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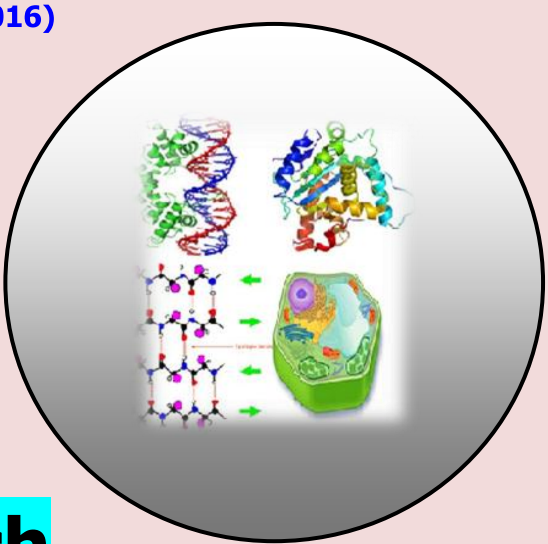

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RESEARCH PAPER

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Effects of Copper Oxide Nanoparticles on the Photosynthesis and Antioxidant levels of Mustard Plants (*Brassica juncea*)

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ABSTRACT

Nanoparticles (NP) have great potential in agriculture fields and plays pivotal role in an increasing number of products and processes of biological systems. The aim of this study was to find out the effects of copper oxide nanoparticles (CuO NPs) given as a soil treatment on photosynthetic rate and antioxidant levels of mustard (Brassica juncea). The surface sterilized seeds of mustard were sown in the pot filled with soil amended organic manure. Different concentration of CuO NPs (0, 2, 4, 8 or 16 PPM) will be given to the soil after the first leaf stage in the plants. At 45 days stages of growth, plants from all the treatments were harvested to find out the various leaf gas exchange traits as well as biochemical parameters. NPs of CuO significantly increased the photosynthetic efficiency along with antioxidant systems. Concentration of 4ppm of CuO NPs proved best and had maximum values for the photosynthetic rate and antioxidant enzymes at both the stages of growth. Concentration of 4ppm of CuO NPs increased the photosynthetic rate (PN), by 32% (45 DAS) Ci by 36% (45 DAS), POX by 54.9%, SOD by 54.08% and CAT by 55%. It is concluded that 4 ppm of CuO NPs can be used through soil to increase the productivity of mustard.
Keywords: Nanoparticles, Copper Oxide, Photosynthetic Rate, Antioxidant and Mustard.

INTRODUCTION

Nanotechnology is the new branch of science which have great value on daily basis due to their diverse application. Materials which have dimension between 1-100 nm is regarded as a nanoparticle. They are present in every product we used in our daily life. Anthropogenic activity releases the nanoparticles into the environment which are not the good sign for human health and plants. Nanoparticles which are released into the environment may get into our food chain and ultimately reaches to our body. In a recent study, it was found that plants weremost easily affected by engineered nanoparticles (ENPs).Nanoparticles may enter into the plants by root systems or adsorb on the cell surface or may be taken up by any micro or macro pole present in the plant which is translocated within plant body (Dietz and Herth, 2011). ZnONPs, TiO_2NPs, and CuONPs are among the most commonly used nanomaterials (Kaweeteerawat et al. 2015). Potential adverse effects of nanoparticles on the environment and human health are being subjectedto study(Monica and Cremonini, 2009).Zhang and Webster, (2009) reported the large number of nanoparticles like, gold (Au), silver (Ag), copper (Cu), zinc (Zn), aluminum (Al), silica (Si), zinc oxide (ZnO), cesium oxide (Ce_2O_3), titanium dioxide (TiO_2) and magnetized iron (Fe) which have great applications in agriculture fields. It was reported that nutrient use efficiency, photosynthetic activity, grain quality and seed yield were increased in the presence of nanoparticles (Batsmanova et al. 2013).Copper is an important essential plant nutrient which worked as a micro nutrient in plant cells. CuO NPs are extensively used in agricultural practices in the form of nano-fertilizer, as a pesticide, herbicide, or as a plant growth regulator. The role of CuO NPs in relation to plant is not specific. It depends on the plant type, concentration and nanoparticles used.

In present experiment was conducted with an aim to study the effect of CuO NPs through soil on the performances of the mustard crop. The hypothesis tested is that application of CuO NPs through soil may enhance the growth and productivity of the crop.

MATERIALS AND METHODS

Plant materials

The seeds of mustard (*Brassica juncea*Czern&Coss*cv.*RGN-48)were procured from Indian Agriculture Research Institute (IARI), New Delhi, India. The healthy looking and uniform size seeds weresurfacessterilized with 1% sodiumhypochlorite solution for 10 min, followed by repeated washing with double distilled water (DDW).

Source of nanoparticles

Nanoparticle purchased directly from the Sigma-Aldrich Division. Required quantities (2, 4, 8, or 16 ppm) of CuO-NPs were dissolved in 10 ml of DDW in a 100-ml volumetric flask and final volume was made up to the mark by using deionized water.

Treatment pattern and experimental design

The experiment was conducted in earthen pots under randomized block design. Sterilized seeds were sown in pots filled with an equal quantity of sandy loam soil mixed with farmyard manure under natural environmental conditions. Twenty-five pots were divided into five sets with five pots in each set. The treatments of CuO NPs (2, 4, 8 or 16 ppm) were given in the soil, after the emergence of the seedling. The sampling was done at 45 DAS to access the various characteristics.

Determination of growth biomarkers and leaf area

The growth biomarkers (shoot and root length, shoot and root fresh and dry mass) were determined at 45 DAS(Khan et al. 2015).The leaf area was measured by using a portable leaf area meter (ADCBio-scientific, UK).

SPAD value and Gas exchange rate

The SPAD values in the leaf were measured under natural conditions by using the SPAD chlorophyll meter (SPAD-502; Konica, Minolta sensing, Inc., Japan).Leaf net photosynthetic rate (P_N), stomatal conductance (gs), transpiration (E) and internal CO_2were measured in the fully expanded leaf between 11:00 and 12:00 h by using an infra-redgas analyser (IRGA) portable photosynthetic system (LI-COR 6400 Lincoln, NE, USA). The instrument was stabilized with the air temperature (25 ^{0}C), relative humidity (85%), CO_2concentration (600 mol mol^{-1}) and photosynthetic photon flux density (PPFD) (800 mol mol^{-2} s^{-1}).

Enzyme Assay

The method given by Dwivedi and Randhawa (1974) and Jaworski (1971) was used to measure the carbonic anhydrase (CA) activity and nitrate reductase (NR) activity respectively in the leaf samples.

The activities of various antioxidant enzymes such as Catalase (CAT), Peroxidase (POX), superoxide dismutase(SOD) were analyzed as described in our previous study(Faizan et al., 2017).

Proline content

Leaf proline content in fresh leaf samples (one plant per replicate) was determined by the method given by Bates et al.(1973).

Statistical analysis

Data were statistically analysed using SPSS, 17.0 for windows (SPSS, Chicago, IL, USA). Standard error was calculated and analysis of variance (ANOVA) was performed on the data to determine the least significance difference (LSD) between treatment means with the level of significance at $P\leq 0.05$.

RESULTS

Growth characteristics

It is deduced from the fig. 1 (A-F) that all the growth parameters (length of root and shoot, fresh and dry mass of root and shoot) increased with the application of CuO NPs through soil at 45 DAS. The 4 ppm of CuO-NPs proved best and increased the value for all the parameters to a maximum level. However, the values obtained in 2 and 16 ppm treatments as well as in control were at par to each other at 45 DAS.

The percent increased due to 4 ppm of CuO NPs at 45 DAS was about 33% (shoot length), 27.7% (root length), 31.7% (shoot fresh mass), 30.3% (root fresh mass), 29.7% (shoot dry mass) and 28.7% (root dry mass) over the control.

Leaf area and chlorophyll (SPAD value)

The value of leaf area and SPAD for chlorophyll increased by the CuO NPs given through soil, irrespective of the concentration used (Fig. 2). The 4 ppm of CuO NPs proved to be best and increased the leaf area by31% and SPAD value by 34% over control at 45 DAS. The other treatment also increased the values for both the parameters at 45 DAS but less than that of 4 ppm of CuO NPs.

Photosynthetic characteristics

All the photosynthetic characteristics [net photosynthetic rate (P_N), internal CO_2 concentration (C_i), stomatal conductance (g_s) and transpiration rate (E)] were increased irrespective of the treatment of CuO NPs (Fig. 2). The values for all the parameters were found to be maximum in the presence of 4 ppm of CuO NPs. The percent increase in the $P_N$was 32%, C_i was 36%, g_s was 36.7% and E was 35% over their respective control at 45 DAS.

Carbonic anhydrase (CA) and nitrate reductase (NR) Activities

The treatment of CuO NPs through soil also increased the CA and NR activity at 45 DAS. The treatment of 4 ppm of CuO NPs through soil proved to be best and enhanced the CA by 30% and NR by 29% respectively over their control at 45 DAS (Fig. 2).

Antioxidant enzymes

It is observed from the figure 3 that out of the treatment, 4 ppm of CuO NPs proved to be the best for all the antioxidant enzymes and increase CAT by and 55%, POX by 54% and SOD by 55.8% over their respective control at 45 DAS.

Proline content

The endogenous level of proline was significantly increased in the plants treated with CuO NPs than control at all the concentrations and maximum was noted at 4 ppm and increased by 42% over the control at 45 DAS (Fig.3).

DISCUSSION

Nanotechnology played important role in agricultural area in recent year due to its huge implication in the crop improvement. Nanoparticles are believed to modify the physiology and biochemical process in plants thus affecting in toxic or positive way. There are several literatures presents which shows the role of nanoparticles in the growth and development of plants. In our study it had been deduced by different parameters that when CuO NPs given as soil treatment it enhances the plant growth and physiological as well as biochemical parameters. Copper is essential micronutrient and play important role in plants. Copper (Cu) act as cofactors in many enzymes like superoxide dismutase (SOD), amino acid oxidase etc ((Mazhoudi et al. 1997). Effect of nanoparticles depends on the size, shape of the nanoparticles and also on the plant species and climatic condition. So, due to these specificity nanoparticles shows variable responses with different study.

Plant performances can be measured by its growth phenomena which is an irreversible process and any changes in its phenotypic character will shows the effect of external stimuli. It is clear from the fig.1 that growth was enhanced when mustard plant treated with different concentration of CuO NPs (Fig 1 A-F). This result was also similar to other finding where nanoparticles improve the plants growth and development (Shende et al., 2017; Yasmeen, 2015). Exogenous application of SiO_2 improved the growth quality and root length of Changbai larch plant (Bao-shan et al. 2004).Rice plant treated with silica coated quantum dots shows the improved root growth than rice treated with bare quantum dots (Wang et al. 2014). Enhanced growth of plant due to nanoparticles reported in several others study. TiO_2improved the growth performances in wheat and spinach plants ((Jaberzadeh et al. 2013; Zheng et al. 2005). Positive effect of nanoparticles may be due their involvement in various biochemical activity which increases the plant growth(Dimetry and Hussein, 2016; Liu et al., 2017; Razzaq et al., 2016). Similar result of nanoparticles to increased plant biomass; shoot and root growth by ZnONPs in *Cyamopsistetra gonoloba* have been reported(Raliya and Tarafdar, 2013). Increase in the plant biomass and total protein reported by the application of mesoporous silica nanoparticles in wheat and lupin (Sun et al. 2016). Increased in the shoot length, leaf area and root dry weight have been reported by the treatment of nano titanium oxide ($nTiO_2$) in broad bean (Abdel Latef et al. 2017).

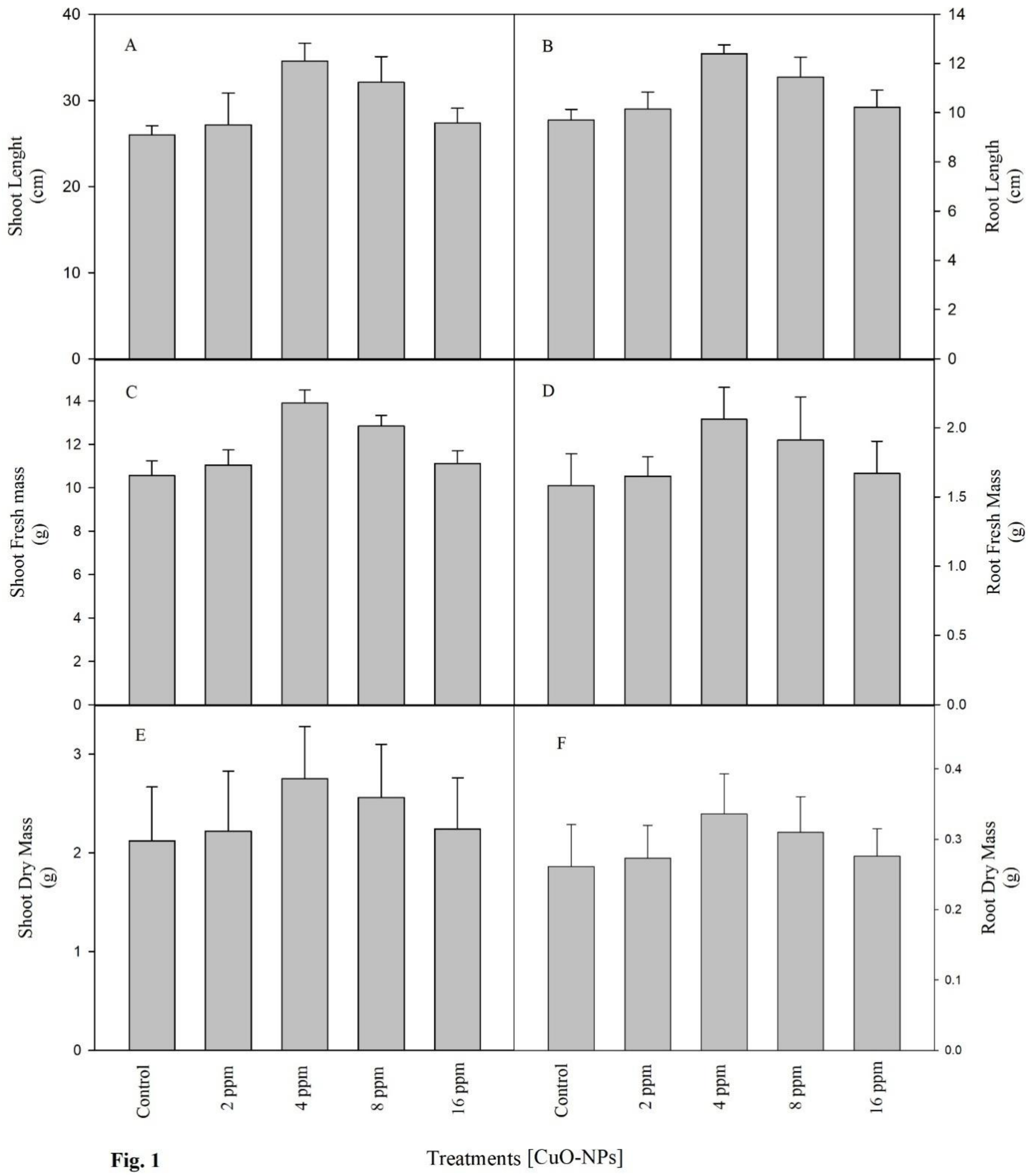

Fig. 1 Effect of nanoparticles (NPs) on the length of shoot(A) and root length(B) fresh mass of shoot(C) and root(D), dry mass of shoot(E) and root(F) of mustard at45 DAS. All the data are the mean of five replicates (n = 5) and vertical bars shows standard errors (±SE).

Increased in the shoot length, root length and fresh weight of mustard seedling by application of silver nanoparticles have been reported by Sharma et al.(2012). All these results are firmly supported our finding under CuO NPs given as a soil treatment where all these parameters increased in mustard plant.

Nitrogen is most important element for plant growth. It is an important constituent of protein, amino acid and nucleic acid. Major source of nitrogen for higher plant is nitrate.

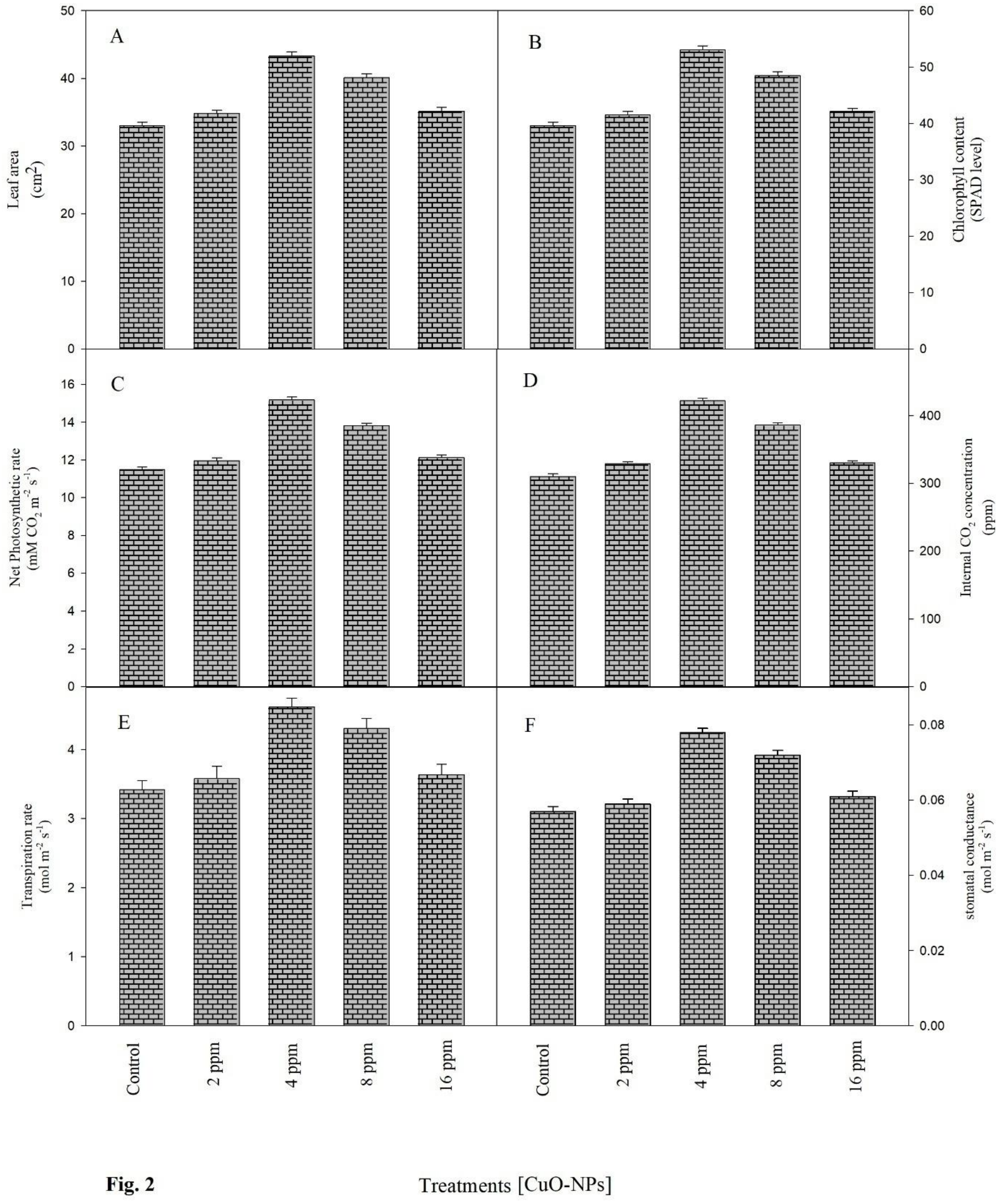

Fig. 2 Effect of nanoparticles (NPs) on the (A) leaf area and (B) chlorophyll content (C) net photosynthetic rate and (D) internal CO_2 concentration (E)transpiration rate and (F)stomatal conductance of mustard at45 DAS. All the data are the mean of five replicates (n = 5) and vertical bars shows standard errors (±SE).

Nitrogen promote the plants vegetative development and increased the yield of plants (Singh et al. 2017). Soil treatment of CuO NPs improved the nitrate reductase activity (Fig.3 B) which may be improved the overall plant growth and photosynthetic attribute. This result is also similar to finding of others in which increases the NR activity due to NPs have been reported(Ahmad et al., 2018).Increased NR activity at 10 ppm of CuO NPs in tomato plants had been found by Singh et al.(2017). However, higher concentration of CuO NPs in the same study reduces the NR activity which revealed that lower concentration of CuO NPs can be used to increase the plant biochemical activity. Application of nano-SiO_2 and nano-titanium dioxide (nano-TiO_2) increased the NR

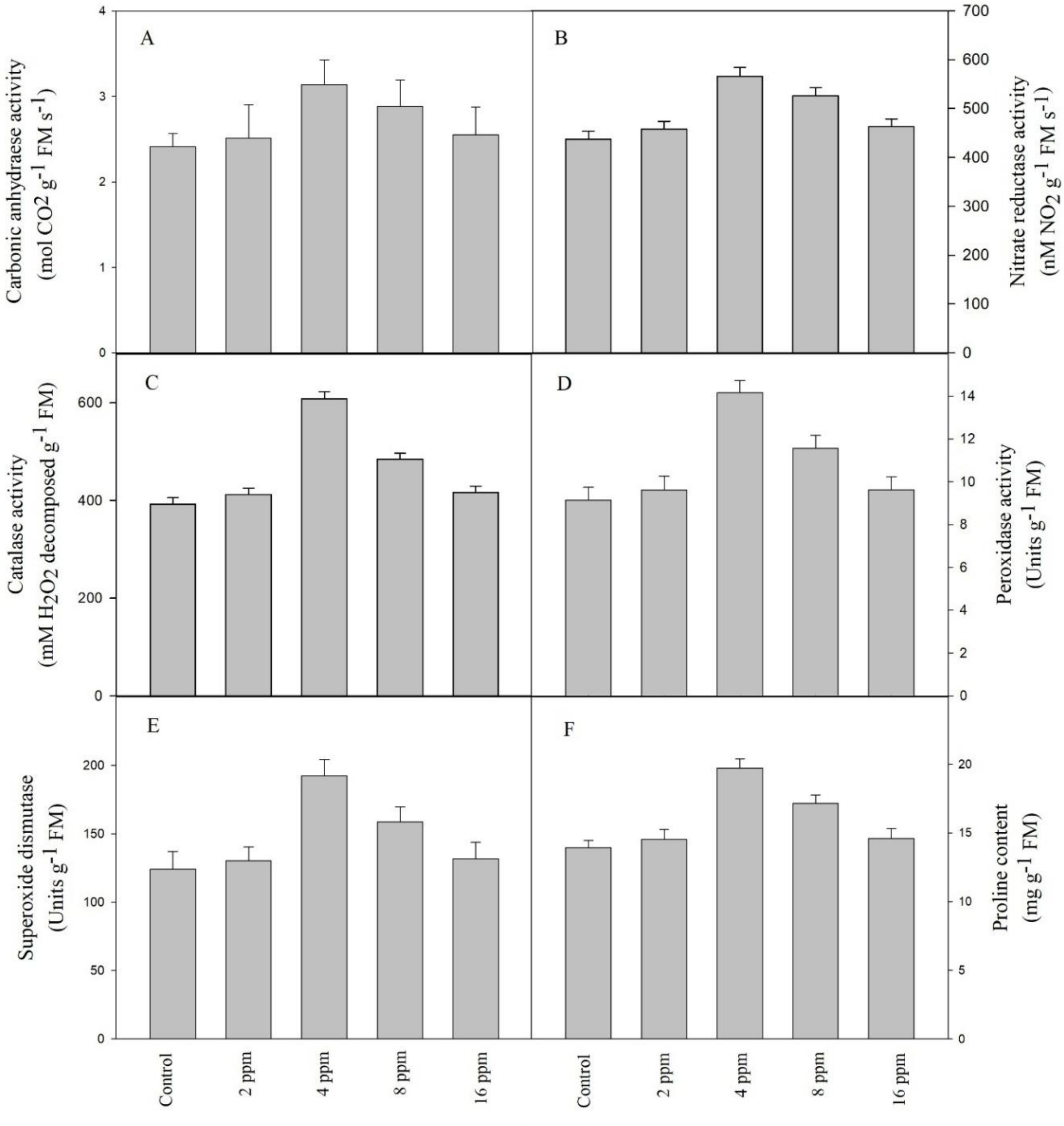

Fig. 3

activity in soybean (Lu et al. 2002).

Fig. 3 Effect of nanoparticles (NPs) on the (A) carbonic anhydrase activity and (B) nitrate reductase activity (C)catalase activity(D)peroxidase activity(E)superoxide dismutase(F) proline of mustard at45 DAS. All the data are the mean of five replicates (n = 5) and vertical bars shows standard errors (±SE).

Productivity of green plants determined by the rate of photosynthesis. Green plants have ability to convert the up-coming solar radiation into chemical energy. Crop yield can be improved by improving the photosynthesis rate of plants. In our study it had been reported that when mustard plant treated with different concentration of CuO NPs given as a soil treatment the rate of photosynthesis is increased (Fig.2). The maximum photosynthetic efficiency was reported in the plants received 4 ppm of CuO NPs. (Fig.2 C-F). Similarly, Govorov and Carmeli, (2007) shows that metal nanoparticles have the ability to increase the photosynthesis efficiency. In another study, Carmeli et al.(2010) reported that metal nanoparticles increase the absorption of light by the protein of photosystem I (PS I). PS I is the most efficient light harvesting complex. So, by increasing the absorption of light by these complex systems photosynthesises is supposed to increased (Fig. 2). Cerium oxide nanoparticles (CeO_2) also increase the photosynthesis and water use efficiency in soybean plant (Cao et al. 2017). All these finding suggest that CuO NPs improve the photosynthetic rate of mustard plant by improving the Rubisco activity or CA activity (Fig. 2).

Antioxidant enzyme play defensive role in plant and mitigate the any adverse effect on plants. CAT, POX and SOD are very important antioxidant enzyme to destroy the reactive oxygen species (ROS) which are released during stress condition. CAT is the first antioxidant enzyme discovered and reported in detoxification of ROS under stress condition(Garg and Manchanda, 2009). Increased activity of these enzyme improved the overall growth of plant by decreasing the ROS and other harmful radicals. In present study it was quite clear that 4ppm of CuO NPs when given to soil increases these antioxidant enzyme activities which further enhances the plant growth and development (Fig. 3 C-E). Our finding is also similar to others study where increased value of antioxidant enzyme under NPs have been reported(Sharma et al., 2012; Siddiqui et al., 2014). Increased activity of SOD and CAT due to NiO_2NPs treatment have been reported in tomato plants (Faisal et al. 2013). CeO_2 and In_2O_3nanomaterials increases the antioxidant enzyme activity and improve the plant growth and defence mechanism in Arabidopsis plant (Ma et al. 2016). Our result are in agreement with the Costa and Sharma, (2016), who reported enhanced activity of SOD at 10 and 100 $mgL^{-1}$by CuO NPs in rice seedling and Regier et al.(2015) who reported increased peroxidase activity due to CuO NPs. Proline is an osmo protectant and work as a non-enzymatic antioxidant system. Proline levels increase under stress condition to hinder the any harmful effect caused by production of ROS during stress. In the present study it has been reported that plant received 4 ppm of CuO NPs possesses maximum levels which improve the plant growth by reducing the stress caused by CuO NPs (Fig.3 F). Similarly, when plant grown in MS media supplemented with ZnO NPs shows the increased proline levels and other antioxidant enzyme activity (Helaly et al. 2014). Tomato seedling treated with different concentration of ZnO NPs shows the enhancement of proline which improve the photosynthetic efficiency (Faizan et al. 2017). Nanoparticles have ability to overcome the abiotic stress. Plant grown under salinity stress shows the increased proline content and antioxidant enzyme by the application of TiO_2 NPs which render the toxic effect of salinity on plant and improve the growth performances of broadbean(Abdel Latef et al. 2017).Siddiqui et al.(2014) reported that $nSiO_2$increased the proline content under salinity stress condition. All these study in agreement with our study and stabilize our result of CuO NPs which can apply to improve the plant growth.

CONCLUSION

It is concluded from the present observation that out of the various concentration, 4 ppm of CuO NPs proved best and enhanced the most of the parameters. Therefore, it is recommended that 4 ppm of CuO NPs can be used as soil treatment for the better performances of mustard plant.

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