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IMPACT OF BORON SEED PRIMING ON THE GROWTH, YIELD, ANTIOXIDANT POTENTIAL, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF *HELIANTHUS ANNUUS* L.VAR AGSUN 5264 MEDIATED WITH INDOLE ACETIC ACID APPLICATION.

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Abstract
Helianthus annuus is an important economic crop which yields high edible oil contents and is feasible to be cultivated in almost all agroclimatic conditions and soils. This plant not only covers the food based energy needs but also known for therapeutic impacts. Majority soils in Pakistan are boron deficient and responsible for low crop yields. Boron is required by plants in very small quantities and the rate of agricultural production is determined by its availability in soil and irrigation water. Several morphological, physiological and biochemical parameters are disrupted due to fluoride sequestration in areas surrounding plant roots and mesophyll cells. In present study, seeds of *Helianthus annuus* L. var. Agsun 5264 were subjected to boron priming treatments. Seeds were primed with five treatment levels of boric acid (0, 0.001%, 0.01%, 0.1% and 0.5%). Seeds were primed overnight in aerated channels. Seeds were sown in earthen pots on the next day. Pots were positioned according to the complete randomized block design. Final harvest was done when plants were fully grown. Various physical parameters i.e, seeds/head, head diameter, root and shoot length, root fresh and dry weight were recorded. The antioxidant activity was determined in terms of DPPH radical scavenging. The phenolic and flavonoid contents were also determined. The Results of boron treatment mediated with indole acetic acid evidently showed that 0.1% and 0.5% solutions of boric acid as a boron source not only improved the yield and growth parameters but also induced high antioxidant response, phenolic and flavonoid production which added a substantial therapeutic and functional aspect in plant for human consumption.

Keywords: *Helianthus annuus,* boron treatment, indole acetic acid, growth, antioxidant, phenolic and flavonoid, phytotheraputic.

Introduction
Among the most important oilseed crops is *Helianthus annuus* which produces higher edible oil and is feasible to be cultivated in almost all conditions and soils. The nutritional properties of sunflower are incredible and are rich in oil content that ranges from 35% to 48%. However, some types may yield upto 50% oil content, protein content 20%-27% and excellent quantities of unsaturated fatty acids that range upto 60% in most types and control the cholesterol levels of blood. The oil, seed and other parts of *Helianthus annuus* (Sunflower) are well known for their therapeutic effects (Rauf et al.,

2020). Among some prime issues about the cultivation and yield enhancement of sunflower is plant balanced nutrition. Other than iron and zinc, boron deficiency is the most harmful to crop. Boron primarily functions in cell wall formation, fixation of nitrogen, phenol metabolism, indole acetic acid (IAA) metabolism and stabilizing membranes (Tanaka and Fujiwara, 2008). Pollen formation and retention of flower is among other parameters to be affected by boron content. In order to meet the requirements, Pakistan is spending huge amounts of foreign exchange to imports. The production of indigenous edible oil should be improved and enhanced in order to minimize the drains on the country's economy. Sunflower being an important non-traditional crop is the second source of oil production after cottonseed (Sarwar, 2015). Vegetative growth of sunflower is least affected by boron deficiency than sexual growth (Ahmad et Al., 2009). Cotton and rice growing areas of Pakistan are reportedly deficient of boron (Rashid and Ryan, 2004). Hence, boron should be applied externally for better yield. Different ways of delivering boron to the crop include foliar spray, seed priming and soil amendments. Application of boron through seed priming is the recent and widely used method in present era (Rehman et al., 2012). Boron is required by plants in very small quantities and the rate of agricultural production is determined by its availability in soil and irrigation water (Tanaka and Fujiwara, 2007). The most common form of boron is boric acid (H_3BO_3) that easily gets leached due to high rainfall leading to deficiencies in plants (Chaudhary, et al., 2019). Contrarily, under low rainfall condition, it does not get leached and accumulates in soil up to toxic concentrations (Reid, 2007). This situation is more common in arid and semi-arid environments where soil already contains high levels of boron. When the water gets evaporated, it leaves back high concentrations of boron in soil that are toxic for plant growth and reduce crop yields (Farooq et al., 2018). The determination of basic role of boron in plants has been a tough challenge since the effects caused due to deficiency of boron are very rapid and of wide variety (Rasool et al., 2019). However, much advances and improvements have been made in understanding the role of boron in plant metabolism. The determination of existence of boron in its chemical forms and the role of boron in plant cell walls and understanding the mechanisms of boron transport across membranes are among these advances. The base of each of the major advances in understanding the biology of boron is based upon the knowledge of chemical and physical characteristics of boron. Stages of development of sunflower are affected by a number of factors like drought, salinity and weather (Reddy et al., 2003). Sunflower can extract water from the depths of soil and can tolerate shorter periods of water unavailability (Vijay, 2004). Regular physiological metabolism, water uptake and cell turgidity is maintained by osmotic balance (Radić et al., 2013). The endogenous growth substances found in the plant may also be the cause of reduction in plant growth under salinity (El-nabarawy, 1994). Proline is one of the osmoprotectants that contribute towards the osmotic adjustments and protect enzymes from oxidative damage under salinity (Gupta and Huang, 2014). The endogenous group of hormones i.e, IAA regulates plant growth and development and is involved in the counter action of stress conditions (Javid et al., 2011). It was demonstrated by Akbari et al., 2007 that IAA application can increase length of hypocotyls, fresh and dry weights of seedlings and dry weight of hypocotyls. The sensitivity of sunflower towards boron is quite evident and can be used in the assessment of boron in soils. Depending upon the content of boron applied as a fertilizer, it can have both negative and positive effects upon the yield and vegetative growth of the plant. Total dry matter was improved to three times in boron sufficient soils than in the boron deficient soils. Symptoms of boron deficiency usually are visible on leaves, stems and reproductive parts hence producing empty seeds that reduce the yield of crop and eventually lead to the corked stem, deformation of capitulum and lower seed yield. It has been demonstrated that boron deficiency can also affect pollen tube growth at flowering stage, consequently causing stamens abortion which may lead to lower seed yield. Boron in sunflower is also associated with sugar translocation, availability of water, metabolism of N, P, fatty acids and carbohydrates. Although boron is essential for optimal plant growth, excessive application of boron can also deteriorate plant growth during early phases. Helianthus annuus is also reported to possess some important medicinal properties and functional food related characteristics due to presence of phytochemicals (Guo et al., 2017). Certain nutrients were reported to improve the medicinal potential and nutritional properties of plants (Pandey et al., 2016). The present study was designed to examine the response of sunflower plants in term of yield and growth to priming application of boron upto a threshold level in boron deficient soil. The changes in antioxidant response and phenolics responsible for therapeutic purposes were also assessed with respect to treatments as an assessment for nutraceutical development and medicinally functional food development.

Materials and methods

Seed of *Helianthus annuus* L. var. Agsun5264 were collected. Healthy seeds were selected for experiment and stored under dry conditions. Seeds showing any disease were discarded carefully. Selected healthy grains were placed in paper bags to be used for experimental work.

Seeds were primed with solutions of boric acid of different concentrations. 5 solutions of boric acid were prepared i.e, 0%, 0.001%, 0.01%, 0.1% and 0.5% and 1 volume of seeds was soaked in 5 volumes of solution with 4 replicates of each treatment, grown in pots. According to the number of replicates, 24 seeds were primed for each treatment (6 seeds per pot).

Weight of 24 seeds = 1.88 g

Volume of solution used for priming = 1.88 x 5 = 9.45 mL

Morphological and yield Parameters

A number of growth and yield parameters were recorded on final harvest. They included root length (cm), shoot length (cm), seeds/head, head diameter, root fresh weight (g) and root dry weight (g)

Preparation of Indole acetic acid solution

Indole acetic acid solution (50ppm) was made and added to each pot. The solution of IAA was supplied as soil drench at 15 days after transplantation. Groundwater was supplied at regular intervals to minimize dehydration throughout the experiment.

Experimental design

The experiment was performed in Botanical Garden, Government College Women University, Sialkot. Plastic pots were placed in wire house in order to prevent entry of animals that might harm plants. Briefly 40 pots made of plastic were placed in wire house of botanical garden. Their size was 10x10 inches. Pots were numbered according to the name of variety, replicate number and were arranged according to Randomized Complete Block Design. 4 replicates of each treatment for both varieties were grown in pots. Pots were filled with sand and placed in natural environmental conditions.

Extraction

The whole shade dried plant material was ground to fine powder and dissolved in methanol. The resultant mixture was shaken on mechanical shaker for 24 hours. After that, the mixture was filtered to remove debris and unnecessary particles. The filtrate was subjected to rotary evaporator to remove excess solvent under vacuum for extract generation. The obtained extracts were stored at low temperature in refrigerator for further analysis (Raza et al., 2018).

Antioxidant activity

The antioxidant activity was determined by already reported method (Raza et al., 2020) with slight changes. About 10 mg of each extract was dissolved in 10 mL of methanol and this dilution was added to diluted DPPH reagent solution and stayed for 30 minutes at room temperature in dark. The absorbance were measured at 520 nm. The butylated hydroxy anisole (BHA) was used as reference standard. The following equation was used for DPPH % scavenging potential of extracts.

$$\text{DPPH radical scavenging (\%)} = [(AB - AE)/AB] \times 100$$

Where, AB is the absorbance of blank and AE is the absorbance of extract.

Total phenolic contents (TPC) and total flavonoid contents (TFC)
The TPC were determined by Folin-Ciocalteu reagent method using Na_2CO_3 and the absorbance of extract containing mixture was noted at 760 nm. Gallic acid was used as standard phenolic compound and results were reported in mg of gallic acid equivalent per gram of extract (mg GAE/gE). Similarly, TFC were determined using standard $AlCl_3$ method for which absorbance was taken at 510 nm. The rutin was used as standard flavonoid and results were reported as mg of rutin equivalent per gram of extract (mg RE/gE)(William et al., 2019).

Statistical Analysis
Experiment was designed by Design- Expert Software-11. Data collected from the experiment was used for the calculation using Duncan's Multiple Range Test followed by one way ANOVA (analysis of variance). The model equation was used to estimate the coefficients, comparison between estimated and observed values and the plots to visualize interaction. Statistical data provided information that helped to observe the individual and synchronic effect of each factor on vegetative and physiological changes of plant with stress.

Results
The results of various understudy parameters and indicators are presented below.

Shoot length
Shoot length In plants of boron group, maximum shoot length was shown by those primed with 0.5% solution of boric acid i.e. 20% increment as compared to control (hydropriming) plants. Decreasing concentration of boric acid solution i.e.,0.01 and 0.1% caused shoot length to increase i.e., 16.7 and 13.4% more than control plant as shown in Fig 1.

Root length
A profound increment in root length was recorded when seeds of boron group were primed with 0.5% boric acid solution over control plants. Similarly, priming with 0.01% boric acid solution caused root length to be enhanced by 7.8% as compared to control plants. Priming treatment with higher concentration of boric acid i.e., 0.1% and 0.5% caused root length to increase by 7 and 12% respectively, over control plants (Fig 2)

Seeds/Head
In boron group plants, maximum seeds per head were observed for those given the priming concentration of 0.5% boric acid solution i.e., 17.5% as compared to control plants. Seeds per head reduced when primed with lower concentrations of boric acid i.e, priming with 0.01 and 0.1% caused only 13and 9.6% increase in seeds per head over control plants. (Fig 3)

Head Diameter
A profound increment in head diameter was recorded when seeds of boron group were primed with 0.01% boric acid solution over control plants i.e, 13%. Similarly, priming with 0.001% boric acid solution caused head diameter to be enhanced by 4.3% as compared to control plants. Priming with 0.1% boric acid solution caused head diameter to be enhanced by 25% over control plants. Priming treatment with higher concentration of boric acid 0.5% caused head diameter to increase by and 36.5% over control plants (Fig 4)

Root Fresh Weight
A profound increment in root fresh weight was recorded when seeds of boron group were primed with 0.01% boric acid solution over control plants i.e, 15%. Similarly, priming with 0.001% boric acid solution caused root fresh weight to be enhanced by13.4 % as compared to control plants. Priming with 0.1% boric acid solution caused root fresh weight to be enhanced by 16% over control

plants. Priming treatment with higher concentration of boric acid 0.5% caused root fresh weight to increase by and 25.5 over control plants (Fig 5)

Root Dry Weight

A non-uniform trend was observed in root dry weight of boron group plants. In plants subjected to priming treatment of 0.001% boric acid solution, dry weight was reduced by 4.6% in comparison to control plants. Similarly, dry weight was reduced by minor percentage i.e, 0.9% in 0.01% treated plants. However, a visible increase was found in the dry weight of roots of plants primed with 0.1 and 0.5% boric acid solution. The increment was recorded upto 9.4 and 16% respectively. (Fig 6)

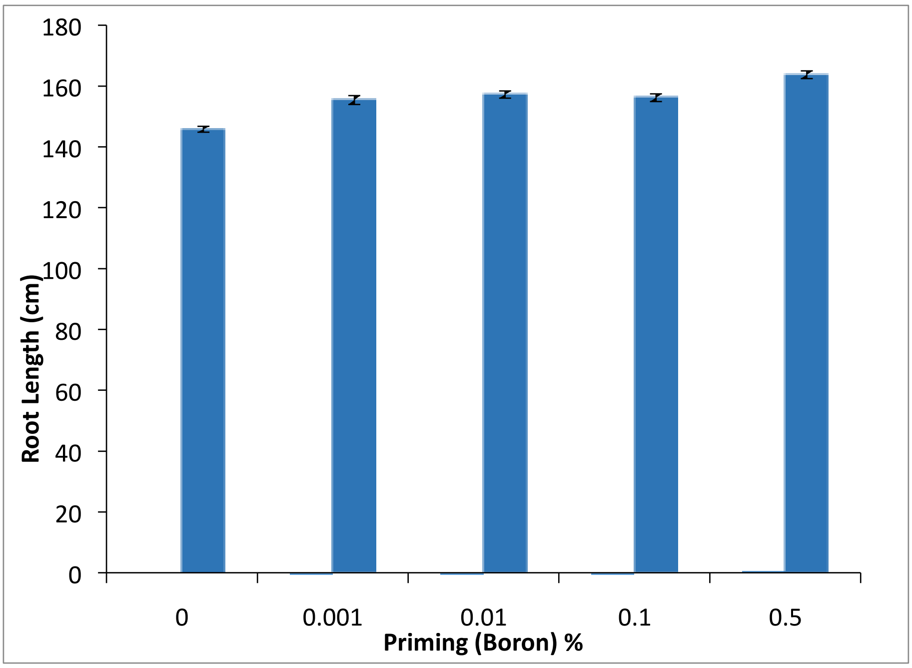

Fig 1: Showing the effect of priming with boric acid (0%, 0.001%, 0.01%, 0.1%, 0.5%) on the root length of *Helianthus annuus* L. var Agsun 5264. Means are statistically different at P ≤ 0.05 (grouped by ANOVA One Way and Duncan's Multiple Range Test)

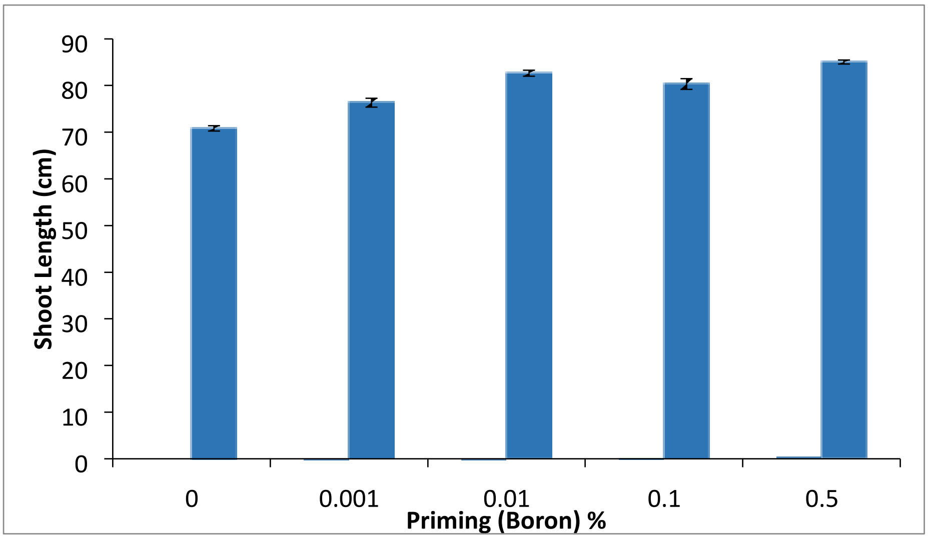

Fig 2: Showing the effect of priming with boric acid (0%, 0.001%, 0.01%, 0.1%, 0.5%) on the shoot length of *Helianthus annuus* L. var Agsun 5264. Means are statistically different at P ≤ 0.05 (grouped by ANOVA One Way and Duncan's Multiple Range Test)

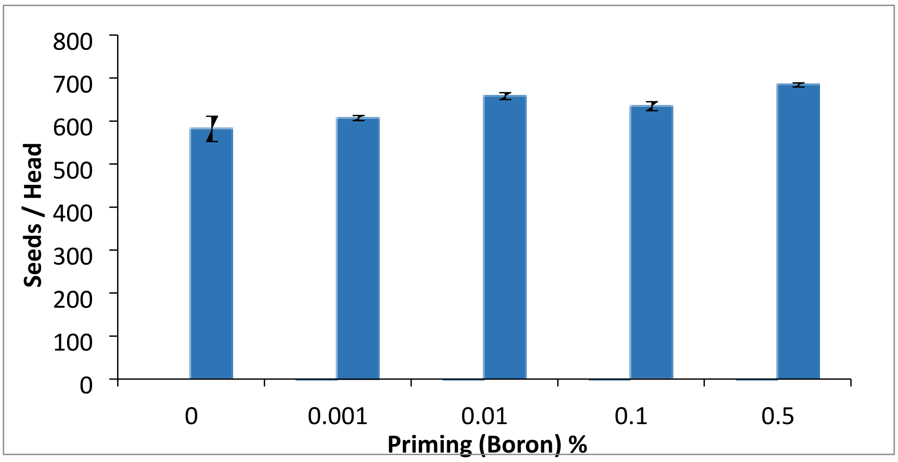

Fig 3: Showing the effect of priming with boric acid (0%, 0.001%, 0.01%, 0.1%, and 0.5%) on the no. of seeds/head of *Helianthus annuus* L. var Agsun 5264. Means are statistically different at P ≤ 0.05 (grouped by ANOVA One Way and Duncan's Multiple Range Test)

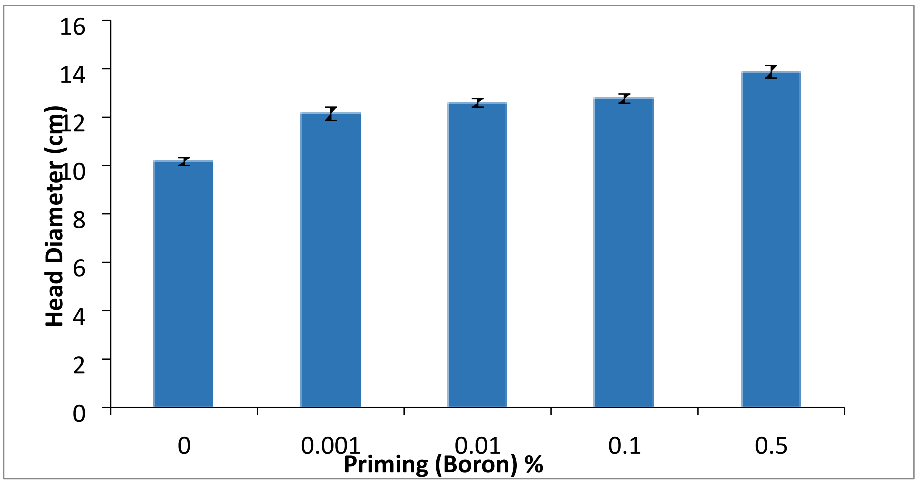

Fig 4: Showing the effect of priming with boric acid (0%, 0.001%, 0.01%, 0.1%, 0.5%) on the head diameter of *Helianthus annuus* L. var Agsun 5264. Means are statistically different at P ≤ 0.05 (grouped by ANOVA One Way and Duncan's Multiple Range Test)

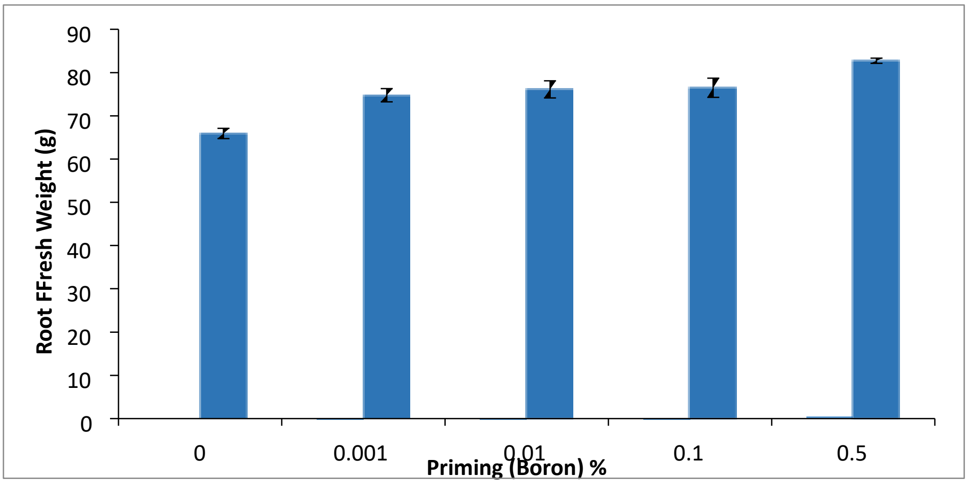

Fig 5: Showing the effect of priming with boric acid (0%, 0.001%, 0.01%, 0.1%, 0.5%) on the root fresh weight of *Helianthus annuus* L. var Agsun 5264. Means are statistically different at P ≤ 0.05 (grouped by ANOVA One Way and Duncan's Multiple Range Test)

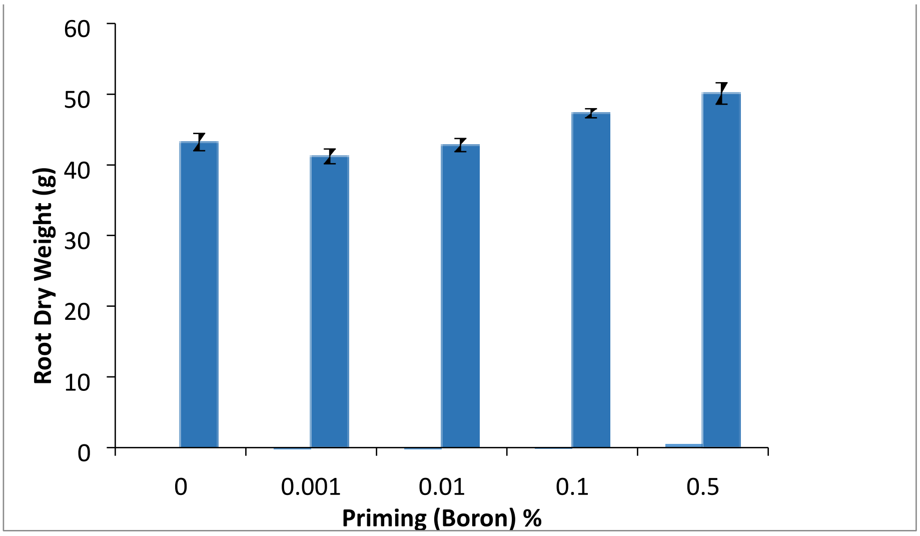

Fig 6: Showing the effect of priming with boric acid (0%, 0.001%, 0.01%, 0.1%, 0.5%) on the root dry weight of *Helianthus annuus* L. var Agsun 5264. Means are statistically different at P ≤ 0.05 (grouped by ANOVA One Way and Duncan's Multiple Range Test)

Antioxidant, TPC and TFC

The impact of treatments on DPPH% activity, TPC and TFC were given in Table 1. The results indicated that 0.5% use of boric acid significantly improved the DPPH% activity, TPC and TFC values.

Table 1: Results of DPPH activity, TPC and TFC with respect to treatments

Treatment	DPPH Scavenging %	TPC (mg GAE/gE)	TFC (mg RE/gE)
0% Boric acid	11.10±0.05DE	9.54±0.10DE	2.13±0.01D
0.001% Boric acid	12.05±0.02D	11.83±0.13CD	2.40±0.01D
0.01% Boric acid	17.36±0.08C	16.28±0.25C	4.17±0.02C
0.1% Boric acid	44.51±1.05B	22.15±1.15B	6.82±0.02B
0.5% Boric acid	56.78±2.12A	26.41±1.11A	9.10±0.04A

The values having different letters were statistically significant.

Discussion

In this study, a consistent increase in growth was observed by an increase in concentration of boron used for priming i.e, from 0.001 to 0.5%. No germination or growth was observed by Rehman et al., (2012) in rice seeds after priming with 0.5% boric acid solution. Better results were drawn from seeds primed with higher concentration of boron i.e., 0.1 and 0.5%. Significant reduction in growth and germination attributes was observed by Ajouri et al., (2004) in barley by applying 0.01M boric acid or any concentration below 0.01M. Nonetheless, germination was observed to be improved in papaya when primed with 2mg/L boron for 6 hours pre-sowing (Deb et al., 2010). According to Shah et al., (2011), germination rate, number of leaves, plant length, number of spikes per plant and spike length in rice plants were greatly enhanced by priming treatments with boron. Soaking for 24 hours produced best results as compared to unprimed seeds. Shoot length and root length in plants were visibly improved in plants at higher concentrations of boron priming. The positive effect of boron on germination of seeds is related to the increment in activity of glutathione peroxidase and glutathioneascorbate cycle activation (Sajedi, 2015). Seed establishment is shown to be slowed down comparatively at lower concentrations of boron as reported by Mershikari, (2012). The growth and maintenance of leaves could better be understood by the eminent role of boron in synthesis of cell walls (Hu and Brown 1994). Two molecules of rhamnogalacturonan II (RG-II) are cross linked to each other by a borate bridge formed by boron and this linkage imparts stability to the cellular matrix. Thus, in the presence of boron, stability and rate of synthesis of cross-linkage increases, which is essential for the growth of plants (O'Neill et al., 2004). The boron application in terms of boric acid as parent compound not only improved growth yield parameters but also enhanced the antioxidant response, TPC and TFC values. The high DPPH activity for extract where 0.5% boric acid was applied was most probably due to the high phenolic and flavonoid contents. The antioxidant properties are directly linked with pharmacologically active ingredients like phenolics and flavonoids (Wadhaya et al., 2023). The relatively higher level of phenolics and flavonoids in sunflower plant was a sign for its potential use in nutraceutical development and functional food perspectives. The boron was also a helping nutrient to induce therapeutic responsive credentials in under study sunflower cultivar which is an additional benefit of phytotherapeutic and socio-economic nature.

Conclusion

The present study revealed that plants primed with 0.1% and 0.5% concentrations of boric acid were better in growth and physiological perspectives. The 0.001% and 0.01% priming do not show any eminent positive effect on the growth and physiological characteristics of plants. Yield parameters were increased at higher concentrations of boron. For better growth and yield, sunflower seeds can be primed with 0.1% or 0.5% solutions of boric acid to enhance the plant growth and hence yield. The most probable reason behind the improvement in yield parameters was the role of boron in plant metabolism to encounter the stress and consequent increase in plant growth. The 0.1% and 0.5% concentrations of boric acid used for priming also improved the antioxidant activity, phenolic and flavonoid contents to a significant level making the plant material usable commodity for therapeutic and functional food development.

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