



Faculty of Sciences

Department of Biological Sciences and Biotechnology

Effects of Bioaccumulation of Green Synthesized Silver Nanoparticles on Early Growth and Metabolism in *Strychnos cocculoides* Baker and *Strychnos spinosa* Lam

By

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DECLARATION AND COPYRIGHT

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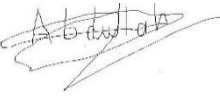
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I, **Professor ABDULLAH MAKHZOUM** hereby confirm that I have inspected the above titled thesis, and, to the best of my knowledge, it is based on the original work of the candidate.

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Date: 3/10/2022

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DEDICATION

I would like to dedicate this to future me, to remind myself of how much I have learnt since attaining the qualification. I would also like to extend the dedication to my parents for their immense emotional and financial support through my study period.

LIST OF ABBREVIATIONS

NPs	Nanoparticles
AgNPs	Silver nanoparticles
Ag	Silver
Ag ⁺	Silver ion
UV-vis	Ultraviolet visible
XRD	X-ray Diffractometer
FTIR	Fourier Transmission Infra-red
SEM	Scanning Electron Microscopy
STEM	Scanning Transmission Electron Microscopy
EDX	Energy Diffraction Dispersive X-ray
FAAS	Flame Atomic Absorption spectroscopy
FW	Fresh Weight
DW	Dry Weight
GAE	Gallic acid Equivalent
QE	Quercetin Equivalent
Tris-HCL	2-Amino-2-(hydroxymethyl) propane-1,3-diol hydrochloride
PMSF	Phenylmethylsulfonyl fluoride
BSA	Bovine serum albumin
DMSO	Dimethyl sulfoxide
MS	Murashige and Skoog
pH	Potential of hydrogen
kPa	Kilopascal
M	Molar
mM	Millimolar
nm	Nanometer
µg/mL	microgram per milliliters

rpm	Revolutions per minute
mL	Milliliters
g/L	Grams/litre
μL	Microlitres
°C	Degree celsius
%	Percentage
g	Grams
xg	Times gravity
SDS-PAGE	Sodium dodecylsulphate polyacrylamide gel electrophoresis
ppm	parts per million
ROS	Reactive Oxygen Species

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ABSTRACT

Nanoparticles are used extensively in agriculture, including in fertilizers to enhance crop quality and strategies to reduce heavy metal contamination. They are also used in nano-enabled platforms for improved agrichemical delivery, plant signal monitoring, and food security. Silver (Ag) is a noble and reliable element, which is why silver nanoparticles are so popular. The increased usage of nanoparticles endangers the environment and causes an ecosystem to become unbalanced. Silver nanoparticles synthesized from silver salts have outstanding properties being antibacterial, antifungal, photochemical, and physicochemical properties. Silver nanoparticles will inevitably be released into the environment through their synthesis, incorporation into products, recycling, and disposal due to their exponential development and expansion. Plants are the first point of entry of nanoparticles into the other trophic levels of the ecosystem through their roots or leaves. According to research in plant biology and biotechnology, depending on the plant species or kind of nanoparticle, nanoparticles can have both positive and negative impacts on plants or have no effect at all. Changes in the morphology, physiology, and metabolism of the plant are signs of the nanoparticle effect on plants. The SEM and STEM examination revealed that the silver nanoparticles made from *S. spinosa* leaf water extract had uniformly dispersed spheres with an average size of 53.4 nm. Additional confirmatory tests using UV-vis, XRD, EDX, FTIR, and Raman spectroscopy supported the presence of these nanoparticles. The nanoparticles were silver with traces of oxygen and carbon (EDX), stable at 427 nm (UV-vis), and had cubic lattice crystals with a crystalline size of 16.75 nm (XRD). The nanoparticles were stabilized by biomolecules with amide and hydroxyl groups (FTIR and Raman). AgNPs (12.5, 25, 50, 100, and 200 µg/ml) were used in varied concentrations to treat *S. cocculoides* and *S. spinosa in vitro* cultures. *Strychnos* seedlings did indeed acquire silver nanoparticles as shown by the rising silver ion content with each rising AgNPs treatment concentration, according to flame atomic absorption spectroscopy. Except for germination percentage and dry weight biomass, silver nanoparticles considerably impacted growth and development overall. The metabolites of the seedlings were also significantly impacted by AgNPs in a dose-dependent manner; total anthocyanin, total flavonoid, total phenol, and total protein content all increased while total chlorophyll content dropped with increasing AgNPs treatment concentration. Silver nanoparticles triggered an accumulation of ROS that resulted in imposing oxidative stress on *Strychnos* seedlings. A reduction in chlorophyll content demonstrated how the accumulation of ROS hampered seedling growth and development. As a crucial component in the process by which plants produce their own food, photosynthesis, chlorophyll decreases the amount of food a plant requires to grow, resulting in reduced growth and development. Seedlings had more phenols and protein than usual because plants have built-in defense strategies against oxidative stress, including overexpression of antioxidative compounds like phenols and proteins. Future research should consider ultramicroscopic examination of the tissues from *Strychnos* seedlings to see how AgNPs affect ultracellular structures. Genomic analysis of genes involved in *Strychnos* is recommended.

CHAPTER 1.0: INTRODUCTION

Nanotechnology is a study that focuses on synthesis of nanomaterials, investigation, and applications of these molecules in different sectors (Ahmed, et al. 2012; Banerjee, et al. 2010). These molecules are nano sized and have a high surface area to volume ratio, thus exhibit novel properties that are not shared with the same bulk material/particles (Ahmed, et al. 2012; Ranu, et al. 2012). These properties include good electrical conductivity, increased efficiency of photochemistry, enhanced surface plasmon resonance, hence the broad applications in areas such as medicine, biotechnology, agriculture, and water treatment (Ma 2016; Sudha, et al. 2018).

Nanoparticles are widely applied in agriculture including nano-enabled platforms for enhanced agrichemical delivery, plant signal monitoring, food security, fertilizers to improve crop quality and strategies to reduce contamination by heavy metals (Ma 2016; Zare-Bidaki, et al. 2022). Carbon, silver, and silicon nanoparticles are explored for their capacity to protect plants against pathogens and enhance their growth as nano-pesticides, nano-herbicides, nano-fertilizers, nano-sensors and nano-molecular carriers for proteins, nuclei acids, plant growth hormones and other molecules needed by the plants (Prasad, et al. 2014).

Silver nanoparticles, synthesized from silver salts have excellent physicochemical, photochemical, antibacterial, and antifungal properties (Kora, et al. 2009; Vance, et al. 2015). Silver(Ag) is a noble and stable material hence the prevalence of silver nanoparticles (Kora, et al. 2009). In 2015, the US market for silver nanoparticles (AgNPs) was reported at \$1 billion and is projected to be at \$3 billion by 2024, and among other nanomaterials, silver nanoparticles comprise of 25% market share of nano-based consumer product (Vance, et al. 2015).

Exponential development of AgNPs results in inevitable discharge of these nanoparticles into the environment during fabrication, integration into products, recycling, and discarding of the products (Handy, et al. 2008; Vance, et al. 2015). Air, soil, and aquatic environments are various reservoirs where there is possible accumulation of nanoparticles over time (Handy, et al. 2008; Klaine, et al. 2008; Zhang, et al. 2018). Numerous studies on effects of nanoparticles to both the terrestrial and aquatic environment are well documented. Discharge of nanoparticles and nanomaterials into the environment imposes potential toxicological risks of nanoparticles to/on living organisms and the ecosystem balance (Künniger, et al. 2014; Vance, et al. 2015; Zhang, et al. 2018).

In plant biology and biotechnology research, nanoparticles have been shown to have both positive and negative effects, or can have no effect at all, depending on the plant species or nanoparticle type (Pacheco and Buzea 2018; Siddiqui, et al. 2015). Some nanoparticles can enhance plant growth and development (Arora, et al. 2012; Delfani, et al. 2014; Rizwan, et al. 2019; Xin, et al. 2020), while some can have an inhibitory role depending on the chemical reaction of the nanoparticles with the plant cell milieu that causes changes to the molecular nature of the plant (Pacheco and Buzea 2018; Pittol, et al. 2017; Siddiqui, et al. 2015).

Nanoparticle effects on plants manifest as changes in the morphology and physiology of the plant. Silver nanoparticles have shown potential to reduce fungal and bacterial contamination during plant *in vitro* culture (Cromwell, et al. 2014;

Mishra, et al. 2014). Indeed, these nanoparticles can also inhibit plant growth and development by weakening and/or damaging various stages of cell division and disintegrating root cortical cells, epidermis, and root caps (Pittol, et al. 2017; Syu, et al. 2014; Zhang, et al. 2018).

Strychnos species are dicotyledonous plants from the Loganiaceae family and are found in Africa and some parts of Asia (Bisset 1988; Mwamba 2006). Interestingly, *Strychnos* has a variety of medicinal benefits owing to their concoction of secondary metabolites. Studies have shown that it has high content of alkaloids, phenols, reducing sugars, terpenoids hence its vast ailment treating potential (Bisset 1988; Mwamba 2006; Phillips 1991). A variety of secondary metabolites found in plant extracts have been investigated for their potential to form nanoparticles. It was observed that when these secondary metabolites are combined with metal ion salts, they serve as reducing agents, producing nanoparticles (Mittal, et al. 2013). *Strychnos* plants were used in this study to synthesize nanoparticles and the investigation of the interactions between *Strychnos in vitro* plants with nanoparticles.

1.1 PROBLEM STATEMENT

Continued use of nanoparticles leads to environmental contamination and has the potential to endanger public health at large. Following mercury, silver is an extremely toxic metal to aquatic ecosystem. Even though AgNPs are stable, upon oxidation they leak silver (Ag^+) ions which are more active, bio-accumulative, and toxic to the environment.

Understanding the effects of AgNPs on plant growth and development is essential for the assessment of any possible environmental threats to food safety and human health imposed by AgNPs since plants are the foundational element of the ecosystem and the most significant source of food for humanity. Terrestrial plants are exposed to AgNPs in the environment through leaking from nano-enabled products, deliberate sub-surface release for environment remediation, irrigation using contaminated water, land application of contaminated biosolids and wastewater effluent discharge. In agriculture, contaminated waters may permeate the fields through fertilizers and irrigation and eventually enter the plant rhizosphere. Plants absorb these nanoparticles from the soil, distribute them internally via translocation and then introduce them into food webs.

1.2 SIGNIFICANCE/JUSTIFICATION

Studies on the phytotoxicity of nanoparticles are crucial for evaluating possible threats to the environment and human health. A conduit for transport in the environment, plants serve as the foundational element (primary producers) of the ecosystem and are a significant source of trophic transfer of pollutants. Though toxic, NPs have potential to elicit growth and secondary metabolite production and accumulation in plants. Silver nanoparticles are widely used as nanoparticles due to the noble nature of the silver metal and are therefore expected to enter the ecosystem thus increasing bioavailability of nanoparticles.

Different organisms respond in different ways when exposed to silver nanoparticles, therefore there is need to understand the mechanisms of AgNPs toxicity in terrestrial plants given the limited studies investigating this matter. AgNPs' potential

toxicity must be better understood to forecast potential hazards to the environment and human health from increased usage of these compounds.

Strychnos species are adopted as the model plant at the plant biotechnology research lab at Botswana International University of Science and Technology in the Biological Sciences and Biotechnology department. As a group of researchers, we have taken a keen interest to explore where these plants can be beneficial starting with documenting its abundance in Botswana. These plants have benefits to people and animals as a source of food. Other species of *Strychnos* such as *S. potatorum* have been investigated for their deflocculation (Rajalakshmi, et al. 2021) and water purification properties (Sarawgi, et al. 2009). We therefore seek to explore how *Strychnos cocculoides* and *Strychnos spinosa* interact with AgNPs, whether these nanoparticles can be an enhancer or inhibitor during growth of the two species.

There is a need to obtain more information and data about trophic contamination in aquatic environments and transfer of AgNPs into terrestrial systems. There is also a need to understand the fate, behavior, and localization of AgNPs in plants. This study therefore is crucial in compiling already done studies, or direct future research that seeks to better understand nanotoxicology as well as to assess the potential implications on *Strychnos* species growth and provide insights on the possibility of using this species to remediate AgNPs in the soil.

Green synthesis of silver nanoparticles from plants is biologically safe as compared to the pathogenic micro-organisms such as *E. coli*, algae, *cyanobacteria* etc. Comparing green synthesis of nanoparticles to other approaches such as chemical and physical nanoparticle synthesis, green synthesis is less toxic to the environment and is cost effective. In accordance with the in-fashion quest to save the environment these study aims to be environmentally sustainable and reduce expenditure on reagents and resources.

1.3 AIM OF STUDY

To evaluate the impact of silver nanoparticles on *Strychnos* growth, development, and metabolism

1.4 OBJECTIVES

- To synthesize and characterize silver nanoparticles from *Strychnos spinosa* leaves by bio-reduction of silver nitrate salt solution with *S. spinosa* leaf extract.
- To analyze silver ion accumulation in *Strychnos* seedlings treated with the green synthesized silver nanoparticles
- To assess the effects of silver nanoparticle on *Strychnos* early growth parameters and metabolism

CHAPTER 2.0: LITERATURE REVIEW

2.1 *Strychnos* Species

Strychnos is a genus belonging to the Loganiaceae family of woody plants, that is pantropical and is made up of 200 species (Mwamba 2006; Struwe, et al. 2018). *Strychnos* plants are widely distributed in tropical and sub-tropical regions in Asia, America, and Africa (Moore 1947; Struwe, et al. 2018). *Strychnos spinosa* Lam and *Strychnos cocculoides* Baker are both trees from Eastern and Southern Africa occupying Sahara and sub-Saharan Africa (Mwamba 2006). These plants grow in sandy soils and can be found along riverbanks hence their adaptation to the tropical and arid regions of Africa (Isa, et al. 2014; Mwamba 2006; Sitrit, et al. 2003; Sunghwa and Koketsu 2009).

Strychnos species are known for their poisonous compounds which have been identified as alkaloids. The alkaloids found in these plants include mainly strychnine and brucine (Neuwinger 1998). In the past, these alkaloids were used in different applications; for instance, curare was used as an arrow and dart poison in South America and Malaysia, to help with hunting (Borgia, et al. 2017; Neuwinger 1998; Phillips 1991). Adding to alkaloids, terpenoids, steroids, tannins, reducing sugars and saponins are some of the other compounds accumulated in different *Strychnos* plants (Isa, et al. 2014; John, et al. 2015; Shoko, et al. 2013; Sudhira, et al. 2015; Sunghwa and Koketsu 2009).

Due to the abundance and variety of secondary metabolites, *Strychnos* is a medicinal plant which is indigenously used to prepare body ailment treatment concoctions. Roots, barks, and leaves are used in different forms, either as powder or in solution to treat venereal diseases, leprosy, diarrhea, fever, and microbial infections (Aremu and Moyo 2022; Isa, et al. 2014). The synergistic activity of *Strychnos* secondary metabolites confer its cytotoxic, antioxidant, anti-inflammatory and antimicrobial properties (Bero, et al. 2009; Chinsembu 2016; Isa, et al. 2014; Mwamba 2006; Tor-Anyiin, et al. 2015). *Strychnos* are also a source of food to both people and herbivores. Fruits of edible *Strychnos* species are rich in vitamin C, zinc and iron and are therefore consumed and processed to by-products such as fruit jams, fruit rolls and juice while leaves are fed on by herbivores (Mwamba 2006; Ngadze, et al. 2017).

Industrial use of *Strychnos* species to decontaminate the environment is less explored yet there is potential for this application to flourish. Environmental contamination is eminent with population growth coupled with development and usage of nanoparticles. Therefore, a search for environmentally friendly practices to circumvent this challenge is necessary. *Strychnos* inclusion into agro-forestry is being explored around the world, and this involves its possible application in bioremediation practices such as phytoremediation (Oppelt, et al. 2005).

Phytoremediation focuses on using plants to avert air, soil, and water pollution. This can occur through absorption, flocculation, coagulation, precipitation, reverse osmosis, evaporation, and absorption (Ahmad, et al. 2012; Alenazi, et al. 2020; Mahmood, et al. 2011; Senthil Kumar, et al. 2013), and these plant species can be investigated to assess their remediation capacity. *Strychnos potatorum* is a popular plant species abundant in India that is being incorporated into phytoremediation. According to the literature, *Strychnos* seeds possess high polysaccharide content (Corsaro, et al. 1995) which are the active remediation components (Vishali and

Karthikeyan 2014), shown to successfully de-flocculate effluent water (Babu and Chaudhuri 2005), remove reactive orange M2-R azo dye from aquatic bodies (Sankar, et al. 2015), adsorb copper, nickel, and cadmium from aquatic water bodies. *S. potatorum* seeds have the capacity to successfully coagulate industrial paint effluent (Senthil Kumar, et al. 2013).

Since this is an indigenous yet useful plant, its propagation is important to preserve its germplasm. In nature, plants perpetuate through seeds, vegetative parts, or special modified organs. Plant tissue culture is a laboratory conditioned technique that is useful in propagating plants (Bhojwani and Razdan 1986). There are different plant tissue culture techniques, categorized into sexual and vegetative propagation used in plant biology research (Cabahug, et al. 2018). Examples of such techniques are embryo culture, meristem culture, suspension culture, callus culture, micro propagation, etc. (Espinosa-Leal, et al. 2018; Smith and Drew 1990; Twaij, et al. 2020). Embryo culture involves extraction of embryos from seed and growing them in sterile plant growth media in small jars (Bhojwani and Razdan 1986; Kundu, et al. 2017; Pierik 1997). This technique has been shown to establish sterile *in vitro* cultures of different *Strychnos* species (Heita 2018; Leite, et al. 2021).

Strychnos spinosa Lam and *Strychnos cocculoides* Baker, figure 2.1 are both trees from Southern Africa occupying Sahara and Sub-Sahara regions and are popularly known as monkey orange trees, because the fruits are round with an orange resemblance and are consumed by monkeys (Mwamba 2006; Sitrit, et al. 2003). These trees are small deciduous shrubs approximately 9 m tall (Sitrit, et al. 2003; Struwe, et al. 2018). *Strychnos spinosa* Lam is characterized by branching from low down; light green leaves, spiny branchlets and grey rough bark while *Strychnos cocculoides* Baker is characterized by a spreading open crown, dark green and hairy leaves, and a thick brown bark (Sitrit, et al. 2003; Struwe, et al. 2018).

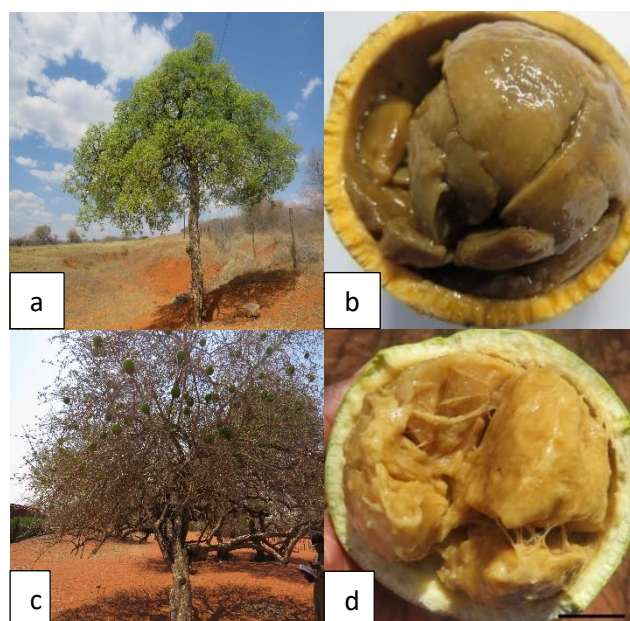


Figure 2.1: a) *S. cocculoides* whole plant and b) mature fruit, c) *S. spinosa* whole plant and d) mature fruit

2.2 NANOPARTICLES APPLICATION IN PLANT BIOTECHNOLOGY

Nanotechnology is a field that is based on studying and evaluating the applications of nanomaterials in various industries such as agriculture, pharmaceuticals, food packaging, and medicine (Ahmed, et al. 2021; Othman 2014; Robles-García, et al. 2016). Nanoparticle-enhanced biotechnology tools applicable to agriculture are focusing on improving food quality and increasing global food production (Kole, et al. 2013; Wang, et al. 2020), on plant protection from pests and diseases (He, et al. 2019), on increasing plant stress tolerance, detection of plant diseases (Wu, et al. 2017) and on monitoring plant growth and development (Adisa, et al. 2018; Liu and Lal 2015; Nandhini, et al. 2019).

Plants take up nanoparticles either by roots or through leaves, and plant cell wall pore sizes affect passage of nanoparticles into the plant (Asli and Neumann 2009; Sabo-Attwood, et al. 2012). Aerosol nanoparticle uptake through the stomatal pathway to the stem and to the other cellular districts mainly affects aerial parts of the plant such as the leaf (Shukla, et al. 2016) while adsorption from the soil prominently affects the root structure as nanoparticles oxidize and form toxic metal ions (Syu, et al. 2014; Ur Rahim, et al. 2021). Selective uptake through the root system enables translocation and biotransformation thus storing nanoparticles in plant cells (Milewska-Hendel, et al. 2019; Sabo-Attwood, et al. 2012).

Novel reactivity of nanoparticles allows for easy interactions with root secretions and specific membrane transporters. Nanoparticles can alter their chemical properties and reaction tendencies in their environments and aggregate to form complexes with organic matter and colloids in soil and water (Sudha, et al. 2018; Zare-Bidaki, et al. 2021). Therefore, their bioavailability for living organisms is subject to abiotic factors such as unregulated release or disposal of silver nanoparticles.

Effects of nanoparticles on plants have been studied in terms of seed germination, plant growth promotion and metabolism. Effects of nanoparticles on plants can either be positive or negative depending on the inherent physicochemical properties of the nanoparticles used as well as their nanoparticle concentration, and varies from one species to another (Ma, et al. 2010; Mohamed and Kumar 2016). Metal nanoparticles and their oxides as well as coated nanoparticles are different classes of nanoparticles usually studied in relation to their effects on seed germination, shoot/root growth, biomass production, and physiological and biochemical activities (Mohamed and Kumar 2016).

Synthesized nanoparticles can exert positive effects on plants by enhancing plant growth and development at the optimum dosage. Increased absorption of solar radiation, uptake of minerals (Fe, Cu, Zn, P, and Mg) and enzyme activities (dehydrogenase, esterase, acid phosphatase, alkaline phosphatase, nitrate reductase) in *Triticum aestivum* L. (wheat) and *Vigna unguiculata* subsp. *unguiculata* (black eyed peas) were observed on application of nano-magnesium at a concentration ranging from 0.2 to 0.5 g/L (Delfani, et al. 2014). According to (Deshpande, et al. 2017; Raliya, et al. 2016), it was observed that upon foliar application of zinc oxide NPs (ZnO-NPs), Zn is released in the acidic stomatal interior and translocated via the phloem with the highest absorption in the leaves followed by the stem, seeds, and shoots.

2.3 NANOPARTICLE MECHANISMS OF ACTION ON PLANTS

Increasing production and applications of nanoparticles in different disciplines results in their eminent release into the environment. Introduction of nanoparticles into the environment by anthropogenic activities is through domestic wastewater, transport and chemical manufacture, environmental remediation efforts as well crop improvement (Medina-Pérez, et al. 2019; Rajendran, et al. 2022). Nanoparticles find their route for bioaccumulation into the food chain by being absorbed by the plant root system from soils and water or uptake through the stomatal pathway in the leaves from the atmosphere (Battke, et al. 2008; Da Silva, et al. 2006; Wang, et al. 2013). The impact of nanoparticles on plant growth and development is influenced by nanoparticle surface area, surface coating, size, concentration, synthesis method used and plant species, genotype, age, development stage and chemical milieu of the plant cells (Cox, et al. 2016; Mehrian and De Lima 2016).

Plants are constantly challenged by heavy metal toxicity which affects cellular and subcellular components altering the plants' physiology. At the core of metal nanoparticle induced stress is the production of reactive oxygen species (ROS) by the plants, due to the release of toxic metal ion (Jalil and Ansari 2019; Rascio and Navari-Izzo 2011; Shahid, et al. 2014; Sharma, et al. 2012). According to Wan, et al. (2019), zinc oxide nanoparticles can enter the plant root system of *Arabidopsis thaliana* by inducing endocytosis as it was observed that plants treated with the nanoparticles had an increased uptake of FM4-64 stain. At the core of metal nanoparticle induced stress is production of ROS by the plants, due to release of toxic metal ion (Jalil and Ansari 2019; Rascio and Navari-Izzo 2011; Shahid, et al. 2014; Sharma, et al. 2012).

Metal nanoparticles undergo redox reactions with plant components and produce free radicals, which then leads to oxidative stress that generates excess ROS with the potential to oxidize proteins, lipids, carbohydrates and nucleic acids, either as a stress signal to trigger the defense system (Tripathy and Oelmüller 2012) or to aggravate cell damage (García-Sánchez, et al. 2015; Kaveh, et al. 2013; Kim, et al. 2015; Nasci, et al. 2000; Pospíšil, et al. 2019; Vannini, et al. 2013). Alterations of the plant macromolecules leads to changes in biochemical function, physiology, cell structure and plant growth. Excessive metals cause oxidative stress in plants either by direct effects of environmental stress or by indirect reactive oxygen species generation and accumulation which damages plant cells before elimination (Czarnocka and Karpiński 2018; Tripathy and Oelmüller 2012).

Reactive oxygen species are natural by-products of cellular metabolism that are generated by metabolic processes in plants as intermediate products of the reduction of ground state oxygen to water (Apel and Hirt 2004; Karuppanapandian, et al. 2011; Pospíšil, et al. 2019). Oxygen is reduced for energy production and in the process the following ROS species are sequentially produced: Singlet oxygen ($^1\text{O}_2$), superoxide (O_2^-), hydroxy peroxy radical (HO_2), hydrogen peroxide (H_2O_2) and hydroxyl ($-\text{OH}$) (Apel and Hirt 2004; Halliwell and Gutteridge 2015; Karuppanapandian, et al. 2011; Tripathy and Oelmüller 2012). These ROS are produced within plant cells particularly the chloroplast, mitochondria, peroxisomes, endoplasmic reticulum, and plasma membrane (Czarnocka and Karpiński 2018; Tripathy and Oelmüller 2012).

Since plants are sessile and cannot move to avoid stressors, accumulation of ROS in plant cells will result in a state of oxidative stress as levels of ROS will be higher than can be handled by the antioxidant defense system (Jalil and Ansari 2019; Tripathy and Oelmüller 2012). Toxicity of nanoparticles can be associated with release of toxicants from the surface such as metal ions or residues after synthesis. To cope with oxidative stress, plants have mechanisms with which to scavenge ROS produced in excess under stress conditions in cells. Biotic and abiotic factors alter the equilibrium between production and removal of ROS which are continuously removed by the plants' enzymatic and non-enzymatic actions in a complex defensive system (Tripathy and Oelmüller 2012).

The enzymatic ROS defense system comprises of different enzymes such as catalase (CAT), guaiacol peroxidase (GPOX), ascorbate peroxidase (APOX), superoxide dismutase (SOD), glutathione reductase (GR) and dehydrogenase reductase (DHAR) and low molecular weight antioxidants including thiols and ascorbates which act as ROS scavenging components (Czarnocka and Karpiński 2018; Tripathy and Oelmüller 2012). Secondary metabolites such as alkaloids, phenols and flavonoids have antioxidant capacity. In fact, antioxidant activity and total phenols increased 2.2-fold in *Raphanus sativa* treated with titanium dioxide nanoparticles (He, et al. 2021).

Studies on nanoparticle interaction with plants pave a way for a comprehensive mechanistic understanding on nanoparticle toxicity. This will enable the development of biomarkers for toxicity as well as evaluating underlying biochemical and molecular mechanisms. Nanoparticles have significant effect on physiology and morphology of plants, biochemical make up as well as gene expression.

2.3.1 PHYSIOLOGICAL AND MORPHOLOGICAL EFFECTS OF NANOPARTICLES ON PLANTS

The whole plant, roots, stems, or leaves have direct interactions with environmental matrices which contain nanoparticles. Interaction of nanoparticles with plants induces several morphological and anatomical changes. Analyzing physiological effects is relevant as it provides comprehension of how nanoparticles affect plant growth and development which in turn interferes with timing of flowering, senescence, fruiting, dormancy, and death (Wan, et al. 2019).

Plant growth, biomass, leaf surface area, germination, root, and shoot length are the aspects assessed to predict the effects of nanoparticles on plant growth and development. Nanoparticle toxicity on plants is dependent on treatment time and concentration. In a study by Boonyanitipong, et al. (2011), it was observed that rice (*Oryza sativa*) treated with zinc oxide nanoparticle had 100% germination, root length reduced at 100 mg/L and was greatly inhibited at 500 mg/L to 1000 mg/L, with longer soaking time inhibiting root growth. Reduced root and shoot length was observed in rice treated with gold nanoparticles as compared to the control while the germination effect was minimal (93%-95%) (Ndeh, et al. 2017).

Multiwalled carbon nanotubes had a positive effect on root and shoot elongation in wheat, maize, peanut, and garlic while germination and biomass were improved at low concentrations (Srivastava and Rao 2014). In a study conducted by Rafique, et al. (2019), TiO₂ enhanced millet (*Pennisetum glaucum*) germination rate as compared to control. Selenium nanoparticles investigated on *Hordeum vulgare* by

Siddiqui, et al. (2021) successfully enhanced morpho-functional parameters of seeds stored for more than 10 years. In their study, Siddiqui, et al. (2021) revealed that shoot and root length as well as germination percentage were enhanced to 13.85 mm, 24.07 mm and 65%, respectively, when treated with 4.65 µg/mL selenium nanoparticles as compared to the control which recorded 7.40 mm, 13.85 mm and 45%, respectively. Wheat germination index, root length and shoot length decreased with increased copper nanoparticle concentration (Essa, et al. 2021).

In a study done by Wan, et al. (2019), it was shown that zinc oxide nanoparticles can enter the plant root system of *Arabidopsis thaliana* by inducing endocytosis because plants treated with nanoparticles had an increased uptake of FM4-64 staining agent. Endocytosis has been observed to be a factor that enables entrance of nanoparticles into the plant root system, observed by disrupted and disorganized actin microfilaments, thereby disturbing transport and localization of plasma proteins leading to a disrupted auxin transport and subsequently repressing root growth and development (Wan, et al. 2019).

Physiological effects of nanoparticles on plant structures are assessed by techniques such as Transmission Electron Microscopy (TEM), light microscopy, and fluorescence microscopy etc. (Rajput, et al. 2019). Ultrastructural deformities of the cell structures such as epidermal walls, cell walls, and chloroplasts and cortical cell layers are affected by plant- nanoparticle interactions visible through these techniques.

Oak (*Quercus robur* L.) chloroplasts treated with 50 mg/L copper and silver nanoparticles exhibited large starch granules and plastoglobules as compared to control plants when visualized with TEM (Olchowik, et al. 2017). Barley seeds treated with silver nanoparticles had deformed chloroplasts that showed fewer grana thylakoid and stroma lamellae, ruptured nuclear envelope and small number of plastoglobuli as compared to the untreated plants (Fayez, et al. 2017). Increased number of peroxisomes and mitochondria with degenerated cristae and condensed nuclei were observed in root cells of tomato seedlings treated with Ni-O nanoparticles (Faisal, et al. 2013). High power optical microscopy and transmission electron microscope used to visualize the effect of fullerene nanoparticles in wheat (*Triticum aestivum* L.) seedlings, revealed that root endothelial cells were squeezed, and inner wall structures were damaged by the extrusion of nanoparticles (He, et al. 2021). Effects of silver nanoparticles on morphology of *Strychnos* species will be assessed in this study.

2.3.2 BIOCHEMICAL EFFECTS OF NANOPARTICLES ON PLANTS

Generation of ROS by nanoparticle oxidative stress has the potential to significantly affect fluidity and membrane permeability and consequently the kinetics of nutrient acquisition in plants. Therefore, transpiration rate, translocation hindrance, and net photosynthetic rate are some biochemical parameters used to assess effects of nanoparticles on plant biochemical functions.

In a state of oxidative stress, activity of anti-oxidative enzymes and non-enzymes such as superoxide dismutase and peroxidase, phenolics, alkaloids etc. are increased (Bhuyan, et al. 2019). In plants, nanoparticles inhibit photosynthesis which is an oxygen producing mechanism and this in turn interferes with food supply in the form of organic matter which forms the basis of food chain (Jiang, et al. 2017;

Zhao, et al. 2021). During oxidative stress there is inhibition in the activity of enzymes key to chlorophyll biosynthesis (Ahanger, et al. 2018; Wang, et al. 2020).

Tomato seedlings treated with 10 mg/L cerium oxide nanoparticles had the least water transpiration as compared to the untreated seedlings (Wang, et al. 2013). Fullerene nanoparticles blocked cell pores of wheat seedlings and resulted in a decreased transpiration rate as compared to untreated seedlings (He, et al. 2021). Titanium oxide nanoparticles had 23% increase on net photosynthesis in *Raphanus sativa*, however beyond 60 hours, net photosynthesis and transpiration rate decreased (He, et al. 2021).

Reactive oxygen species accumulation leads to photo-oxidative damage that is evident through the disintegration of chloroplasts. Nanoparticles have the potential to reduce the fluorescence parameter values, recognized by reduced photosystems efficiency which is concomitant with decrease in chlorophyll content (Tripathi, et al. 2017). These factors combined leads to photosynthesis inhibition. Wheat seedlings treated with silicon dioxide nanoparticles had an increased rate of photosynthesis as it is evident in increased photochemical efficiency of photosystem II, performance index and content of other photosynthesis pigments (Younis, et al. 2020).

Zinc oxide nanoparticles decreased the content of chlorophyll *a* and *b* and reduced photosynthetic efficiency and some other chlorophyll fluorescence parameters in a concentration-dependent manner of 35-day old tomato plantlets (Wang, et al. 2018). The Fe₂O₃ nanoparticles at a concentration of 500 mg/L caused slight increases in the net photosynthetic rate and stomatal conductance of 6 and 20 %, respectively in soybean (Alidoust and Isoda 2013).

Tomato seedlings under 8 mg/L treatments of zinc oxide nanoparticles had a 45% increase in protein content as compared to control plants. In the same study activity of antioxidant enzymes increased under ZnO treatment; CAT, POX, SOD and DAS showed a 69.7%, 65%, 80% and 45% increase respectively and proline also increased by 65% as compared to the control (Faizan et al., 2018).

In a study by Večeřová, et al. (2016), barley seeds treated with cadmium-oxide nanoparticles decreased photosystem II activity by 2%, total chlorophyll content and flavonol content by 14%; however, a significant negative effect was observed on primary metabolites such as amino acids and saccharides while the change was not significant in secondary metabolites at the exposure of cadmium oxide nanoparticles.

2.4 SILVER NANOPARTICLE APPLICATION IN PLANT BIOTECHNOLOGY

Silver nanoparticles (AgNPs) release silver ions which have either positive or negative effects on cells depending on the plant target site. AgNPs unregulated release into the environment affect both terrestrial and aquatic organisms (Haverkamp and Marshall 2009; Rastogi, et al. 2019). Plants that naturally grow in contaminated matrices absorb silver from their environment depending on concentration and chemical species and different oxidative states of silver ions allow for easy uptake and translocation into plants (Miralles, et al. 2012; Tripathy and Oelmüller 2012).

High level enrichment and accumulation of AgNPs in plants can occur when plants are grown in an environment rich in AgNPs and thus the transfer of AgNPs to food chains become inevitable (Tripathy and Oelmüller 2012). Increased AgNPs concentration results in severe reduction of biochemical and molecular parameters while oxidative damage indexes increase with increase in AgNPs concentration (Khan, et al. 2019; Rastogi, et al. 2019).

Silver nanoparticles have both negative and positive impact on plant growth parameters. Seed priming with AgNPs increased growth of wheat seedlings (Anjum, et al. 2013). According to Salama (2012), shoot, root length and leaf area were reduced at lower AgNPs concentration (20-60 mg/kg) while higher concentration (>60 mg/kg) inhibited development of these parameters in common bean and corn plants. *Arabidopsis thaliana* displayed a decrease in fresh weight and root weight upon treatment with 0-1.0mg/L AgNPs (Li, et al. 2018).

It is alleged that AgNPs get accumulated in the intracellular space of plant reducing its translocation capacity (Castro-González, et al. 2019). Microscopic analysis confirmed uptake and translocation of AgNPs in *Stevia*, where they were shown to be present within epidermal stem cells, in vascular bundles, intermembrane spaces as well as in ribs and stomata in leaves (Castro-González, et al. 2019) indicating that AgNPs were transported through the plant.

Upon oxidation of AgNPs the ions released interact with cytoplasmic components and reduce membrane permeability. This phenomenon resembles the activity of ethylene as it helps in extending vase life of cut flower crops by reducing water uptake through the aquaporins (Sreelekshmi, et al. 2021). Treatment of *A. thaliana* with AgNPs significantly decreased petal and pollen viability, reduced pod production and transcription of genes involved in flowering and floral organ development were downregulated by 10-40% compared to the control (Ke, et al. 2020). Flowering stage is a crucial, long, and complex stage in plant development, and it requires numerous genes for flower development therefore its disturbance hinders vegetative growth of the plant (Ke, et al. 2020).

Plant biochemical make up is also affected by AgNPs treatment. Biochemical components in the plant cells function to maintain cell homeostasis as well as helping with osmoregulation under stress (Mohamed, et al. 2017). Proline and total soluble sugar content was increased in wheat seedlings treated with lower concentrations of AgNPs (Mohamed, et al. 2017). According to Tripathi, et al. (2017) comparative total protein content reduction in plants exposed to AgNPs treatment is due to increased generation of reactive carbonyl groups produced under metal stress, this will in turn reduce total protein content in plants. Silver ions bind to thiol groups of proteins or they replaces copper ions and this causes misfolding and damage to proteins (Anjum, et al. 2013; Tripathi, et al. 2017). Due to different nanoparticle sizes, shapes and coating materials, Ag⁺ may interact differently with proteins and the dissolution of Ag⁺ ions increases the effects on proteins (Nangia and Sureshkumar 2012).

Photosynthesis can either be increased or decreased by interaction of plant intracellular environment with AgNPs Anjum, et al. (2013) and Mohamed, et al. (2017) observed that wheat seedlings treated with lower concentrations had high levels of chlorophyll content while at higher concentration chlorophyll content

decreased. Changes in photosystem photochemistry is examined through chlorophyll fluorescence and photosynthetic pigments measurements (Mohamed, et al. 2017; Tripathi, et al. 2017). *Arabidopsis thaliana* seedlings treated with silver nanoparticles had a decreased chlorophyll content as compared to the control, and this resulted in an inhibited photosynthesis efficiency thus reducing the capability of photochemical conversion to enable occurrence of photosynthesis (Li, et al. 2018).

Inhibition of photosynthesis is due to degradation of photosystems and chlorophyll, thylakoid lipids, O₂ evolution (Mohamed, et al. 2017; Tripathi, et al. 2017). Cucumber seedlings treated with AgNPs had decreased content of photosynthetic pigments (carotenoids and chlorophyll) as compared to the control (Tripathi, et al. 2017). Chrysanthemum plants treated with AgNPs had increased carotenoid and decreased chlorophyll a and b content (Tymoszuk and Kulus 2020). Overproduction of ROS leads to inhibited chlorophyll and carotenoid synthesis, which affects developing chloroplasts and causing a decline in chlorophyll content (Tripathi, et al. 2017).

Nanoparticles have the capacity to modulate secondary metabolism in plants by interfering with signaling pathways. Pharmaceutical industry has keen interest in application of AgNPs as a stimulus to improve the quality and quantity of products (Ramezani, et al. 2019). Nanoparticles enter the cells through the apoplast and cross the plasma membrane through endocytosis, influencing gene expression as it enters the nucleus (Ramezani, et al. 2019). In their study, Ramezani, et al. (2019) showed that treatment with 40 mM AgNPs increased transcription level of genes involved in steviol glycoside biosynthesis pathway acting as a strong amplifier of the transcriptional trigger for steviol glycoside biosynthesis pathway genes which have the potential to control the production of steviol glycosides positively.

Safety and toxicity aspects of nanoparticles lags behind the rate at which they are being produced (Ganguly, et al. 2018; Olawoyin 2018). This can be because there are no standards and agreements among researchers that guide study designs or experimental techniques in this area, or it might be because the special features of nanoscale materials which makes it difficult to conduct toxicological evaluations of new substances (Chakravarty and Vora 2021; Olawoyin 2018).

CHAPTER 3.0: MATERIALS AND METHODS

In here, biosynthesis of AgNPs from *S. spinosa*, how AgNPs affect *Strychnos* early growth parameters, metabolism and silver ion accumulation will be studied.

3.1 GREEN SYNTHESIS OF SILVER NANOPARTICLES

Silver nanoparticle synthesis was obtained following a protocol formulated by Rao and Tang (2017) with modifications.

3.1.2 *Strychnos* leaf extract preparation

Fresh *S. spinosa* leaves were collected from the Botswana International University of Science and Technology campus. The leaves were first washed with running tap water, followed by 70% ethanol for 3-5 minutes, rinsed with distilled water 3 times and left to dry for 5 days at 60 °C. The dried leaves were ground into fine powder using a pestle and mortar. A mass of 25 g of the leaf powder was weighed into a 300 mL Erlenmeyer flask and boiled with 200 mL distilled water for 10 minutes. The extract was filtered through a 150 mm filter paper and centrifuged at 4000 rpm for 15 minutes to get a cell free extract.

3.1.3 Silver nanoparticle synthesis

For nanoparticle synthesis, protocols from Mallikarjuna, et al. (2011) as well as Vanaja and Annadurai (2013) were used. Ninety milliliters (90 mL) 100 mM silver nitrate solution was added to 10 mL of the cell free extract obtained in 3.1.2, this was followed by incubation at 25 °C in a shaking incubator for 24 hours. The mixture was centrifuged at 4000 rpm for 15 minutes to separate AgNPs aggregates from the liquid. The obtained AgNPs were washed with distilled water to remove excess silver nitrate, and this was followed by drying the nanoparticles at 60 °C for 6 hours.

3.1.4 Silver nanoparticles characterization

For structural characterization and phase identification, diffraction peaks in the 2 θ scanning mode were recorded using Bruker D8 Advance X-ray diffractometer (XRD), with a CuK α radiation source of $\lambda=1.5406$ Å. The Raman spectrum was measured using a Horiba LabRAM HR Evolution Raman microscope and functional groups of the synthesized silver were investigated through Fourier Transformation Infrared spectroscopy (FTIR) (Bruker vector 22 Spectrophotometer) at transmission range of 400 cm⁻¹ to 4000 cm⁻¹. It is worth to note that during the synthesis, the formation of Ag nanostructures was analyzed through absorbance measurements recorded from a wavelength of 350- 800 nm using a Thermo Scientific Evolution 201 UV-vis spectrophotometer. Silver nanoparticles for Scanning Electron Microscopy (SEM) and Scanning Transmission Electron Microscopy (STEM) analysis were prepared by drop casting the nanoparticles on a carbon coated copper grid. The sample AgNPs were dispersed in double distilled water and a drop of the dispersion was placed on carbon coated copper grids. The excess solution was removed with a blotting paper and the film allowed to dry under a mercury lamp for 3 to 5 minutes. Imaging of the AgNPs was performed with the Zeiss Merlin FEG SEM coupled with a transmission mode detector. The presence of silver element was analysed with SEM coupled to energy Dispersive x-ray detector (Bruker, XFlash 4010) combined with SEM.

3.2 *Strychnos* TISSUE PROPAGATION

3.2.1 Plant sample collection and preparation

Strychnos fruits were collected around Palapye village. *Strychnos cocculoides* fruits were collected at coordinates (S 22°32'34.3" E 027°06' 00.0") while *Strychnos spinosa* was collected at the following coordinates (S 22°32'41.0" E 027°06' 19.5"). The collected fruits were washed with running tap water to remove dirt followed by sequential wash with fungicide (Verkop and vermicide), half strength 3.5% domestic bleach, 70% ethanol and sterile distilled water for 3-5 minutes.

Working aseptically under the laminar flow, fruits were opened using a scalpel to remove the seeds. The seeds were placed in 10% ethanol solution, cut open using a sterile blade and embryos were extracted from the seeds using sterile forceps. The isolated embryos were placed in 10% ethanol while awaiting to be planted. All the materials and equipment used were sterilized by autoclaving at 121⁰ C for 15 minutes at 103kPa.

3.2.2 Plant Growth Medium Preparation

Half strength media was prepared, according to the manufacturer's instructions, by dissolving 15 g sucrose, 8 g micro-agar and 2.215 g of Murashige and Skoog (MS) medium in 1 L distilled water. Concentrated hydrochloric acid and 2 M sodium hydroxide were used to adjust pH to 5.7-5.8. Silver nanoparticles 1 g/L stock solution was prepared by dispersing the nanoparticles in distilled water through ultrasound sonication. Silver nanoparticles were added into half strength MS media to different concentrations (12.5, 25, 50, 100, 200 µg/mL) before adjusting pH. This was followed by autoclaving at 121⁰ C for 15 minutes at 103 kPa. After autoclaving, media was poured into sterile magenta boxes aseptically inside the laminar flow.

3.2.3 Establishing sterile embryo culture

Embryos obtained in 3.2.1 above were cultured in the media prepared in 3.2.2 above aseptically under the laminar flow. Five embryos were cultured in each magenta box, and this was done in triplicates. The cultures were maintained in the dark at room temperature (25 °C) to initiate germination. After germination, the cultures were kept at alternating periods of 16 hours of light and 8 hours of darkness. The cultures were observed daily for germination from the day of culturing (Bian, et al. 2018).

Early growth characteristics such as shoot, and root length were observed and measured with vernier calipers. Fresh and dry weight measured using a balance. An embryo was considered to have germinated when the cotyledons were open and changing color from yellow to green.

3.3 SILVER ION ACCUMULATION ANALYSIS

Samples were prepared by acid digestion for Flame Absorption Absorbance Spectroscopy analysis according to Khan, et al. (2020) with modifications. One gram plant material was dried at 70 °C for 48 hours in an oven. The dried samples were then digested with 8 mL concentrated nitric acid at 80 °C for 1-hour, further digestion was carried at 120 °C for another hour. This was followed by sample decolorization with hydrogen peroxide. The sample was dried at 80 °C to evaporate hydrogen peroxide. Total volume of the digests was adjusted to 25 mL with distilled water followed by filter sterilization through 0.45 µm filters. A standard solution was prepared by dissolving 1.575 g silver nitrate in 100 mL of 2% nitric acid. Different

concentrations were prepared by diluting standard with distilled water to establish a standard curve. The instrument parameters were set to obtain maximum Ag content. Silver ion content in the *Strychnos* samples was analysed with a FAAS contra 700 in a single cycle with five washes.

3.4 METABOLISM ANALYSIS

3.4.1 Biomolecule Decomposition

Strychnos whole seedlings were dried at 70 °C for 48 hours and ground into fine powder. They were then analysed with Bruker VERTEX 70v vacuum FTIR spectrophotometer, with computer-based software-controlled operation and data processing. The following parameters we set to run the samples; 4cm⁻¹ resolution, 64 seconds scan time, 25 seconds measurement time, 300-400cm⁻¹ transmittance wavelength. The spectral data of treated samples was compared with the control spectral data to identify functional groups in the samples. The spectra were analysed with Origin 8.0 software (Origin Lab Corporation, Massachusetts, USA).

3.4.2 Total Chlorophyll Content

A total of 30 mg leaf sample from the plantlets was incubated at 60°C with 5 mL dimethyl-sulfoxide (DMSO) for 60 minutes. This was followed by centrifugation at 4200 g for 10 minutes and absorbance was recorded in triplicates at 665 nm and 648 nm. Chlorophyll a (Chl a) and chlorophyll b (Chl b) were calculated as milligrams of chlorophyll per gram of fresh weight of the leaf divided by volume of DMSO used as shown in equations 3.1 and 3.2. Total chlorophyll was calculated as a sum of Chl a and Chl b (equation 3.3). The protocol was adopted from (Barnes, et al. 1992).

$$Chlo\ a = (14.85 * A_{655nm}) - (5.4 * A_{648nm}) * (FW\ (g)/Volume\ (ml)) \quad \text{Equation 3.1}$$

$$Chlo\ b = (25.45 * A_{648nm}) - (7.36 * A_{655nm}) * (FW\ (g)/Volume(ml)) \quad \text{Equation 3.2}$$

$$Total\ chlorophyll = Chlo\ a + chlo\ b \quad \text{Equation 3.3}$$

3.4.3 Anthocyanin Content

30 mg leaves were added to acidified methanol (1% hydrochloric acid in methanol). This was followed by incubation at 4°C for 4 hours. The mixture was centrifuged at 4200 g for 10 minutes followed by taking absorbance at 530 nm and 657 nm. Anthocyanin content was calculated according to Laby, et al. (2000) and Mancinelli (1984) using equation 3.4 below

$$Anthocyanin = OD_{530\ nm} - (0.25 * OD_{657nm}) * \left(\frac{FW(g)}{Volume(ml)} \right) \quad \text{Equation 3.4}$$

3.4.4 Plant Extraction Preparation

500 milligrams of whole plant seedlings from 3.2.3 above were weighed and pulverized in liquid nitrogen with pestle and mortar. Phytochemicals were extracted with methanol. Secondary metabolites were extracted with 20 mL of the solvent twice using ultrasound technique at 40 °C for one hour. The extracts were filtered through 150 mm Whatman filter paper and dried in the fume hood in darkness at room temperature. After drying the extract was resuspended with (DMSO) to a concentration of 10 mg/mL. Resuspended extract was stored at 4 °C in darkness.

3.4.4.1 Total Phenol Content

To estimate total phenol content a modified protocol was adopted from Ribarova, et al. (2005) and Singleton, et al. (1999). A standard curve was made with gallic acid. Serial dilutions (0, 0.05, 0.1, 0.15 and 0.2 mg/mL) of the standard were made from the stock concentration of 10 mg/mL with methanol as the diluent. To 200 µL gallic acid dilution, 200 µL Folin ciocalteu reagent and 2 mL distilled water was added. This was followed by incubation in the dark for 8 minutes at room temperature. A volume of 600 µL of 20% sodium carbonate was added and incubated for 5 minutes at 60 °C. Absorbance was recorded at 760 nm. To 200 µL of 10 mg/mL plant extract the same procedure was followed. Total phenolics content was calculated as milligrams of gallic acid equivalent per gram of dry weight of the extracted (mg GAE/ dry weight of the extract). Total phenol content was calculated as;

$$\frac{=\text{Extrapolated concentration of th extract (mg/ml)}*\text{volume of extract (ml)}}{\text{mass of extract (g)}} \quad \text{Equation 3.5}$$

3.4.4.2 Total Flavonoid content

Total flavonoid content was estimated according to a protocol developed by (Chang, et al. 2002). Quercetin was used as a standard to make a standard curve. Serial dilutions (0, 25, 50, 100, 125, 150 mg/mL) of the standard were made from the 10 mg/mL quercetin stock with methanol as a diluent. To 200 µL quercetin dilution 200 µL 2% aluminum chloride, 200 µL potassium acetate, 600 µL distilled water was added followed by incubation in the dark at room temperature for 30 minutes. This was followed by taking absorbance at 420 nm. Total flavonoid content was calculated as milligrams of quercetin (QE) per gram of dry weight of the extract (mg QE/dry weight of the extract). Total flavonoid content was calculated as;

$$\frac{=\text{Extrapolated concentration of the extract (mg/mL)}*\text{volume of extract (mL)}}{\text{mass of extract (g)}} \quad \text{Equation 3.6}$$

3.4.5 Total protein Content

Total protein isolation protocol with modifications from Schlereth, et al. (2000) was adopted for this assay. Lysis buffer was prepared with 10 mM, Tris-HCL pH 8.0, 1 mM PMSF, 2% Sodium dodecyl sulfate, 30% sucrose. A total mass of 250 mg fresh leaf samples from each of the concentrations was ground to powder with liquid nitrogen and 10 mL lysis buffer was added to it. The mixture was thoroughly vortexed and centrifuged at 4200 xg for 20 minutes at 4 °C. The supernatant was decanted, and the unstable pellet was extracted again with lysis buffer.

3.4.5.1 Total Protein Content Estimation

Total protein content was quantified using the Bradford method according to Bradford (1976). To make the standard curve the following 2.0 mg/mL bovine serum albumin dilutions were made by aliquoting the following volumes of BSA stock solution: 0, 5, 10, 20, 30, 40, 50 and 100 µL and adjusting the volume to 100 µL with lysis buffer to 100 µL. Five milliliters of the Bradford reagents were added to the dilutions, followed by incubation for 45 minutes and readings were taken at 595 nm with the 200E spectrophotometer. Five hundred µL of the plant protein sample was added to 4.5 mL Bradford's reagent followed by incubation for 45 minutes and absorbance recorded at the same parameters as the standard. The results were recorded in triplicates.

3.4.5.2 Sodium-dodecyl sulphate Polyacrylamide Gel Electrophoresis

Resolution 12% and stacking 4.5% gels were prepared according to a recipe in Appendix a table 1. Isopropanol was added after loading resolution gel to level the surface while waiting for polymerization of the gel. This was followed by washing off isopropanol with distilled water and loading stacking gel. A volume of 10 μ L loading dye was mixed with 10 μ L sample extracts in Eppendorf tubes. The mixture was heated in a heating block for 5 minutes before loading the samples into the SDS page gel. The gel was run for 120 minutes at 120 volts and 400 amps current. *Strychnos* sample protein was run the Bio-rad dual color precision plus protein ladder. The gels were stained with iodine solution for 2 hours at room temperature followed by destaining for 30 minutes. The gel was visualized with Bio-Rad lasec chemi doc MP imaging system. These modified protocol was adapted from (Haider, et al. 2012) and (Jan, et al. 2016).

3.5 Statistical Analysis

Experiments were performed in triplicates and results were expressed as means of triplicates $\pm$ standard error mean (SEM). R-studio 4.0.4 (2021), Graph Pad Prism 9.4.0 and Origin 8-pro analytical softwares were used to analyse the data. Analysis of variance (ONE-WAY ANOVA) in *Strychnos* species responses to treatment with silver nanoparticles was performed and means were compared based on least significant difference (LSD) using Tukey's post-Hoc test at 95% certainty level ($P < 0.05$).

CHAPTER 4: RESULTS

Silver nanoparticles were successfully synthesized and characterized with Ultraviolet visible (UV-vis) spectroscopy, X-ray diffraction (XRD), Scanning electron microscopy (SEM), Scanning transmission electron microscopy (STEM), Energy dispersion X-ray (EDX), Raman spectroscopy and Fourier Transmission Infra-red (FTIR) spectroscopy. *Strychnos* embryos germinated and developed or grew successfully when treated with AgNPs suspension, and this enabled for further analysis as stipulated in chapter 3 above. Silver nanoparticles had a significant effect on early growth parameters and biochemical array of the treated seedlings. Silver ions accumulated in the seedlings as confirmed by Flame atomic absorption spectroscopy (FAAS).

4.1 BIOGENIC SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES

Formation of AgNPs was observed from a color change of extract and silver nitrate suspension from yellow to brown upon addition of silver nitrate solution as shown in figure 4.1. Silver nitrate alone did not show any color change at the same conditions indicating that the reduction of silver nitrate did not occur. Bioreduction of silver nitrate with *S. spinosa* extract was further confirmed with Uv-vis spectroscopy. A distinct peak was detected at 427 nm as shown in figure 4.2.

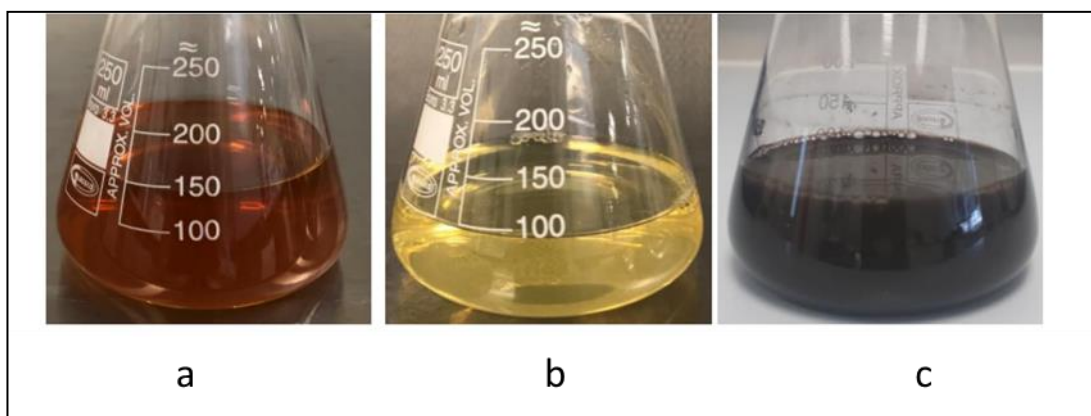


Figure 4.1: Bio-reduction of *S. spinosa* leaf water extract **a)** Leaf extract before adding silver nitrate, **b)** leaf extract with silver nitrate before incubation for 24 hours, **c)** silver nanoparticles in suspension

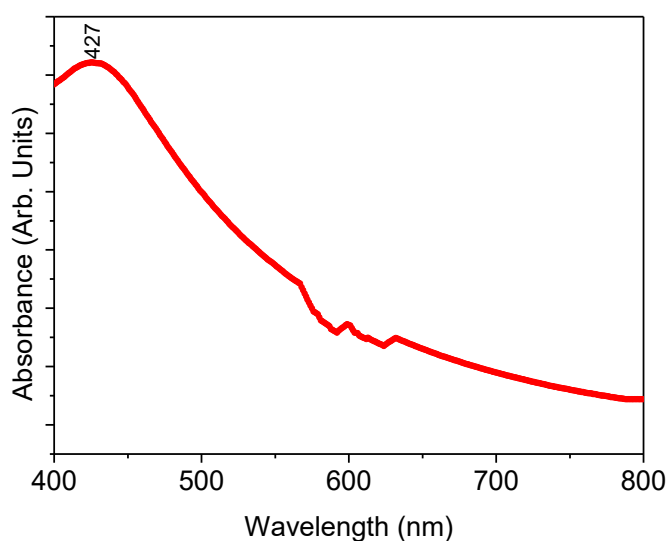


Figure 4.2: UV-vis spectra of the green synthesized silver nanoparticles show absorbance at 427 nm

X-ray diffraction profile of the synthesized silver nanoparticles is shown in figure 4.3. The strong peaks observed at state angles are assigned miller indices of (111), (200), (220), (311) and (222), respectively, as shown in figure 4.3 and table 2 in in Appendix b. The crystalline domain size was calculated by the Debye-Sherrer formula $D = K\lambda / \beta \cos\theta$ where D is the crystallite size in Angstroms, constant K is 1, β is the full width at half maximum, λ is the X-ray wavelength and θ is the diffraction angle.

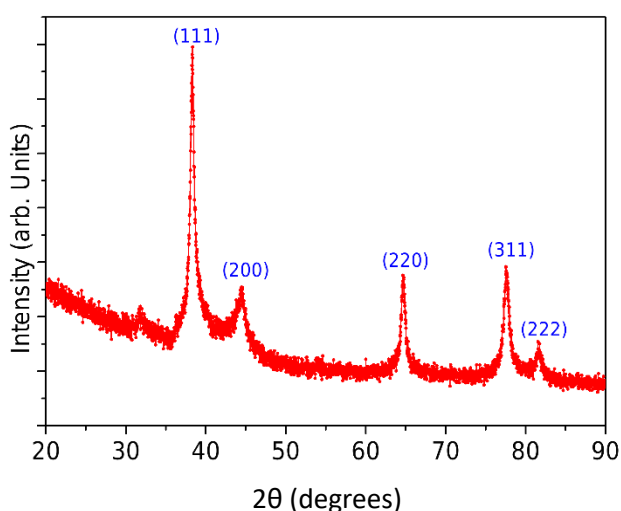


Figure 4.3: XRD peaks of *S. spinosa* water leaf extract synthesized silver nanoparticles correspond to the COD 2013 database ID COD 9004180.

The SEM and STEM images in figure 4.4 revealed a uniform distribution of spherical clusters. The AgNPs are also mono dispersed as shown by the STEM micrograph

(figure 4.4 c and d). The micrographs also showed a monodispersed spherical morphology of the nanoparticles. Figure 4.5 shows the EDX profiles of the AgNPs showing strong signals for elemental silver at 3KeV and other peaks between 0-1KeV are related to oxygen and carbon.

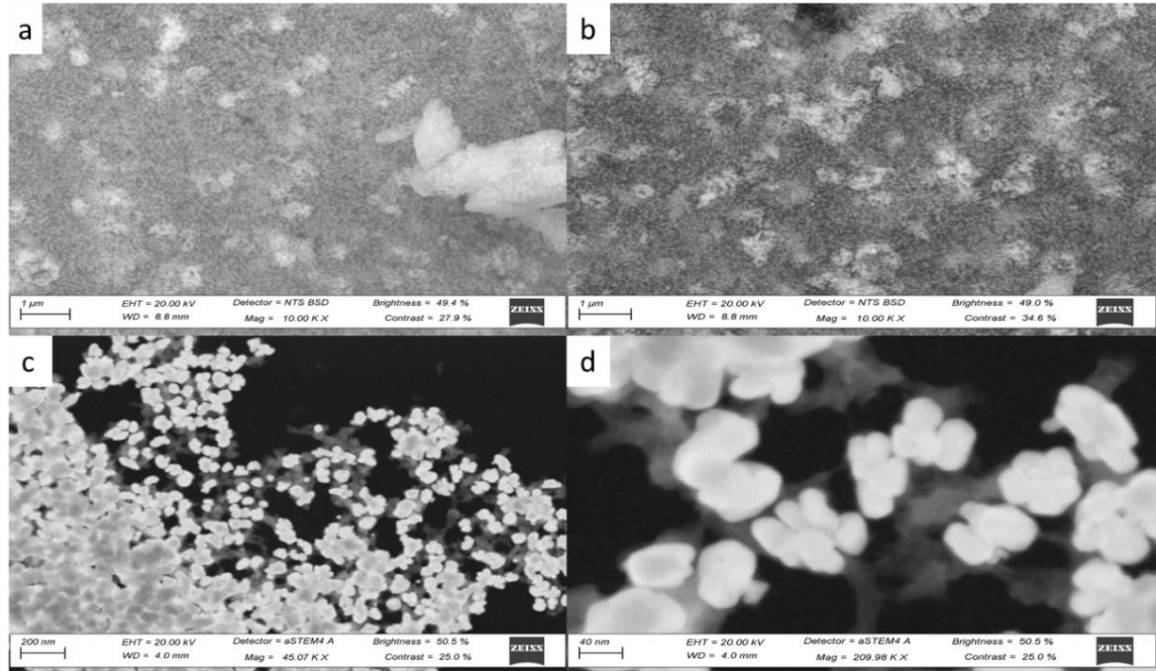


Figure 4.4: SEM (a and b) and STEM (c and d) micrographs of silver nanoparticles synthesized from *S. spinosa* leaf extract and silver nitrate solution

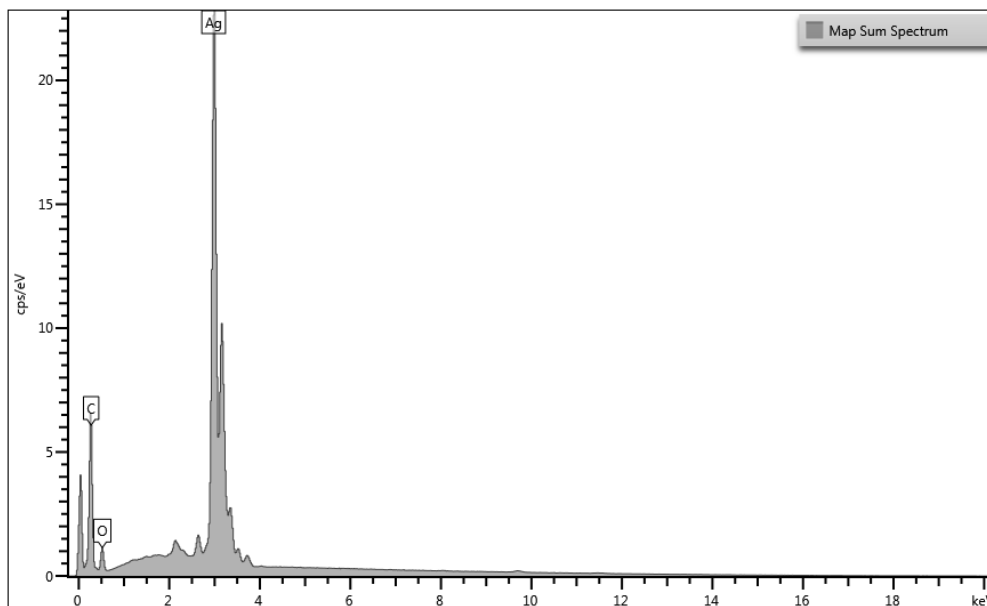


Figure 4.5: EDX spectrum showing elemental composition of silver nanoparticles synthesised by bio-reduction of silver nitrate solution with *S. spinosa* leaf extract.

Fourier Transformer Infrared Spectroscopy was used to identify the biomolecules within the *S. spinosa* plant extracts that are involved in silver nanoparticles synthesis by acting as stabilizers and capping agents. The FTIR spectra revealed peaks from 3728.1 to 950 cm^{-1} as shown in figure 4.6 below. The peaks assigned to O=C-OH/C-OH, C=O, C $\equiv$ O, C=C, C-O-C respectively.

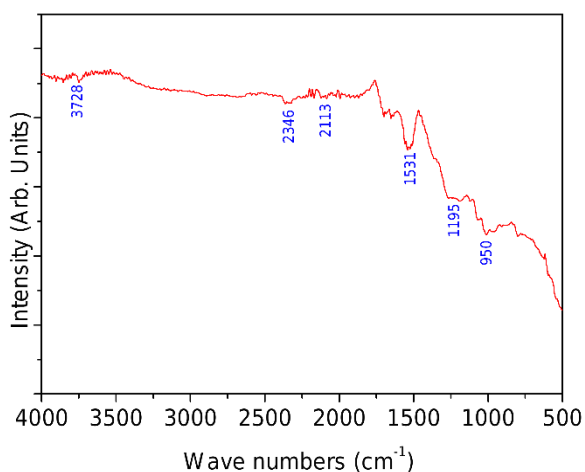


Figure 4.6: Fourier Transformer Infrared Spectroscopy spectra of green synthesised silver nanoparticles.

Raman spectroscopy was used to provide detailed information about the chemical structure phase and polymorph, crystallinity, and molecular interactions. Figure 4.7 shows the Raman spectra with peaks at 229.9 cm^{-1} , 1337.6 cm^{-1} , 1588.2 cm^{-1} and 1899.9 cm^{-1} .

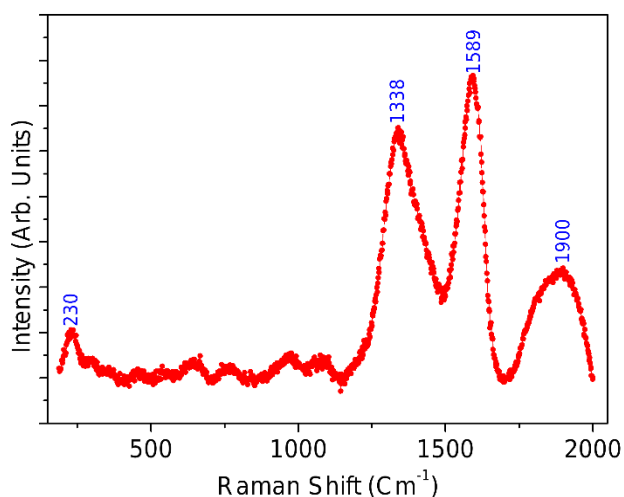


Figure 4.7: Raman spectra of *S. spinosa* water leaf synthesized silver nanoparticles showing different peaks that indicate the presence of different functional groups.

4.3 SILVER ION ACCUMULATION IN *Strychnos* SPECIES

Flame atomic absorption spectroscopy was employed to analyse AgNPs content in *Strychnos* seedlings. Silver nitrate was used for calibration before testing the samples for ion content. In figure 4.8, AgNPs content increased in treated seedlings for both *S. spinosa* and *S. cocculoides* as compared to untreated seedlings, this was evident by a statistically significant difference ($P=0.0001$) of silver ion content at each respective AgNPs treatment concentration as silver ion content increased in a dose dependent manner. The highest accumulated silver ion content was 0.362 ± 0.006 ppm and 0.300 ± 0.012 ppm at 200 $\mu\text{g/mL}$ for both *S. cocculoides* and *S. spinosa* respectively.

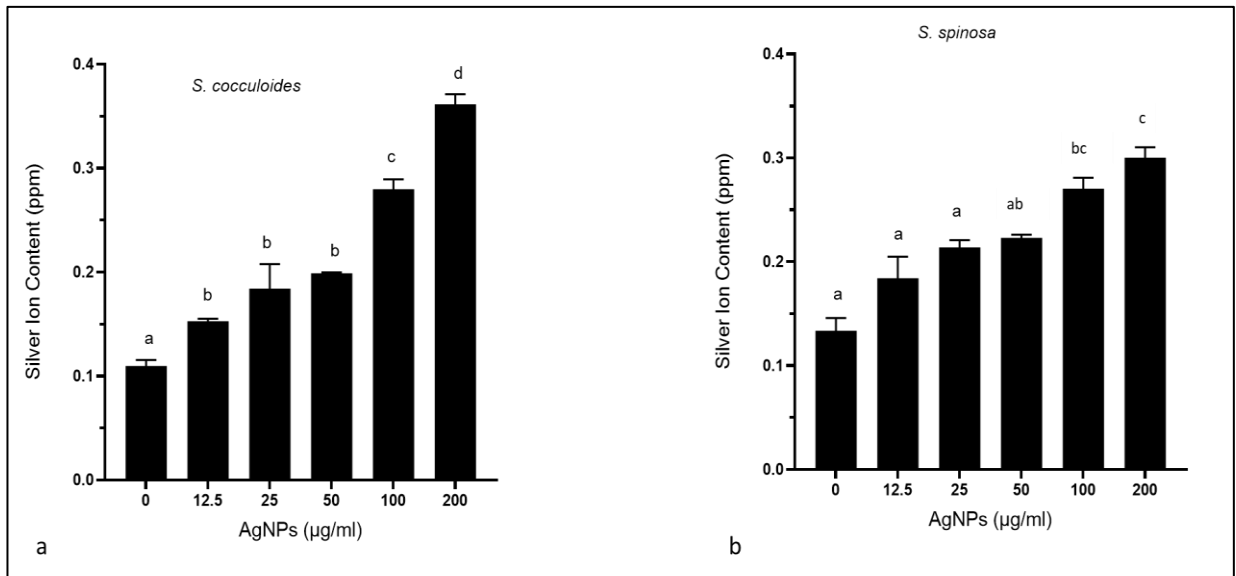


Figure 4.8: Silver ion content in *Strychnos* seedlings, **a)** *S. cocculoides* and **b)** *S. spinosa* cultured in MS media treated with different concentrations of AgNPs.

4.2 EARLY GROWTH PARAMETERS OF THE *Strychnos* SEEDLINGS

Seedlings germinated ten days after their culture on half strength MS growth media. Germination was signaled by opening of cotyledons and radicle elongation of the embryo. High mean germination percentage, 100% and 66.7% occurred at treatment with 50 $\mu\text{g/mL}$ for *S. cocculoides* and 200 $\mu\text{g/mL}$ for *S. spinosa*, respectively. Treatment with AgNPs had no statistically significant difference on germination percentage of *Strychnos* species, $P=0.219$ and $P=0.287$ for both *S. cocculoides* and *S. spinosa* respectively as depicted in figure 4.9.

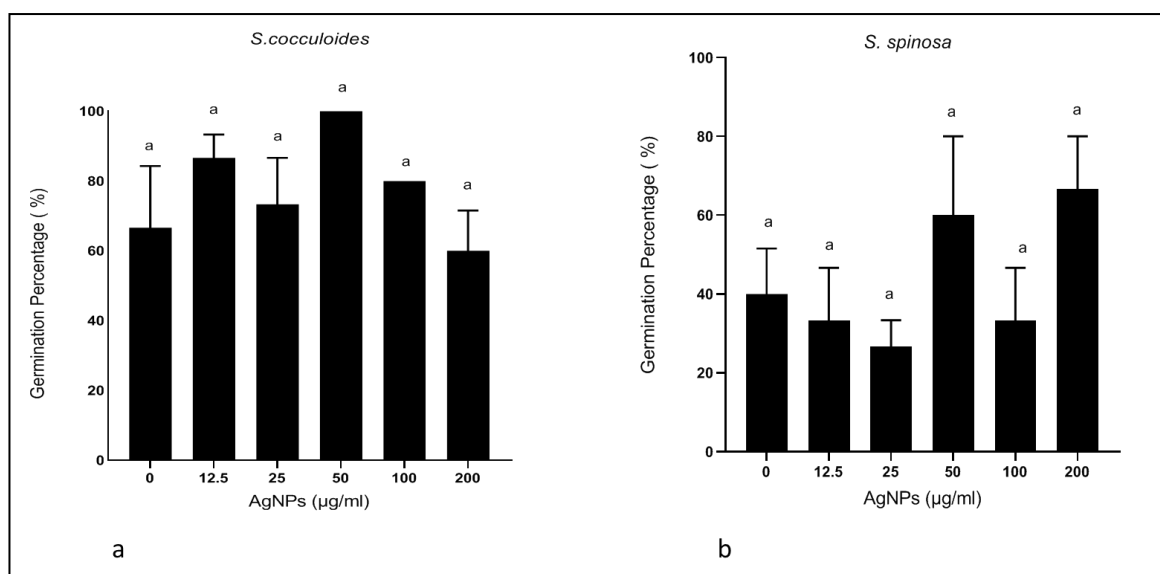


Figure 4.9: Mean germination percentage of *Strychnos* species at each AgNPs concentration and at the control. Lack of statistical difference is indicated by the alphabetical letters.

Silver nanoparticle treatments of the *Strychnos* seedlings had a significant effect on shoot length, $P=0.0012$ and $P=0.0001$ for *S. cocculoides* and *S. spinosa*, respectively, as shown in figure 4.10 and 4.11. Shoot length decreased with increase in AgNPs treatment concentration therefore control seedlings had longer shoots. Control seedlings had the highest shoot length recording of 9.00 ± 0.92 cm for *S. cocculoides* and 10.00 ± 0.72 cm for *S. spinosa* while treatment with $200 \mu\text{g/mL}$ recorded 3.08 ± 0.28 cm and 2.62 ± 0.50 cm for *S. cocculoides* and *S. spinosa*. There was no concomitant relationship between root length and silver nanoparticles concentrations. Root length increased at AgNPs $12.5 \mu\text{g/mL}$ recording 11.1 ± 2.06 cm and 8.30 ± 0.61 cm for *S. cocculoides* and *S. spinosa*.

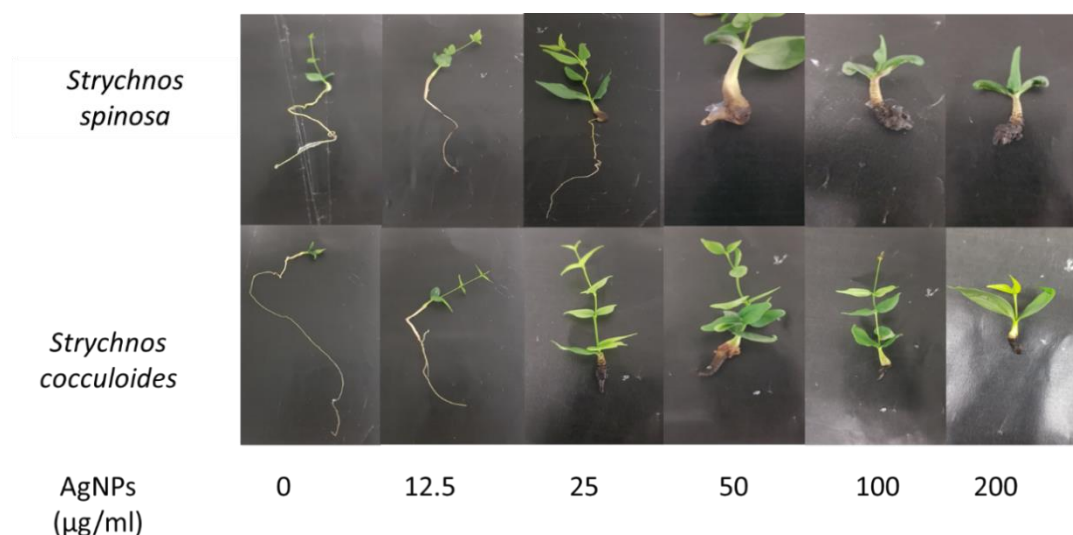


Figure 4.10: *Strychnos* seedlings from treatments with different concentrations of silver nanoparticles.

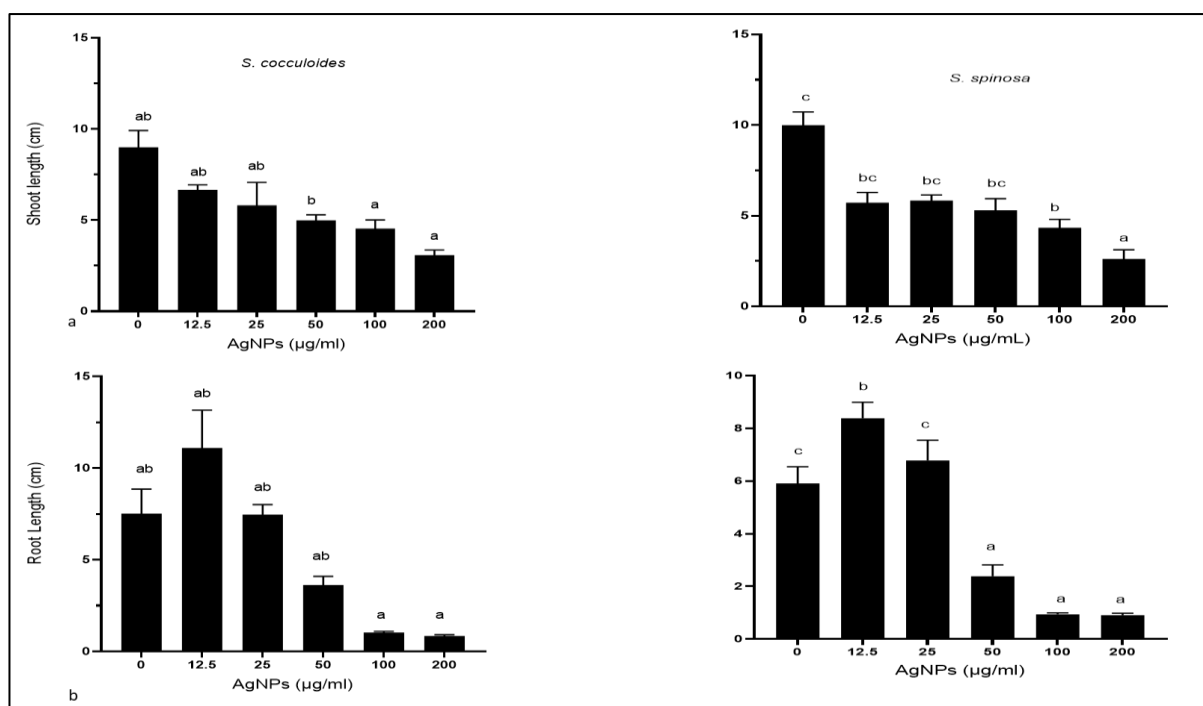


Figure 4.11: Mean $\pm$ standard error of shoot length **a)** and root length **b)** of the *Strychnos* species at each silver nanoparticle treatment and the control (0 $\mu\text{g/ml}$). Different letters indicate statistical difference while similar letters show that there is no statistical difference.

Seedlings treated with AgNPs developed tap roots that darkened and were short while control seedlings developed long tap roots as shown in figures 4.12 and 4.13.



Figure 4.12: **a)** Shoot length of the *Strychnos* seedlings and **b)** thickened stem and root browning in *Strychnos* seedlings under treatment with AgNPs.



Figure 4.13: Seedlings from *in vitro* culture of *Strychnos* embryo. **a)** Long tap roots in untreated seedlings, **b)** seedlings under AgNPs treatment and **c)** root length measuring criteria of *Strychnos* seedlings.

for *S. cocculoides* and *S. spinosa*, respectively, but small fresh weight was observed in treatment with 200 $\mu\text{g/mL}$ for both plant species. *Strychnos cocculoides* had a mean fresh weight of 0.072 ± 0.010 mg while *Strychnos spinosa* mean 0.045 ± 0.01 mg. However, dry weight was not affected neither by AgNPs treatment nor by plant species as there was no statistical difference in dry weight content for both the *Strychnos* species as depicted in figure 4.14.

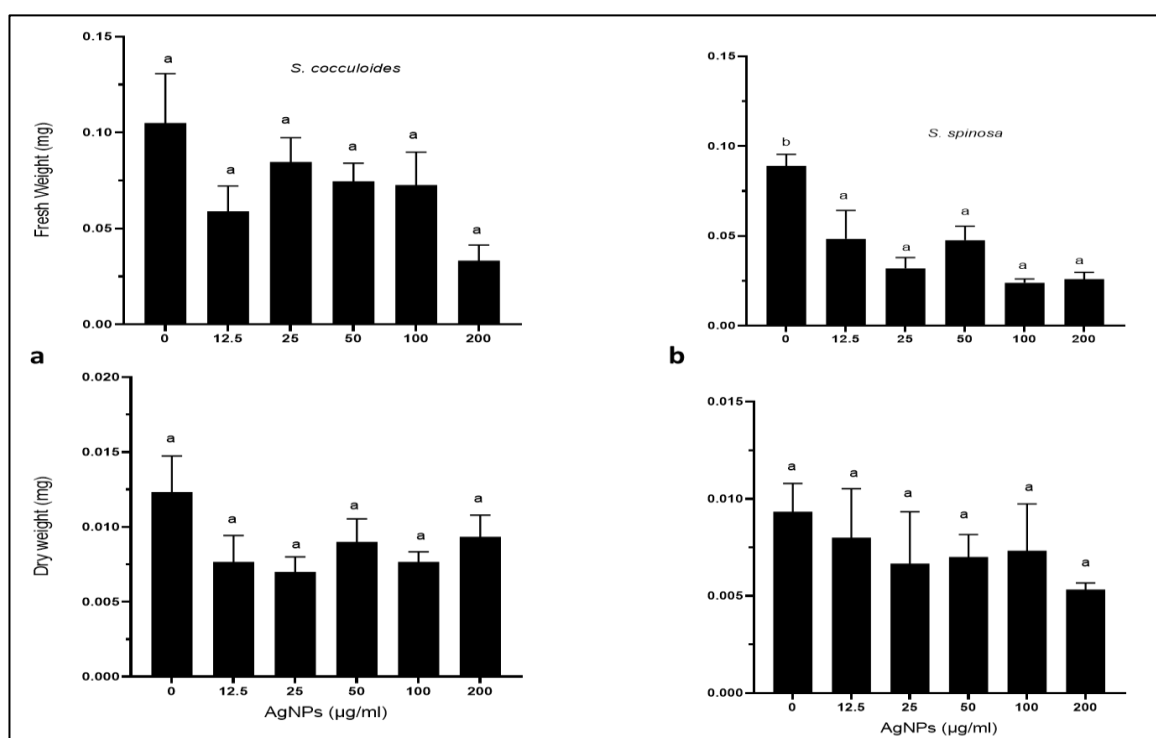


Figure 4.14: Bar graphs with standard error shown depicting mean fresh weight **a)** and mean Dry weight **b)** for *S. spinosa* and *S. cocculoides*. Statistical differences are shown by alphabetical letters.

4.4 *Strychnos* METABOLISM ANALYSIS

The purpose of this analysis was to investigate any shift in bands, which assesses the variability of biomolecules and detect any changes in the chemical bonds of the biomolecules in seedlings treated with silver nanoparticles. For *S. spinosa* in figure 4.14b FTIR spectra shows that 200 µg/mL seedlings had a higher transmittance percentage as compared to the other treated samples and the control seedlings (0 µg/mL). Interestingly *S. cocculoides* seedlings in figure 4.14b showed distinct infrared waves corresponding to 12.5, 25, and 0 (control) µg/mL while other waves were not identified. FTIR analysis revealed different broad and consistent bands for all the samples, both the control and treated seedlings.

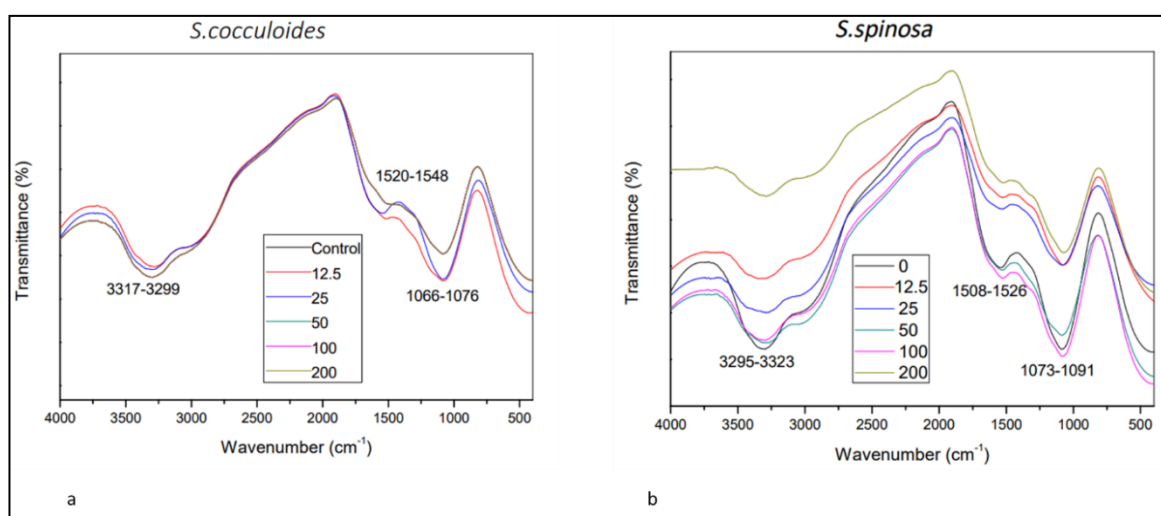


Figure 4.15: FTIR spectra of *S. cocculoides* and *S. spinosa* seedlings treated with different concentrations of silver nanoparticles showing different infrared absorption peaks.

Leaves of seedlings under AgNPs treatment exhibited a color change from green to yellow as shown in figure 4.16. Treatment with AgNPs had a significant effect ($P > 0.0001$) on chlorophyll accumulation in both the *Strychnos* species. Chlorophyll content decreased in a dose dependent manner where content decreased with increase with AgNPs treatment concentrations as depicted in figure 4.17. *Strychnos cocculoides* had a higher chlorophyll content mean (7.84 mg/g FW $\pm$ 1.44) as compared to *Strychnos spinosa* (3.00 mg/g FW $\pm$ 1.04).

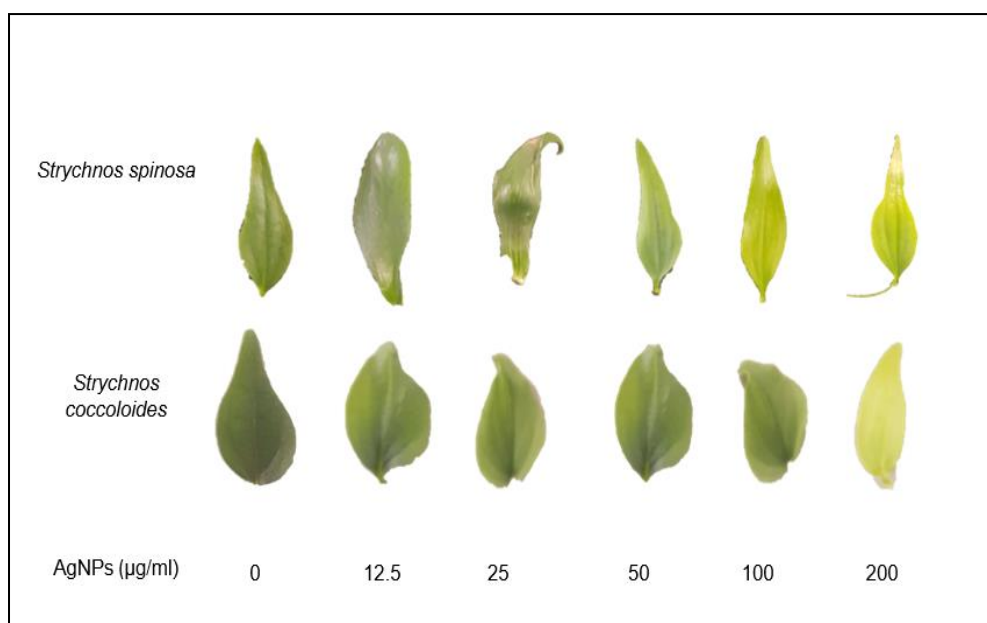


Figure 4.16; Leaves from *Strychnos* seedlings changed color from green to yellow with increase in AgNPs treatment concentration

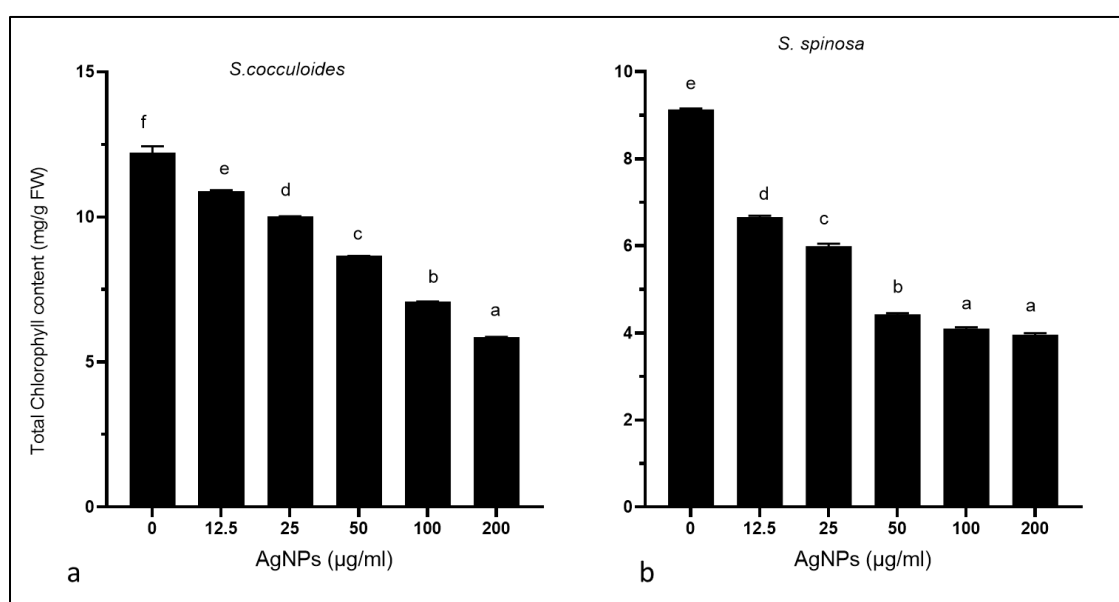


Figure 4.17: Average total chlorophyll content is shown by the bar graphs, different alphabetical letters are showing statistically significant difference in total chlorophyll content in each plant at each treatment as well as the control.

Both the *Strychnos* species had a higher chlorophyll *a* content as compared to chlorophyll *b* as shown in the table 3 in appendix C. *Strychnos coccoloides* had a higher chlorophyll *a* and *b* content as compared to *Strychnos spinosa*. Additionally control seedlings had a higher content of both chlorophyll *a* and *b* content.

Total anthocyanin content increased concomitantly with AgNPs concentration; therefore, silver nanoparticles had a statistically significant effect ($P<0.0001$) on anthocyanin content. Both *Strychnos* species had the same anthocyanin content at all the concentrations of AgNPs treatment with the highest recorded at 100 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$ (figure 4.18).

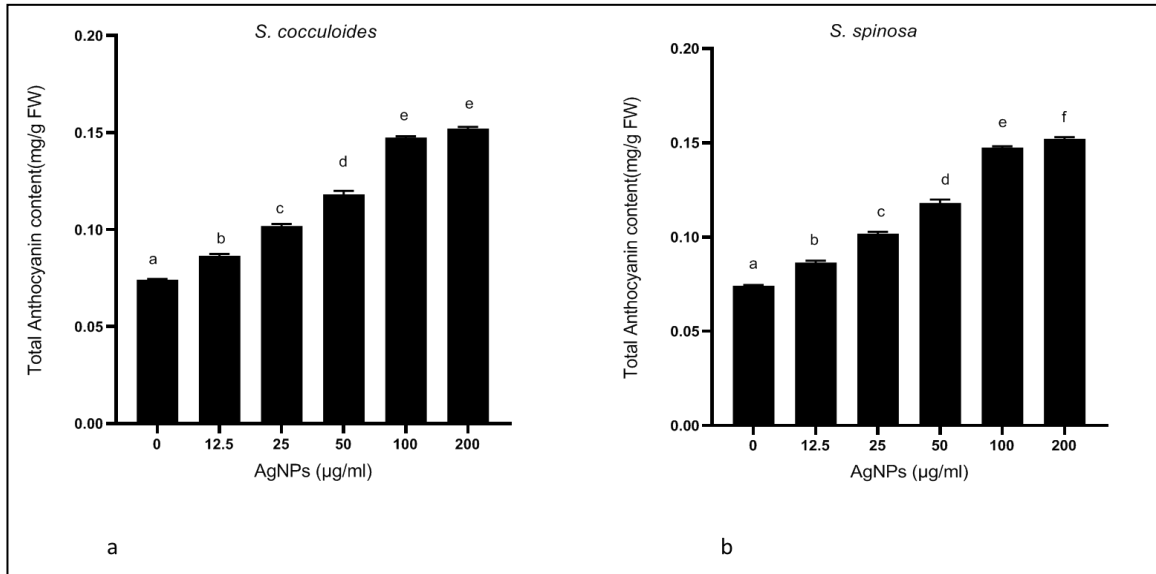


Figure 4.18: Shows bar graph of the mean total anthocyanin content. Differences in statistical significance are shown by alphabets.

Treatment of *Strychnos* seedlings with AgNPs significantly increased ($P<0.0001$) total phenolic content in a dose dependent manner. Control seedlings had 1.4 ± 0.1 mgGAE/g FW and 6.0 ± 0.114 mgGAE/g FW for *S. cocculoides* and *S. spinosa* respectively, whereas treatment with 200 $\mu\text{g/mL}$ AgNPs yielded 8.05 ± 0.087 mgGAE/g FW and 10.44 ± 0.040 mgGAE/g FW for *S. cocculoides* and *S. spinosa* respectively. On average *S. spinosa* had higher total phenol content (8.04 ± 0.097 mgGAE/g FW) as compared to *S. cocculoides* (4.96 ± 0.144 mgGAE/g FW) (figure 4.19).

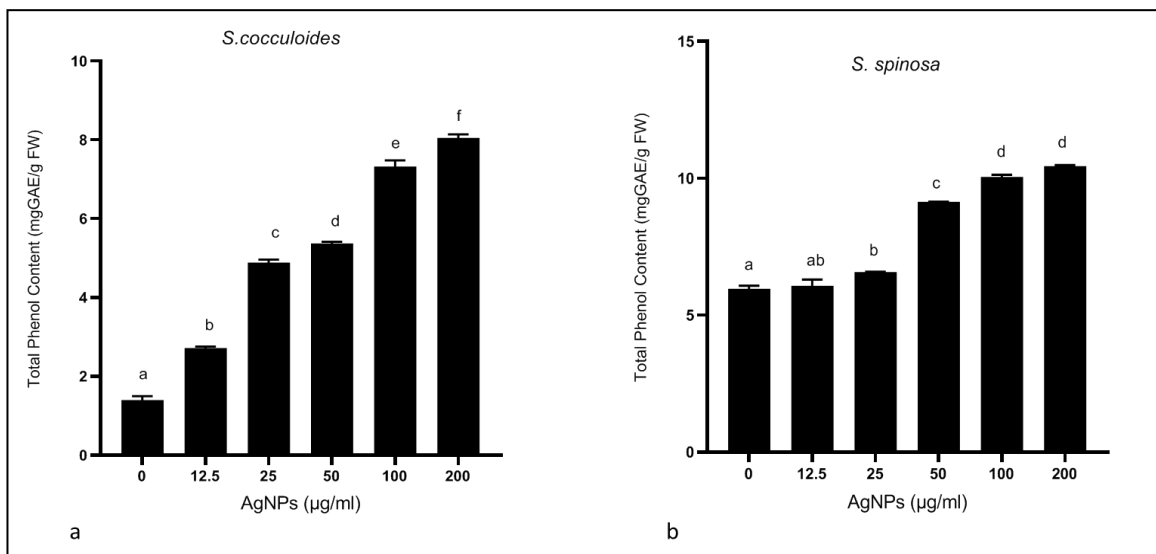


Figure 4.19: Total phenol content, statistically significant difference is depicted by alphabetical letters

The same trend of phenolic accumulation was observed with total flavonoids, where total flavonoid content increased with increase in AgNPs concentration as shown in figure 4.19. However, flavonoids content was higher than phenolic accumulation for both plants, and *S. cocculoides* had a higher total flavonoid content as compared to *S. spinosa* of 1.47 ± 1.08 mgQE/g FW and 1.01 ± 0.009 mgQE/g FW respectively. Treatment of both plants with AgNPs showed a statistically significant effect ($P < 0.0001$) on total flavonoid content (figure 4.20).

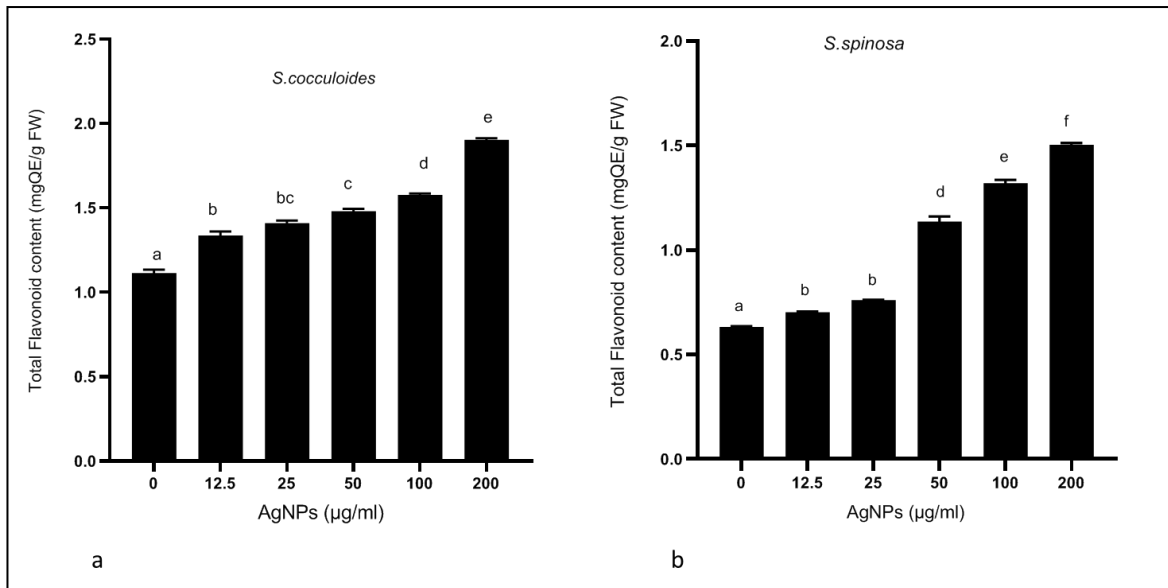


Figure 4.20: a) *S. cocculoides* and b) *S. spinosa* total flavonoid content averages shown in the bar graph with standard errors, alphabetical letters are assigned to statistical significance.

Protein content significantly increased ($P < 0.0001$) with increase of the AgNPs treatment concentrations as shown in figure 21. *Strychnos cocculoides* had an average higher protein content (24.6 ± 0.260 mg/mL FW) than *Strychnos spinosa* (22.7 ± 0.753 mg/mL FW). However, *Strychnos spinosa* had the highest total protein after treatment with 200 µg/mL silver nanoparticles of 52 mg/mL FW ± 1.57) as compared to *Strychnos cocculoides* (41.1 mg/mL FW ± 0.256). These results show that there was a significant difference between *Strychnos* species in terms of total protein accumulation.

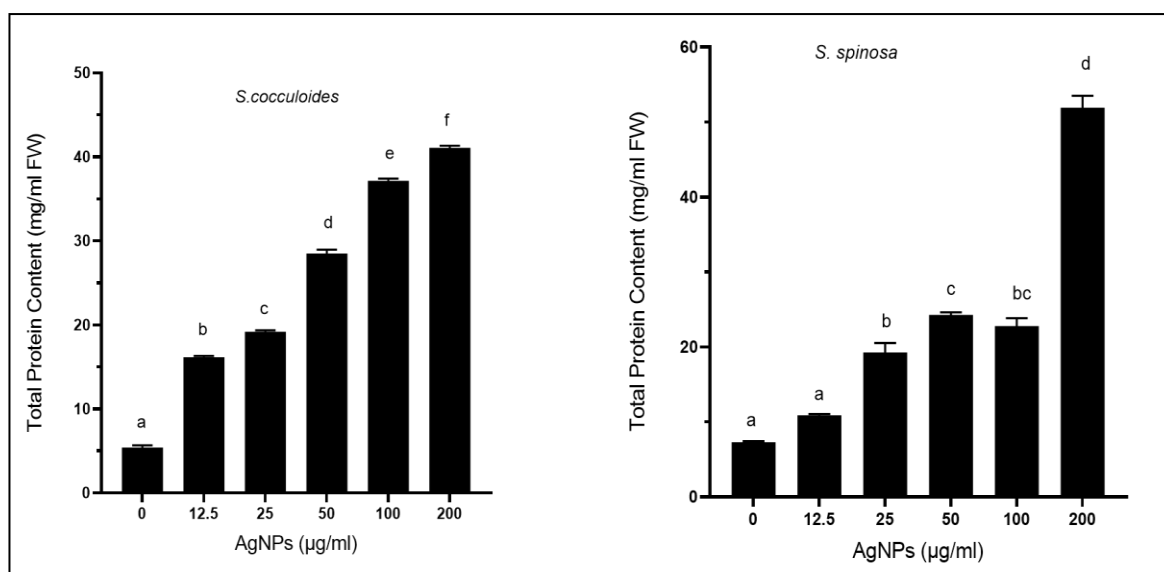


Figure 4.21: Average of the total protein content of *Strychnos* species, statistical differences are depicted by the alphabetical letters

The obtained proteins resolved with SDS Page revealed intense peaks at 10 kD and faint peaks at 150 kD as shown in figure 4.22 below.

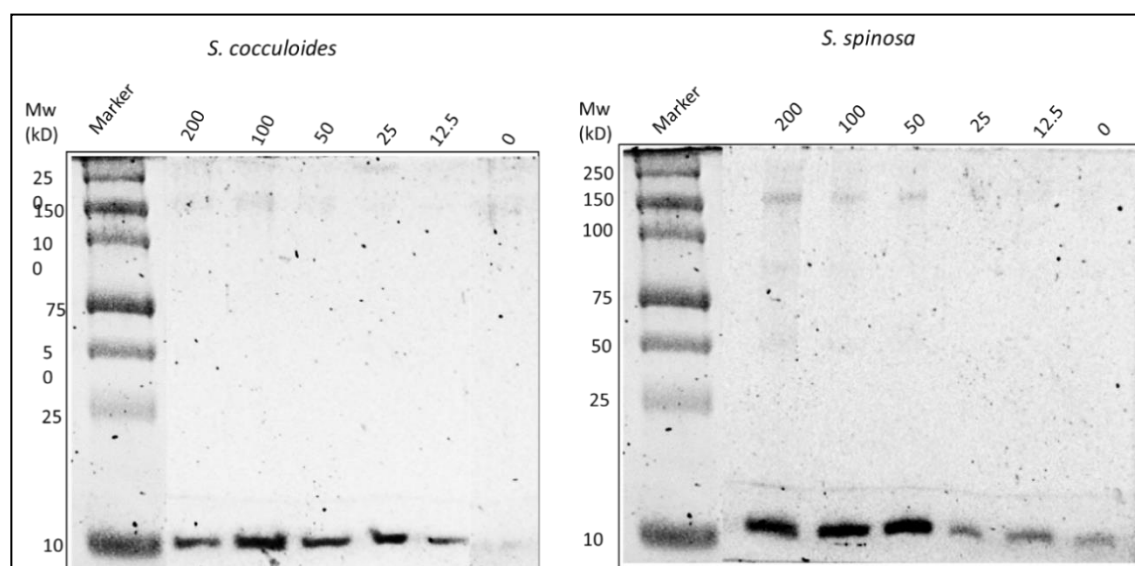


Figure 4.22: SDS-PAGE analysis of *Strychnos* species showing bands at 10kD.

CHAPTER 5: DISCUSSION

The color change of the extract-silver nitrate solution mixture is due to reduction of the silver metallic ions (Ag^+) in silver nitrate solution by the plant extract to AgNPs (Ag^0) (Awwad, et al. 2012). Interesting property of metal nanoparticles is that their optical properties are size and shape dependent. Silver is a noble metal and has excellent optical properties due to excitation of surface plasmon resonance hence the color change as well the characteristic UV-vis band visible at 427 nm (Mittal, et al. 2015).

The characteristic surface plasmon resonance of the synthesized AgNPs was identified at λ 427 nm as shown in figure 4.2, which falls within range of 400nm-450nm (Mittal, et al. 2013) thus confirming stability of the nanoparticles. Free electrons in the AgNPs are excited by absorbing photons and are raised to a higher energy level; however, the electron is unstable in this higher energy state and emits a photon to make a transition to the base energy state (Burda, et al. 2005; Thrall, et al. 2013).

X-ray diffraction peaks match that of COD 2013 database ID COD 9004180, corresponding to the face centered cubic (*fcc*) phase of silver. The average size of crystallites was 16.75 nm and is a cubic lattice. These peaks represent silver as the main constituent. The weaker peak at 32.5° may be attributed to a small size and small atomic distribution of the silver crystals.

The shape and structure of the synthesized AgNPs determined by SEM, STEM and EDX are shown in figure 4. The synthesized AgNPs appeared as spherical clusters uniformly distributed. The mono dispersed aggregates are due to polarity and electrostatic attraction of the AgNPs (Hu, et al. 2016). The size of most of the AgNPs from SEM analysis was 52.0 nm with an average size of 53.4 nm. Elemental composition of AgNPs was analysed with EDX revealed along with it oxygen that could be from organic moieties that were in the leaf extract while carbon could be from coating procedure during AgNPs preparation for SEM analysis.

In the FTIR spectra peaks 3728, 2346, 2113, 1531, 1195 cm^{-1} are assigned to stretching hydroxyl group of $\text{O}=\text{C}-\text{OH}$ and $\text{C}-\text{OH}$ (Tadayon and Jahromi 2020), bending vibrations of $\text{C}=\text{O}$ or ketone carbonyl (Dastan, et al. 2017), triple bond carbon stretching alkyne (Mistry, et al. 2021), stretching $\text{C}=\text{C}$ aromatic ring (Darabdhara, et al. 2016) and asymmetric stretch of $-\text{C}-\text{O}-\text{C}$ group (Tamizifar and Sun 2018), respectively. Phytochemical compounds with these functional groups are known to be good reducing agents with excellent radical scavenging capacity (Brewer 2011). These results show that dry biomass has various functional groups present for binding heavy metal ions.

Raman spectroscopy analysis of the nanoparticle peaks emerging at 229.9 cm^{-1} , 1337.6 cm^{-1} , 1588.2 cm^{-1} and 1899.9 cm^{-1} respectively assigned to $\text{Ag}-\text{O}-\text{C}-\text{O}^-$ (Cai, et al. 2017; Liu, et al. 2010), $\text{C}_\alpha\text{C}_m$ (Kang, et al. 2010) or $-\text{C}=\text{N}$ stretching vibration of guanidine (Khan, et al. 2020). The final peak at 1899.9 cm^{-1} was not identified. The peaks are due to vibrational modes of silver element bonded to different compounds being phenols and alkaloids. The aliphatic chains might be phenolic compounds while the nitrogenous asymmetric are attributed to alkaloids. Mostly the biomolecules found in the spectra are alkanes. The Raman spectra revealed the presence of alkanes, alkaloids and phenols bonded to silver element.

Flame Atomic Absorption Spectroscopy is one of the approaches that can be used to characterize uptake, accumulation, and toxicity mechanisms of AgNPs in *Strychnos*. Silver is taken up by roots, translocated throughout the leaves with substantial transformation or dissolution. Results show that silver ions from AgNPs increased at all concentrations for both the *Strychnos* species as concentration increased from 0.100 ± 0.006 ppm in control plants to 0.362 ± 0.010 ppm in seedlings treated with 200 $\mu\text{g/mL}$. Plants take up AgNPs from the growth media through both the intercellular spaces and vascular tissue, particularly the xylem cells (Tripathi, et al. 2017) and also the uptake and translocation of AgNPs is dependent on their size and shape, permeability and structure of the plant cell (Carlson, et al. 2008; Homaei and Ehsanpour 2015).

Cell wall has a semi-permeable epidermal layer that allows internalization of nanoparticles, in some instances nanoparticles larger than the epidermal pores induce pore formation and enter the plant vascular system and move up the plant through various series of events and processes (Navarro, et al. 2015). Silver nanoparticle uptake into the plant is aided by formation of vesicular membranes which acts as cargo that enfolds AgNPs and transport them to different destinations in the plant (Fabrega, et al. 2009; Ovečka, et al. 2005). Following accumulation of AgNPs into the plant system these nanoparticles will induce physiological, biochemical, and structural effects on plants hence in this study each dose of AgNPs treatment had an increasing or decreasing effects on the early growth or metabolism parameters.

Germination of the embryos was not affected by AgNPs but rather depended on the plant type. These results are consistent with findings reported by Sweet and Singleton (2015). AgNPs have either negative, positive or no effect on germination percentage. Silver nanoparticles enhanced germination in watermelon and zucchini (Almutairi and Alharbi 2015). Consistent with our results are other reports that *Vicia faba* (Abdel-Azeem and Elsayed 2013) and *Arabidopsis thaliana* (Geisler-Lee, et al. 2012) germination parameters were not affected by AgNPs. According to Xiu, et al. (2011), AgNPs have the tendency to aggregate or be complexed by ligands, and this reduces bioavailability of Ag^+ thus decreasing toxicity of Ag^+ on plants. Other reports have stated that lentil (Hojjat and Ganjali 2016; Yadu, et al. 2018) germination parameters were enhanced by treatment with AgNPs. However low concentrations of AgNPs enhanced germination while higher concentrations had detrimental effects on germination percentage in *Pinus sylvestris* (Bayramzadeh, et al. 2019) and *Sorghum bicolor* (Ziotti, et al. 2021).

The results obtained indicated that roots and shoots are more sensitive to AgNPs. This could be because roots are the first tissues in contact with AgNPs and accountable for the continuous uptake of minerals and nutrients from the medium. Root length was higher at lowest concentration and dramatically reduced at high concentration. Treated seedlings had stunted roots with a browning effect and increasing thickness observed by Sweet and Singleton (2015) at the radicles compared to roots of control seedling and those exposed to AgNPs, and this finding concurs with reports (Al-Huqail, et al. 2018; Geisler-Lee, et al. 2012). The browning is anticipated to be due to the highest accumulation of silver at the roots as reported for other plant species and this suggests that AgNPs were adsorbed by the root tips and maybe that was a survival response of roots to AgNPs exposure (Yin, et al. 2012). Decrease in shoot length was dose dependent as they decreased with

increase in AgNPs concentrations as observed by other researchers (Aghdam, et al. 2019; Bagherzadeh Homaei and Ehsanpour 2015; Baskar, et al. 2015; Moustaka, et al. 2018).

Biological material such as plants are highly homogenous, they are made up of biological molecules with different steps of stabilities and reactivities (Luo, et al. 2014). Functional groups of different molecules correspond to specific infra-red-light frequencies and the functional groups interact with transitional metals of which their composition is determined by infrared light adsorption (Jackson, et al. 2006). In here we used FTIR analysis to detect changes in chemical profiles of the *Strychnos* seedlings.

From the results *S. spinosa* seedlings exposed to 200 µg/mL had a higher transmittance percentage while the other samples from different treatment concentrations had a low transmittance percentage. On the other hand, *S. cocculoides* seedlings had a lower transmittance percentage. *S. cocculoides* and *S. spinosa* vibrations were as follows; 3323 – 3295 cm⁻¹ is assigned to -OH stretching vibrations and/or be assigned to N-H stretching vibrations. Bands at 1548-1508 cm⁻¹ are assigned to C-N stretching or N-H in-plane bend. These vibrations could also be assigned to C=O/C-O stretching aliphatic and aromatic compounds while 1091-1066 cm⁻¹ are assigned to -C-O aromatics and symmetric PO₂ phosphate ion (Nandiyanto, et al. 2019).

There was a significant difference in transmittance percentage for the treated *Strychnos* seedlings as compared to the control seedlings, this could imply partial decomposition and change in structure of molecules by silver nanoparticle that changed the chemical environment of the cell (Reheem, et al. 2016). Though the absorption bands were similar for both the species, some were weaker as they appeared at lower transmittance percentages. Evidently the bands pattern is not concomitant with the nanoparticle concentration. Metabolites analyzed (chlorophyll, anthocyanin, phenols, flavonoid, and proteins) were affected by AgNPs concentration as some were increased while others were decreased. These results are correlated by the FTIR spectra in figure 4.15 that shows different transmission percentages of biomolecules at each treatment concentration.

The spectra also shows that there exist the same biomolecules in both the species as shown by the peak's uniformity (bands appear at the same wavelength range) that corresponds to specific function group. FTIR wavenumber revealed peaks with different functional groups that are moieties to plant secondary metabolites. Alkaloids are made up of the amine moiety where nitrogen is bonded to a heterocyclic aromatic ring (Wang 2018). The hydroxyl group is a functional group of alcohols and carboxylic acid, and this reveals the presence of phenols and its derivatives as well as terpenes (Dhivya and Kalaichelvi 2017; Mohandas and Kumaraswamy 2018). Phosphate ions revealed presence of nucleic acids and proteins (Ma and Liu 2020). Therefore, the obtained results show that there was a cationic or metal bonding of biomolecules existing between *Strychnos* and AgNPs that resulted in a changed structure or composition of biomolecules found in the plant samples.

Chlorophyll is an important biomarker that reveals the status of plant growth. Treatment of *Strychnos* seedlings with AgNPs had a negative impact on total

chlorophyll content where chlorophyll content decreased in a dose dependent manner. Chlorosis, which is yellowing of leaves also confirmed the degradation or inhibited synthesis of chlorophyll biomolecule. Treated seedlings had an impaired growth system evident in the short, abscised roots and short shoot length which ultimately disturbed nutrient (nitrogen and magnesium) uptake. The impaired growth can be attributed to interference of photosynthesis because of degraded chlorophyll or inhibition of chlorophyll production (Dias, et al. 2019; Liu, et al. 2019).

Corresponding to results obtained from these research, several reports have indicated that AgNPs reduced chlorophyll content in *Arabidopsis thaliana* (Qian, et al. 2013) *Lycopersicon esculentum* (Song, et al. 2013) and *Oryza sativa* (Nair and Chung 2014). Silver nanoparticles inhibit water uptake hence the yellowing of leaves (Carrillo-López, et al. 2016). The inhibitory effect of AgNPs on chlorophyll is also attributed to chloroplast and thylakoid damage by ROS accumulation which then subsequently affects the process of photosynthesis (Baskar, et al. 2015). Silver nanoparticles enter the *Strychnos* plant system and interact with the chlorophyll metabolism.

AgNPs influence the photophysical behavior activity of plants where the metallic nanoparticles have a quenching effect on chlorophyll fluorescence, in turn decreasing photosynthetic activity. In a study by Falco, et al. (2020), there was an overlap found between chlorophyll fluorescence and AgNPs absorption. In such cases inhibited synthesis and degradation of chlorophyll disturbs/interferes with light harvesting complexes capability to transfer light to photosynthetic reaction centers (Dewez, et al. 2012).

Contradictory to findings in this study, in a study by Park and Ahn (2016) multiwalled carbon nanoparticles and AgNPs increased rate of photosynthesis in carrot. This could be because nanoparticles tend to enter chloroplast membrane and enhance photosynthetic capacity and electron transport chain (Giraldo, et al. 2014), nanosized titanium oxide was also reported to have increased photosynthesis and photosynthetic pigments in maize (Samadi, et al. 2014). Treatment with AgNPs increased chlorophyll content in potato (Homaee and Ehsanpour 2015) confirming that photosynthetic pigment can be used as heavy metal stress biomarkers (Kumar, et al. 2012). According to Giraldo, et al. (2014), it was reported that single walled carbon nanotubes were found in the chloroplast membrane suggesting that nanoparticles can be used to enhance photosynthesis.

Phenols are a diverse group of compounds, inclusive of anthocyanins which are categorized by a free hydroxyl group (Abbasi and Jamei 2019). The free hydroxyl group has antioxidative properties that enables phenols to possess antioxidative activities to scavenge free radicals in living organisms (Bagherzadeh Homaee and Ehsanpour 2015).

Anthocyanins are soluble flavonoids possessing antioxidant properties compounds which relieve stress or protects cells from ROS by scavenging free radicals (Aghdam, et al. 2019; Bagherzadeh Homaee and Ehsanpour 2015; Baskar, et al. 2015; Moustaka, et al. 2018). In this study, treatment of *Strychnos* with AgNPs increased anthocyanin content in a dose dependent manner. Increase in anthocyanin content in plants under heavy metal stress was also observed in *Arabidopsis thaliana* (Nair and Chung 2014), *Brassica rapa* spp. *Pekinensis*

(Baskar, et al. 2015), potato (Homaee and Ehsanpour 2015) and *Echium amoenum* (Abbasi and Jamei 2019). The higher content of anthocyanins in *Strychnos* shoots at high AgNPs concentrations can be attributed to high oxidative stress induced by exposure to AgNPs, of which anthocyanin act against to protect plant from ROS damage (Abbasi and Jamei 2019; Bagherzadeh Homaee and Ehsanpour 2015; Thiruvengadam, et al. 2015). In another study it was observed the *PAL* gene was overexpressed in a progressive manner in *Brassica rapa* spp. *Pekinensis* seedlings treated with AgNPs, making it evident that anthocyanin enhances the plants' response to stress by counteracting ROS activity (Homaee and Ehsanpour 2015). These molecules scavenge free radicals and restrict hydrogen peroxide movement in plant cells (Hatier and Gould 2008).

Treatment of *Strychnos* with AgNPs showed a significant increase in total flavonoid content. *Triticum aestivum* had an increased flavonoid content by 200% (Rastogi, et al. 2019) and *Linum usitatissimum* L. suspension cultures had approximately 6.5-fold total flavonoid (Zahir, et al. 2019) when exposed to different concentrations of AgNPs. These results are therefore in sync with this literature as the accumulation flavonoids was concomitant with increase in silver nanoparticle concentration, indication the oxidative stress AgNPs imposed on *Strychnos* plants. Total flavonoid and total phenol content accumulation is dependent on duration of exposure, (Zahir, et al. 2019) demonstrated that total flavonoid increase in the first phase of culture but declined at a later stage after 20 days of culture. Flavonoids have free radical scavenging activity, can inhibit hydrolytic and oxidative enzymes, and possesses anti-inflammatory activity (Atanassova, et al. 2011).

Silver nanoparticle treatment of *Strychnos* seedlings showed a significant increase in total phenol content in a dose dependent manner. Total phenol content was increased in *Solanum lycopersicum* L. (Girilal, et al. 2018), fenugreek plant (Sadak 2019), *Prunella vulgaris* L calli cultures (Fazal, et al. 2016) and *Cucumis anguria* hairy root cultures (Chung, et al. 2018), *Linum usitatissimum* L. suspension cultures (Zahir, et al. 2019) treated with AgNPs. Phenols have carboxyl and hydroxyl groups that can bind metal ions such as silver hence the elevated increase in their content as concentrations increase (Girilal, et al. 2018). Phenols act as metal chelators and have antioxidant properties that are responsible for reducing reactive oxygen species and lipid alkoxy.

However, AgNPs also have contradicting effects as a decrease in phenol content was noted in rice seedlings (Gupta, et al. 2018) and water hyacinth (Rani, et al. 2016). This phenomenon is corroborated by Zhao, et al. (2010) where it was suggested that elicitation of suspension cultures at an early stage increased flavonoid content while it was decreased at a later stage.

Silver nanoparticles interact with cellular components causing reactive oxygen species formation, a causative agent of oxidative stress (Banerjee, et al. 2010; Rinna, et al. 2015). Phenols, including anthocyanins and flavonoids are the frontline elements functioning as free radical terminators thus essential to plant protection against oxidative stress (Dai, et al. 2006). Silver nanoparticles have the potential to induce oxidative stress on plants that intermittently increases accumulation of phenols and flavonoids as reported in our findings.

Proteins are key players in stress response both as structural proteins and as proteins involved in plant epigenome, transcriptome, and metabolome (Kosová, et al. 2019) (Janmohammadi, et al. 2015). Changes in protein abundance reveal how plants cope with stress (Kosová, et al. 2013). In our study total protein content in *Strychnos* seedlings increased with increase in AgNPs treatment concentration. The increase in protein content is also shown by the intense bands of the proteins at 10 kD in plants. Studies have shown that in AgNPs-induced stress, AgNPs interact with normal cell metabolism such as protein synthesis/degradation and apoptosis, cell defense and primary metabolism (Peharec Štefanić, et al. 2019). Tobacco plants exposed to treatment with AgNPs had differential abundance in total protein content, of which some proteins were increased while others were decreased (Peharec Štefanić, et al. 2019).

In a study by Huang, et al. (2016) it was reported that exposure of *Skeletonema costatum* to 5.0 mg/L AgNPs increased protein content with 16% as compared to the control. However, protein content in wheat grown at different concentrations of AgNPs decreased when compared with the control (Yang, et al. 2018). Treatment of *Brassica sp.* with 3 mM AgNPs reduced protein content by 7% compared to the control (Vishwakarma, et al. 2017) and total soluble protein was decreased in tomato plants (Karami Mehrian, et al. 2015). The reduction in protein content is assumed to be due to conformational changes imposed on the plant's proteome by AgNPs interfering with protein interactions, cell signaling and DNA transcription (Saptarshi, et al. 2013; Vannini, et al. 2013; Vishwakarma, et al. 2017).

Soybean treated with nanoparticles showed both a 10-fold increase and decrease in different proteins, especially aspartyl protease and expansin-like protein (Mustafa, et al. 2015). Lettuce shoots treated with AgNPs 25 ppm and 50 ppm increased protein content by 12% (Hasan, et al. 2021). Plants under stressful conditions alter internal mechanisms of preserving cell from damage by degrading damaged molecules responsible for stress induced response to prevent their accumulation therefore AgNPs can increase accumulation of proteins responsible for degrading these materials (Mirzajani, et al. 2014; Peharec Štefanić, et al. 2019).

Increase in total protein content can be attributed to upregulation and increase of heat shock proteins and certain amino acids secreted by plants in response to the stress induced by AgNPs (Al-Whaibi 2011; Štefanić, et al. 2018). Heat shock proteins have the ability assist other proteins to maintain their integrity or native conformation by stabilizing the partially unfolded states of proteins (Al-Whaibi 2011). As a result, plants have a propensity to release heat shock proteins in response to stress-evoking agents. The secretion of plant proteins would likewise rise under conditions of intense stress (Girilal, et al. 2015; Wang, et al. 2004). Therefore, the increase in total proteins in *Strychnos* seedlings is because of the increase expression of heat shock proteins that counteracted the oxidative stress caused by exposure to AgNPs.

Silver nanoparticles are highly toxic, mostly due to release of the silver ion (Ag^+) during redox reaction with plant intercellular environment, therefore AgNPs-cell- Ag^+ interaction at the cell interface can increase AgNPs toxicity on plants. Silver ion generated is due to the two possible fates of AgNPs when they enter the cell cytoplasm. They can either be oxidized by intracellular ROS, or can inactivate proteins and DNA (Xiu, et al. 2011). Plants differ in their reaction to AgNPs

depending on physicochemical properties of nanoparticles as well morphology and anatomy (Bayramzadeh, et al. 2019) of the plant used as a sample therefore the phytotoxicity of higher plants needs to be investigated to establish a comprehensive toxicity of AgNPs on plants.

CHAPTER 6: CONCLUSION

Strychnos spinosa leaf extract has capping and stabilizing phytochemicals that reduces silver nitrate salt solution to yield stable and pure AgNPs. This study managed to synthesize spherical aggregated of AgNPs with an average of 54.3 nm and their crystals were 16.75 nm. Flame atomic absorption spectroscopy also confirmed accumulation of silver nanoparticles in the seedlings in a dose dependent manner. *S. cocculoides* was amenable to taking up AgNPs as compared to *S. spinosa*. It is therefore concluded that plants have mechanisms of absorbing and internalizing AgNPs. It can also be that plant macromolecules interact with AgNPs and oxidizes them to silver ions.

Accumulation of AgNPs in *S. cocculoides* and *S. spinosa* seedlings influences growth parameters, either negatively or positively, and this is evident in the increased shoot length and fresh weight while root length was greatly reduced. It is therefore concluded that AgNPs uptake alters the growth metabolism of seedlings and in turn interferes with the ultra-cellular structures such as cell wall and cell membrane and the root hair cell. Chlorophyll degradation and biosynthesis is affected by AgNPs accumulation in the two *Strychnos* species. Chlorosis and decreased chlorophyll content is assumed to be due to damaged chloroplast and thylakoid membranes, which are organelles involved in chlorophyll synthesis.

AgNPs accumulation has ability to increase phenolic content, and this is an indication that AgNPs can upregulate production and accumulation of ROS scavenging molecules. Anthocyanin, flavonoid, and phenol content increased in a dose dependent manner. We therefore conclude that administration of silver nanoparticles to *Strychnos* seedlings has a negative impact on early growth parameters except for the dry weight, while metabolism was increased except for chlorophyll content. Silver nanoparticles can indeed be absorbed from the growing media by *Strychnos cocculoides* and *Strychnos spinosa* therefore these plant species can possibly be used to remediate AgNPs contaminated environmental metrics such as soil. Applications of AgNPs to *Strychnos* plants can also increase phenolics accumulation hence useful in increasing the production of these metabolites for commercial purposes.

CHAPTER 7: FUTURE PROSPECTS

To assess the effects of AgNPs on *Strychnos* growth and development, there is a need to assess the relationship between time/duration of exposure to the effect imposed on the seedlings. This will also include assessing germination rate over a set period. For future studies, *Strychnos* species should be cultivated in soil supplemented with AgNPs or in areas contaminated with AgNPs outside the laboratory such as mining areas and areas affected with acid mine drainage as target study areas to simulate environmental exposure of *Strychnos* to AgNPs.

Ultramicroscopic analysis of the *Strychnos* plant tissues is recommended to assess how AgNPs deforms the root structure, the chloroplast, and thylakoid membranes. To deepen this study, gene expression analysis on how AgNPs affect genes involved in total phenol biosynthesis, and chlorophyll degradation and biosynthesis is suggested. Employing more precise and advanced analytical tools such as ICP-MS to assess AgNPs accumulation in *Strychnos* seedlings is also recommended.

To advance the investigation on metabolism, it is a recommendation to identify proteins involved in alleviating ROS activity in *Strychnos* that is caused by exposure to AgNPs. This will help enlighten how proteins are regulated under stress. Assessing the effects of these nanoparticles on other macromolecules that are essential to plant development such as carbohydrates, and nutrient uptake is also recommended.

CHAPTER 8: BIBLIOGRAPHY

- Abbasi F, Jamei R (2019) Effects of silver nanoparticles and silver nitrate on antioxidant responses in *Echium amoenum*. *Russian Journal of Plant Physiology* 66:488-494
- Abbasi F, Jamei RJRJoPP (2019) Effects of silver nanoparticles and silver nitrate on antioxidant responses in *Echium amoenum*. 66:488-494
- Abdel-Azeem EA, Elsayed BA (2013) Phytotoxicity of silver nanoparticles on *Vicia faba* seedlings. *NY Sci J* 6:148-155
- Adisa IO, Reddy Pullagurala VL, Rawat S, Hernandez-Viezcas JA, Dimkpa CO, Elmer WH, White JC, Peralta-Videa JR, Gardea-Torresdey JL (2018) Role of cerium compounds in *Fusarium* wilt suppression and growth enhancement in tomato (*Solanum lycopersicum*). *Journal of agricultural and food chemistry* 66:5959-5970
- Aghdam MS, Moradi M, Razavi F, Rabiei VJSH (2019) Exogenous phenylalanine application promotes chilling tolerance in tomato fruits during cold storage by ensuring supply of NADPH for activation of ROS scavenging systems. 246:818-825
- Ahanger MA, Alyemeni MN, Wijaya L, Alamri SA, Alam P, Ashraf M, Ahmad P (2018) Potential of exogenously sourced kinetin in protecting *Solanum lycopersicum* from NaCl-induced oxidative stress through up-regulation of the antioxidant system, ascorbate-glutathione cycle and glyoxalase system. *PLoS One* 13:e0202175
- Ahmad M, Usman AR, Lee SS, Kim S-C, Joo J-H, Yang JE, Ok YS (2012) Eggshell and coral wastes as low cost sorbents for the removal of Pb²⁺, Cd²⁺ and Cu²⁺ from aqueous solutions. *Journal of Industrial and Engineering Chemistry* 18:198-204
- Ahmed HM, Roy A, Wahab M, Ahmed M, Othman-Qadir G, Elesawy BH, Khandaker MU, Islam MN, Emran TB (2021) Applications of nanomaterials in agrifood and pharmaceutical industry. *Journal of Nanomaterials* 2021:
- Al-Huqail AA, Hatata MM, Al-Huqail AA, Ibrahim MM (2018) Preparation, characterization of silver phyto nanoparticles and their impact on growth potential of *Lupinus termis* L. seedlings. *Saudi journal of biological sciences* 25:313-319
- Al-Whaibi MH (2011) Plant heat-shock proteins: a mini review. *Journal of King Saud University-Science* 23:139-150

Alenazi M, Hashim KS, Hassan AA, Muradov M, Kot P, Abdulhadi B (2020) Turbidity removal using natural coagulants derived from the seeds of *strychnos potatorum*: statistical and experimental approach. In: IOP Conference Series: Materials Science and Engineering. IOP Publishing, p 012064

Alidoust D, Isoda A (2013) Effect of $\gamma\text{Fe}_2\text{O}_3$ nanoparticles on photosynthetic characteristic of soybean (*Glycine max* (L.) Merr.): foliar spray versus soil amendment. *Acta physiologiae plantarum* 35:3365-3375

Almutairi ZM, Alharbi A (2015) Effect of silver nanoparticles on seed germination of crop plants. *J adv Agric* 4:283-288

Anjum NA, Gill SS, Duarte AC, Pereira E, Ahmad I (2013) Silver nanoparticles in soil–plant systems. *Journal of Nanoparticle Research* 15:1-26

Anjum NA, Gill SS, Duarte AC, Pereira E, Ahmad I *IJJoNR* (2013) Silver nanoparticles in soil–plant systems. 15:1-26

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 55:373-399

Aremu AO, Moyo M (2022) Health benefits and biological activities of spiny monkey orange (*Strychnos spinosa* Lam.): An African indigenous fruit tree. *Journal of Ethnopharmacology* 283:114704

Arora S, Sharma P, Kumar S, Nayan R, Khanna P, Zaidi M (2012) Gold-nanoparticle induced enhancement in growth and seed yield of *Brassica juncea*. *Plant growth regulation* 66:303-310

Asli S, Neumann PM (2009) Colloidal suspensions of clay or titanium dioxide nanoparticles can inhibit leaf growth and transpiration via physical effects on root water transport. *Plant, cell & environment* 32:577-584

Atanassova M, Georgieva S, Ivancheva K (2011) Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *Journal of the University of Chemical Technology & Metallurgy* 46:

Awwad AM, Salem NM, Abdeen AO (2012) Biosynthesis of silver nanoparticles using *Olea europaea* leaves extract and its antibacterial activity. *Nanoscience and Nanotechnology* 2:164-170

Babu R, Chaudhuri M (2005) Home water treatment by direct filtration with natural coagulant. *Journal of water and health* 3:27-30

Bagherzadeh Homaee M, Ehsanpour AA (2015) Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under in vitro conditions. *Indian Journal of Plant Physiology* 20:353-359

Bagherzadeh Homaee M, Ehsanpour AA *IJoPP* (2015) Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under in vitro conditions. 20:353-359

Banerjee M, Mallick S, Paul A, Chattopadhyay A, Ghosh SS (2010) Heightened reactive oxygen species generation in the antimicrobial activity of a three component iodinated chitosan– silver nanoparticle composite. *Langmuir* 26:5901-5908

Barnes JD, Balaguer L, Manrique E, Elvira S, Davison A (1992) A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. *Environmental and Experimental botany* 32:85-100

Baskar V, Venkatesh J, Park SW (2015) Impact of biologically synthesized silver nanoparticles on the growth and physiological responses in *Brassica rapa* ssp. *pekinensis*. *Environmental Science and Pollution Research* 22:17672-17682

Baskar V, Venkatesh J, Park SW *JES, Research P* (2015) Impact of biologically synthesized silver nanoparticles on the growth and physiological responses in *Brassica rapa* ssp. *pekinensis*. 22:17672-17682

Battke F, Leopold K, Maier M, Schmidhalter U, Schuster M (2008) Palladium exposure of barley: uptake and effects. *Plant biology* 10:272-276

Bayramzadeh V, Ghadiri M, Davoodi MH (2019) Effects of silver nanoparticle exposure on germination and early growth of *Pinus sylvestris* and *Alnus subcordata*. *Sains Malays* 48:937-944

Bero J, Ganfon H, Jonville M-C, Frédéric M, Gbaguidi F, DeMol P, Moudachirou M, Quetin-Leclercq J (2009) In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. *Journal of Ethnopharmacology* 122:439-444

Bhojwani SS, Razdan MK (1986) *Plant tissue culture: theory and practice*. Elsevier

Bhuyan MB, Hasanuzzaman M, Mahmud JA, Hossain MS, Bhuiyan TF, Fujita M (2019) Unraveling morphophysiological and biochemical responses of *Triticum aestivum* L. to extreme pH: Coordinated actions of antioxidant defense and glyoxalase systems. *Plants* 8:24

Bian F, Su J, Liu W, Li S (2018) Dormancy release and germination of *Taxus yunnanensis* seeds during wet sand storage. *Scientific reports* 8:1-9

Bisset NG (1988) Curare-botany, chemistry, and pharmacology. *Acta Amazonica* 18:255-290

Boonyanitipong P, Kumar P, Kositsup B, Baruah S, Dutta J (2011) Effects of zinc oxide nanoparticles on roots of rice *Oryza sativa* L. In: *Int Conf Environ BioSci*, pp 172-176

Borgia V, Carlin MG, Crezzini J (2017) Poison, plants and Palaeolithic hunters. An analytical method to investigate the presence of plant poison on archaeological artefacts. *Quaternary International* 427:94-103

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* 72:248-254

Brewer M (2011) Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive reviews in food science and food safety* 10:221-247

Burda C, Chen X, Narayanan R, El-Sayed MA (2005) Chemistry and properties of nanocrystals of different shapes. *Chemical reviews* 105:1025-1102

Cabahug RAM, Nam SY, Lim K-B, Jeon JK, Hwang Y-J (2018) Propagation techniques for ornamental succulents. *화훼연구* 26:90-101

Cai Y, Piao X, Gao W, Zhang Z, Nie E, Sun Z (2017) Large-scale and facile synthesis of silver nanoparticles via a microwave method for a conductive pen. *RSC advances* 7:34041-34048

Carlson C, Hussain SM, Schrand AM, K. Braydich-Stolle L, Hess KL, Jones RL, Schlager JJTjopcB (2008) Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. 112:13608-13619

Carrillo-López LM, Morgado-González A, Morgado-González A (2016) Biosynthesized silver nanoparticles used in preservative solutions for *Chrysanthemum* cv. Puma. *Journal of Nanomaterials* 2016:

Castro-González CG, Sánchez-Segura L, Gómez-Merino FC, Bello-Bello JJ (2019) Exposure of stevia (*Stevia rebaudiana* B.) to silver nanoparticles in vitro: Transport and accumulation. *Scientific reports* 9:1-10

Chakravarty M, Vora A (2021) Nanotechnology-based antiviral therapeutics. *Drug delivery and translational research* 11:748-787

Chang C-C, Yang M-H, Wen H-M, Chern J-C (2002) Estimation of total flavonoid content in propolis by two complementary colometric methods. *Journal of food and drug analysis* 10:3

Chinsembu K (2016) Ethnobotanical study of medicinal flora utilised by traditional healers in the management of sexually transmitted infections in Sesheke District, Western Province, Zambia. *Revista Brasileira de Farmacognosia* 26:268-274

Chung I-M, Rajakumar G, Thiruvengadam M (2018) Effect of silver nanoparticles on phenolic compounds production and biological activities in hairy root cultures of *Cucumis anguria*. *Acta Biologica Hungarica* 69:97-109

Corsaro M, Giudicianni I, Lanzetta R, Marciano C, Monaco P, Parrilli M (1995) Polysaccharides from seeds of *Strychnos* species. *Phytochemistry* 39:1377-1380

Cox A, Venkatachalam P, Sahi S, Sharma N (2016) Silver and titanium dioxide nanoparticle toxicity in plants: a review of current research. *Plant physiology and biochemistry* 107:147-163

Cromwell W, Yang J, Starr J, Jo Y-K (2014) Nematicidal effects of silver nanoparticles on root-knot nematode in bermudagrass. *Journal of nematology* 46:261

Czarnocka W, Karpiński S (2018) Friend or foe? Reactive oxygen species production, scavenging and signaling in plant response to environmental stresses. *Free Radical Biology and Medicine* 122:4-20

Da Silva LC, Oliva MA, Azevedo AA, De Araujo JM (2006) Responses of restinga plant species to pollution from an iron pelletization factory. *Water, air, and soil pollution* 175:241-256

Dai LP, Xiong ZT, Huang Y, Li MJ (2006) Cadmium-induced changes in pigments, total phenolics, and phenylalanine ammonia-lyase activity in fronds of *Azolla imbricata*. *Environmental Toxicology: An International Journal* 21:505-512

Darabdhara G, Boruah PK, Borthakur P, Hussain N, Das MR, Ahamad T, Alshehri SM, Malgras V, Wu KC-W, Yamauchi Y (2016) Reduced graphene oxide nanosheets decorated with Au–Pd bimetallic alloy nanoparticles towards efficient photocatalytic degradation of phenolic compounds in water. *Nanoscale* 8:8276-8287

Dastan D, Chaure N, Kartha M (2017) Surfactants assisted solvothermal derived titania nanoparticles: synthesis and simulation. *Journal of Materials Science: Materials in Electronics* 28:7784-7796

Delfani M, Baradarn Firouzabadi M, Farrokhi N, Makarian H (2014) Some physiological responses of black-eyed pea to iron and magnesium nanofertilizers. *Communications in soil science and plant analysis* 45:530-540

Deshpande P, Dapkekar A, Oak MD, Paknikar KM, Rajwade JM (2017) Zinc complexed chitosan/TPP nanoparticles: A promising micronutrient nanocarrier suited for foliar application. *Carbohydrate polymers* 165:394-401

Dewez D, Oukarroum AJT, Chemistry E (2012) Silver nanoparticles toxicity effect on photosystem II photochemistry of the green alga *Chlamydomonas reinhardtii* treated in light and dark conditions. 94:1536-1546

Dhivya S, Kalaichelvi K (2017) UV-Vis spectroscopic and FTIR analysis of *Sarcostemma brevistigma*, wight. and arn. *International Journal of Herbal Medicine* 9:46-49

Dias MC, Santos C, Pinto G, Silva A, Silva SJP (2019) Titanium dioxide nanoparticles impaired both photochemical and non-photochemical phases of photosynthesis in wheat. 256:69-78

Espinosa-Leal CA, Puente-Garza CA, García-Lara S (2018) In vitro plant tissue culture: means for production of biological active compounds. *Planta* 248:1-18

Essa HL, Abdelfattah MS, Marzouk AS, Shedeed Z, Guirguis HA, El-Sayed MM (2021) Biogenic copper nanoparticles from *Avicennia marina* leaves: Impact on seed germination, detoxification enzymes, chlorophyll content and uptake by wheat seedlings. *PloS one* 16:e0249764

Fabrega J, Fawcett SR, Renshaw JC, Lead JRJEs, technology (2009) Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter. 43:7285-7290

Faisal M, Saquib Q, Alatar AA, Al-Khedhairi AA, Hegazy AK, Musarrat J (2013) Phytotoxic hazards of NiO-nanoparticles in tomato: a study on mechanism of cell death. *Journal of hazardous materials* 250:318-332

Falco WF, Scherer MD, Oliveira SL, Wender H, Colbeck I, Lawson T, Caires ARJSotTE (2020) Phytotoxicity of silver nanoparticles on *Vicia faba*: evaluation of particle size effects on photosynthetic performance and leaf gas exchange. 701:134816

Fayez K, El-Deeb B, Mostafa N (2017) Toxicity of biosynthetic silver nanoparticles on the growth, cell ultrastructure and physiological activities of barley plant. *Acta Physiologiae Plantarum* 39:1-13

Fazal H, Abbasi BH, Ahmad N, Ali M (2016) Elicitation of medicinally important antioxidant secondary metabolites with silver and gold nanoparticles in callus cultures of *Prunella vulgaris* L. *Applied biochemistry and biotechnology* 180:1076-1092

Ganguly P, Breen A, Pillai SC (2018) Toxicity of nanomaterials: exposure, pathways, assessment, and recent advances. *ACS Biomaterials Science & Engineering* 4:2237-2275

García-Sánchez S, Bernalles I, Cristobal S (2015) Early response to nanoparticles in the *Arabidopsis* transcriptome compromises plant defence and root-hair development through salicylic acid signalling. *BMC genomics* 16:1-17

Geisler-Lee J, Wang Q, Yao Y, Zhang W, Geisler M, Li K, Huang Y, Chen Y, Kolmakov A, Ma X (2012) Phytotoxicity, accumulation and transport of silver nanoparticles by *Arabidopsis thaliana*. *Nanotoxicology* 7:323-337

Giraldo JP, Landry MP, Faltermeier SM, McNicholas TP, Iverson NM, Boghossian AA, Reuel NF, Hilmer AJ, Sen F, Brew JA (2014) Plant nanobionics approach to augment photosynthesis and biochemical sensing. *Nature materials* 13:400-408

Girilal M, Fayaz AM, Elumalai L, Sathiyaseelan A, Gandhiappan J, Kalaichelvan P (2018) Comparative stress physiology analysis of biologically and chemically synthesized silver nanoparticles on *Solanum lycopersicum* L. *Colloid and Interface Science Communications* 24:1-6

Girilal M, Krishnakumar V, Poornima P, Fayaz AM, Kalaichelvan P (2015) A comparative study on biologically and chemically synthesized silver nanoparticles induced Heat Shock Proteins on fresh water fish *Oreochromis niloticus*. *Chemosphere* 139:461-468

Gupta SD, Agarwal A, Pradhan S (2018) Phytostimulatory effect of silver nanoparticles (AgNPs) on rice seedling growth: An insight from antioxidative enzyme activities and gene expression patterns. *Ecotoxicology and environmental safety* 161:624-633

Haider SR, Reid HJ, Sharp BL (2012) Tricine-sds-page. In: *Protein electrophoresis*. Springer, pp 81-91

Halliwell B, Gutteridge JM (2015) *Free radicals in biology and medicine*. Oxford university press, USA

Handy RD, Owen R, Valsami-Jones E (2008) The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. *Ecotoxicology* 17:315-325

Handy RD, Owen R, Valsami-Jones EJE (2008) The ecotoxicology of nanoparticles and nanomaterials: current status, knowledge gaps, challenges, and future needs. 17:315-325

Hasan M, Mehmood K, Mustafa G, Zafar A, Tariq T, Hassan SG, Loomba S, Zia M, Mazher A, Mahmood N (2021) Phytotoxic evaluation of phytosynthesized silver nanoparticles on lettuce. *Coatings* 11:225

Hatier J-HB, Gould KS (2008) Anthocyanin function in vegetative organs. In: *Anthocyanins*. Springer, pp 1-19

Haverkamp R, Marshall A (2009) The mechanism of metal nanoparticle formation in plants: limits on accumulation. *Journal of Nanoparticle Research* 11:1453-1463

He A, Jiang J, Ding J, Sheng GD (2021) Blocking effect of fullerene nanoparticles (nC60) on the plant cell structure and its phytotoxicity. *Chemosphere* 278:130474

He A, Jiang J, Ding J, Sheng GDJC (2021) Blocking effect of fullerene nanoparticles (nC60) on the plant cell structure and its phytotoxicity. 278:130474

He X, Deng H, Hwang H-m (2019) The current application of nanotechnology in food and agriculture. *Journal of food and drug analysis* 27:1-21

Heita HTN (2018) Evaluating the germination potential of *Pterocarpus angolensis* and *Strychnos cocculoides* with tissue culture techniques. Stellenbosch: Stellenbosch University

Hojjat SS, Ganjali A (2016) The effect of silver nanoparticle on lentil seed germination under drought stress. *Int J Farm Allied Sci* 5:208-212

Homaee MB, Ehsanpour AA (2015) Physiological and biochemical responses of potato (*Solanum tuberosum*) to silver nanoparticles and silver nitrate treatments under in vitro conditions. *Indian Journal of Plant Physiology* 20:353-359

Hu Y, Zhao T, Zhu P, Zhu Y, Liang X, Sun R, Wong CPJCAAJ (2016) Tailoring size and coverage density of silver nanoparticles on monodispersed polymer spheres as highly sensitive SERS substrates. 11:2428-2435

Huang J, Cheng J, Yi J (2016) Impact of silver nanoparticles on marine diatom *Skeletonema costatum*. *Journal of Applied Toxicology* 36:1343-1354

Isa AI, Awouafack MD, Dzoyem JP, Aliyu M, Magaji RA, Ayo JO, Eloff JN (2014) Some *Strychnos spinosa* (Loganiaceae) leaf extracts and fractions have good

antimicrobial activities and low cytotoxicities. BMC complementary and alternative medicine 14:1-8

Jackson M, Mantsch HHJEoacA, theory, instrumentation (2006) Infrared spectroscopy, ex vivo tissue analysis by.

Jalil SU, Ansari MI (2019) Nanoparticles and abiotic stress tolerance in plants: synthesis, action, and signaling mechanisms. Plant Signaling Molecules 549-561

Jan SA, Shinwari ZK, Rabbani MA, Shah SH, Ibrahim MI, Ilyas M (2016) Optimization of an efficient SDS-PAGE protocol for rapid protein analysis of Brassica rapa. J Bio Env Sci 9:17-24

Janmohammadi M, Zolla L, Rinalducci S (2015) Low temperature tolerance in plants: changes at the protein level. Phytochemistry 117:76-89

Jiang HS, Yin LY, Ren NN, Zhao ST, Li Z, Zhi Y, Shao H, Li W, Gontero B (2017) Silver nanoparticles induced reactive oxygen species via photosynthetic energy transport imbalance in an aquatic plant. Nanotoxicology 11:157-167

John KMM, Ayyanar M, Arumugam T, Enkhtaivan G, Jin K, Kim DH (2015) Phytochemical screening and antioxidant activity of different solvent extracts from Strychnos minor Dennst leaves. Asian Pacific Journal of Tropical Disease 5:204-209

Kang Y, Si M, Liu R, Qiao S (2010) Surface-enhanced Raman scattering (SERS) spectra of hemoglobin on nano silver film prepared by electrolysis method. Journal of Raman Spectroscopy 41:614-617

Karami Mehrian S, Heidari R, Rahmani F (2015) Effect of silver nanoparticles on free amino acids content and antioxidant defense system of tomato plants. Indian Journal of Plant Physiology 20:257-263

Karuppanapandian T, Moon J-C, Kim C, Manoharan K, Kim W (2011) Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Australian Journal of Crop Science 5:709-725

Kaveh R, Li Y-S, Ranjbar S, Tehrani R, Brueck CL, Van Aken B (2013) Changes in Arabidopsis thaliana gene expression in response to silver nanoparticles and silver ions. Environmental science & technology 47:10637-10644

Ke M, Li Y, Qu Q, Ye Y, Peijnenburg W, Zhang Z, Xu N, Lu T, Sun L, Qian H (2020) Offspring toxicity of silver nanoparticles to Arabidopsis thaliana flowering and floral development. Journal of hazardous materials 386:121975

Khan I, Raza MA, Awan SA, Shah GA, Rizwan M, Ali B, Tariq R, Hassan MJ, Alyemeni MN, Brestic M (2020) Amelioration of salt induced toxicity in pearl millet by seed priming with silver nanoparticles (AgNPs): The oxidative damage, antioxidant enzymes and ions uptake are major determinants of salt tolerant capacity. *Plant Physiology and Biochemistry* 156:221-232

Khan I, Raza MA, Khalid MHB, Awan SA, Raja NI, Zhang X, Min S, Wu BC, Hassan MJ, Huang L (2019) Physiological and biochemical responses of pearl millet (*Pennisetum glaucum* L.) seedlings exposed to silver nitrate (AgNO₃) and silver nanoparticles (AgNPs). *International journal of environmental research and public health* 16:2261

Khan MA, Wallace WT, Sambhi J, Rogers DT, Littleton JM, Rankin SE, Knutson BL (2020) Nanoharvesting of bioactive materials from living plant cultures using engineered silica nanoparticles. *Materials Science and Engineering: C* 106:110190

Kim J-H, Oh Y, Yoon H, Hwang I, Chang Y-S (2015) Iron nanoparticle-induced activation of plasma membrane H⁺-ATPase promotes stomatal opening in *Arabidopsis thaliana*. *Environmental science & technology* 49:1113-1119

Klaine SJ, Alvarez PJ, Batley GE, Fernandes TF, Handy RD, Lyon DY, Mahendra S, McLaughlin MJ, Lead JR (2008) Nanomaterials in the environment: behavior, fate, bioavailability, and effects. *Environmental Toxicology and Chemistry: An International Journal* 27:1825-1851

Kole C, Kole P, Randunu KM, Choudhary P, Podila R, Ke PC, Rao AM, Marcus RK (2013) Nanobiotechnology can boost crop production and quality: first evidence from increased plant biomass, fruit yield and phytomedicine content in bitter melon (*Momordica charantia*). *BMC biotechnology* 13:1-10

Kora AJ, Manjusha R, Arunachalam J (2009) Superior bactericidal activity of SDS capped silver nanoparticles: synthesis and characterization. *Materials Science and Engineering: C* 29:2104-2109

Kora AJ, Manjusha R, Arunachalam JJMS, C E (2009) Superior bactericidal activity of SDS capped silver nanoparticles: synthesis and characterization. 29:2104-2109

Kosová K, Prášil IT, Vítámvás P (2013) Protein contribution to plant salinity response and tolerance acquisition. *International journal of molecular sciences* 14:6757-6789

- Kosová K, Vítámvás P, Klíma M, Prášil IT (2019) Breeding drought-resistant crops: G× E interactions, proteomics and pQTLs. *Journal of experimental botany* 70:2605-2608
- Kumar A, Prasad M, Sytar O (2012) Lead toxicity, defense strategies and associated indicative biomarkers in *Talinum triangulare* grown hydroponically. *Chemosphere* 89:1056-1065
- Kundu M, Pathak J, Sahni S (2017) Embryo Culture and Endosperm Culture. In: *Plant Biotechnology*. Apple Academic Press, pp 125-141
- Künniger T, Gerecke AC, Ulrich A, Huch A, Vonbank R, Heeb M, Wichser A, Haag R, Kunz P, Faller M (2014) Release and environmental impact of silver nanoparticles and conventional organic biocides from coated wooden façades. *Environmental Pollution* 184:464-471
- Laby RJ, Kincaid MS, Kim D, Gibson SI (2000) The *Arabidopsis* sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. *The Plant Journal* 23:587-596
- Leite JPV, Xavier AA, Batista DS, Vital CE, de Oliveira Ramos HJ, Otoni WC (2021) Embryo culture, callus induction, and flavonoid profile of *Strychnos pseudoquina* A. St.-Hil., an important medicinal species from the Brazilian Cerrado biome. *Plant Cell, Tissue and Organ Culture (PCTOC)* 145:579-589
- Li X, Ke M, Zhang M, Peijnenburg W, Fan X, Xu J, Zhang Z, Lu T, Fu Z, Qian H (2018) The interactive effects of diclofop-methyl and silver nanoparticles on *Arabidopsis thaliana*: growth, photosynthesis and antioxidant system. *Environmental pollution* 232:212-219
- Liu F, Cao Z, Tang C, Chen L, Wang Z (2010) Ultrathin diamond-like carbon film coated silver nanoparticles-based substrates for surface-enhanced Raman spectroscopy. *ACS nano* 4:2643-2648
- Liu N, Zhong G, Zhou J, Liu Y, Pang Y, Cai H, Wu ZJSotTE (2019) Separate and combined effects of glyphosate and copper on growth and antioxidative enzymes in *Salvinia natans* (L.) All. 655:1448-1456
- Liu R, Lal R (2015) Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. *Science of the total environment* 514:131-139
- Luo J, Fang Z, Smith Jr RLJPiE, Science C (2014) Ultrasound-enhanced conversion of biomass to biofuels. 41:56-93

Ma L, Liu J (2020) Catalytic nucleic acids: biochemistry, chemical biology, biosensors, and nanotechnology. *Iscience* 23:100815

Ma X, Geiser-Lee J, Deng Y, Kolmakov A (2010) Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. *Science of the total environment* 408:3053-3061

Ma YJToN (2016) Characterization of nanomaterials in nanotoxicological analyses.

Mahmood T, Saddique M, Naeem A, Mustafa S, Zeb N, Shah K, Waseem M (2011) Kinetic and thermodynamic study of Cd (II), Co (II) and Zn (II) adsorption from aqueous solution by NiO. *Chemical Engineering Journal* 171:935-940

Mallikarjuna K, Narasimha G, Dillip G, Praveen B, Shreedhar B, Lakshmi CS, Reddy B, Raju BDP (2011) Green synthesis of silver nanoparticles using Ocimum leaf extract and their characterization. *Digest Journal of Nanomaterials and Biostructures* 6:181-186

Mancinelli AL (1984) Photoregulation of anthocyanin synthesis: VIII. Effect of light pretreatments. *Plant Physiology* 75:447-453

Medina-Pérez G, Fernández-Luqueño F, Vazquez-Nuñez E, López-Valdez F, Prieto-Mendez J, Madariaga-Navarrete A, Miranda-Arámbula M (2019) Remediating Polluted Soils Using Nanotechnologies: Environmental Benefits and Risks. *Polish Journal of Environmental Studies* 28:

Mehrian SK, De Lima R (2016) Nanoparticles cyto and genotoxicity in plants: Mechanisms and abnormalities. *Environmental Nanotechnology, Monitoring & Management* 6:184-193

Milewska-Hendel A, Zubko M, Stróż D, Kurczyńska EU (2019) Effect of nanoparticles surface charge on the Arabidopsis thaliana (L.) roots development and their movement into the root cells and protoplasts. *International journal of molecular sciences* 20:1650

Miralles P, Church TL, Harris AT (2012) Toxicity, uptake, and translocation of engineered nanomaterials in vascular plants. *Environmental science & technology* 46:9224-9239

Mirzajani F, Askari H, Hamzelou S, Schober Y, Römpf A, Ghassempour A, Spengler BJE, safety e (2014) Proteomics study of silver nanoparticles toxicity on *Bacillus thuringiensis*. 100:122-130

Mishra S, Singh BR, Singh A, Keswani C, Naqvi AH, Singh H (2014) Biofabricated silver nanoparticles act as a strong fungicide against *Bipolaris sorokiniana* causing spot blotch disease in wheat. *Plos one* 9:e97881

Mistry H, Thakor R, Patil C, Trivedi J, Bariya H (2021) Biogenically proficient synthesis and characterization of silver nanoparticles employing marine procured fungi *Aspergillus brunneoviolaceus* along with their antibacterial and antioxidative potency. *Biotechnology Letters* 43:307-316

Mittal AK, Chisti Y, Banerjee UC (2013) Synthesis of metallic nanoparticles using plant extracts. *Biotechnology advances* 31:346-356

Mittal AK, Tripathy D, Choudhary A, Aili PK, Chatterjee A, Singh IP, Banerjee UC (2015) Bio-synthesis of silver nanoparticles using *Potentilla fulgens* Wall. ex Hook. and its therapeutic evaluation as anticancer and antimicrobial agent. *Materials Science and Engineering: C* 53:120-127

Mohamed AKS, Qayyum MF, Abdel-Hadi AM, Rehman RA, Ali S, Rizwan M (2017) Interactive effect of salinity and silver nanoparticles on photosynthetic and biochemical parameters of wheat. *Archives of Agronomy and Soil Science* 63:1736-1747

Mohamed MS, Kumar DS (2016) Effect of nanoparticles on plants with regard to physiological attributes. In: *Plant Nanotechnology*. Springer, pp 119-153

Mohandas GG, Kumaraswamy M (2018) Antioxidant activities of terpenoids from *Thuidium tamariscellum* (C. Muell.) Bosch. and Sande-Lac. a Moss. *Pharmacognosy Journal* 10:

Moore RJ (1947) Cytotaxonomic studies in the Loganiaceae. I. Chromosome numbers and phylogeny in the Loganiaceae. *American Journal of Botany* 527-538

Moustaka J, Panteris E, Adamakis I-DS, Tanou G, Giannakoula A, Eleftheriou EP, Moustakas MJE, Botany E (2018) High anthocyanin accumulation in poinsettia leaves is accompanied by thylakoid membrane unstacking, acting as a photoprotective mechanism, to prevent ROS formation. 154:44-55

Mustafa G, Sakata K, Hossain Z, Komatsu S (2015) Proteomic study on the effects of silver nanoparticles on soybean under flooding stress. *Journal of proteomics* 122:100-118

Mwamba CK (2006) Monkey orange: *strychnos cocculoides*. *Crops for the Future*

- Nair PMG, Chung IM (2014) Assessment of silver nanoparticle-induced physiological and molecular changes in *Arabidopsis thaliana*. *Environmental Science and Pollution Research* 21:8858-8869
- Nair PMG, Chung IM (2014) Physiological and molecular level effects of silver nanoparticles exposure in rice (*Oryza sativa* L.) seedlings. *Chemosphere* 112:105-113
- Nandhini M, Rajini S, Udayashankar A, Niranjana S, Lund OS, Shetty H, Prakash H (2019) Biofabricated zinc oxide nanoparticles as an eco-friendly alternative for growth promotion and management of downy mildew of pearl millet. *Crop Protection* 121:103-112
- Nandiyanto ABD, Oktiani R, Ragadhita R (2019) How to read and interpret FTIR spectroscope of organic material. *Indonesian Journal of Science and Technology* 4:97-118
- Nangia S, Sureshkumar R (2012) Effects of nanoparticle charge and shape anisotropy on translocation through cell membranes. *Langmuir* 28:17666-17671
- Nasci C, Da Ros L, Nesto N, Sperti L, Passarini F, Pavoni B (2000) Biochemical and histochemical responses to environmental contaminants in clam, *Tapes philippinarum*, transplanted to different polluted areas of Venice Lagoon, Italy. *Marine environmental research* 50:425-430
- Navarro E, Wagner B, Odzak N, Sigg L, Behra R (2015) Effects of differently coated silver nanoparticles on the photosynthesis of *Chlamydomonas reinhardtii*. *Chemosphere* 125:8041-8047
- Ndeh NT, Maensiri S, Maensiri D (2017) The effect of green synthesized gold nanoparticles on rice germination and roots. *Advances in Natural Sciences: Nanoscience and Nanotechnology* 8:035008
- Neuwinger H (1998) Alkaloids in arrow poisons. In: *Alkaloids*. Springer, pp 45-84
- Ngadze RT, Linnemann AR, Nyanga LK, Fogliano V, Verkerk R (2017) Local processing and nutritional composition of indigenous fruits: The case of monkey orange (*Strychnos* spp.) from Southern Africa. *Food Reviews International* 33:123-142
- Olawoyin R (2018) Nanotechnology: The future of fire safety. *Safety science* 110:214-221
- Olchowik J, Bzdyk RM, Studnicki M, Bederska-Błaszczuk M, Urban A, Aleksandrowicz-Trzcińska M (2017) The effect of silver and copper nanoparticles

on the condition of english oak (*Quercus robur* L.) seedlings in a container nursery experiment. *Forests* 8:310

Oppelt AL, Kurth W, Jentschke G, Godbold DL (2005) Contrasting rooting patterns of some arid-zone fruit tree species from Botswana—I. Fine root distribution. *Agroforestry Systems* 64:1-11

Othman SH (2014) Bio-nanocomposite materials for food packaging applications: types of biopolymer and nano-sized filler. *Agriculture and Agricultural Science Procedia* 2:296-303

Ovečka M, Lang I, Baluška F, Ismail A, Illeš P, Lichtscheidl IJP (2005) Endocytosis and vesicle trafficking during tip growth of root hairs. 226:39-54

Pacheco I, Buzea C (2018) Nanoparticle uptake by plants: beneficial or detrimental? In: *Phytotoxicity of nanoparticles*. Springer, pp 1-61

Park S, Ahn Y-J (2016) Multi-walled carbon nanotubes and silver nanoparticles differentially affect seed germination, chlorophyll content, and hydrogen peroxide accumulation in carrot (*Daucus carota* L.). *Biocatalysis and Agricultural Biotechnology* 8:257-262

Peharec Štefanić P, Jarnević M, Cvjetko P, Biba R, Šikić S, Tkalec M, Cindrić M, Letofsky-Papst I, Balen B (2019) Comparative proteomic study of phytotoxic effects of silver nanoparticles and silver ions on tobacco plants. *Environmental Science and Pollution Research* 26:22529-22550

Peharec Štefanić P, Jarnević M, Cvjetko P, Biba R, Šikić S, Tkalec M, Cindrić M, Letofsky-Papst I, Balen BJES, Research P (2019) Comparative proteomic study of phytotoxic effects of silver nanoparticles and silver ions on tobacco plants. 26:22529-22550

Phillips O (1991) The ethnobotany and economic botany of tropical vines. *The Biology of Vines* Cambridge University Press, Cambridge 427-475

Pierik RLM (1997) *In vitro* culture of higher plants. Springer science & business media

Pittol M, Tomacheski D, Simões DN, Ribeiro VF, Santana RMC (2017) Macroscopic effects of silver nanoparticles and titanium dioxide on edible plant growth. *Environmental nanotechnology, monitoring & management* 8:127-133

Pospíšil P, Prasad A, Rác M (2019) Mechanism of the formation of electronically excited species by oxidative metabolic processes: role of reactive oxygen species. *Biomolecules* 9:258

Prasad R, Kumar V, Prasad KSJAJoB (2014) Nanotechnology in sustainable agriculture: present concerns and future aspects. 13:705-713

Qian H, Peng X, Han X, Ren J, Sun L, Fu Z (2013) Comparison of the toxicity of silver nanoparticles and silver ions on the growth of terrestrial plant model *Arabidopsis thaliana*. Journal of environmental sciences 25:1947-1956

Rafique M, Jahangir J, Amin BAZ, Tahir MB, Nabi G, Khan MI, Khalid N, Gillani S, Sadaf I (2019) Investigation of photocatalytic and seed germination effects of TiO₂ nanoparticles synthesized by *Melia azedarach* L. Leaf extract. Journal of Inorganic and Organometallic Polymers and Materials 29:2133-2144

Rajalakshmi BS, Vasanthi M, Rajakannan V, Ravindran B, Chang SW, Chandrasekaran M, Alwahibi MS, Ali MA, Albulwi S, Selvi CT (2021) Defluoridation of water with a coagulant, *Strychnos potatorum* L. seed–agglutinin. Environmental Technology & Innovation 24:101983

Rajendran S, Priya T, Khoo KS, Hoang TK, Ng H-S, Munawaroh HSH, Karaman C, Orooji Y, Show PL (2022) A critical review on various remediation approaches for heavy metal contaminants removal from contaminated soils. Chemosphere 287:132369

Rajput VD, Minkina T, Sushkova S, Mandzhieva S, Fedorenko A, Lysenko V, Bederska-Błaszczuk M, Olchowik J, Tsitsuashvili V, Chaplygin V (2019) Structural and ultrastructural changes in nanoparticle exposed plants. In: Nanoscience for sustainable agriculture. Springer, pp 281-295

Raliya R, Tarafdar JC, Biswas P (2016) Enhancing the mobilization of native phosphorus in the mung bean rhizosphere using ZnO nanoparticles synthesized by soil fungi. Journal of agricultural and food chemistry 64:3111-3118

Ramezani M, Asghari S, Gerami M, Ramezani F, Abdolmaleki MK (2019) Effect of silver nanoparticle treatment on the expression of key genes involved in glycosides biosynthetic pathway in *Stevia rebaudiana* B. Plant. Sugar Tech 1-10

Rani PU, Yasur J, Loke KS, Dutta D (2016) Effect of synthetic and biosynthesized silver nanoparticles on growth, physiology and oxidative stress of water hyacinth: *Eichhornia crassipes* (Mart) Solms. Acta physiologiae plantarum 38:58

Rao B, Tang R-C (2017) Green synthesis of silver nanoparticles with antibacterial activities using aqueous *Eriobotrya japonica* leaf extract. Advances in natural sciences: Nanoscience and nanotechnology 8:015014

- Rascio N, Navari-Izzo F (2011) Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? *Plant science* 180:169-181
- Rastogi A, Zivcak M, Tripathi D, Yadav S, Kalaji H, Brestic M (2019) Phytotoxic effect of silver nanoparticles in *Triticum aestivum*: Improper regulation of photosystem I activity as the reason for oxidative damage in the chloroplast. *Photosynthetica* 57:209-216
- Reheem AA, Maksoud MA, Ashour A (2016) Surface modification and metallization of polycarbonate using low energy ion beam. *Radiation Physics and Chemistry* 125:171-175
- Ribarova F, Atanassova M, Marinova D, Ribarova F, Atanassova M (2005) Total phenolics and flavonoids in Bulgarian fruits and vegetables. *JU Chem Metal* 40:255-260
- Rinna A, Magdolenova Z, Hudecova A, Kruszewski M, Refsnes M, Dusinska M (2015) Effect of silver nanoparticles on mitogen-activated protein kinases activation: role of reactive oxygen species and implication in DNA damage. *Mutagenesis* 30:59-66
- Rizwan M, Ali S, Ali B, Adrees M, Arshad M, Hussain A, ur Rehman MZ, Waris AA (2019) Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and cadmium concentration in wheat. *Chemosphere* 214:269-277
- Robles-García MA, Rodriguez-Felix F, Marquez-Rios E, Aguilar JA, Barrera-Rodriguez A, Aguilar J, Ruiz-Cruz S, Del-Toro-Sánchez CL (2016) Applications of nanotechnology in the agriculture, food, and pharmaceuticals. *Journal of Nanoscience and Nanotechnology* 16:8188-8207
- Sabo-Attwood T, Unrine JM, Stone JW, Murphy CJ, Ghoshroy S, Blom D, Bertsch PM, Newman LA (2012) Uptake, distribution and toxicity of gold nanoparticles in tobacco (*Nicotiana xanthi*) seedlings. *Nanotoxicology* 6:353-360
- Sadak MS (2019) Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenum-graecum*). *Bulletin of the National Research Centre* 43:1-6
- Salama HM (2012) Effects of silver nanoparticles in some crop plants, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). *Int Res J Biotechnol* 3:190-197

Samadi N, Yahyaabadi S, Rezayatmand Z (2014) Effect of TiO₂ and TiO₂ nanoparticle on germination, root and shoot Length and photosynthetic pigments of *Mentha piperita*. *Int J Plant Soil Sci* 3:408-418

Sankar MK, Kumar KM, Ranganathan B (2015) Adsorption of anionic azo dye from aqueous solution using *Strychnos potatorum* Linn seeds: isotherm and kinetic studies. *International journal of environmental science and technology* 12:2957-2964

Saptarshi SR, Duschl A, Lopata AL (2013) Interaction of nanoparticles with proteins: relation to bio-reactivity of the nanoparticle. *Journal of nanobiotechnology* 11:1-12

Sarawgi G, Kamra A, Suri N, Kaur A, Sarethy IPJAJow, Environment, Pollution (2009) Effect of *Strychnos potatorum* Linn. Seed extracts on water samples from different sources and with diverse properties. 6:13-17

Schlereth A, Becker C, Horstmann C, Tiedemann J, Muntz K (2000) Comparison of globulin mobilization and cysteine proteinases in embryonic axes and cotyledons during germination and seedling growth of vetch (*Vicia sativa* L.). *Journal of Experimental Botany* 51:1423-1433

Senthil Kumar P, Sai Deepthi A, Bharani R, Prabhakaran C (2013) Adsorption of Cu (II), Cd (II) and Ni (II) ions from aqueous solution by unmodified *Strychnos potatorum* seeds. *European journal of environmental and civil engineering* 17:293-314

Shahid M, Pourrut B, Dumat C, Nadeem M, Aslam M, Pinelli E (2014) Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. *Reviews of Environmental Contamination and Toxicology* Volume 232 1-44

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany* 2012:

Shoko T, Apostolides Z, Monjerezi M, Saka J (2013) Volatile constituents of fruit pulp of *Strychnos cocculoides* (Baker) growing in Malawi using solid phase microextraction. *South African journal of botany* 84:11-12

Shukla PK, Misra P, Kole C (2016) Uptake, translocation, accumulation, transformation, and generational transmission of nanoparticles in plants. *Plant nanotechnology* 183-218

- Siddiqui MH, Al-Whaibi MH, Firoz M, Al-Khaishany MY (2015) Role of nanoparticles in plants. *Nanotechnology and plant sciences* 19-35
- Siddiqui SA, Blinov AV, Serov AV, Gvozdenko AA, Kravtsov AA, Nagdalian AA, Raffa VV, Maglakelidze DG, Blinova AA, Kobina AV (2021) Effect of selenium nanoparticles on germination of *hordéum vulgáre* barley seeds. *Coatings* 11:862
- Siddiqui SA, Blinov AV, Serov AV, Gvozdenko AA, Kravtsov AA, Nagdalian AA, Raffa VV, Maglakelidze DG, Blinova AA, Kobina AVJC (2021) Effect of selenium nanoparticles on germination of *Hordéum Vulgáre* barley seeds. 11:862
- Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: *Methods in enzymology*. Elsevier, pp 152-178
- Sitrit Y, Loison S, Ninio R, Dishon E, Bar E, Lewinsohn E, Mizrahi Y (2003) Characterization of monkey orange (*Strychnos spinosa* Lam.), a potential new crop for arid regions. *Journal of agricultural and food chemistry* 51:6256-6260
- Smith M, Drew R (1990) Current applications of tissue culture in plant propagation and improvement. *Functional Plant Biology* 17:267-289
- Song U, Jun H, Waldman B, Roh J, Kim Y, Yi J, Lee EJ (2013) Functional analyses of nanoparticle toxicity: a comparative study of the effects of TiO₂ and Ag on tomatoes (*Lycopersicon esculentum*). *Ecotoxicology and environmental safety* 93:60-67
- Sreelekshmi R, Siril E, Muthukrishnan S (2021) Role of biogenic silver nanoparticles on hyperhydricity reversion in *Dianthus chinensis* L. an in vitro model culture. *Journal of Plant Growth Regulation* 1-17
- Srivastava A, Rao D (2014) Enhancement of seed germination and plant growth of wheat, maize, peanut and garlic using multiwalled carbon nanotubes. *European Chemical Bulletin* 3:502-504
- Štefanić PP, Cvjetko P, Biba R, Domijan A-M, Letofsky-Papst I, Tkalec M, Šikić S, Cindrić M, Balen B (2018) Physiological, ultrastructural and proteomic responses of tobacco seedlings exposed to silver nanoparticles and silver nitrate. *Chemosphere* 209:640-653
- Struwe L, Gibbons K, Conn B, Motley T (2018) Loganiaceae. In: *Flowering Plants Eudicots*. Springer, pp 511-526
- Sudha PN, Sangeetha K, Vijayalakshmi K, Barhoum A (2018) Nanomaterials history, classification, unique properties, production and market. In: *Emerging*

Applications of Nanoparticles and Architecture Nanostructures. Elsevier, pp 341-384

Sudhira L, Rao SV, Kamakshamma J (2015) Phytochemical Screening, Antioxidant and Antibacterial Activity of *Strychnos colubrina* L. as an important Endangered Medicinal Species in Eastern Ghats. *Journal of pharmaceutical sciences and research* 7:242

Sunghwa F, Koketsu M (2009) Phenolic and bis-iridoid glycosides from *Strychnos cocculoides*. *Natural product research* 23:1408-1415

Sweet MJ, Singleton I (2015) Soil contamination with silver nanoparticles reduces Bishop pine growth and ectomycorrhizal diversity on pine roots. *Journal of Nanoparticle Research* 17:1-8

Syu Y-y, Hung J-H, Chen J-C, Chuang H-w (2014) Impacts of size and shape of silver nanoparticles on *Arabidopsis* plant growth and gene expression. *Plant physiology and biochemistry* 83:57-64

Tadayon F, Jahromi M (2020) A sensitive and selective electrochemical determination of Diazinon based on Au–Pd/rGO–MWCNTs composite: Analytical application in water, fruit and vegetable samples. *Journal of the Iranian Chemical Society* 17:847-857

Tamizifar M, Sun G (2018) Controlled surface functionalization of poly (ethylene terephthalate) fibers with varied vinyl monomers via radical graft copolymerization. *Materials Today Communications* 17:124-132

Thiruvengadam M, Gurunathan S, Chung I-MJP (2015) Physiological, metabolic, and transcriptional effects of biologically-synthesized silver nanoparticles in turnip (*Brassica rapa* ssp. *rapa* L.). 252:1031-1046

Thrall ES, Preska Steinberg A, Wu X, Brus LE (2013) The role of photon energy and semiconductor substrate in the plasmon-mediated photooxidation of citrate by silver nanoparticles. *The Journal of Physical Chemistry C* 117:26238-26247

Tor-Anyiin T, Igoli J, Anyam J, Anyam J (2015) ISOLATION AND ANTIMICROBIAL ACTIVITY OF SARRACENIN FROM ROOT BARK OF *STRYCHNOS SPINOSA*. *Journal of Chemical Society of Nigeria* 40:

Tripathi A, Liu S, Singh PK, Kumar N, Pandey AC, Tripathi DK, Chauhan DK, Sahi S (2017) Differential phytotoxic responses of silver nitrate (AgNO_3) and silver nanoparticle (AgNps) in *Cucumis sativus* L. *Plant Gene* 11:255-264

Tripathi A, Liu S, Singh PK, Kumar N, Pandey AC, Tripathi DK, Chauhan DK, Sahi SJPG (2017) Differential phytotoxic responses of silver nitrate (AgNO₃) and silver nanoparticle (AgNps) in *Cucumis sativus* L. 11:255-264

Tripathi DK, Tripathi A, Singh S, Singh Y, Vishwakarma K, Yadav G, Sharma S, Singh VK, Mishra RK, Upadhyay RJFim (2017) Uptake, accumulation and toxicity of silver nanoparticle in autotrophic plants, and heterotrophic microbes: a concentric review. 8:07

Tripathy BC, Oelmüller R (2012) Reactive oxygen species generation and signaling in plants. *Plant signaling & behavior* 7:1621-1633

Twaij BM, Jazar ZH, Hasan M (2020) Trends in the use of tissue culture, applications and future aspects. *International Journal of Plant Biology* 11:8385

Tymoszuik A, Kulus D (2020) Silver nanoparticles induce genetic, biochemical, and phenotype variation in chrysanthemum. *Plant Cell, Tissue and Organ Culture (PCTOC)* 143:331-344

Ur Rahim H, Qaswar M, Uddin M, Giannini C, Herrera ML, Rea G (2021) Nano-enable materials promoting sustainability and resilience in modern agriculture. *Nanomaterials* 11:2068

Vanaja M, Annadurai G (2013) *Coleus aromaticus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. *Applied nanoscience* 3:217-223

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella Jr MF, Rejeski D, Hull MS (2015) Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. *Beilstein journal of nanotechnology* 6:1769-1780

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella Jr MF, Rejeski D, Hull MSBJon (2015) Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. 6:1769-1780

Vannini C, Domingo G, Onelli E, Prinsi B, Marsoni M, Espen L, Bracale M (2013) Morphological and proteomic responses of *Eruca sativa* exposed to silver nanoparticles or silver nitrate. *PloS one* 8:e68752

Večeřová K, Večeřa Z, Dočekal B, Oravec M, Pompeiano A, Tříška J, Urban O (2016) Changes of primary and secondary metabolites in barley plants exposed to CdO nanoparticles. *Environmental Pollution* 218:207-218

Vishali S, Karthikeyan R (2014) A comparative study of *Strychnos potatorum* and chemical coagulants in the treatment of paint and industrial effluents: an alternate solution. *Separation Science and Technology* 49:2510-2517

Vishwakarma K, Upadhyay N, Singh J, Liu S, Singh VP, Prasad SM, Chauhan DK, Tripathi DK, Sharma S (2017) Differential phytotoxic impact of plant mediated silver nanoparticles (AgNPs) and silver nitrate (AgNO₃) on Brassica sp. *Frontiers in plant science* 8:1501

Wan J, Wang R, Wang R, Ju Q, Wang Y, Xu J (2019) Comparative physiological and transcriptomic analyses reveal the toxic effects of ZnO nanoparticles on plant growth. *Environmental science & technology* 53:4235-4244

Wan J, Wang R, Wang R, Ju Q, Wang Y, Xu J (2019) Comparative physiological and transcriptomic analyses reveal the toxic effects of ZnO nanoparticles on plant growth. *53:4235-4244*

Wang Q, Ebbs SD, Chen Y, Ma X (2013) Trans-generational impact of cerium oxide nanoparticles on tomato plants. *Metallomics* 5:753-759

Wang S (2018) Alkaloid Synthesis via Gold-Catalyzed Carbon-Carbon Bond Formation Using Enamine-type Nucleophile. *Israel Journal of Chemistry* 58:557-567

Wang W-N, Tarafdar JC, Biswas P (2013) Nanoparticle synthesis and delivery by an aerosol route for watermelon plant foliar uptake. *Journal of nanoparticle research* 15:1-13

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in plant science* 9:244-252

Wang X, Li Q, Pei Z, Wang S (2018) Effects of zinc oxide nanoparticles on the growth, photosynthetic traits, and antioxidative enzymes in tomato plants. *Biologia plantarum* 62:801-808

Wang Y, Deng C, Cota-Ruiz K, Peralta-Videa JR, Sun Y, Rawat S, Tan W, Reyes A, Hernandez-Viezcás JA, Niu G (2020) Improvement of nutrient elements and allicin content in green onion (*Allium fistulosum*) plants exposed to CuO nanoparticles. *Science of The Total Environment* 725:138387

Wang Y, Yuan M, Li Z, Niu Y, Jin Q, Zhu B, Xu Y (2020) Effects of ethylene biosynthesis and signaling on oxidative stress and antioxidant defense system in *Nelumbo nucifera* G. under cadmium exposure. *Environmental Science and Pollution Research* 27:40156-40170

- Wu H, Tito N, Giraldo JP (2017) Anionic cerium oxide nanoparticles protect plant photosynthesis from abiotic stress by scavenging reactive oxygen species. *ACS nano* 11:11283-11297
- Xin X, Zhao F, Rho JY, Goodrich SL, Sumerlin BS, He Z (2020) Use of polymeric nanoparticles to improve seed germination and plant growth under copper stress. *Science of The Total Environment* 745:141055
- Xiu Z-M, Ma J, Alvarez PJ (2011) Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. *Environmental science & technology* 45:9003-9008
- Yadu B, Chandrakar V, Korram J, Satnam ML, Kumar M, Keshavkant S (2018) Silver nanoparticle modulates gene expressions, glyoxalase system and oxidative stress markers in fluoride stressed *Cajanus cajan* L. *Journal of hazardous materials* 353:44-52
- Yang J, Jiang F, Ma C, Rui Y, Rui M, Adeel M, Cao W, Xing B (2018) Alteration of crop yield and quality of wheat upon exposure to silver nanoparticles in a life cycle study. *Journal of agricultural and food chemistry* 66:2589-2597
- Yin L, Colman BP, McGill BM, Wright JP, Bernhardt ES (2012) Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants.
- Younis A, Khattab H, Emam M (2020) Impacts of silicon and silicon nanoparticles on leaf ultrastructure and TaPIP1 and TaNIP2 gene expressions in heat stressed wheat seedlings. *Biologia plantarum* 64:343-352
- Zahir A, Nadeem M, Ahmad W, Giglioli-Guivarc'h N, Hano C, Abbasi BH (2019) Chemogenic silver nanoparticles enhance lignans and neolignans in cell suspension cultures of *Linum usitatissimum* L. *Plant Cell, Tissue and Organ Culture (PCTOC)* 136:589-596
- Zare-Bidaki M, Mohammadparast-Tabas P, Peyghambari Y, Chamani E, Siami-Aliabad M, Mortazavi-Derazkola S (2021) Photochemical synthesis of metallic silver nanoparticles using *Pistacia khinjuk* leaves extract (PKL@ AgNPs) and their applications as an alternative catalytic, antioxidant, antibacterial, and anticancer agents. *Applied Organometallic Chemistry* e6478
- Zare-Bidaki M, Mohammadparast-Tabas P, Peyghambari Y, Chamani E, Siami-Aliabad M, Mortazavi-Derazkola S (2022) Photochemical synthesis of metallic silver nanoparticles using *Pistacia khinjuk* leaves extract (PKL@ AgNPs)

and their applications as an alternative catalytic, antioxidant, antibacterial, and anticancer agents. 36:e6478

Zhang H, Du W, Peralta-Videa JR, Gardea-Torresdey JL, White JC, Keller A, Guo H, Ji R, Zhao L (2018) Metabolomics reveals how cucumber (*Cucumis sativus*) reprograms metabolites to cope with silver ions and silver nanoparticle-induced oxidative stress. *Environmental science & technology* 52:8016-8026

Zhang J, Guo W, Li Q, Wang Z, Liu S (2018) The effects and the potential mechanism of environmental transformation of metal nanoparticles on their toxicity in organisms. *Environmental Science: Nano* 5:2482-2499

Zhao J-L, Zhou L-G, Wu J-Y (2010) Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in *Salvia miltiorrhiza* cell cultures. *Applied Microbiology and Biotechnology* 87:137-144

Zhao Z, Xu L, Wang Y, Li B, Zhang W, Li X (2021) Toxicity mechanism of silver nanoparticles to *Chlamydomonas reinhardtii*: Photosynthesis, oxidative stress, membrane permeability, and ultrastructure analysis. *Environmental Science and Pollution Research* 28:15032-15042

Ziotti ABS, Ottoni CA, Correa CN, de Almeida OJG, de Souza AO, Neto MCL (2021) Differential physiological responses of a biogenic silver nanoparticle and its production matrix silver nitrate in *Sorghum bicolor*. *Environmental Science and Pollution Research* 1-14

APPENDIX A

Table 1: Resolution and stacking gel reagent proportions

Reagent	12% Resolution Gel (mL)	4.5% Stacking Gel (mL)
40% Acrylamide	3.13	0.56
Distilled water	4.2	3.73
1.5M Tris-HCl (pH 8.8)	2.5	-
1M Tris-HCl (pH 6.8)	-	0.63
Sodium Dodecyl Sulphate (10%)	0.1	0.1
Ammonium persulphate (10%)	0.05	0.05
TEMED	0.02	0.01

APPENDIX B

Table 2: XRD data of green synthesized AgNPs

D	2θ	I	h	K	L
2.35430	38.196	999	-1	-1	-1
2.03890	44.395	468	-2	0	0
1.44170	64.493	256	-2	-2	0
1.22950	77.587	271	-3	-1	-1
1.17720	81.741	77	-2	-2	-2

APPENDIX C

Table 3: Shows the content of (a), Chlorophyll *a* (b) Chlorophyll *b* in *Strychnos* seedlings treated with different concentrations of AgNPs.

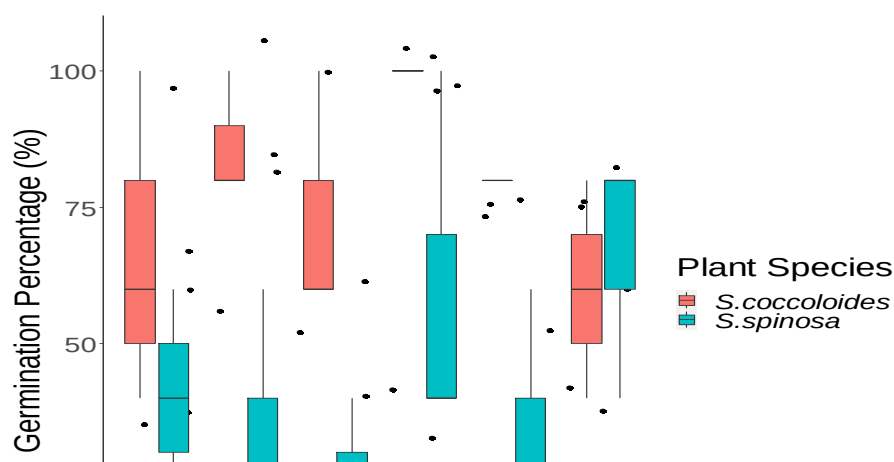
AgNPs (µg/ml)	<i>S. cocculoides</i>	<i>S. spinosa</i>
0	8.10 ± 0.149 ^a	4.06 ± 0.015 ^c
12.5	1.79 ± 0.021 ^b	1.80 ± 0.022 ^d
25	2.67 ± 0.015 ^{ab}	2.68 ± 0.061 ^{cd}
50	1.37 ± 0.010 ^b	1.80 ± 0.025 ^d
100	2.47 ± 0.008 ^{ab}	2.48 ± 0.015 ^{cd}
200	1.57 ± 0.009 ^b	2.03 ± 0.015 ^d

a

AgNPs (µg/ml)	<i>S. cocculoides</i>	<i>S. spinosa</i>
0	14.4 ± 0.009 ^a	5.08 ± 0.005 ^c
12.5	8.21 ± 0.020 ^b	3.99 ± 0.005 ^d
25	7.5 ± 0.005 ^{ab}	3.52 ± 0.009 ^{cd}
50	7.09 ± 0.005 ^b	2.40 ± 0.005 ^c
100	5.28 ± 0.015 ^b	2.30 ± 0.015 ^{cd}
200	4.48 ± 0.009 ^b	2.16 ± 0.026 ^d

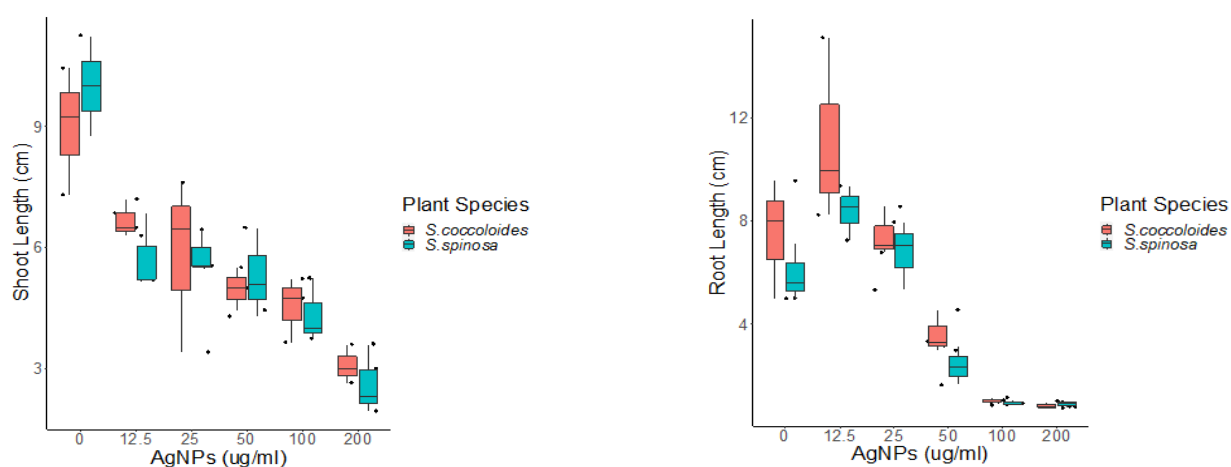
b

APPENDIX D



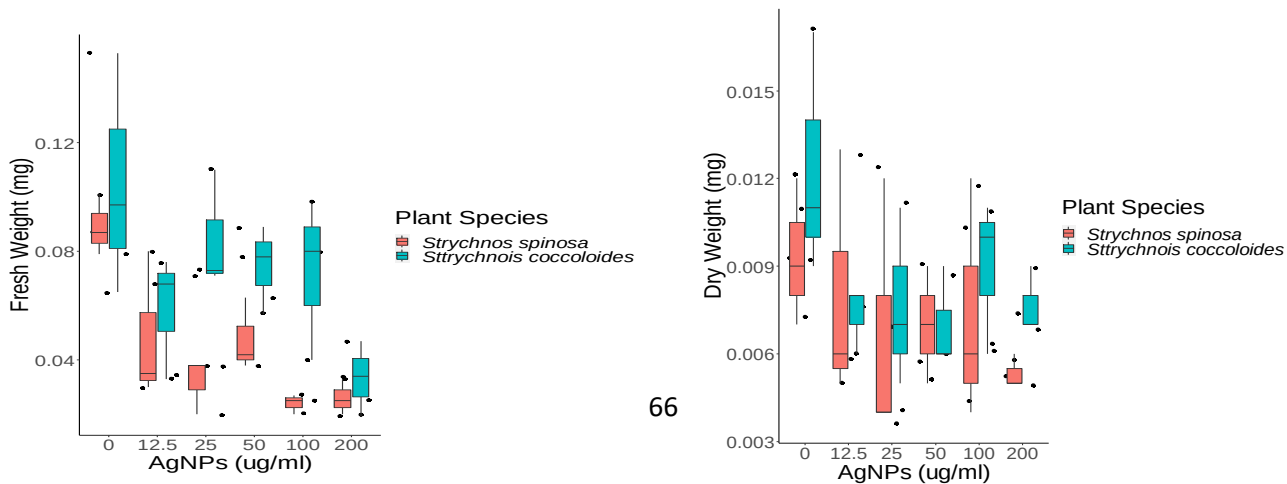
Germination Percentage distribution for *Strychnos* species

APPENDIX C



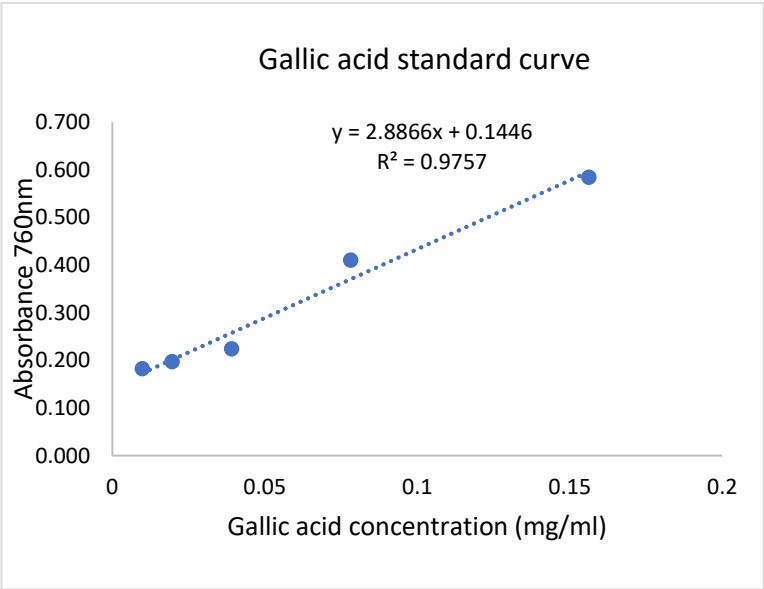
Shoot length and root length distribution of *Strychnos coccoloides* and *Strychnos spinosa*.

APPENDIX D



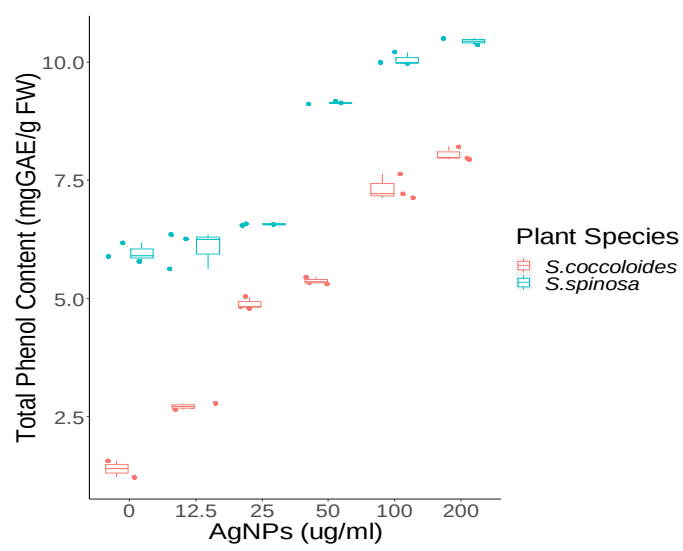
Fresh weight and Dry weight distribution of *Strychnos* species.

APPENDIX E



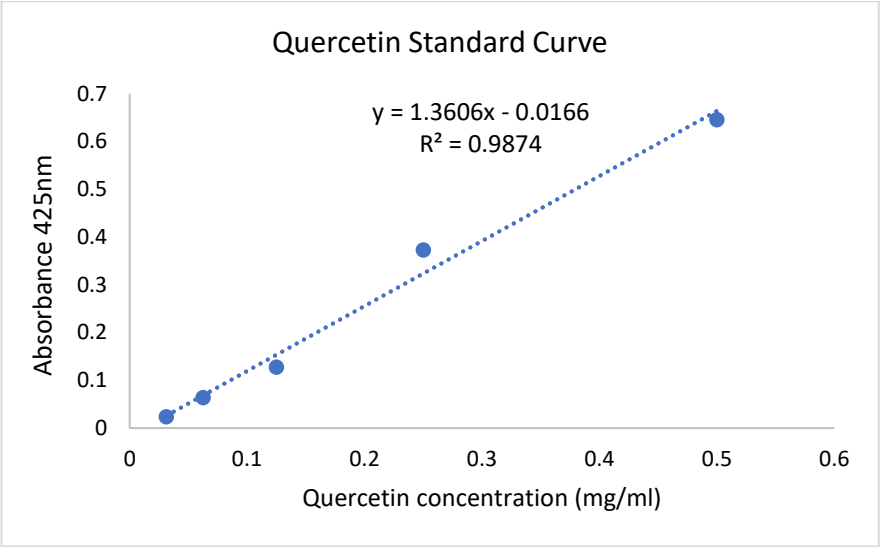
Gallic acid standard curve for total phenol content in *Strychnos* species

APPENDIX F



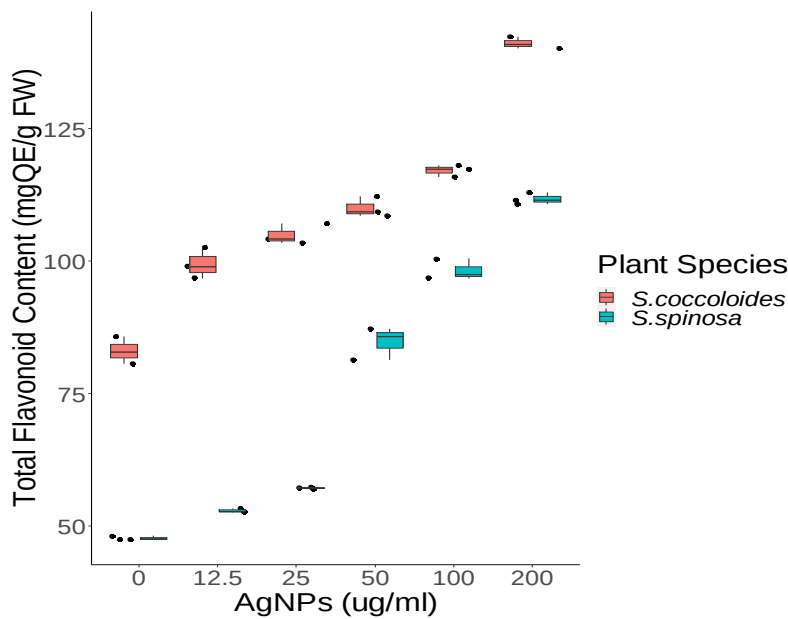
Total Phenol Content distribution for *Strychnos* species

APPENDIX G



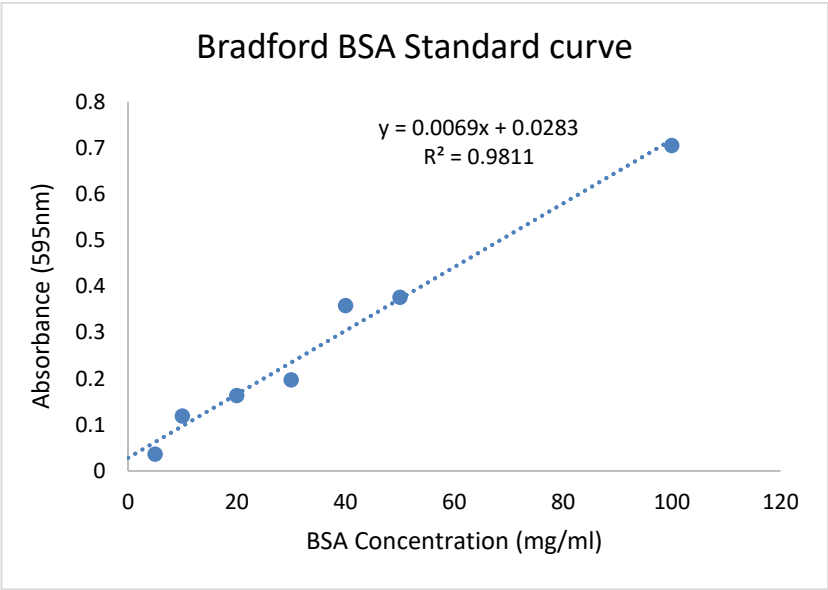
Quercetin standard curve for flavonoid content quantification in *Strychnos* seedlings

APPENDIX H



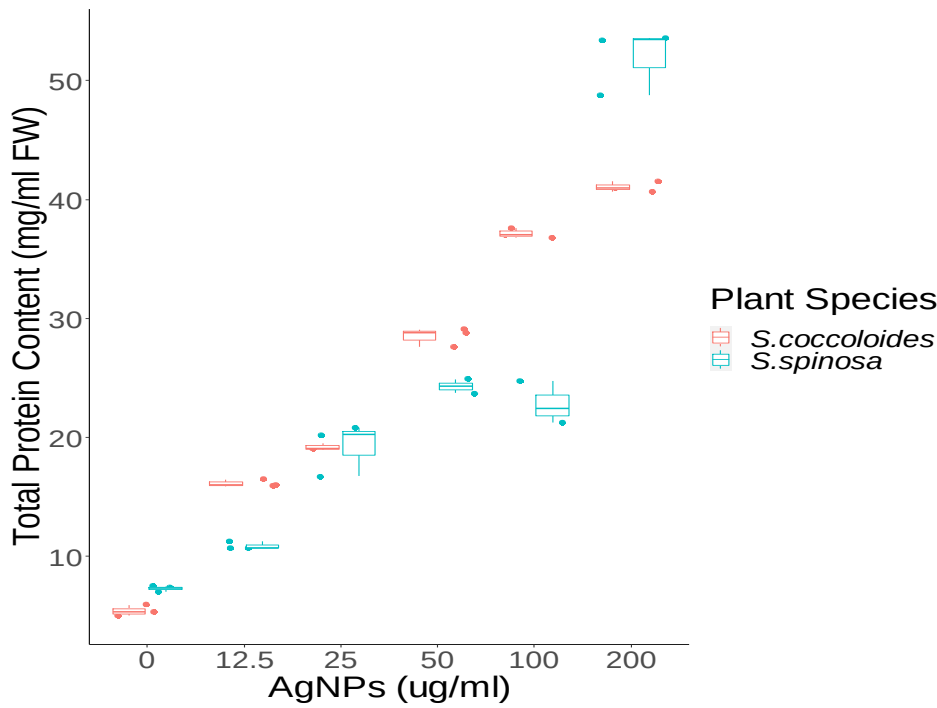
Total Flavonoid content distribution for *Strychnos* species

APPENDIX I



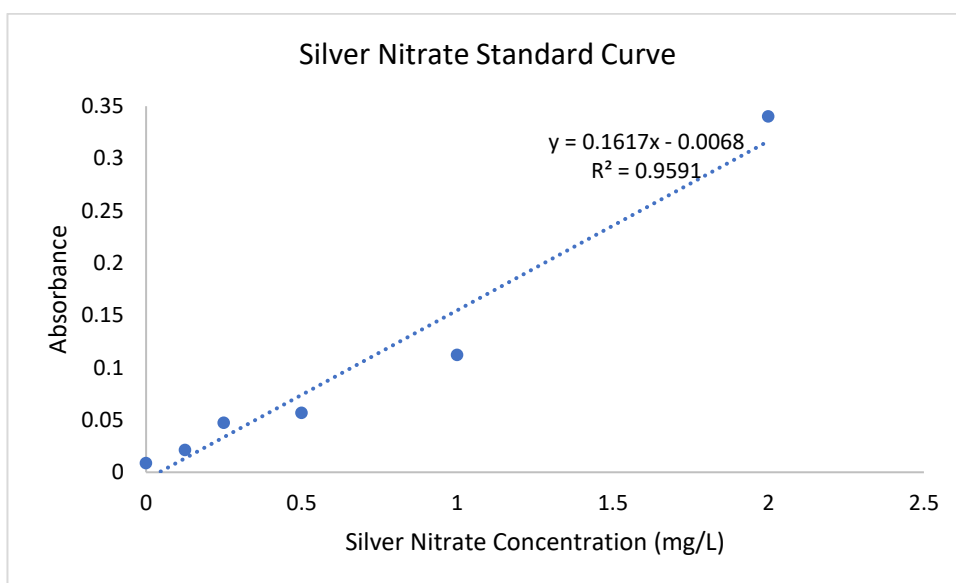
Bovine Serum Albumin calibration curve for quantifying total protein content in *Strychnos* seedlings.

APPENDIX J



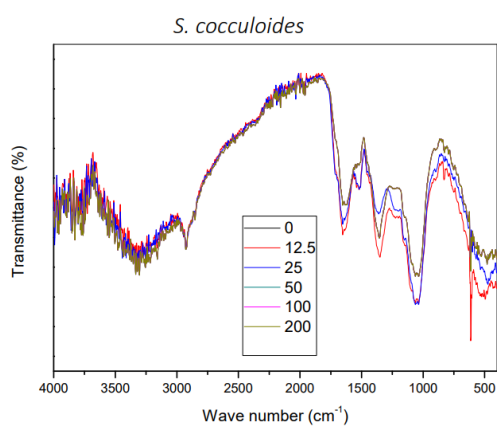
Strychnos total Protein Content Distribution

APPENDIX K

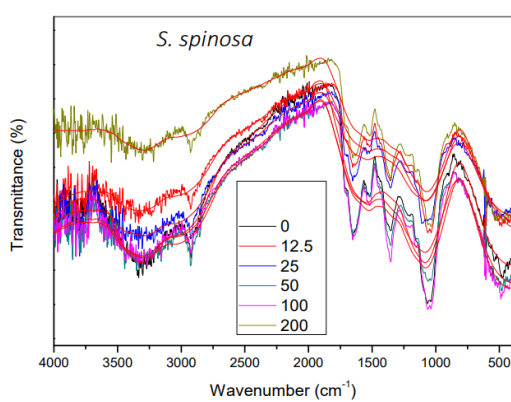


Silver nitrate standard curve for silver ion accumulation in *Strychnos* species

APPENDIX L



a



b

FTIR spectra showing biomolecule composition of (a) *S.cocculoides* and (b) *S. spinosa*