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Nano-Priming with Zinc and Boron Enhances seed Germination and seedling vigour in Sunflower (*Helianthus annuus* L.)

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ABSTRACT

Nanotechnology is a broad and interdisciplinary area of research and development activity that has been growing at a rapid pace worldwide in the past few years. Recent manufacturing advancements have led to the fabrication of nanoparticles of different sizes and shapes. The new dimension in improving nutrient use efficiency is the use of nanoparticles which are used extensively in a wide range of sectors. Most investigations so far have focused on cereals and a very few studies have been done in sunflower. Hence, an attempt was done to standardize the concentration and soaking period by treating sunflower hybrid seeds with nano zinc and nano boron based on germination percentage and seedling vigour. The results showed that nano zinc @100ppm and nano boron @50ppm for one and a half hour soaking period significantly enhanced germination, higher seedling length with noticeably more secondary and tertiary roots over control and bulk nutrients and seedling vigour and there were no significant results at higher concentrations and longer soaking period. The use of nano nutrients can reduce the burden of excess zinc and boron fertilizer to soil.

Keywords Nano boron, Nano particles, Nano zinc, Seedling vigour index, Seed germination, Speed of germination, Sunflower.

INTRODUCTION

Sunflower is the third largest source of vegetable oil worldwide, followed by soybean and groundnut both in terms of area and production. Among oilseeds, it ranked 3rd in 2018 behind soybean and rapeseed (4th after palm, soybean and rapeseed for edible oil) with an average annual world production of about 52 MnT (Oil World Annual 2019).

In India, sunflower is cultivated over an area of 4.006 lakh ha with a production of 2.840 lakh tonnes and productivity of 709 kg/ha (Average of 2014-15 to 2018-19). The average area under sunflower ranged from 21 lakh ha (1990-95) to 19.4 lakh ha (2005-2010) and declined to 2.83 lakh ha during 2017-18 and now stagnant around 2.6 lakh ha (Parveen *et al.* 2020).

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Sunflower is an exhaustive crop and inadequate and imbalanced crop nutrition affects production leading to low productivity due to poor seedling vigour, poor seed setting and high per cent of chaffy seeds in the center of the capitulum (Manjushri *et al*. 2018).

The lower production in sunflower could be due to lack of nutrient availability for plants. Due to traditional agronomic practices like soil application of micronutrient leads to formation of complex and leaching of nutrients in soil leads to unavailability of nutrients to the plants causing micronutrient deficiency in plants results in reduced growth and yield of the crop plants (Farooq *et al*. 2012).

In India among all the micronutrients, zinc (Zn) is considered as fourth most important yield limiting nutrient in agricultural crops. Zinc deficiency in Indian soils is likely to increase from 49 to 63% by 2025 (Arunachalam *et al.* 2013). In addition to zinc, survey revealed that deficiency of boron ranges from 1-84% with a mean of 33% in India (Gupta *et al.* 2008). Boron is involved in nucleic acid metabolism, carbohydrate and protein metabolism, indole acetic acid metabolism, cell wall synthesis, cell wall structure, membrane integrity (Fujiwara *et al.* 2008). Sexual reproduction in plant is more sensitive to low boron than vegetative growth. Boron is necessary for translocation of sugars, increased reproduction and germination of pollens (Benjamin *et al.* 2019). It tends to keep calcium in soluble form within the plant and also act as regulator of potassium ratios (Milka Brdar-Jokanovic 2020).

The greatest challenge in Indian agriculture in the coming decades is to increase production with ecological sustainability and in order to achieve that there is a need for optimum utilization of fertilizers. The new dimension in improving nutrient use efficiency is the use of nano particles which are used extensively in a wide range of sectors. To increase the productivity in sunflower, nano particles (NPs) may be useful for higher plant growth and yield. The nanoparticles are having high surface area (30-50m^2/g), better catalytic activity, rapid chemical reaction, rapidly dispersible and adsorb abundant water (Hamidreza *et al.* 2017).

In order to exploit the potential use of nano nutrients on seedling growth in sunflower the present study was taken up to find out the optimum concentration of nano zinc and nano boron for better seedling growth.

MATERIALS AND METHODS

Commercially available forms of nano nutrients viz., nano zinc oxide (ZnO) with particle size of 30 nm size and nano boron nitride (BN) with 70 nm were used, in addition to borax powder and zinc oxide (ZnO) as bulk nutrients. Experiments were conducted with the sunflower hybrid, KBSH-44 released from University of Agricultural Sciences, Bangalore, India.

To prepare the nano particle solutions for seed treatment, nano materials were suspended directly in distilled water and dispersed by ultrasonic vibration for 30 min. Magnetic bars were placed in the suspensions for stirring before use to avoid aggregation of the particles. Once the particles were fully dissolved, the solution turns to milky (Plate 1). In a similar way different concentration of nano-nutrients were prepared.

A laboratory experiment was conducted by soaking sunflower seeds in 100 ml of different concentrations of nano zinc oxide, nano boron nitride, bulk zinc oxide and borax starting from lower concentrations (25-150 ppm) to higher concentrations (500-1000 ppm) at different soaking durations with half an hour interval from half an hour to two hour soaking period to study the effect of nano nutrients on germination and seedling traits.

Results were compared with control where only water was used. During the experimental period seven days after sowing, observations on speed of germination, germination per centage, root length, shoot length, seedling vigour index I and seedling vigour index II were recorded.

Germination percentage was calculated by taking the ratio of number of seeds sown to the number of seeds germinated in a petri plate in a given time and expressed as percentage. After treating the seeds at different concentrations of nano particles, number of seeds germinated per 50 seeds in four replications was counted on daily basis till all the seeds completed their germination to record the speed of germination.

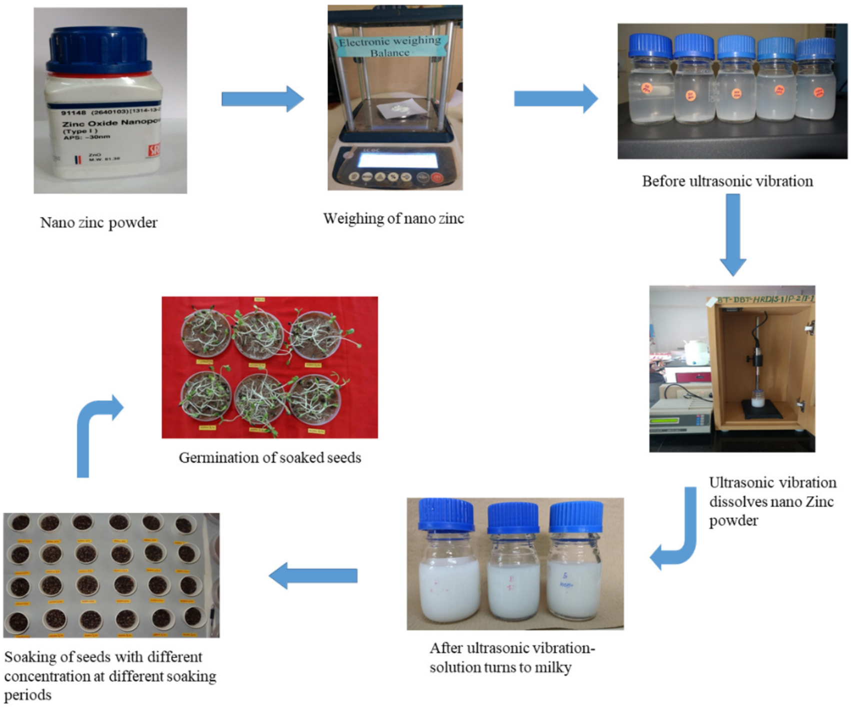

Plate 1. Preparation of nano-nutrient solution for seed treatment.

Shoot length and root length of all the germinated seeds of all the replications was recorded in centimeter with the help of measuring scale to arrive finally at the average length. For seedling dry weight (g), the seven days old seedlings were kept in hot air oven with 70^{o}C for 48 hrs and then the seedling dry weight was recorded using electronic balance.

Seedling vigour index I (SVI-1) was calculated using the formula described by Abdul-Baki and Anderson (1973).

Seed vigour index = Germination % × (root length + shoot length)

Seedling vigour index II (SVI-II) was calculated using the formula described by Kharb *et al.* (1994).

Seedling vigour Index II = Seedling dry weight (g) × Germination percentage

WinRHIZO Scanner of Regent Instruments is an image analysis system, specifically designed for root measurement in different forms. Various root morphological characters viz., length, area, volume, diameter, forks, topology, architecture and color analyses were done. Washed roots of sunflower from various treatments were measured using WinRHIZO root scanner.

RESULTS AND DISCUSSION

Effect of nano zinc seed treatment on seedling traits

Speed of germination (hrs)

Nano particle treated seeds outperformed compared to control in terms of speed of germination (Table 1). Speed of germination was faster at lower concentrations of nano zinc treated seeds with a mean of 21 hrs, whereas in control and higher concentration speed of germination was very slow with 35 hrs and 31 hrs, respectively. As the duration of soaking increases, the

Table 1. Effect of different concentrations of nano zinc with different soaking periods on seedling traits.

Treatments	Half an hour			One hour			One and half hour			Two hours		
	Speed of germination (hrs)	Root length (cm)	Shoot length (cm)	Speed of germination (hrs)	Root length (cm)	Shoot length (cm)	Speed of germination (hrs)	Root length (cm)	Shoot length (cm)	Speed of germination (hrs)	Root length (cm)	Shoot length (cm)
Control	48	3.88	6.97	33	4.07	5.10	30	7.72	7.60	28	7.04	8.31
25 ppm	36	7.13	8.43	21	6.30	8.11	23	7.52	8.38	16	6.6	7.52
50 ppm	19	7.14	8.08	19	7.83	8.62	20	7.42	9.24	18	6.56	7.8
75 ppm	17	6.68	7.87	18	7.02	8.72	23	7.12	9.19	20	8.38	9.85
100 ppm	21	6.41	9.27	21	7.00	8.69	21	8.81	9.53	18	8.26	7.47
150 ppm	22	7.07	8.69	22	6.69	8.03	23	6.58	8.80	22	7.27	8.15
Mean	**23**	**6.89**	**8.47**	**20**	**6.97**	**8.43**	**22**	**7.49**	**9.03**	**19**	**7.41**	**8.16**
500 ppm	25	5.77	8.91	25	6.46	9.87	24	5.33	8.99	24	6.65	9.13
600 ppm	29	5.77	7.50	29	4.69	7.97	30	6.61	8.89	28	7.13	8.92
700 ppm	36	5.63	6.51	34	6.75	9.19	34	7.18	7.94	32	6.19	9.59
800 ppm	36	7.70	8.19	33	7.01	8.70	36	5.84	6.33	30	5.95	8.31
900 ppm	31	6.40	8.17	31	7.18	9.50	34	7.34	9.91	31	7.12	8.74
1000 ppm	36	6.76	8.62	36	7.25	10.68	31	7.44	9.89	32	7.45	9.52
Mean	**32**	**6.34**	**7.98**	**31**	**6.56**	**9.32**	**32**	**6.62**	**8.66**	**30**	**6.75**	**9.04**
CD @5%	7.02	2.02	1.87	15.65	1.85	1.20	3	2.06	1.19	8.98	2.12	1.8
CV (%)	14.98	14.58	10.62	26.85	13.03	6.40	10	13.72	6.37	15.61	14.11	9.81
SEm±	0.77	0.66	0.61	5.08	0.6	0.39	17	0.67	0.39	2.92	0.69	0.59

time taken for germination reduced significantly from 23 hrs to 19 hrs. The reason for rapid germination could be that the nano particles may form new pores on seed coat during penetration facilitating the influx of water inside the seed or NPs may enter into the seed through the cracks present over the surface of the seed and activated the enzymes in early phase thereby enhanced the speed of germination (Sridhar 2012).

Root length (cm)

Among ZnO nano particle treated seeds the lower concentrations of zinc treated seedlings recorded maximum root length at different soaking hours (half an hour, one hour, one and half hour and two hours) with a mean of 6.89, 6.97, 7.49 and 7.41 compared to higher concentrations (6.34, 6.56, 6.62 and 6.75) and control (3.88, 4.07, 7.72 and 7.04). Among all the concentrations and duration, 100 ppm zinc concentration with one and half hour soaking exhibited higher root length (8.81 cm) followed by 75 ppm with two hour soaking (8.38 cm).

Similarly, in cucumber seeds treated with ZnO NPs increased germination and growth rate of seedlings in *Cicer arietinum.* Due to oxygen vacancies or oxygen deficient zinc-rich ZnO NPs increased the level of IAA in roots (sprouts), which in turn indicate the increase in the growth rate of plants as zinc is an essential nutrient for plants (Pandey *et al.* 2006).

Shoot length (cm)

The shoot length was observed more in higher concentrations of nano zinc oxide at different soaking hours with half an hour, one hour, one and half hour and two hour (7.98, 9.32, 8.66 and 9.04) than the lower concentrations (8.47, 8.43, 9.03 and 8.16) compared to control (6.97, 5.10, 7.60 and 8.31). At 1000 ppm concentration with one hour soaking seedlings had more shoot length (10.68) than all other concentrations and soaking periods.

Similar results were also observed by Prasad *et al.* (2012) where, ZnO nanoparticles at a concentration of 1000 ppm improved the germination percentage, root growth, shoot growth in groundnut. ZnO in the nanoscale form is absorbed by plants to a larger extent unlike bulk $ZnSO_4$ leads to activation of enzymes like α-amylase which leads to early breakdown of stored materials in seeds.

Enhanced physiological performance due to nano

Table 2. Effect of different concentrations of nano zinc with different soaking periods on germination percentage and seedling vigour.

	Half an hour			One hour			One and half hour			Two hours		
Treatments	Germination (%)	SVI-I	SVI-II	Germination (%)	SVI-I	SVI-II	Germination (%)	SVI-I	SVI-II	Germination (%)	SVI-I	SVI-II
Control	100	1085	4.50	100	917	5.00	100	1532	7.00	95	1454	7.20
25 ppm	100	1555	7.50	100	1441	6.50	100	1590	6.50	100	1412	7.00
50 ppm	100	1521	6.50	100	1645	7.00	100	1665	7.50	100	1436	7.50
75 ppm	100	1455	6.50	100	1574	6.50	100	1631	5.50	98	1741	8.50
100 ppm	100	1568	7.00	99	1459	7.80	100	1834	11.00	100	1573	6.50
150 ppm	98	1521	6.30	98	1472	6.50	100	1538	7.00	100	1542	7.00
Mean	**100**	**1524**	**6.76**	**99**	**1518**	**6.86**	**100**	**1652**	**7.50**	**100**	**1541**	**7.30**
500 ppm	100	1468	7.50	100	1633	7.50	100	1432	6.00	100	1578	7.00
600 ppm	100	1327	8.00	100	1266	7.00	100	1550	6.00	95	1524	7.60
700 ppm	98	1152	7.10	100	1594	7.50	100	1512	6.50	100	1578	7.20
800 ppm	100	1589	8.00	100	1571	7.50	100	1217	7.00	95	1350	5.70
900 ppm	100	1457	7.50	100	1668	7.00	95	1634	7.60	100	1586	7.50
1000 ppm	100	1538	8.00	90	1793	8.10	100	1733	8.00	95	1617	8.00
Mean	**99.17**	**1422**	**7.68**	**98.33**	**1588**	**7.43**	**99.17**	**1513**	**6.85**	**98**	**1539**	**7.17**
CD @5%	2.2	367.34	1.45	2.5	209.22	1.84	1.88	244.93	2.07	1.64	297.03	1.91
CV (%)	1.11	11.71	9.47	1.2	6.42	12.32	1.03	7.32	13.23	1.26	9.04	12.76
SEm±	0.77	119.22	0.47	0.67	67.9	0.60	0.55	79.49	0.67	0.62	96.4	0.62

particles treatment could be attributed to the quenching of free radicals by the nano particles. Smaller size of the nanoparticles would have easily entered through cracks present on the outer seed surface, reacting with free radicals resulting in enhanced seed vigour (Sengupta *et al.* 2005).

The improvement in root and shoot length of seedlings due to Zn ascribed to the efficient protein synthesis and better source to sink relationship which resulted in better development of seeds giving rise to higher germination and vigour index. These results were in collaborative with Dileepkumar *et al.* (2009) in cowpea, where seeds pelleted with $ZnSO_4$ recorded higher root length (18.51 cm), vigour index (4277) and seedling dry weight (0.595 g).

Germination (%)

Significant difference was not found for germination between the treatments (Table 2). However, at higher concentrations, germination reduced from 99 to 98 % as the soaking duration increased. However, in most of the studies, higher concentration NPs reduced germination per centage and the reason for decreased germination could be the increased absorption and accumulation of these NPs both in extracellular space and within the cells resulted in reduction in cell division, cell elongation and inhibition of the hydrolytic enzymes involved in food mobilization during the process of seed germination (Prasad *et al.* 2012).

Seedling vigour I and II

Lower concentrations with different levels of soaking showed increased seedling vigour compared to control plants. Among all, 100 ppm with one and half hour soaking period showed significantly higher seedling vigour I and II (1834 and 11).

Similarly, Dharam Singh Meena *et al.* (2017) studied different concentrations of nano zinc oxide (800, 1000, 1200, 1400 and 1600 ppm) treated seeds to assess the performance of maize seedling. Among the different concentrations of nano zinc oxide, 1000 and 1200 ppm recorded 100% germination of maize seeds. However, 1200 ppm nano zinc oxide recorded higher root length (6.5 cm), shoot length (3.9 cm) and seed vigour index I (1040) compared to other concentrations because of solubility and penetrability of nanoparticles which help to increase availability of nutrient to the crop plant from applied surface.

A study by Sara *et al.* (2022) indicates that ZnO NPs increase the synthesis of tryptophan, one of the precursors of the IAA leading to increased growth,

Table 3. Effect of bulk zinc with one and half hour soaking period on seedling traits.

Treatments	Germination (%)	Speed of germination (hr)	Root length (cm)	Shoot length (cm)	SVI-I	SVI-II
Control	100	36	4.06	6.20	1026	4.00
25 ppm	100	20	4.46	8.95	1341	5.00
50 ppm	98	24	4.20	8.76	1270	5.00
75 ppm	100	20	4.90	9.10	1400	5.50
100 ppm	100	16	4.94	9.32	1426	5.50
150 ppm	98	24	5.33	9.77	1480	6.00
Mean	**99**	**21**	**4.77**	**9.18**	**1383**	**5.40**
500 ppm	98	28	5.78	9.89	1536	6.50
600 ppm	100	32	6.14	10.06	1620	7.50
700 ppm	96	28	8.91	10.38	1852	9.50
800 ppm	95	24	5.25	9.67	1417	6.00
900 ppm	98	26	4.22	9.04	1299	5.50
1000 ppm	96	24	3.00	7.93	1049	4.50
Mean	**97**	**27**	**5.55**	**9.50**	**1462**	**6.58**
CD @5%	3.21	8..42	0.13	0.34	42.82	1.45
CV (%)	1.85	14.35	1.19	1.69	1.36	1.49
SEm±	0.87	4.25	0.04	0.11	13.9	0.47

germination rate and biomass production. Besides, zinc is one of the essential micronutrients required for plant growth and an important component of various enzymes that are responsible for driving many metabolic reactions in all crops. The improvement of Zn nutritional status also reduces the uptake of harmful heavy metals that hinders its toxicity in plants such as Cd (Adiloglu 2002).

It can be concluded that the Zn nanoparticles applied along with the seed polymer are capable of entering into seeds utilizing the cracks and crevices available on the seed coat during the imbibition into the seeds and would improves the enzymatic activity and free radical scavenging system by quenching the free radicals there by lowering the oxidative damages, eventually promoting viability and vigour of the seeds.

Effect of bulk zinc oxide on seedling traits

Speed of germination and germination per cent was maximum in lower concentration in seeds treated with bulk ZnO (21 hrs and 99%), whereas, root length, shoot length, SVI-I and SVI-II were maximum in higher concentrations i.e., at 700 ppm (Table 3).

Intensive re-translocation of Zn from the Zn pools (e.g. embryo and aleurone) into newly developed roots and coleoptiles. Such high remobilization of Zn influences seed germination, root and shoot length (Moussavi-Nik *et al.* 1997).

Zn is an important metal micronutrient that act as cofactors for the most of the dehydrogenase enzyme complexes involved in respiration and food mobilization in seeds. The increased availability of these micronutrients at nanoscale with increased chemical reactivity resulted in the increase in synthesis and activity of the dehydrogenase enzymes (Vijayalakshmi *et al.* 2013).

Effect of nano boron seed treatment on seedling traits

Speed of germination (hrs)

Lower concentrations of nano boron seed treated seedlings exhibited early germination at different soaking hours with a mean of (27, 21, 21, 20 hrs) compared to control (34, 32, 30 and 38 hrs) and higher concentrations 33, 29, 30 and 23 hrs, respectively (Table 4).

Khodakovskaya *et al.* (2009) reported that nano particles can penetrate thick seed coat and support water uptake by the seeds which could be responsible

Table 4. Effect of different concentration of nano boron with different soaking periods on seedling traits.

Treatments	Half an hour			One hour			One and half hour			Two hours		
	Speed of germination (hrs)	Root length (cm)	Shoot length (cm)	Speed of germination (hrs)	Root length (cm)	Shoot length (cm)	Speed of germination (hrs)	Root length (cm)	Shoot length (cm)	Speed of germination (hrs)	Root length (cm)	Shoot length (cm)
Control	34	4.06	6.20	32	5.81	6.82	30	6.29	6.78	38	6.66	7.81
25 ppm	30	7.09	9.42	21	7.67	9.34	20	8.14	11.05	24	7.68	9.93
50 ppm	26	7.56	8.14	16	6.42	10.55	18	8.85	10.33	16	8.01	10.13
75 ppm	24	6.99	9.18	24	6.94	9.42	22	7.44	8.83	20	8.20	9.72
100 ppm	28	7.53	9.37	26	7.99	10.62	24	7.24	9.75	18	8.07	9.05
150 ppm	20	7.64	10.02	20	7.70	9.67	20	8.00	9.28	22	8.02	9.86
Mean	**26**	**7.36**	**9.23**	**21**	**7.34**	**9.92**	**21**	**7.93**	**9.85**	**20**	**8.00**	**9.74**
500 ppm	32	5.14	10.93	28	6.95	11.61	26	6.82	12.12	24	7.32	12.22
600 ppm	36	5.19	11.32	26	7.53	12.01	30	8.81	10.48	20	8.19	9.30
700 ppm	28	5.15	11.09	24	5.41	11.56	28	7.74	10.76	24	6.62	12.09
800 ppm	30	6.83	11.11	32	4.71	11.07	32	7.12	11.52	26	7.76	11.59
900 ppm	32	5.92	10.93	30	5.23	10.81	34	6.88	11.91	24	5.35	11.24
1000 ppm	36	6.10	11.27	32	6.35	11.52	30	5.67	12.29	20	6.51	11.80
Mean	**32**	**5.72**	**11.11**	**29**	**6.03**	**11.43**	**30**	**7.17**	**11.51**	**23**	**6.96**	**11.71**
CD @5%	10.51	1.77	1.88	8.15	1.87	1.40	6.42	1.65	0.77	10.24	1.44	1.02

for the significantly faster germination and higher biomass production in tomato. Upon seed priming it can rapidly imbibe and revive the seed metabolism, resulting in a higher germination rate.

Root length (cm)

Root length was noticed maximum in lower concentrations of nano boron at all the soaking hours with a mean of 7.36, 7.34, 7.93 and 8.00, respectively. Reduced root growth was seen in higher concentrations of nano boron at different soaking hours.

Boron inhibits root growth primarily through limiting cell elongation rather than cell division (Brown and Shelp 2002). Boron is vital for shoot and root growth development because it involves in cell wall and plasma membrane structure and function (Brown and Shelp 2002).

Boron involved in cell elongation, cell division and development of apical root meristems (Blevins and Lukaszewski 1998) and ameliorating of root elongation.

Shoot length (cm)

Increased shoot length was observed at higher concentrations of nano boron at various soaking hours (11.11, 11.43, 11.51 and 11.71) compared to control (6.20, 6.82, 6.78 and 7.81 cm) and lower concentrations. The significant increase in shoot length of low concentration of boron treated seeds could be due to its involvement in cell elongation or cell division and meristematic growth (Khan *et al.* 2006).

Bonilla *et al.* (2004) and Farr (2010) reported that low concentrations of exogenous boric acid stimulated seed germination and seedling growth, while high concentrations showed an inhibitive effect on these parameters because it has adverse effects on cell division and has capable of complexing intracellular pyridine nucleotide coenzymes.

Boron at lowest concentration of 0.001% to 1% has been found successful for improved lengths and fresh weights of shoot and root in rice cultivar as reported by Rehman *et al.* (2012). Improvement in radicle and plumule lengths and root score and seedling fresh and dry weights seems the involvement of boron in meristematic growth of radicle and plumule primordia, cell wall extensibility and cell division and elongation.

Germination (%)

Significant difference was observed between the lower and higher concentrations of nano boron

Table 5. Effect of different concentrations of nano boron with different soaking periods on germination percentage and seedling vigour.

	Half an hour			One hour			One and half hour			Two hours		
Treatments	Germination (%)	SVI-I	SVI-II	Germination (%)	SVI-I	SVI-II	Germination (%)	SVI-I	SVI-II	Germination (%)	SVI-I	SVI-II
Control	100	1025	4.50	100	1263	5.50	100	1766	5.00	100	1768	6.50
25 ppm	100	1651	6.00	100	1701	6.50	98	1871	5.40	100	1761	7.50
50 ppm	98	1544	5.90	100	1697	7.50	100	1918	8.50	100	1814	6.00
75 ppm	100	1617	5.50	98	1603	5.40	100	1627	6.00	99	1774	5.90
100 ppm	100	1690	6.00	99	1841	6.40	100	1699	5.50	98	1707	5.90
150 ppm	100	1766	6.00	99	1719	5.00	100	1728	6.00	98	1752	5.40
Mean	**100**	**1654**	**5.88**	**99**	**1712**	**6.16**	**100**	**1769**	**6.28**	**99**	**1762**	**6.14**
500 ppm	97	1559	5.30	98	1819	6.80	99	1875	7.00	96	1866	6.60
600 ppm	97	1601	6.60	97	1895	7.50	97	1871	6.90	97	1749	7.00
700 ppm	96	1551	6.90	98	1655	6.60	98	1813	6.40	99	1841	6.80
800 ppm	97	1740	6.80	99	1561	6.60	99	1844	5.80	98	1896	7.20
900 ppm	97	1635	5.60	99	1580	6.10	97	1822	6.70	97	1609	6.10
1000 ppm	98	1702	5.60	97	1731	5.90	98	1752	6.30	97	1776	6.50
Mean	**97**	**1631**	**6.13**	**98**	**1707**	**6.58**	**98**	**1830**	**6.52**	**97**	**1813**	**6.70**
CD @ 5%	2.07	324.66	1.20	1.34	198.35	1.63	2.05	185.86	2.00	2.34	171.22	2.09
CV (%)	3.16	9.37	9.28	2.85	5.44	12.05	3.1	4.85	15.00	2.18	4.46	15.62
SEm±	2.21	105.36	0.39	2.08	64.37	0.53	2.16	60.32	0.65	1.65	55.57	0.68

treated seeds. Lower concentrations of nano boron treated seeds showed higher germination per cent in all the soaking hours with a mean of 100, 99,100 and 99 whereas, higher nano boron concentration treated seeds recorded reduced germination per cent at different soaking hours (Table 5).

Study in maize showed that seed priming with micronutrients viz., zinc, boron and molybdenum improved mean germination time, germination percentage and this was attributed to the involvement of Zn in radicle development and in the early stages of coleoptile growth and in auxin synthesis (Ullah *et al.* 2019).

The low concentration of boron has been reported to activate key enzymes including phosphorylase, α-amylase involved in starch metabolism that led to early emergence of radicle with improved seed germination (Rehman *et al.* 2012).

Similarly, the germination percentage at 0.25mg/l showed difference compared to the control. A significant decrease in germination was observed at boron concentrations higher than 0.5 mg/L. At 8 and 16 mg /L, wheat seed failed to germinate, indicating that germination is totally inhibited at such high concentrations of boron (Ashagre *et al.* 2014).

Boron toxicity results in disruption of cell wall development, metabolic disruption by binding to the ribose moieties of NADPH, NADH and ATP and inhibition of cell division and elongation (Reid *et al.*2004) as well as osmotic imbalances, photo oxidative damage, membrane leakage, lipid peroxidation (Eraslan *et al.* 2007) and inhibition of seed germination and seedling emergence and root elongation (Reid *et al.* 2004).

Seedling vigour index I and II

Seedling vigour was significantly differed between the treatments and soaking hours. Maximum seedling vigour was observed in 50 ppm concentration of nano boron with one and half hour soaking period among all the concentrations and duration of soaking (SVI-I and SVI-II).

Boron is a micronutrient required for plant nutrition. It is necessary for root and meristematic tissue extension, cell wall formation, membrane integrity, cell wall synthesis which results in more root length, shoot length and seedling vigour (Nalini *et al.* 2013).

Effect of bulk boron (borax) on seedling traits

The effect of bulk boron on different seedling traits

Table 6. Effect of different concentration of bulk boron with one and half hour soaking periods on seedling traits.

Treatments	Germination (%)	Speed of germination (hr)	Root length (cm)	Shoot length (cm)	SVI-I	SVI-II
Control	100	38	4.31	5.94	1025	4.00
25 ppm	100	26	8.40	10.16	1856	9.00
50 ppm	100	24	6.07	9.50	1557	7.00
75 ppm	100	22	6.10	8.53	1463	6.50
100 ppm	98	26	4.52	8.04	1256	5.00
150 ppm	96	30	4.35	7.89	1224	5.00
Mean	**99**	**26**	**5.89**	**8.82**	**1471**	**6.50**
500 ppm	98	24	4.25	5.97	1022	4.50
600 ppm	96	20	3.80	5.82	962	4.00
700 ppm	96	24	3.16	5.52	868	3.80
800 ppm	95	26	3.05	4.65	770	3.50
900ppm	95	28	3.10	4.52	762	3.50
1000ppm	95	30	1.98	3.80	578	3.00
Mean	**96**	**25**	**3.22**	**5.05**	**827**	**3.72**
CD @5%	3.24	6.02	0.28	0.22	38.51	1.82
CV (%)	4.76	7.21	2.84	1.48	1.54	8.35
SEm±	1.04	2.26	0.09	0.07	12.5	0.56

has been illustrated in Table 6. Speed of germination with different concentrations of bulk boron where the seeds were soaked for one and half hour did not significantly differ between concentrations of borax but germination percentage was more at lower concentrations. Root length, shoot length and SVI-I and SVI-II were more at 25 ppm concentration of borax.

Seed priming with boron (nutria priming) exhibited beneficial effects for seed germination, mean germination time, germination index, seedling vigor index, chlorophyll content of leaf and shoot to root related parameters. Priming enhanced seed performances are related to the repair and the buildup of nucleic acid, enhanced synthesis of protein, repair of membranes and improves antioxidant system (Hsu *et al*. 2003).

Table 7. Selection of better performing zinc and boron concentration among all treatments based on seedling traits.

Treatments	Root length (cm)	Shoot length (cm)	SVI- I	SVI- II
100 ppm (1 1/2 hr)	8.81	9.53	1833	11.00
75 ppm (2 hrs)	8.38	9.85	1740	7.16
Nano boron				
50ppm (1 1/2hr)	8.85	10.33	1917	8.50
600ppm (2hrs)	8.20	10.30	1749	7.23

Boron is considered to be necessary for hormone metabolism, photosynthetic activities, cellular differentiation and water absorption in plant parts and seed priming triggers the hydrolytic enzymes and altered the physiology of embryos, so that germination metabolism may take place rapidly than normal.

Selection of better performing nano zinc and boron concentrations for seedling traits

From the laboratory study, two each treatment was selected based on their response to different seedling traits (Table 7). Over all data shows that 100 ppm concentration of nano zinc compared to 75 ppm for two hours soaking 100 ppm with one and half hour soaking period recorded higher seedling traits like root length (8.81), shoot length (9.53) and seedling vigour I (1833) and seedling vigour II (11.00) and 75 ppm with two hours soaking period recorded 8.38 cm root length, 9.85 cm shoot length, 1740, SVI-I and 7.16 SVI-I.

In nano boron, 50ppm for one and half hours and 600 ppm for two hours soaking duration showed higher seedling growth compared to all other treat-

Plate 2. Images captured by root scanner indicating difference in root traits.

ments. Among them, 50ppm nano boron with one and half hours soaking recorded higher root length (8.85 cm), shoot length (10.32 cm), SVI-I (1917) and SVI-II (8.50).

CONCLUSION

The speed of seed germination was higher at lower concentrations (25-150 ppm) of nano zinc or boron treated seeds compared to the control and other higher concentrations (500 to 1000 ppm). Soaking of seeds in nano zinc or boron for one and half hour resulted in high speed of germination. There was no gain with longer soaking period.

The root length of seven days old seedlings was highest at 100 ppm nano zinc or 50 ppm of nano boron (Plate 2). The seedling shoot length was significantly higher under high concentration compared to the lower concentration of nano zinc or boron. However, significantly higher SVI-I and SVI-II were observed at lower concentrations compared to the higher concentrations of nano zinc or nano boron.

Effects of nano zinc and nano boron were compared with bulk zinc and boron on seedling vigour characters. The lower concentrations of nano zinc and boron regulate higher SVI-I and SVI-II compared to the bulk zinc or boron. Higher SVI- I was also observed with higher concentrations of bulk zinc. These results indicate that the quantity of zinc or boron would be minimized when nutrients are applied in nano form.

REFERENCES

Abdul-Baki AA, Anderson JD (1973) Vigour determination in soybean seeds by multiple criteria. *Crop Sci* 13: 630-633.

Adiloglu A (2002) The effect of zinc (Zn) application on uptake of cadmium (Cd) in some cereal species. *Archives of Agric Soil Sci* 48:553–556.

Arunachalam P, Kannan P, Prabukumar RG, Govindaraj M (2013) Zinc deficiency in Indian soils with special focus to enrich zinc in peanut. *African J Agric Res* 8(50): 6681-6688.

Ashagre H, Ibrahim AH, Urgecha Fita, Worku Nedes A (2014) Influence of boron on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *Academia J* 8(2): 133-139.

Benjamin Pommerrenig, Kai Eggert, Gerd P Bienert (2019) Boron Deficiency Effects on Sugar, Ionome, and Phytohormone Profiles of Vascular and Non-Vascular Leaf Tissues of Common Plantain *(Plantago major* L.). *Intl J Mol Sci* 20(16): 3882. doi:10.3390/ijms20163882.

Blevins DG, Lukaszewski KM (1998) Boron in plant structure and function. *Annual Rev P Phy Pl Mol Biol* 49: 481–500.

Bonilla I, El-Hamdaoui A, Bolanos L (2004) Boron and calcium increase *Pisum sativum* seed germination and seedling development under salt stress. *Pl Soil* 267: 97-107.

Brown PH, Shelp BJ (2002) Boron mobility in plants. *J Pl Soil* 193:85-101.

Dharam Singh Meena, Jayadeva HM, Chirag Gautam, Hari Mohan Meena (2017) Effects of Nano Zinc Oxide (ZnO) Particles on Germination of Maize (*Zea mays* L.) seeds. *Int J Plant Soil Sci* 16 (1) :1-5

Dileepkumar Masuthi A, Vyakaranahal BS, Deshpande VK (2009) Influence of pelleting with micronutrients and botanical on growth, seed yield and quality of vegetable cowpea, Karn. *J Agric Sc*i 22(4):898-900.

Eraslan F, Savasturk O, Gunes A (2007) Changes in antioxidative system and membrane damage of lettuce in response to salinity and boron toxicity. *J Scientia Hort* 114(11):5-10.

Farooq M, Wahid A, Kadambot HM, Siddique (2012) Micronutrient application through seed treatments. *J Pl Sci Pl Nutr* 12 (1): 125-142.

Farr HJ (2010) Early Growth Tolerance to Boron and Salt in Wheat and Barley. MSc thesis. Curtin University, Agric Techn. Australia, pp 95.

Fujiwara T, Mayuki Tanaka, Kyoko M (2008) Optimization of nutrient transport processes by plants-boron transport as an example. 2010, 19th World Congress of Soil Science, Soil Solutions for a Changing World 1-6 August 2010, Brisbane, Australia., Published on DVD.

Gupta SP, Singh MV, Dixit ML (2008) Deficiency and management of micronutrients. *Ind J Fert* 3: 57-60.

Hamidreza Sadegh, Gomaa AM Ali, Vinod Kumar Gupta, Abdel Salam Hamdy Makhlouf, Ramin Shahryari-ghoshekandi, Mallikarjuna N, Nadagouda, Mika Sillanpaa, Elzbieta Megiel (2017) The role of nanomaterials as effective adsorbents and their applications in wastewater treatment. *J Nanostruct Chem* 7 : 1-14

Hsu CC, Chen CC, Chen JJ, Sung JM (2003) Accelerated aging enhanced lipid peroxidation in bitter gourd seeds and effects of priming and hot water soaking treatments. *Scientia Horti* 98:201-212

Khan R, Gurmani AH, Gurmani AR, Zia MS (2006) Effect of boron application on rice yield under wheat rice system. *Intl J Agri Bio* 8: 805-808.

Kharb RPS, Lather BPS, Deswal DP (1994) Prediction of field emergence through heritability and genetic advance of vigour parameters. Seed Sci and Techn, pp 461-466.

Khodakovskaya M, Enkeleda Dervishi E, Mahmood M, Yang Xu, Zhongrui Li, Fumiya W, Alexandru SB (2009) Carbon nano tubes are able to penetrate plant seed coat and dramatically affect seed germination and plant growth. *ACS Nano* 3 (10):3221–3227.

Manjushri B Kawade, DB Jadhav, SP Arshewar (2018) Effect of micronutrients on growth, yield and quality of sunflower in *kharif* season. *Intl J Cur Micr Appl Sci Special Issue*-6: 2189-2196.

Milka Brdar-Jokanovi (2020) Boron Toxicity and Deficiency in Agricultural Plants. *Int J Mol Sci* 21 : 1424; doi:10.3390/ijms21041424

Moussavi-Nik M, Rengrl Z, Pearson JN, Hollamby G (1997) Dynamics of nutrient remobilization from seed of wheat genotypes during imbibition, germination and early seedling growth. *J Pl Soil* 197(2):271-280.

Nalini P, Bhavana Gupta, Girish Chandra Pathak (2013) Foliar application of Zn at flowering stage improves plants performance, yield and yield attributes of blackgram. *Ind Exptl Bio* 51:548-555.

Oil World Annual (2019) Global analysis of all major oilseeds, oils and oil meals: Supply, demand and price outlook. ISTA Mielke Gmbh, Vol.1.

Pandey N, Girish Chandra P, Chandra Prakash S (2006) Zinc is critically required for pollen function and fertilization in lentil. *J Trace Elements Medical Biology Med Bio* 20: 89–96

Parveen Kumar Nimbrayan, Vivek K Singh, Meenu Punia, Anu, Ajay Kumar (2020) Trends and Growth Rate Analysis of Sunflower in Haryana and India. *Res J Agric Sci*11(3): 686-688.

Prasad TNVKV, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Raja Reddy K, Sreeprasad TS, Sajanlal PR, Pradeep T (2012) Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. *J Pl Nutr* 35: 905-927

Rehman A Ur, Farooq Muhammad F, Nawaz A, Iqbal Saba, Rehman Abdul (2012) Optimizing the boron seed coating treatments for improving the germination and early seedling growth of fine grain rice. *Int J Agri Biol* 14(3): 453-456.

Reid JR, Hayes JE, Post A, Stangoulis JCR, Graham RD (2004) A critical analysis of the causes of boron toxicity in plants. *Pl Cell Environ* 27(11):1405-1414.

Sara Sarkhosh, Danial Kahrizi, Elahe Darvishi, Masoud Tourang, Sohrab Haghighi-Mood, Parviz Vahedi, Sezai Ercisli (2022) Effect of zinc oxide nanoparticles (zno-nps) on seed germination characteristics in two Brassicaceae family species: Camelina sativa and Brassica napus L. J Nanomat. Article ID 1892759, https://doi.org/10.1155/2022/1892759.

Sengupta AK, De BK, Mandal AK (2005) Pre-storage seed in vigouration treatments for the maintenance of vigour, viability and field performance of high vigour onion seed (*Allium cepa* L.). *Seed Sci Technol* 33(3) : 753-760.

Sridhar C (2012) Effect of nanoparticles for the maintenance of tomato seed vigour and viability. MSc (Agric) thesis TNAU, Coimbatore (India).

Ullah A, Farooq M, Hussain M, Ahmad R, Wakeel A (2019) Zinc seed priming improves stand establishment, tissue zinc concentration and early seedling growth of Chickpea. *J Ani Pl Sci* 29 (4): 1046-1053.

Vijayalakshmi S, Venkataraj S, Jayavel R (2013) Characterization of cadmium doped zinc oxide (Cd : ZnO) thin films prepared by spray pyrolysis method. *J Appl Phy* 41: 1-6.