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IMPACT OF CHEMICALLY SYNTHESIZED COPPER NANOPARTICLES ON GROWTH AND BIOCHEMICAL PROFILING OF WHEAT (*TRITICUM AESTIVUM* L.)

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ABSTRACT

The aim of this research was to determine the impact of chemically synthesized copper nanoparticles of five different concentrations (1, 2, 5, 10 and 100pm) on *in vitro*seed germination and biochemical profiling of Wheat PBW677. No significant reduction was observed in germination percentage of wheat at all concentration, however morphological data including shoot length, root length, leaf length found to increase at very low concentration of Cu-NPs (up to 10ppm) whereas average root number showed major increase at 100ppm. Application of Cu-NPs on wheat seedling showed significant changes in its biochemical profiling. Total phenol content (TPC) and Total ascorbic acid (TAC) were maximum at100ppm whereas total flavonoid content (TFC) showed significant increase at 5ppm as compared to control. Chlorophyll content decreases with increase in Cu-NPs concentration in comparison to control. Further maximum carotenoids content was observed at 2ppm concentration of Cu-NPs.

Keywords: Biochemical profiling, wheat, growth parameters, cu nanoparticles, seed germination

INTRODUCTION

Agriculture is the prime source of nutrition for most of developing countries. Wheat (*Triticum aestivum*) being primary commodity for worldwide population of family *Poaceae*, is second most important staple food of India. Besides, its growth in the tropical and sub-tropical zones, it can also be grown in temperate and cold areas of far north. It can also tolerate highly cold conditions and can resume growth with the onset of warm weather during spring season Loamy soil with moderate water holding capacity is consider to be best for wheat cultivation. It contains carbohydrate 78.10%, protein 14.70%, fat 2.10%, minerals 2.10% and considerable proportions of vitamins (thiamine and vitamin-B) and minerals (zinc, iron). Wheat is also a good source of traces minerals like selenium and magnesium, nutrients essential to good health **[1]**. Sustainable agriculture is needed to meet the increasing demand of emerging population. To increase productivities in a resource efficient way agriculture needs to be reinforced and revitalized with innovating science-based technologies **[2]**. Nanotechnology deals with nanoscopic dimensions ranging 1-100nm.With production of nano fertilizers, these nano compounds rapidly and completely absorbed by plants and fix their nutrients shortages and growing needs **[3]**. Nanoparticles of gold (Au), silver (Ag), copper (Cu), zinc (Zn), aluminium (Al), silica (Si), zinc oxide (ZnO), caesium oxide (Ce_2O_3), titanium dioxide (TiO_2) and magnetized iron (Fe) have various applications in agriculture **[4]**. Copper being the micronutrient for plant growth are essential for plant growth and development under their optimum range. Deficiency of this micronutrient results in dieback of stems and twigs, yellowing of leaves, stunted growth, pale green colour of leaves, necrotic spots etc. Copper nanoparticles show positive effects on germination **[5]**. So, this study was carried out to determine the effects of Cu-NPs at different concentrations on *in-vitro* seed germination and biochemical profiling of wheat.

MATERIALS AND METHODS

Collection of materials

Seeds of wheat cultivar PBW677 were collected from Punjab Agricultural University, Ludhiana.

Surface sterilization and application of treatment

Seeds were treated with 15% BENFIL (Carbendazim 50% w/w) for 10-15 min and rinsed three times with distilled water, followed by surface sterilization under aseptic conditions (LAF) with 0.1% $HgCl_2$ solution for 3 min, followed by 3 times washing with autoclaved water. Nanosuspension of copper nanoparticles

(Cu-NPs) synthesized by using methods of **[6]** were prepared by suspending different concentrations (1ppm, 2ppm, 5ppm, 10ppm, 100ppm) of these in distilled water for different treatments. Surface sterilized seeds of Wheat (5seeds/Petri plate) were placed. 10ml of prepared nanosuspension of copper nanoparticles prepared in distilled water of different concentration were added to each plate against control containing distilled water without nanoparticle.

Estimation of morphological parameters

Root and shoot length of germinated seedling were measured with the help of centimetre scale. Triplicates were prepared for each treatment. Petri plates were incubated in light/dark conditions (16/8) at 25±2 °C, Humidity 70 ±10 % in growth chamber. Further germination data for wheat treated with Cu-NPs after seven days was recorded by following formula:

% Seed germination = Number of seeds germinated / total number of seeds x 100

Preparation of extract and Biochemical assays

The leaf extracts of Wheat (PBW677) were prepared by freezing the plantlets, and then by making a fine liquid extract of it, by grinding it in mortar and pestle obtained after different Cu-NPs treatments. 5% Plant extract was prepared by using 0.1M Potassium dihydrogen phosphate buffer. Prepared plant extract was kept in 2ml centrifuge tubes in freezer to perform different biochemical assays.

Estimation of biochemical parameters

Estimation of phenolic content**:** The phenolic content was estimated by folin-ciocalteau methodgiven by **[7]**.

Estimation of Chlorophyll and Carotenoid**:** The chlorophyll and carotenoid content were estimated by acetone method given by **[8]**.

Estimation of total antioxidant: The total antioxidant content was estimated by phospho molybdenum method given by **[9]**.

Estimation of flavonoid: flavonoid content was estimated by aluminium chloride method of **[7]**.

Estimation of Ascorbic acid: The ascorbic content was estimated by 2, 4-DPPH method **[10]**.

Statistical Analysis of data: Data were statistically analysed according to one-way analysis of variance (ANOVA) with significant differences among treatments at $p < 0.05$ determined according to Tukey's test.

RESULTS AND DISCUSSION

Seed Germination assay of wheat seedlings treated with Cu-NPs

The germination of seeds was observed and found to increase for wheat in petri plates having Cu-NPs at concentration of 1, 2,5,10 and 100ppm when compared with the control plate which had only distilled water.100% germination was observed at 1,10 & 100ppm as compared to control. Reduction at 2 and 5 ppm was observed due to fungal contamination in comparison to control. However, in contrast many available studies **[11-13]** observed negative impact of Cu-NPs on percentage seed germination with increase in concentration. The cause of reduction in germination percentage in most of the studies is due to the nature, size, shape, concentration and toxic nature of these particles **[14]** and **[15]**. Toxicity of Cu-NPs may arise due to the formation of metal oxide. Further germination data for wheat treated with Cu-NPs after seven days was recorded.Lack of negative impact of Cu-NPs on % seed germination may be because of the presence of protective seed coat **[16]**.

Shoot length and root length data analysis for wheat treated with Cu-NPs

In present study, there observed maximum positive impact of Cu-NPs at low concentration i.e., at 1ppm,2ppm with maximum mean shoot length along with at 5 & 10ppm too for mean leaf length as well.Further increase in Cu-NPs concentration results in accumulation of Cu-NPs in the shoot as well as in the leaf too resulting in browning of shoot which may be due to the absorption of Cu-NPs via roots. **[17]** and **[18]** found evidence that some plants can uptake NPs and accumulate them in their tissues. **[19]** also recorded increased accumulation of CuFe2O4- NPs via roots of cucumber plants. Better shoot organogenesis in *S. rebaudiana* seeds at 1&10 ppm with maximum average shoot length, mean number of shoots per explant, and fresh weight at 10ppm in comparison to control **[20]**. Increase in leaf length was also observed at same concentration**.** Copper being a micronutrient, act as metal ions for metalloenzymes involved in electron transport, protein and carbohydrate biosynthesis that plays important role in plant regeneration **[21]** thus improving the growth parameters of plants treated with Cu-NPs**.** Growth parameters including shoot length, shoot biomass were reduced when the concentration goes above 1000 mgL^{-1} with respect to control **[22]**.

In our study, significant increase in mean root length was recorded at 1, 10 & 100 ppm for *T. aestivum* **(Table 1)**. Increase in root fresh weight, root dry weight, root length, and root elongation in wheat was observed at 75ppm of Cu-NPs as compared to the control, beyond which it decreases **[23]**. Root number increases with increase in Cu-NPs concentration in present study with hairy root formation at 10 and 100ppm in comparison to control. Maximum average root number was found at 100ppm. In contrast to our results, **[2]** found decrease in root length and root number when the concentration goes beyond 1ppm **[24]**.

Biochemical data analysis of wheat treated with copper nanoparticles

Total Phenolic content (TPC)

Due to redox properties of phenolics, they function as antioxidants. Hence, TPC can be assessed for rapid screening of antioxidant properties of plants. The enzymatic and non- enzymatic antioxidants naturally present in plants actually help them cope with an oxidative stress of metal ions or free radicals **[25]**. **[20]** demonstrated that CuO nanoparticles confer positive effect for in-vitro Stevia growth dynamics and steviol glycoside production.

In present study, TPC was found to be maximum in case of 100ppm (4.74±2.28) Cu-NPs concentration, whereas the minimum phenolic content was found in case of 10ppm (2.4011mg/g) Cu-NPs concentration. Contradicting to our results, are the results of **[26]**, as they found maximum TPC content (6.22 µg GAE/mg) at 10ppm on *Stevia rebaudiana* samples treated with CuO-NPs whereas **[27]**, found that treatment with the application of 250 mg L^{-1} of Cu-NPs on tomato seedlings, generated an increase of 5.43% in relation to the Control, while treatment with 125 mg L^{-1} of Cu NPs showed a decrease of 14.44%. Also, Jalapeno pepper fruits showed an increase in the content of the total phenols (5.9%) when treated with 2.0 mg of Cu NPs + Chitosan-PVA **[28]**. It was evident that the phenolic content increases with the increase in copper nanoparticle concentration, however there was a decrease in phenolic content at 10ppm Cu-NPs concentration and a significant increase at 100ppm Cu-NPs concentration **(Figure 1)**. Phenolics assist as ROS scavengers by neutralizing radicals before damaging cells and thus are important for plant resistance under various stresses **[29]**.

Total flavonoids content

The TFC was found to be maximum in case of 5ppm (0.087±0.00043) Cu-NPs. concentration, whereas the minimum flavonoids content was found in case of 100ppm (0.042±0.0021) Cu- NPs concentration. It was evident that the flavonoids content increases with the increase in copper nanoparticle concentration up to certain extent, however there was a decrease in flavonoid content at 100ppm Cu-NPs concentration and a significant increase at 5ppm Cu-NPs concentration as compared to control **(Figure 2)**.

Results from previous literature confirms that increase in Cu-NPs concentration results in production of ROS, further triggering antioxidant accumulation in plants treated with these particles improving plant nutritional value. **[30]** reported highest content of flavonoids in the tomato fruits and 50 mgL^{-1} of Cu-NPs, exceeding the Control by 36.14%.

Estimation of chlorophyll and carotenoids content

Chlorophyll 'a': Photosynthesis is the basic function determining productivity of green plants. In the variant treated with Cu-NPs, chlorophyll 'a' content was found to decrease with increase in Cu-NPs concentration as compared to control **(Figure 3)**. In the variant with nanoparticles, there observed significant increase in case of 100ppm (0.0060$\pm$0.00114) whereas a drastic drop in chlorophyll 'a' content at 1ppm (0.002425$\pm$0.000275). At 2,5 & 10ppm there observed a constant increase in chlorophyll 'a' content as compared to 1ppm ($p<0.05$).

Chlorophyll 'b': In the variant treated with Cu-NPs, chlorophyll 'b' content was found to decrease with increase in Cu-NPs concentration as compared to control. Maximum chlorophyll 'b' content was found in case of control. In the variant treated with Cu-NPs, chlorophyll 'b' content was found to maximum in case of 10ppm (0.01365$\pm$0.00055) and a drastic drop at 1ppm (0.0063$\pm$0.00077) **(Figure 4)**.

Total chlorophyll content: Total chlorophyll content follows the same trend as in case of chlorophyll 'a'& 'b' content in variants treated with Cu-NPs as compared to control. Maximum total chlorophyll content was found in case of control. In the variant treated with Cu-NPs, total chlorophyll content was found to maximum in case of 10ppm (0.01905$\pm$0.00065) and a drastic drop at 1ppm (0. 0.00901$\pm$0.001) **(Figure 5)**. **[31]** observed in the variant with nanoparticles, low copper concentrations proved to stimulate photosynthesis, with its rate increasing to 130–140% of the control level. **[32]** reported that plant exposure to 100 and 500 mg L^{-1} of CuO NPs has resulted in significant reduction of total chlorophyll and sugar content in the two test plants while 10 mg L^{-1} of NPs slightly increased the pigment and sugar content in tomato plants only.

Carotenoid's content: Maximum carotenoids content was observed at 2ppm concentration of Cu-NPs and drop at 1ppm as compared to control. In the variant treated with Cu-NPs, carotenoids content was found to maximum in case of 10ppm (0.000418$\pm$0.000064) and a drastic drop at 1ppm (0.00027$\pm$0.000054) **Figure 6**.

Total Antioxidant Content: The antioxidant content was found to be maximum in case of 100ppm Cu-NPs concentration as compared to control, whereas in case of variants treated with Cu-NPs, the minimum antioxidant content was found in case of 5ppm Cu-NPs concentration .It was evident that the antioxidant content increases with the increase in copper nanoparticle concentration, however there was a slight decrease in antioxidant content at 5ppm (3.1905±0.1191) Cu-NPs concentration and a significant increase at 100ppm (4.282±0.536) Cu-NPs concentration. **[33]** observed increase in the free radical scavenging or antioxidant activity in leaf extract treated with green synthesised nanoparticles.

Ascorbic acid content: The ascorbic acid content was found to be maximum in case of 1ppm Cu-NPs concentration, whereas the minimum ascorbic acid content was found in case of 2ppm Cu-NPs concentration.

It was evident that the ascorbic acid content increases with the increase in copper nanoparticle concentration, however there was a decrease in ascorbic acid content at 2ppm (37.68±0.579) Cu-NPs concentration and a significant increase at 1ppm (47.56±1.159) Cu-NPs concentration as compared to control **(Figure 7)**. It was reported that at 10mg/L concentration of CuO NPs, there was significant enhancement in TAC (11.9µg AAE/mg) of leaves grown on MS medium containing CuO-NPs in Stevia rebaudiana (Candy leaf) **[20]**.

Table 1: Effect of different concentrations of cu nanoparticles on growth of wheat

Concentration Of Cu-NPs	Mean shoot length (cm) ± SD	Mean root length(cm) ± SD	Mean leaf length (cm) ± SD	Mean root number(cm) ± SD
Control	2.13± 0.50	2.23±0.74	3.30± 0.76	3.75±0.93
1 ppm	2.56± 0.33	3.00± 0.45	3.15 ± 1.00	4.56± 0.63
2 ppm	1.95± 0.33	4.24± 1.07	2.81± 1.01	4.13±0.81
5 ppm	2.03± 0.17	3.73± 0.91	2.45± 0.98	4.25±0.93
10 ppm	1.79±0.34	3.68± 1.28	2.40±0.86	4.63± 0.62
100 ppm	2.30± 0.33	2.72± 0.93	3.18± 0.94	4.75±0.68

For the provided data p<0.05, so this data is statistically significant.

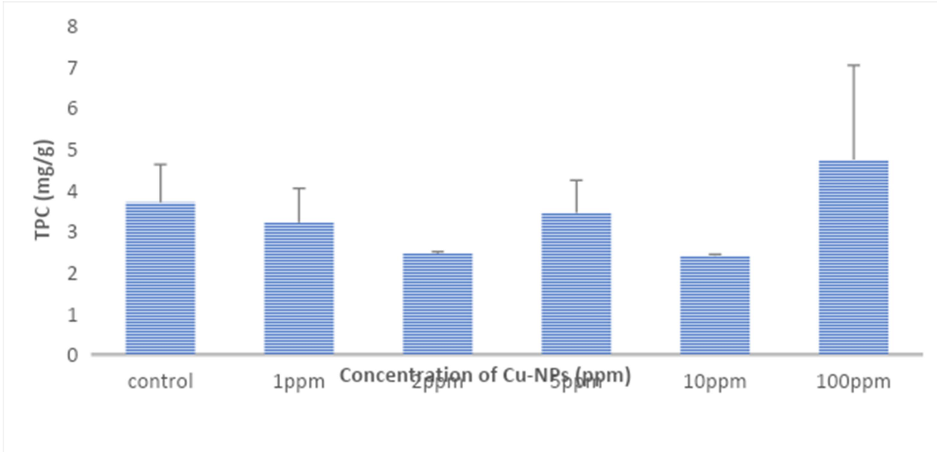

Figure 1: Effect of various concentrations of cu-nanoparticles on total phenol content

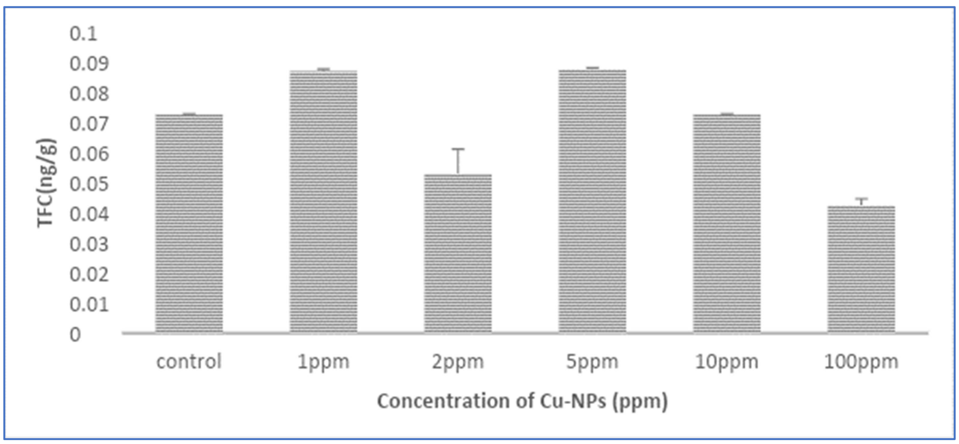

Figure 2: Effect of various concentrations of cu-nanoparticles on total flavonoid content

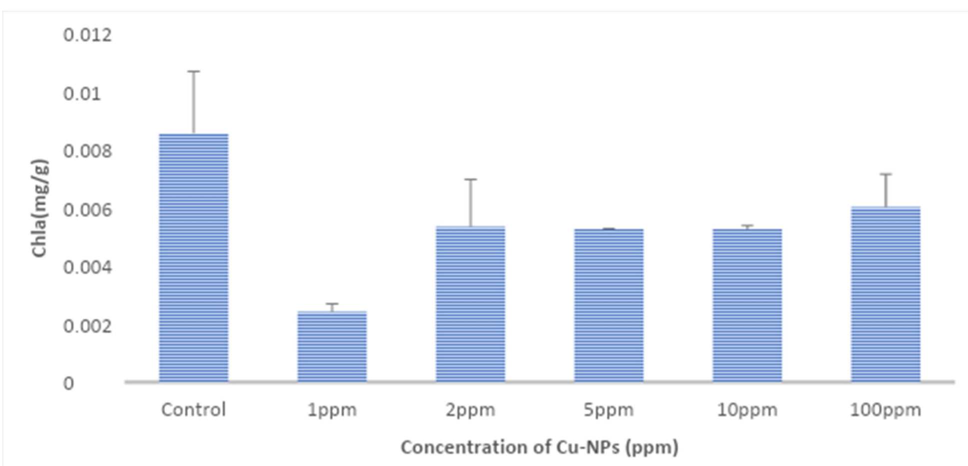

Figure 3: Effect of various concentrations of cu-nanoparticles on chlorophyll a content

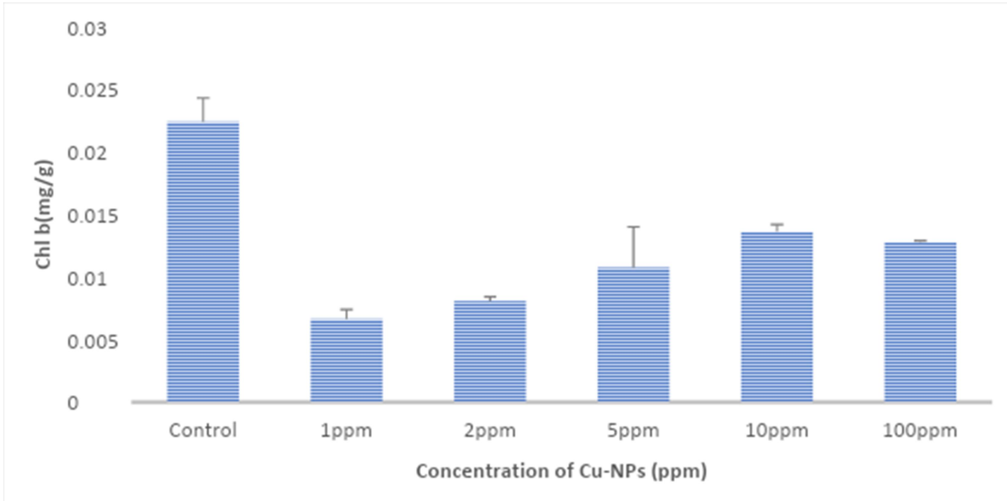

Figure 4: Effect of various concentrations of cu-nanoparticles on chlorophyll b content

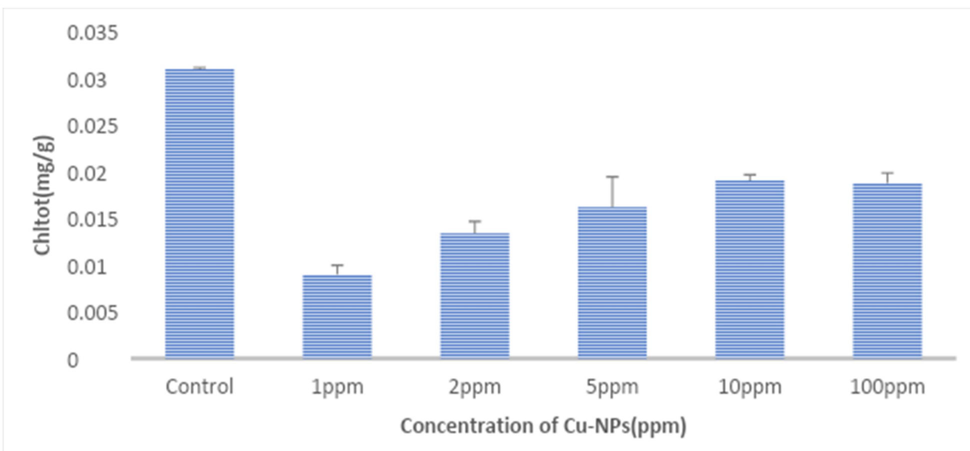

Figure 5: Effect of various concentrations of cu-nanoparticles on total chlorophyll content

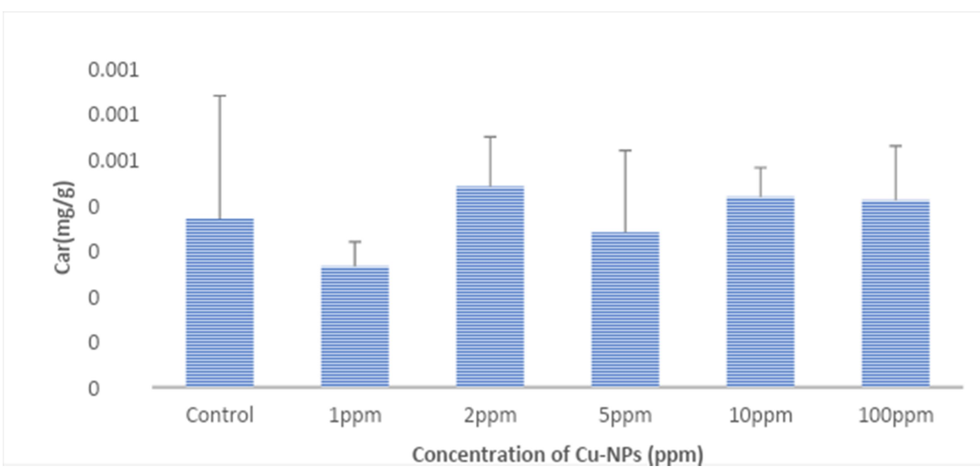

Figure 6: Effect of various concentrations of cu-nanoparticles on carotenoid content

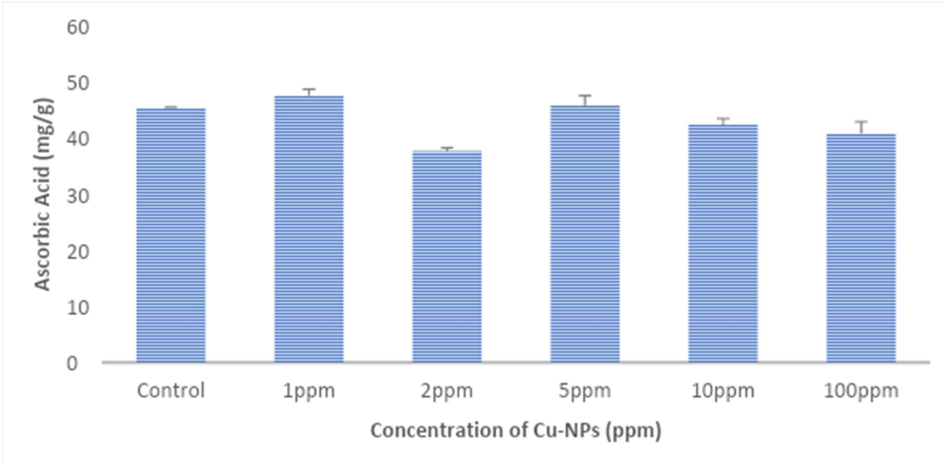

Figure 7: Effect of various concentrations of cu-nanoparticles on ascorbic acid content

CONCLUSION

It may be concluded from the above study that Cu-NPs at low concentration have positive impact of growth parameters of wheat i.e., shoot length, root length, leaf length. Application of Cu-NPs on wheat seedling showed significant changes in its biochemical profiling. Phenolic and ascorbic acid content was maximum at100ppm whereas the production of total flavonoid and carotenoid content was enhanced by application of cu nanoparticles as compared to control. These findings revealed perspectives of use of lower concentrations of cu nanoparticles to improve yield of wheat. The application of nanoparticles at seed level may offer a safer way be avoiding direct applications in soils and hence being less toxic to soil microbiota in comparison to synthetic fertilizers. Further we suggest the research on molecular level because modulation of gene expression by nanoparticles are not totally elucidated, and is extremely important.

Conflict of Interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

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