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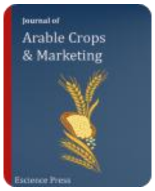

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Foliar Application of Zinc Oxide (ZnO) Nano Fertilizer Improves the Growth and Physiological Attributes of Pumpkin (*Cucurbita moschata*)

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ABSTRACT

Zinc is an essential micronutrient required in trace amounts by all living organisms for their natural growth, development and reproduction. It serves as a cofactor for various enzymes and proteins involved in several physiological and metabolic pathways. Low availability of the soluble form of this micronutrient (Zn2+) causes Zn deficiency in plants and related food chains. Foliar fertilization is an effective way to conquer the problem and improve zinc content in plants. Study was performed to determine the effect of foliar application of zinc oxide nanoparticles on the growth and yield of Cucurbita moschata (var. Banochi). Three-week-old seedlings of the plant were subjected to different doses of zinc oxide nanoparticles (T0= control, T1= 25ppm, T2= 50ppm and T3=100 ppm) four times (in an interval of fifteen days) via foliar spray for 100 days. The results showed that a 50-ppm optimum dose of ZnO NPs favour the plant growth by significantly improving shoot length (6.1 feet), root length (4.3 cm), dry weight (shoot-12.21 g; root – 6.31 g) and seed weight (1.16 times more than control). The maximum photosynthetic rate (1.21 folds), chlorophyll content (1.5 folds), transpiration rate (1.2 folds), and stomatal conductance (1.2 folds) were also recorded at this concentration. However, a higher concentration of nanoparticles (100 ppm ZnO NPs) reduces these attributes. Thus, the finding for the first time suggested the optimum dose of nano zinc to be applied as fertilizers for obtaining maximum growth and yield from pumpkin.

Keywords: Micronutrient deficiency; nano fertilizers; Zinc oxide nanoparticle; foliar spray; *Cucurbita moschata*

INTRODUCTION

Micronutrient starvation also known as hidden hunger is one of the major problems worldwide (Veena and Puthur, 2022). The deficiency is more common in developing countries including Pakistan where cereal crops such as maize, wheat, rice etc. provide maximum calories to more than half of the population and diets are destitute in essential nutrients. Among the four major micronutrient deficiencies (iron, zinc, vitamin A and iodine), zinc has emerged as the most widespread deficiency which globally affect nearly 2 billion population (Younas et al., 2022; Müller and Krawinkel, 2005). In developing nations like Pakistan zinc malnutrition affects more than 37 % of the population and majority of them are pregnant women and young children under five years. Therefore, zinc deficiency is presently the fifth prime cause of illness and mortality in the country (WHO, 2002).

Zinc is an essential micronutrient required in trace quantity by all living organisms for their natural growth, development and reproduction. It serves as a cofactor for various enzymes and proteins involved in several physiological and metabolic pathways. It is the single element, present in all six classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases) (Majumder et al., 2019) which catalyzes the major molecular processes in biological organisms such as DNA replication, transcription, translation, DNA repair and cell signaling (Khan et al., 2022). Zinc is equally important for plant growth and physiology. Zinc is an integral component of carbonic anhydrase and hydrogenase which are involved in carbohydrate metabolism. It also plays an important role in cytochrome synthesis, regulating auxin synthesis, cell membrane integrity ribosomal, fraction stabilization, pollen formation and resistance to plant pathogens. Previous study on exogenous application of zinc to crop plants demonstrated that critical concentration of Zn2+ improves crop yield and quality. Moreover, adequate supply of Zn enhances plant resilience towards environmental stresses by modulating physiological and biochemical functioning of plants, upregulating antioxidants activities and displacing redox active metals ions (iron and copper) (Suganya et al., 2020; Prasad, 2014).

There are multiple factors associated with soil, plants and environment, which are causing Zn deficiency in plants and indirectly humans. The poor availability of soluble form of zinc (Zn2+) in soil is the foremost reason of this micronutrient deficiency in plants and related food chains. Approximately, 1/3rd of the world soil are zinc deficient (Khan, and Malik 2022). Soil factors such as pH, texture, salinity, temperature etc. is the other cause which affects Zn solubility and its uptake by plants. The calcareous and sandy soils having alkaline pH (>6.5) reduces the Zn availability to plants. If zinc fertilizers such as zinc sulphate is applied to such type of soils (having high pH) they easily precipitate to form insoluble complexes like ZnO, Zn(OH)2, ZnS etc. thus, reduces its availability to plants. The precipitation of zinc sulphate to ZnS was studied by Alloway (2008) in paddy field under highly reduced condition. Foliar fertilization technique is an effective way to overcome this problem and improve zinc content in plants which ultimately increase crop yield and productivity. Boonchuay et al. (2013) studied the impact of foliar application of zinc on Oryza sativa. The study observed that foliar zinc significantly enhances the micronutrient content in rice grains and the greater increase was recorded after repeated application (Boonchuay et al., 2013).

Similarly, foliar application of iron, copper and zinc improve the yield of Gossypium herbaceum (Toor et al., 2020). Nanoform of micronutrient is prefered over their bulk form to promote plant growth and nutritional quality due to its improved solubility, bioavailability to plants, slow-release function, controlled supply, high penetration ability, easy to handle and transport. Moreover, nanofertilizers is a great alternative to decrease the over-application of conventional fertilizers. Raliya and Tarafdar (2013) studied the effect of ZnO NP in improvement of growth and phosphatase enzyme activity in Cyamopsis tetragonoloba. Similar effect of Fe NPs in Glycine max was reported by Ghafariyan et al., (2013). Therefore, the present study aimed to assess the impact of foliar application of nano zinc on the growth and physiology of *Cucurbita moschata* (pumpkin).

MATERIALS AND METHODS

Experimental site and its analysis

The present study was conducted at the Research Centre of the College of Agriculture, University of Sargodha (Latitude: 32.1339617 and Longitude: 72.6868086) a semi-arid region in Punjab, Pakistan. For soil analysis, sandy loam soil was collected from the experimental site and processed in the laboratory. Soil impurities were removed by seive, and soil characteristics were analysed by standard protocol. The pH and electrical conductivity (ECe) were estimated by the protocol mentioned in Handbook 60 (1954). The pH and ECe of experimental soil are 8 and 2.11 dS/m respectively. The soil texture was studied by the hydrometer method (Bouyoucos 1962). Moreover, the Zn content in the soil was evaluated by atomic adsorption spectrophotometer, and the amount obtained was 0.51 ppm.

Zinc oxide (ZnO) nanoparticles and their preparation

The size of ZnO NPs was 20-30 nm purchased from Alfa Aesar (Thermo Fisher Scientific). The nanoparticles were highly pure and had a density of 5.606 g/cm3. The solutions of ZnO NPs were prepared in deionized water. For better dissolution, the solutions of nanoparticles were placed on a stirrer for 30-40 minutes.

Plant material and its processing

Seeds of Cucurbita moschata (pumpkin var. Banochi) were purchased from the local market of Sargodha, Pakistan. After surface sterilization, seeds were sown in pots containing two doses of NPK (100 and 50 kg/ha). After three weeks of germination, the ZnO NPs were applied to the seedlings via foliar application in different concentrations (25, 50 and 100 ppm). The seedlings treated with distilled water (0 ppm) were considered as control. The above treatments in the study were denoted as T0= control, T1= 25ppm, T2= 50ppm and T3=100 ppm of ZnO NPs. Foliar application of ZnO NPs were done four times throughout the experiment with an interval of fifteen days. The experiments were conducted in the control chamber at the Department of Soil & Environmental Sciences, University of Sargodha, Pakistan. The plants were harvested after 40 days of treatments to evaluate different growth and physiological parameters.

Growth parameters

Growth parameters were determined by measuring shoot length, root length and dry weight. The length of shoots and roots of both controlled and treated plants were measured in cm using a measuring tape. For calculating the dry weight, fresh shoots and roots after separation were placed at 80°C for 48 h in a hot air oven for drying and the weight of the dried tissue were measured.

Physiological parameters

For the measurement of gas exchange attributes such as net photosynthesis rate (Pn), stomatal conductance (gs), and transpiration rate (E), three fully developed and healthy leaves per plant from each treatment were selected and placed individually in the chamber of the Infrared Gas Analyzer (IRGA; Analytical Development Company, Hoddesdon, England). These physiological attributes were measured during the day time (between 10 AM to 12 noon) at a molar flow of air per unit leaf area of 403.3 mmolm-2s-1, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 bar, PAR at leaf surface was maximum up to 1711 molm-2s-1, leaf temperature ranged from 28.4 to 32.4°C (Zekri 1991). Portable equipment (Model: SPAD-502; Konica Minolta, Japan) was used to calculate chlorophyll content (Almansoori et al. 2021). Total chlorophyll content was measured using Minolta SPAD 501 chlorophyll meter.

Statistical analysis

Data were statistically analysed by one way ANOVA using statistix version 8.1software. Data were considered as mean (± standard deviation) of individual replicas (n=4) of every test conducted separately. To check the reproducibility of results, four independent biological replicates were studied following Completely Randomized Design (CRD). Comparison of means was done using Least Significant Difference (LSD) test (Steel et al., 1997). The signifcant variations among treated and control plants were illustrated at means was compared at $P \leq 0.05$ probability level by using the statix 8.1.

RESULTS

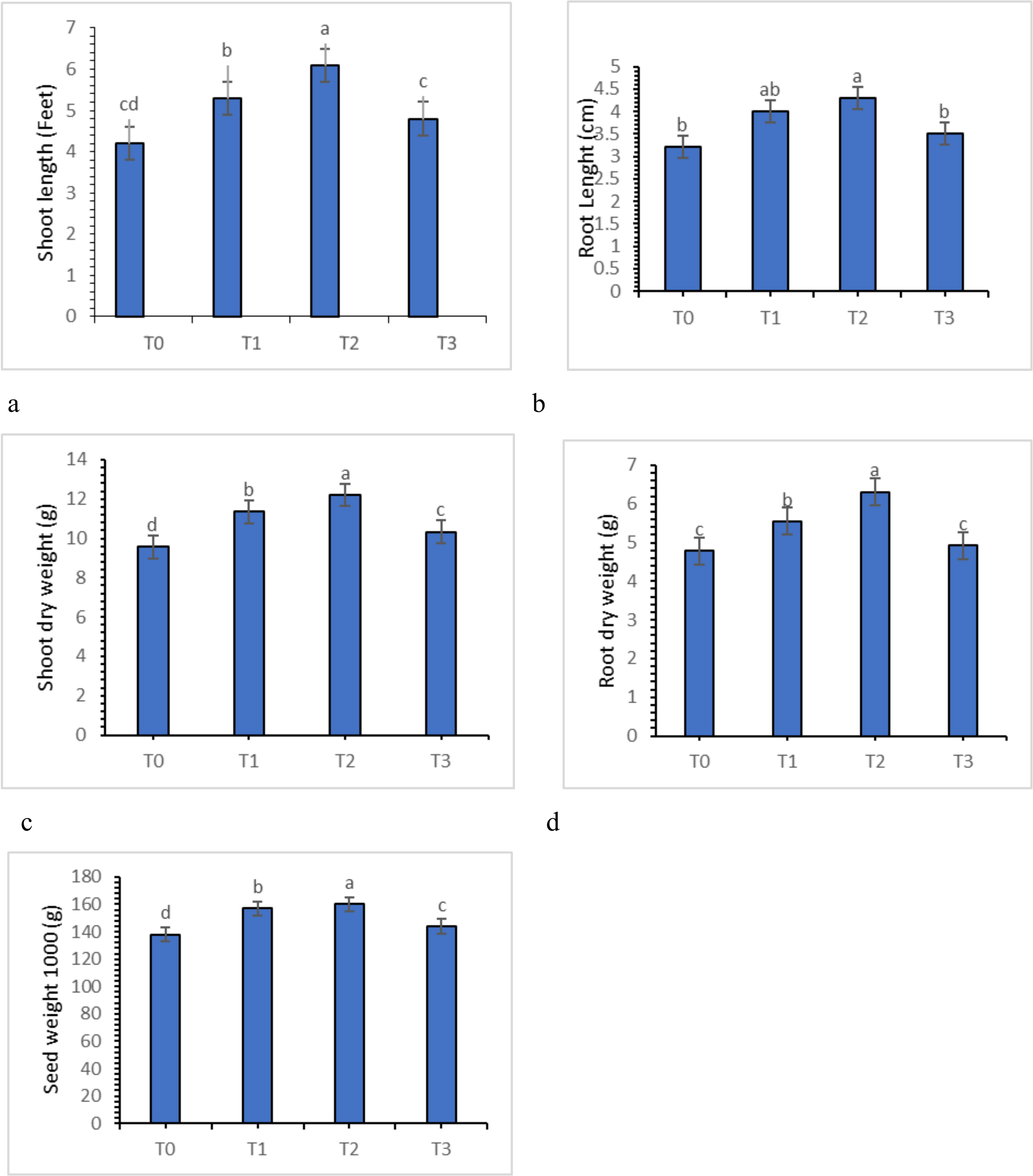
Growth attributes

As evident from Figure 1 growth parameters increases with rise in ZnO NPs dose and the maximum increase was recorded at 50 ppm treatment. Foliar spray of ZnO NPs at 50 ppm concentration was found to enhance the shoot and root length to 6.1 feet and 4.3 cm which were 21 and 20 times more in comparison to control (Figure 1- a&b). Similarly, threshold value of both shoot and root dry weight was recorded in plants treated with 50 ppm of ZnO NPs which were 12.21 g (shoot weight) and 6.31 g (root weight) respectively (Figure 1-c&d).

Effect of foliar application of ZnO nanoparticles on plant yield was also studied by measuring the seed weight. Our results showed that the weight of seeds improve on application of nanoparticles and the maximum increase was recorded at 50 ppm concentration which was 1.16 folds more than control (Figure 1-e).

Physiological attributes

The effect of ZnO NPs on different physiological parameters was shown in Figure 2. Chlorophyll content and photosynthetic rate significantly increase with rise in nanoparticle concentration till 50 ppm and then decreases with further rise in ZnO NPs concentration. At 50 ppm ZnO NPs the chlorophyll content and photosynthetic rate were 1.5 and 1.21 times more than control (Figure 2- a&b). Similar pattern of results were obtained with other photosynthetic parameters (transpiration rate and stomatal conductance). Foliar application of 50 ppm ZnO NPs increases transpiration rate and stomatal conductance by 17.5 % (transpiration rate) and 15.7 % (stomatal conductance) respectively in comparison to control (Figure 2-c&d).



a

b

c

d

e

Figure 1. Impact of zinc oxide nanoparticles on the plant growth parameters of pumpkin a) Shoot length, b) Root length, c) Shoot dry weight d) Root dry weight and e) weight of 1000 seeds (g). Values are mean (n= 4) ± standard deviation. Data points marked with the different letters show significant differences in means at P ≤ 0.05 probability level within treatments.

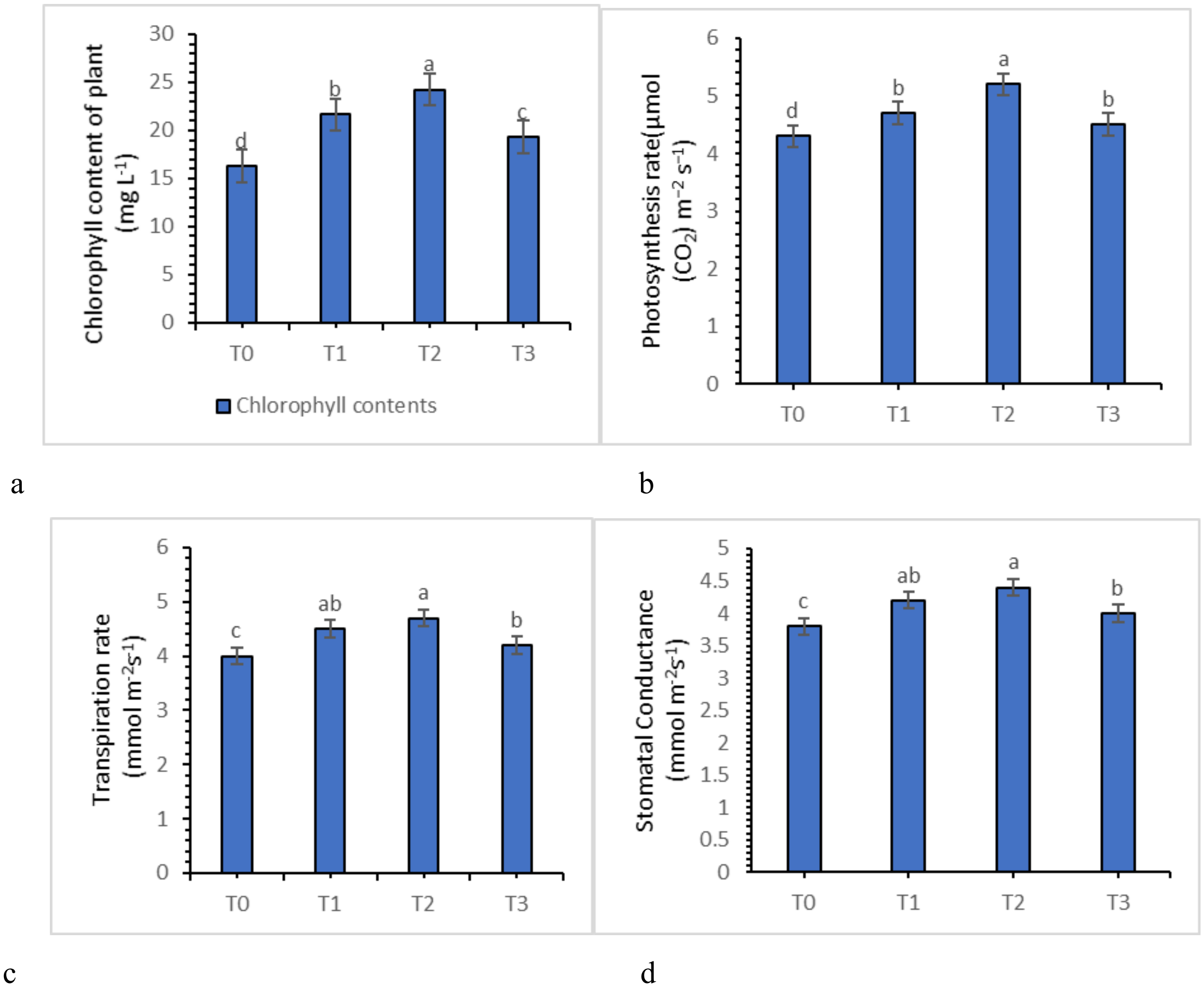


Figure 2. Impact of zinc oxide nanoparticles on photosynthetic parameters of pumpkin. a) Chlorophyll content b) Photosynthesis rate c) Transpiration rate d) stomatal conductance. Values are mean (n= 4) ± standard deviation. Data points marked with the different letters show significant differences within treatments at $P \leq 0.05$ probability level.

DISCUSSION

Nano- fertilizer used in agriculture sector

Over the past few decades, research on the intimation of nanotechnology in the agriculture sector has increased tremendously. (Lowry et al., 2019, Kah et al., 2019). The significant use of nano-based products such as nano fertilizers, nano pesticides and nanotools for improving plant yield, productivity, plant's ability to uptake soil nutrients and maintaining rapid disease diagnosis is a key factor to achieve sustainable agriculture development particularly in developing countries (Adil et al., 2022). The present study is therefore based on the impact of nano zinc fertilizers on growth and yield of Cucurbita moschata.

Effect of ZnO NPs on plant growth

From the results of the recent study, it is observed that foliar application of zinc oxide nanoparticles at appropriate concentration (50 ppm) considerably increase the height, biomass, yield and photosynthetic parameters of the plant compared to control. The other studies conducted on different crop plants found similar effect of nano zinc in improving growth and productivity of plants (Zhang et al., 2021, Ahmad et al., 2020, Dimkpa et al., 2020). Appropriate dose of nanoparticle is necessary for the growth of plant. In the current study, the optimum concentration of ZnO NPs which favors plant growth is 50 ppm, where all the growth and physiological parameters were found to be higher

(Figure 1&2). Further increase in NP treatment results in an overall reduction in plant growth. These outcomes were in accordance with the study conducted by Salachna et al. (2021), which shows a dose–dependent effect of ZnO NPs on the leaf yield and quality of Perilla frutescens. The study showed that lower concentration (50 and 100 ppm) of ZnO NPs improve plant biomass, anthocyanin content, antiradical and bacteriostatic activity while higher concentration reduced these attributes (Salachna et al., 2021). The improved growth in pumpkin plant at 50 ppm ZnO NPs is due to the fact that zinc oxide nanoparticles by foliar spray made slow and target release of fertilizer that enhance the fertilizer utilization efficiency and Zn availability to plants (Adil et al., 2022). Similar enhancement in growth rate due to increase in fertilizer utilization efficiency were recorded in maize cultivars subjected to variable concentrations of ZnO NPs (Azam et al., 2022). The decline in growth rate at higher nanoparticle treatment (100 ppm) might be due to the dissolution of Zn2+ (Mousavi et al., 2015) as high concentration of Zn2+ negatively affected the plant physiology by disturbing the mass flow, chloroplast organization and chlorophyll content (Rao and Shekhawat 2014).

CONCLUSION

Results from the study concluded that zinc nano fertilizer has a positive effect on plant height, shoot and root dry weight, and photosynthetic parameters of pumpkin (*Cucurbita moschata*) at lower concentrations (50 ppm ZnO NPs). However, an increase in concentration decreases these physiological attributes. Hence, our study recommended the optimum dose of ZnO-NPs that can be used as nano fertilizer per acre for pumpkin crops on a commercial basis.

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