FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 4 No. 1, March, 2020, pp 120 - 132

APPLICATION OF BIOSYNTHESIZED NANOPARTICLES IN THE ENHANCEMENT OF GROWTH AND YIELD PERFORMANCES OF RICE (*ORYZA SATIVA* VAR. NERICA) UNDER SALINITY CONDITIONS IN A FERRUGINOUS ULTISOL

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ABSTRACT
The study was conducted to investigate the effects of application of biosynthesized silver nanoparticles (AgNPs) on the growth and yield performances of rice (*Oryza sativa* var. Nerica) under salinity conditions in a ferruginous ultisol. AgNPs were biosynthesized following standard procedure using leaves of *Hibiscus sabderiffa*. Viable rice seeds were sown in soils that were previously moistened with salt solution in 3 concentrations (100, 250 and 400mM). The AgNPs (200ml) was sprayed in five concentrations (5, 10, 15, 20 and 25% v/v) on the test plant for seven weeks. Morphometric parameters such as plant height, root number, leaf length and rice yield parameters were studied. Results showed that salinity significantly ($p<0.05$) impaired growth and yield parameters of rice at increasing salinity levels, leading to death rice plants exposed to 400mM of salt solution. With the application of AgNPs, significant improvements in growth responses of the plants exposed to salinity; especially at low salt stress and low AgNPs concentration. Plants in ferruginous soils showed minimal increases in measured growth parameters (plant height, root number, leaf length and rice yield) compared to salt stressed plants. Number of roots was observed to be highly significant with the application of AgNPs; however, shelling percentage showed lowest response with the application of AgNPs. There was no significant difference in modal periods taken for complete foliar necrosis as well as complete foliar chlorosis. This paper suggests therefore that minimal application of AgNPs improved growth and yield parameters of rice in minimal saline condition as well as under ferruginousity.

Keywords: Silver nanoparticles, Enhancement, Salinity conditions, Ferruginous soil, rice[H1]

INTRODUCTION

Nigeria, one of the most populated country in Africa and the highest producer of rice in Africa, is seriously affected by high level of food insecurity (Obayelu, 2015). Security of economic crops such as rice is very significant in maintaining national food security. Rice (*Oryza sative* L.) is an edible starchy cereal grain that is susceptible to salinity and iron stress (Munns, 2005). It belongs to the family *Poaceae* and consumed by more than 50% of the world population (Maclean *et al*., 2002). It is an annual free tillering grass, which possess adventitious roots, created from the basal nodes with a shallow root system. The stem has solid nodes and hollow internodes and serves as the last part of the vegetative phase of its growth. According to Federal Ministry of Agriculture and Rural Development (FMARD, 2016), rice production in Nigeria hits 15 million tones but yet, not enough to serve the increasing population of Nigeria. In 2016, national rice demand was estimated at 6.3 million metric tons, while domestic supply was put at 2.3 million metric tons (FMARD, 2016). The deficit of 4 million metric tons was expected to be filled by import, showing inability of the nation to produce its total rice demand (Udemezue, 2018).

Factors influencing rice cultivation in Nigeria are multi-dimensional; with the most significant, being the soil fertility challenges (Sanusi, 2014). Soil ferruginousity and salinity has led to reduction in soil fertility, which affect cultivation of economic crops in most part of Nigeria, causing Na, Fe and Cl-toxicity, imbalance in the uptake of ions, deficiency in N,P,K+ and microbial aggregations due to ion uptake imbalance (Pitman and Lauchli, 2002; Munns, 2005; Omoregie *et al*., 2014). These negative soil conditions may be either because of the dry condition of the north that pre-dispose the soil to high saline condition, or perhaps down in the south where is closer to the ocean. So, there's possibility of interferance with brackish water. Salinity stress has been documented to negatively affect chlorophyll index of some economic crops such as rice (Faustino *et al*., 2010). However, at favorable environmental conditions, rice may evolve various mechanisms such as reactive oxygen detoxification mechanism that would help the plant tolerate salt stress to certain level (Artiola and Crimmins, 2019). This is not always constant at every time.

Furthermore, about 58% of agricultural soils in the southern region is ferruginous. Ferruginous soils are acidic soil with high iron and aluminum content, characterized by red coloration (Adnan *et al*., 2018). Literatures has established ferruginousity and salinity as poor growing conditions, affecting cultivation of economic crops and food security in Nigeria (Anumalla *et al*., 2019). With the current closure of Nigerian borders by the government in order to improve local rice production, boost national income and address the increasing challenges of sustainable production and food security, there is need to employ innovative and sustainable agricultural approaches (Dwivedi *et al*., 2016; Kou *et al*., 2018).

Nanotechnology is an innovative approach that deals with the understanding and control of matter at nanoscale (1 - 100 nm) dimensions (Surajyoti *et al*., 2017). It involves the use of nanoparticles, which are organic, inorganic or hybrid materials at nanoscales, having unique physical properties, therefore make novel applications possible (Love *et al*., 2005). At the nanoscale, matter shows extraordinary properties that are not shown by bulk materials (Gogos *et al*., 2012). Nanotechnology has long been introduced in multiple disciplines such as health and electronics. The idea that nanoparticles (NPs) could be of interest in agricultural development is a recent technological innovation, and it is still under progressive development (Gogos *et al*., 2012). Recent advancements in the fabrication of nanomaterials of deferent sizes and shapes have yielded their wide array of applications in medicine, environmental science, agriculture and food processing. Throughout history, agriculture has always benefited from these innovations (Chen *et al*., 2016). In continuation, as agriculture faces numerous and unprecedented challenges, such as reduced crop yield due to biotic and abiotic stresses, including nutrient deficiency and environmental pollution, several nanoparticles such as titanium dioxide (TiO2), zinc oxide (ZnO) and Silver nanoparticles (Ag NP) with the later offering a more promising response for precision agriculture (Sanchez *et al*., 2015). There are several reports indicating that appropriate concentrations of AgNPs play an important role in enhancing seed germination (Barrena *et al*. 2009; Shelar and Chavan 2015) and plant growth (Sharma *et al*. 2012; Kaveh *et al*. 2013; Vannini *et al*. 2013), improving photosynthetic quantum efficiency and chlorophyll content (Sharma *et al*. 2012; Hatami and Ghorbanpour 2013), and increasing water and fertilizer use efficiency (Lu *et al*. 2002).

Over the last two decades, a significant amount of research has been carried out on nanotechnology, emphasizing its numerous applications in agriculture sectors (Lv *et al*., 2018; Chen *et al*., 2012; Prasad *et al*., 2017). Soil properties plays a pivotal role in plant growth and improved agricultural production of crops; however, even though, the world is facing a serious case of environmental pollution, researchers are more concerned with crop improvement at the expense of soil improvement (Prased et a., 2017). Notably, nanomaterials enhance the productivity of crops by increasing the efficiency of agricultural inputs to facilitate site-targeted controlled delivery of nutrients, thereby ensuring the minimal use of agri-inputs (Dwivedi *et al*., 2016; Chen *et al*., 2016). Considering the ferruginousity and salinity profile of most soils in Nigeria and the need to improve national rice productivity through sustainable strategies, this research aims at investigating the influence of silver nanoparticles on salt stressed rice, in a ferruginous soil with respect to morphological parameters.

MATERIALS AND METHODS

The experiment was conducted at the mini botanical garden of the department of plant biology and biotechnology, University of Benin, Benin City. Ferruginous soil was collected from reddish soil portion at the Botanic Garden, Department of Plant Biology and Biotechnology; whereas non-ferruginous soil was collected from mulched garden soil at Faculty of Agriculture, University of Benin. Soils were sun-dried to constant weight, and thereafter 20kg of the soil was placed on different experimental bowls (diameter 32 cm, height 23 cm) amounting to 48 bowls. Care was taken to ensure that each experimental bowl was rid of weed and insects on a constant basis, up till the end of the experiment.

Preparation of saline environment

Three salinity concentrations were adopted for this study - 100mM, 250mM, 400mM salt solution using Laboratory-graded NaCl and prepared following the methods of Tongwei and Shijun, (2019). These salt levels were used to subject the soil to saline condition for 12 weeks. The experimental bowls were divided into 4 groups and moistened with the three salinity concentrations two times a day for seven days. Thereafter, seeds were sown in the respective experimental bowls. The fourth experimental bowl was used as the control.

Sowing of test plant

Rice seeds (*Oryza sativa* var. Nerica) were sowed at the rate of 20 seeds per bowl and were later tilled down to 5 rice plant stand per bowl after 2 weeks.

Biosynthesis and application of silver nanoparticle

Silver nanoparticle (AgNPs) was synthesized following the methods of Anandalakshi and Venugobal (2017), using 10 g of fresh leaves of *Hibiscus sabderiffa*. The leaf samples were washed with de-ionized water and then subjected to crushing in de-ionized water, boiling, filtration through ethanol and centrifugation following the methods of Anandalakshi and Venugobal (2017). 10 mL of 1 mM aqueous solution of silver nitrate (AgNO3) was used in the synthesis of NP using the prepared leaf extract solution and strictly following methods of Anandalakshi and Venugobal (2017). A colour change from light yellow to dark brown colour indicated the formation of Ag-NPs. Thereafter, absorbance spectra of colloidal samples was taken in the range of 200 -800 nm using a UV-VIS Spec at

800nm with distilled water as reference. The absorbance spectra was compared with literature to confirm formation of NP. A measured 200ml of the synthesized AgNPs was made into five (v/v) concentrations (5, 10, 15, 20 and 25%) by diluting with distilled water. This was immediately sprayed on each of the five weeks rice stand from top to bottom using a foliar spray for seven weeks (when the rice plant has achieved its maximum growth). The experiment was allowed in the field under the prevailing environmental conditions possible for the month of May 2018

Maintaining soil moisture

The set up was in the open and as such relied basically on rainfall. However, the soil moisture was always maintained following the methods of USDA (2000).

Morphometric parameters

Plant height was determined periodically on weekly basis. It was taken as the length of plant from the soil level to the meristematic tip using a measuring tape. Number of leaves per plant was physically counted once every week. Leaf length was measured using a measuring tape from where the petiole attaches to the leaf blade up till the leaf tip. Number of primary root branches was the total number of root branches attached directly to the main root at harvest time. Stem girth was done using a venial caliper. Plant dry weight was obtained by carefully uprooting the plant, washing the root in distilled water to remove soil particles and then carefully drying the plant with its shoot and root intact in the oven at 130 ^{o}C for 24 hours following Weiwei *et al*. (2019). Number of tillers was determined by physically counting the tillers. Leaf color was determined weekly and color code charts were used. Charts were previously downloaded from google app store.

Soil physiochemical parameter

Soil pH was determined according tp Luo *et al*. (2013). Soil nitrogen and soil nitrate and exchangeable acidity and exchangeable base were carried out according the methods described by Miller and Kalra (1998); Yan and Schubert (2000) respectively. Soil iron extraction by hydrochloric acid methods was determined in the laboratory. All these were analyzed to obtain the baseline property of the experimental soil.

Statistical analysis

Data obtained from the analysis were subjected to statistical analysis under descriptive statistics, association and inferential statistics. Analysis of variance were conducted on a single factor bases since the soil used in the experiment was homogenized and homogeneity of the entire plot was also assumed. A significant value p= 0.05 was sufficient for the study.

RESULTS AND DISCUSSION

Baseline physicochemical study of the experimental soil

Physicochemical parameters of the experimental soil (Table 1) before exposing the soil to salinity condition implied a pH of 5.27, an electrical conductivity of 30.21 µs/cm, a total organic carbon of 0.49% and a high iron content of 1011.92mg/kg (10.1%). This shows a typically ferruginous property in accordance with (Dao *et al*., 2013; Yanling and Hailin, 2016).

Table 1: Physicochemical properties of soil before application of treatments

Parameters	Soil
pH	5.27
Electrical conductivity (µs/cm)	301.21
Total organic carbon (%)	0.49
Total Nitrogen (%)	0.18
Exchangeable acidity (meq/100 g soil)	0.22
Na (meq/100 g soil)	10.90
K (meq/100 g soil)	1.48
Ca (meq/100 g soil)	14.32
Mg (meq/100 g soil)	12.01
NO^{-}_{2} (mg/kg)	16.43
NO^{-}_{3} (mg/kg)	30.01
Clay (%)	5.13
Silt (%)	7.06
Sand (%)	87.81
Fe (mg/kg)	1011.92

Plant height

An assessment of plant height under salinity conditions and after exposure to concentrations of AgNPs was conducted. The results showed a significant (p= 0.04) reduction in plant height with increasing saline concentration compared to control. The plant from the soil with highest salinity condition (400Mm) had no growth (Fig 1). This shows that increase in salt stress affects plant growth. This observation agree with the report of Prachi *et*

al., (2017) and Achuo *et al*., (2006) who suggested that salt stress impaired plant growth. However when applied with concentrations of AgNPs, the N5(100) plant height was 12cm a week after AgNPs intervention, compare with 9.5cm in the N15(250). The general array showed that saline concentration affected the action of the nanoparticles. Increased nanoparticle concentrations did not affect the height of plants moistened with high concentration of salt compared to the effect the low concentration of nanoparticles had on the plants moistened with low concentration of salt (N5(100)-17, N25(250)-7.9, N25(400)-O after a week of AgNPs inoculation. This shows that the AgNPs enhanced the growth of the plant in little saline soil by activating a faster cell division and improved apical meristem activity at first week of application. This observation agrees with Mahajan *et al* (2011); Mihaela and Dorina (2007); Seif *et al* (2011); Salama (2012); Sharma *et al*. (2012) who observed that AgNPs increased plant growth attributes such as shoot length in *B. juncea*, *P. vulgaris* and *Z. mays*.

However, the result after 12 weeks showed the plant height was better in the plant with no AgNPs intervention (Table 2). Also, plant height in soils with AgNPs was better at the earlier weeks than at post-harvest. These maybe the effect of the ferruginous soil had lessen the activity of the nanoparticle at the 12th week. Lin and Xing. (2008) found that in the presence of ZNO nanoparticles rye grass biomass reduced significantly. Also, Yin *et al*. (2011) also reported that AgNPs reduced the growth of many species from the poaceae family. Gruyer *et al*. (2013) elaborated that the effect of AgNPs on plants can be positive or negative depending upon plant species and soil environmnet. It has been explained previously that AgNPs stimulate shoot growth by jamming ethylene signaling which is a shoot growth inhibitor (Rezvani *et al*., 2012; Syua *et al*., 2014). In this study, we have established that AgNPs improved height of rice in less saline environment at the early stage of sowing (1 to 5 weeks). However, slow or no growth was recorded from 6th week to 12th.

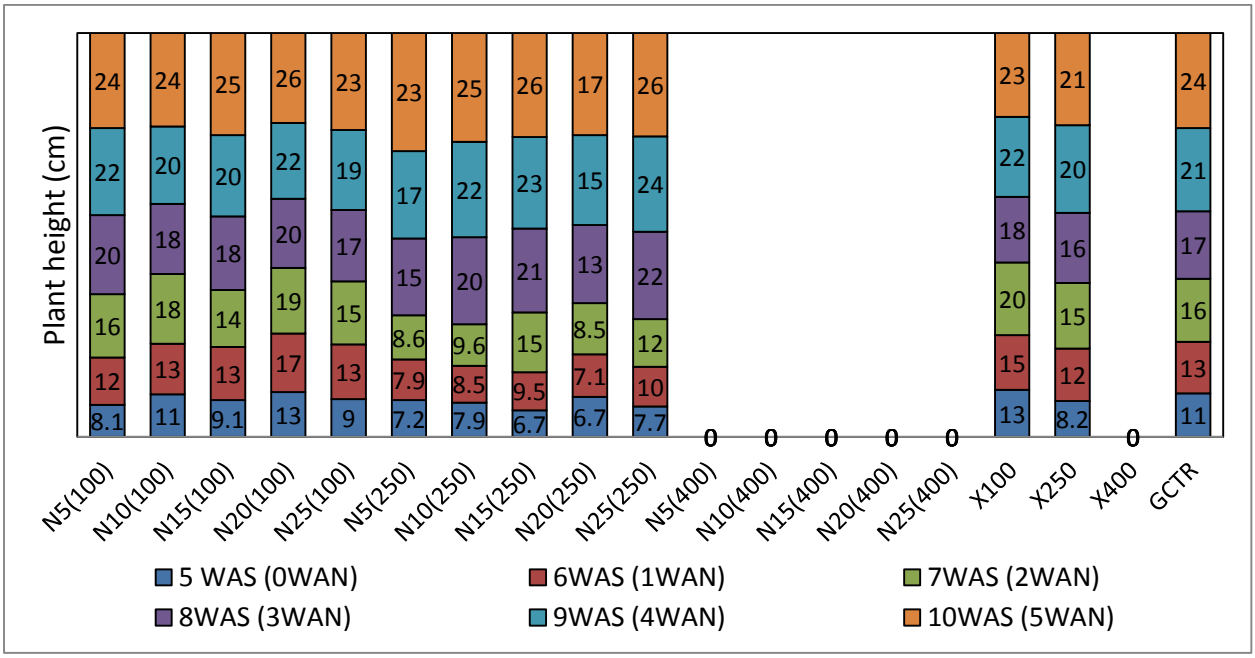

Fig. 1: Assessment of plant height of test plant under salinity condition and after exposure to silver nitrate nanoparticles. Key: WAS= weeks after sowing, X100; WAN= weeks after nano application.

Table 2: Plant growth characteristics of 12-week-old plants under salinity condition and after 7-week exposure to silver nitrate nanoparticles

Treatments	Plant height (cm)	*No. of tillers	*No. of leaf	Leaf length	Flag leaf length	Flag leaf width	Stem girth	*No. of root	*No ligule	Internode (cm)	Length of sheath	Length of ligule (cm)	Length of root (cm)
N5(100)	32.9	3	17	47.2	36.4	1.1	3.2	74	7	12.52	70.1	1.17	38.5
N10(100)	33.5	1	16	42.7	22.2	1.2	2.1	126	7	10.72	75.7	1.37	27.7
N15(100)	34.9	2	20	42.4	29.8	1.1	2.1	92	6	7.77	67.5	1.07	29.5
N20(100)	22.3	4	25	43.8	23.2	1.0	1.3	62	8	13.54	71.4	1.13	28.8
N25(100)	34.7	2	29	39.9	23.5	1.1	2.9	55	5	9.23	70.3	1.01	37.4
N5(250)	22.8	1	15	32.5	0	0	2.2	36	7	7.83	80.7	1.23	41.6
N10(250)	24.7	1	15	37.5	33.9	1.9	2.4	42	4	8.27	78.3	0.87	37.3
N15(250)	31.5	1	17	47.5	28.2	1.5	2.5	92	5	7.14	66.4	1.33	41.7
N20(250)	45.5	2	16	44.7	38.2	1.7	2.1	41	8	4.72	45.2	1.33	21.2
N25(250)	37.4	1	12	42.1	26.6	1.3	2.5	46	5	6.31	59.3	1.03	34.2
N5(400)	-	-	-	-	-	-	-	-	-	-	-	-	-
N10(400)	-	-	-	-	-	-	-	-	-	-	-	-	-
N15(400)	-	-	-	-	-	-	-	-	-	-	-	-	-
N20(400)	-	-	-	-	-	-	-	-	-	-	-	-	-
N25(400)	-	-	-	-	-	-	-	-	-	-	-	-	-
X100	44.5	3	9	53.2	21.4	1.2	4.3	100	5	1.67	72.4	1.03	43.2
X250	39.7	3	8	34.3	29.4	1.6	2.3	72	5	4.83	73.4	1.23	47.4
X400	-	-	-	-	-	-	-	-	-	-	-	-	-
GC	49.3	2	16	45.6	27.9	1.5	2.9	43	7	9.83	76.4	1.03	35.5
p-value	0.106	0.027	0.281	0.823	0.932	0.412	0.076	0.028	0.521	0.029	0.327	0.201	0.142
LSD(0.05)	11.4	1	7	12.8	11.6	1.3	1.9	26	4	3.13	28.4	0.87	18.4

*means have been presented to the nearest whole number. N5(400), N10(400), N15(400), N20(400), N25(400) were deliberately not included in the Table as the plants exposed to 400mM salinity levels (i.e. X400) all died prior to 5th week when NPs were applied to salt-exposed plants

Key: N5-100 Plant applied with 5% nanoparticle solution (NPS) exposed to 100mM salinity level; N10-100 Plant applied with 10% NPS and exposed to 100mM salinity level; N15-100 Plant applied with 15% NPS and exposed to 100mM salinity level; N20-250 Plant applied with 20% NPS and exposed to 250mM salinity level; N25-250 Plant applied with 25% NPS and exposed to 250mM salinity level; N5-400 Plant applied with 5% NPS and exposed to 400mM salinity level; X100, X250, and X400 are plants exposed to 100, 250, and 400 mM salinity levels but no NPS respectively

Number of leaves

The plant with the least salinity content (100mM) treated AgNPs had the highest number of leaves (17-29) compared to plants with highest (400Mm) salinity content. There was reduction in leaf number with increasing salinity content (Table 2). The number of leaves increased with increase in AgNPs concentrations. However, compared to the control (without AgNPs application), a significant reduction in leaf number was observed at the post-harvest stage. This depicts that AgNPs enhanced growth of leaves even though increasing salinity affects leaf number. Also, there was a significant increase in leaf number between salt stress plants treated with AgNPs and those not treated. This study disagrees with Mervat, (2019); Gopinath *et al*. (2014), who reported that AgNPs improved leaf number, in wheat plants under abiotic stress, but with little reduction with increasing salinity. Kumar *et al*. (2013) reported possible reason behind this improvement by explaining that AuNPs altered levels of microRNAs expression that regulates various morphological, physiological, and metabolic processes in plants.

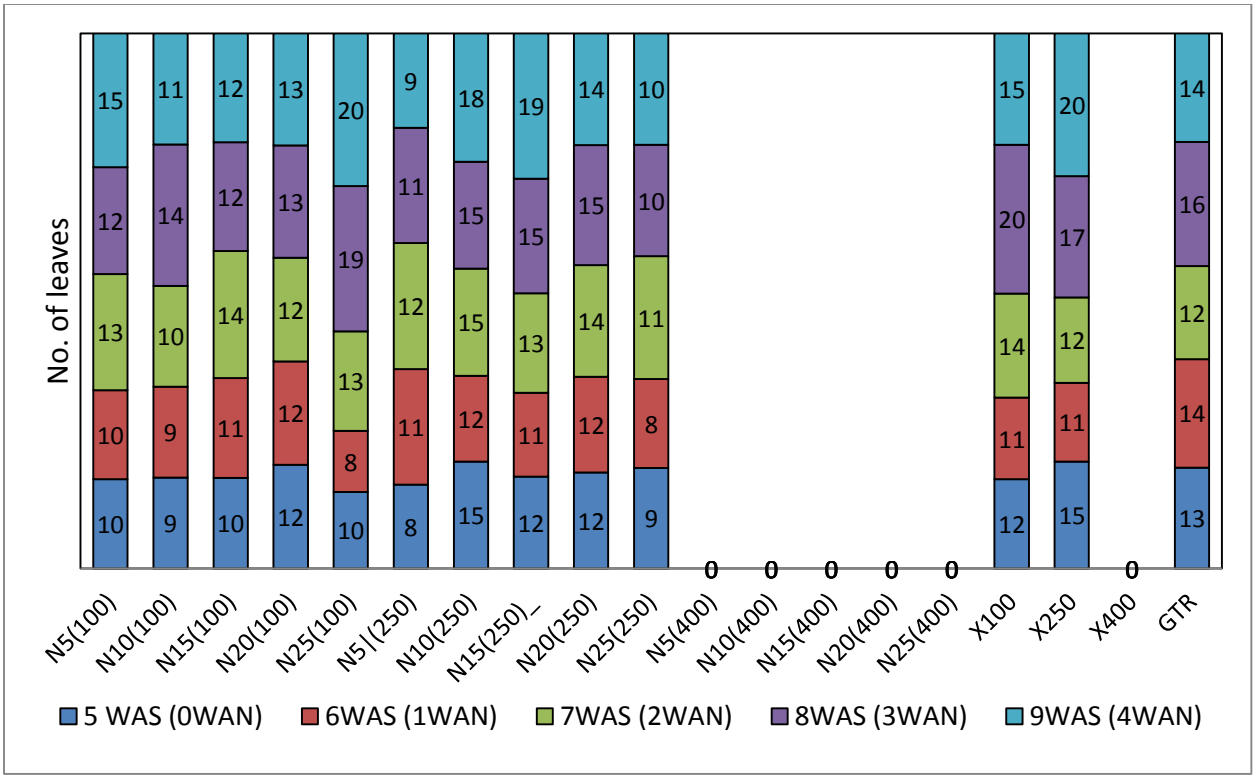

Fig. 2: Number of leaves of test plant under salinity condition and after exposure to silver nitrate nanoparticles.

Root number

Results generally showed that without AgNPs application, there was significant increase in the number of primary roots for salt stressed plant. In X100 for example there was 100 roots, 72 roots in X250 compared to 43 primary root in the control. However, application of nanoparticles to the salt stressed plant significantly enhance the root number in N10(100) [126roots], N15(100)[92 roots]. This result is not consistent with the work of El-temash and Joner (2012) who reported that AgNPs inhibited the seed germination and root number of ryegrass.

Rice yield parameters

Table 3 shows yield characteristics of test plant after salinity control and under 7 weeks exposure to AgNPs. Result showed that all plant in soil moistened with 400mM of salt solution died with or without application of AgNPs. A minimal increase in weight of panicle was observed in X250(1g) and X250(0.7g) compare to the control (0.68g). However, upon application of AgNPs, weight of panicle significantly increased in N15(250) to 2.1g and 1.22g in N5(100). AgNPs increased the yield parameters such as pod height in soyabean (Sheikhbaglon *et al*., 2010).

The weight of rice grain per panicle also showed significant increase upon application of AgNPs in all plants exposed to 100mM and 250mM salt solution except N5(250) when compared with the control. Shelling percent was 69% in the control as well as in rice plant exposed to 250mM of salt solution without AgNPs (X250). However, shelling percent increased to 79% in N15(250), 76 in N20(250) and 73% in both N15(100) and N25(100).

Table 3: Plant yield characteristics of 12-week-old plants under salinity condition and after 7-week exposure to silver nitrate nanoparticles

Treatments	Wt. of panicle (g)	Wt. of rice grain per panicle (g)	Shelling (%)	Tillering success (%)	No. of Seed	No. of spike in plant	Additional spike	No. of spikes	Length of spikelet (cm)	Diameter of spikelet (cm)
N5-100	1.22	0.67	55	100.0	102	3	2	6	1.1	0.5
N10-100	0.84	0.58	69	77.7	65	1	2	6	1.1	0.5
N15-100	0.92	0.67	73	77.7	81	2	2	7	1.5	0.5
N20-100	0.66	0.47	71	44.4	92	2	3	5	1.3	0.4
N25-100	0.6	0.44	73	61.0	73	1	1	7	1.2	0.5
N5-250	0	0	0	0	0	0	0	0	0	0.5
N10-250	1.12	0.7	0	100.0	174	1	3	10	1	0.5
N15-250	2.11	1.65	79	77.7	132	2	2	7	1.5	0.5
N20-250	0.63	0.48	76	100.0	65	3	2	9	1.5	0.5
N25-250	1.3	0.94	72	100.0	75	1	2	5	1.1	0.5
N5-400	-	-	-	-	-	-	-	-	-	-
N10-400	-	-	-	-	-	-	-	-	-	-
N15-400	-	-	-	-	-	-	-	-	-	-
N20-400	-	-	-	-	-	-	-	-	-	-
N25-400	-	-	-	-	-	-	-	-	-	-
X100	1.01	0.52	52	44.4	93	1	1	6	1.5	0.5
X250	0.77	0.53	69	33.3	92	1	2	5	1.4	0.4
X400	-	-	-	-	-	-	-	-	-	-
GC	0.68	0.47	69	100.0	112	2	1	5	1	0.5
p-values	0.746	0.534	0.328	0.634	0.042	0.724	0.519	0.738	0.524	0.351
LSD (0.05)	0.78	0.41	15	59.3	24	2	2	4	0.8	0.32

N5(400), N10(400), N15(400), N20(400), N25(400) were deliberately not included in the Table as the plants exposed to 400mM salinity levels (i.e. X400) all died prior to 5th week when NPs were applied to salt-exposed plants

Key: N5-100 Plant applied with 5% nanoparticle solution (NPS) exposed to 100mM salinity level; N10-100 Plant applied with 10% NPS and exposed to 100mM salinity level; N15-100 Plant applied with 15% NPS and exposed to 100mM salinity level; N20-250 Plant applied with 20% NPS and exposed to 250mM salinity level; N25-250 Plant applied with 25% NPS and exposed to 250mM salinity level; N5-400 Plant applied with 5% NPS and exposed to 400mM salinity level; X100, X250, and X400 are plants exposed to 100, 250, and 400 mM salinity levels but no NPS respectively

To simplify the research objective, Table 4 shows percentage effect in the various rice parameter upon application of AgNPs. The result showed a significant increase in percentage plant height, leaf length, leaf width, stem girth and leaf number with the application of AgNPs. More or less similar data were observed by Salama (2012), who noticed that low concentrations of AgNPs had a stimulating effect on the growth of the common bean and corn plants. Latif *et al*. (2017) showed that AgNPs foliar treatment with different concentrations increased growth parameters of the wheat plant. A minimal increase in these parameters was also observed in the plants grown in untreated soil (GC), compared to the salt stressed plants (X100 and X250). This is consistent with the work of Neelesh and Veena (2015) who suggested that increased salt concentration significantly decrease growth parameters of fenugreek variety RMt-1. This minimal increase between the GC and X100;X250 may be as a result of the poor growing condition of ferruginous soil which was the soil used in the current study. According to Anumalla *et al*., (2019), ferruginous soil is an acidic and poor growing soil that is rich in iron content. Furthermore, increase in the assayed parameters were also inversely proportional to increase in salt concentrations.

Table 4: Comparative view of the percentage parameter at different treatment levels and conditions.

Treatments	Gain in plant ht. (%)	Gain in leaf length (%)	Gain in leaf width (%)	Gain in stem girth (%)	Gain in no. of leaves (%)
N5-100	246.2	168.4	110	77.95	127.6
N10-100	144.6	78.11	76.19	43.81	121.3
N15-100	238.4	121.5	75.4	95.23	138.3
N20-100	130.9	53.49	137.1	72.22	131.3
N25-100	221.6	109.7	156.5	73.81	251
N5-250	408.6	108.2	103.8	89.81	38.87
N10-250	327.5	164.3	179.3	129.2	72.37
N15-250	453	204.6	391.7	93.94	79.93
N20-250	221.7	192.2	325	79.17	108.3
N25-250	390.5	234.5	333.3	109.3	31.27
N5-400	-	-	-	-	-
N10-400	-	-	-	-	-
N15-400	-	-	-	-	-
N20-400	-	-	-	-	-
N25-400	-	-	-	-	-
X100	214.1	130.1	69.8	113.1	143.2
X250	118.5	94.57	66.2	108.8	68.3
X400	-	-	-	-	-
GC (no nano-particles)	217.4	145.3	84.4	88.9	55.86
p-values	0.002	0.028	0.031	0.027	0.031

N5(400), N10(400), N15(400), N20(400), N25(400) were deliberately not included in the Table as the plants exposed to 400mM salinity levels (i.e. X400) all died prior to 5th week when NPs were applied to salt-exposed plants

Key: N5-100 Plant applied with 5% nanoparticle solution (NPS) exposed to 100mM salinity level; N10-100 Plant applied with 10% NPS and exposed to 100mM salinity level; N15-100 Plant applied with 15% NPS and exposed to 100mM salinity level; N20-250 Plant applied with 20% NPS and exposed to 250mM salinity level; N25-250 Plant applied with 25% NPS and exposed to 250mM salinity level; N5-400 Plant applied with 5% NPS and exposed to 400mM salinity level; X100, X250, and X400 are plants exposed to 100, 250, and 400 mM salinity levels but no NPS respectively

Table 5 shows the presentation of prominent necrotic and chlorotic predisposition of the test plants and experimental conditions. Although there was no significant difference in modal periods taken for complete foliar necrosis as well as complete foliar chlorosis. Generally apart from plants exposed to 400mM of salt solution, the period taken for complete foliar necrosis and chlorosis did not significantly differ from the control respectively ($p=0.75$). Generally, it took between 88 and 288 hours for a complete leaf to become chlorotic and between 112 to 168 hours for a complete leaf to become necrotic. It should be noted however, that differences in time was reported because of the presence of leave in different length. For longer leaves, it took longer times to be necrotic and vice-versa. Percentage foliar necrosis shows the percentage number of leaves that turned necrotic.

Table 5: Presentation of prominent necrotic and chlorotic predisposition of the test plants under experimental condition.

Treatments	Modal Period taken for complete foliar chlorosis (hr)	Modal Period taken for complete foliar necrosis (hr)	Percentage foliar necrosis (%)
N5(100)	200[a]	168[ab]	25.6[a]
N10(100)	160[ab]	144[ab]	29.37[a]
N15(100)	88[b]	152[ab]	35.56[a]
N20(100)	160[ab]	168[ab]	26.75[a]
N25(100)	136[ab]	152[ab]	30.33[a]
N5(250)	144[ab]	128[ab]	50.00[a]
N10(250)	96[b]	144[ab]	40.81[a]
N15(250)	144[ab]	112[a]	29.53[a]
N20(250)	152[ab]	168[ab]	31.85[a]
N25(250)	128[ab]	152[ab]	50.92[a]
N5(400)	-	-	-
N10(400)	-	-	-
N15(400)	-	-	-
N20(400)	-	-	-
N25(400)	-	-	-
X100	200[a]	128[ab]	25.28[a]
X250	160[ab]	192[b]	31.83[a]
X400	-	-	-
GC	208[a]	160[ab]	20.83[a]
p-values	0.6230	0.3830	<0.001

Key: N5-100 Plant applied with 5% nanoparticle solution (NPS) exposed to 100mM salinity level; N10-100 Plant applied with 10% NPS and exposed to 100mM salinity level; N15-100 Plant applied with 15% NPS and exposed to 100mM salinity level; N20-250 Plant applied with 20% NPS and exposed to 250mM salinity level; N25-250 Plant applied with 25% NPS and exposed to 250mM salinity level; N5-400 Plant applied with 5% NPS and exposed to 400mM salinity level; X100, X250, and X400 are plants exposed to 100, 250, and 400 mM salinity levels but no NPS respectively

Fig 4 shows loading on the principal component analysis showing variability among selected growth and yield parameters of test plant under experimental conditions. The result shows that the number of root presented the highest variability in the study. This shows that soil conditions impacted the roots more than other plant parts. This is consistent with the work of Gaida *et al*, (2017) that roots are the most active part of plant growth and development. The parameter with the lowest variability is shelling percentage. Generally one yield parameter which included shelling, weight of rice grain per particle and foliar yield all presented low variability compared to number of roots(Fig 4)

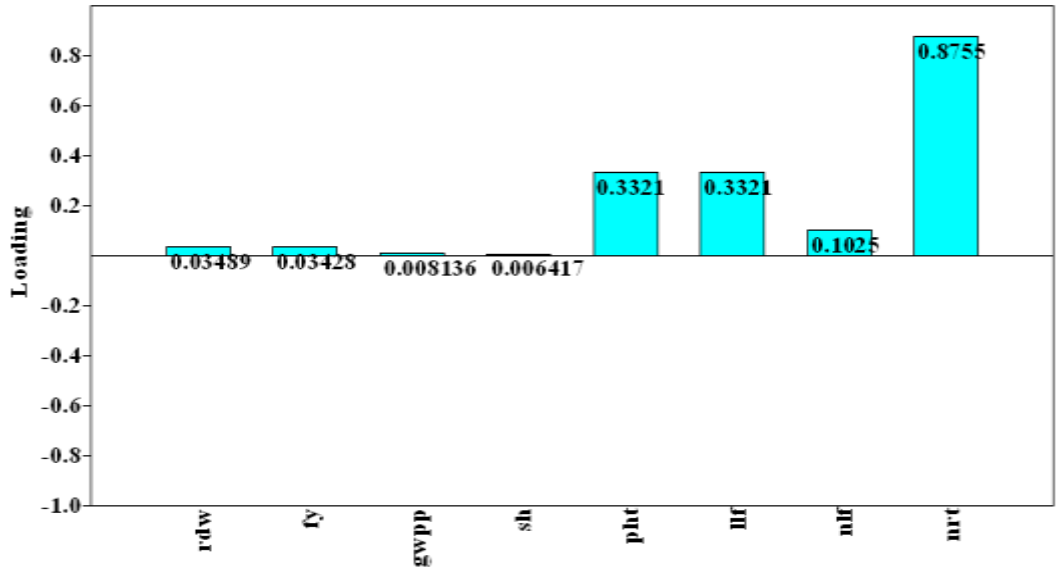

Fig. 4: Loadings on the principal component analysis showing variability of selected growth and yield parameters of test plant under experimental conditions. rdw - Root dry wt., fy -Foliar yield, gwpp - Wt. of rice grain per panicle, sh –Shelling, pht - Plant height, nlf - number of leaf, llf - Leaf length, nrt - No. of root

A correspondence analysis was recorded to show association between treatment and selected growth and yield parameters of test plant under experimental conditions. Results as presented in Fig 5 Shows close association between N5 (100) and weight of rice grain per panicle. The implication may be that this is the most likely to enhance this parameters. This may mean that in ferruginous soil under 100mM salt stress, AgNPs of 200ml diluted with 5% distilled water may improve rice growth parameters than other treatments.

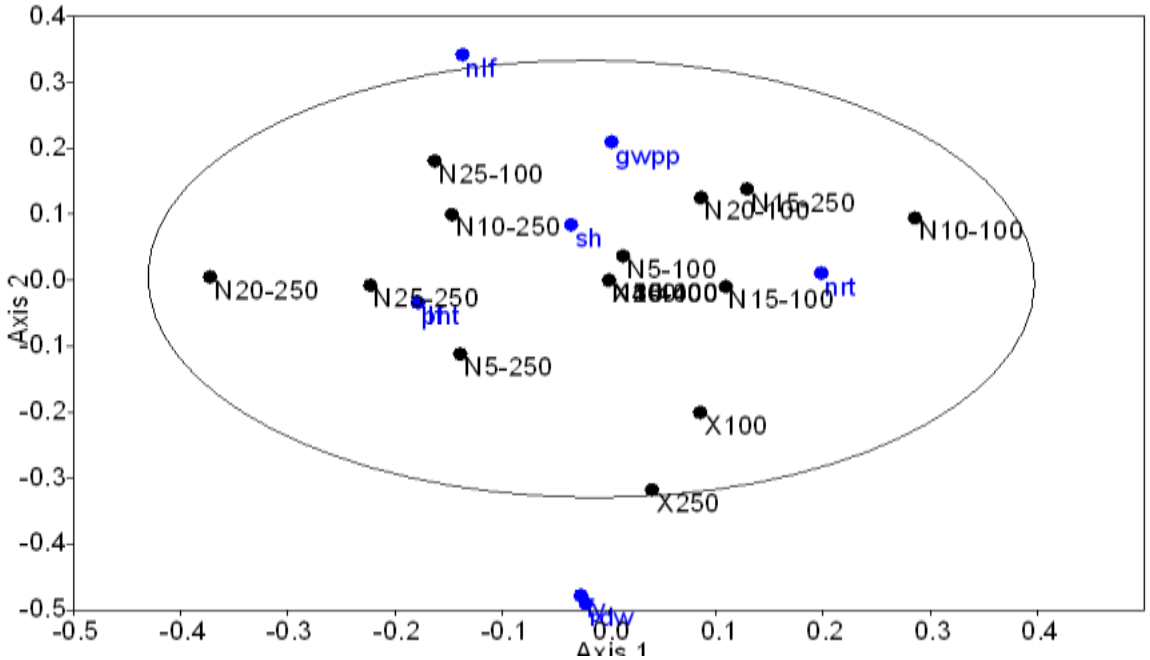

Fig. 5: Correspondence analyses showing association between treatments and selected growth and yield parameters of test plant under experimental conditions. rdw - Root dry wt., fy -Foliar yield, gwpp - Wt. of rice grain per panicle, sh –Shelling, pht - Plant height, nlf - number of leaf, llf - Leaf length, nrt - No. of root

CONCLUSION

The use of AgNPs to enhance the growth and yield performances of salt-stressed rice in a ferruginous soil was investigated. Both yield and total biomass of rice were enhanced upon exposure to AgNPs. As both plant parameters are important yardsticks for underscoring plant-based contributions to positive economic development, the application of AgNPs may become, if properly harnessed, one of many routes to achieving sustainable agriculture and food security. It is however suggested that mechanisms of action of AgNPs in plants be further evaluated with a view to ascertaining possible accumulations of silver elements in harvestable plant parts.

AUTHOR CONTRIBUTIONS

Beckley Ikhajiagbe designed and executed the study. Musa Saheed Ibrahim analyzed the data. Musa Saheed Ibrahim prepared the drafts. Beckley Ikhajiagbe and Musa Saheed Ibrahim wrote the final manuscript.

ACKNOWLEDGEMENTS

The authors sincerely appreciate the immense contributions of Miss M. Asia, Mr. F.A. Igiebor, Mr. O. Kadesh, Miss S. Archibong and Miss M. Sommer during this study.

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