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Original article

Effect of calcium oxide, zinc oxide nanoparticles and their combined treatments on growth and yield attributes of *Solanum lycopersicum* L.

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ABSTRACT

Tomato (*Solanum lycopersicum* L. Syn *Lycopersicon esculentum* Mill.) occupies a significant position among vegetable crops. With the increasing population, the food supply is going to be short and will be causing a major problem. Therefore, the enhancement of crop production is necessary for food security. To enhance nutrient efficiency and yield in an agricultural field, nanotechnology can play a key role. In the present study, zinc oxide (ZnO) and calcium oxide (CaO) nanoparticles (NPs) were synthesized by following green synthesis i.e., by using water extract of *Nigella sativa* seeds. These particles were characterized by UV visible spectroscopy and particle size analyzer. A UV–visible spectrum of ZnO and CaO NPs showed absorption peaks at 230 nm and 220 nm respectively. The particle size of 50–60 nm and 5 to 10 nm was observed for ZnO and CaO nanoparticles respectively. Sterilized seeds of tomato were germinated and grown in pots, which were treated with foliar spray of ZnO, and CaO nanoparticles in various concentrations (50, 100, 200 ppm) with three replications separately. The effect of ZnO, CaO, and CaO + ZnO NPs was observed by measuring vegetative growth parameters (i.e. plant length, number of leaves/plant, leaf area/plant, fresh and dry weight of the whole plant) and biochemical parameters (i.e. titratable acidity, total phenolics and zinc and calcium metal content). Results revealed that nano-spray of CaO and ZnO NPs gave positive results on the physiological parameters of tomato plants. A combined effect of ZnO and CaO on *S. lycopersicum* was significantly higher as compared to the separate treatments of ZnO and CaO. The combined effect of ZnO + CaO with the concentration of 50 ppm gave positive results in shoot length, number of shoots, number of roots, yield/plant, fruit weight, and leaf area. Whereas, root length, plant weight, and fruit diameter were higher at 200 ppm of ZnO + CaO. Biochemical parameters were also improved by using the nano-spray application as CaO < ZnO < ZnO + CaO respectively. The research proved to be cost-effective, less toxic, and eco-friendly which will also be beneficial for future perspectives.

1. Introduction

A higher agricultural yield is a demand for the persistently rising population of the world and advanced agricultural strategies are also the major requirement for maintaining agricultural output. World agricultural system uses a great variety of fertilizers, herbicides, and pesticides for obtaining large production per unit area, but these chemicals also lead to severe problems such as environmental pollution. On the other hand, there is also a major issue to sustain agricultural output for the continually growing world population (Umesha et al., 2018). In the future, there is a need to adopt

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some methods which help us to overcome these problems. Nano fertilizers, pesticides, and herbicides are the better replacement in the agricultural systems for better nutrient and pest management because these nano-size particles have the high surface area and penetration capacity which keeps away such environmental residues as these nanoforms can be used in controlled amounts (Joseph and Morrison, 2006). The field to manipulate the atoms at the nanoscale to achieve beneficial products is "Nanotechnology". The reduction of environmental problems, lesser energy consumption, and reduced cost are also some other positive points of nanotechnology. The present scenario of food demand worldwide, urges scientists to develop new methods for nanoparticle synthesis; among which green method integrated biological principle (oxidation/reduction) by plant phytochemicals or microbial enzymes with different methods are acceptability (Shehata et al., 2016).

Calcium (Ca) plays an important role in maintaining the cell function, and constituents of plant tissues (Elmar et al., 2007). Calcium ions are also responsible for the regulatory effect of fruit ripening (Singh et al., 2007). In plants, calcium content can be enhanced, by direct contact with calcium in a faster way because it is an immobile element and cannot move from part to part easily in plants (Manganaris et al., 2005). There are reports of application of nanocalcium phosphate on various plants but there is not reported work on the application of nano-calcium on tomato (*S. lycopersicum*) as nanospray till date. Furthermore, the present study also includes the combined treatments of two nano-supplements to plants and studying their responses. It also increases the worth of present research.

Zinc (Zn) is also a vital micronutrient in all plants, which takes part in the synthesis of proteins, chlorophyll, and enzymes; it is also a part of numerous cellular processes and also responsible for the synthesis of phytohormones i.e. auxin, cytokinin, gibberellin, ethylene, and abscisic acid (Singh et al., 2018). Zinc nanoparticles are the most commonly used nano-sized particles in many fields, especially in medicine, cosmetology, and agriculture.

Solanum lycopersicum L. (tomato) is a member of the family Solanaceae (nightshade). It is grown worldwide due to its nutritional and medicinal value. It is rich in nutrients like potassium, carbohydrates, and ascorbic acid along with various antioxidants like lycopene and tocopherols. Tomato fruit has many health benefits including a reduction in the risk of heart disease, and cancer (Renna et al., 2018). According to an estimate, 61.1% of tomato is produced in Asia, but the growing population of this region and the decrease in agricultural land has created real problems for this crop. It is also a fact that the quality of tomato fruit and its metabolite composition is significantly affected by its growing conditions (Diouf et al., 2018). Production of tomato overall is suffering from the problem of climate change and various resultant stresses, due to which production of tomatoes is decreasing but its demand is increasing. Therefore, there is a demand for methods and techniques to increase the production of tomatoes within the present soil and land resources.

In this field, nanoparticles can play their role as they have a smaller size and larger surface area as compared to their respective bulk metals and metal oxides. Iron, zinc, and calcium salts are already in use to reclaim various problems of soil. In future, replacement of these metal salts with their respective nanoparticles can increase their efficiency to give more crop production with better nutrient profile of fruits, thus increasing the value of fruits produced. Furthermore, these nanoparticles when efficiently absorbed by plants maybe a part of their fruit and can work as functional food, as iron, zinc, and calcium, etc. are also essential for normal human growth and balanced human metabolism. Zinc is a vital element, essential for the function of almost 300 enzymes. The use of ZnO NP's improves the eye vision, reduce the risk of cancer, treatment of diabetes, and mend the impaired immune function. Calcium is basic mineral of bone, teeth and muscle health. Adequate calcium intake ensures the healthy metabolism of other nutrients like phosphorous, vitamin D and protein. Calcium has also been reported to reduce the aging in human (Skalnaya and Skalny, 2018; Beto, 2015).

The present study was, therefore, aimed to check the effects of bio-fabricated nanoparticles (ZnO and CaO) individually and in combined form, on enhancing the growth and yield of tomato plants.

2. Materials and methods

The study was conducted in the Biochemistry lab and Botanical Garden of the Botany Department, Lahore College for Women University, Lahore, Pakistan. The seeds (*Nigella sativa* L.) were bought from a local market in Lahore, and properly washed with distilled water to remove fine dust particles, dried for 3 to 4 days, then ground into a fine powder which was further used for extraction. Tomato seeds were purchased from a local nursery in Lahore. Surface sterilization for seeds was done by soaking them in 1% sodium hypochlorite solution (NaClO) for 5 min, then rinsed with deionized water 2 to 3 times and were used for further experiment.

2.1. Bio-fabrication of nanoparticles

For the synthesis of metallic nanoparticles, plant extract was obtained by using microwave-assisted extractor (Saneo, MDS-6G). Seeds were extracted by microwave-assisted extraction using water as a solvent, solvent system and other parameters were opted by slightly modifying the method of Ma et al (Ma et al., 2020). The resultant mixture was filtered with the help of the Whatman filter paper. The extract with maximum phytochemicals was selected for zinc and calcium nanoparticle synthesis, which was stored in the refrigerator for future use.

For the synthesis of nanoparticles, 0.1 M stock solution of zinc sulfate was prepared. The *Nigella* seed extract was mixed in zinc sulfate solution at a volume ratio of 3:1 with continuous stirring. The resultant solution was poured into centrifuge tubes and centrifuged at 12000 rpm for 15 min. The supernatant was discarded and ZnO NPs pellets were washed with distilled water and dried overnight at room temperature. Same procedure was followed for CaO NPs synthesis by using $CaSO_4 \cdot 2H_2O$ solution and *Nigella* seed extract as raw materials. The characterization of nanoparticles was done by UV–visible spectroscopy (Hitachi U2900) within the range of 200–700 nm and particle size analysis was done by using the Beta size analyzer (BT 90).

2.2. Seed germination and pot experiment

The seedlings were grown in the Botanical garden at Lahore College for Women's University, Lahore (December 2020 to April 2021). The seeds were sown into pots (4–5 inches) for germination. Healthy and uniform seedlings were transferred to larger pots for further growth. A pot experiment was completely based on a Randomized design with three replications. In each pot, 3–5 seedlings were grown.

2.3. Treatment of nanoparticles (Foliar application)

The pots were treated with ZnO and CaO nanoparticles. The experiment consisted of three groups with different concentrations and doses which are as follows (pH of foliar spray was maintained between 4.5 and 4.8 as it ensures the optimized absorbance of foliar sprays like that of ZnO NPs).

Group I: Foliar spray of ZnO nanoparticles was applied with the concentration of 50, 100, and 200 ppm.

Group II: Foliar spray of CaO nanoparticles was applied with the concentration of 50, 100, and 200 ppm.

Group III: A combination of both ZnO + CaO was applied by foliar spray with the concentration of 50 + 50, 100 + 100, and 200 + 200 ppm.

Group I, II, and III were treated along with positive control ($ZnSO_4$ and $CaSO_4$) and negative control (distilled water). The first foliar spray was given after 20 days of seedling emergence, and repeated after every 10th day. Sprays were given to plants at dawn time. After 100 days of growth, plants were observed for vegetative and biochemical parameters. Loamy soil was used for growing tomato plants where NPK was used at the time of sowing of seedlings and after 50 days of growth. Plants were watered regularly according to the requirement.

2.4. Analysis of morphological traits

Different morphological parameters were measured and recorded during plant growth. Traits that were measured included height of the plant, leaf area/plant, plant weight, number of shoots and roots, root length, yield per plant, and fruit weight. Three replicas were selected from each treatment and measured by using standard methods (Awan et al., 2021).

2.5. Biochemical analysis

Following biochemical analysis was performed.

a. Total Phenolic Content

The determination of total phenols was done by using a standard Folin-Ciocalteau (FC) assay with slight modifications. The phenols were measured by mixing 1.40 mL of FC reagent with 7.0 mL of methanol extract. 7.0 mL of $NaHCO_3$ was also added to the solution after three minutes. Then the solution was allowed to stay for 30 min at room temperature. The absorbance was measured at 750 nm by UV–vis spectrophotometer. The results were expressed in mg of Gallic acid equivalents (GAE)/ gm of the sample (Awan et al., 2021).

b. Determination of Calcium and Zinc Content in Fruits

Microwave-assisted digestion method with a standard protocol was followed for calcium and zinc analysis of tomato. 2.0 g of grounded fruit sample was weighed and added in 6 mL of 65% HNO_3 and 1 mL of 30% H_2O_2. Then poured into a Teflon vessel and placed in the microwave extractor. The microwave was set to the required temperature of 130, 150, and 180℃, power level (600 W), and time (10, 5, and 10 min) and started to digest the material. After digestion, the sample was filtered with the help of whattman filter paper into the flask and kept for metal analysis. Atomic absorption spectroscopy (AAS) was used for the detection of Zn and Ca content from tomatoes.

c. Titratable Acidity (TA)

The material was measured through the titration of fruit juice via 0.1 N NaOH with pH up to 8.2, using 1 mL juice diluted with 10 mL of distilled water, and expressed as the percentage of malic acid (Ranjbar et al., 2020).

$$\%acid = [NaOH\,vol]x[NaOHnormality]x[milliequi\,valentfactor]x[100]/sample\,vol$$

2.6. Statistical analysis

The data was interpreted as the mean standard deviation (±SD). Data obtained was analyzed by analysis of variance (ANOVA-One way) by using SPSS software. Each experimental value was compared with the control. Duncan's multiple test was used to analyze and describe the significant difference in mean values.

3. Results and discussion

3.1. Characterization of ZnO and CaO nanoparticles:

Initial characterization of synthesized nanoparticles with the help of a UV–visible spectrophotometer is quite interesting as their optical properties are proportional to their size and shape. In Fig. 1a and b, the UV–visible spectrum of ZnO nanoparticles is shown with the highest absorbance peak of 3.86 visible at 230 nm while the highest peak of the CaO NPs with an absorbance value of 3.25 visible at 220 nm. Our results are in line with our previous reports to optimize nanoparticles that were used as fertilizers for *Brassica oleracea* Italica (Awan et al., 2021). Tondey *et al.* reported a sharp peak of ZnO NPs at 210 nm purposing that ZnO NPs have monodispersed nature (Tondey et al., 2021). Similarly, Elrefaey et al., reported ZnONPs absorption peak at 269 nm (Elrefaey et al., 2022). In the same way, the green synthesis of calcium oxide nanoparticles absorption spectrum was reported by Eram *et al.*, at 267 nm within the range of 200 to 800 nm (Eram et al., 2021).

This may be attributed to the electron transition from the valence to the conduction band. It is suggested that free electrons present in nanoparticles absorb light and jump into higher energy shells, where they are quite unstable. Therefore, they release a photon and come back to their stable low-energy state. This resonance frequency of nanoparticles also depends upon their size, nature, and shape. The absorption at lower wavelength ranges indicates the formation of small-size nanoparticles (Aldalbahi et al., 2020).

These nanoparticles were also characterized by a laser beam particle size analyzer. The results of particle size analysis for zinc oxide nanoparticles showed that 24% of particle distribution was in the range of 500–600 nm and a few were in the size range of less than 50 nm (Fig. 1c). It also indicated that most of the particles in the aqueous dispersion had uniform nano-scale dimensions. The results of particle size analysis for calcium oxide nanoparticles showed that 35% of particle distribution was in the range of 5–10 nm and a few less than 50 nm as shown in (Fig. 1d). When metal and metal oxide nanoparticles are formed purely from metals, their large interactive surface area possesses great characteristics but they also cause agglomeration of particles to each other and result in an increase in the size of particles. In such a situation, stabilizing agents are required to cap the surface of nanoparticles to keep them in small nano size. Plant secondary metabolites here work as capping agents or stabilizing agents which cap the large and highly interactive surfaces of nanoparticles to make them stable (Gur et al., 2022).

3.2. Evaluation of growth parameters

Foliar application of ZnO, CaO, and ZnO + CaO nanoparticles on tomato plants had different effects at different concentrations as shown in Table 1 and Fig. 2. In CaO NPs treated plants, shoot length varied from 12 to 13.5 cm whereas in ZnO NPs treated plants, shoot length ranged from 11 to 12.9 cm. Overall, CaO NPs had a positive effect on the shoot length of *S. lycopersicum.* The effect of nanofertilizers was ZnO < CaO < ZnO + CaO respectively. Combined

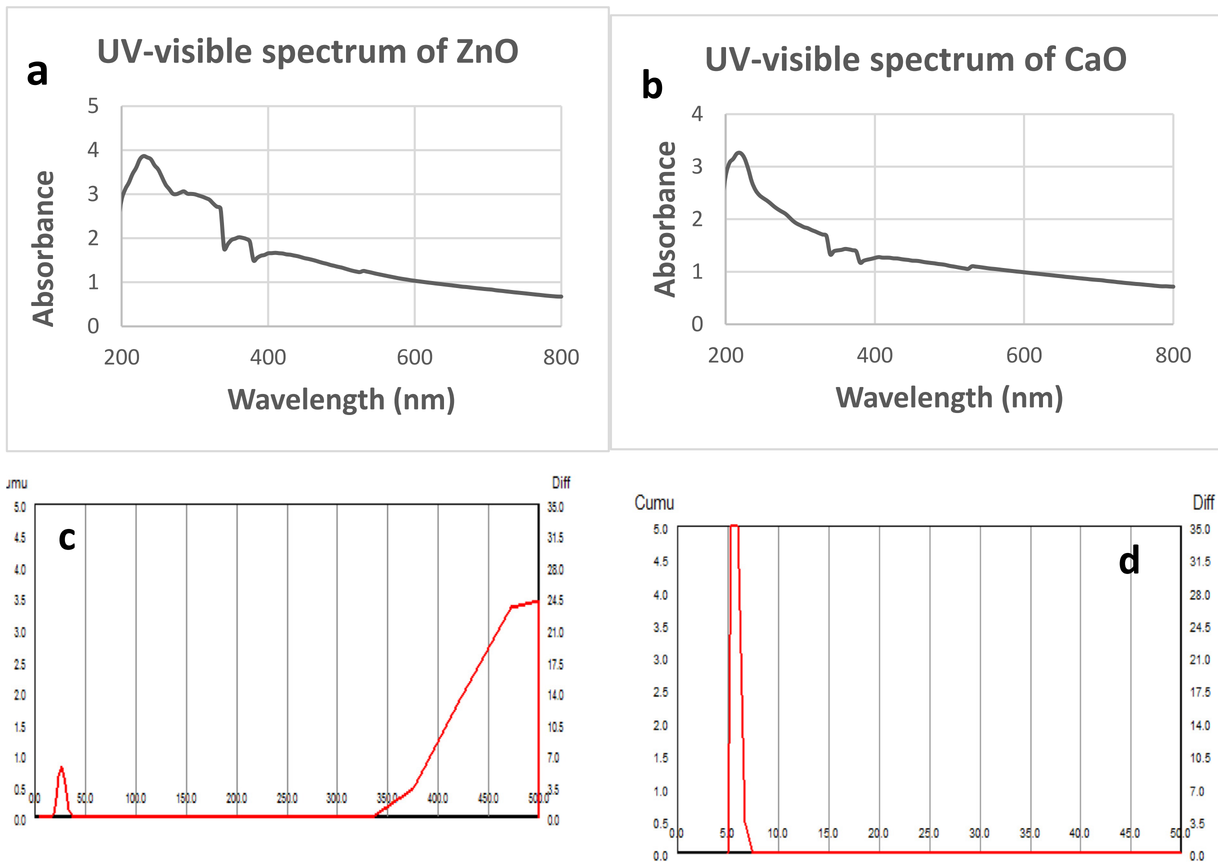

Fig. 1. UV–visible spectrum of a) ZnO NPs b) CaO NPs; Particle size of c) ZnO NPs d) CaO NPs.

Table 1
Effect of ZnO NPs on growth of tomato plant.

Sr. No.	Treatment	Shoot length (cm)	Root length (cm)	No. of shoots	No of roots	Leaf area (cm^2)	Fresh plant weight (g)	# of fruits per plant	Fruit Diameter (cm)	Weight of tomatoes/plant (g)
ZnONPs	50	$12.4^{ef} \pm 0.4$	$9.33^{cde} \pm 0.6$	$11.3^{c} \pm 0.6$	$35.7^{de} \pm 1.5$	$13.8^{b} \pm 0.7$	$31.1^{d} \pm 1.01$	$4.0^{e} \pm 0.1$	$3.4^{bc} \pm 0.21$	$86.7^{e} \pm 5.8$
	100	$12.1^{f} \pm 0.3$	$10.5^{bcd} \pm 0.5$	$9.3^{ef} \pm 0.6$	$35.3^{de} \pm 1.5$	$13.0^{bc} \pm 0.8$	$31.1^{d} \pm 1.03$	2.3 ± 0.6	$3.4^{bc} \pm 0.5$	$98.3^{de} \pm 9.6$
	200	$12.6^{cde} \pm 0.6$	$11.5^{bcd} \pm 0.5$	$7.3\ ^{g} \pm 1.5$	$38.0^{b} \pm 1.0$	$12.8^{bc} \pm 0.1$	$32.3^{d} \pm 2.1$	2.7 ± 1.2	$3.8^{b} \pm 0.3$	$103.0^{d} \pm 9.5$
Zn salt		$12.0^{cde} \pm 0.1$	$10.7^{bc} \pm 1.5$	$10.3^{cde} \pm 0.6$	$36.7^{b} \pm 1.2$	$12.1^{a} \pm 0.4$	$30.0^{c} \pm 1.7$	$2.3^{d} \pm 1.5$	$3.1^{bc} \pm 0.3$	$96.7\ ^{cd} \pm 5.3$
CaONPs	50	$12.5^{def} \pm 0.5$	$8.5^{de} \pm 0.5$	$9.7^{ef} \pm 0.6$	$33.0^{e} \pm 2.0$	$10.5^{d} \pm 1.1$	31.3 ± 1.5	$6.0^{c} \pm 1.7$	$3.2^{c} \pm 0.4$	$207.7^{b} \pm 8.3$
	100	$12.8^{cde} \pm 0.3$	$9.5^{cde} \pm 1.3$	$10.7^{cde} \pm 0.6$	$33.3^{e} \pm 1.5$	$11.3\ ^{cd} \pm 1.1$	31.3 ± 1.2	$3.7^{e} \pm 1.5$	$3.0^{c} \pm 0.1$	$157.8^{bc} \pm 21$
	200	$13.1^{ab} \pm 0.4$	$10.0^{bcd} \pm 1.0$	$9.0^{f} \pm 1.0$	$37.0\ ^{cd} \pm 1.7$	$10.1^{d} \pm 0.4$	$32.0^{d} \pm 2.0$	$3.3^{ef} \pm 0.6$	$3.3^{c} \pm 0.4$	$130.3^{c} \pm 7.5$
Ca salt		$12.0^{cde} \pm 0.2$	$9.0^{b} \pm 0.5$	$9.3^{b} \pm 0.6$	$31.0^{ab} \pm 1.7$	$10.0^{c} \pm 0.14$	$30.0^{c} \pm 4.0$	$3.0^{e} \pm 1.0$	$3.2^{c} \pm 0.3$	$122.4^{c} \pm 6.5$
ZnO CaO NPs	50	$13.8^{a} \pm 0.8$	$12.5^{bc} \pm 0.5$	$13.3^{a} \pm 0.6$	$44.7^{a} \pm 2.5$	$10.6^{d} \pm 0.4$	$34.3^{c} \pm 4.5$	$8.3^{a} \pm 0.6$	$3.7^{b} \pm 0.15$	$273.3^{a} \pm 21$
	100	$13.2^{abc} \pm 0.3$	$14.3^{a} \pm 0.2$	$10.0^{def} \pm 1.0$	$43^{a} \pm 1.0$	$11.6\ ^{cd} \pm 0.7$	$37.0^{b} \pm 1.5$	$7.0^{b} \pm 1.0$	$3.4^{bc} \pm 0.5$	$220.5^{b} \pm 10$
	200	$13.5^{ab} \pm 0.5$	$14.8^{a} \pm 0.3$	$11.7^{bc} \pm 1.5$	$37^{bc} \pm 1.0$	$12.0^{c} \pm 0.8$	$40.0^{a} \pm 2.0$	$7.0^{b} \pm 1.0$	$4.0^{a} \pm 0.2$	$213.3^{b} \pm 15.3$
Ca + Zn salt		$12.5^{abc} \pm 0.5$	$8.01^{e} \pm 0.5$	$9.0^{f} \pm 1.0$	$35^{de} \pm 1.0$	$9.8^{e} \pm 0.3$	$38.0^{b} \pm 2.5$	$5.0^{d} \pm 1.0$	$3.1^{b} \pm 0.45$	$134.4^{c} \pm 3.8$

Each value is a mean of 5 replicates with ± SD where means in a column sharing the same superscript are not significantly different at Pless than 0.05.

ZnO + CaO with a concentration of 50 ppm gave 86.25% higher shoot length measurements as compared to the control group. In the same way, root length in combined treatment of ZnO + CaO NPs (200 ppm) was observed significantly higher as compared to all other concentrations/treatments of nanoparticles. Other parameters such as the number of shoots, leaf area, fresh weight, yield per plant, fruit diameter, and fruit weights were also significantly enhanced. However, the combined effect of Ca and ZnO nanoparticles was found more effective in increasing the growth parameters as compared to the sole application of each nano-nutrient as a foliar spray (Table 1). Zinc nano-spray treatment of tomato plants enhanced shoot length, root length, no. of shoots, no. of roots, leaf area, fresh plant weight, number of fruits per plant, fruit diameter, and weight of tomatoes per plant by 5, 15, 9.7, 3.5, 14.1, 7.7, 73.9, 22.5, and 6.5% respectively as compared to conventional zinc salt treated tomato plants. Whereas Ca nano-spray treatment of tomato plants enhanced shoot length, root length, no. of shoots, no. of roots, leaf area, fresh plant weight, number of fruits per plant, fruit diameter, and weight of tomatoes per plant by 9.2, 11.1, 15.1, 19.3, 13, 6.6, 100, 6.6, and 69.6 % respectively as compared to conventional calcium salt treated tomato plants. While a combined treatment of ZnO and CaO nano-spray treatment of

Fig. 2. The pictorial description of the effect of various applied treatments of nano-sprays on growth and yield parameters of *S. lycopersicum.*

tomato plants enhanced shoot length, root length, no. of shoots, no. of roots, leaf area, fresh plant weight, number of fruits per plant, fruit diameter, and weight of tomatoes per plant by 10.4, 84.7, 11.1, 27.7, 22.4, 5.3, 66, 29, and 100% respectively as compared to combined treatment of conventional zinc salt and calcium salt spray to tomato plants.

ZnO NPs application enhanced the photosynthetic property of plants thus increasing the cell division and producing greater plant biomass (Rai-Kalal and Jajoo, 2021). The positive effects of ZnO NPs on morphological parameters (root length, shoot length, and plant biomass) of various plants have been reported by various researchers like on broccoli, coffee, wheat, rice, and pepper (Rai-Kalal and Jajoo, 2021; Garcia et al., 2019; Rossi et al., 2019). Calcium nanoparticles have also a history of being used for the betterment of crops like rice, wheat, grapes, beans, etc. These types of nanoparticles have also shown their role in the reduction of various stresses like salinity, water, and heavy metal stress (Nasrallah et al., 2022; Mustafa et al., 2021). Ca is directly involved in improving photosynthesis which results in higher leaf number (Akhtar et al., 2010). The Ca improves leaf area by activating enzymes, photosynthesis, and carbohydrate metabolism (Akhtar et al., 2010; Bergmann, 1992). Similarly, Ayyub et al. (Ayyub et al., 2012) also reported an increase in tomato vegetative characteristics with the foliar application of calcium. The increment in the studied parameters of plants by application of calcium oxide nanoparticles can also be attributed to the fact that calcium is an essential constituent of the plant cell walls and plays a significant function in cell division and enlargement (Ayyub et al., 2012).

The number of tomato fruits per plant was also found highest (8 tomatoes per plant on average) in plants treated with a combined dose of ZnONPs, and CaONPs as compared to other treatments. Same results were noticed for fruit diameter and fruit weight on average. The yield of fruits has also been reported to be higher with the application of zinc oxide and calcium oxide nanoparticles (Gao et al., 2019; Faizan et al., 2020). ZnONPs can affect the development of fruit in dose-dependent manner as zinc is a precursor of tryptophan which is required for the biosynthesis of indole acetic acid. Thus zinc help in cell division and cell elongation. This is the reason zinc as a nutrient when supplied to plants can help to enhance yield (Faizan et al., 2020). But calcium has a very direct role in fruit development and its post-harvest health. Calcium treatment has been reported to help in maintaining fruit quantity and quality (Zhang et al., 2019). Therefore, its combination with ZnO NPs gave the higher yield attributes of tomato plants in the present study. Lower concentrations of CaONPs worked well as a higher concentration of calcium in plants or fruits can cause toxicity.

Nanoscale materials have a large surface area-to-volume size ratio and hence, the application of Zn and calcium oxide nanoparticles either as a foliar spray or as a root placement can facilitate easy transportation potentially in the plant system (Mustafa et al., 2021; Faizan et al., 2020) and can facilitate optimized growth patterns. Both types of metal oxide nanoparticles are a requirement for the main metabolism of the plant, therefore, giving positive effects on the growth of tomato plants.

3.3. Total phenolic content of fruits

Total phenolics in *S. lycopersicum* were evaluated under the effect of different concentrations of foliar application of nanofertilizers i.e., ZnO NPs, CaO NPs, and their combination. ZnO NPs (200 ppm) treated plants showed the highest amount of phenolics (77.72 μg/g) while the lowest amount (49.44 μg/g) of phenolics was observed in ZnO + CaO NPs treated plants (200 + 200 ppm). Results showed the increasing phenolic content order of tomato plants under the effect of NPs treatment was ZnO + CaO < CaO NPs < $ZnSO_4$ < Control < $CaSO_4$ < ZnO NPs as shown in (Fig. 3a). In earlier studies, it was reported by Gracia *et al.* (2018) that total phenolics in *Capsicum annum* (nightshade) were enhanced with an increase of ZnO NPs doses because it enhanced the accumulation of zinc content and ultimately phenolic content (Garcia et al., 2018; Raghib et al., 2020).

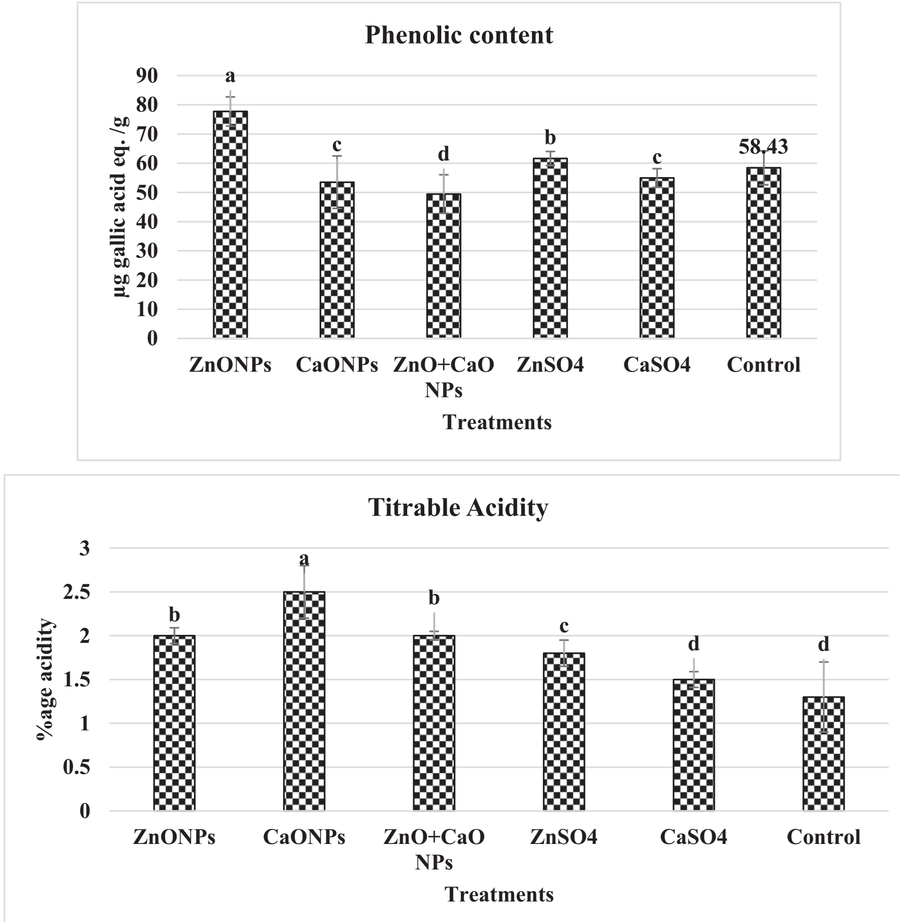

Fig. 3. **(a):** Effect of different NPs treatments on total phenolics of *S. lycopersicum* **(b):** Effect of different NPs treatments on %age acidity of *S. lycopersicum.*

Various protective mechanisms have been developed by plants to protect against oxidative damage, caused by ROS through the production of antioxidants such as phenols, carotenoids, and antioxidant enzymes (Faizan et al., 2020; Zhang et al., 2019; Lopez et al., 2018). Phenolic compounds play a prominent role in the detoxification mechanisms of ROS as electron donors in organelle structures and can directly eliminate the molecular species of active oxygen, mainly due to their redox properties. They act in the absorption and neutralization of free radicals, extinction of singlet and triplet oxygen, or decomposition of peroxides (Faizan et al., 2021; Ahmad et al., 2020; Shah et al., 2020). This behavior explains the highest accumulation of phenols and flavonoids in the fruits from plants exposed to treatments with ZnO NPs whereas lowest the stress was found with combined treatment of both nanoparticles.

3.4. Total titratable acidity

Total titratable acidity (TTA) is a measure of the volume of acids that are present in tomato fruit. Titratable acidity is a better indicator of fruit quality and its acceptance by consumers. The highest percentage acidity was shown in the control group as represented in Fig. 3b. The percentage acidity in the control group was 2.44 and the lowest acidity was observed in the calcium oxide NPs treated plant that was 1.2. The titratable acidity was increased with the order of Control < $CaSO_4$ < $ZnSO_4$ < ZnONPs < CaO + ZnONPs < CaO NPs. Lopez et al. (Lopez et al., 2018) reported the total titratable acidity of Cu NPs treated tomatoes that were higher for higher concentrations of NPs used (Lopez et al., 2018). Garcia et al. (Garcia et al., 2019) also reported the work on titratable acidity by ZnO-treated *Capsicum chinense* Jacq. that was higher in NPs-treated plants as compared to the control (Garcia et al., 2018).

TTA is increased by applied zinc treatment as zinc causes an increase in the rate of photosynthesis which ultimately causes an increase in plant metabolism. This causes an enhancement in the TTA of fruits (Kazemi, 2014). Whereas calcium is known to have not any significant effect on the TTA of fruits (Akhtar et al., 2010). Meanwhile, it is also reported that calcium treatment reduces the rate of respiration which in turn slows down the conversion of acids to sugars (Shehata et al., 2021). This zinc and calcium in nano size have the larger surface area that acts more rapidly and efficiently on the TTA of tomato fruits in the present study.

3.5. Zinc and calcium analysis by atomic absorption spectroscopy

Zinc and calcium analysis was done in NP-treated plants (Table 2) and the highest calcium content was found in CaO NPs treated plants (7.34 mg Ca /g of plant tissue). The highest concentration of zinc metal was observed to be 2.65 mg/g of plant material in ZnONPs treated plants. In *S.lycopersicum*, according to Akanbi et al. (Akanbi et al., 2019), high zinc translocation in the shoot was found as compared to the control and highest in root as compared to the shoot (Akanbi et al., 2019). Ali et al. (Ali et al., 2019) reported the accumulation of zinc in ZnO NPs treated *Oryza sativa* L. that the amount of zinc increased with increasing ZnO NPs levels by the foliar spray method and decreased with biochar application (Ali et al., 2019).

Table 2
Metal Analysis by Atomic Absorption Spectroscopy.

Sr. #	Sample	Zn content mg/g of plant tissue	Ca content mg/g of plant tissue
1	ZnO NPs treated plant	$2.65^{a} \pm 0.12$	$00^{b} \pm 0.00$
2	ZnO + CaO NPs treated plant	$1.96^{b} \pm 0.08$	$5.31^{a} \pm 0.11$
3	$ZnSO_4$ treated plant	$2.02^{b} \pm 0.07$	$00^{b} \pm 0.00$
4	CaO NPs treated plant	$0.09^{c} \pm 0.00$	$7.34^{a} \pm 0.91$
5	$CaSO_4$ treated plants	$0.08^{c} \pm 0.00$	$0.12^{b} \pm 0.01$

Each value is a mean of 5 replicates with ± SD where means in a column sharing the same superscript are not significantly different at Pless than 0.05.

4. Conclusion

It was concluded that the nano ZnO and CaO can be synthesized by using *Nigella* seeds extract by following the green principle of nanotechnology. Application of nano-fertilizers (CaO, ZnO, and Combined ZnO + CaO) is advantageous over conventional fertilizers. This research will be beneficial for future research work by following cost effective and eco-friendly green synthesis of nanoparticles.

CRediT authorship contribution statement

Ayesha Farooq and **Asma Ahmad:** Experimental work and Writing – original draft preparation. **Sumera Javad** and **Anis Ali Shah:** Validation, and Supervision. **Khajista Jabeen:** Conceptualization. **Adnan Noor Shah:** Writing – review and editing. **Mohammed Alyemeni** and **Walid F.A. Moosa:** Statistical analysis. **Asad Abbas:** Proof reading and final analysis of NPs. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102647.

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