Prospective response of *Phaseolus vulgaris* seeds primed in silver nanoparticles and aqueous phycocyanin extracted from *Spirulina platensis*

Abdel-Fattah S. Soror, Mai. W. Ahmed and Samia S. Saffan

Department of Botany and Microbiology, Faculty of Science, Zagazig University, El-Gamaa Street 1, 44519 Zagazig- Sharkia- Egypt.

Abstract:

The metabolic effects of silver nanoparticles AgNPs prepared by phycocyanin extracted from *Spirulina platensis* on metabolic activities of economic plant *Phaseolus vulgaris* including seed germination, pigments(chl. a, chl. b and carotenoids) , carbohydrates, total lipids, amino acid contents, proline, protein banding, malondialdehyde as well as the antioxidant enzymes activities, superoxide dismutase [SOD], peroxidase [APX], and non-enzymatic antioxidants such as glutathione reductase (GR), catalase [CAT] have been investigated. The results demonstrated that all studied parameters seed germination, pigments, carbohydrates, and total lipids were stimulated by low concentrations of AgNPs [5-30 ppm]. On the other hand, the high concentrations of AgNPs [40–50 ppm] exhibited an inhibitory effect .The three antioxidant enzymes SOD, CAT, and GR were increased in a dose-dependent manner as a result of the increase in AgNPs concentrations. The total amino acids progressively increased under the same treatment. Electrophoretic polypeptide banding patterns consists of 159 bands with a molecular weight range of 27 to 145 KDa. Ten bands—represented polymorphic loci with the value of 6.21 %, whereas 6 bands—represented monomorphic loci with value of 3.77%. The most polypeptide bands [14 bands] were discovered in phycocyanin treatments at lane 5 with molecular weights ranging from 27 to 129 KDa as well as AgNPs treatments at lanes 9, 11 and 13 with a value reaching 8.80% and molecular weights ranging from 27 to 145 KDa.

Keywords: Amino acids, antioxidant enzymes, Phycocyanin, *Phaseolus vulgaris*, protein banding, silver nanoparticles

Introduction

There is a higher demand for food because the global population is growing swiftly and less land available for agriculture. The implementation of novel and imaginative technologies is required to resolve this problem in modern agricultural practices. One of them is nanotechnology (**Singh *et al.*, 2021**). Moreover, **Leslaw *et al.* (2022)** claimed that nanoparticles are extremely small

*Corresponding author: email: mainasr2018@gmail.com (ISSN: 1110-8649)

particles that have special qualities in terms of their physical, chemical, or biological properties.

Plants are an important part of an ecosystem and the main source of food for humans. It was found that the way of **nanoparticles (**NPs) affect plants depends on their chemical makeup, size, shape, coating agents, concentration, species of plant, and developmental stage (**Zhao *et al.*, 2021**). AgNPs infiltrated plant cells by passing through the wall of the plant root, leaves, and seeds (**Wang *et al.*, 2023**).

In this regarding, small-sized AgNPs can pass through the cell wall's pores because it is a porous network of polysaccharide fiber matrices that serves as a natural sieve (**Zhang *et al.*, 2023**). AgNPs release ions as soon as they enter plants through vascular routes, which causes the production of reactive oxygen species (ROS), which changes the metabolic profile of all plant tissues, **Durairaj *et al.* (2020)**. Moreover, **Khan *et al.* (2023)** found that low concentrations of AgNPs promoted the growth of common bean and maize plants. In addition, **Latif *et al.* (2017)** showed that applying AgNPs to the leaves of wheat plants in a variety of doses improved plant growth parameters. Also, AgNPs greatly support photosynthesis and are associated with a modification of nitrogen metabolism. This was a result of AgNPs' capacity to interfere with ethylene signaling (**Zhang *et al.*, 2023**). The beneficial stimulatory effects of AgNPs on plants are improved seed germination, enhanced seedling growth, facilitated water and fertilizer absorption, higher activity of antioxidant enzymes like catalase and superoxide dismutase, and increased photosynthesis pigments (**Khan *et al.*, 2021**).

Meanwhile, **Dipak and Gagon (2023)** reported that the functions of antioxidant enzymes involved in hormonal metabolism may be directly impacted by changes in phytohormones, which may then have an impact on how signaling activities or chemical levels that control growth are regulated, these changes boost

the amount of metal compounds, suggesting improving quality and yield. Moreover, **Roman e*t al.* (2023)** asserted that in particular, levels of chlorophyll, carbohydrates, protein, and antioxidant enzymes, as well as the growth profile and biochemical characteristics of *Brassica juncea*, common bean, and maize plants were improved by the presence of AgNPs. On the other hand, AgNPs may harm plants and crops where they exist in excessive concentrations (**Matras *et al.*, 2022**). It became obvious that both silver ions and AgNPs had a dramatic effects on plant cells .They induce toxicity including cellular dysfunction caused by cell membrane damage. Also, the process of oxidative stress led to the production of reactive oxygen species (ROS) and an increase of free radicals, which destroys the structure of inter-cellular molecules DNA, proteins, and lipids (**Leslaw *et al.*, 2022**).

Materials and Methods

Phaseolus vulgaris L. (cv.Giza 3) seeds were obtained from the Horticulture Research Institute Centre in Egypt in a healthy state, alive, free of infection, uniform in size and shape, and ready for planting during February 2022 for 14 days.

Spirulina platensis biomass preparation

Spirulina platensis (Gomont) Geitler (strain MIYE 101) was acquired from the Phycology Lab, Faculty of Science, Zagazig University, Egypt. An inverted divert light microscope was used to identify the microalga up to species using the keys of (Vymazal, 1995). The width of the trichomes consists of cylindrical cells that are shorter than broad cells, with a diameter of 8 to 10μm and

length of tens to hundreds of μm, and coiled cells with a diameter of 5-6μm. The *S. platensis* was cultivated on a standard Zarrouk culture medium (**Zarrouk, 1966**). Two-liter culture flasks were inoculated with 250mL of *S. platensis* culture, aerated with air pumps, and incubated at 25±3ºC with fluorescent light tubes at 50 μEm-2s-1. The pH was adjusted to be suitable for this *S. platensis* growth (9.0±0.2). The culture was supplied with an air pump (97% O2 and 3% CO2) to accelerate *S. platensis* growth. The biomass was harvested by centrifugation at 5000rpm for 15min. The cell pellets were cleaned four times and resuspended in sterile H2 O to remove traces of growth medium. The suspension was then centrifuged at 6000rpm for 20min. The collected biomass of *S. platensis* was dried in the air for 4 days, powdered by hand mortar, and stored at 5ºC until used.

Phycocyanin Isolation and Purification

Phycocyanin (C-PC) was extracted from the blue-green alga, *Spirulina platensis*, according to **Boussiba and Richmond (1979)**. Two grams of experimental algae were stirred in 200 mL of a phosphate buffer (0.1 M, pH 7.2) containing 100 μg/ml lysozyme and 10 mM EDTA in a shaking water bath at 30 ˚C for 24 h to test the enzymatic breakdown of the cell wall. The cell remnants were eliminated, and the slurry was centrifuged for one hour at 10,000 rpm, providing a bright blue supernatant of C-PC. C-PC crude extracts were centrifuged at 10,000 rpm for 30 min without being cooled. The supernatant containing the C-PC solution was precipitated twice by ammonium sulfate precipitation at two levels (50% and 75% $(NH_4)_2SO_4$ (w/v) at pH 7.2 for 6 h). Ten milliliters of ammonium sulfate extract were dialyzed against the extraction buffer using a Dialysis membrane-70. The sample was dialyzed twice against one liter of extraction buffer, first at room temperature and then overnight at 4 ˚C. The

extracted solution was recovered from the dialyzed membrane and filtered through a 0.45 m filter.

The phycocyanin was purified using anion exchange chromatography using a DEAE Cellulose column (30 × 2 cm) equilibrated with 150 mL of acetate buffer (pH, 5.10). Ten milliliters of dialyzed, filtered material were deposited on the column. The column was developed using a linear gradient of acetate buffer with a pH range of 3.76 to 5.10; the eluate was collected in 5 mL fractions, and the buffer flow rate was set to 20 ml h^{-1}. A spectrophotometer (Analytik, Jena GmbH, Jena Germany) was used to scan the sample in the 300–750 nm range for absorbance assessment. The purified phycocyanin was identified by HPLC (**Kumar *et al.*, 2014**).

Preparation of Phycocyanin

Five grams of dried phycocyanin powder were mixed with 100 mL of distilled water and incubated overnight in a rotary evaporator incubator at 30 ˚C and 150 rpm. The samples were filtered using Whatman's No. 1 filter paper and kept at 4 ˚C for further use.

Biosynthesis of *Spirulina Platensis* Phycocyanin Silver Nanoparticles (SPAgNPs)

Approximately 0.17 g of $AgNO_3$ was dissolved in 1 L of sterilized deionized water to obtain the $AgNO_3$ solution (1 mM), then 10 mL of aqueous phycocyanin filtrate was added to 90 mL of $AgNO_3$ and placed in optimized conditions of pH 5, a temperature of 30 ˚C, a reaction time of 5 h, and an agitation speed of 150 rpm **Saad et al. (2021)** until the color changed to a reddish brown.

Seed germination experiment

Phaseolus vulgaris seeds were surface sterilized with a 0.1% mercuric chloride solution and then immersed overnight in cold water. AgNPs and aqueous phycocyanin extract were used to prime seeds at various concentrations [5, 10, 20, 30, 40, and 50 ppm], as previously indicated (**Soror *et al.,* 2022**), in addition to control. Twenty seeds from each concentration were taken out and placed in three replicates in Petri-dishes with moist Whatman No. 1 filter paper at a temperature of 25 ºC in the laboratory. The germination percentage was computed based on measurements taken at 12-h. intervals for 7 days according to **Berena *et al.* (2009**).

Pigment analysis

The photosynthetic pigments (Chl. *a*, Chl. *b*, and carotenoid) were estimated according to the method modified by **Metzner *et al.* (1965)**.

Determination of total carbohydrates

Total carbohydrates extracted using the method of **Said and Naguib (1964)** and it was quantitatively evaluated as glucose according to **Nelson (1944)** as modified by **Naguib (1964)**.

Estimation of free amino acids

Amino acids were extracted and their amounts were evaluated using an amino acid analyzer, according to **Bailey (1967)** and **Steven *et al.* (*1*989)**.

Estimation of proline content

Proline was estimated according to the method of **Bates *et al.* (1973)**.

Determination protein profile (protein print) using SDS-PAGE:

Protein profile was carried out according to **Laemmli (1970)**.

Determination of total lipids

Total lipids determined using the approach outlined by **Varma and Tiwari (1967)**.

Determination of lipid peroxidation

Malonaldehyde (MDA) measured according to the method developed by **Health and Paker (1968)**.

Determination of antioxidant enzymes activity

Sueperoxide dismutase [SOD] was assayed according to the method of **Dhindsa *et al.* (1981)**. Glutathione reductase [GR] was estimated according to the method of described by **Goldberg and Spooner (1983)**. Ascorbate peroxidase [APX] activity was measured according to **Kumar and Khan (1982)** and catalase [CAT] was assayed as method of **Kar and Mishra (1976)**.

Results

The results in Fig. (1) indicated that germination of *Phaseolus vulgaris* seeds in various concentrations of silver nanoparticles AgNPs and aqueous phycocyanin extracted from *Spirulina platensis* [5, 10, 20 and 30 ppm] had a stimulatory impact regarding seed germination. Maximum percentage of growth was attained at 30 ppm AgNPs after 7 days, since it was 90%, as compared with aqueous phycocyanin and control [80 and 75%], respectively. However, the high AgNPs concentrations, [40–50 ppm], exhibited a deterrent effect on seed germination after 7 days. The percentages of decline were 20 and 10%, respectively.

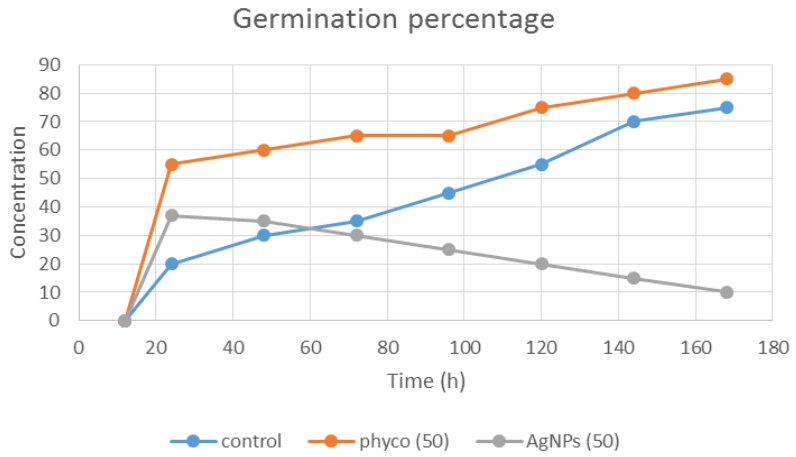

Fig. 1. Germination percentage of *P. vulgaris* seeds primed in AgNPs and aqueous phycocyanin extracted from *Spirulina platensis*

Regarding total pigment Fig. (2) clarified that treatment of *Phaseolus vulgaris* seeds with different concentrations of phycocyanin extract (5-50 ppm) resulted in gradual increase in total pigments if compared with untreated seeds. The highest increase in total pigments was recorded at 50 ppm; it was 22 mg/g fresh weight, when compared with their corresponding control (16.86 mg/g). On the other hand, lower concentrations of AgNPs (5 -30ppm) promoted the total pigments contents in *Phaseolus vulgaris* seeds**.** The highest increase was 22.21 mg/g fresh weight and recorded at AgNPs concentration 30 ppm. While the high AgNPs concentrations (40 and 50 ppm) were accompanied with the decrease in total pigments. It reached 11, 7.03 mg/g fresh weight in 40, 50 ppm AgNPs respectively.

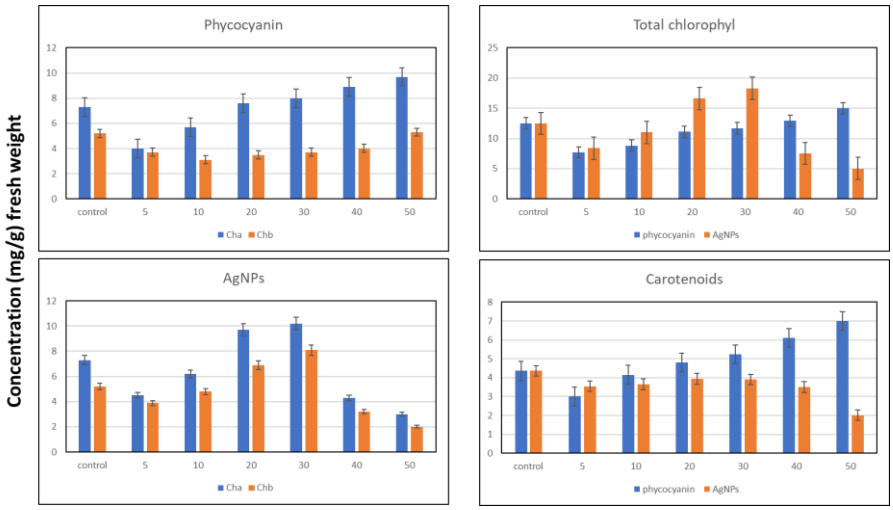

Figure 2: Effect of different concentrations of AgNPs and aqueous phycocyanin extracted from *Spirulina platensis* on pigment contents (mg\g fresh weight).

The amount of total carbohydrates in *Phaseolus vulgaris* seeds presoaked in different concentrations of AgNPs and phycocyanin extract were analyzed in the range of 5-50 ppm [see Fig. 3.] It was noticed that the total carbohydrates were gradually increased in seeds treated with phycocyanin extract, reaching its maximum value at concentration 50 ppm, since it was 554.8 mg/g dry weight. On the other hand, in case of AgNPs, only the lower concentrations (5-30 ppm) were associated with a gradual increase in total carbohydrate. The highest value was recorded at 30 ppm AgNPs; it was 528.45mg/g dry weight if compared with its corresponding value in phycocyanin value in phycocyanin extract (421.22 mg/g). On the other hand , the higher AgNPs concentrations (40 and 50 ppm) led to decrease the amount of total carbohydrates if compared with its corresponding values in phycocyanin extract, since they were 304.8, 271.42 and 504.33, 554.8 mg/g dry weight respectively.

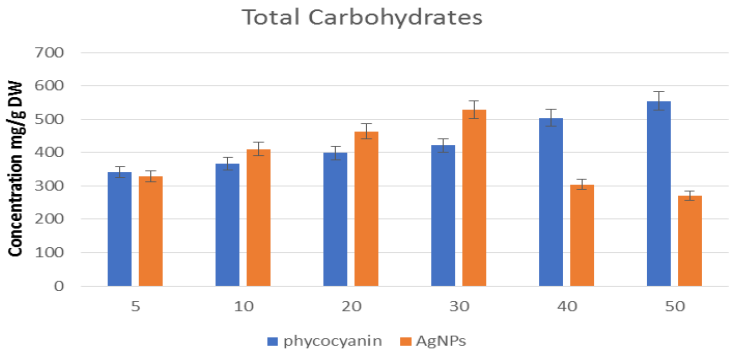

Figure 3 : Effect of different concentrations of AgNPs and aqueous phycocyanin extracted from *Spirulina platensis* on total carbohydrates contents (mg\g dry weight)

In case of total lipid contents Fig. (4) showed that the *Phaseolus vulgaris* seeds primed with phycocyanin extract showed an increase in total lipids. The percentages of total lipid enhancement were found to be a dose-dependent manner. The highest proportion of total lipids was 46% when the phycocyanin extract concentration was 50 ppm. Nevertheless, with AgNPs, the percentage of total lipids was only increased at lower dosages [5–30 ppm], reaching its maximum value of 77.5% at 30 ppm AgNPs. Despite a decline in total lipid percentage at higher AgNPs concentrations (40–50 ppm) with a percentage of decrease reached to 25 and 20% respectively.

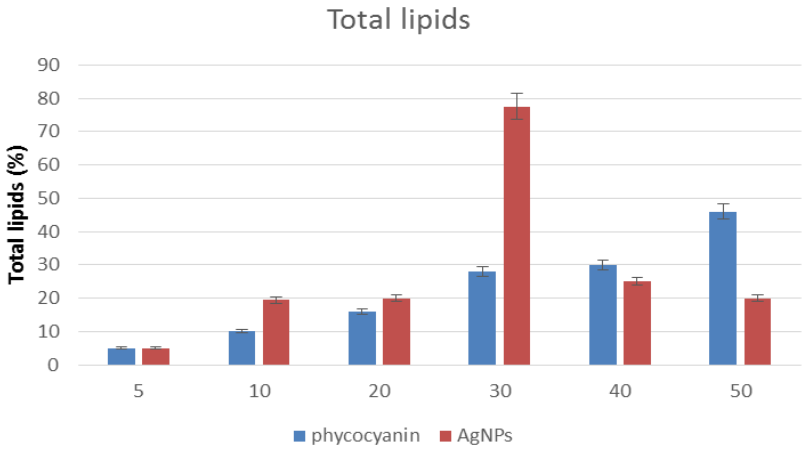

Figure 4: Effect of different concentrations (ppm) of AgNPs and aqueous phycocyanin extracted from *Spirulina platensis* on percentage of total lipids contents.

With regard to free amino acids Fig. (5) showed that *Phaseolus vulgaris* seeds contain 14 different amino acids in varied concentrations. It was shown that all phycocyanin extracts boosted the synthesis of amino acids when compared to their control. The most significant increase in total amino acids was observed at a phycocyanin extract concentration of 50 ppm since it reached [215.9g/100 g dry weight seeds] if compared with their corresponding control [47.8g/100 g dry weight seeds] .This improvement was brought about by an increase in glutamic acid [29.2], aspartic acid [26.3], glycine [15.3], alanine [13.5], and phenyl alanine [12.4], in that order, respectively. Additionally, presoaking *Phaseolus vulgaris* seeds in a different concentrations of AgNPs , particularly the low ones [5-30 ppm], was associated with a progressive enhancement in total amino acids, which peaked at a concentration of 30 ppm AgNPs and was measured [175.7 g/100 g dry weight seeds]. Such improvement was due to the increases in glutamic acid [26.4], aspartic acid [23.5], glycine [12.4], alanine [10.6], and phenyl alanine [9.5] g/100 g dry weight seeds respectively.

The results of proline contents in *phaseolus vulgaris* seeds presoaked in high concentration of phycocyanin extract and AgNPs Fig. (6) revealed that proline contents was gradually increased in case of phycocyanin and AgNPs if compared with control. The rate of increase in AgNPs was higher than its increase in phycocyanin at higher concentrations (40 and 50 ppm). Since it recorded 17.6, 21.3 and 10, 16.6 mg/g dry weight in AgNPs and phycocyanin receptively if compared with their irrespective control (4mg/g dry weight).

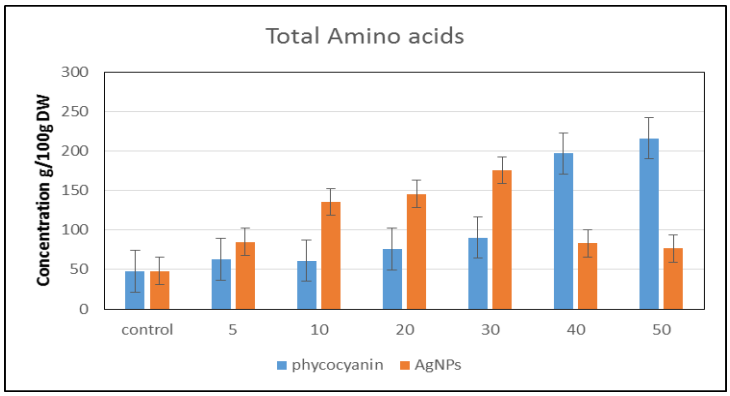

Figure 5: Effect of different concentrations (ppm) of AgNPs and aqueous phycocyanin extracted from *Spirulina platensis* total amino acids contents g/ 100 g dry weight seeds.

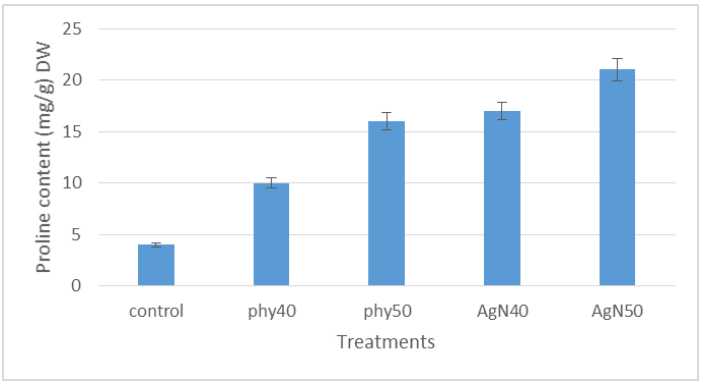

Figure 6: Effect of different concentrations (ppm) of AgNps and aqueous phycocyanin extracted from *Spirulina platensis* on proline contents.

Electrophoretic polypeptide banding patterns generated by SDS-PAGE were investigated to determine genetic variations in *Phaseolus vulgaris* seeds treated with phycocyanin and AgNPs **(**Photo 1). These electrophoretic banding patterns produced by SDS-PAGE gave satisfactory results with many quantitative and qualitative alterations in polypeptide banding pattern of protein profiles at different treatments. Photo (1) revealed that total of 159 polypeptides bands with different molecular weights ranging from 27 to 145 KDa were revealed, of which 10 bands represented polymorphic loci with value of 6.21%. The level of protein polymorphism observed by SDS-PAGE was moderate and reached 62.5% based on the molecular weight (KDa) of polypeptide bands and their fractionation, type (polymorphic and monomorphic bands), band number, band intensity, appearance of polypeptide bands, and absence of others. The maximum number of polypeptide bands (14 bands) was found in phycocyanin treatments at lane5 with molecular weights ranging from 27 to 129 KD as well as AgNPs treatments at lanes 9, 11 and 13 with a value reached 8.80% and molecular weights ranging from 27 to 145 KDa, while the minimum number of polypeptide bands (8) was found in AgNPs treatments at lanes 12 with a value of 5.03% with molecular weights ranging from 34 to 95 KDa. The number of polymorphic bands was 10 with value of 6.29 % while the number of monomorphic bands was 6 polypeptide bands with value of 3.77 % therefore, polypeptide polymorphism reached 62.5%.

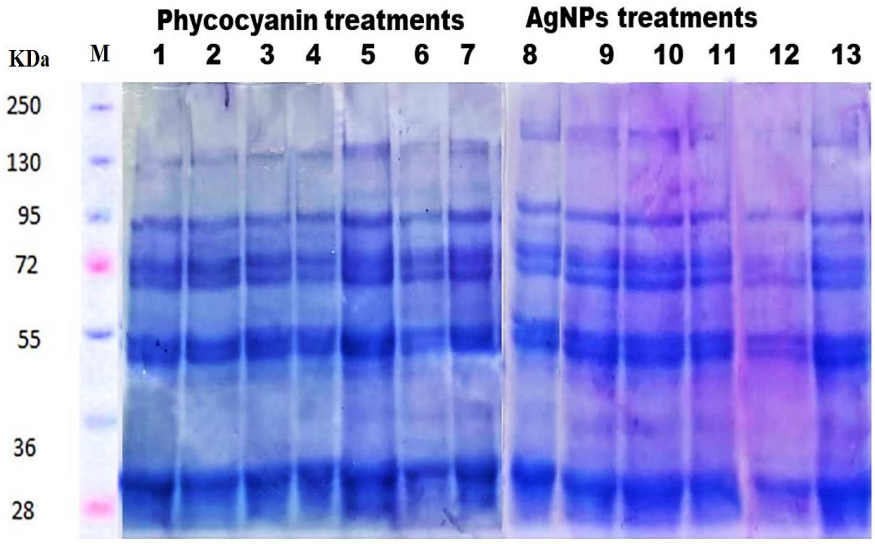

Photo 1: Protein banding pattern of polypeptide bands generated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in non-treated and treated *Phaseolus vulgaris seeds* with phycocyanin and AgNPs.

Regarding antioxidant enzymes, superoxide dismutase [SOD] , catalase [CAT] , ascorbate peroxidase[APX] and glutathione reductase [GR] , Fig. (7) revealed that soaking of *Phaseolus vulgaris* seeds in high concentrations of AgNPs [40 and 50 ppm] was accompanied with an increase in the activities of antioxidant enzymes especially at 50 ppm followed by 40 ppm . since they were [25.6,21.3 mg/g protein] for SOD, [69.31 , 68.45, mg/g protein] for catalase, [6.5, 4.6 mg protein / min] for peroxidases and [20.51,19.97 mg/g protein] for GR respectively.

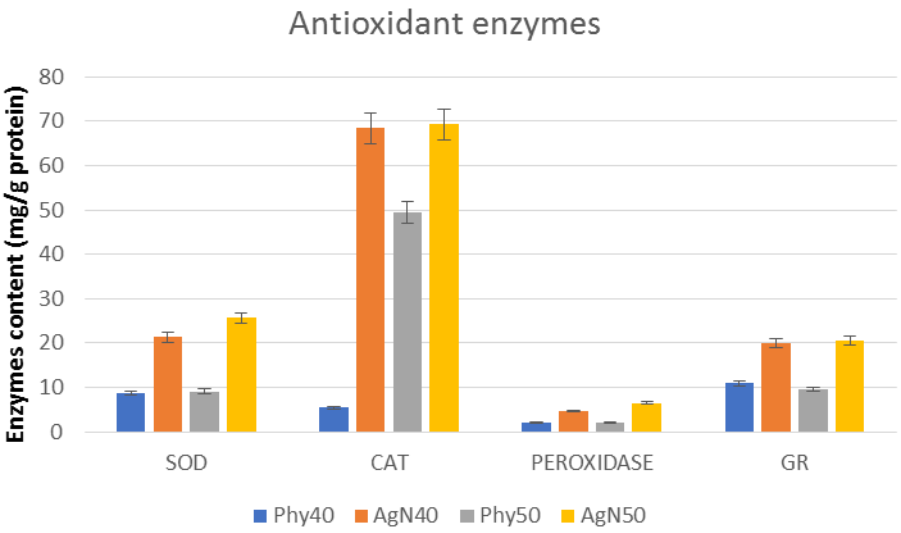

Figure 7 : Effect of different concentrations (ppm) of AgNPs and aqueous phycocyanin extracted from *Spirulina platensis* on antioxidant enzymes.

In case of oxidative stress caused by AgNPs Fig. (8) the current findings demonstrated that the augmentation of MDA was significantly correlated with the priming of *Phaseolus vulgaris* seeds in high concentrations of AgNPs [40 and 50 ppm]. Since it reached 26.74 and 27.45 mg^{-1} when compared to the 13.13 and 13.67 mg^{-1} of the aqueous phycocyanin extract respectively. This can be a sign that the treatment increased lipid peroxidation.

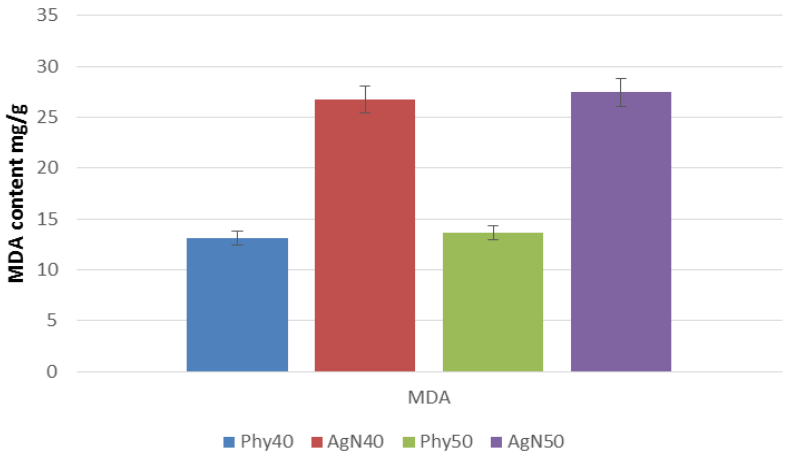

Figure 8: Effect of different concentrations of AgNPs and aqueous phycocyanin extracted from *Spirulina platensis* on Malondialdehyde content.

Discussion

These results of seed germination showed that AgNPs' effects were dose- and time-dependent. The findings are consistent with **Asma *et al.* (2019)**, who found that seeds treated with silver nanoparticles [AgNPs] had significantly greater germination rates and seedling growth than seeds that weren't treated. This could be attributed to the effective water and nutrient uptake by the treated seeds. Additionally, there has been a decrease in the total phenol levels as well as an

increase in the biosynthesis of protein and carbohydrates **(Asma *et al.*, 2019)**. Such stimulatory effect of [AgNPs] explained by **Yan and Chen (2019)**, who mentioned that AgNPs penetrate cell wall, creating more new pores that continue to be beneficial in efficiently transporting nutrients, resulting in a rapid germination development rate. **Shruti *et al.* (2019)**, claimed that the lower concentrations of AgNPs induced increases in root length, shoot length, seed germination, and surface area. While high concentrations had an inhibitory effect that caused reduction of these parameters. **Rastogi *et al.* (2019)** reported that the phytotoxicity of AgNPs, may impede photosynthetic activities by destroying photosystems and impairing PSI electron transport as well as assimilation rate was decreased .

In the term of total pigments, the findings supported by **Sibi *et al.* (2017)** asserted that using low doses of nanoparticles have allegedly stimulated algal growth and pigment levels. However, **Tripathi *et al.* (2017)** claimed that the loss of chlorophyll is a reliable indicator of the phytotoxicity of AgNPs to plants. AgNPs causing thylakoid membrane disruption in *Arabidopsis* leaves and a reduction in chlorophyll content. Moreover, **Liang *et al.* (2018)** found that *Physcomitrella patens* leafy gametophytes exposed to AgNPs had altered thylakoid structure and had less chlorophyll b.

With respect to total carbohydrates, these findings are in accordance with **Mehmood and Murtaza (2017)** assert that seeds treated with low amounts of AgNPs boosted their protein and carbohydrate content. Consequently, AgNPs have the potential to change agriculture in the future. Despite that, a high AgNP concentration has a phytotoxic effect on the overall carbohydrate content. These results are consistent with **Vishwa Karma *et al.* (2017)**, which showed that AgNPs may accumulate in Brassica sp. seedlings and significantly impair photosynthesis. Additionally, **Mura *et al.* (2015)** asserted that the conversion of AgNPs to Ag^{+} heavy metal could have deleterious consequences on a variety of

organisms. **Sigfridsson (1998)** concluded that Ag^+ interfered with photosynthesis by competitive substituting Cu^+ in phycocyanin [PC], a soluble copper-binding protein. It is located in the thylakoid lumen of the chloroplast and acts as an electron carrier for the transmission of electrons from cytochrome b6/f to photosystem 1 [PSI] and this photosynthetic electron transport is disabled (**Yuki *et al.*, 2020**).

In term of total lipids the results obtained are consistent with the findings of **Kang *et al.* (2014)** demonstrated that the interaction between AgNPs and algae resulted in the production of (ROS), with a synergistic harmful effects. So, the algal metabolic route was switched from the growth pathway to the generation of hydrocarbons (carbohydrates or lipids) as storage molecules. Additionally, **Sir Jumisa *et al.* (2016)** reported that AgNPs are used to break down the cell walls of *C. vulgaris* in order to liberate lipids and carbohydrates for the production of biofuel. **Sibi *et al.* (2017)** predicted that the usage of modest quantities of nanoparticles allegedly caused an increase in lipid production, pigment content, and algal biomass. **Shanab *et al.* (2019)** discovered that cell proliferation was hampered by the augmentation of lipid synthesis at lower AgNPs concentrations.

Regarding amino acids contents of *Phaseolus vulgaris* seeds presoaked in different concentrations of AgNPs and phycocyanin extract. These results were confirmed by **Kocsy *et al.* (2011)** who suggest that polyamines and free amino acids might function as natural antioxidants, share in a number of metabolic activities, giving a resistance to abiotic stressors. Furthermore, according to **Yan *et al.* (2020),** to detoxify metal ions within plant tissues, this require specific ligands to chelate them. Amino acids are important in metal chelation process, so the plants become tolerant and detoxify heavy metals. Moreover, **Sanaa *et al.* (2019)** stated that, most physiological functions are improved at lowered AgNPs concentrations. A higher content of total amino acids was discovered as well after soaking *Phaseolus vulgaris* seeds at high doses [40–

50 ppm]. Amino acid content may have increased as a result of tolerance to environmental challenges. These results concur with those of **Khan *et al.* (2020)**. Many mechanisms, including functioning as compatible solutes against osmotic alterations **Hasegewa *et al.* (2000)** and through modulating k+ transport (**Demidchick, 2015**), also it have been hypothesized that the role of amino acids in removing stress conditions, enhances the stability of proteins and membranes (**Csonka, 1989)** , the turnover, synthesis, and incorporation of nitrogen into high molecular components (**Rolletschek *et al.*, 2001)**. It is worth mentioning that during stress conditions, both proline, Polyamines, sugars, and amino acids are accumulated. These findings are consistent with those of **He *et al.* (2012)**, who claimed that plants can respond to stress by producing higher osmotic potential as a result of accumulating more osmolytes, such as proline, polyamines, sugars, and amino acids, which are essential for osmotic adjustment and ROS scavenging.

In case of proline, the current findings demonstrated that the augmentation of MDA was significantly correlated with the priming of *Phaseolus vulgaris* seeds in high concentrations of AgNPs (40 and 50 ppm). These findings are correlated with **Rai (2002)** claimed that proline is regarded as an indicator of environmental stress, performs a crucial protective role. According to **Alia and Saradhi (1991)**, contact with heavy metals promotes proline to accumulate heavy metals.

These proteins electrophoretic banding patterns produced by SDS-PAGE gave satisfactory results with many quantitative and qualitative alterations in polypeptide banding pattern of protein profiles at different treatments. These results could be explained according to **Abdelhaliem *et al.* (2016)**. The transcriptional events that may alter plant metabolism by modifying proteins and increasing their susceptibility to "proteolytic degradation" leading to oxidative protein lesions are reflected in the alterations observed in protein banding patterns that are induced by varying levels of oxidative stress produced by phycocyanin

and AgNPs treatments. The high levels of protein polymorphisms seen by SDS-PAGE may be caused by changes in the amino acid sequences of proteins or by the addition, insertion, or deletion of amino acids between altered sites of protein bands (**Galani *et al.*, 2011**). On the other hand, variations in the number of polypeptide bands seen between the treated and control samples may be caused by changes to the nitrogenous bases of DNA, protein sites, amino acid sequences, or frame shift mutations (**Mondini *et al.*, 2009**).

The current findings demonstrated that the augmentation of MDA was significantly correlated with the priming of *Phaseolus vulgaris* seeds in high concentrations of AgNPs. These results are consistent with those of **Gawei *et al.* (2004)** who demonstrated that the considerable rise in malondialdehyde [MDA] levels was evidence of lipid peroxidation. **Muraadoglu *et al.* (2015)** claimed that free radicals are generated in excess and accumulate in cells when plants are established in stressful conditions. Moreover, **Tripathi *et al.* (2017)** showed that the main mechanism causing AgNPs' phytotoxicity is the overproduction of (ROS), which results in oxidative stress in plant cells. Finally, the reduction in plant development and cell death may result from polyunsaturated fatty acid peroxidation, which also disrupts the permeability of cell membranes and directly affects the structure of cells by harming protein and DNA (**Gupta *et al.*, 2009**). Also, **Samberg *et al.* (2011)** stated that AgNPs may infiltrate the plasma membrane through cell wall pores, connect to various cell organelles, and increase (ROS), which could affect the metabolic processes taking place inside algal cells. Also, **Taylor *et al.* (2016)** claimed that AgNPs bind to cell membranes, change their permeability or ion-transport abilities interfere with cellular phosphate regulation, prevent DNA synthesis by rupturing hydrogen bonds, which causes ribosome denaturation, and deactivate enzymes and proteins by binding to their active site.

The results of antioxidants enzymes run parallel with **Rico *et al.* (2015)** who claimed that oxidative stress contributes to AgNPs phyotoxicity by producing [ROS]. Therefore, producing [ROS] by oxidative stress assists in AgNPs phytotoxicity. As a result, plant cells activate many antioxidant defense systems to combat the adverse impacts of ROS. In order to protect plant cells from oxidative stress, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and peroxidase are all involved. Following exposure to AgNPs, the enzymatic antioxidants' activities are increased in plant tissues in a dose-dependent manner (**Bagherzadeh and Ehsanpour, 2016**) to protect the cells from oxidative stress (**Zou *et al.*, 2016**). Moreover, **Navarro *et al.* (2008)** reported that algae may release a metal chelator upon contact with nanoparticles, repressing the availability of metal ions secreted by AgNPs. **Miao *et al.* (2009)** found that algae may produce chemicals to make nanoparticles more susceptible to flocculation, which could decrease their availability. **Taylor *et al.* (2016)** showed that algal cells may also release organic carbon compounds that inactivate AgNPs toxicity. **Huang *et al.* (2016)** proved that an algal defense system generates low molecular weight antioxidant compounds and enhances the production of antioxidant enzymes.

Conclusion

The current study outlines and illustrates the toxic effect of AgNPs especially at high concentrations [40 and 50 ppm] while it had a stimulatory effects regarding all biochemical characters ,the morphological and physiological levels when compared with their control.Also it cleared the phytotoxicity process by which AgNPs cause their toxicity on plants. Additionally, the processes of

tolerance that underlie the survival strategy used by plant to deal with the detrimental effects of AgNPs was studied .On the other hand, aqueous phycocyanin extracted from *Spirulina platensis* had a stimulatory effects regarding all tested biochemical contents either at low or high concentrations .

References

A**bdelhaliem, E., Abdalla, H. M., Bolbol, A. A. and Shehata, R. S. (2016).** Assessment of protein and DNA polymorphisms in corn (*Zea mays*) under the effect of non-ionizing electromagnetic radiation. *Caryologia*, **75(4)**.

Alia and Pardha Saradhi, P. (1991). Proline accumulation under heavy metal stress. *J Plant Physiol*, **138, 554–558**.

Asma, N., Crispin, H. and Mudassar, T. (2019). Impact of AgNPs on seed germination and seedling growth. A focus study on its antibacterial potential against C*lavibacter michiganensis* subsp. Michigonensis infection in *Solanum lycopersicum.* J. *of Nanoparticles*, **e2019,** Article.ID 6316094 https://doi.org/10.1155/2019/6316094.

Bagherzadeh, H. M. and Ehsanpour, A. A. (2016). Silver nanoparticles and silver ions: oxidative stress responses and toxicity in potato (*Solanum tuberosum* L.) grown in vitro. *Horticulture, Environment, and Biotechnology*, **57, 544-553**.

Bailey, G. B. (1967). Purification and properties of an α-dialkyl amino acid transaminase. *Biochemistry*, **6(5), 1526-1533**.

Bates, L. S., Waldern, R.P. and Teare, I. D. (1973). Rapid determination of free proline of water stress studies. *Plant and Soil*, **39(1), 205-207**.

Berena, R., Casals, F. Colon, F., Font, X., Sahchez, A. and Puntes, V. (2009). Evaluation of the ecotoxicity of model nanoparticles. C*hemosphere* , **75 (7), 850-857**.

Boussiba, S.; Richmond, A.E. (1979). Isolation and characterization of phycocyanins from the blue-green alga *Spirulina platensis*. Arch. Microbiol, **120, 155–159**.

Csonka, L. N.(1989). Physiological and genetic responses of bacteria to osmotic stress. *Microbiological reviews*, **53(1), 121-147**.

Demidchik, V. (2015). Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. *Environmental and Experimental Botany*, **109 , 212-228**.

Dhindsa, R.S., Plumb, D. P. and Thorpes, T.A. (1981). Leaf senescence: correlation with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot*, **126, 93-101**.

Dipak, K.S. and Gagon, B.N.C. (2023). Chapter Eight - Hormone-linked redox status and its modulation by antioxidants.*Vitamins and Hormones*, **121, 197-246**.

Durairaj, K., Roy, B., Chandrasekaran, N., Krishnan, S.P., Mukherjee, A. (2020). Silver nanorods induced oxidative stress and chromosomal aberrations in the *Allium cepa* model. IET Nanobiotechnol, **14(2), 161–166**.

Galani, S., Naz, F., Soomro, F., Jamil, I., Azhar, A. and Ashraf, A. (2011). Seed storage protein polymorphism in ten elite rice (*Oryza sativa* L.) genotypes of Sindh. *African Journal of biotechnology*, **10(7), 1106-1111**.

Goldberg, D.M. and Spooner, R.T. (1983). Glutathione reductase. In: Pergmeyer HU Bergmeyer I. GraBiM, (ed). *Methods of enzymatic analysis*, Verlag Chemie, Weinhein., **111 (3), 258-265**.

Gupta, H. V., Kling, H., Yilmaz, K. K. and Martinez, G. F. (2009). Decomposition of the mean squared error and NSE performance criteria: Implications for improving hydrological modelling. *Journal of hydrology*, **377(1-2), 80-91**.

Hasegawa, P. M., Bressan, R. A., Zhu, J. K. and Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. *Annual review of plant biology*, **51(1), 463-499**.

He, D., Darantes-Aranda, J.J. and Waitee, T.D. (2012). Silver nanoparticle algae interaction: oxidative dissolution reaction oxygen species generation and synergistic toxic effects. *Environ. Sci. Technol*, **46 (16), 8731-8738**.

Health, R. L. and Packer, L. (1968). Photoperoxidation in isolated chloroplants T. kinetics and stoichiometry of fatty acids peroxidation. Arc*h. Biochem. Biophys*, **125,189-198**.

Huang, J., Cheng , J. and Yi, J. (2016). Impact of silver nanoparticles on marine diatoms *Skeletonema ostatum*. *J. App. Toxicol*, **36 (10), 1343-1354**.

Kang, N.K., Lee, B., Choi, G.G., Moon, H., Park, M.S., Lim, J. and Yang, I.W. (2014). Enhancing lipid productivity of *Chlorella vulgaris* using oxidative stress by TiO2 nanoparticles. *Korean J. Chem. Eng*, **31 (5), 861-867**.

Kar, M. and Mishra, D. (1976). Catalase, peroxidase, polyphenol oxidase activities during rice leaf senescence. *J. Plant Physiol*, **57, 315-319**.

Khan, I., Raza, M. A., Awan, S. A., Shah, G. A.,Rizwan, M., Ali, B. and Huang, L. (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., Samrah, A. A., Muhammad, A.R., Muhammad, R., Rezwan, T. R., Ali,S., and Huang, L.(2021). Silver nanoparticles improved the plant growth and reduced the sodium and chlorine accumulation in pearl millet: a life cycle study. Environ Sci Pollut Res Int, **8(11), 13712-13724**.

Khan,S., Zahoor, M., Khan,S.R. and Ikram, M. (2023). The impact of silver nanoparticles on the growth of plants: The agriculture applications. Heliyon **9(6),e16928**.

Kumar, K.B. and Khan, P.A. (1982). Peroxidase and polyphenol oxidase in excised fungi (*Fleusine coracana* cv. PR 202) leaves during senescence. *Indian J. Exp. Bot*, **20, 412-416**.

Kumar, D., Dhar, D.W., Pabbi, S., Kumar, N., Walia, S. (2014). Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). *Indian J. Plant Physiol*, **19, 184–188**.

Latif, H.H., Gharib, M. and Abu-Tahon, M. (2017). Photosynthesis of silver nanoparticles using leaf extracts from *Ocimum basicium* and *Mongifira indica* their effect on some biochemical attributes of *Triticum aestivum*. *Gesunde Pflanzen* , **69, 39-46**.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of Bacteriophage T_4. *Nature (London)*, **227, 680- 685**.

Leslaw, B.L., Joanna, S.P., Katarzyna, G., Karolina, S., Viorica, R. P., Marcin, H., Pawel, P. and Bogustaw, B. (2022). The effect of bio synthesized silver nanoparticles on germination, early seedling development, and metabolism of wheat (*Triticum aestivum* L.). *Moleciles*, **27, 2303**.

Liang, L., Tang, H., Deng, Z., Liu, Y., Chen, X. and Wang, H. (2018). Ag nanoparticles inhibit the growth of the bryophyte, *Physcomitrella patens. Ecotoxicology and environmental safety* , **164, 739-748**.

Matras, F., Gorczyca, A., Pociecha, F., Przemieniecki, S.W. and Ocwieja, M. (2022). Phytotoxicity of silver nanoparticles with different surface properties of monocots and dicots model plants. *J. Soil Sci. Plant Nutr*, **2022, 1-18**.

Mehmood, A. and Murtaza, G. (2017). Impact of biosynthesized silver nanoparticles on protein and carbohydrate contents in seeds of *Pisum sativum* L. *Crop Breeding and Applied Biotechnology* , **17, 334-340**.

Metzner, H., Rau, H., and Senger, H. (1965). Untersuchungen zur Synchronisier barkeepein Zelner pigment Mango I Mutanten Von *Chlorella. Planmta* , **56 ,186-194**.

Miao, A. J., Schwehr, K.A., Xu, C., Zhang, S.J., Luo, Z., Quigg, A. and Santschi, O.H. (2009). The algal toxicity of silver engineered nanoparticles and detoxification by exopolymeric substances. *Environ. Pollut* , **157 (11), 3034.-3041**.

Mondini, L., Noorani, A. and Pagnotta, M.A. (2009). Assessing plant genetic diversity by molecular tools. *Diversity*, **1, 19- 35**.

Mura, S., Greppi, G., and Irudayaraj, J. (2015). "Latest developments of nanotoxicology in plants," in *Nanotechnology and Plant Sciences,* eds Siddiqui M. H., Al-Whaibi M. H., Mohammad F. (Cham: Springer International Publishing), **125–151**.

Muraadoglu , F., Gundogd, U.,Ercisli, S., Encu, T., Balta, F., Jaafar, H.Z.E. and Zia Zu-Haq, M. (2015). Cadmium toxicity affects chlorophyll a and mineral nutrient accumulation in strawberry. *Biol. Res*, **48(4), 1-7**.

Naguib, M.I. (1964). Effect of sever on carbohydrates and nitrogen metabolism during the germination of cotton seeds. *Ind. J. Exp. Biol*, **2, 149-152**.

Navarra, E., Piccapietra, F., Wagner, N., Marconi, F., Faeg, R.and Odzak, N. (2008). Toxicity of silver nanoparticles to *Chlamydomonins srein*. *Environ Sci Technol*, **42 (23), 8959-8964**.

Nelson, N. (1944). Photometeric adaptation of some method for the determination glucose. *J. Biol. Chem*, **153, 275-281**.

Rai, V. K. (2002). Role of amino acids in plant responses to stresses. *Biologia plantarum* , **45 (4)**, 481-487.

Rastogi, A., Tripathi, D. K., Yadav, S., Chauhan, D. K., Živčák, M., Ghorbanpour, M. and Brestic, M. (2019). Application of silicon nanoparticles in agriculture. *Biotech,* **3 (9), 1-11.**

Rico, C.H., Perolta-Videa, J.R. and Gardea-Torresdey, J.L. (2015). Chemistry, biochemistry of nanoparticles and their role in antioxidant defense system in plants. In: Seddiqui, M.H., Al-Whaib, M.H., Mohammad, F., editor. Nanotechnology and plant sciences, Nanoparticles and their impact in plants. *Springer, Chem. Switzerland,* **pp. 1-17.**

Rolletschek, A., Chang ,H., Guan, K., Czyz, J., Meyer, M. and Wobus, A. M. (2001). Differentiation of embryonic stem cell-derived dopamine neurons is enhanced by survival-promoting factors. *Mechanisms of development,***105 (1-2), 93-104**.

Roman, P., Agata, S., Anna,K., Stawomir ,M. and Marta ,A.(2023). Impact of Ag nanoparticles on seed germination and seedling growth of green beans in normal and chill temperatures. *Agriculture,* **10(8), 312**.

Saad, A.M., El-Saadony, M.T., El-Tahan, A.M., Sayed, S., Moustafa, M.A., Taha, A.E., Taha, T.F., Ramadan, M.M. (2021). Polyphenolic extracts from pomegranate and watermelon wastes as substrate to fabricate sustainable silver nanoparticles with larvicidal effect against *Spodoptera littoralis*. *Saudi J. Biol. Sci,* **28, 5674–5683**.

Said, A. and Naguib, M.I. (1964). Sucrose determination as means of estimation of the draw, back tax exported Halawa Tehinia. *Bull. Fac. Sci,* Cairo. Univ, **39, 207-216**.

Samberg, M.E., Orndorff, P.E. and Monterio-Riviere, N.A. (2011). Antibacterial efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods. *Nanotoxicol,* **5 (2), 244-253**.

Shanab, S. M. M., Partila, A. M., Ali, H. E. A. and Abdullah, M. A. (2019). Characterization and Impact of Silver nanoparticles on cell growth, lipid, carbohydrate and fatty acids of *Chlorella vulgaris* and *Dictyochloropsis splendida*. *Beilstein Archives*, **2019(1), 91**.

Shruti, V. C., Jonathan M. P., Rodriguez-Espinosa P. F. and Rodríguez-González F. (2019). Microplastics in freshwater sediments of atoyac river basin, puebla city, Mexico. *Science of the Total Environment,* **654, 154-163**.

Sibi, G., Kumar, D.A., Gopel, T., Marinath, K., Baniupria, S. and Chaitra, S. (2017). Metal nanoparticle triggered growth and lipid production in *Chlorella vulgaris* . *Int. J. Sci. Res. Environ. Sci. Toxicol*, **2 (1), 1-8**.

Sigfridsson, K. (1998). Plastocyanin, an electron-transfer protein. *Photosynthesis research*, **57, 1-28**.

Singh, R.P., Handa, R. and Manchanda, G. (2021) Nanoparticles in sustainable agriculture: An emerging opportunity. *J. Control Release*, **329, 1234-1248**.

Sir Jumisa, A.P., Duraarasan, S. and Mani, V. (2016). Biosynthesis of silver nanoparticles and its application in cell wall disruption to release carbohydrate and lipid from *C. vulgaris* for bio-fuel production. *Biotechnol. Rep*, **11, 70-76**.

Soror, A. F. S., Ahmed, M. W., Hassan, A. E., Alharbi, M., Alsubhi, N. H., Al-Quwaie, D. A. and Abdalla, H. (2022). Evaluation of green silver nanoparticles fabricated by *Spirulina platensis* phycocyanin as anticancer and antimicrobial Agents. Life, **12(10), 1493.**

Stevens, D. L., Tanner, M. H., Winship, J., Swarts, R., Ries, K. M.;Schlievert, P. M. and Kaplan, E. (1989). Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. *New England journal of medicine*, **321(1), 1-7**.

Taylor, C., Matzke, M., Kroll, A., Read, D.S., Svendsen, C. and Crossley, A. (2016). Toxic interaction of different silver forms with fresh water green algae and cyanobacteria and their effects on mechanistic endpoints and the production of extracellular polymeric substances. *Environ. Sci. Nano*, **3 (2), 396-408**.

Tripathi, D.K., Tripath, A., Singh, S., Singh, V.K., Mishra, R.K., Upadhyay, R.G., et al. (2017). Uptake accumulation and toxicity of silver nanoparticle in autotrophic plants and heterotrophic microbes: A concentric review. *Front. Microbiol.* Do: 10:33891 fmicb. 2017.00007.

Varma, A. K. and Tiwari, P.N. (1967). Rhizobium inoculation and oil content and soybean seeds (*Glycine max*). *Curr Sci* , **20, 275**.

Vishwa karma, K., Upadhyay, N., Sing, J., Liu, S., Singh, V. P., Prasad, S. M. and 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**.

Vymazal, J. (1995). "Algae and Element Cycling in Wetlands". CRC Press, Inc., Boca Raton, Florida, USA, **689p**.

Wang, X., Xie,H., Wang, P. and Yin ,H. (2023). Nanoparticles in plants: uptake, transport and physiological activity in leaf and root. *Materials (Basel)***. 16(8), 3097**.

Yan, A. and Chen ,Z. (2019). Impacts of Silver Nanoparticles on Plants: A Focus on the Phytotoxicity and Underlying Mechanism. Int. J. Mol. Sci., **20(5)**.

Yuki, O., Leonardo, B., Toshiharu, S. and Ken, M. (2020). Cyclic Electron Transport around PSI Contributes to Photosynthetic Induction with Thioredoxin. *Plant Physiol,* **184 (3),1291–1302**.

Zarrouk, C. (1966). Contribution a l'etude d'une Cyanophycee. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima.* PhD. Thesis. University of Paris, France.

Zhang, N.A., Sun, J.,Yin,L., Liu, J., Chen,C. (2023). Silver nanoparticles: From in vitro green synthesis to in vivo biological effects in plants, *Advanced Agrochem*, https://doi.org/10.1016/j.aac.**2023.**08.004.

Zhao, J., Lin, M., Wang, Z., Cao, X. and Xing, B. (2021). Engineered nanomaterials in the environment. Are they safe? *Crit. Rev. Environ. Sci. Technol* , **541, 1443-1478**.

Zou, Y., Wang, X., Chen, Z., Yao, W., Ai, Y., Liu, Y. and Wang, X. (2016). Superior coagulation of graphene oxides on nanoscale layered double hydroxides and layered double oxides. *Environmental pollutio*n, **219, 107-117**.

الإستجابة المستحسة لبذور الفاصوليا فولجاريس المعاملة بجسيمات الفضة النانوية والفيكوسيانين المائى المستخلص من سبيرولينا بلاتنسيس

عبدالفتاح صلاح سرور، مى وليد أحمد ، سامية السيد سعفان

قسم النبات والميكروبيولوجي - كليه العلوم - جامعه الزقازيق - مصر

أجرى هذا البحث لدراسة التأثيرات الأيضية لـ جسيمات الفضة النانوية بواسطة phycocyanin المستخلصة من طحلب سبيرولينا بلاتنسيس على الأنشطة الأيضية لأحد النباتات الاقتصادية نبات فاصوليا فولجاريس بما في ذلك إنبات البذور والأصباغ، والكربوهيدرات، والدهون الكلية، ومحتوى البذور من الأحماض الأمينية، والبرولين، أنماط باندات متعدده الببتيد، والمالوند يالدهيد بالإضافة إلى نشاط الإنزيمات المضادة للأكسدة فوق أكسيد ديسموتاز [SOD]، وبيروكسيداز [APX]،الكاتلاز [CAT] ومضادات الأكسدة غير الأنزيمية مثل الجلوتاثيون المختزل . أظهرت النتائج أن إنبات البذور، والأصباغ، والكربوهيدرات، والدهون الكلية قد زادت بالتركيزات الضعيفة من (جسيمات الفضة النانوية 5-30 جزء في المليون). ومن ناحية أخرى، أظهرت التركيزات العالية من [40 AgNPs-50 جزء في المليون] تأثيرًا مثبطًا. ولقد أوضحت النتائج زيادة الإنزيمات المضادة للأكسدة SOD وAPX وCAT معتمدة على التركيزات الضعيفة المستخدمة من جزيئات النانوية .كما لوحظ زيادة كمية الأحماض الأمينية تدريجياً مع زيادة تركيزجسيمات الفضة النانوية AgNP. أظهرت انماط باندات متعدده الببتيد أن إجمالى عدد باندات البروتين بإستخدام SDS-PAGE كان 159 باند بأوزان جزيئيه مختلفه تراوحت من 27 إلى 145 KDa و بلغ عدد الباندات المتعددة الأشكال 10 بقيمة 6.29٪ بينما كان عدد الباندات أحادية الشكل 6 بقيمة 3.77٪ .