



Effects of ZnO-NPs Treatments on Fruit Characteristics of Capsicum

Gitika Bhardwaj^{a++*} and Anjali Chauhan^{a#}

^a Department of Soil Science and Water Management, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, 173230, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

To evaluate the effects of zinc oxide nanoparticles (ZnO-NPs) treatments: applied via seed treatment and foliar spray; on post-harvest quality attributes of capsicum fruits, including texture score, specific gravity, maximum penetration and shearing force, cuticle thickness, moisture content, shelf life, and capsaicin content. A two-year pot experiment using a completely randomized design with seven treatments (control, three seed treatment concentrations, and three foliar spray concentrations of ZnO-NPs) and three replications; was conducted in net-house conditions at Department of Soil Science and Water Management, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India. Capsicum seeds were soaked (T2, T4, T6) in ZnO-NPs suspensions (50, 100, 150 mg/L) for 24 hours before sowing (seed treatment); and sprayed (T3, T5, T7) with corresponding suspensions 15 days post-transplanting (foliar). T1 was taken as control (without application of ZnO-NPs). After harvest, uniform fruits were assessed for various fruit characteristics. The results revealed an increment in texture score (8.08),

⁺⁺Junior Research Fellow;

[#]Associate Professor;

^{*}Corresponding author: E-mail: bhardwajgitika93@hotmail.com;

specific gravity (0.84), penetration force (11.98 N), shearing force (80.70 N), cuticle thickness (38.9 µm), moisture content (93.7%), shelf life (6.9 days), and capsaicin (0.21%) content, under treatment comprising seed treatment of capsicum with 50 mg/L ZnO-NPs. With increased concentrations or foliar application, most attributes decreased; however, at 150 mg/L foliar spray, performance frequently returned to control levels. A low-dose ZnO-NP seed treatment (50-100 mg/L) significantly improved the pungency, post-harvest quality, and mechanical integrity of capsicum fruits. Based on the results, which highlight the significance of dosage and application technique, capsicum fruit quality can be considerably improved by targeted early-stage root uptake of ZnO-NPs.

Keywords: *Capsicum*; *fruit characteristics*; *ZnO*; *nanoparticles*; *seed treatment*; *foliar spray*.

1. INTRODUCTION

In India, capsicum is grown all year round and is a member of the Solanaceae family (Asaduzzaman et al., 2010). It is cultivated as a cash crop, spice, and vegetable. In Indian states of Tamil Nadu, Maharashtra, Himachal Pradesh, Karnataka, and the mountainous regions of Uttar Pradesh, capsicum is grown. Around the world, capsicum is grown in temperate and subtropical climates. In addition to being a vegetable that can be eaten raw or cooked, capsicum contains antioxidants that help prevent cardiovascular illnesses and cataracts (Maldonado et al., 2002).

The need for agriculture is rising daily as a result of the unchecked population increase in the world. Therefore, it is imperative that crop yield be increased using appropriate modern technologies. These days, nanotechnology is in demand since it may be used to increase sustainability and production. According to Rico et al. (2011), nanotechnology is a rapidly developing technology in all fields. It has become a cutting-edge technology that offers the best nutrient delivery and improved environmental conditions (Jha et al., 2011). Modern technologies such as nanotechnology can improve the efficacy of insecticides and macro and micronutrients (Mazzaglia et al., 2012).

Micronutrients are extremely important for plant metabolism and are required in trace amounts. A zinc deficiency is frequent in many crops. Zinc is an essential micronutrient and it is necessary for tryptophan synthesis, cell division, and other regulatory processes, it is also implicated in the activities of dehydrogenase, aldolase, isomerase, and transphosphorylase (Marschner, 2012). As a micronutrient element, zinc also plays a role in a number of physiological functions in plants, such as energy production, protein synthesis, and membrane integrity maintenance (Hänsch and Mendel, 2009; de Oliveira et al., 2018). It is essential for the manufacture of plant growth

hormones (Mansour, 2014). Additionally, germination, photosynthetic pigments, biomass buildup, and enzymatic activity were all significantly impacted by zinc oxide nanoparticles (ZnO-NPs). Zinc oxide nanoparticles also have a role in the plant's defence mechanism, which eventually influences the plant's morphology, physiology, and histology (Mantoan et al., 2016). The significance of zinc as a micronutrient for plant growth and development makes ZnO valuable in the field of agricultural research (Seleiman, 2019). According to Munir et al. (2018), the application of ZnO nanoparticles has the potential to regulate plant growth and, alleviate zinc deficiencies in plants. Whether nanoparticles are hazardous or helpful depends on the type of nanomaterial being employed (Monica and Cremonini, 2009). Plants absorb zinc as divalent cations, which are essential for plant growth and development. Nanoparticles' main benefit is their ability to be applied at very low concentrations, which also yields better outcomes than traditional fertilizers (Mazaheri et al., 2019). Keeping this in view, the current research was carried out to examine the effective concentration as well as application method of ZnO (nanoparticles) treatment on various fruit characteristics of capsicum.

2. MATERIALS AND METHODS

The two-year pot experiments were carried out in 2020 and 2021 at the net house of Department of Soil Science and Water Management, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India. The experiment comprised of seven treatments viz., T1-Control, T2-50 mg/L ZnO as Seed treatment, T3-50 mg/L ZnO as Foliar spray, T4-100 mg/L ZnO as Seed treatment, T5-100 mg/L ZnO as Foliar spray, T6-150 mg/L ZnO as Seed treatment, T7-150 mg/L ZnO as Foliar spray. The experiment was replicated thrice and was laid out in completely randomized design. For this experiment, potting

mixture was prepared by mixing unsterilized soil, farm yard manure and sand in ratio of 2:1:1 on volume basis, this mixture was filled in 21 pots. Each pot was filled with 10 kg of this potting mixture. The potting mixture was then analyzed for various physico-chemical properties and the result revealed that potting mixture was neutral in reaction, electrical conductivity was in safe limit and available NPK was in medium range.

Zinc oxide nano powder with size <50 nm particle size (BET), purity >97% was taken for the experiment. For seed treatments, three concentrations *i.e.*, 50, 100 and 150 mg/L were prepared by mixing powder in ethanol and then prepared final volume in distilled water. Seeds were soaked in respective concentration for over night before sowing. Same concentrations were prepared for the foliar application of the ZnO-NPs. For foliar spray, every time fresh solution was prepared. One month old healthy seedling of capsicum with 4-5 leaves were chosen for the experiment. One foliar spray was done after 15 days of transplanting of seedling in treatments comprising foliar spray only. The fruits from second harvest were taken for the estimation of various fruit characteristics of capsicum.

The texture score of capsicum (bell pepper) is a sensory or instrumental assessment of the fruit's texture properties, usually used in post-harvest quality evaluation. For estimation of texture score, only ripe, uniform, and healthy capsicum fruits were used. To guarantee uniformity, the fruits were cleaned with distilled water, let to air dry, and then cut into 2 x 2 cm rectangular slices. All samples were equilibrated to room temperature ($25\pm 2^\circ\text{C}$) before evaluation. A 9-point hedonic scale (1 =very poor and 9 being outstanding) was employed to score each attribute. Texture score was calculated as the mean of the scores. The ratio of the capsicum's fresh weight to volume was used to determine the fruit specific gravity. The maximum penetrating force and shearing force of each fruit were measured using a TMS-Pro texture analyzer testing frame (TMS-PRO, FTC, USA). In order to determine the thickness of the cuticle, pericarp section of fruits was cut and promptly fixed the tissue in a formalin-acetic acid alcohol buffer. After being dried using a gradient ethanol series, the samples were submerged in paraffin, embedded, sectioned, and stained. Sections were seen under a microscope, and Image J software was used to determine their thickness (Liu et al., 2022). The fresh weight was estimated, and then the capsicum was dried to a

constant weight at 80°C in an oven, the dry weight was determined and the moisture content was computed. In order to estimate shelf life, sample fruits were picked individually based on treatment, and the number of days that passed between fruit harvest and the point at which the fruits shriveled and lost their firmness was noted. shelf life was then estimated in days. The capsaicin content was determined by Folin-Dennis method. The pungent principle reacts with Follin-Dennis reagent to give a bluish complex, which was estimated calorimetrically (Mathew et al., 1971).

The data generated from the above parameters from the two-year pot experiment were tabulated and analysed by complete randomized design (Panse and Sukhatme, 2000) and Analysis of Variance (ANOVA) were used to determine significance at $P\leq 0.05$ using Microsoft Excel and OPSTAT packages (Sheron et al., 1998). The least significant difference (LSD) was used to determine significant differences among the means at $P\leq 0.05$. The significant difference between mean treatments were separated using Duncan's Multiple Range Test (DMRT) at 5% level of significance and denoted by alphabets. Superscript letters signify statistical difference between the mean. Means followed by the same letters do not differ significantly from one another and if the mean values have different letters, they are significantly different.

3. RESULTS AND DISCUSSION

3.1 Texture Score

As given in Table 1, a notable difference was observed in the texture scores of the capsicum fruits across the treatments. Significantly higher than the control (5.45), the 50 mg/L ZnO-NP seed treatment had the highest score (8.08), followed by the 100 mg/L seed treatment (7.93) and 150 mg/L seed treatment (7.08). However, 150 mg/L foliar spray had the lowest value (5.19). Overall, the foliar application of ZnO-NPs had lower texture score than seed treatments. One important fruit quality attribute is texture, which is frequently impacted by the pericarp's cellular makeup and biochemical makeup. In order for cell walls to form and remain stiff, zinc is necessary for both protein synthesis and enzyme activation. In instance, at 50 and 100 mg/L, the texture score significantly improved with ZnO-NP seed treatments, indicating improved physiological and biochemical processes during fruit development. A phytotoxic

impact or an imbalance in nutrient availability may be the cause of the lower score at 150 mg/L, particularly under foliar spray. In the present study, capsicum fruits with low to moderate concentrations of ZnO-NPs especially when used as seed treatments have better textures, most likely as a result of enhanced turgidity and cell structure. The significance of dosage and application technique is underscored by the potential adverse effects of elevated concentrations, especially when applied through foliar application on fruit texture.

Similar studies were also carried out by Rameshaiah et al. (2015), in their research, they highlighted the role of zinc-based nanoparticles on fruit quality, especially texture, by improvement in delivering essential nutrients to the plants. Another study by Kole et al. (2013), noted that fruit quality attributes including smoothness, appearance of the fruit were greatly enhanced by ZnO-NPs, which were linked to better texture.

3.2 Specific Gravity

The application of ZnO-NP significantly increased specific gravity, a metric associated with fruit density and interior composition as presented in Table 1. The 50 mg/L seed treatment had the highest specific gravity (0.84), followed by the 100 mg/L seed treatment (0.80), while the control had the lowest value (0.57). The range of values for foliar sprays was 0.72 (50 mg/L) to 0.62 (150 mg/L). Better buildup of dry matter, sugars, and other solutes is often indicated by a rise in specific gravity. Improved assimilate translocation to fruits may result from zinc's involvement in photosynthetic activity and metabolic control. The findings show that ZnO-NPs may have improved fruit growth, particularly when used at lower concentrations through seed priming. Reduced physiological efficiency or stress reactions may be the cause of lower readings at higher doses and in foliar application. In our study, the enhanced specific gravity, the interior fruit quality is positively impacted by the application of ZnO-NPs. The superior root absorption and systemic translocation of zinc during the early stages of growth probably account for the particularly high effectiveness of seed treatments at 50-100 mg/L. In a similar way, ZnO-NPs also enhanced nutritional content and physiological development, which was associated with higher fruit density and specific gravity (Tarafdar et al., 2014; Dimkpa et al.,

2019). High doses of foliar spray seem less beneficial, either as a result of detrimental feedback effects or restricted translocation.

3.3 Maximum Penetration Force (N)

All ZnO-NP treatments showed a substantial increase in maximum penetration force as compared to the control (6.64 N). The seed treatment containing 50 mg/L had the maximum penetration force (11.98 N), followed by the one containing 100 mg/L (10.66 N). Among the treatments, 150 mg/L foliar spray had the minimum penetration force (7.82 N), which was nevertheless more than the control as shown in Table 1. Fruits' ability to retain water and the strength of their cell walls are directly correlated with firmness. Zinc increases the firmness of fruit by aiding in the formation of pectin and structural proteins. The significant increase in maximum penetration force at lower concentrations implies that ZnO-NPs enhanced turgor and cell wall formation. The marginal advantage at greater doses, however, could point to a barrier that prevents further zinc contribution or that could disrupt other metabolic processes. In our results, ZnO-NP treatments, particularly 50 mg/L seed treatment, greatly increase fruit firmness. This improvement may result in improved shelf life and post-harvest quality. According to the research, foliar sprays at greater dosages are less advantageous than early-stage zinc availability achieved by seed treatment. In a similar study carried out by Subbaiah et al. (2016), showed that ZnO-NPs promoted calcium/zinc synergy and reinforced cell walls, which increased stiffness. Also, ZnO-NPs improve firmness and texture by influencing physiological and structural characteristics (Nair et al., 2010).

3.4 Maximum Shearing Force (N)

Shearing force is a crucial factor in assessing the quality of fruit handling and storage. Better tissue integrity and firmness are suggested by the rise in shearing force with ZnO-NP treatments, which is probably the result of increased lignification and structural development impacted by zinc. The improved outcomes from seed treatments highlight the effectiveness of zinc absorption and utilization depending on roots. Higher foliar concentrations with lower values might indicate oxidative stress impacting tissue strength or restrictions in foliar absorption.

Table 1. Effects of zinc oxide nanoparticles on texture score, specific gravity, maximum penetration force and maximum shearing force in capsicum

Treatments	Texture score	Specific gravity	Maximum penetration force (N)	Maximum shearing force (N)
T1-Control	5.45±0.01 ^f	0.57±0.01 ^g	6.64±0.06 ^g	48.83±0.43 ^f
T2-50 mg/L ZnO as Seed treatment	8.08±0.01 ^a	0.84±0.01 ^a	11.98±0.03 ^a	80.70±0.53 ^a
T3-50 mg/L ZnO as Foliar spray	6.65±0.01 ^d	0.72±0.01 ^d	9.25±0.09 ^d	67.51±0.85 ^c
T4-100 mg/L ZnO as Seed treatment	7.93±0.01 ^b	0.80±0.01 ^b	10.66±0.17 ^b	71.01±0.39 ^b
T5-100 mg/L ZnO as Foliar spray	6.55±0.01 ^e	0.67±0.01 ^e	8.65±0.05 ^e	62.97±0.88 ^d
T6-150 mg/L ZnO as Seed treatment	7.08±0.01 ^c	0.75±0.01 ^c	9.57±0.05 ^c	71.64±1.07 ^b
T7-150 mg/L ZnO as Foliar spray	5.19±0.01 ^g	0.62±0.01 ^f	7.82±0.11 ^f	56.24±1.00 ^e
LSD (P≤0.05)	0.03	0.02	0.29	2.38

In comparison to the control (48.83 N), the shearing force was markedly elevated in all ZnO-NP treatments in present research. The 50 mg/L seed treatment had the maximum shearing force (80.70 N), followed by the 100 mg/L seed (71.01 N) and the 150 mg/L seed (71.64 N) treatments. Foliar treatments demonstrated moderate gains; the foliar spray with 150 mg/L had the lowest shearing force (56.24 N) of any treatment. Applying ZnO-NP, particularly through seed treatment at 50 mg/L, greatly improved the mechanical quality of capsicum fruits as measured by shearing force. This affects customer preferences as well as post-harvest treatment. Nonetheless, the decreasing pattern at greater foliar dosages highlights the necessity of careful nanoparticle utilization. Similarly, ZnO-NP treatments significantly increased shearing force and tensile strength in fruits by improving cell wall thickness and rigidity, which are directly linked to higher shearing force (Azeez et al., 2020; Raliya and Tarafdar, 2013).

3.5 Cuticle Thickness (μm)

When compared to the control (16.84 μm), the cuticle thickness of capsicum fruits increased considerably with ZnO-NP treatments as given in Table 2. 50 mg/L seed treatment had the highest thickness (38.88 μm), which was closely followed by 100 mg/L seed treatment (35.32 μm) and 150 mg/L seed treatment (30.55 μm). In general, foliar sprays were less effective; the 150 mg/L foliar spray had the lowest thickness (20.00 μm) among the treatments. A crucial characteristic associated with fruit firmness, resistance to mechanical damage, and shelf life is cuticle thickness. Zinc is essential for the production and integrity of cell walls, and delivery methods based on nanoparticles increase its availability during crucial phases of development. The greater performance of seed treatments was probably caused by early and persistent zinc availability, which encouraged improved pericarp cell proliferation and growth. The reduction in cuticle thickness at higher foliar treatment dosages (150 mg/L) may indicate phytotoxic effects or less than ideal absorption. ZnO-NPs dramatically improved cuticle growth, especially when applied as a seed treatment at 50-100 mg/L. This suggests that early-stage root-mediated zinc absorption is more advantageous. The necessity to optimize dosage and delivery mechanism is highlighted by the decreasing trend at greater foliar concentrations, which suggests a threshold impact. Similarly, increased pericarp thickness and cell proliferation in tomato

fruits improved their quality and structural characteristics. was further seen with the use of zinc oxide nanoparticles. Because of improved nutrient digestion, ZnO-NP seed treatments produced thicker pericarp and harder fruit texture (Kole et al., 2013; Subbaiah et al., 2016).

3.6 Moisture Content (%)

The perusal of data presented in Table 2 revealed that application of ZnO-NP significantly raised the moisture content. The 50 mg/L seed treatment had the highest moisture content (93.71%), whereas the control had the lowest (76.87%). The moisture content of foliar treatments was moderate; among treated groups, 150 mg/L foliar spray had the lowest moisture content (80.19%). Fruit juiciness, freshness, and post-harvest quality all depend on moisture retention. Through the regulation of stomatal function and membrane integrity, zinc improved the osmotic balance and water-holding capacity of plant tissues. The observed rise in moisture content, especially under seed treatments, points to better water absorption and retention made possible by effective root system growth. At greater foliar concentrations, decreased moisture may result from oxidative stress caused by zinc that impairs membrane function. In our results, ZnO-NPs applied to seeds at concentrations of 50-100 mg/L greatly increase fruit hydration, which can improve marketability and sensory quality. Higher dosages of foliar spray, however, could not have the same effect and might even somewhat hinder moisture conservation under some situations. Similar increase in overall moisture content of crop by the usage of zinc oxide nanoparticles was also studied by Tarafdar and Raliya (2011) and Raliya et al. (2016). They also concluded that nano fertilizers improve nutrient-mediated water retention, which raises the water content of leaves and fruit.

3.7 Shelf Life (Days)

The data presented in Table 2 showed that all ZnO-NP treatments had longer shelf lives than the control which had 4.58 days. 50 mg/L seed treatment had the greatest shelf life (6.92 days), followed by the 100 mg/L seed treatment (6.79 days). Foliar treatments had a shorter shelf life; 150 mg/L of foliar spray was comparable to the control (4.85 days). The strength of the pericarp, respiration rate, and moisture loss are some of the physiological and biochemical factors that affect shelf life. Through cellular membrane

Table 2. Effects of zinc oxide nanoparticles on cuticle thickness, moisture content, shelf life and capsaicin content in capsicum

Treatments	Cuticle thickness (μm)	Moisture content (%)	Shelf life (Days)	Capsaicin content (%)
T1-Control	16.84 \pm 0.27 ^g	76.87 \pm 0.27 ^f	4.58 \pm 0.15 ^c	0.16 \pm 0.00 ^d
T2-50 mg/L ZnO as Seed treatment	38.88 \pm 0.69 ^a	93.71 \pm 0.59 ^a	6.92 \pm 0.15 ^a	0.21 \pm 0.01 ^a
T3-50 mg/L ZnO as Foliar spray	27.11 \pm 0.24 ^d	85.77 \pm 0.49 ^c	5.54 \pm 0.13 ^b	0.18 \pm 0.00 ^{bc}
T4-100 mg/L ZnO as Seed treatment	35.32 \pm 0.43 ^b	92.31 \pm 0.49 ^a	6.79 \pm 0.18 ^a	0.19 \pm 0.01 ^b
T5-100 mg/L ZnO as Foliar spray	23.96 \pm 0.26 ^e	82.28 \pm 0.48 ^d	5.62 \pm 0.16 ^b	0.17 \pm 0.01 ^{cd}
T6-150 mg/L ZnO as Seed treatment	30.55 \pm 0.60 ^c	87.93 \pm 0.52 ^b	5.73 \pm 0.15 ^b	0.19 \pm 0.00 ^b
T7-150 mg/L ZnO as Foliar spray	20.00 \pm 0.44 ^f	80.19 \pm 1.13 ^e	4.85 \pm 0.24 ^c	0.17 \pm 0.00 ^{cd}
LSD ($P \leq 0.05$)	1.38	1.89	0.51	0.02

stabilization and antioxidant activity promotion, zinc is known to postpone senescence. ZnO-NP seed treatments probably result in longer shelf lives because they improve fruit hardness, pericarp thickness, and moisture retention. But too much zinc from foliar treatment might upset the metabolic equilibrium and counteract the positive benefits. ZnO-NP seed treatments, particularly at 50 mg/L, reduce moisture loss and metabolic degradation, which may be the reason why they prolong the shelf life of capsicum. It appears that focused early-stage zinc administration is more dependable for post-harvest management, while higher foliar dosages are either less helpful or even harmful. Similar studies were also carried out by Nair et al. (2010) and Azeez et al. (2020), they noted that ZnO-NPs increase antioxidant activity and decrease degradation, delaying fruit senescence and extending shelf life. ZnO-NP treatments improved the capsicum fruits' microbiological resistance and structural integrity.

3.8 Capsaicin (%)

In comparison to the control (0.16%), the 50 mg/L seed treatment had the greatest capsaicin content (0.21%) as given in Table 2. Although there was an increase above control in all treatments, the rise was less noticeable in treatments at 100 and 150 mg/L, particularly in foliar sprays. The primary bioactive ingredient in capsicum that gives it its pungency and therapeutic properties is capsaicin. Secondary metabolic pathways that are impacted by stress signals and nutritional availability control its production. By increasing enzymatic activity and stress-responsive gene expression, zinc, particularly in nanoform, can promote secondary metabolism at lower concentrations. Higher dosages may decrease biosynthesis because of oxidative stress or feedback inhibition, but the increased capsaicin in the 50 mg/L seed treatment may indicate balanced metabolic stimulation. Moderate exposure to ZnO-NP, especially at 50 mg/L by seed treatment, increases capsaicin accumulation, which may increase capsicum's nutritional and economic value. When it comes to nanoparticle-based inputs, more is not necessarily better, since higher concentrations might not raise capsaicin content any further. These results are in conformity with the results of García-López et al. (2019) who noted that Zn nanoparticles stimulated secondary metabolism, which had a favorable effect on capsaicin production in chili. Through the modification of enzymatic pathways,

ZnO-NPs considerably raised capsaicin levels at ideal doses.

4. CONCLUSION

The current study shows that zinc oxide nanoparticles (ZnO-NPs) greatly improve the post-harvest quality of capsicum, especially when used as a seed treatment at a concentration of 50 mg/L. Texture score, maximum penetration and shearing force, specific gravity, cuticle thickness, moisture content, shelf life, and capsaicin content all significantly improved as a result of this treatment. These improvements show that fruit structure, storability, and nutritional quality can all be improved by ZnO-NPs at low concentrations. Early exposure to nano-zinc through seed treatment ensured improved cellular development, structural integrity, and utilization of nutrients in the seedlings. Increased fruit firmness and cuticle thickness resulted in extended shelf life and less moisture loss, which is essential for reducing losses after harvest. Furthermore, higher capsaicin content following this therapy points to greater antioxidant and metabolic activity, which may have been brought about by better zinc availability throughout crucial growth stages. Foliar applications, on the other hand, were less successful or possibly detrimental, particularly at higher dosages (150 mg/L), suggesting possible phytotoxicity and low absorption efficiency through older leaves. This emphasizes how crucial application technique and dose optimization are to the safe and efficient use of nanomaterials in agriculture. The results demonstrate that administering 50 mg/L of ZnO-NPs to seeds is a viable, affordable, and eco-friendly method of enhancing the nutritional value and marketability of capsicum fruits. It promotes sustainable post-harvest management techniques and reduces the need for frequent foliar treatments. To confirm these findings, investigate molecular mechanisms, and evaluate potential ecological effects, more investigations and long-term research are suggested. All things taken into account, there is great potential for incorporating ZnO-NP seed treatment into precision nutrient control in vegetable production systems.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image

generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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