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NUTRACEUTICAL PROFILING OF WHEAT SEEDLING AFTER SEEDS PRIMING TREATMENT OF NANO FERTILIZER BIO RECOVERED FROM ORE TAILING

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Abstract

Background: Ore Tailing, a rich source of minerals stimulates researchers to microbially recover valuable metals from this toxic waste to usable form such as nano fertilizer in an eco-friendly manner. application of nano fertilizer (NF) as macro / or micro nutrients resulted in slow and feasible nutrient release with maximum utilization of nutrient resulting in higher production of food healthy for man in comparison to the commercial fertilizer

Objective: This study was carried out to explore the potential benefits of biosynthesized valuable nano fertilizer bio recovered from ore tailing to produce antioxidant rich crop of wheat after seed priming treatment.

Methodology: Ore tailing was procured and pretreated. *A. niger* strain was collected, purified and used to bioleach metals from ore tailing under optimized conditions according to orthogonal array design. Synthesis of bio recovered nanoparticles were characterized using atomic absorption spectroscopy (AAS), Ultraviolet-visible spectroscopy (UV/VIS) and Zeta sizer analysis (DLS). BNPs with various concentrations were evaluated for their efficacy on seed germination and antioxidant potential of wheat (*Triticum aestivum* L.) by seed priming technique.

Results: *Aspergillus niger* metabolites exhibited good potential for metal ions solubilization and conversion into nano formulation. Seedlings displayed pronounced seed germination (100 % ± 0.00) and seedling growth in terms of root and shoot length (8.31 ± 0.18 &7.46 ± 0.15) with the outcome of phenolics (95.4 ± 0.95) and flavonoids (42.48 ± 0.4) in the seeds treated with 1.00 ppm of biosynthesized nanoparticles. Minimum growth was observed in water as control representing germination % (96.48 ± 0.13) and seedling growth (4.36 & 4.81 cm) with the exhibition of phenolics and flavonoids (59.46 ±1.15.&19.7 ± 0.23).

Conclusion: BNP has stimulatory effect on wheat seeds as progressive increase in their germination and antioxidant capacity were observed. Biosynthesized nanoparticles could be an initiative for the development of nano-biofertilizers of potential importance in nutritional attributes.

Keywords: Bio recovery; biosynthesized nanoparticles; seed priming; wheat, antioxidants

INTRODUCTION:

The rapid growth in population and increasing demand for resources resulted in increased generation of waste by excessive dumping of tailings and inappropriate handling of waste **(1).** Tailings are commonly stored in deposits surrounded by constructing tailing dams. The materials used for constructing tailing dams may be geological burrow material or tailings waste **(2).** Due to the toxicity, tailings pose a number of risks on communities and the surrounding environment if not managed properly. In addition, the bulk quantities in which they are produced put extra pressure on managing such waste. Bioleaching is considered as a special hydrometallurgical process in which metals are extracted with the help of biological organisms such as bacteria, fungi, and yeasts, from their ores and mineral compounds. Recently, this method has been used to treat industrial and municipal wastes, as well as to recover important metals from low-grade ores and concentrates. Myco nano mining is considered as a safe and eco-friendly technique for the conversion of metal based bulk material into nanoparticle form **(3).** There are many reports in which fungi are being used as agents for bioleaching to dissolve valuable metals from the tailing waste **(4, 5).** The capability of these microorganisms to withstand high metal concentrations and transform waste metals into nanoparticles form have motivated the scientists to utilized them for the biosynthesis of nanoparticles as eco-friendly nano factories **(6, 7).**

Nanoparticles are materials which range from 1-100 nm in size **(8).** Increasing use of nanotechnology in every field of life necessitates finding their potential to enhance food production in order to fulfill the needs of growing population. Traditionally, nanoparticles were produced by various physical and chemical methods. However, these methods were costly, complicated, out dated and threat to environment as produces hazardous toxic waste which is harmful to environment as well as humans **(9).** Plants, algae, fungi, and yeast, which are made up of biomolecules, act as a large "bio-laboratory" in nature. These naturally occurring biomolecules have been exploited to play a key role in the production of nanoparticles of various shapes and sizes. As a result, it acts as a driving factor in the development of safer, greener, and ecologically friendly nanoparticle production techniques. In the recent years, fungi have been used in the biosynthesis of nanoparticles referred as myco-nanotechnology which has a substantial potential and great demand due to economic viability and ease in handling biomass **(10).** Nanotechnology has the potential to improve agriculture by increasing quality food production in eco-friendly manner and because of their small size, vast surface area, and slow-release rate, nanoparticles may help plants to enhance their nutrient usage efficiency which facilitate the plant to absorb most of the nutrients without any waste **(11).**

In the field of agriculture and horticulture, nanoparticle applications play a comprehensive role in maintaining healthy growth of plants, thus health food for man. Iron is considered as an essential micronutrient for crops. Plant roots absorbed iron in the form of Fe^{+2} and Fe^{+3}. Fe is the major component of cytochrome, porphyrin molecules, leg-hemoglobin, Fe-S protein and heme protein. It is also involved in the process of respiration and photosynthesis, in chlorophyll biosynthesis, as catalytic agents and for some enzyme activity such as nitrogenase. Adequate range of Fe in plant is 50-250 ppm and below 50 ppm, symptoms of deficiency appears on young leaves as interveinal chlorosis and leaves turn completely white and necrotic in severe conditions **(12).** Iron increases the nutrient absorption and photosynthetic efficiency of plant therefore, can be used as fertilizers **(13).** In modern agriculture, nanotechnology proves to exhibit tremendous potential in resolving nutrient shortages and leaching losses **(14).** It was revealed by various researchers that the application of nano fertilizer (NF) as macro / or micro nutrients resulted in slow and feasible nutrient release with maximum utilization of nutrient resulting in higher production of food healthy

for man in comparison to the commercial fertilizer (CF) **(13).** The potential benefits of phenolic substances for human health have gained a lot of attention. It has been shown that phenolic contents exhibited antioxidant, antiviral, antiallergic, antiplatelet, and anti-inflammatory properties. Because phenolic have the capacity to chelate metals, inhibit lipoxygenase and scavenge free radicals, their antioxidant behavior may be linked to their bioactivity **(15).**
Wheat is considered as one of the most important staple food crop of world. Production of wheat is a difficult trait as it is controlled by various (genetic, management and environmental) factors. In order to increase the valued production of wheat crop, new, cheaper, and efficient target oriented technologies have to be employed **(16).** Flavonoids are considered as phytochemical compounds which are present in many fruits, vegetables, plants and leaves and have widespread applications in medicinal chemistry. Flavonoids exhibited many medicinal benefits like antioxidant, anticancer, antiviral and anti-inflammatory agent. Researchers have suggested that a diet rich in flavonoids reduces the risk of diabetes, cardiovascular diseases and some form of cancer as they have cardio protective and neuroprotective effect on human health **(17).**
In this regard nanotechnology may offer incredible potential to increase agricultural yield in order to achieve food requirements of humans. The technique of seed priming has been used in agriculture in recent years to increase seed quality, which affects crop productivity even in adverse conditions **(18).** It has been reported that priming treatment significantly increases the rate of seed emergence as well as germination by triggering the metabolic activities generally activated during the early phases of germination. This treatment helps the seeds to withstand under different abiotic stresses. However, for different crop species, the priming solution may have variable qualities and efficiency **(19).** It has been reported that various metal-based (Cu, Fe, Zn, TiO_2, Au and Ag) nanoparticles have been applied as seed priming agent that resulted in the production of nutrient rich crop **(20).** In recent reports, wheat seeds when primed with multi walled carbon nanotubes not only enhance the early germination and root hairs growth but also higher yield of wheat with rich antioxidant potential **(21).**

To fulfill the ever-increasing food demand of an expanding population, agricultural production must be increased on a regular basis. Fertilizer consumption is increasing day by day, posing a threat to human health as well as environment. Producing more and better food in an environmentally sustainable manner is one of the century's most difficult challenges. Agriculture must be supplemented with innovative science-based technology in order to improve productivity in a resource-efficient manner. Current study was carried out to explore the potential benefits of biosynthesized valuable nano fertilizer bio recovered from ore tailing to produce antioxidant rich crop of wheat after seed priming treatment.

MATERIALS AND METHOD:

Study Design:

The research work was performed in the Natural product and synthetic chemistry (NPSC) Laboratory, Department of Chemistry, Government College University, Faisalabad, Pakistan.

Plant Collection and Authentication:

Seeds of wheat (*Triticum aestivum* L) variety Faisalabad 2008 were used in this study and were collected from Ayub Agricultural Research Institute (AARI) Jhang Road, Faisalabad, Pakistan. The seeds were identified and verified through authentication at the Government college university, department of Botany, Faisalabad.

Chemicals and Reagents:

All the chemicals were purchased from Sigma-Aldrich Industries Ltd. Chemicals include: Folin-Ciocalteu’s reagent, NaOH, $AlCl_3$, $NaNO_2$, Na_2CO_3, PDA. Before usage, potato dextrose agar (PDA) was autoclaved at 121^0 C for 15 min at 15 psi for sterilization.

Collection, pretreatment and metallogeny of ore tailing:
Ore tailing was procured from the Chakwal Mineralogical Center, Pakistan. Ore sample was pretreated by drying in an oven at 103^0C and ground to 200 mesh size. After digestion and filtration, the sample was subjected to elemental analysis by atomic absorption spectroscopy (AAS) Model (Perkin Elmer, A Analyst300) **(22).**

Fungal growth, bioleaching and biosynthesis of nano fertilizer from ore tailing:
A. niger strain was collected from microbial laboratory, Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. Fungal strain was purified on slants of Potato Dextrose Agar (PDA 3.9 % w/v) and multiplied in liquid media (Vogel's media) after incubation at 30^oC for three days in growth chamber (Incubator, Sanyo, Germany). Liquid culture was further enriched and used to bioleach metals from ore tailing under optimized conditions such as pH (5.5), substrate concentration (5%), temperature (30°C), pulp density (10%) and agitation speed (160 rpm) according to orthogonal array design **(22).** Bioleached metals were further converted into nano fertilizer **(21)**

Characterization of biosynthesized nano fertilizer:
Different characterization methods, such as Atomic absorption spectroscopy (AAS), Ultraviolet-visible spectroscopy (UV/VIS) and Zeta sizer (DLS) were used to analyze biosynthesized nano fertilizer.

Analysis by Atomic absorption spectroscopy (AAS):
A flame atomic absorption spectrometer (AAS) with PerkinElmer type analyzer was used for the determination of total metals content such as iron, zinc, copper and nickel present in the ore tailing. Initially, a blank and standard solution was prepared with 1.00% nitric acid (HNO_3) for AAS analysis. All the samples were diluted from 0.05 mg/L to 3mg/L to set the measuring limit of AAS and then distilled water was added to it with 1.00% nitric acid (HNO_3). AAS analysis was performed in triplicates for each sample within 15 seconds time intervals and mean value was calculated **(23).**

Analysis by UV-visible spectroscopy Technique:
It is a general and most commonly used methodology for the characterization of raw ore sample and biosynthesized nano fertilizer. The metals in raw sample and formation of bioleachate nano metals were monitored for spectrophotometric measurements using UV-visible absorption spectroscopy in order to confirm the formation of biosynthesized nano fertilizer at a resolution of 1 nm. The absorbance spectrum of the sample was obtained at the wavelength range of 190-800 nm using a UV-visible spectrometer with distilled water as a reference in order to study the absorption spectra of nanoparticles as well as raw sample **(24).**

Analysis by Dynamic Light Scattering (DLS):
Size distribution analysis of biosynthesized nano fertilizer was monitored by Zeta sizer using Dynamic light scattering (DLS) technique. In this method, particle size of nanoparticles was determined by measuring the random changes in the intensity of light scattered from a solution. Samples were prepared by dilution of 1 mL of the nano formulated suspension with distilled water to determine the particle size of the sample U-type tube at 25°C, using a zeta sizer (3000HS Malvern Instruments, UK) **(25).**

Antimicrobial activity of nano fertilizer:
Antibacterial activity of biosynthesized fertilizer was tested against two bacterial strains (*Escherichia coli*) and (*Staphylococcus aureus*) by well diffusion method **(26).**

Cytotoxic analysis:
Hemolytic (RBCs) lysis activity of biosynthesized nano fertilizer was analyzed by the protocol described by Kuzmin *et al.* **(27)** with little modifications.

Preparation of solutions for riming treatment:
Biosynthesized metallic nano formulations were used as a soaking agent for wheat seeds as a priming material for seeds pre-germination in petri plates. All the treatments were applied in different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 ppm) along with water as control on wheat (*T. aestivum*.) seeds.

In vitro germination of seeds:
The experiment consisted of various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 ppm) of bio recovered metallic nano formulations and water. Five petri dishes were taken for each treatment having diameter of 9 mm in three replicates and labeled as 0.2, 0.4, 0.6, 0.8, 1.0 ppm and control. A double layer of Whatsmann filter paper No 1 was used in each petri dish and ten seeds of wheat were sown in 10 mL of bio recovered metallic nano fertilizer and water. All the petri dishes were kept at room temperature in the laboratory.

Measurement of physiological parameters in wheat seedling:
Germination of wheat seeds were observed after the emergence of the radical during the first 48 h of growth in control and treated solution. Seeds having coleoptile longer than 2mm were considered to be germinated and their germination percentage was calculated by the formula given below:

$$\text{Germination percentage} = \frac{\text{Number of seed germinated}}{\text{Total number of seeds}} \times 100$$

Shoot and root length:
On the seventh day following priming treatment, the seedlings in petri dishes were harvested, and with the use of a ruler, the root and shoot length of seedlings that had been growing for one week was measured in centimeters.

Measurement of non-enzymatic antioxidant activities of wheat seedling:
Non-enzymatic antioxidant activities of wheat seedlings were calculated by various methods given as; Determination of TPC (Total phenolic contents).
Determination of TFC (Total flavonoid contents).

Total Phenolic contents (TPC):
The number of Total phenols in wheat seedlings was determined following the Folin-Ciocalteu (FC) calorimetric method of Petropoulos *et al*. **(28).** 200 µL of sample were taken in test tubes.1.0mL of Folin-Ciocalteu's reagent and 0.8 mL of 7.5 % $NaCO_3$ was added in test tube. The test tubes were mixed thoroughly and allowed to stand for 30 min. The absorbance was noted after 1 hour at 765nm (Perkin-Elmer λ15 UV-Vis spectrophotometer, Norwalk, CT) and the calibration curve was plotted by taking absorbance as a function of concentration. Total content of phenolic compounds in plant extracts in gallic acid equivalents (GAE) were calculated by the following formula.

$$\mathbf{T = C \times V / M}$$

Were
T = total contents of phenolic compound in mg GAE/g plant extract.
C = concentration of gallic acid calculated from calibration curve in mg/mL
V = volume of plant extract in mL
M = weight of plant extract in grams.

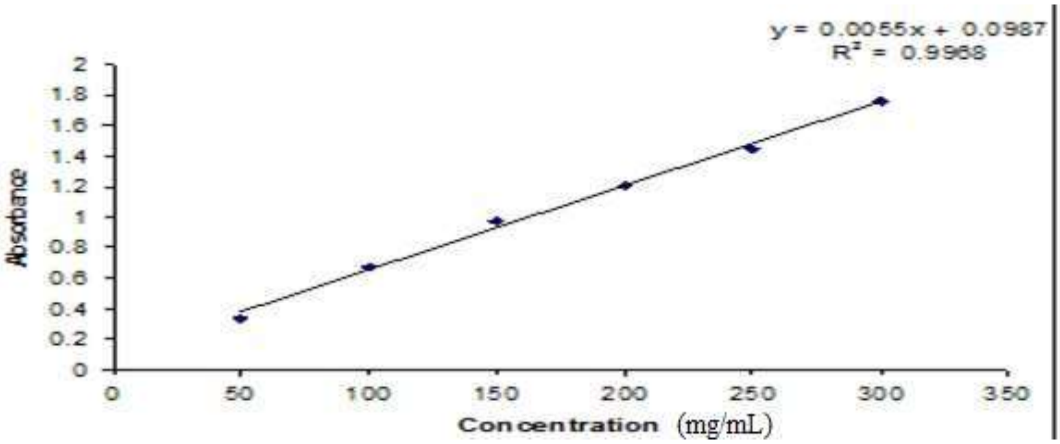


Fig 1: Gallic acid calibration curve for the determination of TPC

Total Flavonoid Contents (TFC):

Total flavonoid contents (TFC) of wheat seedlings were determined according to the method reported by Liu *et al.* **(29).**1 mL of plant extract was mixed with 2 mL of deionized water, 0.5 mL of 5 % $NaNo_2$ solution and 0.5 mL of 10% $AlCl_3$ and incubate the reaction mixture for 5- 6 minutes at 37°C.Then add 2.5 mL of (1M) NaOH solution and further incubated for 15 min. Total volume of the reaction mixture was made up to 9 mL by the addition of deionized water. Absorbance of the reaction mixture was noted at 510 nm. Total flavonoid contents (TFC) of the wheat seedlings were expressed as catechin equivalents from the linear regression curve of catechin.

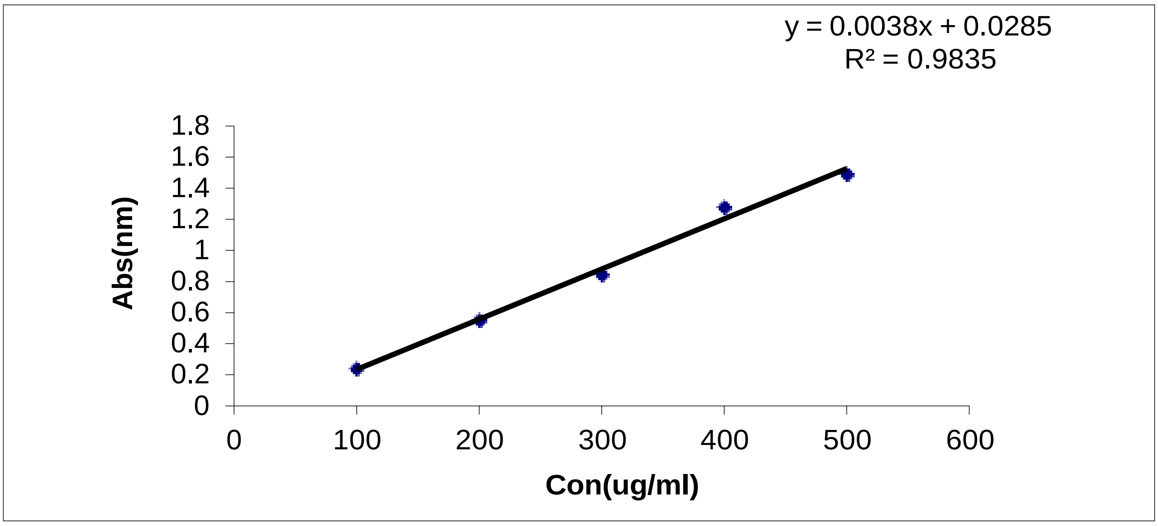


Fig 2: Calibration curve of catechin for determination of TFC

Statistical analysis:

Each treatment was conducted with three replicates, and the results were presented as mean standard deviation. The experimental data were statistically evaluated by one-way analysis of variance (ANOVA) utilized the SPSS 20 and Excel software and the mean values for each treatment were compared at $P < 0.05$ confidence level. Each of the experimental values was compared to its corresponding control.

RESULTS AND DISCUSSION:

Fungal growth, bioleaching and biosynthesis of metals nanoparticles from ore tailing:

In petri plates, dark colored beads of *A. niger* of variable sizes were found in large number just after 20 hours of incubation and further multiplied in liquid media (Vogel's media).

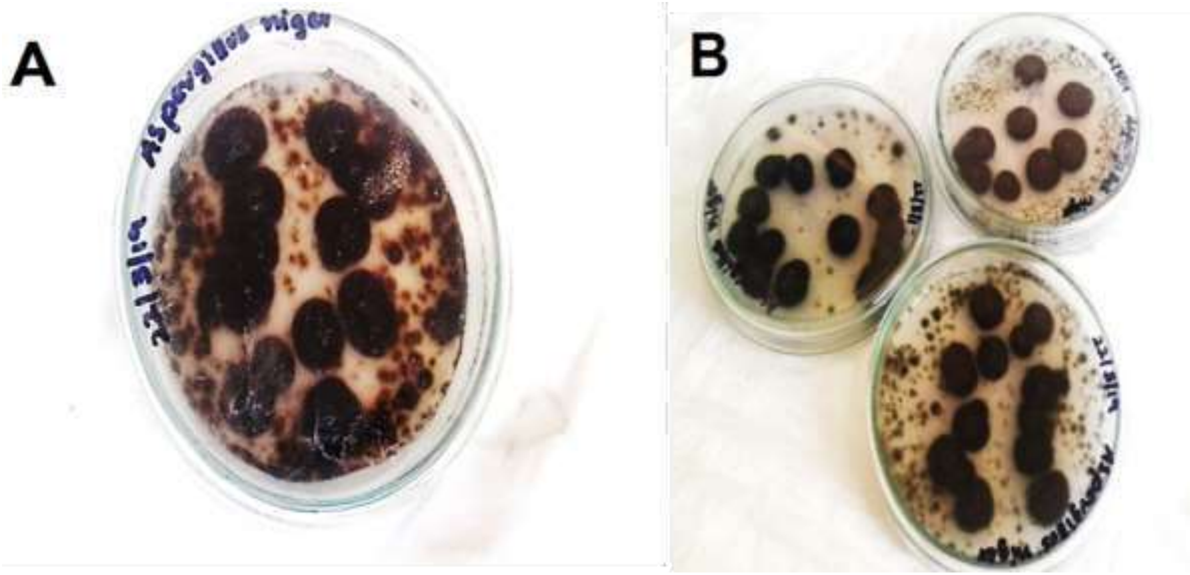


Fig 3: (A, B) Colony morphology of *Aspergillus niger* on solid media slants of PDA.

Elemental analysis of raw as well as bioleached sample:

The metal ion contents of raw as well as bioleached sample were analyzed by the technique of atomic absorption (AAS). Results are displayed in Table 1.

Liquid culture was further enriched and used to bioleach metals from ore tailing under optimized conditions such as pH (5.5), substrate concentration (5%), temperature (30°C), pulp density (10%) and agitation speed (160 rpm) according to orthogonal array design. Under the optimum conditions, percentage of bio recovered metal ions in cell-free extract of *A. niger* were Fe (80.89 %), Cu (79.47 %), Zn (68.97 %) and Ni (42.10 %) as analyzed by atomic absorption spectroscopy (AAS). The bio leachate from the reaction mixture was collected into new flask and stirred at 35°Cfor 3 h for the complete nucleation of metals with fungal strain secretome followed by the addition of liquid ammonia (pH 8) to precipitate nanoparticles. The biosynthesized metal nanoparticles were harvested using centrifugation at 5000 rpm for 10 min followed by washing three times with sterile distilled water. The characteristics change of the mixture color to brown color confirmed the biosynthesis of metal nanoparticles. The dried bio recovered nanoparticles powder was stored in an air-tight bottle at room temperature under dry and dark conditions for further use.

Table1: Elemental analysis of ore tailing and bio recovered metals Results are presented as mean ± SD of samples measured in triplicates.

Metal	Concentration (mg/Kg)	Bio recovered metals under optimum conditions (mg/Kg)	Percentage of bio recovered metals (%)
Iron	247.22 ± 2.03	200 ±2.0	80.89 %
Copper	59.53 ± 1.24	47.31 ± 1.0	79.47 %
Zinc	5.77 ±0.31	3.98 ± 0.15	68.97 %
Nickel	0.76 ±0.01	0.32 ± 0.01	42.10 %

It was investigated that iron ore tailing contain heavy metals including iron, copper, zinc and nickel. The most abundant metal was iron (247.22 mg/kg) followed by copper and zinc (59.53 and 5.77 mg/kg respectively). Whereas, nickel was present in trace amount (0.76 mg/kg). Current results showed higher iron proportionality as compared to other metals. In consistent to our results, Mulligan *et al*. **(30)** reported similar trend of heavy metals in low grade ore in which metal such as iron was detected as the most abundant metal followed by copper and zinc whereas nickel was found in trace amount (7245, 26470, 201 and 27 mg/kg respectively). Similarly, Anjum *et al***. (22)** reported that black shale as low grade ore mainly composed of iron (3761), copper (39.1), zinc (31.9) and nickel (8.3) mg/kg. Heterogeneity was also observed when data was compared with other investigators as Ilyas *et al.* **(31)** who reported the composition of mine tailing as Fe (18375 mg/kg), Ni (42 mg/kg), Zn (25.5 mg/kg), Cu (20.1 mg/kg), Mg (20 mg/kg) and Co (15.3 mg/kg) respectively. In another study, Bedi et al. **(3)** investigated the chemical composition of iron ore tailing and reported that iron (41,191 mg/kg) was the most abundant metal found in ore tailing followed by aluminum (34,530 mg/kg), silica (12,316 mg/kg), manganese (9614 mg/kg) and zinc (435 mg/kg). Nickel (3.78 mg/kg) was also present in minor concentration. This slight variation in the metal contents compared with other investigation might be caused by a number of physical factors, such as temperature, sampling site and various other environmental factors as reported by **(3).**

Characterization of Biosynthesized nanoparticles:

UV-visible spectroscopy:

Fig 4 represents the UV-visible absorption spectra of biosynthesized nanoparticles showing characteristics peaks at 238 nm and 265 nm which might be attributed due to the excitation of surface plasmon vibrations of iron nanoparticles present in biosynthesized nanoparticles. The size of nanoparticles was confirmed by analyzing sample on zeta sizer and it was found in the range of 50-1000nm.

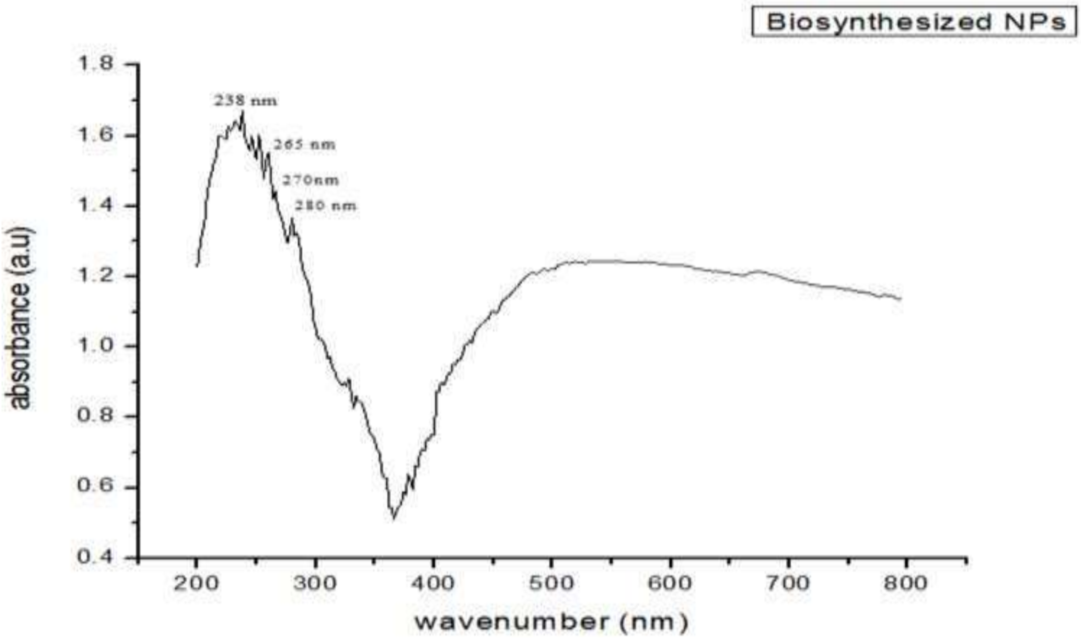


Fig 4: UV-spectra of biosynthesized nano fertilizer from cell free extract of *A. niger*

These results are in accordance to the findings of Mohamed *et al.* **(32)** who reported identical peaks of biosynthesized iron nano fertilizer at 238 nm and 265 using fungus *Alternaria alternate.* The peaks between 270-280 nm represents aromatic acids such as tyrosine, tryptophan and phenylalanine that was used for end capping of nanoparticles in the fungal metabolites as proteins and peptides in the residues might be released by the fungus under stress conditions during the bioleaching period and some of these proteins may bound on the surface of nanoparticles **(33).** Similarly, Sytar *et al.* **(34)** investigated nearly peaks of iron oxide NPs at 230 nm and 272 nm.

Toxicological evaluation of biosynthesized nano fertilizer:

For the assessment of nanoparticles as novel therapeutic agents, cytotoxic screening is central requirement. In this regard, biosynthesized nanoparticles have been assessed for their cytotoxic screening through hemolytic assay.

Cytotoxicity/ Hemolytic assay:

In vitro hemolytic assay of biosynthesized nanoparticles was performed to assess the nonspecific cytotoxicity of the test samples. This procedure allows investigating the potential of nanoparticles to disrupt the membrane of red blood cells. Destruction of erythrocytes membrane resulted in significant increase in the absorbance of reaction mixture due to the increase in release of hemoglobin. (PBS) was used as positive control which shows no hemolytic activity. Whereas, Triton-X-100 was used as reference standard (negative control) showing 90% of the lysis of human red blood cells. Fig 5 indicated that biosynthesized nano fertilizer showed lysis of erythrocytes less than 20% and were considered safe for human use. The process of hemolysis may occurs due to the destruction of red blood cells (major target of free radicals) membrane activated by free radicals **(35).**

Biosynthesized nano fertilizer exhibited very low hemolytic activity which may be due to the presence of several flavonoids and phenolics as flavonoids have been reported for their capability to prevent lipid peroxidation present in erythrocyte membrane and increase the integrity of membrane against lyses by attaching to it **(36).** Similarly, phenolic compounds have also been reported for causing interruption in the spreading of free radicals and consequently decreasing or breaking the free radicals chain reaction **(37).**

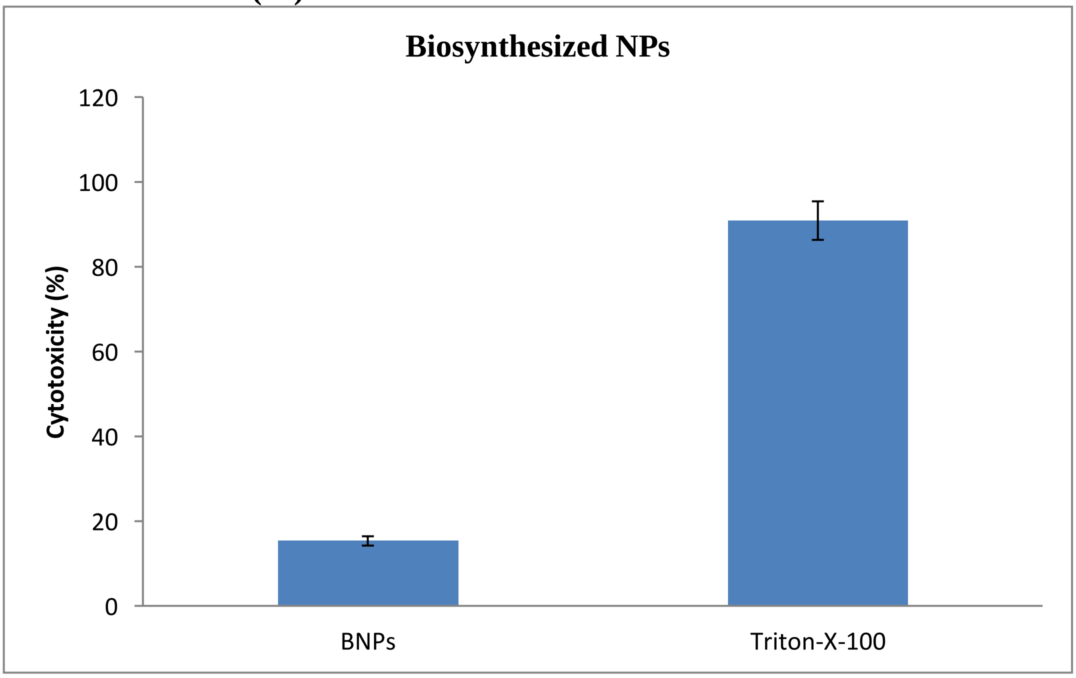


Fig 5: Graphical representation of cytotoxicity of biosynthesized NPs

Antibacterial activity of biosynthesized nanoparticles against *E.coli* and *S.aureus*:

The antibacterial potential of biosynthesized nanoparticles were investigated against two pathogenic bacterial strains i.e, Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli)* by using agar well diffusion method which is considered as the most common method for the analysis of antimicrobial activity. Ciprofloxacin was used as positive control in this method. The zone of inhibition (ZOI) exhibited by nanoparticles were measured in terms of diameter (mm) and their results are tabulated in Table. The synergistic effect of biosynthesized nanoparticles was tested against two pathogenic bacteria and showed strong antibacterial potential against both the tested pathogens. It was evaluated that biosynthesized nanoparticles showed maximum zone of inhibition against *S.aureus* (16 mm) and (12 mm) against *E. coli*. This pronounced antibacterial activity of biosynthesized nanoparticles might be due to the interaction between bacterial cell membrane and nanoparticles. The constituents of nanoparticles may causes changes in the membrane of bacterial cells by taking up their ions which in turn hinders the respiratory enzymes and facilitates the formation of reactive oxygen species (ROS) which damage cells resulting in cell death.

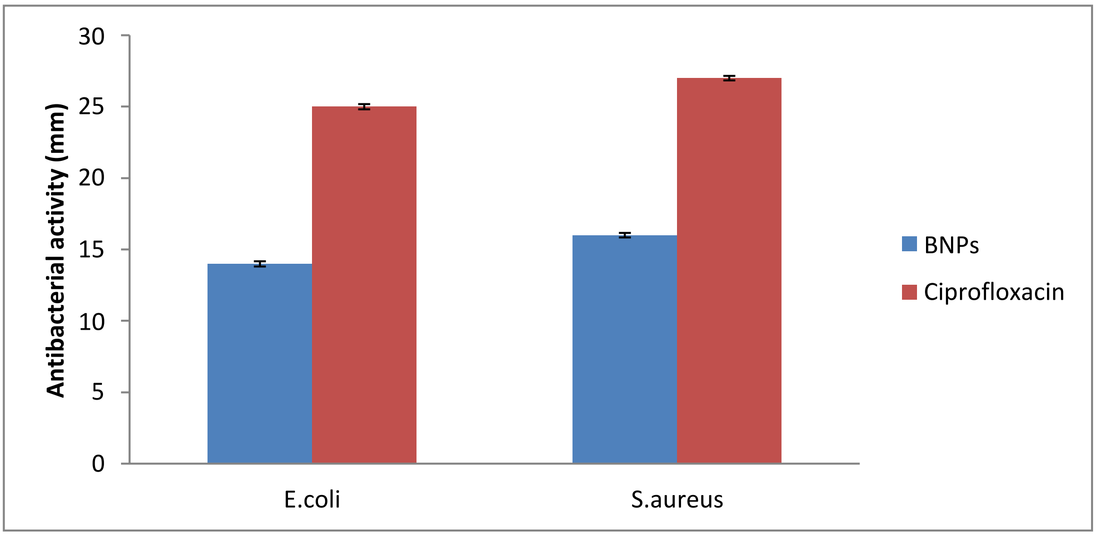
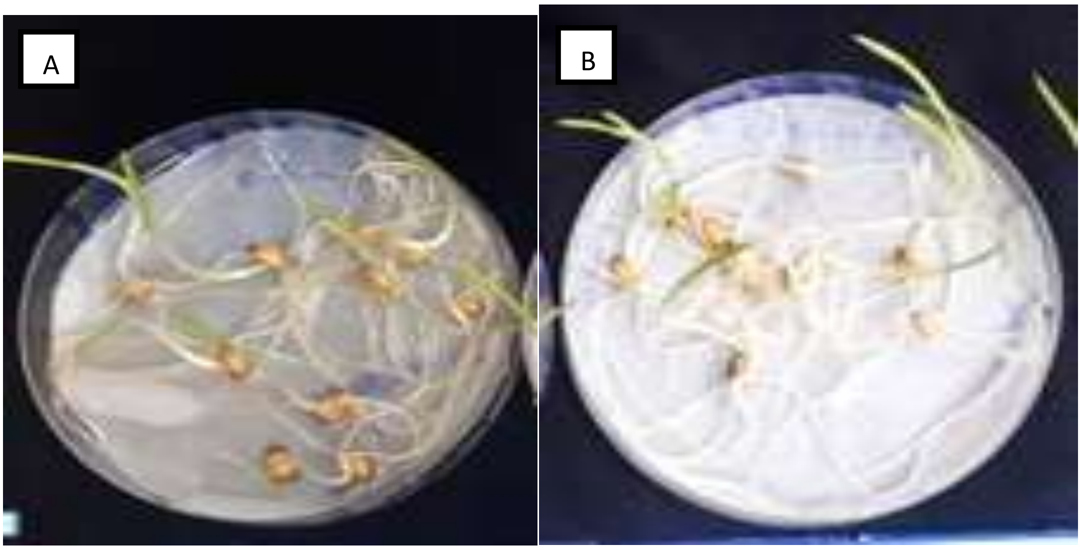


Fig 6: Antibacterial activity of biosynthesized NPs against *E.coli* and *S.aureus*.

Effect of various concentrations of biosynthesized nano fertilizer on seed priming:

The present research tried to evaluate the effect of different dosages of biosynthesized nanoparticles on wheat seed germination and seedling growth by the technique of seed priming as shown in Fig 7 (A-F). Results demonstrated that priming treatment with various doses of biosynthesized nanoparticles before germination significantly improves germination rate and root and shoot growth at all concentrations as compared to water. It was evaluated that seed priming effect was strongest at 1.00 ppm concentrations of biosynthesized nanoparticles. This pronounced effect might be due to the presence of nano Fe, Cu and Zn as nutritive elements present in biosynthesized nanoparticles. All these nanoparticles are essential for plant as Fe is involved in photosynthetic process, RNA synthesis and activates many enzymes, Zn plays an essential role in various biochemical reaction of plants such as formation of carbohydrates and chlorophyll. Cu in trace amount is a component of regulatory proteins and is also involved in electron transport in photosynthesis and respiratory chains **(38).** Fig 7 has shown that the seed priming impact grew as the seedling growth stage expanded. It was further investigated that germination percentage, root length, and shoot length was significantly increased with the increased administration of bio recovered nanoparticles in comparison to water.



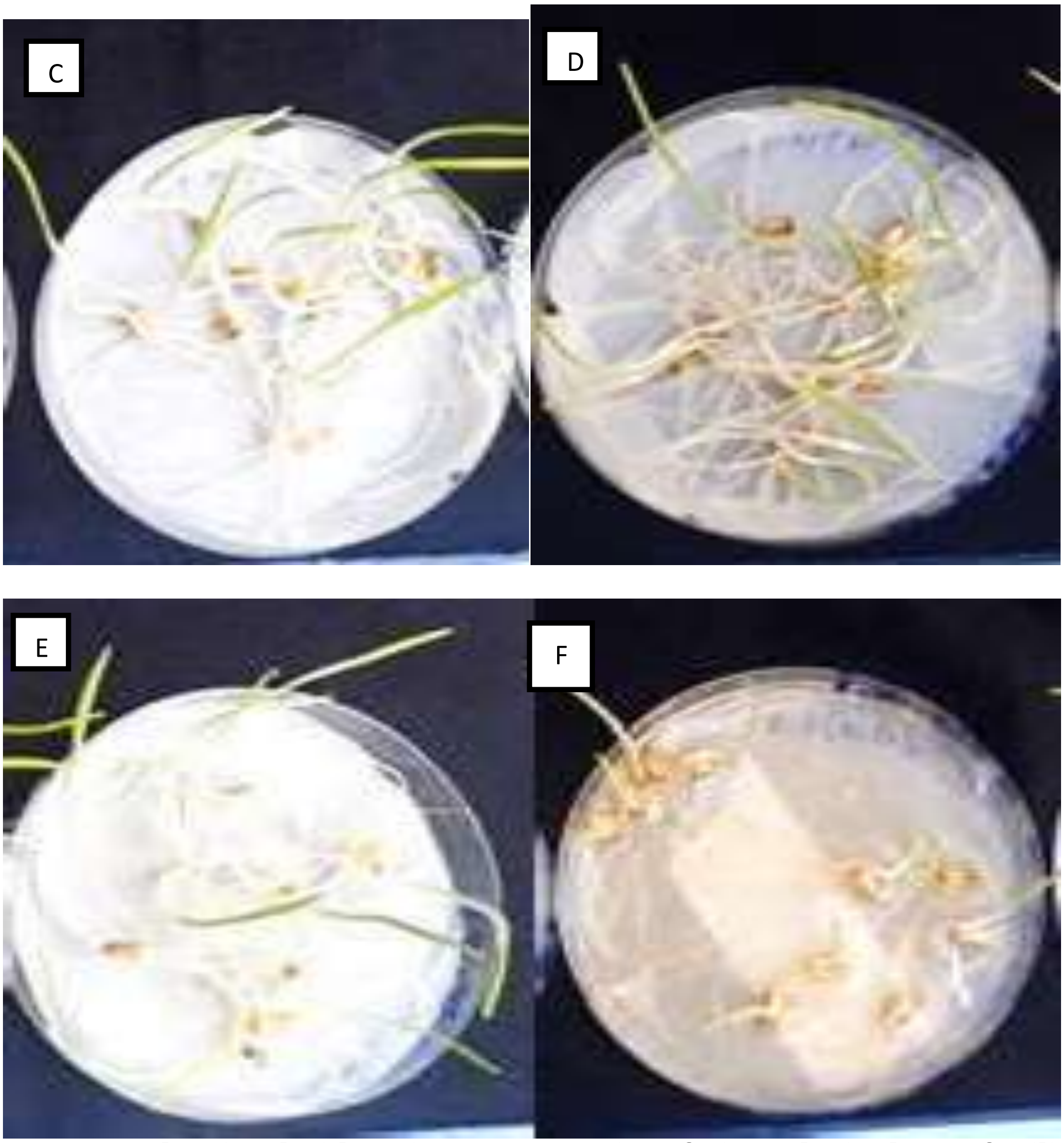


Fig 7: (A - F). Seeds primed with various concentration of Biosynthesized nano fertilizer (A) 0.2ppm, (B) 0.4ppm, (C) 0.6 ppm, (D) 0.8 ppm (E) 1.00 ppm and (F) water.

Effect of biosynthesized nano fertilizer concentration on germination % of wheat seedlings:
The results indicated that wheat seeds when exposed to various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 ppm) of biosynthesized nanoparticles had a positive effect on germination % as compared to water. All the treatments of biosynthesized nanoparticles showed pronounced effect on germination percentage and seedling growth as shown in Fig 8. All the treatments of biosynthesized nanoparticles showed pronounced effect on germination percentage and seedling growth as seeds primed with 1.00 ppm of biosynthesized nanoparticles had the highest germination % (100 %). However, seeds that were sown in water (control) showed the least amount of germination (96.48%). The reason for increase in germination could be due to the fact that nanoparticles may form new pores on seed coat facilitating the penetration of water inside the seed or nanoparticles may enter into the seed through the cracks present over their surface and trigger enzymes in the early phase thereby increasing the germination % **(39).** Furthermore, during germination, nanoparticles would trigger oxidation-reduction reactions through superoxide-ion-radical, resulting in the quenching of free radicals in the germinating seeds **(40).**

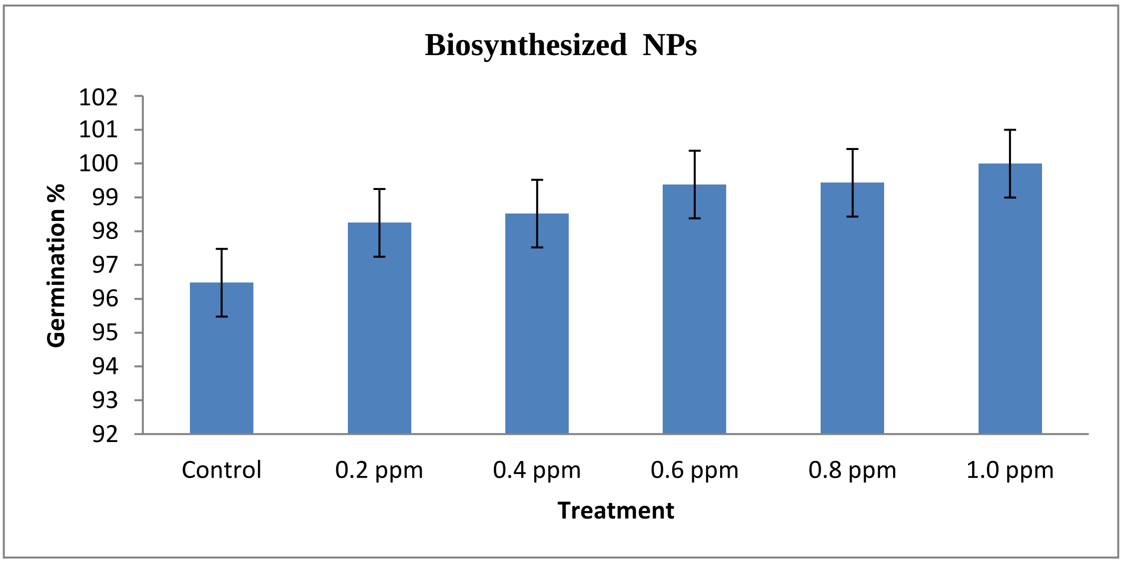


Fig 8: Effect of various concentrations of Biosynthesized nanofertilizer on germination % of wheat seeds.

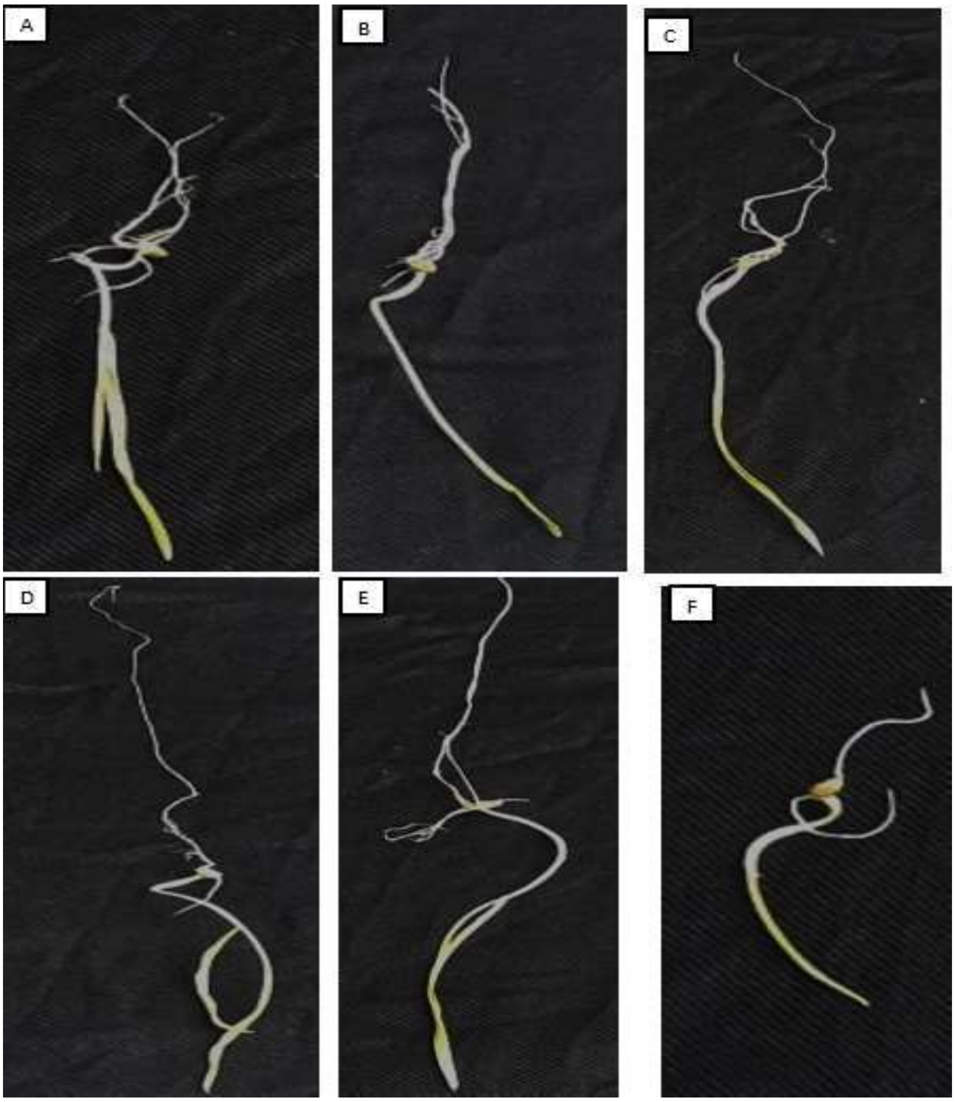


Fig 9: (A - F). Root and shoot length of one week old wheat seedling treated with different concentrations of biosynthesized nano fertilizer (A) 0.2ppm, (B) 0.4ppm, (C) 0.6 ppm, (D) 0.8 ppm (E) 1.00 ppm and (F) water.

Effect of various concentrations of biosynthesized nano fertilizer along on root length of wheat seedling:

The physical parameters of wheat seedlings treated with various concentrations of biosynthesized nanoparticles were recorded in terms of root length (cm) as shown in the Fig 10. Results indicated that there was a significant increase in root length (cm) of wheat seeds when treated with various concentrations of biosynthesized nanoparticles as compared to water. The highest root length (8.31± 0.18 cm) was observed in the group treated with 1.00 ppm of biosynthesized nanoparticles and it was significantly higher than that observed in seeds treated with water. This increase in root length might be due to synergistic effect of biosynthesized metal nanoparticles with better absorption and translocation in plant due to more specific surface area or increased reactivity of these area on the particle surfaces. However, lowest growth in terms of root length (4.36 ± 0.17 cm) was observed in wheat seeds treated with water. Our results are consistence with the findings of Rahman *et al.* **(41)** who studied the effect of mixed nano fertilizer on various parameters including nutrient efficiency, productivity and nutritive value of tomato fruits. It was evaluated from the study that plant height, photosynthetic pigment, root length as well as leaf mineral increases significantly ($p < 0.05$) by the application of mixed nano fertilizer and commercial fertilizer (MNF_f $+CF_s$) as compared to commercial fertilizer (CF_s).

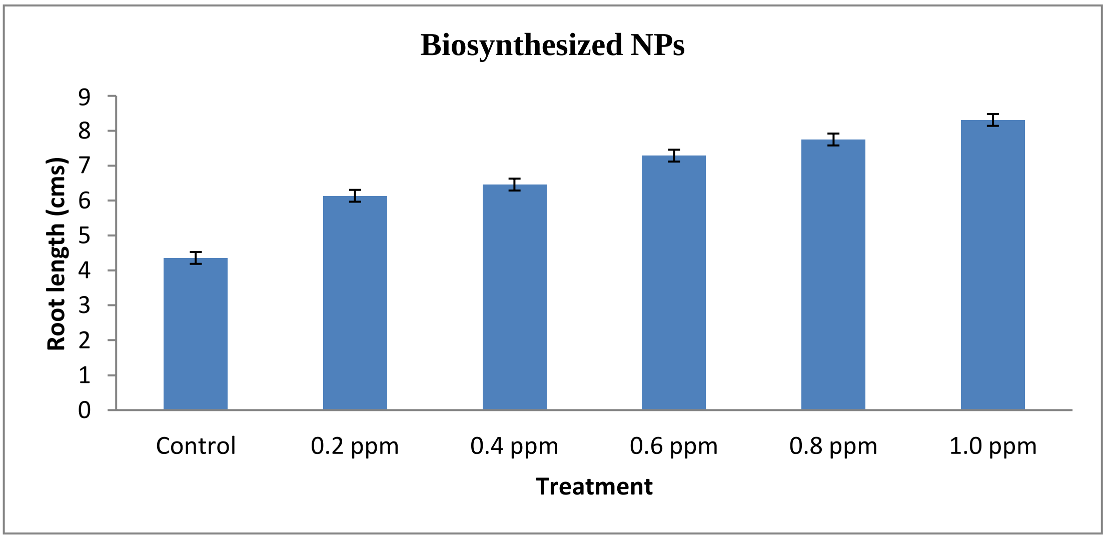


Fig 10:Effect of Biosynthesized nano fertilizer on root length of wheat seeds under various concentrations.

Effect of various concentrations of biosynthesized nano fertilizer on shoot length of wheat seedlings:

Shoot length of wheat seeds treated with various concentrations of biosynthesized nano fertilizer and water was determined as shown in the Fig 11. Results indicated that there was a significant increase in shoot length (cm) of wheat seeds treated with various concentrations of biosynthesized nanoparticles over control. This might be due to synergistic effect of biosynthesized metal nanoparticles that results in better absorption and translocation of nanoparticles in plant due to nano from and more specific surface area or increased reactivity of these area on the particle surfaces. The highest shoot length (7.46 ± 0.15cm) was observed in the group treated with 1.00 ppm of biosynthesized nanoparticles. However, lowest growth in terms of shoot length (4.81 ± 0.15cm) was observed in wheat seeds treated with water. A similar agreement was found by Dhoke *et al.* **(38)** who investigated the effect of nano-ZnCuFe-oxide, nano-FeO and nano-ZnO particles on seedling growth of mung (*V. radiata*) and concluded that nano-ZnCuFe-oxide exhibited pronounced effect in terms of root and shoot growth and biomass followed by nano-FeO and nano-ZnO. Similar trend was investigated by Yasmeen *et al.* **(42)** who reported the effect of iron, copper and silver nanoparticles on wheat (*T. aestivum*) seeds and concluded that enhanced growth in terms of root and shoot length was recorded in seeds treated with treated with iron nanoparticles. However, silver and copper nanoparticles showed reduction in root and shoot length.

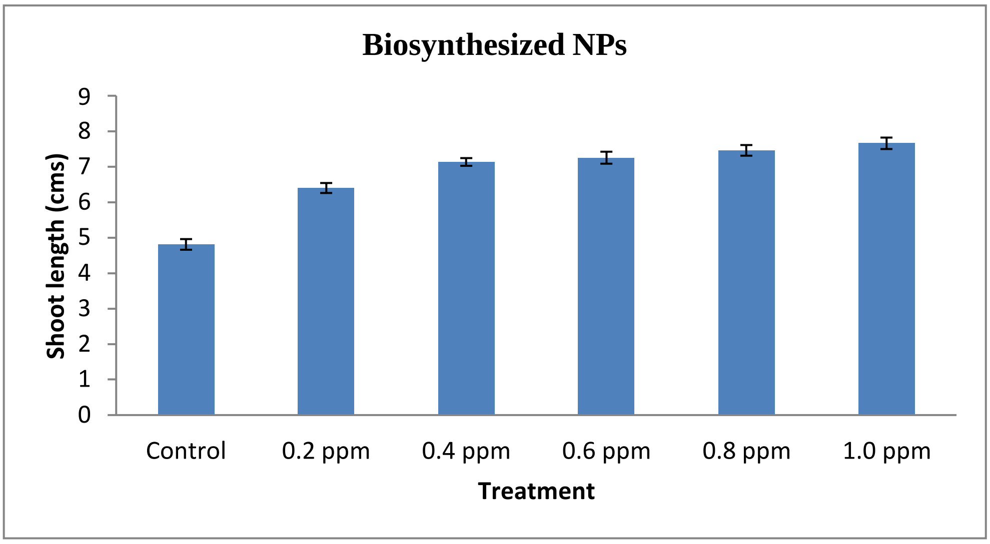


Fig 11: Effect of various concentrations of Biosynthesized nano fertilizer on shoot length of wheat seeds.

Effect of Biosynthesized nano fertilizer non-enzymatic antioxidant activities of wheat seedlings:

Total phenolic contents (TPC):

Trend of total phenolic contents of wheat seeds treated with various concentration of biosynthesized nano fertilizer was represented in Fig 12. The phenolic contents was positively influenced by nanoparticles treatments. Compared to control, all the treatments of biosynthesized nanoparticles led to an increase in total soluble phenolic contents. From the Figure, it was depicted that the treatment with biosynthesized nanoparticles significantly increased the leaf phenolic content of wheat plant with the increasing concentration as wheat seeds that was treated with 1.00 ppm concentration of biosynthesized nanoparticles have highest (95.4 ±0.95) phenolic contents. However, lowest phenolic contents (59.46 ±1.15) were recorded in control group.

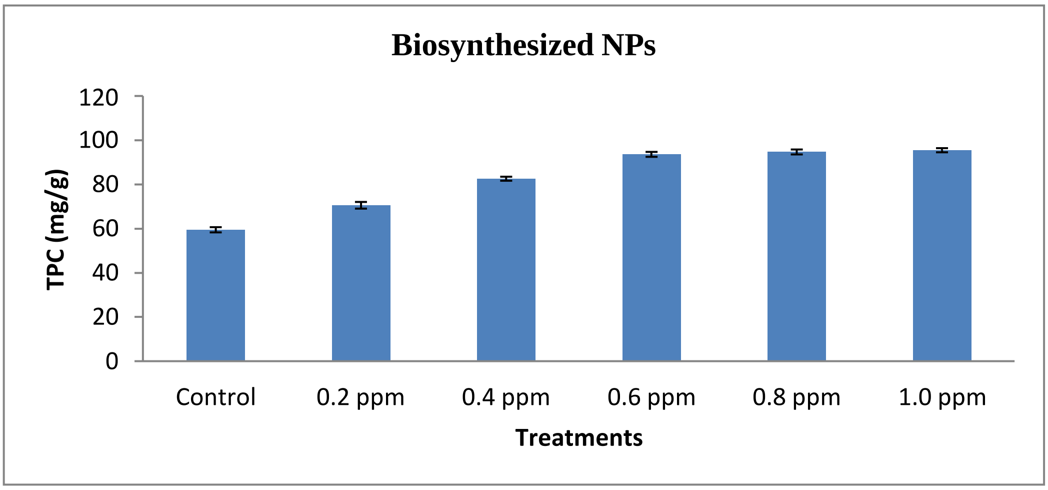


Fig 12: Total phenolic contents in wheat seedlings estimated with respect to various concentration of biosynthesized nano fertilizer

Total flavonoid contents (TFC):

Trend of total flavonoid contents of wheat seeds treated with various concentration of biosynthesized nanoparticles was represented in Fig 13. The flavonoids contents were positively influenced by nanoparticles treatments. All the treatments with biosynthesized nanoparticles increased the leaf flavonoid content as compared to water as control, where in the highest increase was recorded in the group treated with 1.00 ppm of biosynthesized nanoparticles showing (42.48 ± 0.4) flavonoid contents. However, lowest flavonoid contents was recorded in control group having (19.7 ± 0.23) flavonoids content.

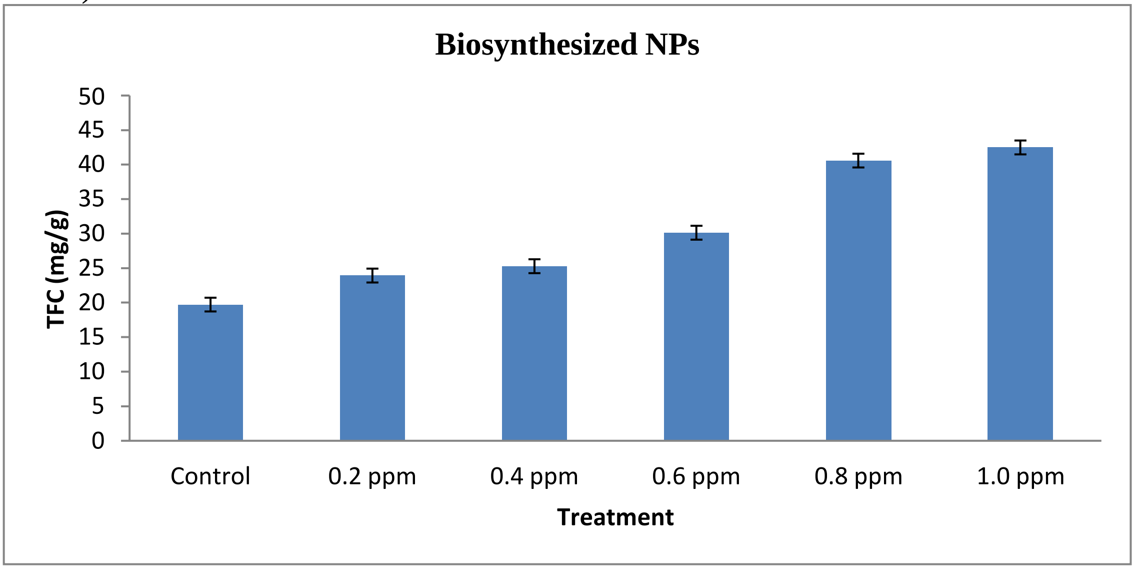


Fig 13: Graph of total flavonoid contents wheat seedlings estimated with respect to various concentration of biosynthesized nano fertilizer.

CONCLUSION:

In the present study, biosynthesized metal nanoparticles were obtained from ore tailing sample using *A. niger* which is environment friendly and cost effective approach. These metal nanoparticles were further characterized by AAS, UV-visible and DLS techniques. It was investigated that bio recovered metallic nanoparticles have great potential to promote earlier plant germination and seedling growth in terms of root and shoot length and non-enzymatic antioxidant activities (TFC and TPC) of wheat seed variety Faisalabad 2008 by the technique of seed priming. Seeds treated with biosynthesized nanoparticles displayed pronounced growth over water representing a positive effect of the biosynthesized nanoparticle treatment on plant growth. It was found that various concentrations (0.2-1.0 ppm) of biosynthesized nanoparticles significantly ($p < 0.05$) increased the germination rate and early seedling growth by seed priming treatment before germination of the wheat seeds and also enhances the shoot and root proliferation. Biosynthesized nanoparticles effect was highest at 1.0 ppm which might be due to the nano form and synergistic effect of biosynthesized nanoparticles and easy penetration through seed coat which provide better absorption and utilization of these particles by seeds. However, lowest germination and seedling growth was recorded in seeds treated with water used as control. According to the present study, biosynthesized nanoparticles could be an initiative for the development of nano bio fertilizer of potential importance in agriculture sector.

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