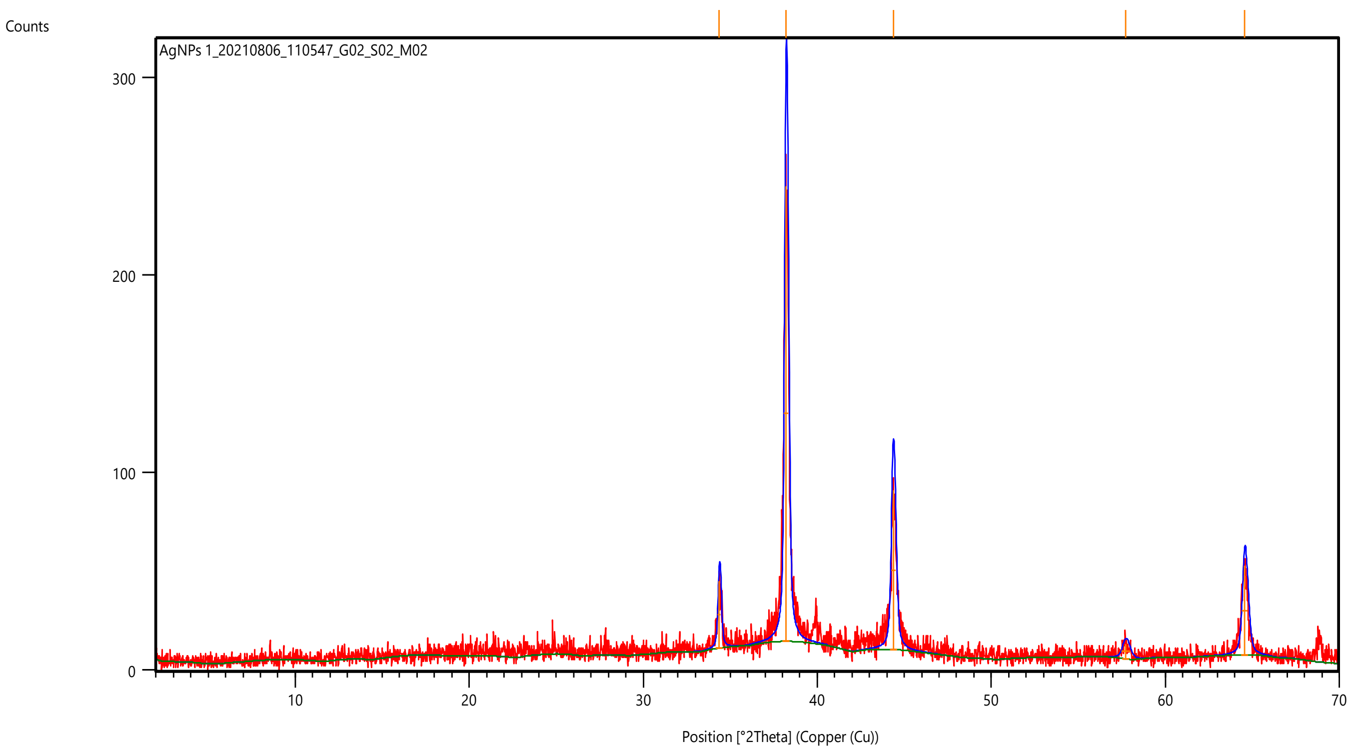

Figure 8. XRD sSpectrum for green synthesized silver nanoparticles from *Azadirachta indica* leaf extract.

were found at 34.37150, 38.21830, 44.36510, 57.71860 and 64.55620. The silver nanoparticle's XRD pattern shows a face-centred cubic structure with an average crystallographic size of 32.8 nm (Figure 8). The acquired result was in agreement with the results reported by Raut *et al*. (2009), whereby XRD analyses revealed the presence of polydisperse silver nanoparticles with an average size of 27 nm. The XRD pattern, as reported in Vivek *et al.* (2011), revealed the average size of the silver nanoparticles that were made from *Gelidiella acerosa* extract.

Gas-Chromatography Mass Spectrometry (GC-MS)

The GC-MS analysis of synthesized silver nanoparticles

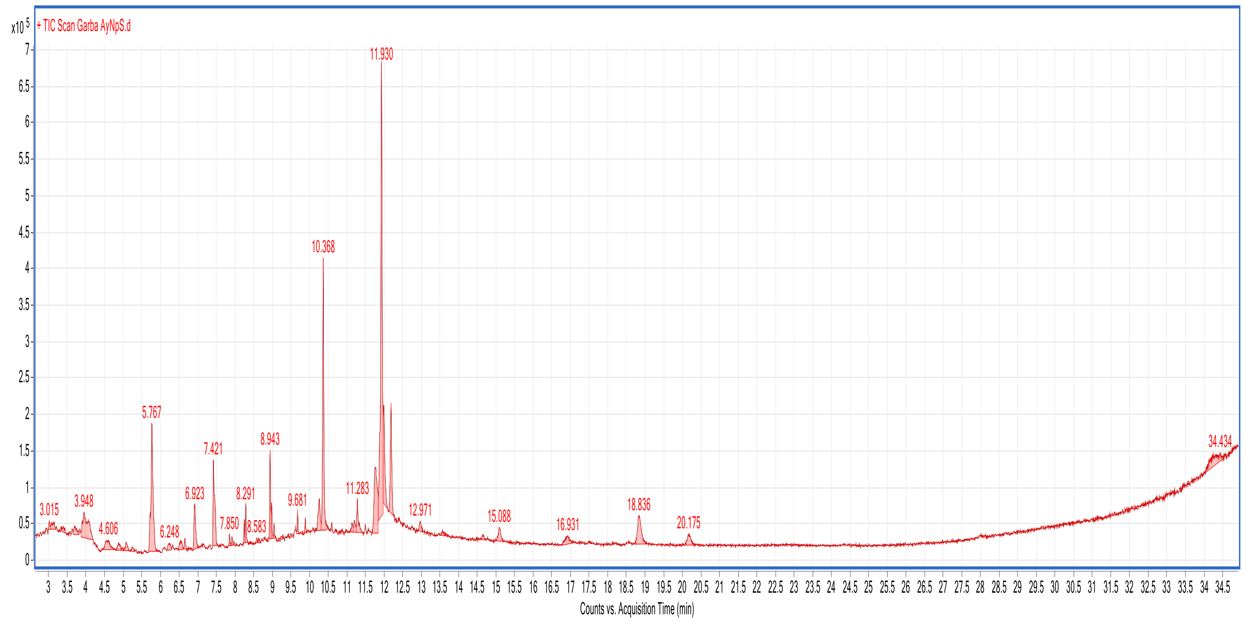

Figure 9. GC-MS spectrum of silver nanoparticles.

from *Azadirachta indica* aqueous leaf extract has shown four different peaks among which peak (10) octadecanoic acid (ethyl ester) is one of the metabolites for drought stress (Figure 9). The GC-MS analysis revealed several peaks but four compounds were recorded. However, there was one prominent peak in the chromatography (peak 10) namely octadecanoic acid (ethyl ester) identified to play a vital role in mitigating drought stress tolerance in rice. This result is in agreement with the result obtained by Zahratul *et al.* (2021) and Ullah *et al.* (2017).

Growth and physiological parameters

The application of various AgNPs concentrations to *Oryza glaberrima* L. plants resulted in a considerable improvement in growth metrics in both stressed and non-stressed plants when compared to control plants. This outcome is consistent with that of Latif et al. (2017), who demonstrated that applying AgNPs foliar treatment at several doses enhanced the growth characteristics of wheat plants. The observed growth increase generated by varying doses of AgNPs, particularly at 40 mg/l, may be attributed to AgNPs' known function in inhibiting ethylene signalling in fenugreek plants (Rezvani *et al.,* 2012). The results for plant height, flag leaf area, tiller count and stem diameter are displayed in Table 1. In Faro 67 treated with 20 mg/L AgNPs under non-stressed conditions, the maximum plant height (18.0 cm) was recorded, but under drought stress, 20 mg/L AgNPs displayed the lowest mean (12.4 cm) for plant height. With the exception of stem diameter, all assessed growth parameters (plant height, flag length and number of tillers) exhibited significant interaction effects, with p <=0.042, 0.006, and 0.024) respectively (Table 1).

The 20 mg/L concentration was noticed to have an impact on Faro 67 having the highest mean (24.6 cm^2) for flag leaf under non-stressed conditions and the lowest mean (16.6 cm^2) was observed in Faro 60 treated with 20 mg/L under drought-stressed (Figure 10).

For tiller numbers, at 10 and 20 mg/L AgNPs treatment Faro 67 under the non-stressed condition exhibited more tillers than under the stressed condition, and this trend was observed under all the treatments (Figure 11).

Faro 67 exhibited the highest mean (1.55 cm^2) in almost all the morphological parameters measured under the stressed and non-stress conditions with the exception of its low performance in stem diameter under the 20 mg/L stressed condition (Figure 12).

Physiological parameters

Physiological parameters of the rice plants in terms of relative water content, chlorophyll content and chlorophyll stability index were determined in response to various concentrations of *Azadirachta indica* leaves extract-mediated AgNPs under drought stress. RWC is an important physiological parameter that measures the water level of plants and is involved in the performance of metabolic activities of plant tissues.

According to Savicka and Škute (2010), plant membrane stabilization plays an important role in maintaining the plant’s cell integrity. Drought stress decreases the plant's physiological parameters. It was reported by Wang *et al*.

Table 1. Effect of the induced drought treatments on growth parameters observed among the varieties.

Varieties	Treatment	Plant height (cm)	Flag leaf area (cm^2)	Tiller numbers	Stem diameter (cm^2)
	Control	8.98±1.06	9.69±1.79	2.58±0.351	0.775±0.0783
	10mg/L WW	14.8±0.650	22.5±1.59	3.25±0.207	1.29±0.0532
Faro 60	10mg/L DS	12.9±0.608	17.3±1.33	2.39±0.153	1.04±0.0446
	20mg/L WW	12.7±0.647	18.1±1.26	2.93±0.192	1.15±0.0539
	20mg/L DS	12.4±0.682	16.6±1.37	2.47±0.172	1.06±0.0573
	Control	13.0±1.23	16.7±2.15	3.67±0.480	1.11±0.105
	10mg/L WW	16.9±0.761	23.7±1.68	4.26±0.289	1.31±0.0613
Faro 67	10mg/L DS	15.5±0.739	18.0±1.07	3.03±0.215	1.21±0.0579
	20mg/L WW	18.0±0.738	24.6±1.49	4.40±0.266	1.55±0.182
	20mg/L DS	16.8±0.710	22.5±1.39	3.63±0.234	0.0783±0.0493
	Variety	<.001***	<.001***	<.0.001***	0.008**
P >value	Treatment	<.001***	<0.01**	<0.05*	<.001***
	Varieties*Treatment	<0.042	0.006**	<0.024	0.104

Footnote. * p < .05, ** p < .01, *** p < .001, WW- well watered, DS- drought stress.

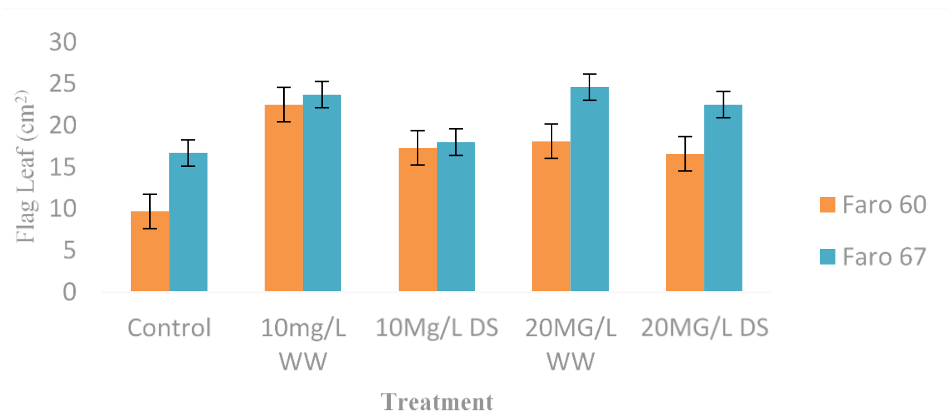

Figure 10. Effect of the induced drought stress on flag leaf area (cm^2) of the varieties.

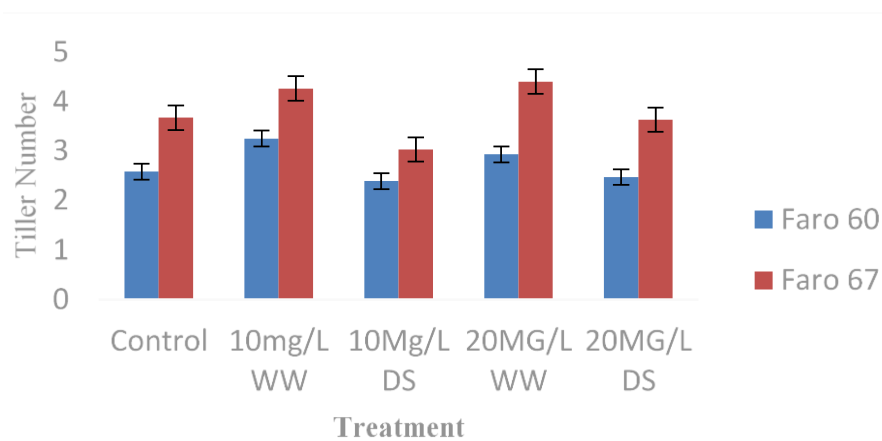

Figure 11. Effect of the induced drought stress on number of tillers of the varieties.

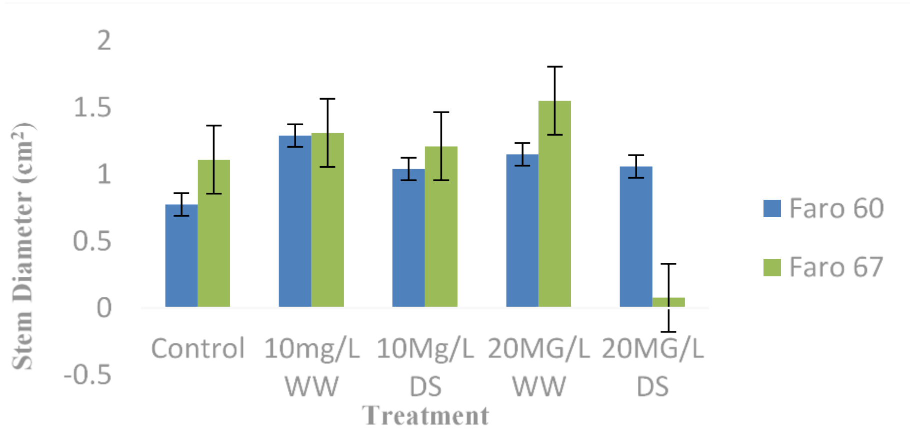

Figure 12. Effect of the induced drought stress on stem diameter of varieties among treatments.

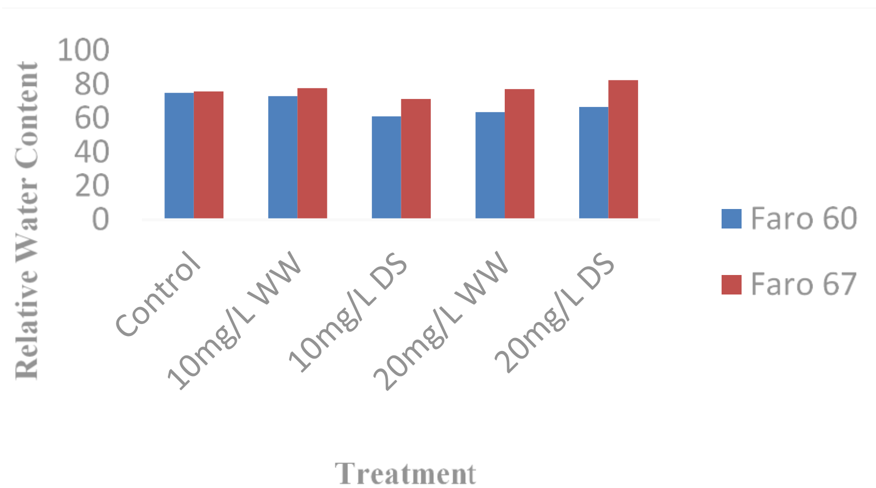

Figure 13. Relative water content of leaves of the different rice varieties

(2010) earlier that wheat plants under drought stress have decreased water content due to a decrease in the water potential of the plant. The application of 20 mg/L AgNPs resulted in the highest RWC (82.35%) by the Faro 67 rice plants exposed to drought stress and the lowest RWC (61.11 %) was seen in Faro 60 treated with 10 mg/LDS compared to the control plant (Figure 13). The drought stress affects the photosynthetic apparatus, which in turn alters the physiological parameters of plants (Salama, 2012). The determination of the chlorophyll content plays a significant role in determining the efficiency of plants to produce carbohydrate metabolites.

The result for chlorophyll content in Figure 14 shows the highest chlorophyll content (1.08 µg-g) in Faro 67 treated with 10 mg/L AgNPs and the lowest chlorophyll content (0.77 µg-g) in Faro 60 with 20 mg/L AgNPs in well-watered plants. In stress plants, the highest chlorophyll content (0.81 µg-g) was observed in Faro 67 treated with 10 mg/L AgNPs and the lowest chlorophyll content (0.54 µg-g) was achieved with Faro 60 treated with 20 mg/L AgNPs in contrast with control.

The synthesis of chlorophyll a and b decreases under stress conditions due to the injuries to the thylakoid, which results in reducing the exposure area of the leaf (Mostajeran and Rahimi-Eichi, 2008). For this study, there is an increase in chl a (0.65) and chl b (0.43) in Faro 67 (WW) with 10 mg/L AgNPs, chlorophyll stability index (93 %) with Faro 60 treated by 10 mg/L drought-stressed and rate of transpiration (1.2 ml) in Faro 67 treated with 20 mg/L AgNPs under non-stressed condition.

The colour separation and result in Figures 15 and 16 respectively indicated the highest CSI (91%) recorded in Faro 67 treated 20 mg/L AgNPs and the lowest CSI (30 %) was observed in Faro 60 treated 20 mg/L AgNPs.

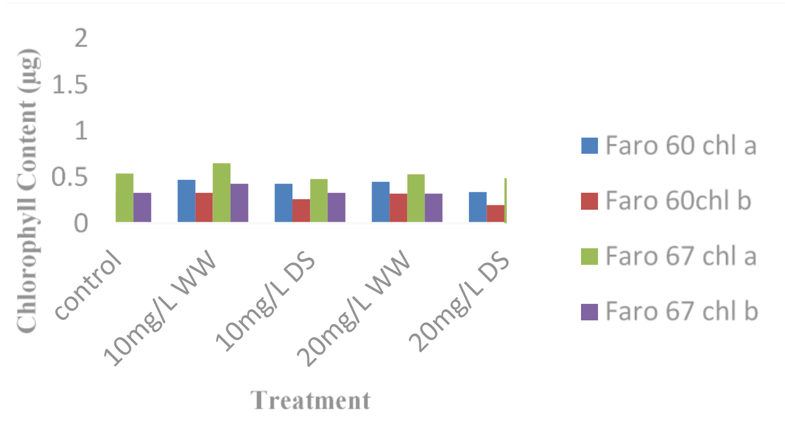

Figure 14. Effect of the induced drought stress on chlorophyll content in the treatment.

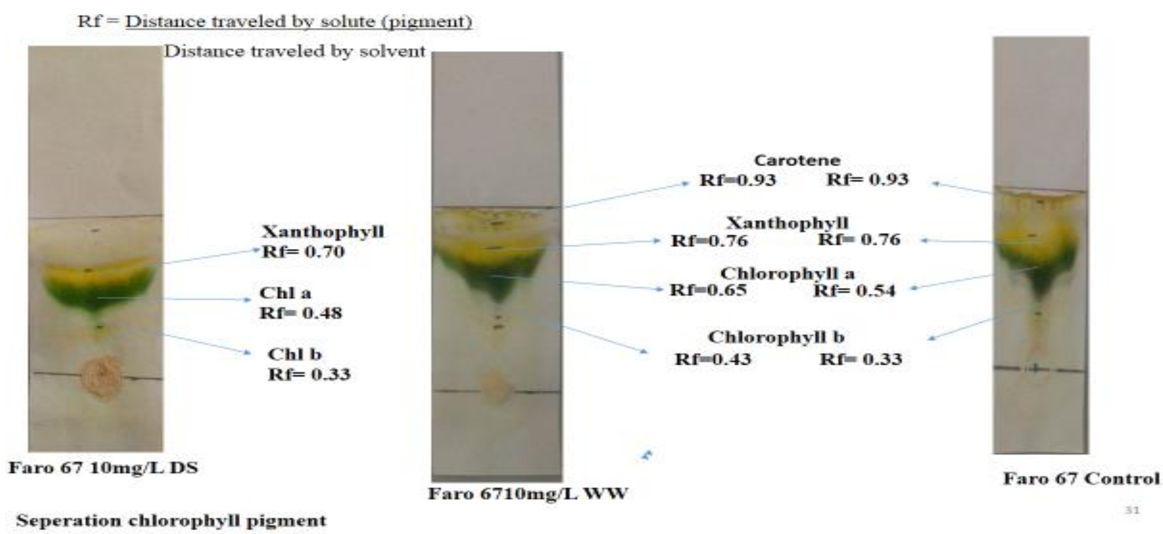

Figure 15. Chlorophyll content separation of the pigments of the leaf samples of the rice varieties.

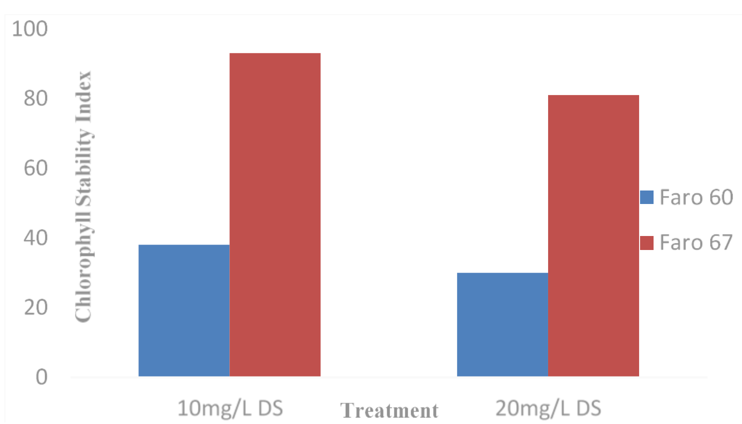

Figure 16. Effect of the induced drought stress on chlorophyll Stability Index of the varieties.

Table 2. Effect of the induced drought treatments on yield parameter observations among the varieties.

Varieties	Treatment	Spike length	Spikelet per spike	No. of seeds per spike	1000 Seed weight
	Control	20.5±2.08	9.50±1.00	92.8±21.6	25.0±5.7
	10mg/L WW	16.3±4.62	8.00±1.00	85.7±13.6	30.0±0.00
Faro 60	10mg/L DS	21.3±1.53	10.3±1.53	101±3.00	23.3±5.77
	20mg/L WW	20.7±1.15	11.3±0.58	92.7±32.1	30.0±0.00
	20mg/L DS	20.3±1.15	9.33±0.58	97.0±1.00	20.0±0.00
	Control	22.8±3.0	10.3±1.00	10.4±29.6	27.5±5.8
	10mg/L WW	23.7±4.62	11.3±1.00	108±13.6	30.0±0.00
Faro 67	10mg/L DS	19.7±1.53	8.7±1.53	75.7±3.00	20.0±5.8
	20mg/L WW	25.0±3.61	10.0±0.00	112±13.3	30.0±10.0
	20mg/L DS	19.7±4.2	10.0±1.00	93.0±20.2	30.0±0.00
	Varieties	0.031	0.385	0.625	0.263
P-value	Treatment	0.356	0.409	0.918	0.018
	Varieties*Treatment	0.068	0.006	0.5	0.150

Morphological/ yield parameters: Spike Length (cm)

Drought stress has led to an excessive buildup of intermediate chemicals, such as reactive oxygen species (Ashraf and Harris, 2013), which led to oxidative damage to DNA, lipids and proteins and as a result, there is reduced plant development and production. The results for yield metrics and total dry biomass showed the impact of applying various concentrations of AgNPs to *Oryza glaberrima* L. plants, particularly 20 mg/L, which displays a considerable increase in yield parameters compared to their controls.

The outcome is comparable to that of Iqbal *et al.* (2019) application of CuNP and AgNPs, which had a significant impact on the morphological characteristics of wheat plants. Additionally, this outcome is consistent with certain earlier scientific studies that have been published (Sallam et al., 2019; Ahmed *et al.,* 2011; Cumplido-Nájera *et al*., 2019; Malea *et al*., 2019) where it was observed that the effect of AgNPs at 25 and 50 ppm on the number of grains/spike, 100-grain weight and grain production per pot in wheat was quite beneficial.

The result revealed the highest spike length (25.0 cm) with 20 mg/L AgNPs in Faro 67 under a non-stressed condition and the lowest spike length (16.3 cm) was achieved with 10 mg/L AgNPs in Faro 60 under the non-stressed condition in comparison with control (Table 2).

The highest number of spikelets per spike (11.3) was observed with 20 mg/L AgNPs in Faro 60 and 10 mg/L AgNPs in Faro 67 under non-stressed conditions while the lowest number of spikelets per spike (8.0) was observed with 10 mg/L AgNPs in Faro 60 under well-watered condition compared with control (Figure 17). The highest number of seeds per spike (112) was observed with 20 mg/L AgNPs in Faro 67 under non-stressed conditions while the lowest number of seeds per spike (75.7) was observed with 10 mg/L AgNPs in Faro 67 under stressed conditions compared to their control (Figure 18).

A 1000 seed weight of 30.0 was achieved with all AgNPs treatments in both varieties under non-stressed conditions as the mean for the highest seed weight while the lowest seed weight was observed with Faro 60 20 mg/L AgNPs and Faro 67 10 mg/L AgNPs compared to their controls (Figure 19). The highest total dry root and shoot biomass mean (38.46 g bush- 1) was achieved with the application of 10 mg/L AgNPs in Faro 67 under non-stress. And the lowest biomass (7.55 g bush-1), was observed in Faro 60 with application of 10 mg/L AgNPs under drought stress compared to the control (Figure 20).

Based on the reviewed literature, we can forecast that, at ecologically acceptable concentrations, AgNPs will not significantly harm crop plants and that there is little chance that soil Ag will reach the edible parts. However, it must be noted that to draw a reliable conclusion about the effects of AgNPs on the environment and human health, more information is required regarding the phytotoxicity and bioaccumulation of AgNPs in crop plants, such as rice, wheat, maize, and soybean, grown in actual soil and cultivated throughout their entire life cycle (Chen *et al*., 2023). Furthermore, it has been discovered that AgNPs even positively stimulate plant development, which may have uses in agriculture. To improve agricultural uses of AgNPs, more research is needed to identify the process of stress formation, the real exposed form, and the threshold of the stress that could stimulate or inhibit plant development.

Microscopy

Fully developed aerenchyma was observed in well-watered roots. The results indicate that rice roots under

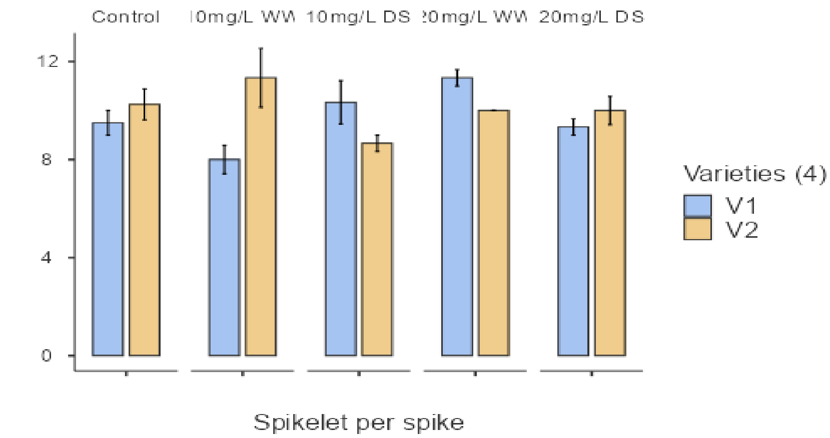

Figure 17. Effect of the induced drought stress on spikelets per spike among varieties.

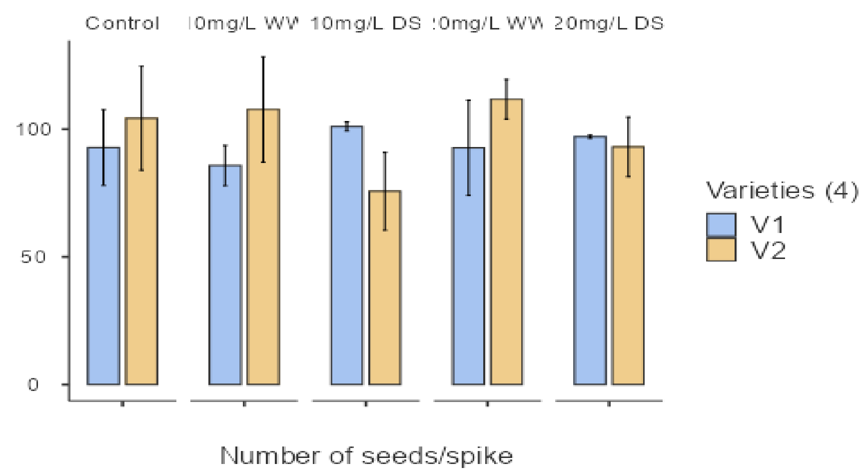

Figure 18. Number of seeds per spike of the varieties.

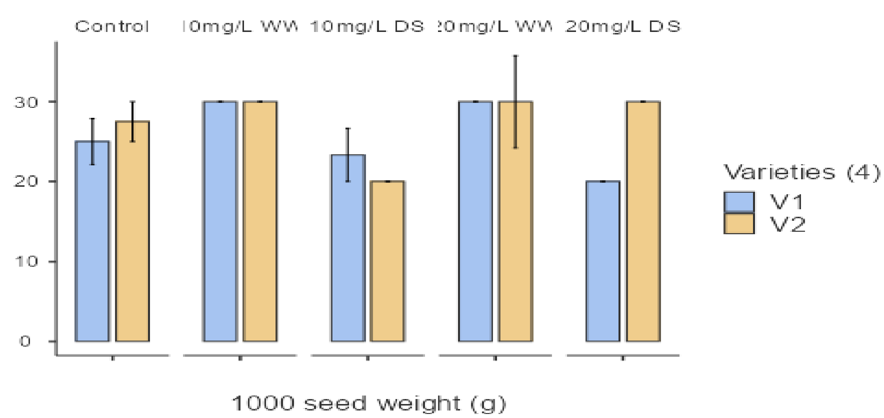

Figure 19. Effect of the induced drought stress on 1000 seed weight of the varieties.

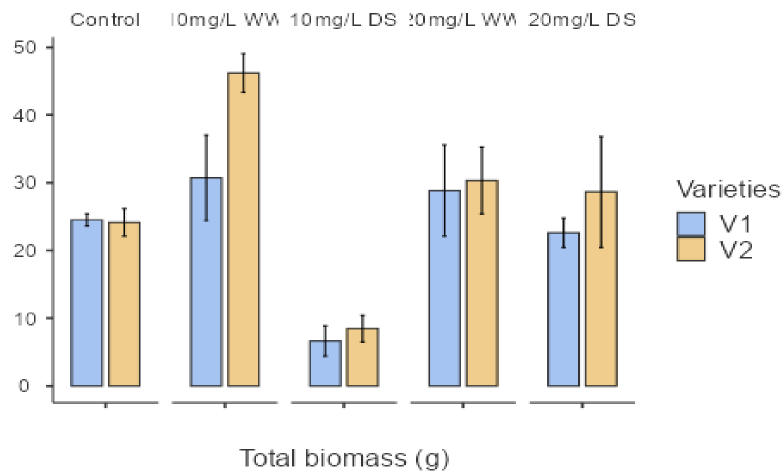

Figure 20. Effect of the induced drought stress on total dry root and shoot biomass among varieties.

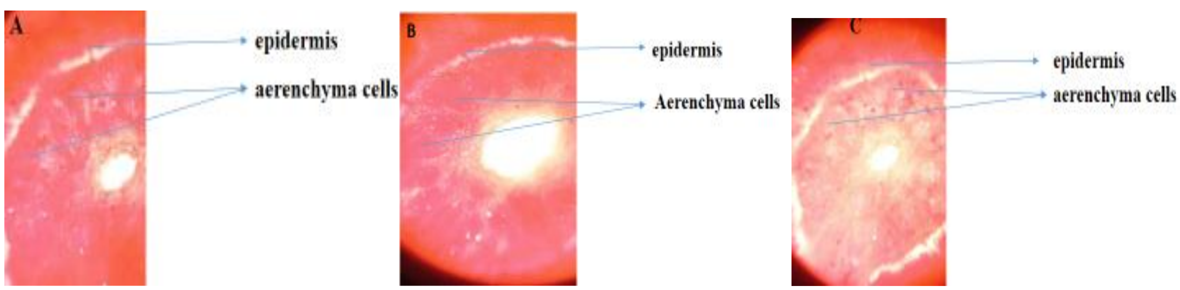

Plate 1. Aerenchyma formation showing numerous aerenchyma cells in (A) control, and (B) AgNPs treated well watered and less aerenchymatous cell in (C) drought stressed varieties treated with the AgNPs.

well-watered conditions consist of more aerenchyma and air spaces than plants under drought-stress conditions (Plate 1). Although the development of aerenchyma is seen as a desirable trait in well-watered conditions, it might appear as a substantial weakening component in drought-stress conditions (Mostajeran and Rahimi-Eichi, 2008).

The main cause of mechanical stress is the continual wetting and drying cycles of the soil, which causes swellings and shrinkages. AgNPs treated roots under drought stress in the current study had less root aerenchyma than control and AgNPs treated roots in well-watered conditions, which had more aerenchyma. This conclusion is comparable to that of Yazdanpanah *et al.* (2001).

Conclusion

The synthesis of silver nanoparticles (AgNPs) from Azadirachta indica leaf extract has been successfully demonstrated in this study, with various characterization techniques confirming their formation and properties. The application of AgNPs, particularly at a concentration of 20 mg/L, significantly enhanced the growth and physiological parameters of *Oryza glaberrima* L. under drought stress. The findings suggest that AgNPs not only contribute to improved plant resilience against abiotic stress but may also play a role in promoting key metabolites associated with drought tolerance.

Recommendations

1. Field trials: Conduct field experiments to evaluate the long-term effects of AgNPs on crop yield and quality under varying environmental conditions.
2. Optimal concentration studies: Investigate a broader range of AgNP concentrations to determine optimal dosages for different crop varieties and stress conditions.

3. Mechanistic studies: Further research should focus on understanding the underlying mechanisms by which AgNPs enhance drought tolerance, including their interaction with plant metabolic pathways.
4. Safety assessments: Assess the environmental impact and potential toxicity of AgNPs to ensure sustainable agricultural practices.
5. Integration with other technologies: Explore the combination of AgNP application with other agronomic practices, such as irrigation management and soil amendments, to maximize crop resilience and productivity.

By addressing these recommendations, future research can further enhance the applicability of AgNPs in sustainable agriculture, especially in regions vulnerable to drought conditions.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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REFERENCES

Ahmed, M., Hassen, F. U., Qadeer, U., & Aslam, M. A. (2011). Silicon application and drought tolerance mechanism of sorghum. *African Journal of Agricultural Research*, *6*(3), 594-607.

Ashraf, M., & Harris, P. J. (2013). Photosynthesis under stressful environments: An overview. *Photosynthetica*, *51*(2), 163-190.

Burlando, B., & Cornara, L. (2014). Therapeutic properties of rice constituents and derivatives (Oryza sativa L.): A review update. *Trends in food science & technology*, *40*(1), 82-98.

Chen, S., Yan, X., Peralta-Videa, J. R., Su, Z., Hong, J., & Zhao, L. (2023). Biological effects of AgNPs on crop plants: environmental implications and agricultural applications. *Environmental Science: Nano*, *10*(1), 62-71.

Cumplido-Nájera, C. F., González-Morales, S., Ortega-Ortíz, H., Cadenas-Pliego, G., Benavides-Mendoza, A., & Juárez-Maldonado, A. (2019). The application of copper nanoparticles and potassium silicate stimulate the tolerance to Clavibacter michiganensis in tomato plants. *Scientia Horticulturae*, *245*, 82-89.

Cutler, D. F. (1978). *Applied plant anatomy*. Longman, London, 103p.

Davies, J. C. (2009). Nanotechnology oversight: an agenda for the new administration. Project on emerging technologies. Woodrow Wilson International Center for Scholars, Washington, DC.

Dimetry, N. Z., & Hussein, H. M. (2016). Role of nanotechnology in agriculture with special reference to pest control. *International Journal of PharmTech Research.*, *9*(10), 121-144.

García-Mata, C., & Lamattina, L. (2001). Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiology*, *126*(3), 1196-1204.

Gole, A., Dash, C., Ramakrishnan, V., Sainkar, S. R., Mandale, A. B., Rao, M., & Sastry, M. (2001). Pepsin− gold colloid conjugates: preparation, characterization, and enzymatic activity. *Langmuir*, *17*(5), 1674-1679.

Igwe, O. U., & Ekebo, E. S. (2018). Biofabrication of cobalt Nanoparticle odorata and their potential. *Research Journal of Chemical Science*, *8*(1), 11-17.

Iqbal, M., Raja, N. I., Mashwani, Z. U. R., Wattoo, F. H., Hussain, M., Ejaz, M., & Saira, H. (2019). Assessment of AgNPs exposure on physiological and biochemical changes and antioxidative defence system in wheat (Triticum aestivum L) under heat stress. *IET Nanobiotechnology*, *13*(2), 230-236.

Islam, M. Z., Khalequzzaman, M., Bashar, M. K., Ivy, N. A., Haque, M. M., & Mian, M. A. K. (2016). Variability assessment of aromatic and fine rice germplasm in Bangladesh based on quantitative traits. *The Scientific World Journal*, *2016*(1), 2796720.

Javed, B., & Nadhman, A. (2020). Optimization, characterization and antimicrobial activity of silver nanoparticles against plant bacterial pathogens phyto-synthesized by Mentha longifolia. *Materials Research Express*, *7*(8), 085406.

Ji, K., Wang, Y., Sun, W., Lou, Q., Mei, H., Shen, S., & Chen, H. (2012). Drought-responsive mechanisms in rice genotypes with contrasting drought tolerance during reproductive stage. *Journal of Plant Physiology*, *169*(4), 336-344.

Kaveh, R., Li, Y. S., Ranjbar, S., Tehrani, R., Brueck, C. L., & Van Aken, B. (2013). Changes in Arabidopsis thaliana gene expression in response to silver nanoparticles and silver ions. *Environmental Science and Technology*, *47*(18), 10637-10644.

Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Molecular Biology*, *59*(1), 1-6.

Latif, H., Ghareib, M., & Tahon, M. (2017). Phytosynthesis of silver nanoparticles using leaf extracts from Ocimum basilicum and Mangifira indica and their effect on some biochemical attributes of Triticum aestivum. *Gesunde Pflanzen*, *69*(1), 39-46.

Lemire, J. A., Harrison, J. J., & Turner, R. J. (2013). Antimicrobial activity of metals: mechanisms, molecular targets and applications. *Nature Reviews Microbiology*, *11*(6), 371-384.

Malea, P., Charitonidou, K., Sperdouli, I., Mylona, Z., & Moustakas, M. (2019). Zinc uptake, photosynthetic efficiency and oxidative stress in the seagrass Cymodocea nodosa exposed to ZnO nanoparticles. *Materials*, *12*(13), 2101.

Manna, I., & Bandyopadhyay, M. (2019). A review on the biotechnological aspects of utilizing engineered nanoparticles as delivery systems in plants. *Plant Gene*, *17*, 100167.

Mishra, A. K., & Singh, V. P. (2010). A review of drought concepts. *Journal of Hydrology*, *391*(1-2), 202-216.

Mostajeran, A., & Rahimi-Eichi, V. (2008). Drought stress effects on root anatomical characteristics of rice cultivars (*Oryza sativa* L.). *Pakistan Journal of Biological Sciences*, *11*(18), 2173-2183.

Prasad, P. V. V., Pisipati, S. R., Momčilović, I., & Ristic, Z. (2011). Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. *Journal of Agronomy and Crop Science*, *197*(6), 430-441.

Preeti, R. (2017) Green-synthesis of silver nanoporticles, their characterization and mukicrobial potential. Phd. Thesis (published) submitted to the department of molecular and cellular engineering. Jacob institute of biotechnology and bioengineering samhigginbottom university of agriculture, technology and science, Allahabad-211007, U.P., India.

Rai, R. V., Parthiban, K. T., & Kumaravelu, G. (1995). Studies on the drought tolerance of Eucalyptus at seedling stage. *Journal of Tropical Forest Science*, *8*(2), 155-160.

Rahman, M. S., Chakraborty, A., Kibria, A., & Hossain, M. J. (2023). Effects of silver nanoparticles on seed germination and growth performance of pea (Pisum sativum). *Plant Nano Biology*, *5*, 100042.

Raut, R. W., Lakkakula, J. R., Kolekar, N. S., Mendhulkar, V. D., Kashid, S. B. (2009). Photosynthesis silver nanoparticle using *Gliricidia sepium* (Jacq.). *Current Nanoscience*, *5*, 117-122.

Reiss, C. (1994). *Experiment in plant physiology*. Englewood Cliffs, NJ, USA; Prentice Hall.

Rezvani, N., Sorooshzadeh, A., & Farhadi, N. (2012). Effect of nano-silver on growth of saffron in flooding stress. *International Journal of Agricultural and Biosystems Engineering*, *6*(1), 11-16.

Salama, H. M. (2012). Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (Zea mays L.). *International Research Journal of Biotechnology*, *3*(10), 190-197.

Sallam, A., Alqudah, A. M., Dawood, M. F., Baenziger, P. S., & Börner, A. (2019). Drought stress tolerance in wheat and barley: advances in physiology, breeding and genetics research. *International Journal of Molecular Sciences*, *20*(13), 3137.

Savicka, M., & Škute, N. (2010). Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). *Ekologija*, *56*(1), 26-33.

Savithramma, N., Rao, M. L., & Devi, P. S. (2011). Evaluation of antibacterial efficacy of biologically synthesized silver nanoparticles using stem barks of Boswellia ovalifoliolata Bal. and Henry and Shorea tumbuggaia Roxb. Journal of Biological Sciences, 11(1), 39-45.

Serraj, R., McNally, K. L., Slamet-Loedin, I., Kohli, A., Haefele, S. M., Atlin, G., & Kumar, A. (2011). Drought resistance improvement in rice: an integrated genetic and resource management strategy. *Plant Production Science*, *14*(1), 1-14.

Shankar, S. S., Ahmad, A., Pasricha, R., & Sastry, M. (2003). Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. *Journal of Materials Chemistry*, *13*(7), 1822-1826.

Shelar, G. B., & Chavan, A. M. (2015). Myco-synthesis of silver nanoparticles from Trichoderma harzianum and its impact on germination status of oil seed. *Biolife*, *3*(1), 109-113.

Suriyathana, M., Janeesha, K. J., Mani Kandan, M., Sandhya, M., & Ashok ram, K. (2018). Synthesi of iron nanoparticles and characterization of macroty loma uniflorum seed extract. *International Journal of Science and Engineering Research*, *7*(3), 513-519.

Swamy, B. M., & Kumar, A. (2013). Genomics-based precision breeding approaches to improve drought tolerance in rice. *Biotechnology advances*, *31*(8), 1308-1318.

Tamilselvan, A., Srividhya, P., Karuthapandian, S., & Mehalingam, P. (2016). Plant-Driven to Synthesis of Silver Nanoparticles using Crataeva religiosa Hook & Frost and its Utility in Detecting Antimicrobial and Antioxidant Assay. *International Journal of Green and Herbal Chemistry*, *5*(4), 390-402.

Ullah, N., Yüce, M., Neslihan Öztürk Gökçe, Z., & Budak, H. (2017). Comparative metabolite profiling of drought stress in roots and leaves of seven Triticeae species. *BMC Genomics*, *18*, 1-12.

Veerasamy, R., Xin, T. Z., Gunasagaran, S., Xiang, T. F. W., Yang, E. F. C., Jeyakumar, N., & Dhanaraj, S. A. (2011). Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *Journal of saudi chemical society*, *15*(2), 113-120.

Vivek, M. Kumar, P. S., Steffi, S., & Sudha, S (2011). Biogenic silver nanoparticles by Gelidiella acerosa extract and their antifungal effects," *Avicenna Journal of Medical Biotechnology*, *3*(3), 143-148.

Wang, G. P., Hui, Z., Li, F., Zhao, M. R., Zhang, J., & Wang, W. (2010). Improvement of heat and drought photosynthetic tolerance in wheat by overaccumulation of glycinebetaine. *Plant Biotechnology Reports*, *4*, 213-222.

Yazdanpanah, M. J., Banihashemi, M., Pezeshkpoor, F., Khajedaluee, M., Famili, S., Tavakoli Rodi, I., & Yousefzadeh, H. (2011). Comparison of oral zinc sulfate with systemic meglumine antimoniate in the treatment of cutaneous leishmaniasis. *Dermatology Research and Practice*, *2011*(1), 269515.

Zahratul, M., Muhamad, S., & Wahyuning, A. S. (2021). Metabolomic analyses to evaluate the effect of drought stress on chili pepper (*Capsicum annuum* L.) at vegetative stage. *Russian Journal of Agricultural and Socio-Economic Sciences*, *111*(3), 48-60.