The Pharma Innovation Journal 2023; 12(5): 1038-1042

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(5): 1038-1042

www.thepharmajournal.com
Received: 14-02-2023
Accepted: 22-03-2023

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Influence of nano nutrients on seed yield and yield attributing characters in sunflower (*Helianthus annuus* L.)

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Abstract
One of the major constraints in sunflower production is the deficiency of micronutrients like zinc and boron which can be overcome by improving nutrient use efficiency using nano nutrients. Hence, the present study was carried out to study the effect of nanoparticles on growth and yield of sunflower with several combination of treatments with nano zinc and nano boron comparing the results with control and bulk nutrients. Results indicated application of nano boron was significantly superior compared to application of nano zinc in terms of yield and yield attributes. The nano boron seed treatment enhanced seed yield by 42.3% (30.55g/pl) and additional foliar spray with 50ppm nano boron resulted in 86.8% higher yield (39.51g/pl) compared to control. Therefore, the application of nano boron to seeds treatment and foliar application is highly recommendable for increasing the productivity of sunflower.

Keywords: Boron, gas exchange parameters, growth parameters, nanoparticles, zinc

Introduction

Soil application was the most prevalent method of micronutrient addition but the cost involved and difficulty in obtaining high quality micronutrient fertilizers are major concerns in developing countries. Micronutrient foliar spray and seed treatments, which include seed priming and seed coating, are attractive and alternative methods. Micronutrient application through seed treatments improves the crop stand, establishment, advances phenological events and increases yield. In most cases, micronutrient application through seed treatment and foliar application performed better or similar to other application methods. Being an easy and cost effective method of micronutrient application, seed treatment and foliar spray offer an attractive option for resource poor farmers (Farooq *et al.*, 2012) [11].

Among all the nutrients, zinc and boron play major roles in increasing growth and seed yield in oilseed crops. In India, Zn is one of the multinutrient deficiencies that are causing poor crop yields as the zinc is involved in most of the plant growth functions. It helps to produce auxins and is a growth promoting substance that controls the development of the shoot also forms enzyme systems, which regulate plant life (Nalini *et al.*, 2013) [18].

Boron is essential for plant growth *i.e.,* cell wall strength, cell division, fruit and seed development and sugar transport. Though the boron requirement for optimum plant nutrition is low compared with the primary nutrients, the need for boron is especially significant in flowering and seed development (Mahmoud *et al.*, 2006) [16]. Boron deficiency is the most widespread micronutrient deficiency around the world and it affects vegetative and reproductive growth of plants, resulting in inhibition of cell expansion, death of meristem and reduced fertility.

In recent years, there has been mounting interest in management of fertilizer application in soils to obtain higher fertilizer use efficiency. To address the issues relating to increase fertilizer use efficiency, development of new agricultural technologies such as nanotechnology will be crucial in meeting the ecological needs and in achieving the anticipated food demands of the growing population in the near future. In this context, an attempt was made to enhance the growth and yield of sunflower using nano zinc and nano boron by seed treatment and foliar application.

Materials and Method

The field experiment was conducted with sunflower hybrid KBSH-44 in RCBD design with three replications and nine treatments which included nano seed treatment, nano foliar spray, combination of nano seed treatment and foliar spray, bulk foliar spray for both zinc and boron nanoparticles.

Based on results of laboratory experiment 100ppm concentration of nano zinc oxide and 50ppm concentration of boron nitride with 1 ½ hr soaking were selected for seed treatment. Seed treatment was done at the time of sowing. Foliar spray was given two times i.e. @30DAS and 60DAS. Same concentration of nano zinc oxide and boron nitride were used for foliar spray, where as bulk zinc (0.6%) and boron (0.2%) were sprayed as per recommendation in package of practice.

The soil is red sandy clay loam with slightly acidic (pH 6.7) and the electrical conductivity was normal (0.22 m mhos/cm at 25 °C). The nitrogen content in the soil was low (340.0 kg/ha), whereas the phosphorus was high (40.5 kg/ha) and the potash was medium (239.0 kg/ha). Micronutrient like zinc and boron available in soil was 1.25 and 0.2 mg/kg^{-1}.

Results were compared with control where plants were grown according to package of practice. Growth parameters and physiological parameters were recorded during crop growth @ 60DAS and yield parameters were recorded after harvest of the crop. Soil analysis was done before and after harvesting of the crop, initial nitrogen (355 kg/ha), phosphorus (50.49 kg/ha), potassium (243 kg/ha), zinc (0.8) and boron (0.8 mg/kg). The treatment details are as follows;

T_1: Control (as per package of practices)
T_2: Nano zinc seed treatment @100ppm
T_3: Nano zinc foliar spray @100ppm
T_4: Nano zinc seed treatment @ 100ppm + foliar spray @100ppm
T_5: Bulk zinc foliar spray @0.6% (as per package of practices)
T_6: Nano boron seed treatment @50ppm
T_7: Nano boron foliar spray @50ppm
T_8: Nano boron seed treatment @50ppm + foliar spray @50ppm
T_9: Bulk borax foliar spray @0.2% (as per package of practices)

The observations on plant height (cm) was measured by taking height of the main stem from the ground level to the tip of the plant at 60DAS and at the time of harvest and expressed in cm per plant. To record total leaf area per plant all the leaves from each selected plant at flowering was measured @ 60DAS by following factor method reported by Nanja Reddy *et al.*, (1995) [19]. Chlorophyll content was measured using SPAD chlorophyll meter in the fully expanded green leaf from the top of the plant.

The photosynthetic rate (µmol CO_2 m^{-2} s^{-1}), Transpiration rate (mmol H_2O m^{-2} s^{-1}), Stomatal conductance (mmol m^{-2} s^{-1}) and Water use efficiency (mmol of CO_2 per mmolH_2O) were measured using Infra Red Gas Analyser (IRGA) at different time intervals *i.e.* @35DAS, 45DAS and 60DAS. Measurements were made from 9 am to 12 noon when the weather was clear.

Yield parameters such as seed yield per plant (g/plant) was measured by taking the total quantity of seeds from threshing after harvest and dried completely and seed yield was recorded in 10 plants in each treatment in three replications weighed and expressed in grams. Hundred dried seeds were counted using seed counter and counted seeds were weighed and expressed in grams.

After harvesting, seeds, leaves, petiole, stem and thalamus were separated individually and dried them properly. Dry weight of each parameter was noted down and all the values were added to get total dry matter per plant and expressed as g/plant.

Harvest index is defined as the ratio of economic yield to total biological yield (Donald, 1962) [10] and expressed in percentage. The harvest index of sunflower was worked out using the following formula,

Harvest index (%) = {(Economic yield (g)/Total biological yield (g)} × 100 and expressed in percentage.

Kernel was separated from husk of 100 seeds and their weights were recorded separately to get kernel to husk ratio by dividing kernel weight by husk weight.

Results

Higher fresh weight was noticed in seeds treated with nano zinc @100 ppm (9.76 g and 48.19 g) and dry weight (0.68 g and 3.02 g) on 13th and 25th DAS. However, nano boron treated seeds at 50ppm showed higher fresh weight (8.16 and 38.63g) and dry weight (0.55 and 2.81g) compared to control (5.91 g and 17.99g) (Table 1). With reference to growth parameters as mentioned in Table 2, the nano and bulk treatments were similar, but additional foliar spray of nano zinc or boron at 30DAS resulted in higher plant height, leaf area, SPAD etc.

Combination of nano zinc seed treatment @ 100 ppm + foliar spray @ 100 ppm recorded maximum plant height at 60DAS (191 cm) followed by nano boron seed treatment @ 50 ppm + foliar spray @ 50 (185 cm) compared to control (165 cm). The increase in plant height may be due to fundamental role of Zn in maintaining structural stability of cell membranes (Welch *et al.*, 1982) [28] and use in protein synthesis, membrane function and cell elongation (Cakmak, 2000) [9]. Improvement in plant height with Boron application indicates involvement of boron in cell elongation and/or cell division (Mouhtaridou *et al.*, 2004) [17], photosynthesis and transpiration (Brown & Hu, 1996) [5] and also in meristematic growth (Bohnsack and Albert, 1977) [6].

Table 1: Vigour of plants in the field @13th DAS and 25th DAS

Treatments	13th DAS		25th DAS	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
T1	5.91	0.34	17.99	1.31
T2	9.76	0.68	48.19	3.02
T3	6.04	0.33	18.34	1.42
T4	8.19	0.74	46.87	3.07
T5	6.00	0.35	18.87	1.33
T6	8.16	0.55	38.63	2.81
T7	5.93	0.34	17.94	1.38
T8	5.96	0.56	37.03	2.86
T9	6.00	0.34	18.25	1.35
CD @5%	0.90	0.05	2.60	0.22
CV (%)	7.58	6.50	5.16	6.24
S.Em±	0.30	0.02	0.87	0.07

Total photosynthetic leaf area was found to be significant between the treatments (Table 3). Among the treatments, nano boron seed treatment @ 50 ppm (2774.33), nano boron foliar spray @ 50 ppm (2762.04) and nano boron seed treatment @ 50 ppm + foliar spray (2893.26) recorded maximum leaf area compared to nano zinc treatment and control (1570.90). Larger leaf area was due to expansion of epidermal cells coinciding with formation of intercellular airspaces in mesophyll cells (Jiao, 2001) [14]. Boron also enhances the growth of the plant by supplementing the soil available nutrients and role of nutrients in various physiological processes there by delaying senescence and abscission.

Chlorophyll content varied between the treatments of nano zinc and boron. Nano zinc treated plants recorded highest chlorophyll content. Among the treatments, combination of seed Treatment @ 100 ppm + foliar spray @ 100 ppm recorded maximum chlorophyll content (40.0) followed by nano zinc seed treatment @ 100 ppm (38.33) compared to control (33.46).

Increased chlorophyll content is due to zinc which acts as a structural and catalytic component of proteins, enzymes and as co-factor for normal development of pigment biosynthesis (Balashouri, 1995) [3]. Boron is also necessary for increased chlorophyll content.

Table 2: Effect of nano zinc and boron treatments on growth parameters

Treatments	Plant height (cm)	Leaf area (cm^2/pl)	SPAD Chlorophyll content	Stem girth (cm)
T1	165	1570.90	33.46	3.1
T2	183	1929.54	38.33	2.6
T3	184	2015.24	37.67	2.4
T4	191	2477.19	40.00	2.6
T5	177	2182.46	33.21	2.5
T6	180	2774.33	37.00	2.2
T7	182	2762.04	36.39	2.1
T8	185	2893.26	36.33	2.3
T9	173	2708.04	32.63	2.3
CD @5%	13.80	438.65	7.75	0.25
CV (%)	4.43	10.70	12.40	5.41
S.Em±	4.60	146.30	2.58	0.08

The physiological traits *viz.*, photosynthetic rate, stomatal conductance and transpiration were higher at 30 DAS in seed treatment with nano zinc and boron. However, at 45 and 60DAS, seeds treated with nano alone or bulk zinc or boron were similar. However, additional spray of nano zinc or boron enhanced the gas exchange traits.

The gas exchange parameters like photosynthetic rate, stomatal conductance and transpiration rate was differed significantly between the treatments at 30 DAS, 45DAS and 60 DAS. Photosynthetic rate, stomatal conductance and transpiration rate was maximum in nano boron treatment at 30, 45 and 60 DAS than the nano zinc treatment and control plants.

Positive effect of boron/nitrogen interaction associated with nitrogen enhancement for the photosynthetic activity in presence of adequate boron contents. Boron involved both in nitrogenase activity and as protection against O_2 diffusion (Rizk and Abdo, 2001) [25].

Boron has a vital role in the process of transpiration for the movement of potassium to the stomata of the leaf. Boron also maintains stable balance between sugars and starches, pollination and seed production (Gupta *et al.*, 2008) [13]. Increase in photosynthetic activity with increased exogenously applied B concentration specifically at ray floret stage confirmed the findings of Gonzalez *et al.*, (1993) [12] and Liu *et al*., (2005) [15] who explored direct proportionality between B application and photosynthetic rate in crop plants.

Table 3: Effects of nano zinc and boron treatments on gas exchange parameters

Treatments	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})			Stomatal conductance (mmol m^{-2} s^{-1})			Transpiration rate (mmol H_2O m^{-2} s^{-1})		
	30DAS	45DAS	60DAS	30DAS	45DAS	60DAS	30DAS	45DAS	60DAS
T1	12.16	17.58	21.31	0.23	0.3	0.49	2.53	4.85	4.98
T2	15.2	18.32	21.36	0.15	0.23	0.35	2.24	4.47	4.63
T3	12.67	18.21	22.16	0.21	0.38	0.36	2.57	5.43	5.42
T4	15.04	18.06	22.04	0.17	0.35	0.31	2.29	5.29	4.68
T5	12.08	16.94	21.57	0.21	0.26	0.37	2.46	4.72	4.88
T6	13.56	17.18	23.54	0.25	0.47	0.51	2.55	4.07	6.62
T7	12.13	18.44	23.44	0.21	0.38	0.65	2.92	4.06	5.75
T8	13.6	18.21	23.67	0.28	0.33	0.75	2.59	3.94	5.33
T9	11.98	18.7	22.28	0.24	0.28	0.55	2.43	3.9	5.31
CD @5%	0.53	0.47	0.28	0.03	0.04	0.09	0.22	0.43	0.28
CV (%)	1.58	1.39	0.83	0.24	0.12	0.28	0.65	1.27	0.83
S.Em±	7.3	3.28	2.15	15.78	18.29	32.45	9.77	12.26	9.07

Liu, *et al.*, (2005) [15] explained that boron enhanced photosynthesis efficiency of soybean by membrane maintenance and photosynthesis products translocation, chlorophyll stability and enlarging leaf area for photosynthesis. Interaction of boron/nitrogen associated with nitrogen enhancement for the photosynthetic activity in the presence of adequate boron. Similar results were observed by Ayada *et al.*, (2011) [2] that boron foliar application resulted in increased leaf area and enhanced photosynthesis activity, resulting in an increase in seed yield.

Total dry matter, seed yield, harvest index, 100 seed weight and kernel to husk ratio were recorded maximum in boron nanoparticle treatments than the control and nano zinc seed treatment (Table 4). Total dry matter was recorded more in control (81.30 g) followed by combination of nano boron seed treatment @ 50 ppm and foliar spray @ 50ppm (73.53 g). Similar findings were recorded by Niaz Ahmed, *et al.*, (2011) [21] where dry matter and yield increased significantly with B up to 2.0 kg ha^{-1} and biological yield increased linearly with increasing of doses of boron fertilizer up to addition of 2.0 kg B ha^{-1}however; response was flattened beyond this dose of fertilizer.

Seed yield was maximum in combination treatment of nano boron seed treatment @ 50 ppm and foliar spray @ 50ppm (39.51 g) followed by combination of nano zinc seed treatment @ 100 ppm and foliar spray @ 100ppm (32.77 g). Boron applied in small amounts is a critical component of membranes in pollen tubes (Bolanos *et al.,* 2004) [4]. As growth of pollen tubes requires rapid synthesis of cell wall and plasma membrane (Taiz and Zeiger, 2010) [27], B deficiency decreases the growth of pollen tube and fertilization thus causing failure of grain setting (Rerkasem *et al.,* 1993) [24]. Improved seed setting and seed filling were due to enhanced pollen germination and fertilization through boron and improved photo assimilation and translocation to the reproductive organs of economic value.

Proper and adequate supply of Zn increased the uptake of N during the grain formation stage and ultimately improved the yield (Siddiqui *et al.,* 2009) [26]. More number of seeds filled per plant may be due to timely availability of B for pollen tube growth and increased assimilation of sugars as found with simultaneous increase in seed yield and decrease of total dry weight with foliar and soil B supply Hussain *et al.,* (2008) [22].

Combination treatment of nano boron seed treatment @ 50ppm + foliar spray @ 50 ppm recorded maximum harvest index (34.99) followed by nano zinc seed treatment @100 ppm + foliar spray @ 100 ppm (34.77). Overall harvest index was more in plants treated with the nano boron treatments.

Improvement in harvest index with boron nutrition might be due to better starch utilization causing higher seed setting and translocation of assimilates to developing seeds increasing the seed yield, number of seeds per siliqua and harvest index of mustard (Hussain *et al.,* 2008) [22].

Increase in harvest index resulted from boron application due to better starch utilization that results in higher seed setting and translocation of assimilates to developing grains, which increases the grain size and number of grains per panicle by maintaining considerable mobility of phloem sap to reproductive parts from older leaves (Rashid *et al.,* 2004) [23].

Exogenous application of boron to the developing head was shown to increase the transport of photosynthates from leaf to the developing head that leads to increase harvest index as it involved in partitioning of pre-anthesis assimilates from vegetative to reproductive sinks during seed development.

Table 4: Effect of nano zinc and boron on yield parameters like total dry matter, seed yield, harvest index and 100 seed weight

Treatments	Total dry matter (g/plant)	seed yield/plant (g)	Harvest index	100 Seed weight (g)
T1	81.30	21.17	20.67	4.33
T2	63.93	24.97	28.08	5.15
T3	58.93	25.99	30.60	5.13
T4	61.60	32.77	34.72	5.83
T5	66.14	22.53	25.39	4.85
T6	67.87	30.55	31.21	5.73
T7	64.85	29.20	31.04	6.08
T8	73.53	39.51	34.99	6.20
T9	74.89	24.51	24.70	5.74
CD @ 5%	8.27	2.30	2.08	0.40
CV (%)	7.01	4.76	4.14	4.25
S.Em±	2.76	0.76	0.69	0.13

It has been reported that B plays an essential role in the structure and function of cell walls, cellular membranes, translocation of sugars, fruit and seed development (Cakmak and Romheld, 1995) [7]. External application of boron was found to increase the vegetative and reproductive growth of the sunflower plant (Asad *et al*., 2002) [1]. Application of boron (2 kg/ha) at ray floret stage increased the HI to an extent of 29% and the seed yield by 53%, as boron involved in mobilization of photosynthates from vegetative parts to head so that improves HI and thus seed yield (Nanja Reddy *et al*., 2003).

The 100 seed weight was observed more in nano boron seed treatment @ 50 ppm + foliar spray @ 50 ppm (6.20 g) followed by nano boron foliar spray @ 50 ppm (6.08 g). Boron application affirms its role in development of anthers and pollen and pollen germination during anthesis and fertilization that leads to decrease in chaffyness of the seeds (Cheng and Rerkasem, 1993; Noppakoonwong *et al.,* 1997) [24, 20].

Conclusion

Study reveals that improved seed setting and seed filling were due to enhanced pollen germination and fertilization through boron and improved photo assimilation and translocation to the reproductive organs of economic value. The seed treatment and foliar application of nano ZnO and boron at the vegetative and reproductive stage helps in good crop growth and also the translocation of Zn and boron to the seed further increasing yield and yield parameters.

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