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Silver nanoparticles, synthesis, characterization and applications

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ABSTRACT **: Recently, the scientists are attracted towards cyanobacterial components . These components are potential low-cost and biological reagents used for the green synthesis of silver nanoparticles . This article deals with the green synthesis of silver nanoparticles using a protenaceous pigment Phycocyanin extracted from *Spirulina platensis* as a reducing agent .The green synthesis is an alternative harmless and environ- mentally friendly method for producing nanoparticles. Also Silver nanoparticles (AgNPs) are one of the most significant and fascinating nanomaterials. The invented nanoparticles were confirmed by using an advanced devices; spectrophotometer; transmission electron microscope (TEM); fourier transform inferred spectroscopy (FT-IR);zeta potential analysis and finally EXD. AgNPs have received particular attention due to their potential for use in the treatment and diagnostics of cancer. Moreover, they have immense applications such as breast cancer cell detection; basal cell carcinoma fingerprinted detection; antibacterial and antifungal. Finally, the great concern is that the developing nanotechnology-based therapy should be better than available technologies, and it should overcome the limitations of existing treatment techniques. Also, it has to provide a safe, reliable, and viable treatment of diseases with high accuracy in a patient-friendly manner.**

KEYWORDS: Nanoparticles; synthesis of nanoparticles; advanced devices in confirmation of nanoparticles, its applications.

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INTRODUCTION

Silver nanoparticles (AgNPs) are being employed in a variety of industries, including those involving food, medicine, consumer goods, and industry due to their distinctive physical and chemical characteristics. High electrical conductivity, biological characteristics, optical, electrical, and thermal properties are a few of them Li et al ., (2010) , Mukherjee et al ., (2001) and Gurunathan et al.,(2015) Due to their peculiar characteristics, they have been used for a variety of purposes, including as antibacterial agents, in industrial, household, and healthcare-related products, in consumer products, medical device coatings, optical sensors, and cosmetics, in the pharmaceutical and food industries, in diagnostics, orthopedics, drug delivery, and as anticancer agents, ultimately enhancing the tumor-killing effects of anticancer medications Chernousova and Epple.,(2013).A lot of fabrics, keyboards, wound dressings, and biomedical devices have recently used AgNPs often Sondi and Salopek-Sondi (2004), Li et al ., (2010) and Li et al ., (2014) . Due to their uniqueness and ability to significantly alter physical, chemical, and biological properties due to their small size and high surface-to-volume ratio, metallic nanoparticles have been used for a variety of applications Li et al .,(2001) and Sharma et al ., (2009).

Several synthesis techniques have been used to produce AgNPs in order to meet the demand. Conventional physical and chemical procedures generally appear to be extremely expensive and dangerous Gurunathan et al ., 2009) and Gurunathan et al.,(2015) . It's interesting to note that biologically produced AgNPs exhibit good yield, solubility, and stability (Gurunathan et al ., 2015).Among the several synthetic techniques for AgNPs, biological techniques appear to be straightforward, quick, non-toxic, trustworthy, and environmentally friendly ways that can generate well-defined size and morphology under ideal circumstances for translational research. The production of AgNPs using green chemistry has great potential, in the end. Precise particle characterization is required following synthesis since a particle's physicochemical characteristics may have a great impact on those particles' biological characteristics. It is vital to describe the manufactured nanoparticles before use in order to solve the safety issue and utilize the full potential of any nano material for human welfare, in nanomedicines, or in the healthcare industry, etc. Pleus ,(2012) and Lin et al ., (2014) .Before determining toxicity or biocompatibility, it is necessary to examine the distinctive properties of nanomaterials, such as size, shape, size distribution, surface area, form, solubility, aggregation, etc. (Murdock et al ., 2008). Numerous analytical methods have been employed to evaluate the synthesised nanomaterials, including scanning electron microscopy (SEM), transmission electron microscopy

(TEM), atomic force microscopy (AFM), ultraviolet visible spectroscopy (UV-vis spectroscopy), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), and others. The surface chemistry, size, size distribution, shape, coating/capping, agglomeration, dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type all play an important role in the biological activity of AgNPs. The type of reducing agents used to create AgNPs is also a key factor in determining cytotoxicity (Carlson et al ., 2008). The physicochemical characteristics of nanoparticles improve the bioavailability of therapeutic agents following systemic and local administration (Staquicini et al ., 2011) and (Jo et al ., 2015); however, they can also have an impact on cellular uptake, biological distribution, penetration through biological barriers, and the subsequent therapeutic effects (Albanese et al ., 2012) and (Duan and Li , 2013). For numerous biological applications, it is therefore imperative to generate AgNPs with regulated structures that are consistent in size, shape, and functioning Gurunathan et al .,(2009) , Panáček et al ., (2009),Wong et al ., (2009), Zodrow et al ., (2009) and(Sriram et al ., (2010) . Cancer is a sophisticated, multifactorial disease that has the distinctive trait of uncontrolled growth and spread of aberrant cells. It is controlled by using a variety of treatments, including chemotherapy, hormone therapy, surgery, radiation, immunological therapy, and targeted therapy (American cancer society 2015).Determining efficient, affordable, and sensitive lead compounds that are more sensitive and have a cell-targeted selectivity is therefore challenging. Because of the therapeutic uses of AgNPs as anticancer medicines, in diagnostics, and in probing, there has recently been a great deal of interest in these molecules. The antibacterial, antifungal and anti-cancer properties of AgNPs in a single platform were the main topics of this review, which took into account the literature. We also focused on recent developments in synthesis, characterization, properties, and bio-applications.The mechanism of anticancer action, therapeutic strategies, and the difficulties and constraints of nanoparticles in cancer therapy are also highlighted in this review. This review concludes with a discussion on AgNPs' future prospects.

Synthesis of nanoparticles

A wide range of bulk materials can be used to create nanoparticles, and that the chemical makeup, size, and shape of the particles can affect how they behave (Brunner et al ., 2006). Chemical reduction (Yu , 2007), electrochemical reduction (Liu and Lin , 2004) ,photochemical reduction (Henglein , 1998), gamma ray irradiation Temgire and Joshi , (2004), Remita et al ., (2005) , El-Batal et al ., (2013)and El-Batal et al ., (2014), UV irradiation (Cheng et al ., 2005), and microwave radiation (Tsuji et al ., 2007) are all methods for creating nanoparticles. Moreover, water in particular is a crucial solvent for bioreduction and has been employed in ultrasonic organic solvents for the environmentally friendly creation of nanoparticles (Wu et al ., 2008). A safer and more environmentally friendly alternative to traditional nanoparticle production is known as "green synthesis" (Wang et al ., 2006).

Green synthesis of nanoparticles

The green synthesis methods are the most promising of the many synthesis techniques to create nanoparticles without creating any harmful byproducts or even much waste. Researchers are therefore very interested in using algae as well as using its components to create inorganic nanoparticles (Dahoumane et al., 2017). Using algae, there are three main methods for synthesizing nanoparticles. In addition to directly using live algae cells for nanoparticle synthesis, there are two additional common techniques: harvesting nanoparticles from the supernatants of the algal broth and lysis of algal cells followed by extraction using various downstream process techniques like centrifugation and filtration (Dahoumane et al., 2017).

Using of phycocyanin in preparing of nanoparticles

Silver ions can be reduced extracellularly by polysaccharides and phycocyanin. The phycocyanin derived from Limnothix sp. 37-2-1 produced spherical and elongated AgNPs, and the phycocyanin extracted from Spirulina sp. produced spherical shape AgNPs, according to (Patel et al., 2015)

Algae-Assisted Synthesis

New and emerging species employed for the synthesis of nanomaterials include algae, particularly microalgae. More promising than other living things or biomaterials, algae is a good alternative for a nanomaterial-synthesizing agent. Depending on the algae species and method of operation, nanoparticles can be synthesised intracellularly or extracellularly. A variety of algae types and the nanoparticles discovered. For the creation of metallic nanoparticles, algae of any kind can be employed. Several techniques were employed by the researchers to cultivate diverse algae species, including open cultivation systems (such as open ponds, tanks, and raceway ponds) and closed cultivation systems (such as photo bioreactors) (Narala et al., 2016). It has been suggested that polysaccharides, reducing carbohydrates, proteins, peptides, or other reducing substances present in the algal culture are responsible for the potential formation of extracellular metallic nanoparticles by precipitating reducing metal ions to nanoparticles

(Gahlawat and Choudhury, 2019). Algal respiration and photosynthesis both result in the reduction of metallic ions, which in turn causes the intracellular generation of metallic nanoparticles. In addition, reducing agents like NADPH or NADPH-dependent reductase have been suggested to be essential for the reduction of metallic ions to nanoparticles in cyanobacteria through energy-producing reactions within the electron transport system and redox reactions occurring at the thylakoids, cell membranes, and in the cytoplasm (Oza et al., 2012).

Phycocyanin and phycoerythrin contain varying concentrations of carbohydrates, vitamins, nutrients, oil, fats, polyunsaturated fatty acids, bioactive substances like antioxidants (polyphenols and tocopherols), pigments like carotenoids (carotene and xanthophyll), chlorophylls, and phycobilins. Theoretically, these active substances have been described as reducing and stabilising agents for the creation of nanoparticles (Fawcett et al., 2017).The aggregation of the nanoparticles can be prevented by the hydrophobic and hydrophilic contacts between the nanoshells. Algal cells have lower food requirements and maintenance costs for growth parameters like light and temperature than prokaryotic and eukaryotic species. Many algae that are able to withstand huge amounts of metal or non-metal pollutants can be seen flourishing in a polluted environment with both. Algae have successfully developed defense-related mechanisms to reduce and improve their survival in increasing concentrations of metals and the damaging effects of metal ions. Due to their abundance in both freshwater and saltwater, cost-effectiveness, reusability, and high metal sorption capacities, algae are ideal bio-sorbents. Microalgae maintained their ability to synthesize nanoparticles. By removing biomolecules from their cells, a number of micro-algal organisms have been employed in the production of nanomaterials. Utilization of silver nanoparticles was seen in C. vulgaris conditioned media that had a colour shift from bright yellow to dark brown (UV-Vis absorbance at 415 nm) (da Silva Ferreira et al., 2017).

Characterization of silver nanoparticles

1.UV-Visible Spectroscopy UV-vis spectroscopy

It is a very useful and reliable technique used for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of AgNPs (Sastry et al ., 1998). AgNPs have unique optical properties which make them strongly interact with specific wavelengths of light (UV/VIS/IR Spectroscopy Analysis of Nanoparticles, 2012). In addition, UV-vis spectroscopy is fast, easy, simple, sensitive, selective for different types of NPs, needs only a short period time for measurement, and finally a calibration is not required for particle characterization of colloidal suspensions (Tomaszewska et al ., 2013). In AgNPs, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band, occurring due to the collective oscillation of electrons of silver nano particles in resonance with the light wave Kreibig and Vollmer, (1995), Taleb et al ., (1998) Link and Ei-Sayed , (2003), Nath and Gope, (2007), Noginov et al.,(2007) and Das et al ., (2009),. The absorption of AgNPs depends on the particle size, dielectric medium, and chemical surroundings (Link and EL-Sayed , 2003). Observation of this peak—assigned to a surface plasmon is well documented for various metal nanoparticles with sizes ranging from 2 to 100 nm (Sastry et al ., 1998). The stability of AgNPs prepared from biological methods was observed for more than 12 months, and an SPR peak at the same wavelength using UV-vis spectroscopy was observed. The wave lengths used for AgNps formation lied in uv-visible spectra between 100-700nm.

2. X-ray Diffraction (XRD) X-ray diffraction (XRD)

It is used for the analysis of both molecular and crystal structures Das et al .,(2009) and Waseda et al ., (2011), qualitative identification of various compounds(Ivanisevic, 2010) , quantitative resolution of chemical species (Cabral et al ., 2013), measuring the degree of crystallinity (Dey et al ., 2009) , isomorphous substitutions (Ananias et al ., 2013), particle sizes (Singh et al ., 2013), etc. When X-ray light reflects on any crystal, it leads to the formation of many diffraction patterns, and the patterns reflect the physico-chemical characteristics of the crystal structures. In a powder specimen, diffracted beams typically come from the sample and reflect its structural physico-chemical features. Thus, XRD can analyze the structural features of a wide range of materials, such as inorganic catalysts, superconductors, biomolecules, glasses, polymers, and so on (Robin, 2009). Analysis of these materials largely depends on the formation of diffraction patterns. Each material has a unique diffraction beam which can define and identify it by comparing the diffracted beams with the reference database in the Joint Committee on Powder Diffraction Standards (JCPDS) library. The diffracted patterns also explain whether the sample materials are pure or contain impurities. XRD is a primary technique for the identification of the crystalline nature at the atomic scale Cantor,(1980), Sapsford et al ., (2011), Waseda et al ., (2011) and Lin et al ., (2014). The working principle of X-ray diffraction is Bragg's law Cantor,(1980) and Waseda et al ., (2011) . Finally , crystalline nature of AgNps was confirmed from x-ray diffraction (XRD) analysis . The average crystalline size of AgNps can be calculated using Debye-Scherrer equation $D = K\lambda/\beta \cos\theta$

Where K is Scherrer constant and its value is 0.94.

λ is the wavelength of X-ray.

β is the full width of full maximum.

θ is the Bragg angle.

3.Fourier Transform Infrared (FTIR) Spectroscopy FTIR

FTIR spectroscopy is frequently used to find out whether biomolecules are involved in the synthesis of nanoparticles, which is more pronounced in academic and industrial research Shang et al ., (2007), Perevedentseva et al .,(2010), Gurunathan et al ., (2014) and Lin et al ., (2014). Furthermore, FTIR has also been extended to the study of nano-scaled materials, such as confirmation of functional molecules covalently grafted onto silver, carbon nanotubes, graphene and gold nanoparticles, or interactions occurring between enzyme and substrate during the catalytic process Barth and Zscherp, (2002), Baudot et al ., (2010) and Gurunathan et al ., (2014). Furthermore, it is a non-invasive technique. Finally, the advantages of FTIR spectrometers are rapid data collection, strong signal, large signal-to-noise ratio, and less sample heat-up (Kumar and Barth , 2010) . Recently, further advancement has been made in an FTIR method called attenuated total reflection (ATR)-FTIR spectroscopy Harrick and Beckmann ,(1974) ,Goormaghtigh et al ., (1999) and Hind et al ., (2001) . Using ATR-FTIR, we can determine the chemical properties on the polymer surface, and sample preparation is easy compared to conventional FTIR Demathieu et al ., (1999), Acosta et al ., (2005) ,Kazarian and Chan , (2006) ,(Liu and Webster, 2007) , Johal ,(2011) and Lin et al ., (2014),.

4. Scanning Electron Microscopy(SEM)

SEM is a surface imaging method, fully capable of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales Ranter et al ., 2004),(Hall et al ., (2007), Johal , (2011) ,Fissan et al ., (2014) and Lin et al ., (2014). Using SEM, we can probe the morphology of particles and derive a histogram from the images by either by measuring and counting the particles manually, or by using specific software (Fissan et al ., 2014). The combination of SEM with energy-dispersive X-ray spectroscopy (EDX) can be used to examine silver powder morphology and also conduct chemical composition analysis. The limitation of SEM is that it is not able to resolve the internal structure, but it can provide valuable information regarding the purity and the degree of particle aggregation. The modern high-resolution SEM is able to identify the morphology of nanoparticles below the level of 10 nm.

5.Transmission Electron Microscopy TEM

It is a valuable and important technique used for the characterization of nanomaterials, used to obtain quantitative measures of particle and/or grain size, size distribution, and morphology Joshi and Bhattacharyya ,(2008), Williams and Carter, (2009) and Lin et al ., (2014). TEM has two advantages over SEM: it can provide better spatial resolution and the capability for additional analytical measurements Hall et al ., (2007), Williams and Carter, (2009) and Lin et al ., (2014) .The disadvantages include a required high vacuum, thin sample section Hall et al ., (2007), Joshi and Bhattacharyya ,(2008) and Lin et al ., (2014) and the vital aspect of TEM is that sample preparation is time consuming. Therefore, sample preparation is extremely important in order to obtain the highest-quality images possible.

Properties of AgNPs

Major physicochemical properties of AgNPs

AgNPs' size (surface area), shape, surface charge and coating, aggregation, and dissolution rate are some of its key physicochemical characteristics that are crucial for predicting how they will interact with and affect biological systems. Smaller particles have a bigger surface area and a higher potential for toxicity (Johnston et al.,2010). The physical and chemical characteristics of silver nanostructures can be significantly influenced by their shape. Rycenga et al., (2011) stated that the interaction of AgNPs with living systems can be impacted by the biological effects of AgNPs depend on the various surface charges of their coatings (Powers et al., 2011). The majority of manufactured nanoparticles are known to aggregate. AgNPs have been demonstrated to clump together in culture media as well as in the cytoplasm and nucleus of HepG2 cells (Kim et al ., 2009) . Ionic silver is created when AgNPs dissolve as a result of surface oxidation. The size, chemical, and surface characteristics of the particle, as well as its rate of dissolution, are all influenced by the surrounding media (Misra et al.,2012).

Localized surface plasmon resonance of AgNPs

The sizes, shapes, and chemical compositions of nanoparticles all directly affect their Surface Plasmon Absorbance (SPA) characteristics. The increase in particle size cause changes in the SPA absorption spectrum's intensity and wavelength (Naghavi et al ., 2010). Silver nanostructures have extraordinary optical capabilities. Free electron oscillation leads to either radiative decay, which produces a high visible light scattering, or nonradiative decay, which transforms photon energy into heat energy. These two decay mechanisms have been widely used in biodiagnostic, imaging, and therapeutic applications (Austin et al.,2014). AgNPs' LSPR is influenced by the size, shape, dielectric environment, and electromagnetic interactions between nearby particles (Ren and Tilley ,2007).

Application of nanoparticles

A.Impact of nanoparticles on plant

According to Gruère et al.,(2011) and Prasad et al. (2014), the properties of the nanoparticles can be used to protect plants, increase crop growth and yield, detect plant diseases, increase global food production, and improve food quality. Nanoparticles are prospective candidates to improve plant metabolism due to their distinctive physiochemical characteristics (Giraldo, et al., 2014).

Nanoparticles are taken in by plants through their natural nano- or micrometer-scale apertures after adhering to plant surfaces. Furthermore, throughout growth, plants take in a lot of both essential and non-essential substances, some of which may be poisonous at certain concentrations. Beneficial or hazardous substances can be passed through the food chain to consumers after being stored inside plants.

The edible fruit of the common bean (Phaseolus vulgaris L.), an annual herbaceous plant, is exported all over the world. The straw is utilized as animal feed, while the leaf serves as a vegetable. Beans are a staple food for people of all socioeconomic levels because they provide nutritional protein, vitamins, fiber, complex carbs, and bioactive substances with antioxidant properties Kutoš et al ., (2003) and Granito et al., (2008) . Higher plants are predicted to be impacted by nanoparticle exposure due to their strong interactions with their atmospheric and terrestrial environments and the rising generation of artificial nanoparticles (NPs). Silver, including silver nitrate (AgNO3), silicate, and a water-soluble polymer, is a potent promoter of plant growth (Kumar et al ., 2010) . In agricultural soils and hydroponic systems, silver kills undesirable microorganisms. It is applied as a foliar spray to protect plants from fungi, rot, mould, and various other diseases. Moreover, silver ions in AgNO3 salt have been shown to block the action of ethylene (Chamani et al ., 2005) . Silver is a key component of many agricultural goods as well as detergents, polymers, and textiles due to its antibacterial characteristics Blaser and Scheringer (2007), Benn and Westerhoff ,(2008) . Nonetheless, considerable NP inputs on plant leaves may be anticipated as a result of air particle deposition or the use of herbicides that contain NPs (Larue et al ., 2014) . Certain plants have the ability to absorb and store artificial nanoparticles (ENs). Plant cells' interactions with ENs alter plant gene expression and related biological pathways, which ultimately have an impact on the growth and development of plants Khiew et al ., (2011) and Feizi1 et al ., (2013) .

Phytotoxicity of silver nanoparticles

Oryza sativa was directly exposed to liquids containing silver nanoparticles in order to study its phytotoxicity. The root cells have deposits of different particle sizes. The root cell's cell wall and vacuoles were found to have been injured by particles as they entered the root cell. It might be brought on by big particles penetrating via tiny gaps in cell walls (Harajyoti and Ahmed, 2011).

1-Seed germination

AgNPs' effect on higher plants appears to be influenced by the species, age of the plants, particle size and concentration, and experimental settings (Khiew et al ., 2011) .At concentrations of 10 mg, AgNPs inhibit Hordeum vulgare seed germination and Linum usitatissimum and Hordeum vulgare shoot length, while Eruca sativa seedlings exhibit enhanced root elongation at concentrations of 10 mg Ag L-1 of either PVP-AgNPs or AgNO3 El-Temsah and Joner, (2012) , Vannini et al ., (2013) .At 25 and 50 ppm silver nanoparticle, a considerable improvement in the development of Brassica juncea seedlings was observed (Sharma et al ., 2012) . However, at 100 ppm, AgNPs have no discernible impact on Cucumis sativus and Lactuca sativa seed germination (Barrena et al ., 2009). Castor bean seedling growth, even at 4,000 mg L1, whereas bulk silver hindered seed germination (Yasur and Rani., 2013). On the other hand, an increase in nanosilver concentration from 20 to 60 ppm enhanced the Borage seed yield (Seif et al ., 2011), increased the Phaseolus vulgaris and Zea mays shoot and root lengths, (Rezvani et al ., 2012). Moreover, spraying silver ions reduced the abscission of flowers and flower buds in orchid plants (Uthaichay et al ., 2007) and reduced the abscission of flowers and flower buds in alstroemeria plants by 100% within the first two days. Currently, one of the most frequently employed nanomaterials in commerce is silver nanoparticles (AgNPs) (Chen and Schluesener, 2008). Meyer et al. (2010) looked at the toxicity and intracellular absorption of three different-sized silver nanoparticles in caenorhabditis elegans. At low mg/l doses, they saw growth inhibition from all AgNPs. The impact of nanosilver and silver nitrate on seed output and abscission in borage is examined by Seif et al. (2011). They demonstrated that the seed yield decreased as silver nitrate concentration rose from 100 to 300 ppm. On the other hand, an increase in nanosilver concentration from 20 to 60 ppm has resulted in an improvement in seed output. Moreover, control plants had the lowest quantity of seed output. By directly exposing Oryza sativa to liquids containing silver nanoparticles, the plant's phytotoxicity was investigated.

2- Pigment content

In response to treatment with various concentrations of AgNPs, AgNps significantly increase the contents of all photosynthetic pigments (chlorophyll a, chlorophyll b, carotenoids, and total pigments). According to Farghaly and Nafady (2015) and Latif et al. (2017), AgNPs considerably improve photosynthesis, and this is directly tied to a shift in nitrogen metabolism. Moreover, Racuciu and Creange (2007) noted that maize plants' chlorophyll content rose when exposed to low concentrations (10–50 l/l) of AgNP, however it was observed to be suppressed at larger concentrations of NPs. Metal nanoparticles, according to (Govorov and Carmeli, 2007), can increase the effectiveness of chemical energy synthesis in photosynthetic systems. Yet, higher concentrations of photosynthetic pigments, such as chlorophyll a, chlorophyll b, and carotenoids, would speed up photosynthesis. As a result, there would be more photosynthesis taking place, which would boost plant weight and growth. Many organisms, including marine and freshwater microalgae Miao et al ., (2009) , Oukarroum et al ., (2012), aquatic plants , Jiang et al ., (2012), Jiang et al ., (2017) and Zou et al .,(2017) , crop plants Nair and Chung et al ., (2014), Tripathi et al ., (2017) , Vishwakarma et al ., (2017), Das et al ., (2018) and Pardha-Saradhiet al ., (2018), as well as a model plant Arabidopsis Qian et al ., (2013), Nair and Chung , (2014), Sosan et al ., (2016) and Ke et al ., (2018), have shown decreased chlorophyll content as a measure of AgNP phytotoxicity. AgNP toxicity in different species shown a link between the lower chlorophyll fluorescence production and the AgNP-induced decrease in chlorophyll content Jiang et al ., (2012), Oukarroum et al ., (2012), Jiang et al ., (2017), Tripathi et al ., (2017) and Li et al ., (2018). Several investigations have demonstrated a correlation between the rise in ROS formation and the detrimental effects of AgNP exposure on photosynthetic indices Oukarroum et al ., (2012), Nair and Chung , (2014), Sosan et al ., (2016) , Jiang et al ., (2017), Tripathi et al ., (2017) , Das et al ., (2018) and Li et al ., (2018).

3-Carbohydrates

In our diets, carbohydrates play a crucial role. 43% of the carbohydrates in pea seeds are stored. The demand for pea cultivars with high carbohydrate contents is rising daily. According to this study, adding silver nanoparticles caused P. sativum seeds' carbohydrate content to rise significantly. The highest seed carbohydrate concentrations were found in response to 60 ppm applications of silver nanoparticles, which was significantly different from 90 ppm and 30 ppm applications. Following seed treatment and foliar spray, PF-400 collected the greatest carbohydrate contents among the different cultivars. In Phaseolus vulgaris and Zea mays plants, an application of 60 ppm silver nanoparticles raised the carbohydrate content by 57 and 62% over the control, respectively, according to Salama's (2012) study. Nonetheless, there were noticeable drops in the carbohydrate content at 80 and 100 ppm. According to Liu et al (2010). Applying zinc nanoparticles reduced the amount of carbohydrates in Abelmoschus esculentus from 100 ppm to 500 ppm. This demonstrates that plant species as well as nanoparticle type determine the toxicity of nanoparticles.

4-Amino acids

Free amino acids and polyamines act as natural antioxidants and participate in a number of metabolic activities as well as abiotic stress defense (Kocsy et al. 2011). It is commonly acknowledged that chelation by the proper ligands is typically necessary for metal ion detoxification within plant tissues. Amino acids are thought to be important in the process of metal chelation, which is how plants become tolerant to and detoxify heavy metals (Hall 2002). Moreover, amino acids play a variety of roles in plants, including regulating ion transport, stomatal opening, enzyme production and activity, gene expression, and redox homeostasis (Rai 2002). One crucial amino acid, proline (Pro), which is also regarded as a sign of environmental stress, plays a crucial protective role. According to Alia and Saradhi (1991), exposure to heavy metals causes Pro to accumulate. According to certain theories, nano-silver can result in protein corona development, protein unfolding, and changed protein function (Saptarshi et al. 2013).

5.Protein and protein banding

Silver nanoparticle treatment was observed to boost seed protein levels. The application of silver nanoparticles increased the protein levels, according to earlier investigations. Studies on the impact of AgNPs on the protein content of Phaseolus vulgaris and Zea mays produced substantial findings (Salama ,2012). According to Liu et al .,(2010) found that the addition of ferric oxide nanoparticles increased the protein content.

The administration of either GA-AgNP or $AgNO_3$ at 10 and 60 ppm, respectively, affected the protein patterns in the leaves of the two bean varieties as compared to the corresponding controls, according to the SDS-electrophoresis protein banding patterns. In order to achieve this, new polypeptides with molecular weights of 50.72 and 47.00 kDa (in Nebraska) and 25–26 kDa (in Bronco) are expressed, while control polypeptides with a molecular weight of 28.40 are lost in the two bean types. These conditions might make it easier for the two bean kinds to adapt to various pressures. Osmotin, a protein of Mr 26 kDa, was more frequently induced as cells continued to adapt to salinity (El-Enany, 1995) in Citrus sinensis cells contains a 25 kDa protein (Benhayyim et al ., 1993) .By the production of more suitable solutes or the development of certain antioxidative enzymes, plants that overexpress osmotin neutralise reactive oxygen species (ROS) (Anil Kumar et al ., 2015). A universal stress protein, four glutathione-S-transferases, an isoflavone reductase, and a number of important latex proteins all showed elevated abundance in response to $AgNO_3$. The plastidial and mitochondrial ATP synthase subunits, carbonic anhydrase, and aconitate hydratase were all specifically increased by these proteins, which are leitmotifs in the detoxification of metals (Duressa et al ., 2011). Its up-regulation may aid cells in producing more reducing power to speed up the reaction to $AgNO_3$ stress (Vannini et al ., 2013).

6-Antioxidant enzymes

Antioxidant enzymes are now understood to play a crucial role in controlling AgNP-induced oxidative damage. Antioxidative enzymes, such as guaiacol peroxidase (POX), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and alternative oxidase, as well as other low-molecular substances like polyphenols, vitamins, and minerals, are in charge of preventing the formation of too many reactive oxygen species (ROS) or removing them from plant organisms Chew and Park , (2004), Hatami and Ghorbanpour, (2014). Numerous plants, including Oryza sativa Panda et al ., (2011) and Mirzajani et al ., (2013), Alium cepa (De et al ., 2016), Brassica juncea Rao and Shekhawat , (2016) , Sharma et al ., (2020), Arabidopsis Tthaliana (Liu et al ., 2010), and Nicotina tabacum (Dai et al ., 2018), have shown increased ROS generation levels when exposed to nanoparticles. The production and qualitative and quantitative content of secondary metabolites in plants may be impacted by the oxidative stress caused by metal nanoparticles Moharrami et al ., (2017) and Hussain et al ., (2018) . According to studies by Oloumi et al. [43,77] for Glycyrrhiza glabra, Sharafi et al.,(2013) and Javed et al ., (2018) for Hypericum perforatum, and Lee et al ., (2008) and Zhang et al.,(2013) for Artemisia anuua, metal nanoparticle elicitation has a positive effect on the production of physiologically active chemicals in plants. In cultures of Prunella vulgaris (Fazal et al ., 2016), Stevia glycosides (Golkar et al ., 2018), Cucumis anguria (Chung et al ., 2018), and Caralluma tuberculata (Ali et al ., 2019), silver and gold nanoparticles have been utilised as elicitors. Recent research indicates that silver nanoparticles have a considerable impact on the metabolite profile of Arabidopsis thaliana (Kruszka et al ., 2020)Thus, antioxidant enzymes have become more active in plants, which is one way in which their defensive mechanism is manifested Sharma et al ., (2014) and Harish et al ., (2018). Antioxidative enzyme activity may be a reliable indicator of how harmful the external environment is to an organism. The effect of AgNPs on the activity of antioxidant enzymes in Lycopersicon esculentum was investigated by (Mehrian et al., 2016). It was discovered that the activity of SOD, CAT, and POX in the shoots and roots rose as the concentration of silver nanoparticles in the plant tissue medium increased. In the callus tissue of sugar cane (Saccharum spp. cv CP-77,400) cultured on media with the addition of 20 to 60 AgNPs (Barbasz et al.,2016), the enhanced activity of CAT, SOD, and POX was also noted. In a study, Iqbal et al.,(2017) looked at how wheat (Triticum aestivum L.) under heat stress responded to silver nanoparticles (AgNP) on the physiological, biochemical, and antioxidant parameters. AgNPs were discovered to have a protective impact on plant tissues under stress; wheat plants treated with AgNPs displayed a large increase in dry matter along with a parallel rise in SOD, POX, CAT, APX, and GPX activity. All of the examined antioxidative enzymes, with the exception of CAT, showed a significant impact of the applied nanoparticles on their activity, according to the study. Using modest quantities of nanoparticles, it was found that the activity of these three antioxidative enzymes increased significantly. The observed effect varied depending on the type and concentration of the nanoparticles. However, the activity of these enzymes was noticeably decreased with the increased concentration of metal nanoparticles in the culture media (above 100 mg dm1).

B-Antimicrobial effect of AgNPs:

The ability of AgNPs to combat antibiotic resistance makes them a popular choice for antimicrobial agents. Both gram-positive and gram-negative bacteria are successfully combatted by them. According to reports, AgNP interacts with and penetrates the bacterial cell wall. This results in substantial disruptions in cellular function, which lead to cell death Prabhu and Poulose, (2012), Yan et al ., (2018) and Dawadi et al ., (2021).Size, shape, concentration, surface charge, and colloidal state are a few of the physicochemical factors that influence AgNPs' antibacterial activities (Burdușel et al ., 2018) .These physicochemical characteristics mostly depend on the process employed to create the nanoparticle. (Sondi et al.,2004) demonstrated that AgNP attaches to the Escherichia coli cell wall and forms membrane-piercing holes that ultimately cause cell death (Sondi and Salopek-Ssondi ,2004). Moreover, they showed that the size of silver nanoparticles affects their antibacterial action, with smaller particles being more effective than bigger ones (Sondi and Salopek-Sondi ,2004) . AgNPs have bactericidal and bacteriostatic effects on organisms that produce biofilms. According to Raffi et al.,(2008) AgNP may effectively kill E. coli at a concentration of 60 g/ml or more, demonstrating its bactericidal capabilities (Raffi et al ., 2008). The antimicrobial activity of AgNPs is due to their combined effects of binding of Ag ions to the cell walls, inactivation of the membrane-associated enzymes, accumulation within the cells, interference with the essential biomolecules of the bacterial cells, denaturation of the cell envelope, and the formation of reactive oxygen species. The bacterial cells are adversely affected by each of these occurrences Kim et al., (2011) , Prabhu and Poulose, (2012), Xu et al ., (2020)and Dawadi et al ., (2022) . It has been experimentally established that suspension of AgNPs displays antibacterial efficacy against reference and hospital-isolated bacterial strains of Pseudomonas aeruginosa, which were resistant to the numerous antibiotics (Salomoni et al., 2017). Due to their extraordinarily huge surface area, which improves contact with microbes, silver nanoparticles have superior antibacterial characteristics over other nanoparticles (Logeswari et al ., 2015). Several studies have shown that Ag-NPs are effective against MRSA (methicillin-resistant Staphylococcus aureus) strains even at extremely low doses. They experience cell wall disruption due to AgNPs. It can be utilized as a powerful stand-in for the treatment of infections linked to medical devices and brought on by bacterial strains that are drug-resistant Prabhu and Poulose ,(2012), Ansari , (2018).

The harmless inorganic antibacterial agent silver has been used for millennia. It is non-toxic and well known for its ability to kill roughly 650 different types of disease-causing microbes (Drahansky et al ., 2016). The ability of silver to exert a substantial potential for a variety of biological applications, including preventing infections, healing wounds, and acting as an anti-inflammatory even at very low concentrations, has led to the description of silver as being oligodynamic (Elechiguerra et al ., 2005).Recent research has demonstrated that the majority of microorganisms, including viruses, fungi, gram-positive and gram-negative bacteria, drug-resistant bacteria, and even some pathogens, are strongly inhibited by silver nanoparticles and are shielded by them. Sahayaraj et al ., (2012) and Govindan et al ., (2020).

C-Cytotoxicity effects of AgNPs:

AgNPs are a common therapeutic drug utilized nowadays in the detection and management of cancer. This is due to the fact that silver nanoparticles are more poisonous to malignant cells than bulk materials are. AgNPs have been found to inhibit the growth of tumor cells, acting as an antitumor drug. The inhibition of multiple signaling cascades by nanoparticles, which are necessary for the aetiology and progression of cancer, may be the likely cause of this. Remarkably, normal cells are able to withstand the toxicity of nanoparticles (Gomathi et al ., 2019). Several human cancer cell lines, including endothelium cells, IMR-90 lung fibroblasts, U251 glioblastoma cells, and MDA-MB231 and MCF-7 breast cancer cells, are being studied for the potential anticancer effects of silver nanoparticles Thapa et al ., (2017), Dawadi et al ., (2021) and Gopu et al ., (2021). Tumor cells need constant nutrition and oxygen to thrive, which necessitates a dense network of blood vessels that are created by the angiogenesis process. According to reports, AgNP prevents angiogenesis, which slows the tumor's growth. HeLa cells were successfully killed by AgNPs created using Ecklonia cava extract, with an IC50 value of about 59 g/ml Venkatesan et al ., (2016) and EL-Naggar et al ., (2017). After cardiovascular disorders, cancer ranks as the second leading cause of death (Hay et al ., 2015). A novel approach to fighting cancer is presented via the development of anticancer agents using nanotechnology. AgNPs have a cytotoxic effect on cancer cells, and their toxicity is dependent upon their size, surface functionalization, and concentration (Yakop et al ., 2018), according to (Quan et al., 2021). Nanoparticles are strong possibilities for minimally invasive direct drug administration to a specific area in target cancer cells. Nanoparticles may enter cells through non-specific absorption and cell processes such adhesion, cytoskeleton organization, migration, proliferation, and death, according to (Huang et al.,2010) . These processes are influenced by the shape and size of nanoparticles. The size and shape of metallic nanoparticles are important because they allow for the greatest accumulation in malignant tumors, according to (Yakop et al.,2018). The pharmacodynamics and pharmacokinetics of nanoparticles are influenced by other factors (Sathishkumar et al ., 2015). The cytotoxicity of Hela cell lines increased with increasing AgNPs concentrations, according to (Jeyaraj et al., 2013). (Mfouo-Tunga et al.,2014) also demonstrated that lower viability, increased cytotoxicity, and proliferation—all of which led to apoptosis via programmed cell death—were the causes of AgNPs' enhanced cytotoxicity towards MCF7 cells.

Toxicity of AgNPs

1.Impact on human health

Patients may be exposed to AgNPs in a variety of ways, including cutaneous contact, oral ingestion, inhalation, and blood circulation. The first cells that AgNPs will come into contact with in the human body are macrophages (Pratsinis et al ., 2013). It is well known that the AgNP's size determines its mechanism of cytotoxicity (Ag^+ ion-specific and/or particle-specific) for murine macrophages. The liver is the primary target organ for AgNPs (10nm) toxicity, with the spleen, lungs, kidney, and liver rounding out the top five. One study found that Wistar-derived WU rats treated with 6 mg/kg body weight dosages of 20 nm and 100 nm AgNPs saw an increase in spleen weight, and the clinical chemistry parameters also indicated liver injury (De Jong et al ., 2013). The neutral mucins in the respiratory mucosa of Sprague-Dawley (SD) rats exposed to AgNPs at concentrations of 0.5-61 g/m3 were affected by AgNPs, according to a different investigation on the inhalation toxicity of AgNPs, but this effect was not toxicologically significant (Hyun et al., 2008). Importantly, a different investigation revealed that AgNPs had no effect on the lungs and nasal cavities (Lee et al ., 2009). Also, it was noted that the levels of silver recorded from workers producing nanomaterials exposed to concentrations of silver of 0.35-1.35 g/m3 were only 0.0135-0.034 mg/m3 for blood and 0.043 mg/m3 for urine, with no discernible effects on their health (Lee et al., 2012). Even though many toxicological studies involving AgNPs have been published, it is still challenging to determine with certainty how harmful they are. We can infer that varied synthesis processes, their varying sizes, the presence or absence of capping agents, distinct species, and/or culture cells may cause AgNPs to have different toxicological features. As a result, their hazards should be evaluated on an individual basis.

2. Impact on the environment

The chemical make-up of AgNPs and the availability of free silver ions determine how harmful they are to the environment. AgNPs are disseminated in the environment in many ways after being released, which changes their characteristics and affects their transit, destiny, and toxicity. According to a study by Blaser et al., (2008), silver can leak into wastewater that is either processed in a sewage treatment plant (STP) or immediately discharged into natural waters, as well as solid waste that is disposed of in solid waste landfills or burned in thermal waste treatment (TWT). Agricultural soils, solid waste dumps, or TWT are all used to dispose of sewage sludge. . Silver may contaminate groundwater and subsoil as a result of landfilling solid waste. STP effluents, untreated wastewater, and silver found in sewage sludge that is spread out over agricultural fields are additional sources of immediate release to the environment. AgNPs are most likely to interact with sulphide, chloride, or other natural compounds and change from what they were originally made of. Due to the decreased solubility of silver sulphide, Levard et al. discovered that even a very modest level of sulfidation of AgNPs can cause a discernible reduction in their toxicity (Levard et al ., 2013). Moreover, Tiede et al.,(2010) found that >90% of AgNPs were eliminated during the treatment of wastewater. The toxicity of Ag+ in the presence of Cl among different species of fish has been researched (Nichols et al ., 2006), despite the fact that the dissolution of AgNPs in the presence of chloride in aqueous solution has not been completely investigated. In general, nothing is known about how AgNPs specifically affect the ecosystem. As a result, it is currently impossible to determine with accuracy the environmental dangers connected to the creation and application of AgNPs.

Mechanism of AgNPs-induced toxicity

Among the most important topics pertaining to AgNP-induced toxicity are the interactions between nanomaterials and cells, cellular absorption, and the consequent toxic response of the cell. Endosomes and lysosomes are the primary target organelles for AgNP uptake in the majority of cells, which is mostly dependent on time, dose, and energy AshaRani et al ., (2009) and Zhang et al ., (2014). If exposed to the acidic environment of lysosomes, nanoparticles can directly trigger the generation of reactive oxygen species (ROS) (Chang et al ., 2012). Furthermore, Singh et al. showed that Ag+ builds up in lysosomes (Singh and Ramarao, 2012). Superoxide anions (O2), hydroxyl radicals (•OH), and hydrogen peroxide are all components of ROS (H2O2). Ag+ release in vivo is hypothesised to be caused, in part, by interactions between H2O2 and AgNPs. 2Ag + H2O2 + 2H+ 2Ag+ + 2H2O E 0 = 0.17 V (AshaRani et al ., 2009) is a potential chemical reaction. Contact with proteins in the cytoplasm or cell culture media can trigger the response. Additionally, ROS are highly reactive and cause mitochondrial dysfunction in addition to oxidative damage to proteins and DNA. AgNPs and Ag+ ions have the ability to escape from lysosomes, which causes an even greater rise in intracellular ROS. AgNPs and released Ag+ ions prefer to bind with molecules that are present in the cytoplasm's thiol groups. , mitochondrial inner membrane, and cell membrane, which may produce lipid peroxide and promote penetration of the mitochondrial and cell membrane systems Almofti et al ., (2003) and Zhang et al ., (2014). . While lysosomal membrane rupture initiates lysosome-mediated apoptosis, damage to the cell membrane causes the release of cytoplasmic contents and eventually necrosis. Furthermore, mitochondrial damage hinders electron transmission, triggering mitochondrion-dependent apoptosis (Arora et al ., 2008). Furthermore, it has been shown that AgNPs can easily enter and move around the nucleus through nuclear pore complexes, causing ROS to develop, which in turn causes DNA damage and chromosomal abnormalities (AshaRani et al ., 2009). In addition to harming mitochondria and causing ROS generation, recent investigations have shown that Ag+ can directly cause DNA damage (Guo et al ., 2013).

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