

Effect of gibberellin, nano-nutrition with titanium, zinc and iron on yield and some physiological and qualitative traits of white beans

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Abstract

Plant nutrition has a vital role in crop production. This study was performed to investigate the effects of different application methods of some nutrients (nano Fe, Zn, and Ti), and gibberellin on yield, some morphophysiological and grain protein of white beans in 2018 as a factorial experiment in a randomized complete block design with four replications. Experimental factors included seed priming (hydropriming, gibberellin priming, titanium nano dioxide, and nano-Zn priming) and micronutrient foliar spraying (zinc, iron, and zinc + iron). The results illustrated that seed priming and foliar application significantly affected yield, yield components and chlorophyll content. Plant height increased in seed priming treatment with gibberellin and foliar application of zinc + iron by 13% compared to the control. Furthermore, this treatment enhanced the number of sub-branches per plant by 32% compared to the control. Grain yield components such as the number of pods per plant and 100-grain weight were also affected by seed priming with nano-Zn, and the simultaneous spraying of iron and zinc that grain yield by 18%, so that grain yield by 2649 kg ha⁻¹ in hydropriming treatment reached to 3211 kg ha⁻¹ in nano-Zn priming with simultaneous application of zinc and iron. Nano-Zn priming with iron foliar application caused the highest biological yield (9011 kg ha⁻¹), which increased by 19% compared to control. Nano-Zn priming increased grain protein percentage by 21%. This treatment along with the foliar application of zinc + iron, significantly enhanced leaf chlorophyll content compared to other treatments. Therefore, to increase the yield of white beans, priming treatment with nano-Zn as well as foliar application of zinc + iron can be used.

Keywords: chlorophyll; grain yield; nano-nutrition; nano-titanium; nano-Zn

Introduction

Bean (*Phaseolus vulgaris* L.) is an annual thermophilic plant of the legume family, which provides a rich source of protein, vitamins, and minerals. Bean is rich in essential amino acids such as lysine, but lacks methionine; so, it can supplement cereal grain protein. Therefore, this plant is cultivated in many developing countries to provide calories and protein (Nazeri *et al.*, 2010). In 2019, the global area sown of beans was 33066183 hectares with about 28902672 tons of production, and in the same year, the area sown of beans in Iran was about 60027 hectares with 122789 tons of production (FAO, 2019).

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Priming is one of the ways to improve seed efficiency. Primed seeds germinate faster and more uniformly over a wider temperature range. During priming, some water is provided to the seed that induces pre-germination metabolic activities, but root emergence is prevented (Hasanuzzaman and Fotopoulos, 2019). Various priming methods such as priming with water, nutrients, microorganisms, plant hormones, salt solutions as well as nanomaterials were suggested for seed priming. Seed hydropriming was proved to increase the germination rate, drought resistance, increase pod number as well as cowpea seed dry weight (Singh *et al.*, 2014).

Crop production is threatened by various environmental stresses and the reduction of resources. A novel revolution in crop production is needed to increase the productions and guarantee the quality and safety of food, in a sustainable way. Nanotechnology can play an important role in this revolution. Seed nano-priming can change seed metabolism and signaling pathways, affecting not only germination and seedling establishment but also the entire plant lifecycle. Studies have shown various benefits of using seed nano-priming, such as improved plant growth and development, increased productivity, and a better nutritional quality of food. Nano-priming modulates biochemical pathways and the balance between reactive oxygen species (ROS) and phytohormones, resulting in the promotion of stress and diseases resistance resulting in the reduction of pesticides and fertilizers (do Espirito Santo Pereira *et al.*, 2021). Seed priming using nanomaterials should be carefully conducted, otherwise not only does not increase the germination rate but can also impair its performance (Xiang *et al.*, 2015).

Understanding the mechanism of reaction at the molecular level between nanoparticles and biological systems is somewhat unknown (Alpana *et al.*, 2019). Nevertheless, there are reports of the positive effects of these compounds on cropping systems. Therefore, the discussion of the biological effects of nanoparticles requires valuable scientific findings. Numerous reports indicate the improvement of germination behavior and related indicators, including germination rate, seed vigor, root length, shoot length, and early establishment of seedling (Lee and Kim, 2000). The accelerated germination in primed seeds can be attributed to increased activity of degrading enzymes such as alpha-amylase, increased bioenergy level in the form of increased ATP, increased RNA and DNA synthesis, simultaneously increased number, and improved yield of mitochondria (Bewley *et al.*, 2013; Hasanuzzaman and Fotopoulos, 2019). Seed priming KCl₂ in pinto beans improves growth (Hajikhani *et al.*, 2011). It has also been stated that seed priming with nano-Zn has improved the physiological characteristics and thus the yield of peanut plants (Prasad *et al.*, 2012).

The application of titanium nanoparticles affects the biochemical and physiological properties of plants (Mishra *et al.*, 2014). In addition to nano-Zn, the application of titanium at the nano level has also positively affected bean seed germination. Alpana *et al.* (2019) stated that priming bean seeds with nano-titanium caused a significant increase in seed germination percentage and other traits associated with seed germination. They reported that bean seed germination enhanced more by increasing nano-titanium concentration. Furthermore, it was found that the titanium nanoparticle's application increased the final grain yield in wheat (Jaberzadeh *et al.*, 2013).

Providing the essential nutrients for the plant is one of the important aspects of crop management to achieve maximum quantitative and qualitative yield. Applying trace elements in the form of seed priming and foliar spraying can be useful in the cropping system. Micronutrients, especially zinc, are essential for higher plants growth, involving various biochemical activities. Zinc plays a key role in the synthesis of proteins, RNA, and DNA (Marschner, 2011; Taiz *et al.*, 2014). Although plants need little zinc, if the plant face zinc deficiency, physiological stresses will reduce plant growth and yield due to the inefficiency of multiple enzyme systems and other metabolic functions related to zinc (Baybordi, 2006).

Iron is also one of the essential elements for plant growth. Wheat and barley, among crop species, are resistant to iron deficiency, while some cultivars of soybean, peanut, and bean are sensitive to it (Tehrani and Malakouti, 2000). Iron deficiency reduces the number of seeds per pod and the number of pods in plants of the *Fabaaceae* family, this phenomenon can be explained by the vital role of this element in nitrogen fixation and reduction (Brear *et al.*, 2013). It is important to maintain sufficient zinc in the soil during seed germination

and early seedling development (Syta *et al.*, 2019). Foliar spraying and nutri-priming are effective methods to meet the nutritional needs of plants for trace elements.

This study firstly aimed at the application of gibberellin to accelerate germination and use the benefits of this rapid and uniform germination. Secondly, to investigate the possibility of addressing the zinc and iron deficiency through priming and foliar application of these elements; and thirdly, the evaluation of titanium nanoxide priming effect on quantitative and qualitative yield and some physiological traits of Pak white beans cultivar.

Materials and Methods

Experimental site, design and materials

The factorial experiment was conducted in a randomized complete block design with four replications on white beans of Pak cultivar in a research farm located in Dorud city, Lorestan province (33° 40' N, 48° 70' E) with an elevation of 1650 meters above sea level in 2018. Before planting, samples were taken from 0-30 cm soil depth and mixed. The properties of soil are listed in Table 1.

Each plot consisted of 6 m six rows, with plant spacing between and per rows 50 and 5 cm, respectively. The distance between the plots was 1m, and the spacing of the blocks was 2 m. The seeds of Pak bean cultivar were inoculated with bacteria (*Rhizobium phaseoli*) then were sowed. Experimental factors included priming treatments (hydropriming, gibberellin 250 ppm, nano titanium dioxide 30 ppm, and nano-zinc 5 ppm), all of them were performed for 12 hours at 25 °C. Aquarium pump was used for aeration. Foliar spraying treatments included (5 ppm zinc in the form of zinc sulfate, 5 ppm iron in the form of iron sulfate, and 5 ppm zinc and iron combination) were applied in two stages before flowering and pod formation at 8 to 10 am. Plastic protection was used to prevent the spread of spraying to adjacent plots.

Table 1. Properties of the initial soil in the experiment

Depth (cm)	Soil texture	EC (dS m ⁻¹)	pH	OC (%)	Total N (%)	OM (%)	P (ppm)	K (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
0-30	Loam (38, 37 and 25%, silt, sand and clay, respectively)	0.3	7.97	1	0.1	1.7	13	246	6.7	0.95	0.61	5.43

Measurement of chlorophylls b, a, and a+b

Measurement of leaf chlorophyll based on Arnon (1949) was determined using a spectrophotometer at 663 and 645 nm. Half of a gram of fresh leaves in the early stages of flowering was grounded using liquid nitrogen in a pestle and mortar; then, transferred into 15 ml test tubes. Next, 10 ml of acetone was added to it and kept in the dark for 2 hours; centrifuged for 15 min at 8000 rpm at 15 °C and the supernatant was used. Then, the adsorption rate was measured to determine the concentration of chlorophyll a, b, and a + b at the associated wavelengths using the following equations (1), (2), and (3) (Arnon, 1949):

$$\text{Chl a (mg/g FW)} = [12.7(\text{ABS}_{663}) - 2.69(\text{ABS}_{645})] \times V/W \quad (1)$$

$$\text{Chl b (mg/g FW)} = [22.9(\text{ABS}_{645}) - 4.68(\text{ABS}_{663})] \times V/W \quad (2)$$

$$\text{Chl a+b (mg/g FW)} = [20.2(\text{ABS}_{645}) + 8.02(\text{ABS}_{663})] \times V/W \quad (3)$$

Where, ABS is the absorption rate at the desired wavelengths (nm), V is the volume of acetone consumed, and W is the weight of fresh leaf.

Field plant sampling for nodules, yield, yield components and harvest index

Plant height and number of sub-branches per plant were measured using an average of ten plants after physiological maturity in each plot. The number of nitrogen-fixing nodules per plant was measured at the start of the podding stage. To count the nitrogen-fixing nodules, the roots were removed from the soil to a depth of 50 cm, and after washing, the active nitrogen-fixing nodules (those with pink color after cutting) were counted. Furthermore, after harvest, the number of pods per plant, the number of seeds per pod, and 100-seed weight were determined by random selection of ten plants from each plot. Finally, after removing the marginal effects, grain yield and biological yield were measured based on each plot's 2 m² harvesting area. Using the following formula, harvest indices were also calculated:

$$\text{Harvest index (HI)} = (\text{Grain yield/Biological yield}) \times 100$$

Grain protein analysis

A NIR instrument (DA 7250, Pertent, Sweden) was used for determination the grain protein percent.

Statistical analysis

Data were analyzed using SAS software version 9.1, and the means were compared using Duncan's multiple range test. Graphs were drawn with Excel software.

Results

A brief result from ANOVA about all traits

The results of analysis of variance showed that the effect of priming treatment on plant height, number of sub-branches per plant, number of nodules per plant, 100-seed weight, grain yield, biological yield, and harvest index were significant, but there was no significant effect on other traits. Besides, the foliar application significantly affected plant height, number of sub-branches per plant, 100-seed weight, grain yield, biological yield, and harvest index but it had no significant effect on other traits. The interaction effect of priming and foliar application on plant height, number of sub-branches per plant, 100-seed weight, grain yield, biological yield, and harvest index were significant (Table 2). The results showed that seed priming significantly affected grain protein, Chl a and Chl b, and total chlorophyll contents. The effect of fertilizer foliar application and the interaction between seed priming and fertilizer foliar treatment on Chl. a, Chl. b and total Chl was significant (Table 3).

Table 2. ANOVA (mean of squares) for yield and yield components of white bean affected by priming treatments and foliar application of Fe and Zn

S.O.V	DF	Plant height	No. of sub-branches per plant	No. of nodules per plant	Pods per plant	Pod length	100 grain weight	Grain yield	Biological yield	HI
Replication	2	19.9 ^{ns}	6.02*	0.08 ^{ns}	1.44*	2.9 ^{ns}	96*	587**	438*	0.046 ^{ns}
Priming (P)	3	26.29**	43.9**	10.69**	58.7**	27.6**	28.7**	253639**	3255526**	11.84**
Foliar spray of Fe and Zn (F)	2	15.86**	7.4**	6.58**	18.7**	1.35 ^{ns}	16.88*	42326**	210706**	0.4**
P*F	6	1.89**	5.14**	0.69 ^{ns}	3.29 ^{ns}	3.25 ^{ns}	2.72**	12875**	121267**	3.19**
Error	22	0.32	1.3	0.41	1.41	1.85	0.09	261	56047	0.02
CV (%)		5.3	6.4	5.41	8.31	14.6	1.11	5.2	8.1	14.8

ns, *, and **, represent non-significant, significant at 5% and 1%, respectively.

Table 3. ANOVA (mean of Squares) for grain protein percentage, Chl. a, Chl. b and Chl. a+b of white bean affected by priming treatments and foliar application of Fe and Zn

S.O.V	DF	Grain Protein (%)	Chl. a	Chl. b	Chl. a+b
Replication	2	2.86	0.023*	0.0023	0.08*
Priming (P)	3	16.23**	0.054**	0.0029**	0.222**
Foliar spray of Fe and Zn (F)	2	4.69	0.014**	0.0002**	0.034**
P*F	6	1.83	0.003**	0.0006**	0.026**
Error	22	2.22	0.00003	0.00006	0.00006
CV (%)		6.3	14.4	12.1	13.6

ns, *, and **; represent non-significant, significant at 5% and 1%, respectively.

Plant height

Mean comparison showed that the highest plant height (51 cm) was obtained in the priming treatment with gibberellin and foliar application of zinc + iron, while the lowest height (44.8 cm) was associated with hydropriming + zinc foliar application (Table 4). The plant height increased in co-treatment of gibberellin priming and simultaneous foliar application of zinc and iron, with a 13% increase compared to the lowest height. This phenomenon can be explained by the hormonal effect of gibberellin on growth. Although priming with gibberellin at the level of simultaneous foliar co-application of zinc + iron and iron only increased the plant height more than other experimental treatments, but it was observed that priming with zinc nanoparticles was placed following this treatment, especially foliar application with iron and zinc + iron caused higher plant height than others by 49 and 49.3 cm, respectively.

The number of sub-branches per plant

The highest number of sub-branches per plant (21.3) was obtained from nano-Zn priming + zinc and iron spraying, while the lowest one was observed in hydro-priming and separate iron and zinc spraying (Table 4). The priming treatment with nano-Zn and simultaneous foliar spraying of zinc and iron increased plant height and enhanced the number of sub-branches per plant by nearly 32% compared to the control.

Nodule per plant

The results showed that the number of nodules per plant in titanium dioxide and gibberellin priming treatment (13 and 12 nodules per plant, respectively) was higher than other priming treatments. It was also found that the hydropriming treatment had the lowest number of nodules per plant (11 nodules per plant) (Figure 1A). The results showed that simultaneous foliar application of zinc and iron had a more positive effect on the number of nodules, and using this treatment, the number of nodules per plant was higher than others (13 nodules per plant). Although the difference between foliar co-application of zinc and iron did not significantly affect nodules per plant, but findings illustrated that iron foliar application had the lowest number of nodules (11 nodules per plant) (Figure 1B).

Pods per plant

The main effect of seed priming and foliar application treatments significantly affected the number of pods per plant, while their interaction was not significant. The results illustrated that nano-Zn priming had the highest number of pods per plant (17 pods). Besides, there was no significant difference between gibberellin and titanium dioxide primers, and plant pods in hydropriming were significantly less than others (Figure 2A). Co-application of zinc + iron produced the highest number of pods per plant (16 pods) (Figure 2B). The number of pods per plant with simultaneous application of zinc and iron along with nano-Zn priming was higher than others, and it was found that nano-Zn priming increased the number of pods by nearly 40% compared to hydropriming.

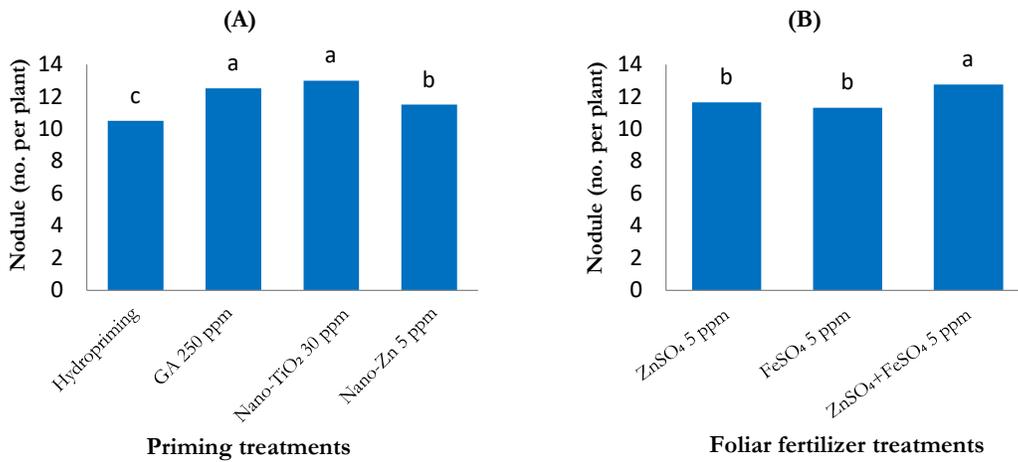


Figure 1. Effect of priming treatments (A) and foliar application of Zn and Fe (B) on number of nitrogen's fixing nodule in white bean

Means with at least one common letter are not significantly different according to Duncan's multiple range test (P=0.05). GA represents gibberellin.

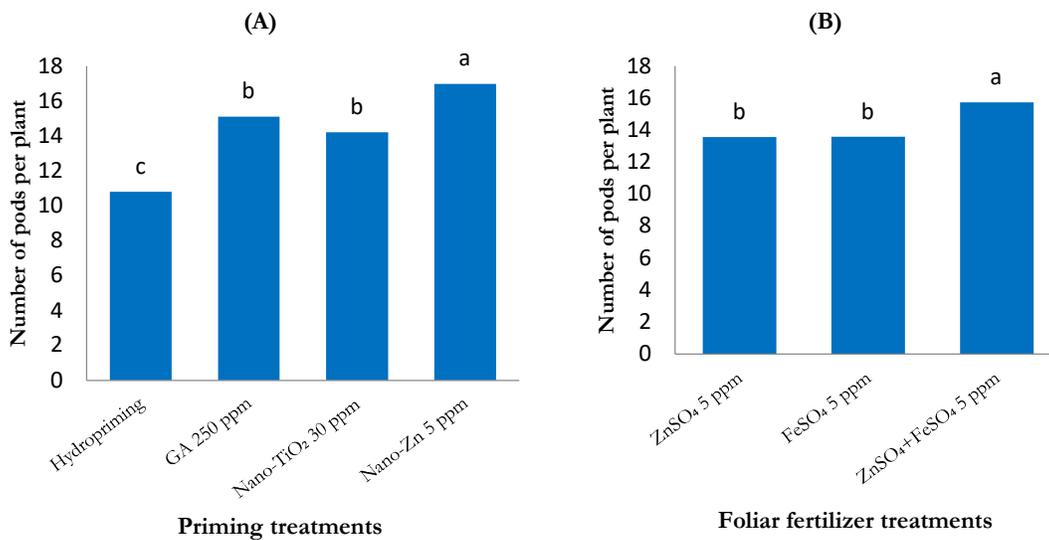


Figure 2. Effect of priming treatments (A) and Foliar application of Zn and Fe on number of pod per plant in white bean

Means with at least one common letter are not significantly different according to Duncan's multiple range test (P=0.05). GA represents gibberellin.

Pod length

The pod length was only affected by seed priming treatment. Pod length under nano-Zn and nano-titanium priming were longer than gibberellin and hydropriming treatments. There was no significant difference between gibberellin priming and hydropriming in terms of pod length (Figure 3).

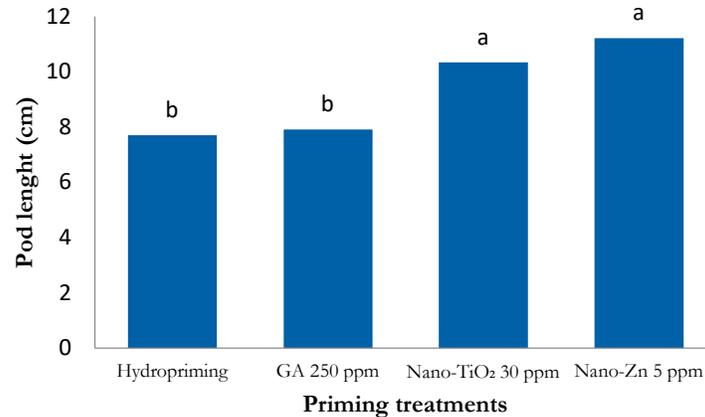


Figure 3. Effect of priming treatments on pod length of white bean

Means with at least one common letter are not significantly different according to Duncan's multiple range test ($P=0.05$). GA represents gibberellin.

100-grains weight

Nano-Zn priming + foliar application of zinc + iron spraying resulted in a 100-grain weight increase that the highest one was 29.4 g. The lowest 100-grain weight was associated with the priming with titanium dioxide + zinc foliar application (Table 4). Simultaneous application of zinc and iron along with nano-Zn priming increased the 100-grains weight by 25%. Foliar spraying treatments accompanied with gibberellin priming resulted in an increase in 100-grain weight of bean compared to hydropriming (Table 4).

Grain yield

The grain yield was strongly affected by the simultaneous foliar application and seed priming. So that, priming with nano-Zn along with the simultaneous application of zinc and iron increased grain yield (3211 kg ha^{-1}). The lowest grain yield was achieved by hydro priming and foliar application of zinc (Table 4). Priming with nano zinc and foliar application of zinc and iron increased grain yield by 18% compared to the control. Gibberellin priming showed a superior effect on grain yield rather than hydropriming (Table 4).

Biological yield

Biological yield was affected by priming and foliar application treatments. Priming treatment with nano-Zn, foliar application of iron resulted in the highest biological yield by 9647 kg ha^{-1} (Table 4). Nano-Zn priming increased the biological yield by about 19% compared to hydropriming. Nano-Zn priming and foliar application with iron were the superior treatment, which led to a 19% increase in biological yield compared to hydro-priming. In addition to the simultaneous foliar spraying of zinc and iron, nano-Zn priming further increased the biological yield. Biological yield in priming treatment with gibberellin was higher than hydropriming at all different foliar treatments.

Harvest index

The highest harvest index (36.7%) was achieved in gibberellin priming + foliar application of iron and zinc, also titanium dioxide priming + zinc foliar application. The lowest one was found in nano-Zn priming + iron foliar application (Table 4).

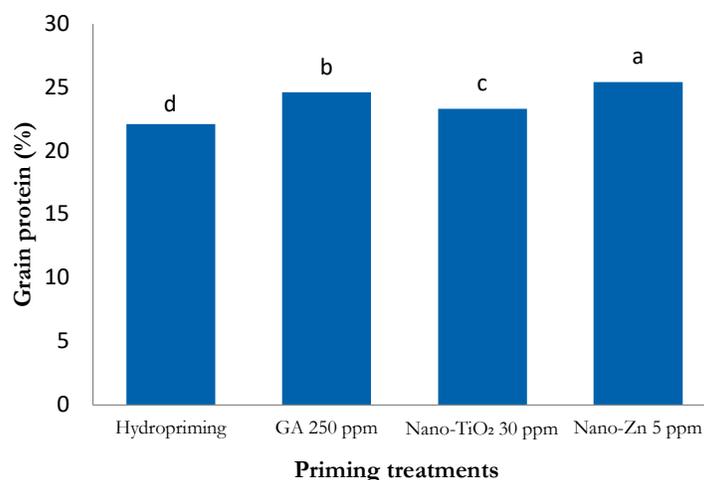
Table 4. Effect of seed priming and foliar of Zn and Fe on yield and yield components of white bean

Priming treatments	Fertilizer (foliar)	Plant height (cm)	No. of branches per plant	100 grain weight (gr)	Grain yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)	Harvest Index (%)
Hydropriming	Zn 5 ppm	44.8g ^a	14.6g	23.8f	2649h	8752l	33.87f
	Fe 5 ppm	45.1g	14.6g	25.1e	2656g	8790k	36.33b
	Zn+ Fe 5 ppm	45.7g	16.3efg	25.5e	2869f	8119j	36.32b
GA 250 ppm	Zn 5 ppm	46.6e	17def	27d	3009e	8325i	36.1bc
	Fe 5 ppm	49.4b	19bcd	28.2b	3099d	8433h	36.7a
	Zn+ Fe 5 ppm	51a	20abc	27.8bc	3101d	8563g	36.2bc
TiO ₂ 30 ppm	Zn 5 ppm	46.1f	18.3cde	22.2g	3201ab	8708f	36.7a
	Fe 5 ppm	46.9e	15.3fg	25.2e	3179bc	8831e	36.0c
	Zn+ Fe 5 ppm	47.7d	17def	27.1d	3102ab	9011d	35.53d
Nano-Zn 5 ppm	Zn 5 ppm	46.9e	18.6cd	27.3d	3127d	9129c	34.25e
	Fe 5 ppm	49c	21ab	27.7bc	3161c	9647a	32.77g
	Zn+ Fe 5 ppm	49.3bc	21.3a	29.7a	3211a	9330b	34.42e

* Means in each column with at least one common letter are not significantly different according to Duncan's multiple range test (P=0.05). GA represents gibberellin.

Grain protein

Considering the significant effect of seed priming treatment on the percentage of grain protein, it was found that priming with nano-Zn had a more positive effect on this trait (24%), while the lowest one was observed in hydropriming treatment (Figure 4).

**Figure 4.** Effect of priming treatments on grain protein of white bean

Means with at least one common letter are not significantly different according to Duncan's multiple range test (P=0.05). GA represents gibberellin.

Chlorophyll a

Mean comparison demonstrated that the highest content of Chl a was obtained in the priming with nano-Zn and foliar application of zinc + iron (Table 5).

Chlorophyll b

Priming with nano-titanium and foliar application of iron had the highest chlorophyll b content. The lowest content of chlorophyll b was related to hydropriming and foliar application of iron (Table 5).

Chlorophyll a+b

The maximum Chl a+b was observed in priming with nano-Zn accompanied with nano-Fe + nano-Zn foliar application. The lowest one was in the hydropriming treatment with foliar application of zinc (Table 5).

Table 5. Effect of seed priming and foliar of Zn and Fe on Chl a, Chl b and Chl a+b of white bean

Priming treatments	Fertilizer (foliar)	Chl a	Chl b	Chl a+b
Hydropriming	Zn 5 ppm	0.47i*	0.18f	0.65i
	Fe 5 ppm	0.49h	0.17g	0.66i
	Zn+ Fe 5 ppm	0.5g	0.19e	0.69h
	Zn 5 ppm	0.54f	0.22b	0.76g
Gibberellin 250 ppm	Fe 5 ppm	0.51g	0.2d	0.71h
	Zn+ Fe 5 ppm	0.55e	0.23a	0.79cf
	Zn 5 ppm	0.58d	0.22b	0.8e
TiO ₂ 30 ppm	Fe 5 ppm	0.6c	0.23a	0.83d
	Zn+ Fe 5 ppm	0.69b	0.2d	0.89c
	Zn 5 ppm	0.58d	0.2d	0.78f
Nano-Zn 5 ppm	Fe 5 ppm	0.69b	0.21c	0.91b
	Zn+ Fe 5 ppm	0.7a	0.22b	0.93a

*Means in each column with at least one common letter are not significantly different according to Duncan's multiple range test (P=0.05)

Summarized results including all treatments and major traits is presented as a graphical abstract in Figure 5.

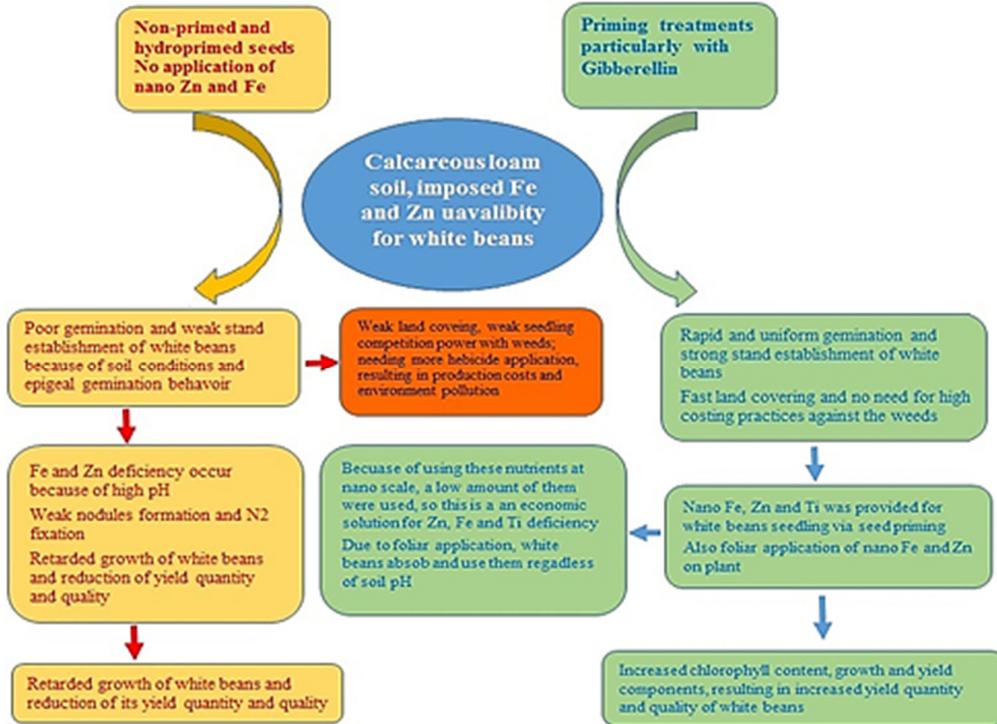


Figure 5. Graphical abstract of the current research

Discussion

Plant height

Increasing the plant height by gibberellin can be explained by the hormonal effect of gibberellin on growth. Pouryousef Miandoab and Esmacilzadeh (2017) stated that gibberellin priming caused an enhancement in plant height. By gibberellin, the height of dwarf plants such as corn, peas, and beans return to the normal situation (Itoh *et al.*, 1999). Since zinc is an essential trace element, it regulates plant growth by interfering with the formation of indole acetic acid and activating many enzymes, as well as it increases plant height by synthesizing chlorophyll and producing carbohydrates, and transporting them to growth points (Shafiee *et al.*, 2015). However, zinc deficiency reduces plant growth (Tehrani and Malakouti, 2000). Seed priming with zinc nanoparticles leads to improved seed germination and better plant establishment, which leads to a final increase in plant height by increasing plant growth (Laware and Raskar, 2014). On the other hand, our findings showed that foliar spraying of iron performed better than zinc and increased plant height. Further increase in plant height under iron foliar application can be due to the vital role of iron in chlorophyll synthesis; thus, the availability of photosynthetic iron and the final growth of the plant will be affected (Jin *et al.*, 2008).

The number of sub-branches per plant

The nano-Zn priming and foliar spraying of zinc and iron increased enhanced the number of sub-branches per plant. The production of indole acetic acid regulates plant growth by activating many enzymes; ultimately, the number of sub-branches per plant increases (Shafiee *et al.*, 2015). A decrease in plant growth occurred due to zinc deficiency; subsequently, the number of sub-branches per plant reduced (Tehrani and Malakouti, 2000). Foliar application of zinc and iron has resulted in the availability of micronutrients during vegetative and reproductive growth, and also this process combined with nano-Zn priming has led to a further increase in the number of sub-branches in the plant. The increased number of sub-branches can be attributed to further plant growth as well as enhanced photosynthetic activities of the plant along with increased leaf area. It has also been suggested that the application of nano-Zn elements leads to further induction of photosynthesis and the availability of photo-assimilates to increase plant growth (Gorczyca *et al.*, 2015).

Nodule per plant

Although co-application of zinc and iron caused more nodules along with more vegetative growth, titanium dioxide priming had a more positive effect on the number of bacterial nodules per plant rather than other priming levels. Increased growth by zinc availability has led to the expansion of roots in the plant rhizosphere, and titanium dioxide priming had more effect on increasing the growth of plant roots; therefore, more expansion of roots in the plant rhizosphere increased the number of nodules per plant. The use of zinc in the co-application of zinc and iron increased vegetative growth in the plant (Hajikhani *et al.*, 2011); and subsequently, the number of nodules per plant increased. However, it should be noted that the increased number of nodules per plant in priming treatments with gibberellin and titanium dioxide might be due to vegetative growth increase, and expanded root growth in the rhizosphere leads to more production of bacterial nodules per plant. The effectiveness of titanium in an increased number of nitrogen-fixing nodules per plant can be explained by the more facilitated passage of nano-sized elements from cell walls and their influence on specific target genes when plants were primed with these elements (Tymoszuk and Wojnarowicz, 2020).

Pods per plant

The zinc application can cause pollination improvement in plants that led to better fertility; thus, it increases the number of reproductive organs in the plant; which ultimately, positively affects the number of pods per plant. In a study on soybeans, Bank (1982) found that zinc foliar treatment increased the number of pods per plant and the number of seeds per pod, which confirms the results of this study. The number of pods per plant was affected by seed priming treatment (Hajikhani *et al.*, 2011), which accords with the findings of

this study. The zinc nanoparticles increased the number of pods per plant (Makarian *et al.*, 2017). Furthermore, zinc significantly affected chlorophyll synthesis and also increases energy production, the metabolism of lipids, carbohydrates, proteins, and phosphorus in the plant; and thus, has a positive effect on plant reproductive activities (Eisvand *et al.*, 2018). It is also illustrated that the zinc nanoparticles increase reproductive organs; and thus, enhance production. The production increase in plants using nano forms of zinc is more than the forms of zinc sulfate and zinc chelate (Prasad *et al.*, 2012).

Pod length

The increase in pod length can be explained by the increase in plant vegetative growth resulting from seed priming treatments. Other researchers reported increased vegetative growth due to seed priming (Hajikhani *et al.*, 2011). Application of nano-Zn and nano-titanium increased pod length compared to gibberellin and hydropriming. This increase can be due to the nano-titanium effects on the metabolic activities before seed germination; thus, it will affect the germination process and plant establishment that ultimately leads to improved plant growth characteristics (Shah *et al.*, 2021).

100-grains weight

The higher efficiency of zinc nanoparticles may be due to the structure of nanoparticles and their very small dimensions, so they have a high specific surface area, which leads to higher reactivity and mobility in the plant. It seems that all of the mentioned reasons can improve the yield components and protects the plant against serious damage, especially under abiotic stress (Nair *et al.*, 2010). A study indicated that the developmental stage from pod formation to full seed set significantly affects 1000-grain weight. The supply of nutrients at this stage can increase the number of grains per plant. At different levels of priming, co-application of zinc and iron increases the 100-grains weight largely. A possible explanation for this increase can be the key role of zinc and iron in photosynthetic processes and carbohydrate accumulation (Bahre and Dehnavi, 2012). An increase in 1000-grain seed caused by gibberellin and auxin can be attributed to the increase in sink strength. Using gibberellin, the cell division speed and the number of storage cells in seeds may have increased (Taiz *et al.*, 2014).

Grain yield

The zinc increases grain yield by affecting leaf chlorophyll content, indole acetic acid concentration, and increasing photosynthesis (Ravi *et al.*, 2008). Since nano fertilizers release nutrients gradually, so they are superior to conventional fertilizers to provide the required elements of plant in both methods (leaf or root absorption) (Würth, 2007). A study suggested that nano-Zn has led to increased grain yield in mung beans (Dhoke *et al.*, 2013). It has also been stated that seed priming with nano-Zn has improved the physiological and functional characteristics of the peanut (Prasad *et al.*, 2012).

Zinc involves the formation of pollen tube which leads to increased pollination and fruit and seed formation (Makarian *et al.*, 2017), which increases yield by the number of seeds per plant. The zinc oxide nanoparticles are more available to the plants and play a key role in seed formation in the pod, that is because of their stability and durability; thus, increase the final grain yield. In this study, although zinc nanoxide priming increased the grain yield, it was observed that this increase in the co-application of zinc and iron is more than the foliar application of each of these elements solely. High grain yield in titanium nano oxide priming can be due to titanium oxide's positive effect on increasing plant fertility, and the more reproductive organs, the more final grain yield (Jaberzadeh *et al.*, 2013). Another reason for increasing grain yield in nano-titanium priming treatment is that bean seed priming with nano-titanium improves seed germination characteristics (Alpana *et al.*, 2019) and increases seedling establishment speed, thus yielding. Seeds also increased as plant growth improved. Mahmoodzadeh *et al.* (2013) studied rapeseed and found that nano-titanium improves root and shoot growth in germinated seeds, and ultimately, the final grain yield increases by improving plant growth.

It seems that the improvement of growth rate by gibberellins may be due to the effective leaf area increase, stimulation of photosynthesis, activation of some enzymes, and changes in the distribution of the photosynthetic material. By stimulating the activity of some protease enzymes, they convert proteins into amino acids, including tryptophan, which is an auxin precursor (Pouryousef Miandoab and Esmacilzadeh, 2017).

Biological yield

As zinc plays a key role in enzymatic activities and the participation of iron in chlorophyll production, plant's biological performance has increased by improving photosynthesis and production of the essential nutrients (Tehrani and Malakouti, 2000). Because nano-Zn has a very fine particle size with a higher specific surface area. Therefore, this specific feature can improve the physiological functions of plant through enzymatic activities which ultimately leads to increased grain and biological yields (Bhattacharjee and Mukherjee, 2002). Using zinc significantly increased biological yield (Rengel, 2001). Gibberellin priming increases biological yield due to increase leaf area and enhance photosynthetic performance. It also increases CO₂ fixing by opening stomata, increasing Rubisco and sucrose phosphate synthetase activities (Ashraf and Foolad, 2005).

Harvest index

Treatments that increase the ratio of grain yield to biological yield will increase the harvest index. Titanium dioxide increases the number of grains per plant by enhancing the fertility process and has a higher yield, following that increases the harvest index (Jaberzadeh *et al.*, 2013). It seems that the increase in harvest index at different levels of foliar application of micronutrients and titanium dioxide treatment was more than other ones. Hence, iron and zinc foliar treatment illustrated a higher increase than others did.

Grain protein

The increase in seed protein content with nano-Zn priming is probably attributed to the role of zinc in enzymatic processes; ultimately, it leads to increased protein production. Madadi *et al.* (2016) found that the nano-priming treatment resulted in a significant increase in the protein percentage in black seed.

Chlorophylls

Iron is the main element involved in the production of chlorophyll. One of the main reasons for the increase in chlorophyll content can be the synthesis of new chlorophyll and prevent its degradation via the inhibition of chlorophyllase. By iron and zinc foliar application as well as nano-Zn priming, the activity of some antioxidant enzymes such as catalase increased; this enzyme can prevent chlorophyll degradation via inhibition of chlorophyllase, and accelerate DNA synthesis (Farhoudi and Sharifzadeh, 2006). According to Rengel (2001), zinc is required for chlorophyll production in plants, and its application significantly increase the chlorophyll content, which was consistent with the results of this study. Simultaneous application of iron and zinc, especially with nano-Zn priming, further increased the content of chlorophyll a, b, and Chl a+b. The reason for this phenomenon can be the limitation in the amount of delta-aminolevulinic due to iron deficiency (Kiani, 2012), so the simultaneous foliar application of iron and zinc provides iron availability, and therefore, the chlorophyll content increased. Ru *et al.* (2018) also stated that the simultaneous application of iron and zinc increased the chlorophyll content of wheat. In current study, it was found that the use of iron solely compared to the separate zinc application led to a further increase in chlorophyll a, b, and a+b content. This can be explained by the main role of iron in increasing chlorophyll synthesis (Jin *et al.*, 2008). Some other studies indicate an increase in leaf chlorophyll content due to foliar application of iron, which is consistent with the findings of this study (Mohamed Amanullah *et al.*, 2012; Sharifi *et al.*, 2016).

Increasing the content of Chl a, b and a+b by the titanium nanoparticles treatment can be attributed to the titanium nanoparticles' roles in stabilizing chloroplast membrane and protecting the chloroplast from aging. They also can improve the chlorophyll structure and pigments' light absorption (Morteza *et al.*, 2013).

Sartip and Sirousmehr (2017) reported that titanium nanoparticles increase chlorophyll synthesis by the improvement of nitrogen uptake and metabolism.

Conclusions

The improvement of grain yield, biological yield chlorophyll content, grain protein, and some other physiological and morphological traits of white bean by nutri-priming and hormonal priming along with foliar application of important micronutrients such as zinc and iron as well as titanium (Figure 5) indicate that we can use such nutritional treatments to improve yield quantity and quality of this crop. Using nutrients at nano size will be efficient and economic because in such way, a low amount of nutrients is required.

Authors' Contributions

Conceptualization: HRE and SMA; Investigation: NA and AM; Methodology: HRE, AM, and NA; Formal analysis: AI; Writing-original draft: SMA; and Writing-review and editing: HRE and SMA. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Alpana P, Priyankar, R., Akhila Nand D, Brijmohan P (2019). Effect of nano-titanium dioxide polymorphs priming on seed germination and seedling growth of French bean (*Phaseolus vulgaris* L.). International Journal of Agriculture, Environment and Biotechnology 12(2):121-127. <https://doi.org/10.30954/0974-1712.06.2019.7>
- Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24(1):1-15. <https://doi.org/10.1104/pp.24.1.1>
- Ashraf M, Foolad MR (2005). Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. In: Advances in Agronomy, Academic Press, pp 223-271.
- Bank L (1982). Effects of timing of foliar zinc fertilizer on yield components of soyabeans. Australian Journal of Experimental Agriculture 22(116):226-231. <https://doi.org/10.1071/EA9820226>
- Baybordi A (2006). Zinc in soils and crop nutrition. First Ed., Parivar Press.
- Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013). Seeds physiology of development, germination and dormancy. Third Ed., Springer, New York, Heidelberg, Dordrecht, London.
- Bhattacharjee S, Mukherjee A (2002). Salt stress-induced cytosolute accumulation, antioxidant response and membrane deterioration in three rice cultivars during early germination. Seed Science and Technology 30:279-287.
- Brear EM, Day DA, Smith PMC (2013). Iron: an essential micronutrient for the legume-rhizobium symbiosis. Frontiers in Plant Science 4:359-359. <https://doi.org/10.3389/fpls.2013.00359>

- Dhoke SK, Mahajan P, Kamble R, Khanna A (2013). Effect of nanoparticles suspension on the growth of mung (*Vigna radiata*) seedlings by foliar spray method. Nanotechnology Development 3(1):e1. <https://doi.org/10.4081/nd.2013.e1>
- do Espirito Santo Pereira A, Caixeta Oliveira H, Fernandes Fraceto L, Santaella C (2021). Nanotechnology potential in seed priming for sustainable agriculture. Nanomaterials 11(2):267. <https://doi.org/10.3390/nano11020267>
- Eisvand HR, Kamaei H, Nazarian F (2018). Chlorophyll fluorescence, yield and yield components of bread wheat affected by phosphate bio-fertilizer, zinc and boron under late-season heat stress. Photosynthetica 56(4):1287-1296. <https://doi.org/10.1007/s11099-018-0829-1>
- FAO (2019). FAOSTAT. <https://www.fao.org/faostat/en/#data>
- Farhoudi R, Sharifzadeh F (2006). The effects of NaCl priming on salt tolerance in canola (*Brassica napus* L.) seedlings grown under saline conditions. Indian Journal of Crop Science 1:74-78.
- Gorczyca A, Pocięcha E, Kasprowicz M, Niemiec M (2015). Effect of nanosilver in wheat seedlings and *Fusarium culmorum* culture systems. European Journal of Plant Pathology 142(2):251-261. <https://doi.org/10.1007/s10658-015-0608-9>
- Hajikhani S, Habibi H, Shekari F, Fotoukian MH (2011). The effect of seed priming on grain yield and its components of spotted bean cultivars under water deficit stress. Iranian Journal of Field Crop Science 42(1):191-197.
- Hasanuzzaman M, Fotopoulos V (2019). Priming and pretreatments of seeds and seedlings: Implication in plant stress tolerance and enhancing productivity in crop plants. Springer, Singapore.
- Itoh H, Tanaka-Ueguchi M, Kawaide H, Chen X, Kamiya Y, Matsuoka M (1999). The gene encoding tobacco gibberellin 3beta-hydroxylase is expressed at the site of GA action during stem elongation and flower organ development. Plant Journal 20(1):15-24. <https://doi.org/10.1046/j.1365-313x.1999.00568.x>
- Jaberzadeh A, Moaveni P, Tohidi Moghadam HR, Zahedi H (2013). Influence of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat subjected to water deficit stress. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 41(1):201-207. <https://doi.org/10.15835/nbha4119093>
- Jalil Shesh Bahre M, Movahedi Dehnavi M (2012). Effect of zinc and iron foliar application on soybean seed vigour grown under drought stress. Journal of Crop Production 5(1):19-35.
- Jin Z, Minyan W, Lianghuan W, Jianguo W, Chunhai S (2008). Impacts of combination of foliar iron and boron application on iron biofortification and nutritional quality of rice grain. Journal of Plant Nutrition 31(9):1599-1611. <https://doi.org/10.1080/01904160802244803>
- Kiani S (2012). Effects of iron on efficiency and map of photosystem II photochemical yield of rose flower using chlorophyll fluorescence imaging. Journal of Soil and Plant Interactions 2(4):25-35.
- Laware S, Raskar S (2014). Influence of zinc oxide nanoparticles on growth, flowering and seed productivity in onion. International Journal of Current Microbiology and Applied Sciences 3(7):874-881.
- Lee S, Kim JH (2000). Total sugars, alpha-amylase activity, and germination after priming of normal and aged rice seeds. The Korean Journal of Crop Science 45:108-111.
- Madadi M, Khomari S, Javadi A, Sofalian O (2016). Effect of black cumin seed priming with calcium nitrate and nano-zinc oxide on germinability and seedling growth under salinity stress. Journal of Plant Process and Function 5(15):169-180.
- Mahmoodzadeh H, Nabavi M, Kashefi H (2013). Effect of nanoscale titanium dioxide particles on the germination and growth of canola (*Brassica napus*). Journal of Ornamental Plants 3(3):25-32.
- Makarian H, Shojaei H, Damavandi A, Nasiri Dehsorkhi A, Akhyani A (2017). The effect of foliar application of zinc oxide in common and nanoparticles forms on some growth and quality traits of mungbean (*Vigna radiata* L.) under drought stress conditions. Iranian Journal Pulses Research 8(2):166-180. <https://doi.org/10.22067/ijpr.v8i2.51644>
- Marschner P (2011). Marschner's mineral nutrition of higher plants. Academic Press, London, UK.
- Mishra V, Mishra RK, Dikshit A, Pandey AC (2014). Interactions of nanoparticles with plants: An emerging prospective in the agriculture industry. In: Ahmad P, Rasool S (Eds). Emerging Technologies and Management of Crop Stress Tolerance. Academic Press, pp 592.
- Mohamed Amanullah M, Archana J, Manoharan S, Subramanian KS (2012). Influence of iron and AM inoculation on metabolically active iron, chlorophyll content and yield of hybrid maize in calcareous soil. Journal of Agronomy 11:27-30. <https://doi.org/10.3923/ja.2012.27.30>

- Morteza E, Moaveni P, Farahani HA, Kiyani M (2013). Study of photosynthetic pigments changes of maize (*Zea mays* L.) under nano TiO₂ spraying at various growth stages. Springerplus 2(1):247. <https://doi.org/10.1186/2193-1801-2-247>
- Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS (2010). Nanoparticulate material delivery to plants. Plant Science 179(3):154-163. <https://doi.org/10.1016/j.plantsci.2010.04.012>
- Nazeri P, Kashani A, Khavazi K, Ardakani MR, Mirakhori M, Pour siah bidi M (2010). The effect of biofertilizer and phosphorus fertilizer banding with Zinc on white bean (*Phaseolus vulgaris* L.). Journal of Agroecology 2(1):175-185. <https://doi.org/10.22067/jag.v2i1.7617>
- Pouryousef Miandoab, M, Esmacilzadeh F (2017). The effect of foliar application of growth stimulants and priming on yield and grain oil content of flax (*Linum usitatissimum* L.). Journal of Crop Ecophysiology 10(4):874-857.
- Prasad, TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Sreepasad TS, Sajanlal PR, Pradeep T (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of Plant Nutrition 35(6):905-927. <https://doi.org/10.1080/01904167.2012.663443>
- Ravi S, Channel HT, Hebsur NS, Patil BN, Dharmatti PR (2008). Effect of sulphur, zinc and iron nutrition on growth, yield, nutrient uptake and quality of safflower (*Carthamus tinctorius* L.). Karnataka Journal of Agricultural Sciences 21(3):382-385.
- Rengel Z (2001). Genotypic differences in micronutrient use efficiency in crops. Communications in Soil Science and Plant Analysis 32(7-8):1163-1186. <https://doi.org/10.1081/CSS-100104107>
- Ru K, Hl S, Kunjadia B (2018). Effect of zinc and iron application on leaf chlorophyll, carotenoid, grain yield and quality of wheat in calcareous soil of Saurashtra region. International Journal of Chemical Studies 6:2092-2096.
- Sartip H, Sirousmehr A (2017). Effect of titanium nano particles and different irrigation levels on photosynthetic pigments, proline, soluble carbohydrates and growth parameters of Purslane. Journal of Plant Ecophysiology 9(28):79-90.
- Shafiee A, Sajedi N, Changizi M (2015). The effects of different treatments of seed priming and foliar application of nano particle and zinc sulphate on agronomic traits in safflower. Iranian Journal of Seed Science and Technology 4(2):71-80.
- Shah T, Latif S, Saeed F, Ali I, Ullah S, Abdullah Alsahli A, Jan S, Ahmad P (2021). Seed priming with titanium dioxide nanoparticles enhances seed vigor, leaf water status, and antioxidant enzyme activities in maize (*Zea mays* L.) under salinity stress. Journal of King Saud University - Science 33(1):101207. <https://doi.org/10.1016/j.jksus.2020.10.004>
- Sharifi R, Mohammadi K, Rokhzadi A (2016). Effect of seed priming and foliar application with micronutrients on quality of forage corn (*Zea mays*). Environmental and Experimental Biology 14(4):151-156. <https://doi.org/10.22364/ceb.14.21>
- Singh A, Dahiru R, Musa M, Sani Haliru B (2014). Effect of osmopriming duration on germination, emergence, and early growth of cowpea (*Vigna unguiculata* L.) in the Sudan Savanna of Nigeria. International Journal of Agronomy 841238. <https://doi.org/10.1155/2014/841238>
- Sytar O, Kumari P, Yadav S, Brestic M, Rastogi A (2019). Phytohormone priming: regulator for heavy metal stress in plants. Journal of Plant Growth Regulation 38(2):739-752. <https://doi.org/10.1007/s00344-018-9886-8>
- Taiz L, Zeiger E, Moller I, Murphy A (2014). Plant physiology and development. 6 ed. Sinauer Associates, Sunderland, CT.
- Tehrani MM, Malakouti MJ (2000). The role of micronutrients in increasing yield and improving the quality of agricultural products "Microelements with macro impact". Tarbiat Modares University, Iran.
- Tymoszuk A, Wojnarowicz J (2020). Zinc oxide and zinc oxide nanoparticles impact on in vitro germination and seedling growth in *Allium cepa* L. Materials 13(12):2784. <https://doi.org/10.3390/ma13122784>
- Würth B (2007). Emissions of engineered and unintentionally produced nanoparticles to the soil. an exposure assessment for Switzerland. ETH Zurich Department of Environmental Sciences.
- Xiang L, Zhao HM, Li YW, Huang XP, Wu XL, Zhai T, Yuan Y, Cai QY, Mo CH (2015). Effects of the size and morphology of zinc oxide nanoparticles on the germination of Chinese cabbage seeds. Environmental Science and Pollution Research International 22(14):10452-10462. <https://doi.org/10.1007/s11356-015-4172-9>



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