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Research Paper

"Effect of gold and zinc oxide nanoparticles on morphological parameters and nutritional content of *Spinaciaoleracea*"

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ABSTRACT
Green synthesis of gold (AuNPs) and zinc oxide (ZnO NPs) nanoparticles from Psidiumguajava plant. AuNPs and ZnO NPs were synthesized from P. guajava and characterized. UV-Vis spectrophotometer showed the highest peak at 529 nm of AuNPs and 355 nm of ZnO NPs which confirmed the synthesis of gold and zinc oxide nanoparticles. In SEM the crystallite size of AuNPs was observed in the range of 36.82 nm to 55.90 nm and the crystallite size of ZnO NPs was observed in the range of18.79 nm to 27.15 nm, these values confirmed that nanoparticles were successfully synthesized. EDS graphs confirmed that Au and ZnO nanoparticles were successfully synthesized. The spinach plants were sprayed with graded concentration of gold nanoparticles and zinc oxide nanoparticles (0, 100, 500, 1000 ppm) after 14 days of sowing. The morphological parameters like height of spinach, number of leaves per plant and leaf surface and nutritional contents like protein, carbohydrate and total chlorophyll in leaf samples were recorded at the time of maturity (45-50 days). As the concentrations of NPs increased there was increase in height of plant (14cm,12.8cm), number of leaves per plant (6±1,4±1), leaf surface area ($13cm^{-1}$,$13cm^{-1}$) and protein (0.60 mg/ml, 0.68 mg/ml), carbohydrate (1.31 mg/ml, 0.96 mg/ml), and total chlorophyll (11 µg/ml, 17.9 µg/ml).Hence, concluded that the zinc oxide nanoparticles showed the more effective results than the gold nanoparticles on morphological and nutritional content of spinach.

KEYWORDS: *Psidiumguajava,spinach,AuNPs, ZnONPs,SEM, EDS, FTIR*

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I. INTRODUCTION

Green nanotechnology has been described as the development of clean technologies, to minimize potential environmental and human health risks associated with the manufacture and use of nanotechnology products and to encourage replacement of existing products with new nano products that are more environmentally friendly throughout their lifecycle [17]. Nanoparticles are particles between 1 and 100 nm in size. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties. Particles are further classified according to diameter. Nanoparticles are of great scientific interest as they are bridge between bulk materials and atomic or molecular structures[9].NPs have started being considered as nano antibiotics because of their antimicrobial activities. Nanoparticles have been integrated into various industrial, health, food, feed, space, chemical, and cosmetics industry of consumers which calls for a green and environment-friendly approach to their synthesis[6]. Nanoparticle synthesis is conducted by physical, chemical and biological or green method. A physical and chemical technique of synthesizing nanoparticles has proved to be quite expensive and potentially hazardous to the environment. Toxic and perilous chemicals involved in the synthesis of nanoparticles in chemical synthesis technique possess various biological risks and are responsible for several health diseases [14].Gold nanoparticles so far have been synthesized from a wide array of plant systems. The protective and reductive activity of plant biomolecules is accountable for the reduction of gold ions[12]. Gold nanoparticles exhibit novel optical and catalytic properties. They are also non-toxic and biocompatible. They have attracted considerable interest in a range of applications including electron microscopy marker, DNA sequencing, catalysis, wave guider and in biomedical science [16].AuNPs can be synthesized in a range of size and shape distributions via different techniques such as citrate reduction of $HAuCl_4$ in water, seed-mediated growth method, metal vapour synthesis, electrochemical method through gold ionization and reduction. It has been reported that AuNPs synthesized by physical and chemical methods aggregates in physiological conditions hindering its in *vivo* applications [11]. Zinc oxide is a multifunctional material with its unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photo stability.ZnO is non-toxic and self-cleansing. It is also compatible with skin, is anti-bacterial, dermatological and has UV radiation blocking property; hence it is used in cosmetics and many biomedical fields. Furthermore, ZnO NPs also act as good antimicrobial agent as they show antimicrobial activity. ZnO NPs are less toxic and safe so they find increased applications in industries like food where they are used in packaging and processing of meat and vegetables. The utilization of plants in synthesis of zinc oxide nanoparticles (ZnO NPs) is quite novel technique and does not require consent with the preparation, which leads to eco-friendly green chemistry approach. The biological methods of nanoparticles synthesis were reported to have advancement over chemical and physical methods[7].Zinc oxide nanoparticles have been used to eliminate sulphur, arsenic from water because bulk ZnO cannot remove arsenic because nanoparticles have great surface area than bulk material[18]. Application of micronutrient in the form of nanoparticles (NPs) is an important route to release required nutrients gradually and in a controlled way, which is essential to mitigate the problems of soil pollution caused by the excess use of chemical fertilizers[5]. Ultraviolet (UV) spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample (a quantity known as the extinction, which is defined as the sum of absorbed and scattered light) [2]. A scanning electron microscope (SEM) is an instrument that allows the observation and characterization of materials within an mm to nm scale. In most cases, the SEM is connected to an energy-dispersive spectrometer (EDS), so there is the possibility of an elemental analysis showing chemical characteristics of the samples [3]. The FTIR has infrared range of mid-IR. The term "infrared" generally refers to any electro-magnetic radiation falling in the region from 0.7 mm to 1000 mm. However, the region between 2.5 mm and 25 mm (4000 to 400) is the most attractive for chemical analysis[1]. Guava is a medicinal plant and is represented by approximately 120-150 species. Guava is a well-known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. In India, decoction of the leaves and bark of guava is used to cure diarrhea, dysentery, vomiting and sore throats, and to regulate menstrual cycles. Guavas are free from fat and cholesterol. They are also an excellent source of fiber, potassium and vitamin A. It is a native of Central America but is now widely cultivated, distributed and the fruits enrich the diets of millions of people in the tropics of the world[8]. Spinach is a green-leafy vegetable belongs to family *Amaranthaceae.* It is often recognized as one of the functionalfoodsfor its wholesome nutritional, antioxidants and anti-cancer composition. The major micronutrients in spinach are vitamins A (from β-carotene), C, K and folate, and the minerals, calcium, iron and potassium [5].

II. MATERIALS AND METHODS

Collection of samples

Psidiumguajava leaves were collected from Balaji Nursery, Darwali, Paud, Pune. Spinach seeds of "All Green" variety were collected from KrishiBhavan, Seed Research Laboratory, Shivajinagar, Pune-411005.

Preparation of leaf extract

Psidiumguajava leaves weighing 25g separately and were thoroughly washed in distilled water for 5 min, dried, cut into fine pieces and were boiled in a 500 ml Erlenmeyer flask with 100 ml of sterile distilled water up to 15 min and were filtered for the removal of dust [16].

Synthesis of Gold nanoparticles (AuNPs)

1ml of *P.guajava* leaf extract was added to a vigorously stirred 10 ml of 1mM $HAuCl_4$ solution [16].

Synthesis of Zinc oxide nanoparticles

0.02M zinc acetate (50ml) solution was taken and 2ml of *Psidiumguajava* leaf extract was added dropwise and this mixture was stirred for 10mins. The pH of the mixture was maintained at 12 by adding 1M NaOHdropwise. A pale white precipitate resulted which was washed with distilled water 2-3 times followed by ethanol, filtered and dried at 50^0C overnight in oven. Pale white powder of zinc oxide nanoparticles was store for characterization[4].

Foliar spray of *P. guajava*AuNPs and ZnO NPs

Dilutions of 0, 100, 500, and 1000 ppm were prepared. These dilutions were prepared by directly suspending the nanoparticles in deionized water dispersed by ultrasonic vibration (100 W, 40 KHz) for 1 hour. Magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles. After 14 days of sowing, plants were sprayed with the different concentrations of AuNPs and ZnO NPs. The leaves samples were harvested at 45^{th} day of sowing and were further processed for proximate analysis [5].

Morphological analysis

Plant height was measured from the ground level to the growing point and expressed in cms. The numbers of leaves present in each plant were counted [19]. Surface area of spinach leaf (sq. cm) was measured by grid paper method[10].

Biochemical analysis

Sample was extracted by using extraction buffer 0.12gm of the leaf sample was homogenized in 1.5 ml of the buffer contained in chilled mortar and pestle. Then sample was centrifuged at 13,000 rpm for 10 mins at 4^0C and supernatant was used for protein estimation. Protein was estimated by folinlowry method.The amount of protein in the samples were determined fromthe standard graph [15].Carbohydrate estimated by anthrone method and calculated the amount of carbohydrate present in the sample tube from following formula [15];

$$\text{Carbohydrate (mg/100gm)} = \frac{\text{Mg of Glucose}}{\text{Volume of test sample}} \times 100$$

Total chlorophyll was estimated by arnon method. The absorbance of the solution was read at 645 nm and 663 nm against the solvent (80%acetone) blank and calculated total chlorophyll content on following formula [13];

$$\text{Total Chlorophyll (µg/ml): } 20.2(A_{645}) + 8.02(A_{663})$$

Characterization of the synthesized AuNPs and ZnO NPs

UV-Vis spectrophotometry analysis

The sample was observed under UV-Vis spectrophotometer (UV-2450, Shimadzu) for its maximum absorbance and wavelength to confirm the synthesis. Spectral analysis was carried out between 400-800 nm for gold nanoparticles [16] and 300-800 nm for zinc oxide nanoparticles [4].

Scanning electron microscopy (SEM), Energy Dispersive Spectroscopy (EDS)

The microstructure and composite homogeneity of the obtained samples were investigated using SEM-EDS. NPs were centrifuged at 10,000 rpm for 30 min. The pellet was re-dispersed in 10 ml ethanol and washed 3 times with sterile distilled water to obtain the pellet. The pellet was dried in an oven and thin films of dried samples (10 mg/ml) were used for compositional analysis [11][4].

Fourier-Transform Infrared Spectroscopy (FT-IR)

Infrared Spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence an important technique for identification and characterization of a substance. The particles were analyzed under FT-IR for the size conformation [16].

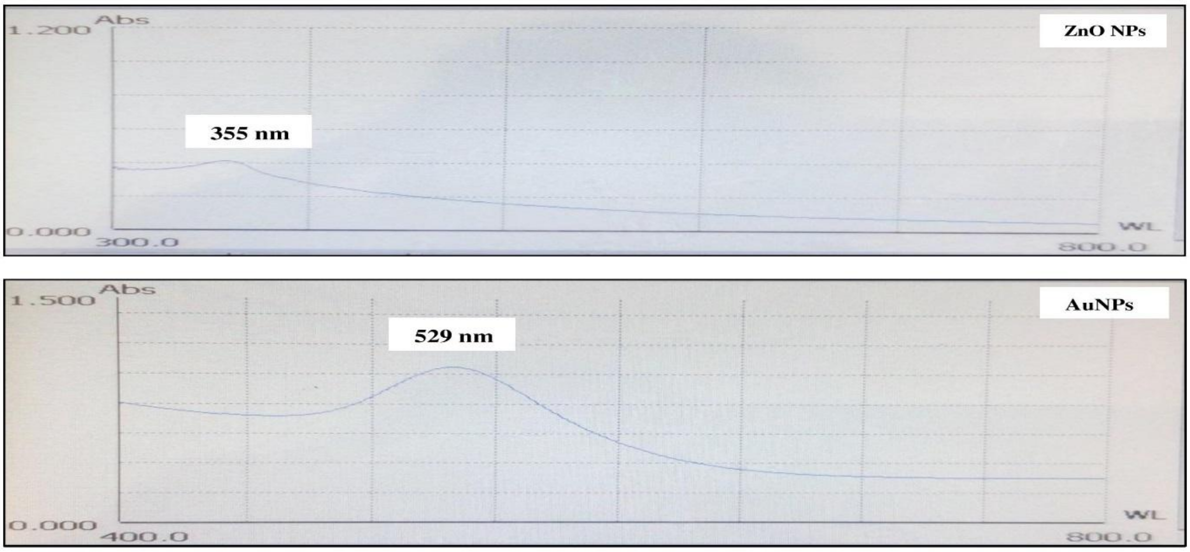
III. RESULTS AND DISCUSSION

Visual detection of AuNPs and ZnO NPs

The *Psidiumguajava* leaf extract sample was found to show change in color from pale yellow to ruby-red indicated synthesis of AuNPs and pale yellow to pale white indicated synthesis of ZnO NPs.

UV-Vis spectrophotometry analysis

UV-Visible spectroscopy carried out for the confirmation of synthesis of nanoparticles. Absorbance was taken between 400-800 nm for gold nanoparticles and 300-800 nm for zinc oxide nanoparticlesin which highest peak was observed at 529 nm which indicated synthesis of gold nanoparticles and another highest peak was observed at 355 nm which indicated synthesis of zinc oxide nanoparticlesfrom *Psidiumguajava*.The graph obtained is shown in graph1.



Graph No. 1 UV-Vis Spectroscopy for *Psidiumguajava*AuNPs andZnO NPs.

SEM-EDS analysis

Scanning Electron Microscopy was carried out for the confirmation of size of gold nanoparticles and zinc oxide nanoparticlessynthesized from *Psidiumguajava*.It was observed that AuNPs were synthesized in range from 36.82 nm to 55.90 nm and ZnO NPs were synthesized in range from 18.79 nm to 27.15 nm.The SEM images obtained are shown in figure 1 and 2.

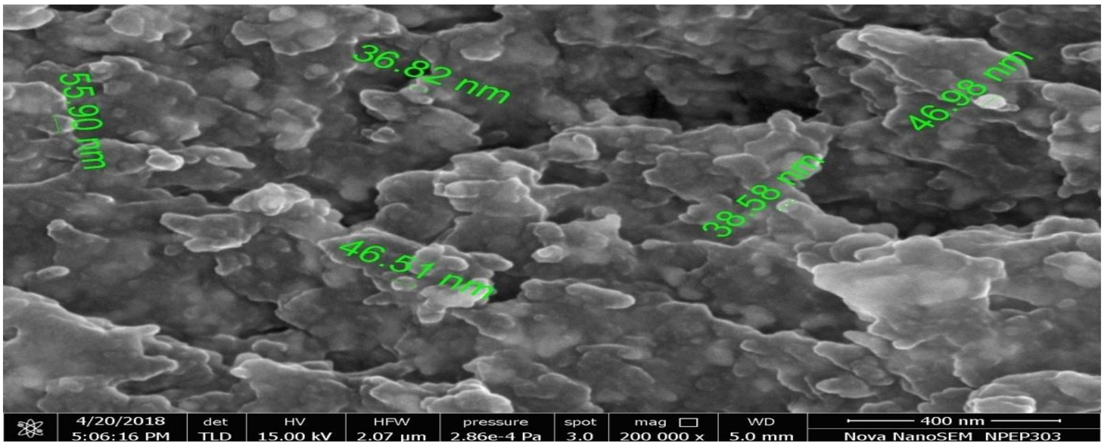


Figure. 1 Image indicating the size and shape of AuNPs

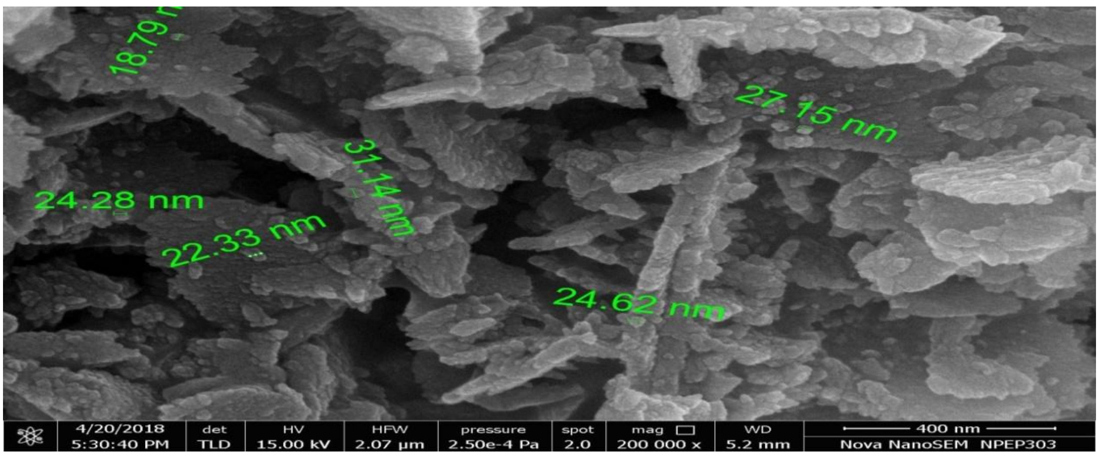
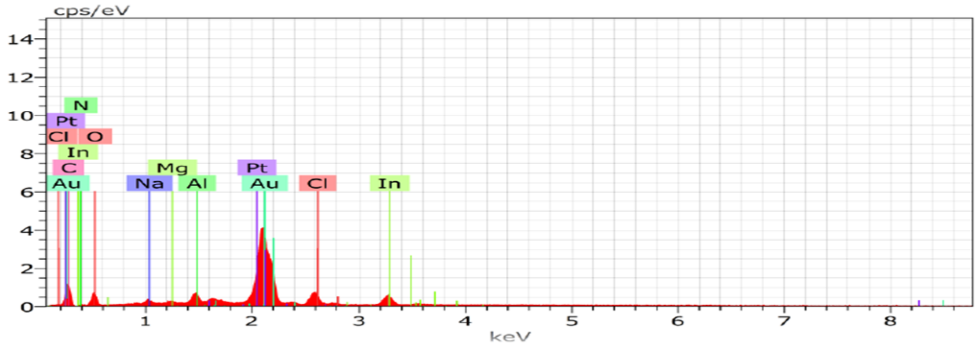
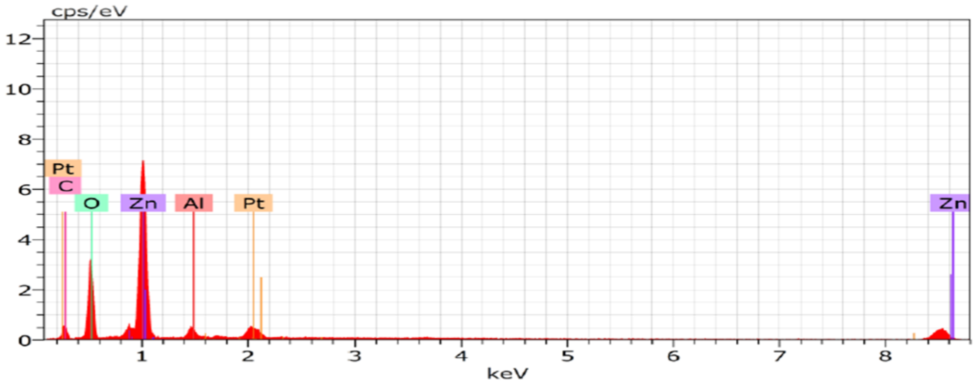


Figure. 2 Image indicating the size and shape ofZnO NPs

In EDS spectra it was confirmed that elemental composition of AuNPs with Nitrogen, Platinum, Oxygen, Magnesium, Aluminium, Chlorine, Indium, Sodium, Carbon elements and ZnO NPs with Platinum, Oxygen, Aluminium and Carbon. From EDS spectra it was observed that weight percent of Au in the prepared sample was 50.16 and atomic percent was 10.03. Atomic percent of carbon was highest i.e. 45.82% and lowest was of sodium i.e. 1.97% and weight percent of Zn in the prepared sample was 42.37 and atomic percent was 58.22. Atomic percent of carbon was highest i.e. 25.20 % and lowest was of aluminium i.e. 3.13 %.The graphs obtained are shown in graph2 and 3.



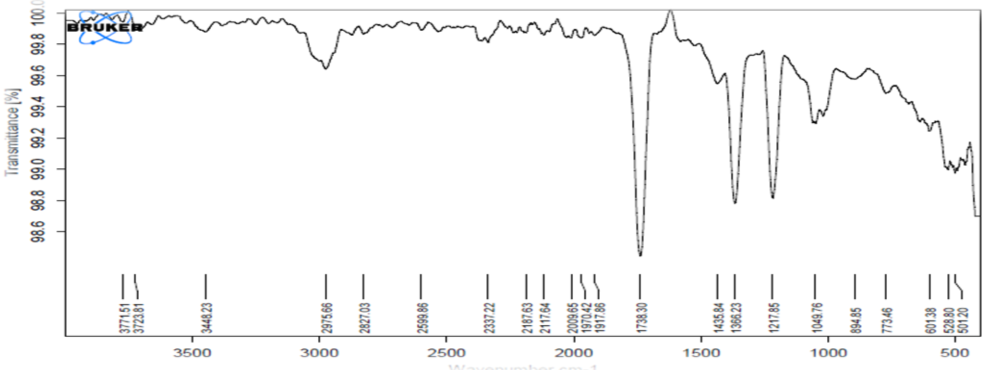
Graph. 2 Image indicating the elemental composition of AuNPs



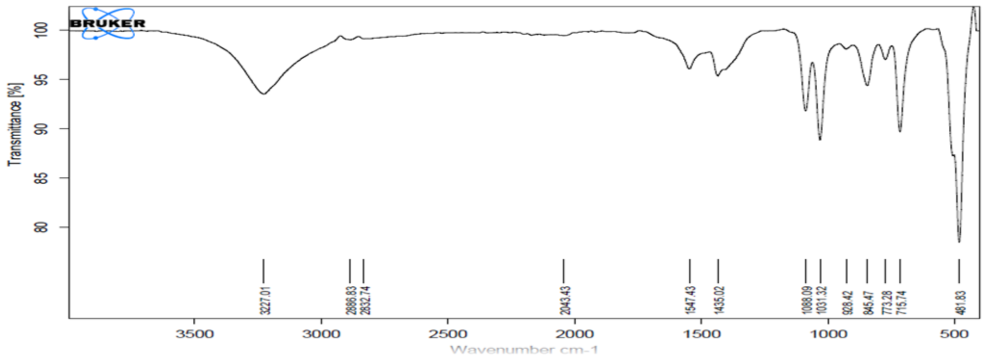
Graph. 3 Image indicating the elemental composition of ZnO NPs

FTIR of *P. guajava* AuNPs and ZnO NPs

FTIR showed the absorption bands at 2975 cm^{-1}, 1738 cm^{-1}, 1366 cm^{-1}, 1217 cm^{-1},1049 cm^{-1} and 601 cm^{-1} which showed methyl, aldehydes, gem-dimethyl, amines, aliphatic phosphates and aliphatic iodo functional groups. These groups were present in *P. guajava* leaf extract which played a major role in reduction and stabilization of Au. The absorption bands at 3227 cm^{-1}, 1547 cm^{-1}, 1435 cm^{-1}, 1031 cm^{-1}, 845 cm^{-1}, 715cm^{-1} and 481 cm^{-1} which showed the signatures of alkynes, secondary amine, carbonate ion, alcohols, aliphatic chloro compounds, methylene and polysulfides functional groups. These functional groups were present in *P. guajava* leaf extract which played a major role in reduction and stabilization of ZnO.The graphs obtained are shown in graph4 and 5.



Graph 4 FT-IR image of the synthesized AuNPs



Graph 5 FT-IR image of the synthesizedZnO NPs

Morphological analysis

Morphological analysis of spinach was carried out after 45 daysof foliar sprayand it was observed the gold and zinc oxide nanoparticles showed positive results, as the concentration increased of NPs increased in height of the plant, number of leaves per plant and surface area of leaf. The highest height of spinach plant was recorded 14 cm at 1000 ppm concentration of AuNPs, than control 9.1 cm and 12.8 cm at 1000 ppm concentration of ZnO NPs than control 7.2 cm. The number of leaves per plant were observed highest (06) at 1000 ppm of AuNPs than control (03) and another highest (04) at 1000 ppm of ZnO NPs than control (03). The surface area of leaves were observed highest (13 cm^{-1}) at 1000 ppm of AuNPs than control (05 cm^{-1}) and another highest (13 cm^{-1}) at 1000 ppm of ZnO NPs than control (4.6 cm^{-1}).

Biochemical analysis

Morphological analysis of spinach was carried out after 45 days of foliar spray and it was observed the gold and zinc oxide nanoparticles showed positive results, as the concentration increased of NPs increased in protein, carbohydrate, and total chlorophylls contents of spinach. Protein content was found to be highest in 1000 ppm concentrations of ZnO NPs which was 0.68 mg/ml and AuNPs which was found to be 0.60 mg/ml. Carbohydrate content was found to be highest in 1000 ppm concentrations of AuNPs which was 1.31 mg/ml and ZnO NPs which was found to be 0.96 mg/ml. Total chlorophylls content was found to be highest in 1000 ppm concentrations of ZnO NPs which was 17.9 µg/ml and AuNPs which was found to be 11 µg/ml. The morphological and biochemical characters obtained are shown in table 1.

Table No. 1 Morphological and Biochemical analysis with AuNPs and ZnO NPs

NPs	Conc. of NPs	Morphological characters			Biochemical characters		
		Height (cm)	No. of leaves	Surface are(cm^{-1})	Proteins (mg/ml)	Carbohydrates (mg/ml)	Total chlorophylls (µg/ml)
AuNPs	0 ppm	9.1	3±1	5	0.52	0.64	8.8
	100 ppm	11.5	5±1	7.6	0.53	0.66	9.0
	500 ppm	11.8	5±1	7.6	0.54	0.80	9.4
	1000 ppm	14	6±1	13	0.60	1.31	11
ZnO NPs	0 ppm	7.2	3±1	4.6	0.60	0.71	11.3
	100 ppm	11.5	4±1	8.6	0.61	0.81	11.8
	500 ppm	11.5	3±1	10.6	0.66	0.94	14.1
	1000 ppm	12.8	4±1	13	0.68	0.96	17.9

Hence, present study concluded that the zinc oxide nanoparticles showed the more effective results than the gold nanoparticles on morphological and nutritional content of spinach.So, it is recommended to use of zinc oxide nanoparticles which are cost-effective and beneficial to the growers than the gold nanoparticles.

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