



## Research article

# Foliar application of biosynthesised zinc nanoparticles as a strategy for ferti-fortification by improving yield, zinc content and zinc use efficiency in amaranth<sup>☆</sup>

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## ABSTRACT

Deficiency in zinc is widely prevalent in developing countries. Ferti-fortification is one of the easiest and quickest options for improving the zinc content in food. Consumption of such food can provide zinc in adequate amounts to the individual. Nanotechnology is now envisioned as the future of agriculture owing to the immense advantages of nanoparticles over bulk materials. In this work, the effect of zinc nanoparticles (Nps) synthesized via biological route using moringa leaves extract was studied on seed germination, its growth parameters, zinc content and nutrient use efficiency in amaranth crop. *Moringa* leaves are rich in plant metabolites such as amino acids, alkaloids, flavonoids, sugars and fatty acids as confirmed by the UPLC-MS system analysis. The XRD studies show that the biosynthesized Nps were hexagonal crystals with an average size of 23.69 nm. The particle size as indicated by scanning electron microscopy was between 15 to 30 nm, and by DLS was 22.8 nm. Foliar application of 10 ppm biosynthesized zinc Nps, resulted in the highest plant height and fresh weight. Although, an increase in concentration of zinc applied through foliar route led to higher zinc content in the plant biomass, the nutrient use efficiency indices indicated that zinc Nps at 10 ppm concentration resulted in better nutrient recovery, improved yield and productivity with respect to the nutrient input. This reflects the advantage of biologically synthesized Nps over the bulk counterparts. These results show that the biologically synthesized Nps can be an attractive alternative to conventional fertilizers for nutrient biofortification and better crop yields.

## 1. Introduction

Micronutrients are required for overall balanced growth in plants as well as in humans. Deficiency of micronutrients is known to cause malnutrition and growth impairment in children. Children and lactating women are most susceptible to such deficiencies. According to a UNICEF report, over 80% adolescents suffer from hidden hunger in India [1], and almost one third of the global population is under the threat of hidden hunger. Hidden hunger refers to the deficiency of essential vitamins and micronutrients in humans (<https://ourworldindata.org/micronutrient-deficiency>). Possible measures to mitigate the micronutrient deficiencies in the diet include supplementation with minerals, consumption of fortified commercial food products, biofortification, etc. Whereas taking external supplements with the diet is a lucrative option to rapidly address the deficiencies, it is not an economical option for individuals from certain social backgrounds. Biofortification is the process by which

the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology. Plant breeding for selection of germplasm with enhanced nutrient content is time and labour intensive approach. Agronomic method of biofortification, called ferti fortification of crops with micronutrients is envisaged as a fast and easy way to address micronutrient deficiencies [2]. Ferti fortification includes application of nutrients to plants externally in order to increase the content of these nutrients in plants which on consumption can provide adequate amount of nutrition to the individual. However, the success of agronomic fortification is limited by low availability of micronutrients to plants in the soil. Although, the micronutrients may be present in adequate amount, the biochemical properties of soil, such as pH and their reaction with soil renders them in forms that are unavailable to the plants. Farmers and crop growers without understanding this relation take up excessive application of nutrients to soil leading to an unchecked accumulation of these nutrients in soil. Thus,

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there is a need to develop ways to improve crop nutrition without disturbing the natural balance of soil through sustainable use of chemicals.

Nanotechnology is now envisioned as the future of agriculture owing to the immense advantages of nanoparticles (Nps) over their bulk counterparts. Nps has strikingly different properties over their bulk counterparts. As the size decreases, there is change in the properties, like increased surface area, surface to volume ratio, and altered surface properties. These impart characteristics enabling the nanomaterials to acquire unique and beneficial properties as compared to bulk materials. Nanotechnology has been applied in the field of agriculture to increase plant growth and yield using nanofertilizers, to increase nutrient use efficiency through synthesis of control release fertilizers, to protect plants from pesticides using sustained release nano-formulations of herbicides, to develop nano-sensors for monitoring soil quality and pesticides for pest and disease management, and to deliver DNA molecule or chemicals into plant cell for gene manipulation [3]. Various reviews have summarized the application of nanotechnology in plant nutrition [4, 5, 6], in plant protection [7, 8], as control release fertilizers [9], nanomaterials delivery to plants [10] and other applications [11, 12, 13]. Nanotechnology based applications have mainly delved into the synthesis of macronutrient Nps wherein the focus has been improving nitrogen use efficiency by developing controlled release urea [14, 15]. There are studies on chemically synthesised iron, zinc and copper based Nps and their positive as well as antagonistic effect on plant growth and soil physiology [5]. However, these chemical protocols of synthesis tend to be toxic, expensive and may involve the use of toxic chemicals. Therefore nanotechnology has now centred on synthesising these metal Nps using plant extracts and microbial cells which comes out as clean, non-toxic, biologically compatible, and eco-friendly method [16, 17]. Use of plant extract to synthesise Nps is an active area of research. Plants are rich in metabolites like alkaloids, phenols, flavonoids, which act as reducing as well as stabilizing agents.

Zinc is a vital nutrient for humans and acts majorly as catalytic, structural and regulatory ion. Zinc plays essential role in maintaining homeostasis in immune function, oxidative stress, apoptosis, and aging. Zinc deficiency is associated with a number of diseases [18]. Prevalence of zinc deficiency in developing countries is very common, and 61% of the population is at an increased risk of low dietary zinc intake. Almost 4% of child mortality and disability-adjusted life years (DALYs) have been associated with zinc deficiency. The Comprehensive National Nutrition Survey for 2016–18 in India reported the national prevalence of low serum zinc among preschool children (17%), school children (16%) and in adolescents (30%) [19]. Economically weaker sections of society are at much greater risk as their diet is not inclusive of food rich in zinc, such as pulses, nuts, meat, eggs and animal-derived food. Thus, ferti-fortification of staple diet like rice, wheat, green leafy vegetables is being proposed as an attractive way to combat zinc deficiency. Agronomic fortification of various crops using zinc containing fertilizers via soil, foliar and soil + foliar routes are well documented [20, 21, 22, 23, 24, 25, 26, 27].

This work aims at developing a nano based formulation of zinc fertilizer for zinc fortification of plants. We have employed moringa (*Moringa oleifera* Lam.) leaves extract for synthesis of zinc based liquid nano-suspension wherein the Nps formed after the reaction of plant extract and zinc salt are directly used for foliar treatment. This gives the advantage of avoiding the time consuming and energy intensive steps of precipitating the Nps and/or drying them at high temperature, which are conventionally followed for biologically synthesized Nps. Moreover moringa leaves extract is known to have plant growth stimulating properties as it is rich in amino acids, minerals like potassium, calcium, Iron, ascorbate, and growth regulating hormones like zeatin, and therefore have been studied for plant growth enhancing abilities [28, 29, 30, 31, 32]. While these studies have established moringa leaves extract as a plant bio-stimulant, in this study, we have used the moringa leaves extract to synthesize Zn Nps. The application of Nps as a foliar spray is expected to provide the plant with the dual advantages- that of an easily bio-available plant micronutrient as well as a bio-stimulant.

Amaranth is a C4 dicotyledonous herbaceous plant. It is rich in proteins with essential amino acids, such as methionine and lysine, dietary fibres, minerals, phytopigments, and bioactive compounds, such as betacyanin, chlorophyll, betaxanthin, carotenoids,  $\beta$ -carotene, vitamin C, phenolic compounds, and flavonoids. It is grown throughout the year and is an easily available economical vegetable. In this work, we have studied the effect of biosynthesised zinc Nps and zinc sulphate on seed germination, growth parameters, zinc content in amaranth, and nutrient use efficiency.

## 2. Materials and methods

### 2.1. Biosynthesis of zinc Nps using moringa leaf extract

#### 2.1.1. Preparation of leaf extract

The moringa leaves were collected from the BITS Pilani Goa Campus. The leaves were washed with distilled water and air dried. 10 g leaves were weighed and ground in 100 ml distilled water to obtain 10% leaf extract. The extract was filtered through muslin cloth and filtrate was centrifuged at 7000 rpm to obtain clear extract which was stored in refrigerator.

#### 2.1.2. Phytochemical composition profile of moringa leaf extract

The water extract of moringa leaves was qualitatively analysed for plant metabolites using Ultra-performance liquid chromatography (UPLC) system (Thermo Fischer Scientific) coupled to Q-Exactive Plus Orbitrap mass spectroscopy (MS) system. The extract was filtered with a syringe filter (0.22  $\mu$ m) and injected into the UPLC system at flow rate of 0.3 ml/min. Chromatographic separation was performed using a Hypersil Gold column (3  $\mu$ m, 100  $\times$  2.1 mm). Mobile phase composition was varied between 0.1% formic acid in water (A) and methanol (B) and gradient was used as follows: 0–2 min, 5% B; 2–20 min, 5% B; 20–25 min, 95% B; 25–26 min, 95% B; 26–30 min, 5% B; 30–35 min, 5% B. The parameters for mass spectrometer analysis were as follows: spray voltage, 3 kV; capillary temperature, 300  $^{\circ}$ C; auxiliary gas (N<sub>2</sub>), 9 a. u.; sheath gas (N<sub>2</sub>), 37 a. u.; AGC target, 1e6; mass range, 70–1000 m/z, resolution, 70,000. Data acquisition and processing was done using Version 4.2.28.14 (Thermo Scientific Xcalibur) and Compound Discoverer 3.2 SP1 respectively. mzCloud and Chemspider were used for identification of metabolites.

#### 2.1.3. Synthesis of zinc Nps

200 mM zinc stock solution was prepared in deionised water using zinc sulphate heptahydrate. *Moringa* leaf extract and zinc stock solution were mixed in 1:1 ratio under continuous stirring condition. The solution was stirred overnight. There was no visible precipitate indicating that the Nps formed were in the suspension. The suspension was stored in refrigerator.

### 2.2. Characterization of phytosynthesized zinc Nps

Biosynthesised zinc Nps were characterized morphologically by Field Emission Scanning Electron Microscope (FESEM) and energy dispersive X-ray spectroscopy (EDS) for surface elemental analyses (Quanta FEG 250). X-ray diffraction (XRD) patterns of the Nps were recorded using X-ray diffractometer (Bruker D8 Advanced). Particle size analyser (Nano-Plus 3 HD by Particulate Systems) was used for size measurement and confirmation of nanoparticles size distribution.

### 2.3. Effect of biosynthesised zinc Nps on the amaranth seeds

Amaranth seeds were used for studying the effect of biosynthesised zinc Nps and commercial zinc sulphate on seed germination and vigour index. The seeds were obtained from local market in Margaon (Salcete taluka) of Goa, India. Biosynthesised zinc Nps and zinc sulphate suspension was prepared at 10, 50, 100, 250, and 500 mg/l (ppm). *Moringa*

leaves extract and distilled water served as the controls. Seeds were cleaned with liquid soap solution and distilled water to remove the dust and other surface adherents. The cleaned and dried seeds (25 seeds per treatment) were taken in a 50 ml falcon tube and treated with 25 ml of treatment solution. This was shaken gently and incubated overnight at room temperature. The germination of the seeds was determined by using "between papers" method [33]. The overnight treated seeds were placed between two layers of moist germination papers. The germination papers were rolled carefully ensuring that no excess pressure was placed on the seeds. These rolls were put in plastic bags to avoid drying and for retention of moisture and kept in the dark at 28–30 °C. After 7 days the seeds were evaluated for normal, abnormal seedling, un-germinated and dead seeds. The experiment was performed in triplicates.

The germination percentage and Vigour Index were calculated as follows:

$$\text{Germination \%} = \frac{\text{No of seeds germinate}}{\text{Total number of seeds}} \times 100 \quad (1)$$

$$\text{Vigour Index} = \text{Germination \%} \times \text{Seedling height (Roots + shoots)} \quad (2)$$

#### 2.4. Effect of biosynthesised zinc Nps on the growth parameters and zinc content in amaranth plants

For the experiment, the surface soil was collected from the vegetable farm in Pernem, Goa. The physiochemical properties of the soil were as follows; pH: 5.8, E.C.: 0.086 mmhos/cm, available nitrogen as N: 172 kg/ha, phosphorus as P<sub>2</sub>O<sub>5</sub>: 114 kg/ha, potassium as K<sub>2</sub>O: 613 kg/ha, organic carbon: 1.03%, iron as Fe: 3.44 ppm, zinc as Zn: 0.42 ppm, copper as Cu: 0.25 ppm. The amaranth seeds were sown at 1 cm depth in pots. After germination the seedlings were thinned to three per pot. The pots were in completely randomized positions and re-positioned weekly to minimize any effect of uneven environmental factors. The experiment was carried out in a shade-net house in BITS Pilani Goa Campus. The plants were irrigated with Hoagland's solution without zinc on alternate days. Once the plants reached the four leaves stage, the pots were treated with following treatments; zinc sulphate solution and biosynthesised zinc Nps suspension at 10, 50, and 100 mg/l (ppm). The concentrations were decided based on our preliminary studies during which burnt patches were observed at higher concentrations of 250 and 500 ppm. The mode of application was via foliar treatment. Water and moringa leaves extract served as controls. The plants were harvested 30 days after treatment. Plant height (cms) and fresh weight (g) were recorded. The zinc content in plant aerial parts was determined using atomic absorption spectrometer (Shimadzu 7000 A) [34]. For this, the aerial parts of the plants were dried in hot air oven. The samples were ground into powder and digested in 2:1 nitric acid and perchloric acid and heated on a sand bath. The sample was then cooled and 1:1 Hydrochloric acid was added. This solution was filtered and clear residue free filtrate was used for determination of zinc content.

#### 2.5. Effect of biosynthesised zinc Nps on zinc use efficiency indices

Zinc use efficiency indices: agronomic efficiency, partial factor productivity, apparent recovery efficiency and physiological efficiency were calculated based on the zinc input, yield and concentration of zinc in the dry biomass [35].

$$\text{Agronomic efficiency (mg mg}^{-1}\text{)} = \frac{Y - Y_0}{F} \quad (3)$$

$$\text{Partial factor productivity (mg mg}^{-1}\text{)} = \frac{Y}{F} \quad (4)$$

$$\text{Apparent recovery efficiency (\%)} = \frac{U - U_0}{F} \times 100 \quad (5)$$

$$\text{Physiological efficiency (mg mg}^{-1}\text{)} = \frac{Y - Y_0}{U - U_0} \quad (6)$$

Where, Y: fresh weigh of biomass (mg) of treatment, Y<sub>0</sub>: fresh weight of biomass (mg) of control, F: nutrient applied, U: zinc content in treated plants, U<sub>0</sub>: zinc content in control plants.

#### 2.6. Statistical analysis

A Shapiro-Wilk's test (p > 0.05) showed that the data was approximately normally distributed. A Levene's test indicated the homogeneity of variance (p > 0.05). Data was statistically analysed using analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) at 5% probability level, and was used for comparison of treatment's means. All analysis was carried out through statistical software IBM SPSS 25.

### 3. Results and discussions

#### 3.1. Phytochemical composition profile of moringa leaf extract

The UPLC-MS analysis revealed that the extract is rich in amino acids, alkaloids, flavonoids, sugars, fatty acids and other metabolites (Supplementary data. 1). The presence of amino acids and plant hormones makes moringa leaves extract an excellent plant bio stimulant. Though the exact mechanism of how plant extracts aid in NPs synthesis is not well elucidated, the proposed hypothesis suggests that the plant metabolites like alkaloids, flavonoids help as reducing and capping agents [36]. The moringa leaves extract is rich in flavonols like kaempferol, trifolin; the alkaloid trigonelline and organic acids such as quinic acid, malic acid and citric acid, which could be involved in reducing the zinc salts and act as capping and stabilizing agents for the synthesised Nps.

#### 3.2. Characterization of biosynthesised zinc Nps

As seen from Figure 1(a) FESEM micrographs revealed that the biosynthesised zinc Nps are spherical in shape with the diameter ranging from 15 to 30 nm. The slight heterogeneity in the size is expected during biological synthesis of Nps as several plant metabolites contribute towards Nps synthesis and stabilization. The EDAX analysis confirmed the presence of zinc in Nps Figure 1(b). The presence of other elements like carbon, oxygen is attributed to biomolecules present in the plant extract used for nanoparticle synthesis. Figure 2 illustrates the XRD pattern for biosynthesised Nps. The diffraction peaks obtained at 2 theta values of 28.24°, 28.64°, 29.43° were assigned to zinc sulphide structure with hexagonal crystal system (JCPDS no: 01-73-6009). The average crystal size was calculated using Debye Scherrer formula (Eq. (7)):

$$D = \frac{0.9\lambda}{\beta \cos\theta} \quad (7)$$

Where, D is crystal size, λ is X-ray wavelength, β is full width half maxima and θ is Bragg's angle in radians. The average crystal size obtained for biosynthesised zinc Nps was 23.69 nm. Particle size distribution was analysed by dynamic light scattering (DLS). The average particle size (based on volume distribution) was found to be around 22.8 nm (Figure 3).

#### 3.3. Effect of biosynthesised zinc Nps on the amaranth seeds

As seen in Figure 4(a), germination percentage calculated using Eq. (1) is influenced by varying concentration of zinc. Germination percentage was not affected significantly from 10 to 250 ppm concentrations in case of both

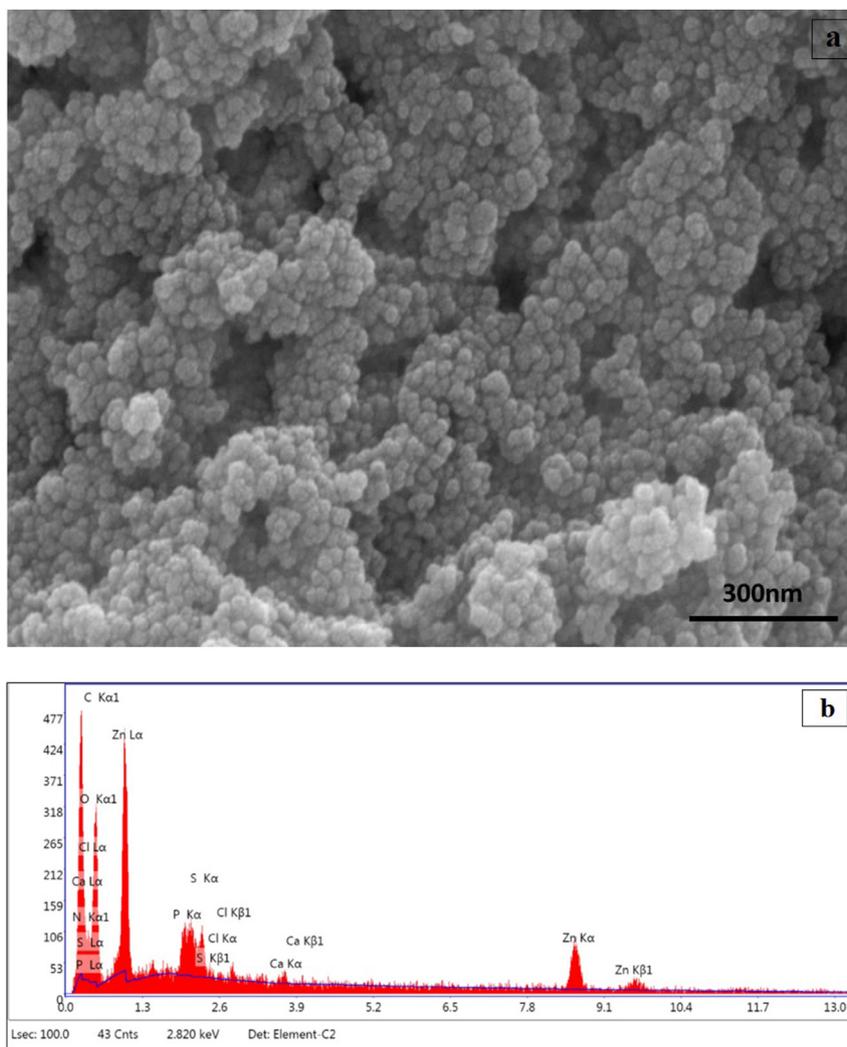


Figure 1. SEM Micrograph (a) and EDAX spectrum (b) of biosynthesised zinc nanoparticles.

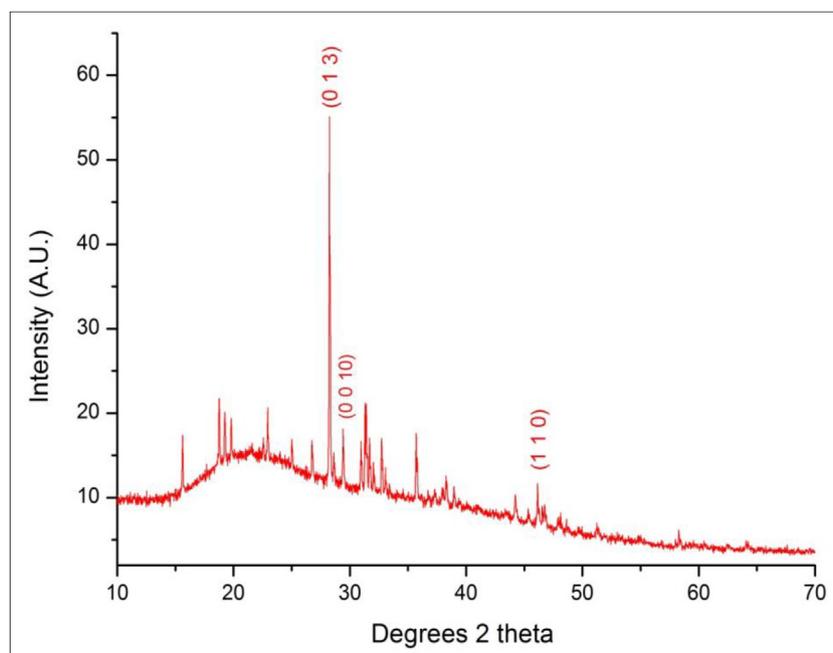


Figure 2. XRD pattern of biosynthesised zinc nanoparticles.

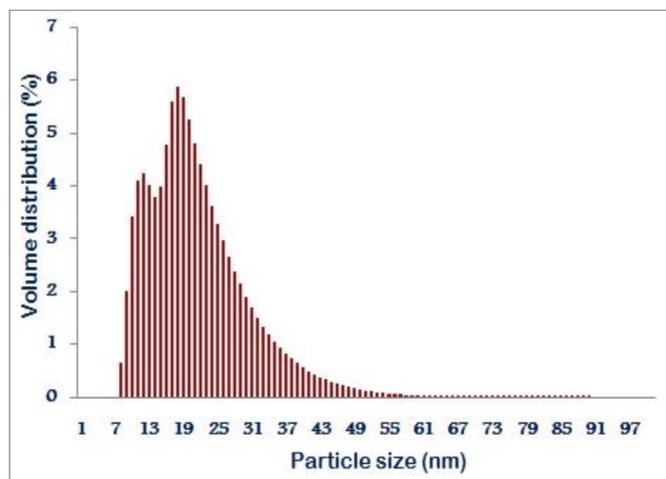


Figure 3. DLS analysis of zinc nanoparticles.

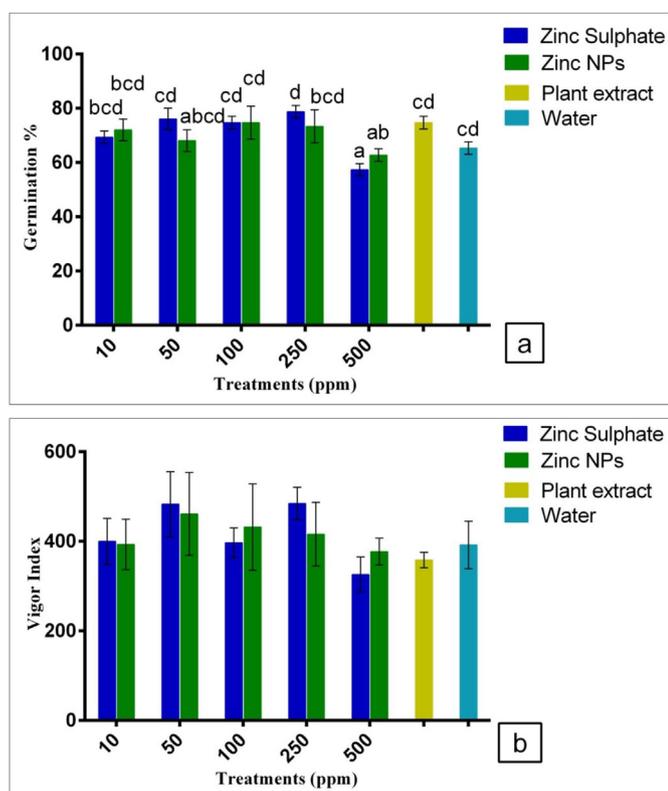


Figure 4. Effect of zinc sulphate and biosynthesised zinc Nps on Germination Percentage (a) and Vigour Index (b) of Amaranth seeds. (Similar letters indicate no statistical significance).

zinc sulphate and biosynthesised zinc Nps, however at 500 ppm, the germination percentage decreased significantly; this is possibly due to zinc toxicity at higher concentration. Germination percentage in case of plant extract was also comparable to 10–250 ppm concentration of zinc sulphate and biosynthesised zinc Nps; this could be due to the stimulatory effect of moringa leaves extract as it is rich in amino acids, minerals like K, Ca, Fe, ascorbate phenols, flavanoids, and growth regulating hormones like zeatin. It is known that antioxidant compounds like ascorbic acid can increase phenolic content, free radical scavenging enzymes like catalases (CAT), peroxidases (POD) and superoxide dismutases (SOD) which are involved in improving the plant growth behaviour under stressful environments. Studies have demonstrated that priming of maize seeds with moringa leaves

extract resulted in increased seedling emergence rate which is attributed to the increased phenolic content in the seedling [28]; priming of wheat seeds with moringa leaves extract led to an increase in radical scavenging enzymes activity, thereby increasing seedling vigour in wheat [37]. A study reported reduced germination percentage at higher dose of  $\text{Fe}_2\text{O}_3$  Nps wherein 50–200 ppm exhibited the best result while 400–800 ppm led to reduction in germination percentage [38]. Numerous studies are available for the effect of zinc oxide (ZnO) Nps on seed germination of various plant cultivars [39, 40, 41, 42]. It is evident from these studies that the effects of nano-materials on plant growth and development are dependent upon the type of Nps, the concentration, and the plant species involved. ANOVA analysis of vigour index as calculated using Eq. (2) (Figure 4 (b)) showed variation in the indices for different treatments; however the effect was not statistically significant (Fc treatment = 2.009; df = 11 and  $p = 0.074$ ).

It is known that interplay between reactive oxidative species (ROS), radical scavenging system and phytohormone signalling is essential for breaking seed dormancy and initiation of germination. However, the exact mechanism of how crosstalk between ROS, phytohormones giberellic acid (GA) and abscisic acid (ABA) aids in seed germination and dormancy is not known. It is postulated that ROS probably is involved in activation of GA synthesis, mobilization of storage proteins and hydrolysis of polysaccharides in seed endosperm which help in seed germination [43]. Plants respond to external stimuli by ROS generation and their activity is regulated by the antioxidant system. Thus the antioxidant system is indirectly associated with seed germination [44]. Interestingly, Nps are recognized as foreign agents by seeds and on internalisation, result in accumulation of ROS which leads to activation of antioxidant enzyme system and therefore may have a role in enhancing seed germination. In a study on the effect of nanopriming by phytosynthesized silver Nps and silver nitrate ions at 5 and 10 ppm concentrations on aged rice seeds, it was observed that nanopriming resulted in increased germination percentage and seedling vigour as compared to unprimed, hydroprimed or seeds primed with silver nitrate. Treatment with silver Nps resulted in an up-regulation of aquaporin genes, enhanced  $\alpha$ -amylase activity leading to higher soluble sugar content essential for supporting the growth of seedlings, and increased ROS in seeds which was essentially within an oxidative window that allows ROS signalling required for germination [45]. Previous studies have also established that increased levels of ROS and several phytohormones regulate expression of various plant aquaporins [46, 47]. Nanopriming of seeds has a role in regulating aquaporins, thereby improving water uptake and retention, aiding in seed germination. It has been observed in a study that tomato seeds treated with carbon nano tubes (CNTs) (10–40  $\mu\text{g}/\text{ml}$ ) contained 19% more water and showed higher germination rate as compared to untreated seeds. Here, it is proposed that the CNTs create channels to permit entry of water in the seed coats [48].

#### 3.4. Effect of biosynthesised zinc Nps on the growth parameters and zinc content in amaranth plants

As observed in Figures 5 and 6(a) and (b), plant height and fresh weight was seen to be highest in plants treated with 10 ppm biosynthesised zinc Nps. Plant height and fresh weight decreased with increase in treatment concentration in case of biosynthesised zinc Nps. This could be attributed to excessive zinc content which is toxic to the plant. However, in case of zinc sulphate, maximum height and fresh weight was observed at 50 ppm. Plant height and fresh weight following treatment was in the order of 10 ppm biosynthesised zinc Nps > 50 ppm biosynthesised zinc Nps > 50 ppm  $\text{ZnSO}_4$  > 100 ppm  $\text{ZnSO}_4$  > 100 ppm biosynthesised zinc Nps > 10 ppm  $\text{ZnSO}_4$  > plant extract > water. Biosynthesised zinc Nps at 10 ppm showed significant improvement in plant height and fresh weight as compared to zinc sulphate at 10 ppm. However, no significant difference was observed between bulk zinc sulphate and biosynthesised zinc Nps at 50 and 100 ppm treatments. Our findings are similar to a study on foliar treatment of ZnO Nps in Pearl Millet where significant improvement in plant height, dry biomass, and grain yield as compared to the bulk ZnO treatment was noted at 10 ppm [49]. An



Figure 5. Amaranth Plants on treatment with control, zinc sulphate and biosynthesised zinc Nps.

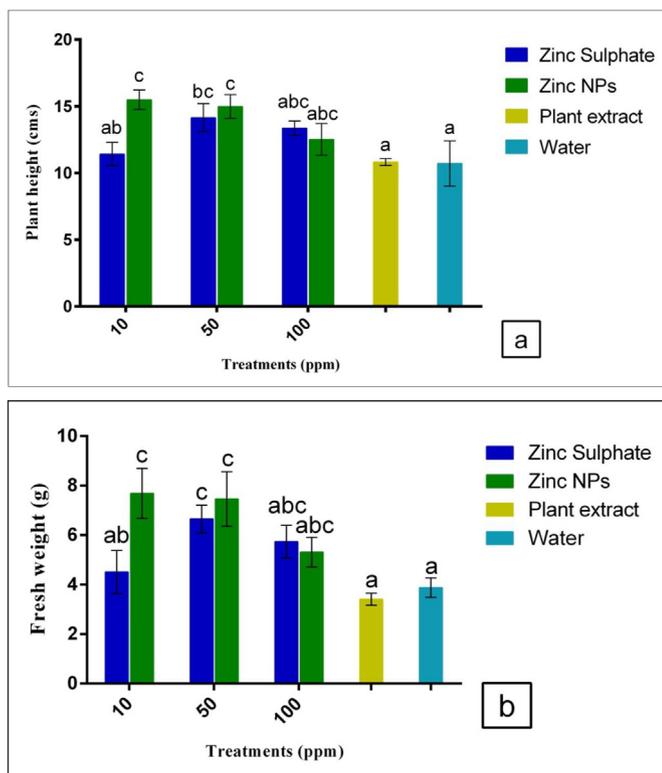
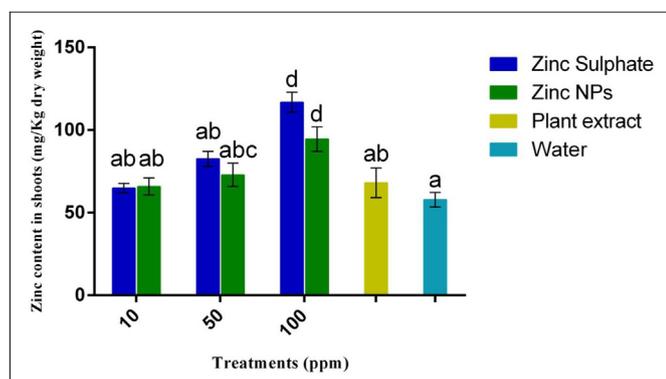


Figure 6. Effect of zinc sulphate and biosynthesised zinc Nps on Plant height (a) and fresh weight of Amaranth plants. (Similar letters indicate no statistical significance).

increase in plant height and biomass is also reported in case of Clusterbeans, and moong crop on application of 10 ppm ZnO Nps as a foliar spray [50, 51]. Other studies have reported comparative effect of bulk

and nano zinc on various crops. A comparative study on the effect of nano ZnO and bulk zinc sulphate at 25, 50, 75, 100 ppm on *Caesalpinia bonducella* (L) Fleming, showed that for all concentrations, the plant height and fresh weights of plants were higher with nano zinc treatment as compared to bulk zinc sulphate [52]. The growth parameters increased with increase in treatment concentration in both zinc sulphate and nano ZnO treated plants. However, our finding suggested that at concentrations higher than 100 ppm there was a decrease in plant height and biomass. This difference in the findings could be attributed to the nature of the plant used for the study, as the amaranth plant is soft and sensitive, and is probably susceptible to burning at high concentration of zinc salt. It has also been reported that the plant height and growth of soyabean plant was better when nano zinc was applied to the soil as compared to bulk zinc chloride [53]. An increased grain zinc content with foliar application of nano zinc at 40 ppm which was 10 times lower than bulk zinc application (zinc sulphate 400 ppm) was reported in wheat [54]. As stated earlier, in our study we found that biosynthesised zinc Nps resulted in the best yield parameters at 10 ppm whereas 50 ppm of bulk zinc sulphate was required to obtain similar plant height and yield. This could be explained by understanding the difference between the stomatal and cuticular pathway for uptake of aqueous solutes and water-suspended Nps applied through foliar route. While the stomatal pathway is mainly limited by the size of the particles [55], for cuticular pathway water repellence by adaxial and abaxial leaf surface becomes the limiting factor [56, 57]. Zinc sulphate ions being highly water soluble might have some hindrance in penetrating the lipophilic cuticle and may be acting as a limiting factor in the case of zinc sulphate. But Nps coated with moringa leaves extract are in a suspension form which is oily in nature, and thus exhibit less hydrophilicity and being more dispersible in lipophilic substances compared to zinc sulphate can penetrate through the leaf surface. Also the low retention of zinc sulphate in plant system reduces the bioavailability of zinc at lower concentration [58].

The zinc content in shoots increased in a dose dependent manner, which is with increase in concentration of zinc applied through foliar route. The biomass zinc content increased in the plants, both for zinc sulphate and



**Figure 7.** Effect of zinc sulphate and biosynthesised zinc Nps on zinc content Amaranth plants. (Similar letters indicate no statistical significance).

biosynthesised zinc Nps (Figure 7). The highest zinc content was observed in case of 100 ppm zinc sulphate treatment. There was no significant difference in the zinc content of biomass between biosynthesised zinc Nps and bulk zinc sulphate at 10 and 50 ppm concentration. Our findings are in agreement with various other studies which have reported an increase in the nutrient content in biomass with increase in concentration of micronutrient application. Comparison between different zinc sources, i. e, zinc sulphate heptahydrate, zinc chloride and zinc nitrate hexahydrate, on citrus, applied as 50, 100, 150 and 250 ppm foliar spray, showed one to four times increase in the zinc content of the leaves, with the best effect observed with zinc chloride and zinc nitrate hexahydrate [59]. An increase in the zinc content in plant biomass of maize was observed with increase in zinc concentration used for the treatments [60]. An interesting observation of our study is that though the fresh weight at 10 and 50 ppm treatment is higher in case of Nps treatment, the zinc content in the shoots is similar to that of bulk zinc sulphate. This can be explained as the result of a phenomenon in plant nutrition called dilution effect [61]. Increases in dry-matter accumulation, resulting from application of fertilizers under optimal environmental conditions, often will be accompanied by decrease in plant mineral concentrations. This inverse relationship between growth and mineral concentration, termed the dilution effect, occurs when dry-weight accumulation increases at a faster rate than mineral-nutrient accumulation.

Nps applied through foliar routes are taken up by the leaves via stomata or cuticle, and transported either via symplastic route (in case of smaller particles of 10–50 nm) or apoplastic route (in case of larger particles of 50–200 nm) through phloem sieve tubes along with flow of phloem sap and distributed bi-directionally accumulating in shoots, roots, fruits and grains [6]. Upon entering the plant system, it is still not very well understood as to how Nps help in improved growth and development in plants. Several studies have demonstrated that the possible mechanism is through protein-coding and miRNA gene expression regulation or mediate in different reactive oxidative pathways resulting in an oxidative burst [62]. Nps also affect other attributes such as photosynthesis and biochemical properties in plants [63, 64, 65]. In pearl millet treated with biologically synthesized Zn Nps, an increase in the activities of enzymes such as phytase, dehydrogenase, acid and alkaline phosphatase as compared to bulk-Zn treatment, has been observed [49]. Nps are also reported to alleviate stress conditions like drought and salt stress in various plants. Foliar application of chemically synthesised Zn Nps and green synthesised Zn NPs on spinach [66] and faba bean [67] respectively was seen to alleviate salt stress when grown in soil containing 100–150 mM sodium chloride. Foliar spray of biosynthesized ZnO Nps using *Coleus forskohlii* Briq. leaf extract enhanced growth of tomato plants under drought stress [68]. The Nps treatment lead to a decrease in concentration of hydrogen peroxide, malondialdehyde (MDA), and anthocyanin contents, and an increase in soluble proteins, chlorophyll contents, ascorbic acid, sugars, total phenolic contents and osmolytes like proline, betaine, glycine that could help plants overcome the salinity and drought stress. There are few studies available which report the comparative effects of chemically and biologically

synthesised Nps on plant growth and other attributes. A study reported the effect of foliar application of ferric chloride salt ( $\text{FeCl}_3$ ), chemically and biologically synthesised ferric oxide Nps on *Zea mays L.* grown under hydroponic condition. The biologically synthesised iron oxide Nps using moringa leaves extract showed better plant growth with increased leaf surface area, number of leaves per plant, enhanced chlorophyll and nitrate content and anti-oxidant activity at 50 mg/l, while the  $\text{FeCl}_3$  salt and chemically synthesised Nps showed toxic effect on plants even at lower dosage of 25 mg/l [69]. In another report, bio-synthesized silver Nps using cucumber leaves and rice husk extracts and chemically synthesized Ag Nps were assessed for their antibacterial potency and toxicity to plants. While both the chemically synthesized and biosynthesized Ag Nps exhibited a strong antibacterial activity against *Escherichia coli*, chemically Nps were seen to be phytotoxic as they over-induced ROS system, MDA content and down regulated the antioxidant enzymes [70]. These studies emphasise the importance of biological routes for synthesis of Nps that are less toxic and biocompatible for their application in agriculture.

### 3.5. Effect of biosynthesised zinc Nps on zinc use efficiency indices

Nutrient use efficiency refers to the amount of nutrient recovered by the plant from the amount of nutrient applied to the plant [71]. Table 1 presents the zinc use efficiency indices for biosynthesized zinc Nps and zinc sulphate at different concentrations. The agronomic efficiency, partial productivity factor, apparent recovery efficiency and physiological efficiency were highest in case of 10 ppm biosynthesised zinc Nps treatment. Agronomic efficiency (AE) is the measure of yield improvement due to nutrient input, and partial factor productivity (PFP) addresses the productivity of cropping system in comparison to nutrient applied, as seen in Eqs. (3) and (4). AE and PFP reflect the impact of nutrient on economic yield. In this study, biosynthesized zinc Nps resulted in the highest AE and PFP at 10 ppm, and was about 3 times higher than that of zinc sulphate at the same concentration. Although AE and PFP for bulk zinc sulphate at 10 ppm was lower as compared to biosynthesized zinc Nps, it was significantly higher than 50 and 100 ppm of bulk as well as nano zinc suggesting that the foliar application of zinc at 10 ppm is adequate for the improvement in the yield. Reduced AE and PFP at 50 and 100 ppm also shows that application of fertilizers at higher concentrations leads to wastage of nutrients and highlights the fact that the optimization of zinc concentration is essential for economic and yield benefit. While AE and PFP relate the yield to the nutrient input, physiological efficiency (PE) is the correlation between the yield and the nutrient content taken up by the plants from the applied nutrient, as seen in Eq. (6). Often the plants are unable to take up applied nutrients in adequate amount, especially during soil application as leaching, volatilization, fixing by soil particles, etc. results in lower nutrient recovery [72]. In this study, PE for biosynthesised zinc Nps was almost 4 times higher than that for bulk zinc sulphate at 10 ppm, attributed to lower yield obtained for plants treated with zinc sulphate at 10 ppm.

Eq. (5) shows that the apparent recovery efficiency (ARE) index is the measure of the ability of plant to take up nutrient from the input. ARE

**Table 1.** Effect of foliar application of biosynthesised zinc Nps and zinc sulphate on zinc use efficiency indices of amaranth (Values with similar letters suggest no significant difference at  $p < 0.05$ . AE: Agronomic efficiency, PFP: Partial factor productivity, PE: Physiological efficiency, ARE: Apparent recovery efficiency.).

| Zinc concentration (ppm)    | AE ( $\text{mg mg}^{-1}$ ) | PFP ( $\text{mg mg}^{-1}$ ) | PE ( $\text{mg mg}^{-1}$ ) | ARE (%) |
|-----------------------------|----------------------------|-----------------------------|----------------------------|---------|
| Biosynthesised zinc Nps 10  | 381 b                      | 769 c                       | 593.81 b                   | 80.25   |
| Biosynthesised zinc Nps 50  | 71.6 a                     | 149.2 a                     | 263.36 a                   | 30      |
| Biosynthesised zinc Nps 100 | 14.33 a                    | 53.13 a                     | 37.03 a                    | 36.69   |
| Zinc sulphate 10            | 103.33 a                   | 491.33 b                    | 151.76 a                   | 70.43   |
| Zinc sulphate 50            | 55.33 a                    | 132.9 a                     | 112.78 a                   | 49.34   |
| Zinc sulphate 100           | 18.6 a                     | 57.4 a                      | 30.82 a                    | 58.9    |

decreased in case of biosynthesised zinc Nps as well as zinc sulphate with increase in the concentration. Such a trend is observed when the nutrient input surpasses the nutrient demand by the crop [73]. In this study zinc is supplied in adequate amounts at 10 ppm through either biosynthesised zinc Nps or bulk zinc sulphate hence, high ARE was obtained at 10ppm; and it was seen to decrease at 50 and 100 ppm zinc concentration. ARE is known to be affected by the route of application, and for zinc recovery via soil application, it has been reported to be in the range of 3.8–4.6% [74], 3.5–5.3% [24] for rice and 1.2–1.4% for wheat [75]; whereas in case of foliar route it is reported to be in the range of 26–62% for rice [24] and 25–27% for wheat [75]. The values obtained in this study were in the range of 30–80% indicating that foliar application of fertilizer for amaranth is an efficient route for application of micronutrient fertilizer.

It is evident from this study that with increase in concentration of zinc treatment, there is a significant decrease in the indices. This could be attributed to the fact that zinc being a micronutrient is required by plants in small quantity [76] and higher concentrations tend to be toxic to the plants, affecting the yield and accordingly the efficiency indices. The findings of this study are supported by various other studies which have reported higher nutrient use efficiency indices with lower doses of nutrients for rice, barley with the application of zinc fertilizers [74, 77, 78].

The use of biologically synthesized Nps for agricultural applications can be an economical and eco-friendly alternative to chemically synthesised Nps as the synthesis protocols are simple and do not involve use of toxic chemicals and energy intensive processes. In this study, it has also been observed that Zn Nps synthesized using moringa leaves extract were effective at a lower dose resulting in higher plant biomass indicating that there might be a synergistic effect due to phytochemicals present in the leaf extract and Zn Nps. *Moringa* is a native crop to Indian subcontinent making it easy for sourcing the raw material. Moreover, since the synthesis protocol directly leads to the formation of the nanoparticles solution which can be applied as a foliar spray, large scale production is not a challenge.

#### 4. Conclusion

The present work aimed at studying zinc bio fortification of Amaranth plant using zinc nanoparticles synthesized via a green route using moringa leaves extract. Biosynthesized zinc Nps resulted in the highest plant growth and yield at 10 ppm concentration as opposed to higher concentrations of 50 ppm zinc which was required when bulk zinc sulphate was added as fertilizer. The findings of the study support the hypothesis that application of nanoparticles as fertilizers can help in improving yield and nutritional quality of the crops at concentrations lower than their bulk counter parts. As apparent from the nutrient use efficiency indices, foliar application of green synthesized nanoparticles can be an economical alternative for improving crop yield and efficient recovery of input nutrient resulting in fewer losses to the environment. With intensive field trials, exhaustive research and careful considerations, nano fertilizers could be envisioned as potential candidates for yield improvement and nutrient fortification of crops.

#### Declarations

##### Author contribution statement

Reshma Zakane: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted data; Wrote the paper.

Meenal Kowshik: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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##### Data availability statement

No data was used for the research described in the article.

##### Declaration of interest's statement

The authors declare no conflict of interest.

##### Additional information

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