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Nano-zinc oxide foliar application at the tuber initiation stage enhances the starch synthesis and zinc bioaccumulation in potato tubers

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Abstract
Crops cultivated on alkaline soils are normally deficient in micronutrients, therefore among others, zinc deficiency is a widespread problem in Pakistan. By using a novel approach, we have shown that agronomic biofortification of potato tubers can be achieved through application of nano-zinc oxide during the tuber initiation stage. Field experimentation was performed during the potato growing season (2021-2022) with five different concentrations of nano-zinc oxide (0, 25, 50, 100, and 250 mg L^{-1}) applied to 55-d old plants at tuber initiation stage, and after 40 days of spray, potato tubers were harvested for the analysis of various biochemical and agronomic traits and changes in bioavailable concentration of Zn in potato tubers. An increase in growth and yield traits was recorded due to spray of 25 and 50 mg L^{-1} nano-zinc oxide. Starch fraction in tubers was increased in response to all nano-zinc oxide concentrations. Interestingly, over 50% increase in starch was recorded due to application of 25 mg L^{-1} nano-zinc oxide, while higher doses up to 250 mg L^{-1} increased tuber starch by ~34%. Soluble sugars increased progressively up to 250 mg L^{-1} reaching maximum values than the control. Above all, maximum concentration of Zn in potato tuber was recorded in response to 250 mg L^{-1} spray of nano-zinc oxide ranging up to 58.2 - 65.5 µg g^{-1} dry weight. Overall, exogenous application of nano-zinc oxide to potato plants can improve potato tuber quality by enhancing starch contents and contributing to translocation of Zn in potato tubers leading to agronomic biofortification.

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Introduction

Zinc deficiency in soils has been recorded for 70% of cultivated land in Pakistan, and it is widespread globally influencing crop nutrition, edible quality, and most importantly nutrient contents of the edible parts (Henriques et al., 2012; Adhikari et al., 2016; Shaikh and Saraf, 2017; Raza et al., 2022). Soils with lower pH exhibit higher Zn availability in plants (Sturikova et al., 2018), since Zn^{2+} ions get precipitated and soil -OH functional groups make it immobile for plants (Hacisalihoglu et al., 2001; Cao et al., 2004; Broadley et al., 2007). Therefore, poor Zn^{2+} availability in high-pH soils is an important issue to improve crop nutrition, which in turn affects human nutrition. It is pertinent to mention here that Zn deficiency in humans is also a widespread problem among inhabitants of Asia including Pakistan leading

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to several medical and health-related issues (Khalid et al., 2014; Rehman et al., 2020).

Of different ways to counter zinc deficiency in plants is biofortification which can be obtained in a variety of ways such as biotechnology, plant breeding, and fertilization strategy. Plant breeding is an inexpensive, efficient, powerful, and simplest strategy that is used to enhance plant zinc usage as well as the content of zinc in grains. In different countries, improvement in zinc efficacy has become an important challenge for plant breeding (Kabir et al., 2014). Another biofortification strategy to control the deficiency of zinc in plants is zinc supplements through fertilizers, which is referred to as agronomic biofortification (Rehman et al., 2020; Szerement et al., 2021; Barman et al., 2023). Zinc can be added directly to the soil in the form of inorganic (Zn oxide, Zn oxysulpate) and organic (Zn-EDTA). However, the extremely used inorganic Zn form is Zn sulphate, because it is inexpensive and highly soluble in water. Zn-EDTA is more effective as compared to inorganic Zn-containing fertilizers. Although Zn-EDTA application is limited in cereal cultivation because of being cost-intensive (Cakmak, 2008).

Nanoparticles have distinct physiological features and the ability to improve plant metabolic reactions (Giraldo et al., 2014). Agriculture and food processing are both being transformed by nanotechnology and the use of nano-ZnO has increased substantially (Rouhi et al., 2013; Liu et al., 2022; Nekoukhou et al., 2022; Sun et al., 2023) In this instance, foliar-applied nZnO could enhance seed germination, growth, and nutritional value of plants (Rai-Kalal and Jajoo, 2021; Nile et al., 2022; Mazhar et al., 2023). Alternatively, these could be used for the increasing Zn-bioaccumulation in potatoes.

Potato (*Solanum tuberosum* L.) is the fourth most essential food crop after wheat, rice, and maize in terms of human consumption (Ezekiel et al., 2013; Lachman et al., 2013). Moreover, it is a good source of nutrients, water, carbohydrates, lipids, and vitamins (Struik, 2007; Haverkort et al., 2022). More than five thousand varieties of potato are present worldwide, and most of them are found in South America. Furthermore, the potato crop is popular in Pakistan and worldwide based on its nutritional value, the potential for a variety of uses (in both raw and processed form), and easy accessibility for less-income consumers (Zaheer & Akhtar, 2016). Zinc is an essential micronutrient, and its inadequate bioavailability is the key factor in increasing potato growth and yield (Nekoukhou et al., 2022). Keeping this in view, we tested the possibility of using nano-zinc oxide (nZnO) for enhancing Zn-bioaccumulation in potato tubers when applied during vegetative stages. Here, we report that nZnO improved both the tuber yield and Zn-nutrition in the edible part.

Materials and Methods

Experimental site

Influence of the foliar application of zinc oxide nanoparticles on growth, yield, and zinc bioaccumulation in potato (*Solanum tuberosum* L.) was studied in a field experiment conducted at Mauza Rakh Danoya, Tehsil Ferozwala, District, Sheikhupura (31°40’9”N; 74°16’26”E) during potato autumn growing season 2021-2022. The experiment was laid out in a randomized complete block design (RCBD) with five replications per treatment. The field was divided into five plots and each plot size was 4 m^2 (2 m length × 2 m width). Two ridges were made in each plot. Seed tubers (35-50 mm) were sown to the raised beds with 30 cm plant-to-plant distance and a 90 cm row-to-row distance. Moreover, in each plot 10 to 15 plants were planted.

Foliar application of nZnO

After 55 days of seed germination (at tuber initiation stage), foliar application of nZnO crystals was applied at 0, 25, 50, 100, and 250 mg L^{-1} concentrations. Tween-20 (0.1%) was added to enhance the penetration and absorption of liquid on the leaf surface. Changes in morphological, yield and biochemical attributes were recorded after 40 days of foliar treatment at the tuber maturity stage (95 days old plants).

Determination of growth and yield attributes

At maturity (95 d), plants were harvested and different growth parameters like number of shoots per plant, shoot length, shoot fresh weight, root length, root fresh weight, and leaf area were recorded after 40 days of foliar treatment of nZnO. Subsequently, various yield attributes like the number of tubers ($plant^{-1}$), tuber diameter (cm), tuber fresh weight (g $plant^{-1}$), tuber dry weight (g $plant^{-1}$), average tuber fresh weight (g $tuber^{1}$), stem end fresh weight of tuber (g), bud end fresh weight of tuber (g) and pith fresh weight of tuber (g) were determined.

Biochemical parameters and analyses of zinc content

The dried potato material (0.1 g) was extracted with 10 mL methanol (80%) and kept at room temperature for 24 h. After that, it was used for the determination of flavonoids (Pękal and Pyrzynska, 2014), total phenolics (Bray and Thorpe 1954), total soluble sugars (Dubois et al., 1956), and total free amino acids (Hamilton and Van Slyke, 1943). Moreover, soluble starch was determined according to Bahdanovich et al. (2022). The analyses of zinc were determined after wet acid digestion (Wolf, 2008). For this purpose, the dried potato material (0.1 g) was digested in H_2SO_4 for two days and then heated on a hot plate magnetic stirrer for 30 min with periodic addition of hydrogen peroxide until the material was clear. The extract was filtered and made up to 50 mL final volume using distilled water. Afterward, zinc analyses were performed by an atomic absorption spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan).

Data analyses

For all the variables, one way-ANOVA was performed using Statistix 8.1 and the differences among treatments were evaluated using the least significant difference (LSD) test. The figures were prepared using MS Excel 365 using mean values (n = 5 ± SE).

Results

Potato growth in response to foliar application of nano-zinc oxide

Potato growth traits varied considerably due to zinc oxide nanoparticle treatments (**Figure 1**). Maximum improvement in shoot parameters was evident in plants sprayed with 25 mg L^{-1} nano ZnO.

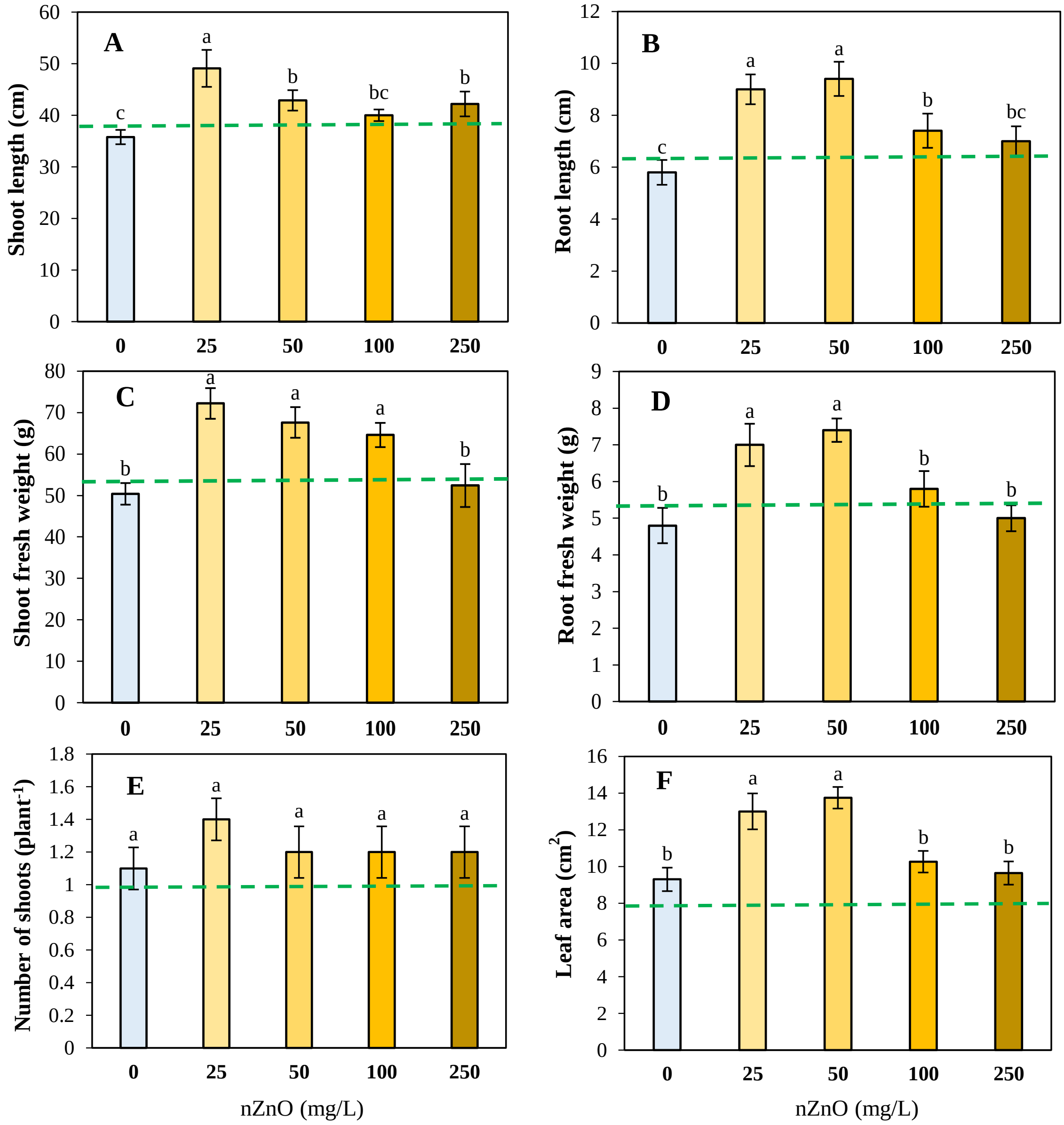

Figure 1 A-F: Changes in morphological parameters of potato due to exogenous application of nZnO. The bars above the green line indicate changes with reference to control (Mean ± SE). Bars bearing same letters do not differ significantly at *P* < 0.05.

Likewise, root parameters and leaf area were recorded highest in response to 50 mg L^{-1} dose and also statistically the same at 25 mg L^{-1} nZnO. Foliar application of 25 mg L^{-1} nZnO increased shoot length, shoot fresh weight, and number of shoots by 37%, 43%, and 27%, respectively compared to those of the un-sprayed control plants. Moreover, the 50 mg L^{-1} dose enhanced root length, root fresh weight, and leaf area by 62%, 54%, and 47%, respectively, as compared to the control (**Figure 1**).

Potato yield traits

A lower concentration of nZnO improved tuber yield in potatoes. Foliar treatment of 25 mg L^{-1} nZnO significantly increased the number of tubers, tubers fresh weight, tuber dry weight, stem end fresh weight of tuber, bud end fresh weight of tuber, and pith fresh weight of potato by 68%, 48%, 95%, 85%, 87% and 38%, respectively, whereas average tuber fresh weight and tuber diameter increased non-significantly by 51% and 13%, respectively, compared to those of water sprayed control plants (**Table 1**).

Table 1: Yield attributes of potato in response to foliar-applied nano-zinc oxide

nZnO	NT	TD	TFW	TDW	ATFW	SFWT	BFWT	PFWT
0	4.40^{c}	4.00	247.4^{b}	12.19^{c}	64.60	24.8^{c}	24.0^{c}	3.6^{b}
25	7.40^{a}	4.52	367.6^{a}	25.44^{a}	98.07	46.0^{a}	45.0^{a}	5.0^{a}
50	6.80^{ab}	4.12	337.4^{a}	21.16^{b}	82.67	39.2^{ab}	40.8^{ab}	4.0^{b}
100	6.20^{b}	4.06	333.4^{a}	18.82^{b}	75.07	38.4^{b}	36.6^{b}	4.4^{ab}
250	6.00^{b}	4.28	346.0^{a}	18.34^{b}	74.27	38.6^{ab}	34.4^{b}	3.8^{b}
F-value	14.41^{*}	2.06^{ns}	5.66^{*}	15.17^{*}	1.53^{ns}	9.46^{*}	8.32^{*}	3.71^{*}

Abbreviations: nZnO = nZnO levels (mg L^{-1}); NT = Number of tubers ($plant^{-1}$); TD = Tuber diameter (cm); TFW = Tuber fresh weight (g $plant^{-1}$); TDW = Tuber dry weight (g $plant^{-1}$); ATFW = Average tuber fresh weight (g $tuber^{-1}$); SFWT = Stem-end fresh weight of tuber (g); BFWT = Bud-end fresh weight of tuber (g) PFWT = Pith fresh weight of tuber (g). Means within each column sharing same alphabets do not differ significantly at $P < 0.05$; *significant at $P < 0.05$.

Potato quality traits

Foliar application of nZnO significantly increased all the quality attributes of potatoes. Foliar application of 50 mg L^{-1} nZnO improved the concentration of total phenolics (**Figure 2A**) and flavonoids

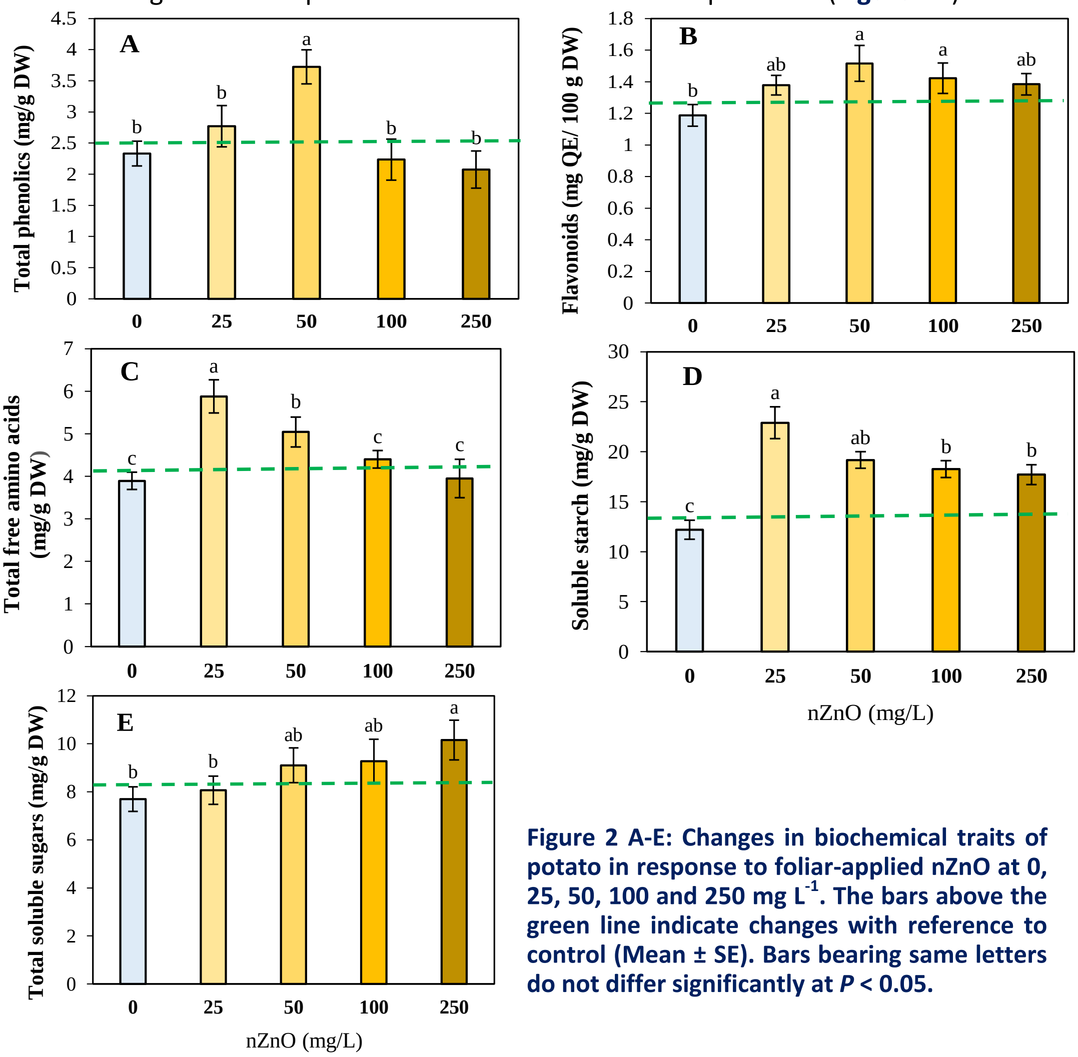

Figure 2 A-E: Changes in biochemical traits of potato in response to foliar-applied nZnO at 0, 25, 50, 100 and 250 mg L^{-1}. The bars above the green line indicate changes with reference to control (Mean ± SE). Bars bearing same letters do not differ significantly at *P* < 0.05.

(**Figure 2B**) by 59% and 27%, respectively, when compared to the control. Similarly, total free amino acids and soluble starch content increased substantially by 50% and 87% in response to the foliar spray of 25 mg L^{-1} nano-ZnO (**Figure 2C; Figure 2D**). Moreover, total soluble sugars increased progressively by increasing the concentration of nZnO. Accordingly, the highest total soluble sugar content was recorded at 250 mg L^{-1} nZnO by 31% than that of the control group (**Figure 2E**).

Tuber zinc content in response to nano-zinc oxide spray

Zinc internalization in the tuber increased gradually by increasing the foliar-applied nZnO dose. Correspondingly, the maximum zinc content in tuber (65.5 µg g^{-1} DW) by 35% was observed in plants treated with 250 mg L^{-1} nZnO, although tuber zinc concentration in the control plants was 48.3 µg g^{-1} DW (**Figure 3A; Figure 3B**).

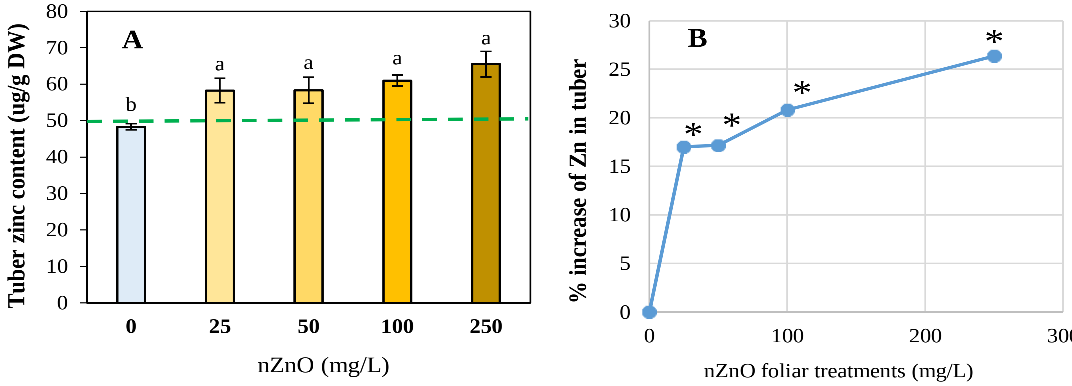

Figure 3: Mean zinc content (A) and percent increase in Zn content (B) in tuber in response to foliar-applied nZnO at 0, 25, 50, 100 and 250 mg L^{-1}. The bars above the green line indicate changes with reference to control (Mean ± SE). Bars bearing same letters do not differ significantly at $P < 0.05$.

Discussion

Zinc oxide nanoparticles have a broad range of impacts on the morphological, physiological, yield, and quality parameters of plants, and various studies have reported the beneficial effects of nZnO on plant growth and productivity (Sturikova et al., 2018; Liu et al., 2022). In our study, foliar treatments of nZnO increased all the growth attributes of potato plants. Potato shoot length, shoot fresh weight, and number of shoots increased by 37%, 43%, and 27%, and root length, root fresh weight, and leaf area increased 62%, 54%, and 47% at 25 and 50 mg L^{-1} of nZnO, respectively. Likewised, in mung bean plants sprayed with 20 mg L^{-1} nZnO, maximum increments in growth of shoot and root were noticed by 97% and 42%, respectively, as compared to the control (Dhoke et al., 2011). Moreover, application of 40 mg L^{-1} nZnO enhanced shoot length, root length, shoot fresh weight, and root fresh weight of flax plant when compared to the control (Sadak and Bakry, 2020). Similarly, shoot growth in coffee (*Coffea arabica* L.) was recorded to be increased due to the foliar application of nZnO (Rossi et al., 2019). Moreover, in *Solanum lycopersicum,* foliar treatment of zinc oxide nanoparticles resulted in a significant increase in shoot and root growth due to enhancement in the inter-nodal length (Raliya et al., 2015). It was reported that Zn increased plant growth by stimulating plant growth hormones like indole acetic acid which plays an important role in shoot and root development (Liu et al., 2015). This enhancement in growth could have been also due to the zinc efficiency to increase the absorption of macronutrients (phosphorus, nitrogen, potassium, etc.) from soil which promote growth and development (Wang et al., 2017).

An increase was recorded in the number of tubers per plant, tuber fresh weight per plant, average tuber fresh weight and related attributes in response to nZnO application. Likewise, recently Seleiman et al. (2023) have reported enhanced potato tuber yield due to exogenously applied zinc. Another study reported an increase in potato tuber yield per plant by 46% due to 25 mg L^{-1} Zn-application (Dhakal and Shrestha, 2019). Earlier, Sati et al. (2017) reported that Zn-mediated enhancement in the number of tubers per plant was linked to regulation of endogenous phytohormonal levels of IAA, GA and cytokinins. These phytohormones are believed to act as a developmental switch to regulate different growth stages of potato growth (Aksenova et al., 2012; Saidi and Hajibarat, 2021). It is reported that both GA and cytokinins regulate the meristematic activity in potato and affect tuber initiation (Hartmann et al., 2011). However, the tuber initiation is positively linked with the hormonal levels of IAA, cytokinins and ABA, whereas GA exhibits a suppressive role in tuber initiation (Cenzano et al., 2003). Nonetheless, it has been shown that Zn treatment could positively affect tuber development and its fresh weight (Kumar et al., 2008). Not only this, the beneficial effects of Zn application on various other crops have been reported.

In case of *Triticum aestivum,* nZnO application at 80 mg L^{-1} concentration also resulted in a maximum grain yield compared to the control (Sheoran et al., 2021). Therefore, our results regarding the positive effects of lower concentrations of nZnO are consistent with the earlier-mentioned several reports.

Foliar application of nZnO induced improvements in the potato quality traits. An increase in the soluble starch and total free amino acids by 87% and 50%, total phenolics and flavonoids content by 59% and 27%, and total soluble sugars by 31% at 25, 50, and 250 mg L^{-1} nZnO, respectively, was recorded. Like our findings, Sadak and Bakry (2020) also reported an increase in free amino acids in flax plants due to ZnO nanoparticles application (0-60 mg L^{-1}). Likewise, broccoli plants treated with zinc oxide nanoparticles had enhanced phenolic and sugar contents to a significant level (Awan et al., 2021). Similar findings were also reported earlier by other researchers (Singh et al., 2013; Salama et al., 2019).

Numerous researchers have been trying to enhance growth of different plants through different mechanisms including application of Fe- and Zn-fertilizers (Kromann et al., 2017). It is also reported that priming of potato tubers with 10 mg mL^{-1} Zn for 12 h could be an effective approach to enhance Zn-fraction in edible tubers (Carmona et al., 2019). Another study concluded that the foliar application of bulk ZnO and $ZnSO_4$ improved Zn status of potato tubers, and therefore foliar application of Zn fertilizers could be a promising strategy for agronomic biofortification of Zn in potato tubers (White et al., 2017).

As a step forward, we tested the effectiveness of nZnO in improving the Zn-fraction of the tubers and whether and to what extent nZnO could alter agronomic biofortification. Our results indicated that tuber Zn concentration was significantly enhanced by increasing the concentration of zinc oxide nanoparticles. To be more precise, nZnO spray at 25, 50, 100, and 250 mg L^{-1} enhanced the tuber zinc concentration by 20%, 20%, 26%, and 35% when compared to the control. The highest value of tuber zinc content by 35% was noticed at 250 mg L^{-1} nZnO. In consistent with our findings, foliar application of mycogenic ZnO nanoparticles caused improvements in Zn-fortification in tomato and potato crops (Singh et al., 2023). Additionally, nZnO have been effectively used to enhance Zn concentration in vegetables, cereals, and many other crops (Umar et al., 2020; Sahoo et al., 2021; Velázquez-Gamboa et al., 2021; Parashar et al., 2023). Overall, it can be concluded that nZnO application to potato plants enhances plant performance and translocation of Zn to potato tubers.

Conclusion

Exogenous application of nano-zinc oxide to potato plants could improve quality traits. Our findings inferred that the foliar spray of nano-zinc oxide was very effective in enhancing the potato biochemical traits, primarily starch accumulation. Another major finding includes substantial bioaccumulation of Zn in potato tubers. Also, we believe that both the smaller size and stage-specific timing of nano-fertilizer application contributed to effective translocation of Zn in the potato tubers by the source-sink relationship (from leaves to tubers) leading to agronomic biofortification.

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Declaration of Author(s), Editor(s) and Publisher

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No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no competing financial interests.

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Contribution of authors
Conceptualization and designing of the study: TN, SA, AM, FS. Conduction of experiment and collection of data: TN, AM. Analytical work: TN, SA, FS. Written first draft of the manuscript: TN, SA, FS. Helped to prepare figures and tables: AM, SA. Statistical analysis of data: TN, SA. Final draft reviewed and read by all authors.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is needed.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with care during collection and experimental procedures. After completion of experiment, all materials were properly discarded to minimize any types of bio-contamination(s).

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher. These are available with the corresponding author and/or with other author(s) as declared by the corresponding author of this manuscript.

Authors' consent
All authors contributed in designing and writing the entire review article. All contributors have critically read this manuscript and agreed for publishing in IJAaEB.

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