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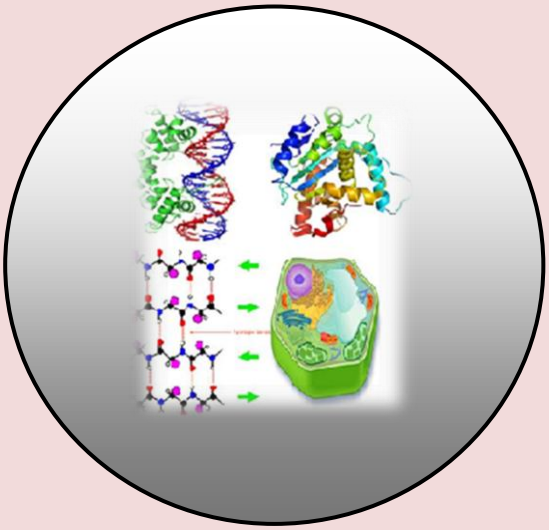

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# Impact of gold nanoparticles on germination, physiochemical and morphological characteristics of *Cicer arietinum* Linn.

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## ABSTRACT
***The purpose of this study was to determine the impact of tobacco gold nanoparticles (GNPs) on physiological and biochemical attribute of Cicer arietinum (chickpea). Different concentrations 25, 50, 100, 200 and 400µM of tobacco GNPs were used as a part of each treatment in chickpea seed to see tobacco GNPs impacts on seed germination, seedling growth, physiochemical and morphological attributes. Results showed that seed treated with lower concentrations (50-200µM) of tobacco GNPs improve germination percentage, photosynthetic efficiency and mitigate the production of reactive oxygen species (ROS).Whereas, high concentration (400 µM) of tobacco GNPs induced production of ROS which cause oxidative stress at cellular level and affect the plant growth and its photosynthetic efficiency.***
***Keywords: chickpea, chlorophyll, germination, gold nanoparticles and tobacco.***

## INTRODUCTION
Among pulses, *Cicer arietinum* (chickpea) is most significant food legume plants due to its wider adaptability, low production cost, capacity to nitrogen fixation, and flexibility to fit as diverse crop cycles in sustainable agricultural systems (Singh 1997).Chickpea are an important source of nutrition for millions of people in underdeveloped countries and are commonly referred "poor man's meat"(Merga and Haji 2019).The chickpea production in India, year 2019-20 as rabi was 12.61 million tonnes (Ministry of agriculture and farmer welfare). In comparison to other edible legumes grain it has high protein content (20–22%) and is free of anti-nutritive components and thus, it is considered as functional food. In addition to proteins, it is abundant in fibers, minerals and unsaturated fatty acids (Williams and Singh 1987).

Along benefits, low soil fertility is a major challenge that hampered chickpea production in India.

Nanotechnology, a subset of technology, has recently attracted the interest of scientists. Several researches have been implemented on synthesis of nanoparticles (NPs) from plants, their application on the physiochemical and morphological attributes of plant (Siddiqi *et al*., 2017). Shah and Belozerova (2009) reported that gold nanoparticles (GNPs) cause hindrances in plants by interfering with the functioning of aquaporin channels. Lei *et al*. (2008) reported the impacts of GNPs on photosystem enzymes, photosynthetic activity, and antioxidant expression in plants. Also, GNPs enable the coordinated release of essential nutrients with plant uptake efficiency, which is why GNPs based fertilizers have recently gained popularity (Solanki *et al*., 2016). GNPs are also utilised to treat wastewater in the agricultural industry because to their high adsorption capacity (Graily-Moradi *et al*., 2020) and preferred because of their interaction with surface functional group, antibacterial capabilities and low toxicity (Noruzi 2015).

In this study, we have exposed chickpea seeds with different concentrations of tobacco gold GNPs to evaluate the effect on germination pattern and plant development, along with other physiochemical parameters. Our observations provide helpful information to understand the metal NPs application in agriculture field.

## MATERIALS AND METHODS

### Preparation of nanoparticles solution, seed treatment and growth conditions

Tobacco GNPs, average size 30 to 50nm (Jalil *et al*., 2019) were synthesized and dissolved accordingly in double distilled water (DDW) to make 25, 50, 100, 200 and 400 µM concentration of GNPs. Chickpea seeds (RSG374) were chosen and surface sterilization were done through 0.01% mercuric chloride ($HgCl_2$), washed with DDW several times to remove $HgCl_2$. Five different concentrations of 25, 50, 100, 200 and 400µM of tobacco GNPs were used as a part of each treatment. Thirty seeds of Chickpea were placed in petri plates and supplemented with solution of each treatment and incubated for 16 hours, while control was treated with DDW. After treatment seeds were rinsed twice with DDW, transplanted to fresh petri plates and maintained at 25°C for 48 hours. After that the seedlings of similar size were moved into 4 inches pots.

### Estimation of morphological parameters

### Germination assay

Five different concentrations of 25, 50, 100, 200, 400µM of tobacco GNPs were used in every experiment. To accomplish germination assay thirty seeds of chick pea were retained in sterile petri plates, surface sterilization were done through 0.01% $HgCl_2$, washed several times with DDW to remove $HgCl_2$ and supplemented with solution of particular petri plates and incubated for 16 hours, while control was treated with DDW. After that again washed twice with DDW and transferred to new petri dishes. Every petridish were incubated at 25±3 °C for 72 hours. The seed was deemed to be germinated after the radicle appeared rupturing the seed coat. Germination percentage of seeds was calculated by the equation 1(Hajra and Mondal 2017).

$$\text{Germination (\%)} = \frac{\text{Number of Seed Germinated } \times 100}{\text{Total Number of Seeds}} \quad (1)$$

**Seedling length and biomass**

A manual centimeter scale was used for the measurement of root and shoot lengths of germinated seedlings. Shoot length measured from the appearance of shoot to the apex and root length was taken from the appearance of root up to root tip. For the measurement of fresh biomass both shoot and root was separated, cleaned and weighed on a digital scale. Both the root and the shoot were then dehydrated at 40°C for 48 hours and then dried out at 70°C for 72 hours to determine their dry biomass (Taibi *et al*., 2016).

**Estimation of biochemical parameters**

**Determination of chlorophyll and carotenoid**

For chlorophyll (Chl) measurement, 5 (g) fresh leaf of tobacco GNPs treated plants were washed with DDW and homogenised 80% (v/v) chilled acetone using pestle-mortal. After that, centrifuge for 10 minutes at 10,000 revolutions per minute (rpm).Optical density (OD) of sample was measured at wavelength of 663 and 645 nm using UV-VIS (Shimadzu-1601) dual-beam spectrophotometer (Arnon1949)**.** The carotenoid content was estimated by taking OD at 510 and 480 nm (MaClachlan and Zalik 1963).

**Estimation of total protein**

Shoot (5g) of Chickpea were washed thoroughly with DDW and homogenizedin20 ml Tris-buffer (100 mM, pH 7.4) using pestle-mortal. The extract was then subjected for centrifugation at 15,000 rpm for 12 minutes, and the supernatant was collect tube. The partial purification was achieved by investigating different concentrations (20, 40, 50, 60, 80, and 95%) of ammonium sulphate $\{(NH_4)_2SO_4\}$ with constant stirring at 4°C and then retained for 8 hours. The protein extract after complete precipitation, were again subjected to centrifugation at 15,000 rpm for 15 minute. Now precipitated protein was dissolve in 500 µl of phosphate buffer (50 mM, pH 5.0) at 4°C. Protein was calculated by using standard curve derived with bovine serum albumin (BSA), concentration range of 50µg-1000 µg $ml^{-1}$(Bradford1976).

**Malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) content**

For shoot lipid peroxidation, thiobarbituric acid (TBA) assay was employed, which identify MDA as the final product of lipid peroxidation. 0.5 (g) shoot sample were homogenized in 5 ml of 1% trichloroacetic acid (TCA) and centrifuged for 12 min at 12,000 rpm. Now, supernatant was mixed with 3ml of 0.5% TBA (w/v) prepared in 20% TCA and the mixture was incubated at 95°C for 30 min. After that reaction was rapidly stopped up in icebath and again centrifuge for 5 min at 10,000 rpm. OD of the supernatant was measured at 532 nm. The correction of nonspecific OD the value was subtracted by 600nm (Hodges *et al*., 1999). MDA-TBA complex were calculate from the extinction coefficient of 155 $mM^{-1}cm^{-1}$. For $H_2O_2$ estimation an aliquot (3mL) of the cold acetone extracted solution of shoot samples was mixed with 1 mL of 0.1% titanium dioxide prepared in 20% (v/v) sulphuric acid and mixture was centrifuged for 12 min at 6000rpm. Supernatant OD was measured at 415nm (Mukherjee and Choudhuri 1983). A standard curve was calibrated in range of 100–1000 µmol of $H_2O_2$ to compute the concentration.

**Root ion leakage**

Plant root 0.5 (g) was carefully cleaned with DDW from both treated and untreated plant samples. In a test tube, the root sample was incubated in 20 mL of DDW for 5 minutes at 25°C.

The solution's electrical conductivity ($EC_0$) was then determined using a conductivity meter. After that again incubated for 10 hours and the electrical conductivity ($EC_1$) was evaluated. Further the tube was placed in a water bath at 95°C for 25 minutes and electrical conductivity ($EC_2$) of the sample was measured again after cooling. Equation 2 was used to compute relative conductivity, which indicates root ion leakage (Lutts *et al*., 1995).

$$\text{Relative conductivity (RC)} = \left[\frac{EC_1 - EC_0}{EC_2}\right] \times 100 \quad (2)$$

## RESULTS AND DISCUSSION

### Assessment of tobacco GNPs on growth and physiological parameter of plant

### Impact on seed germination

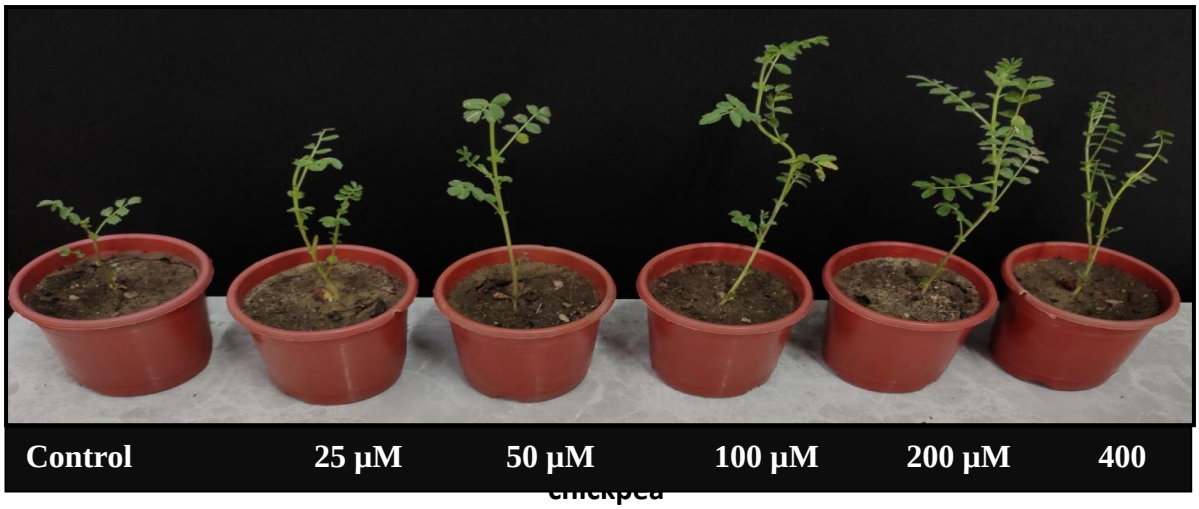
To see the impact of tobacco GNPs on germination, we have calculated the germination percentage of chickpea seeds in solution of different concentration of tobacco GNPs (25, 50, 100, 200, and 400 µM), the germination percentage of 100 µM treated seeds was maximum (91.33 ± 3.61%) in comparison to control (56.67 ± 2.25%), while 25 and 50 µM treated seeds have lower germination rate (71.67 ± 2.89 and 84.33 ± 2.73%, respectively) than 100µM treated seed but higher when compared to control. At higher concentration 200 and 400 µM germination percent was reduced (68.67 ± 3.61 and 62.00 ± 2.37%) in comparison with 25, 50, and 100 µM treatment of tobacco GNPs and found minimum in control (Table1).As a result, the study's findings demonstrated that GNPs improve germination. The findings were nearly identical to those published by Jalil *et al*. (2019), which highlighted the positive effect of GNPs on tobacco seed germination. Also, previous research has shown that treatment of 62 µg $ml^{-1}$GNPs has positive effect on germination (Barrena *et al*., 2009). Lower germination at higher doses might be attributed to increase accumulation absorption of these NPs in extracellular spaces as well as inside the cells, ensuing cell division reduction, cell elongation and inhibition of hydrolytic enzymes engaged in process of seed germination (Korishettar *et al*., 2016).

### Impact on biomass and seedling growth

To investigate the effects of tobacco GNPs on shoot and root length seedlings were removed from the pots following 15 days of treatment and analysed using a standard scale. The shoot length of chick pea *(Cicer arietinum*), of 200µM treated seeds was highest (9.23±0.37cm) than that of control (6.17±0.27 cm), followed by 100, 50 and 25µM tobacco GNPs. In contrast at higher concentration 400µM of tobacco GNPs reduce shoot length (6.77±0.31cm) but higher than control (Table 1). Similarly the root lengths were also increased gradually with increasing concentration and were highest in 200µM treated seeds (5.28±0.21cm) than that of control (3.52±0.15cm) followed by 100, 50 and 25µM of tobacco GNPs. In contrast, a higher concentration of 400µM tobacco GNPs reduced root length (4.04±0.14 cm) but higher than control and 25µM (Table 1). In fraction to growth of seedling, fresh biomass was also increased and decreased (Fig 1).The infiltration of NPs across cell walls and plasma membranes of epidermal layers as well as accumulation inside the vascular tissues may explain the decrease in seedling length at high doses (Korishettar *et al*., 2016). Hence, at higher concentration overall seedling development is reduced.

**Table 1. Changes in morphological parameters of chickpea plant under tobacco GNPs treatment.**

| Parameters | Control | 25 µM | 50 µM | 100 µM | 200 µM | 400 µM |
|---|---|---|---|---|---|---|
| Germination (%) | 56.67± 2.25 | 71.67± 2.88 | 84.33± 2.73 | 91.33± 3.61 | 68.67± 3.61 | 62.20± 2.37 |
| Shoot length (cm) | 6.17± 0.27 | 7.07±0. 26 | 8.00± 0.18 | 8.57± 0.27 | 9.23± 0.37 | 6.77± 0.31 |
| Root length (cm) | 3.52± 0.15 | 3.87± 0.17 | 4.57± 0.10 | 4.90± 0.15 | 5.28± 0.21 | 4.04± 0.14 |
| Shoot fresh weight (g) | 0.54± 0.01 | 0.59±. 08 | 0.67± 0.09 | 0.75± 0.017 | 0.84± 0.018 | 0.57± 0.03 |
| Root fresh weight (g) | 0.12± 0.05 | 0.17±0. 07 | 0.24±0. 01 | 0.265± 0.06 | 0.29±0. 09 | 0.15±0. 05 |




chickpea

**Estimation of biochemical parameters**

**Impact on photosynthetic pigments**

Total Chl content of 200µM tobacco GNPs treated plant was much higher (1.02 ± 0.04 mg $g^{-1}$) than that of control (0.5 ± 0. mg $g^{-1}$), followed by 25, 50, 100µM treated seed (0.7 ± 0.02, 0.79 ± 0.07 and 0.85 ± 0.02 mg $g^{-1}$). In contrast, a higher concentration of 400 µM reduced the chlorophyll content (0.61 ± 0.02 mg $g^{-1}$) but higher in contrast to control (Fig. 2A). The adverse consequence of NPs on Chl may be due to NPs being incorporated into chloroplasts, where they engage in catalytic oxidation-reduction events, speeding the evolution of oxygen and electron transport (Hong *et al*., 2005). The carotenoid content with respect to control was lowest at 200 µM (0.16 ±0.015) followed by 100, 50 and 25µM tobacco GNPs (Fig. 2B). But, at higher concentration (400 µM) does not support further decrease in the carotenoid content. This is maybe due to carotenoid biomolecules interaction with GNPs. However, (Wang *et al*., 2016) acknowledged that genes are mostly responsible for pigment enrichment.

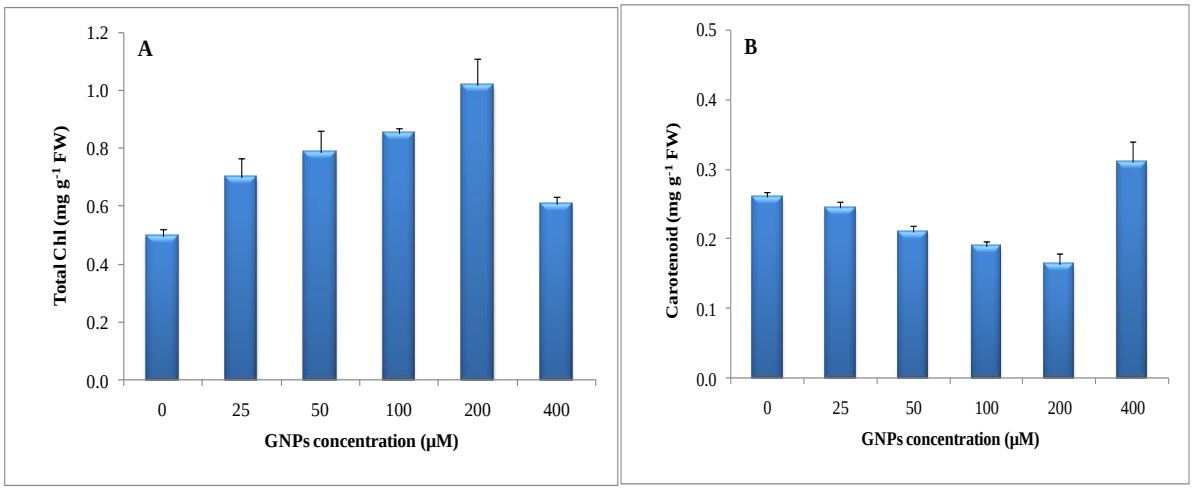



**Figure 2. Impact of different concentration of tobacco GNPs on photosynthetic pigments (A) Total chlorophyll (B) Carotenoid content. Data presented as mean ± SD of three replicates.**

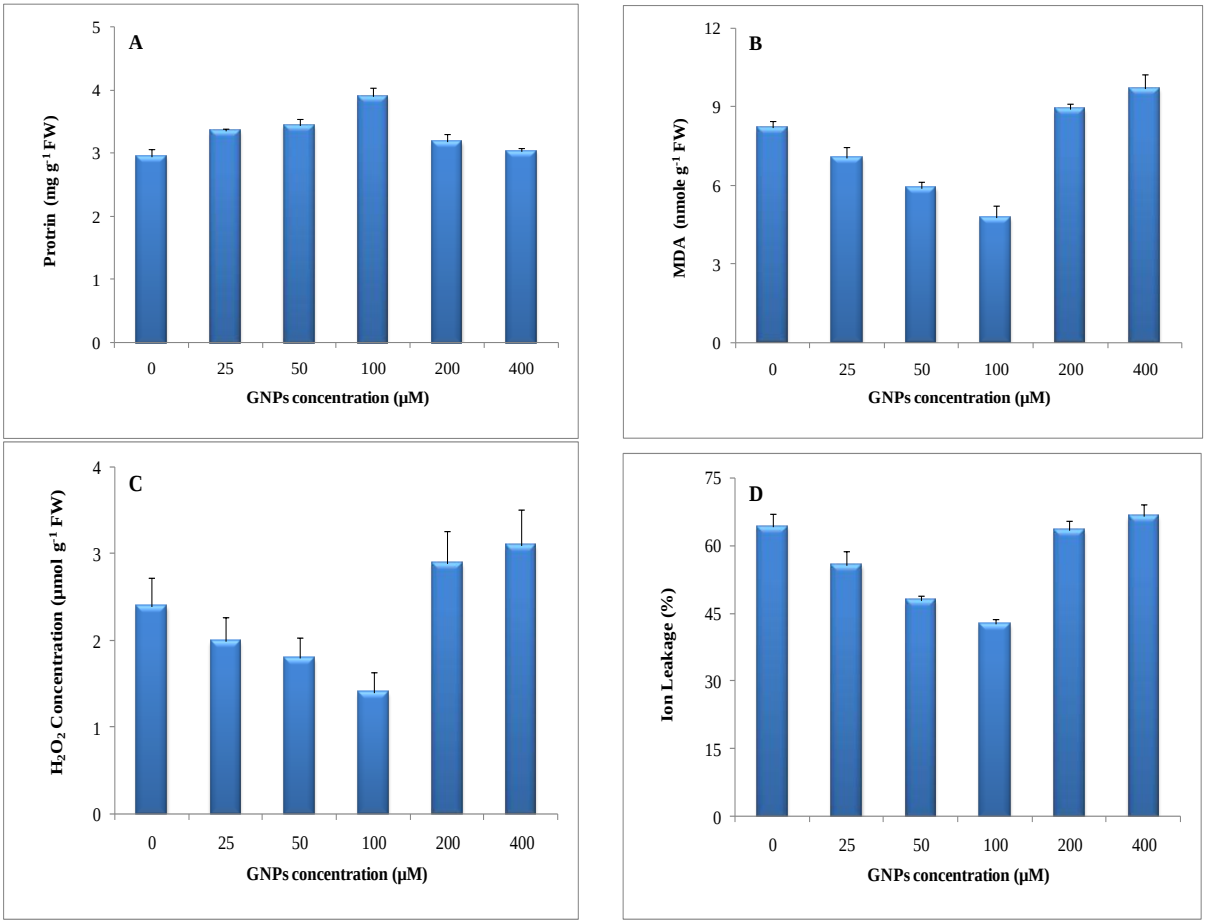



**Figure 3. Impact of different concentration of tobacco GNPs on biochemical attributes (A) protein content, (B) MDA content, (C) root ion leakage(D)$H_2O_2$ content. Data presented as mean ± SD of three replicates.**

**Impact on total protein**

The protein content of 100µM tobacco GNPs treated plant was highest (3.91 ± 0.14 mg $g^{-1}$) than that of control (2.95 ± 0.13 mg $g^{-1}$), followed by 50 and 25µM treated seed (3.45 ± 0.09 and 3.35 ± 0.03 mg $g^{-1}$). However, at higher concentration 200 and 400µM protein content was reduced (0.3.19 ± 0.11 and 3.03 ± 0.06 mg $g^{-1}$) but more than control (Fig. 3A).

**MDA and $H_2O_2$ content**

The MDA content was lowest at 100 µM (4.77 ±0.45 nmol$g^{-1}$FW) with respect to control (8.2±0.25) followed by 50 and 25µM tobacco GNPs. But at higher concentration (200 and 400µM)becomes increased to 8.90±0.21 and 9.70±0.55nmol$g^{-1}$FWrespectively(Fig. 3B).The $H_2O_2$ content was lowest at 100 µM (1.4 ±0.24 µmol g-$^{1}$FW) with respect to control (2.4±0.32) followed by 50 and 25µM tobacco GNPs. But at higher concentration (200 and 400µM) becomes increased to 2.90±0.21 and 3.10±0.41 respectively (Fig. 3C).Such high level of MDA and $H_2O_2$ under NPs interface is possibly due to production of ROS (Mohammadi *et al*., 2013).

**Root ion leakage**

The ion leakage was lowest at 100 µM (42 ±0.97 %) with respect to control (64.28±2.8) followed by 200, 50 and 25µM tobacco GNPs. But at higher concentration (200 and 400µM) it becomes increased (63.37±2.23 and 66.47±2.54) gradually (Fig. 3D).Plant membrane degradation results in peroxidation of lipid molecules and leakage of ions. From study results, it can be suggested that at higher concentration tobacco GNPs caused membrane disruption in chick pea. This is most likely owing to the fact that roots are the primary entry point for NPs (Anjum *et al*. 2013).Hatami and Ghorbanpour (2014) stated that NPs elicit oxidative stress and electrolytic leakage which affect membrane firmness.

## CONCLUSION

This study created a strategy for examining the effects of different concentration of tobacco GNPs on seed germination, morphological and various physio-chemical parameters of seedling during plant development. Studies have shown that the low-dose tobacco GNP supplementation increased seed germination, controlled different morphological and physiological responses in plants, and boosted free radical scavenging capability. Whereas, high concentration of tobacco GNPs induced ROS production and cause damage at cellular level, which affect photosynthetic efficiency and plants growth under short-term exposure. Finally, it has been concluded that application of low concentration of tobacco GNPs helped to improve the photo-system activities and plant responses. However, more research is needed to conclude the presence of NPs in various organs, tissues, and cells, as well as to analyse proteins that interact with NPs and to quantify chemicals in nanoparticle metabolic processes in plant cells.

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