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Plant Response on Exposure to Ag Nanoparticles: A Study with *Vigna subterranea*

Eucharia Oluchi Nwaichi[1,2]* and Emmanuel Okogbue Anosike[1,2]

[1]*The University of Nottingham, Nottingham, UK*
[2]*University of Port Harcourt, Port Harcourt, Nigeria*

Abstract

Effects of silver nanoparticles (Ag NPs) exposure on two geographical cultivars of *Vigna subterranea* were investigated. After inoculation in half strength Hoagland medium amended with Ag NPs for 15 days, both physiological and biochemical responses were evaluated. Exposure significantly decreased plant growth by up to 85%. Interestingly, Ag NPs exposure significantly decreased mean shoot biomass in all treatments but increased root mass (34% and 66%) in relation to control. Chlorophyll production was reduced by approximately 46% (in the more tolerant) and 86% (in the more sensitive) for the two cultivars and observed catalase activity was about 50% of the activity in NPs stressed root tissues for the sensitive cultivar. It may be perceived that the inherent stress is associated with observed surge in catalase activity across all cultivars. Also, the observed increase in catalase activity is positively correlated at 99.9% level (r=0.9571, n=10) with decreasing chlorophyll content on exposure. Recorded ascorbate peroxidase activity was higher in leaf tissues. Statistical analysis revealed marked difference between superoxide dismutase activities of *V. subterranea* cultivars and also between treatments. Time trend of transpiration rate revealed a decreasing order throughout growth period.

Keywords: Antioxidant enzymes; *Vigna subterranea*; Silver nanoparticles exposure; Nanotoxicity; Transpiration rate

Abbreviations: Ag NPs: Silver Nanoparticles; NPs: Nanoparticles; RL: Root Length (cm); PW: Plant Weight (g); NL: Number of Leaves; LW: Leaf Width (cm); LL: Leaf Length (cm); 67 C: Cultivar 67 Spiked With Zero Silver Nanoparticles; 67 Ag NPs: Cultivar 67 Spiked With Silver Nanoparticles; 01 C: Cultivar 01 Spiked With Zero Silver Nanoparticles; 01 Ag Nps: Cultivar 01 Spiked With Silver nanoparticles; S: Shoot Tissues; R: Root Tissues; USDA: Plant Genetic Resources Conservation Unit of USDA Griffin Georgia USA; UI: The Plant Resources Conservation Unit of the University of Ilorin, Nigeria

Introduction

Nanoscience and nanotechnology are the study and application respectively of materials at the nanoscale to leverage their amplified properties such as lighter weight, improved strength and reactivity and greater regulation of light spectrum, in comparison to the larger-scale counterparts. Although many new materials and devices with a vast range of applications, such as in medicine, electronics, biomaterials energy production, military operations, agriculture and consumer products are dividends of nanotechnology, toxicity and environmental impacts of nanomaterials [1] like in any other new technology remain a concern. Nano-sized particles exist in nature and can be fashioned from a variety of products, such as carbon or minerals like silver, but nanomaterials as defined by NIEHS [2] must have at least one dimension that is less than approximately 100 nanometres. Gaharwar et al. [3] and Cho et al. [4] chronicled recent application of nanomaterials to include a range of biomedical applications, such as tissue engineering, drug delivery, and biosensors (sensor development for toxic substances detection for example). Also, the catalytic activity of these nanomaterials may imply potential risks in their interaction with biomaterials and the overwhelming number of new manufacturer-identified nanotech products [5] will only heighten existing safety concerns.

Better understanding of NPs fate and effects on biota has been challenging given the potential for food chain contamination and for an uncharacterized pathway of human exposure [6]. Rico et al. [7] highlighted the unknown interactions of engineered nanomaterials agricultural systems that are recipients of a number of organic chemicals and inorganic amendments. Effort targeted at circumventing any potential threat in the production, use, or disposal of nanoscale products and devices will amount to preparedness in this booming and emerging field.

Widespread presence of silver nanoparticles in consumer products such as antimicrobial coatings, and many textiles, keyboards, Silver Nanowires , wound dressings, biomedical devices, photonic devices, conductive inks, pastes and fillers, has attracted many researchers in a quest to understand how they interact with biological systems and the environment. In their product description, Oldenburg [8] described the unique and desirable properties of Ag NPs to include: diagnostic applications as they are used in biosensors and innumerable assays where the silver nanoparticle materials can be used as biological tags for quantitative detection; antibacterial applications in their incorporation in apparel, footwear, paints, wound dressings, appliances, cosmetics, and plastics for their bactericidal property; conductive applications as they are used in conductive inks and integrated into composites to enhance thermal and electrical conductivity; and optical applications in their ability to efficiently harvest light and for amplified optical spectroscopies including metal-enhanced fluorescence and surface-enhanced Raman scattering.

Nwaichi et al. [9] reported the socioeconomic role played by an indigenous African legume, *V. subterranea* in the semi-arid regions of Africa. They also submitted that the crops can grow in marginal, low-input environments, and occur in the local environment. Also,

***Corresponding author:** Eucharia Oluchi Nwaichi, The University of Nottingham, Nottingham, UK, Tel: 234(0)8056031989; E-mail: nodullm@yahoo.com

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the seeds make a complete food, as it contains sufficient quantities of proteins, carbohydrates and lipid, and these may predispose this crop to anticipated implication with increasing use and disposal of nano products; hence the rationale for choice in this study.

Scarce data, however, are available on the response in important crops subjected abiotic stress due to nanoparticles, as well as on the involvement of antioxidant enzymes in this type of stress in anticipation to the rising patronage of nanoproducts.

This study aims to evaluate nanotoxicity of Ag NPs exposure on selected cultivars of *V. subterranea* at the physiological level and investigate the activity of antioxidant defence system there-of.

Methods

Materials and conditions of growth

Silver nanoparticles (99.99% purity; <20 nm) was obtained from US Research Nanomaterials, Inc. (Houston, TX US). Using an ultrasonic probe, prepared 250 mg L^{-1} and 500 mg L^{-1} Ag NPs solutions were agitated for 10 mins and left overnight thereafter, to determine particle characteristics in water. Afterwards, the solutions were centrifuged (3000 rpm, 10 mins) and an aliquot of the supernatant taken for particle size and ζ-potential determination (Malvern, Zetasizer Nano ZS90). Respective average particle size and ζ-potential values were 98 nm and −16.7 mV 68 nm at 250 mg L^{-1} and 90 nm and −20.8 mV at 500 mg L^{-1}. Seeds of seven cultivars (Table 1) of Bambara beans (*Vigna subterranea*) were sourced from ARS Plant Genetic Resources Conservation Unit of USDA Griffin Georgia US and Plant resources conservation unit of the University of Ilorin Nigeria. Seeds were scarified and sown in racks containing Vermiculite to raise seedlings for Hoagland charged hydroponic growth in the CNS greenhouse of the University of Massachusetts MA US. Wide range test was done to determine critical concentration (250 mg L^{-1} and 500 mg L^{-1}) chosen thereof. The most consistent tolerant (PI 378867 labelled as cultivar 67) and sensitive (UI 14401 labelled as cultivar 01) species were selected for the exposure study.

Exposure assay

Clean and clear 170 mL jars (Fisher Scientific Pittsburgh PA) were adapted to be fitted with growth and air pipes (for connection to air pump). Transplanting of single 10 d old pre - germinated seedling (after the emergence of the first composite bean leaf) was gently done into each half strength Hoagland – filled jars. Two weeks of acclimatization was put in place prior to the 15d experiment while measuring growth parameters and respiratory rate in the 10 – replicated experiment. These jars were carefully replaced with vigorously shaken 250 mg L^{-1} and 500 mg L^{-1} Ag NPs spiked half strength Hoagland solutions. Control replicates were prepared in the absence of Ag NPs nanomaterials. Exposure study was conducted in CNS Greenhouse University of Massachusetts under ambient light and temperature. Replicate jars were top watered as needed with half strength Hoagland solution (to avoid depletion of nutrients and drying) for a 15 d growth period and harvested on day 16. Ten replicate plants were grown for each treatment and measurements were not taken on the Flag Day (first day) to minimize disturbance after spiking. Plant tissues were washed in distilled water to remove loose dirt and thereafter separated into roots and shoots and the tissue mass was determined. Tissues for enzyme assay were stored temporarily in liquid N2 and transferred to -80°C Refrigerator (REVCO) on arrival to the laboratory. Leaves for pigment assay were however processed immediately following protocol outlined below.

Item	Inventory	Accession	Plantid	Country	Source
1	PI 241982 01 SD	PI 241982	Kahemba	Africa	USDA
2	PI 244969 01 SD	PI 244969		Malawi	USDA
3	PI 378867 01 SD	PI 378867	Ditloo	South Africa	USDA
4	PI 414629 01 SD	PI 414629	Potgietersrus 2	South Africa	USDA
5	PI 414638 01 SD	PI 414638	Swazi Homelands 6	South Africa	USDA
6	PI 414645 01 SD	PI 414645	V.6	South Africa	USDA
7	UIH 001 169	UIH 0011		Nigeria	UI
USDA denotes Plant Genetic Resources Conservation Unit of USDA Griffin Georgia USA; UI denotes the Plant resources conservation unit of the University of Ilorin Nigeria.					

Table 1: Collected cultivars of *V. subterranea* for the study.

Chlorophyll content assay

50 mg of fresh plant leaves were soaked in 15 mL tubes containing 10 mL 95% ethanol each. Absorbance values were measured spectrophotometrically at 645 nm and 663 nm for chlorophyll a (chl a) and chlorophyll b (chlb) respectively. The tubes were wrapped with foil and put in a dark room at temperature of 25 ± 3°C for 3 days after which absorbance values were taken.

Enzyme extraction and assay

Catalase assay: Plant tissues weighing 100 mg (rapidly ground in liquid N_2) were homogenized using 1 mL of phosphate buffer (25 mM KH_2PO_4 at pH 7.4) and was centrifuged at 4°C for 20 min at 4,000 rpm in a refrigerated centrifuge (Eppendorf Centrifuge 5810R). The clear supernatant was used as the enzyme source. A modified method of Chance and Maehly (1995) was adopted as follows: One millilitre of the assay mixture included 950 µL of 10 mM H_2O_2 and 50 µL of enzyme extract. A blank (buffer) was run in same way. A unit of catalase activity is defined as the amount of enzyme that degrades 1 µmole of H_2O_2 min^{-1} under the assay conditions prescribed. ThermoScientific Evolution 60S UV – Visible Spectrophotometer was employed for reading at 240 nm and 30 secs interval for 2.5 mins. The specific activity of catalase was expressed as units mg^{-1} protein min^{-1}.

Ascorbate peroxidase assay: Plant tissues weighing 300 mg (rapidly ground in liquid N_2) were homogenized using 1.5 mL of extraction buffer (mixture of 0.1 mM EDTA, 100 mM Phosphate buffer at pH 7.0, 2% (v/v) β – Mercaptoethanol and 0.1 mM Ascorbate) and was centrifuged at 4°C for 20 min at 4,000 rpm in a refrigerated centrifuge (Eppendorf Centrifuge 5810R). The clear supernatant was used as the enzyme source. A modified method of Chance and Maehly [10] was adopted as follows: 2.2 mL of the assay mixture included 50 mM phosphate buffer pH 7.0, 0.1 mM EDTA, 0.6 mM Ascorbate and 10 mM H_2O_2 and 0.2 mL of enzyme extract. Absorbancy was taken at 290 nm and 1 min interval for 3.0 mins. The specific activity of APx was expressed as units mg^{-1} protein min^{-1}.

Superoxide dismutase assay: Plant tissues weighing 100 mg (rapidly ground in liquid N_2) were homogenized using 1.0 mL of extraction buffer (mixture of 0.1% (w/v) Bovine Serum Albumin, 50 mM Phosphate buffer at pH 7.8, 0.05% (w/v) β – Mercaptoethanol and 0.1% (w/v) Ascorbate) and was centrifuged at 4°C for 20 min at 4,000 rpm in a refrigerated centrifuge (eppendorf Centrifuge 5810R). The clear supernatant was used as the enzyme source. In a foil wrapped beaker, 3.0 mL of the assay mixture containing 50 mM phosphate buffer pH 7.8, 9.9 mM L – Methionine, 57 µM Nitrobluetetrazolium, 0.025% (w/v) Triton X - 100 and 0.0044% (w/v) Riboflavin.

In the dark at room temperature (25 ± 3°C), 0.05 mL of enzyme extract was added to 1.45 mL of assay mixture. Absorbance (440 nm) values for test and blank (assay mixture only), were taken at the 20th minute. The specific activity of SOD was expressed as units mg^{-1} protein min^{-1}.

Protein content assay: One microliter of supernatant was read off at 280 nm using Thermo Scientic NANODROP 2000 Spectrophotometer connected to VOSTRO DELL Computer. Buffer and Hygromycin B were used as blank and reference protein respectively. Hygromycin B from Streptomyces hygroscopicus (potency of 1210 U mg^{-1}) was purchased from Phytotechnology Laboratories. Manufacturer's protocol was followed.

Statistical analysis

Data collected from ten replications per treatment were presented as mean ± S.E. and subjected to statistical test of significance using the Student's t - test. Correlation co-efficient was used to determine the association among cultivars under study at 95% confidence level. Each treatment was compared to its control experiment.

Results and Discussion

Effect of silver nanomaterials on plant mass and other growth indices

Over 70% of the cultivars exposed to 500 mg L^{-1} Ag NPs dried up between days 6 and 9 and hence were completely excluded in the final assays. The cultivars with concentration, 250 mg L^{-1} Ag NPs were therefore reserved for analysis. The observed drying may be due to loss of leaves' cuticle leading to widening of the stomatal pores and poor desiccation tolerance. At the end of the 15 d experiment, a marked decrease in shoot and root biomass (Figure 1) was recorded. However, an inverse order between the shoot and root tissues were the case when percentage of total mass contributions were calculated. Shoot tissues decreased in percentage of total mass from 84% and 82% (control) to 79% and 70% (Ag NPs exposed) for cultivars 01 and 67 respectively. Shoots for G. Max and C. pepo decreased in their percentage total biomass contribution from 77% and 84% to 59% and 81% respectively in the exposure (500 mg mL^{-1} Ag NPs) study by De La Torre-Roche et al. [11]. On the other hand, root tissues increased in their% total biomass contribution from 16% and 18% (control) to 21% and 30% (Ag NPs exposed) for cultivars 01 and 67 respectively. Similarly, a decrease from 23% and 16% to 41% and 19% respectively for G. Max and C. pepo, were reported by De La Torre-Roche et al. [11]. Besides reduced biomass, De La Torre-Roche et al. [11] observed 47% and 25% increment in root contribution to total plant biomass for G. Max and C. pepo respectively in relation to their respective controls. These calculations gave 26% for the sensitive cultivar 01 and 67% for the more tolerant cultivar 67 from our results.

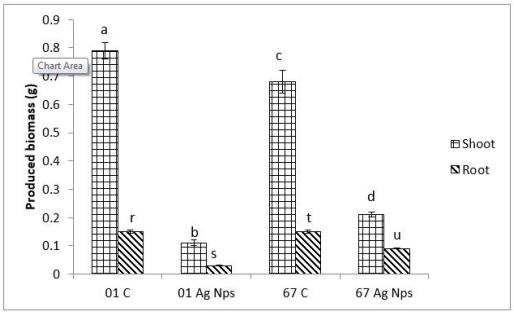

Figure 1: Effect of Ag NPs exposure on two cultivars of *V. subterranea*. Mean values for each part, marked with different letters, are significantly different at p<0.05 for n=10. 01 C, 01 Ag Nps, 67 C, and 67 Ag Nps, represent cultivar 01 spiked with no Silver nanoparticles, cultivar 01 spiked with Silver nanoparticles, cultivar 67 spiked with no Silver nanoparticles, cultivar 67 spiked with Silver nanoparticles, respectively. Vertical bars represent mean ± S. E.

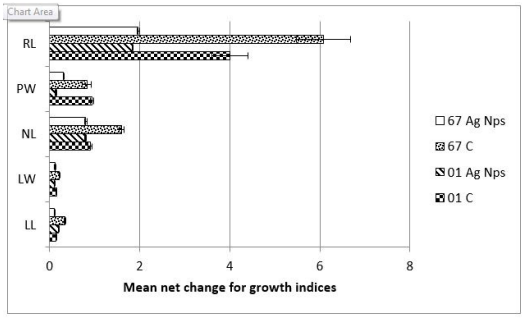

Figure 2: Mean growth change from 0 d to 15 d for studied cultivars. RL, PW, NL, LW and LL represent root length (cm), plant weight (g), number of leaves, leaf width (cm) and leaf length (cm) respectively. 67 C, 67 Ag Nps, 01 C and 01 Ag Nps represent cultivar 67 spiked with zero Silver nanoparticles, cultivar 67 spiked with Silver nanoparticles, cultivar 01 spiked with zero Silver nanoparticles, and cultivar 01 spiked with Silver nanoparticles respectively. Vertical bars represent mean ± S. E. for n=10.

Sample	Catalase A_{240}	APx A_{290}	SOD A_{440}
	IU/mg protein/min	IU/mg protein/ min	IU/mg protein/min
67 C S	61.248 ± 4.886**	2.973 ± 0.004**	3.401 ± 0.007**
67 C R	135.264 ± 6.699*	2.452 ± 0.041*	1.044 ± 0.003*
67 Ag Nps S	108.713 ± 5.874**	3.460 ± 0.008**	1.134 ± 0.003**
67 Ag Nps R	141.897 ± 5.774*	2.479 ± 0.009*	0.806 ± 0.002*
01 C S	81.819 ± 7.996**	2.361 ± 0.007	5.603 ± 0.038**
01 C R	77.405 ± 4.885*	3.234 ± 0.051	3.056 ± 0.013*
01 Ag Nps S	103.056 ± 5.001**	2.298 ± 0.008	1.251 ± 0.004**
01 Ag Nps R	134.304 ± 6.779*	2.941 ± 0.010	1.019 ± 0.005*

** and * indicate significant difference between shoot and root tissues respectively with same cultivar and same parameter. Observed catalase activity was about 50% of the observed activity in nanoparticle stressed root tissues in sensitive cultivar 01 from Nigeria. Values represent mean ± S. E. for n=10. S and R appended to existing abbreviations denotes shoot and root tissues respectively.

Table 2: Mean Catalase, Ascorbate peroxidase (APx) and Superoxide dismutase (SOD) activities in studied crop samples. Treatments were replicated ten times. A unit of SOD is the quantity of enzyme required to inhibit the reduction of Cyt C by 50% in a 1.5 ml reaction volume per minute.

A reduction in leaf length, leaf width, and number of leaves formed were noted with progressive leaf necrosis (Figure 2) and inward curling with time. These changes however affected the older leaves more. Naranjo et al. [12] concluded that necrotic spots are typical for plant–pathogen interactions especially when they are incompatible (avirulent). Although NPs – induced toxicity reduced root length as a growth parameter, there was remarkable increase in the number of roots in spiked treatments.

Similarly, our results demonstrate that growing *V. subterranea* crops in the presence of Silver nanoparticles causes a marked decrease in the fresh biomass typical of studied cultivars, suggesting induced nanotoxicity to both cultivars.

Screening for the expression of the H_2O_2 scavenging enzymes

Catalases and peroxidases are two major systems for the enzymatic removal of H_2O_2 in plants [13]. Table 2 represents the changes of catalase (CAT), ascorbate peroxidase (APx) and superoxide dismutase (SOD) activities. Alterations in studied antioxidant enzymes were noted.

Sample	SOD Specific activity Shoot	APx Specific activity Shoot	SOD / APx
67 C	3.401 ± 0.007**	2.973 ± 0.004**	1.144 ± 0.017**
67Ag Nps	1.134 ± 0.003**	3.460 ± 0.008**	0.328 ± 0.003**
01 C	5.603 ± 0.038**	2.361 ± 0.007**	2.373 ± 0.094**
01AgNps	1.251 ± 0.004**	2.298 ± 0.008**	0.544 ± 0.012**
$CSOD_{67}$:SOD_{01}	0.607	-	-
$TSOD_{67}$:SOD_{01}	0.906	-	-

** indicates significant difference between shoot tissues respectively with same cultivar and same parameter. Statistical analysis revealed marked difference between SOD activities of *V. subterranea* cultivars and also between treatments ($P \leq 0.05$). Values represent mean ± S. E. for n=10. SOD_{67}, SOD_{01}, C and T denote SOD for cultivar 67, SOD for cultivar 01, control (non – exposed) and test (Ag NPs exposed) respectively.

Table 3: Comparison of SOD and APx activities in shoot tissues.

Sample	SOD Specific activity Root	APx Specific activity Root	SOD / APx
67 C	1.044 ± 0.003*	2.452 ± 0.041*	0.426 ± 0.001
67Ag Nps	0.806 ± 0.002*	2.479 ± 0.009*	0.325 ± 0.001
01 C	3.056 ± 0.013*	3.234 ± 0.051*	0.945 ± 0.004*
01AgNps	1.019 ± 0.005*	2.941 ± 0.010*	0.346 ± 0.002*
$CSOD_{67}$:SOD_{01}	0.342	-	-
$TSOD_{67}$:SOD_{01}	0.267	-	-

* indicates significant difference between root tissues respectively with same cultivar and same parameter. Statistical analysis revealed marked difference between SOD and APx specific activities of *V. subterranea* cultivars and also between treatments ($P \leq 0.05$). Values represent mean ± S. E. for n=10. SOD_{67}, SOD_{01}, C and T denote SOD for cultivar 67, SOD for cultivar 01, control (non–exposed) and test (Ag NPs exposed) respectively.

Table 4: Comparison of SOD and APx activities in root tissues.

CAT, a frequently used and very important enzyme in protecting the cell from oxidative damage by reactive oxygen species, gave heightened activity for both cultivars under stress and compares with the findings of Velikova et al. [14] where observation was attributed to H_2O_2 reduction. Highest catalase activity of 141.897 IU mg $protein^{-1}$ min^{-1} was recorded in root tissues for cultivar 67. However, in comparison to equivalent activity in control samples, shoot tissues from cultivar 67 gave highest incremental of 77% CAT activity with exposure (Table 2). More sensitive cultivar gave higher CAT activity in root tissues and maintains higher activity incremental (73%) in root over shoot (26%) under stress. Observed catalase activity was about 50% of the observed activity in nanoparticle stressed root tissues in sensitive cultivar 01 from Nigeria.

Results showed that APx activity was higher in Ag NPs - treated more tolerant cultivar 67 tissues than its equivalent in control. In their studies on the impact of acid rain on *Phaseolus vulgaris*, Velikova et al. [14] made similar observation with peroxidase activity showing increased leaf levels in relation to control. They postulated that increased activity of peroxidase in acid rain treated plants suggests the protective role of the enzyme in the system. The increment (Table 2) in this study was however, much higher in the shoot (17%) than in the root (1.5%) in relation to their respective controls. A significant activation of peroxidase during pH 2.2 treatment was found in two tobacco cultivars by other researchers [15]. The reverse was the case for the more sensitive cultivar 01. APx activity slightly decreased by 3% and 10% respectively for shoot and root tissues in response to posed nanotoxicity in cultivar 01 but were not marked. Spermidine, a polyamine compound found in ribosomes and living tissues, and having various metabolic functions within organisms, decreased peroxidase activity in acid – rain exposed *P. vulgaris* [14]. In their role as longevity agent and growth regulators [16], it could be inferred that Ag nanotoxicity impaired regulation of cell growth in studied cultivars in varied degrees.

SOD (an enzyme that alternately catalyses the dismutation (or partitioning) of the superoxide (O_2–) radical into either less damaging ordinary molecular oxygen (O_2) or H_2O_2), an important antioxidant defence in nearly all living cells exposed to oxygen, decreased in the presence of Ag NPs for all cultivars and was marked (Table 2). They may have been partly denatured by formed superoxides (superoxide toxicity). In higher plants, SOD acts as antioxidants and protect cellular components from being oxidized by reactive oxygen species [17]. No wonder recorded CAT activity with Ag NPs exposure was high as damaging H_2O_2 requires degradation. Abundance of SOD in shoot in all treatments could be attributed to compartmentalization. Of the total plant SOD activity, shoot contributed 77%, 58%, 65% and 55% for non – exposed cultivar 67, exposed cultivar 67, non – exposed cultivar 01, and exposed cultivar 01 respectively.

The ratio of SOD and APX specific activity (Tables 3 and 4) rather than the total activity of each enzyme could be an important factor in determining the level of oxidative stress protection [18]. Observed ratio averaged 1.444 and 2.373 in shoot tissues of cultivars 67 and 01 but decreased to 0.328 and 0.544 respectively. It averaged 0.426 and 0.945 and decreased to 0.325 and 0.346 in the same order for root tissues. The higher SOD: APX ratio in control plants, compared with Ag NPs – exposed plants, is due to both an increase in SOD specific activity and a drop in APX specific activity (Table 4). Gupta et al. [18] concluded that other factors like maturation of other quenching systems that may include antenna pigments and pools of antioxidant compounds such as ascorbate, GSH, and tocopherols, could have contributed to resistance to oxidative stress given a stable SOD activity as SOD: APx value increased from 0.45 at rosette stage to 0.75 at bolting stage in their study. Given higher biomass, root length and leaf width (Figure 2) observe in cultivar 67 under stress, they may have attained higher maturity and may have influenced protection. The ratio of shoot SOD activity for cultivar 67 to 01 was higher in spiked samples but the reverse was the case in root tissues and may be related to compartmentalization.

Chlorophyll content in *V. subterranea* under Ag NPs stress

There was accompanying loss of protein content with chlorophyll content. A positive correlation at 99.9% level (r=0.9429, n=10) between protein content and Chlorophyll content during phytotoxicity was observed. Similarly, a positive correlation (r=0.9571, n=10) was observed between increase in catalase activity at the 99.9% level with the decrease of the chlorophyll content during exposure. A significant decrease in the content of chlorophylls a and b was observed for both studied cultivars, and the content of photosynthetic pigments varied between 46% and 86% for the more tolerant South African and it Nigerian sensitive counterpart respectively.

Formation of reactive oxygen species due to foreign toxic substance [19] and possibly nutrient depletion may have led to observed leaf dryness from day 5 in the more sensitive cultivar 01 and day 11 in the more tolerant cultivar 67. Formed superoxide may have shut down energy harvest, in addition to formation of precursors of certain amino acids as well as the reducing agent NADH that is used in numerous other biochemical reactions by inactivating citric acid cycle enzyme aconitase, hence poisoning energy metabolism, with attendant release of potentially toxic iron. There was complete dryness of leaves in most replicates for cultivar 01 under Ag NPs stress.

Observed transpiration pattern in the face of Ag NPs stress

V. subterranea plants were each irrigated with an average of 26.2 mL

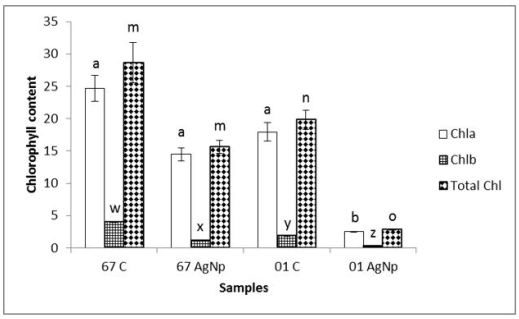

Figure 3: Chlorophyll content recorded for studied samples. 67 C, 67 AgNp, 01 C and 01 AgNp represent cultivar 67 spiked with no Silver nanoparticles, cultivar 67 spiked with Silver nanoparticles, cultivar 01 spiked with no Silver nanoparticles, and cultivar 01 spiked with Silver nanoparticles respectively. F. test compared samples across parameter types and same alphabets indicate no significant difference at α=0.05. Vertical bars represent mean ± S. E. for n=10.

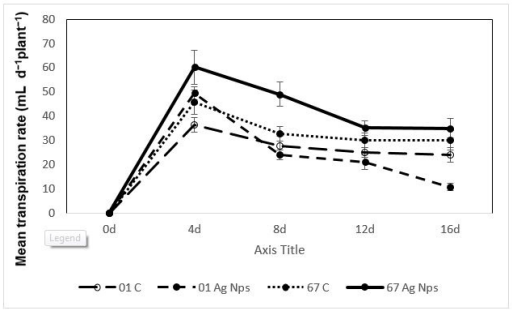

Figure 4: Mean transpiration rate for studied cultivars. 67 C, 67 Ag Nps, 01 C and 01 Ag Nps represent cultivar 67 spiked with no Silver nanoparticles, cultivar 67 spiked with Silver nanoparticles, cultivar 01 spiked with no Silver nanoparticles, and cultivar 01 spiked with Silver nanoparticles respectively. Note that n=10.

(21.8 – 36.3mL); 36.3 mL (27.8 – 49.6 mL); 35.8 mL (26.7 – 55.8mL) and 39.8 mL (31.7 – 60.2 mL) every four days (Figure 3) during the 15 d of growth for Cultivar 01 under no exposure; Cultivar 01 under Ag NPs exposure; Cultivar 67 under no exposure and Cultivar 67 under Ag NPs exposure respectively. Individual replicate irrigation volumes varied based on plant size, growth rate, and transpiration volume. The time trend of transpiration rate revealed a decreasing trend throughout the growth period of studied crop (Figure 4). Evidence of interference of Ag NPs with nutrient and water uptake under hydroponic conditions were visible. Peak mean transpiration rate of 49.6 and 60.2 mL $d^{-1}plant^{-1}$ was recorded for cultivars 01 and 67 respectively on day 4. There was uniform reduction thereafter and these findings demonstrate the complex interactions of Ag NPs with crop plants and may open up new search. Surendar et al. [20] in their studies on effect of stress on transpiration rate observed that 4 of the 12 cultivars and hybrids of bananas identified as tolerant to water stress gave highly accelerated transpiration rate by water stress treatment over control. Similar pattern was observed in this study with more tolerant cultivar 67. Transpiration rate on the 8th day was least (21 mL $d^{-1}plant^{-1}$) for more sensitive cultivar 01 which may go with stomatal closure [21] in response to water stress. In their report, reduction in transpiration rate under water stress led to reduced photosynthetic rate by inhibition of CO_2 entry into the chloroplast through the stomata [20]. This may explain similar trend obtained in this study with least chlorophyll content and transpiration rate for sensitive cultivar 01 under Ag NPs stress.

Such generated data may trigger operators, farmers and households to apply measures to prevent and reduce nanocontamination as low as reasonably achievable in order to protect public health. This may include good agronomic management practice to alleviate contaminant uptake and biomagnification [22]. It also calls for nanotoxicological acceptable limits to be set following rigorous experiments and validations by relevant regulatory bodies.

Techniques used in this work offer unique insights into changes to some physiological and biochemical processes inside of a crop plant under Ag NPs stress. An important consideration when studying the crop plants and interpreting results is that one specie does not represent the high variability and diversity of phylla present in the plant kingdom. However, this work demonstrated that even same plant species can behave in dramatically different ways under the same experimental conditions. This may proffer explanations to some observed variations within the same conditions.

Conclusion

Our study highlights the relevance of studying physiological and biochemical responses of crop plant under Ag NPs exposure given observed alterations. The rising use of nanomaterials in commercially available products like consumer products and agrichemicals such as pesticides and fertilizers has resulted to environmental nanocontamination, including agricultural systems. Nevertheless, attendant consequence is not inevitable given heightened interest in exposure and risk assessment, fate and footprints of NPs in food and environment. Here, significant changes in anti-oxidant enzymes and physiological parameters demonstrated that the cell integrity of studied cultivars have been injured to a varied extent due to Ag NPs stress. It was also observed that Ag NPs hampered the photosynthetic ability of tested cultivars and may hence hamper food production. Also, the time trend of transpiration rate revealed a decreasing trend throughout the growth period of the crop under Ag NPs stress. A used and presented approach here offer a novel combination of indicators for identifying NP-induced perturbances within the crop plant and has contributed to data pool in the field.

The current study demonstrates that the response of markers of oxidative stress varies greatly between cultivars and sensitivity of interest plant. The potential implications of findings on fate of Ag NPs are a topic currently under keen investigation.

Ethics Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable

Availability of Data and Materials

Authors will make available any other raw data that may be requested by researchers through email to the corresponding author.

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Competing Interest

The authors declare that they have no competing interests.

Disclosure Statement

The authors acknowledge that is no conflict of interest or benefit arising from the direct applications of their research.

Author's Contribution

EO^1 carried out growth experiment and laboratory analyses and drafted the manuscript. EO^2 conceived of the study and participated in its design. EO^1 and EO^2 jointly performed the statistical analysis. All authors read and approved the final manuscript.

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