Turkish Journal of Agriculture - Food Science and Technology, 10(10): 1966-1971, 2022
DOI: https://doi.org/10.24925/turjaf.v10i10.1966-1971.5432

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Exploring Zinc and Boron Chemo-Priming Effects on Low-Vigour Seed Germination and Seedling Establishment of Sunflower (*Helianthus annuus* L.)

Negar Ebrahim Pour Mokhtari[1,a], Ferhat Kızılgeçi[2,b,*], Raees Ahmed[3,c], Muhammad Aamir Iqbal[4,d]

[1]*Department of Animal and Plant Production, Islahiye Vocational School, Gaziantep University, 27010 Gaziantep, Türkiye*
[2]*Department of Animal and Plant Production, Kızıltepe Vocational School, Mardin Artuklu University, 47500 Mardin, Türkiye*
[3]*Department of Plant Pathology, Faculty of Agriculture, University of Poonch Rawalakot, Pakistan*
[4]*Department of Agronomy, Faculty of Agriculture, University of Poonch Rawalakot, Pakistan*
[*]*Corresponding author*

A R T I C L E I N F O

Research Article

Received : 11/08/2022
Accepted : 23/09/2022

Keywords:
Seed priming
Micro-nutrients
Root length
Seed invigoration
Germination

A B S T R A C T

Poor germination and suboptimal seedling growth constitute as prime factors in lowering the achene yield and seed oil content of sunflower (*Helianthus annuus* L.), while chemo-priming with micronutrients might serve as a biological viable strategy provided source and dose optimization are performed. A trial was executed to appraise the comparative impact of seed priming with different doses of micronutrients like boron (B) and zinc (Zn) on sunflower germination and seedling growth traits of sunflower. The experiment was comprised of six treatments of B (0, 0.2, 0.4, 0.8, 1.6, 3.20 ppm) and Zn (0, 4, 8, 12, 16 and 20 mM) each, while seed germination, root and seedling growth related traits were taken as experimental variables. The results revealed that all treatments remained ineffective regarding seed germination and seed vigour of sunflower, while B and Zn doses of 0.2 ppm and 12 mM respectively, enhanced seedling emergence rate and vigour. The Zn (8 mM) significantly improved root length along with their fresh and dry weights, while all doses of B imparted antagonistic effects on root attributes. Likewise, Zn (8 mM) remained superior for shoot length, fresh and dry weights, while higher doses of B remained contra-productive for shoot growth of sunflower.

[a] n_mokhtary@yahoo.com https://orcid.org/0000-0002-2307-5756
[b] ferhatkizilgeci@artuklu.edu.tr http://orcid.org/0000-0002-7884-5463
[c] fergokibo@hotmail.com https://orcid.org/0000-0002-9673-8610
[d] muhammadaamir@upr.edu.pk https://orcid.org/0000-0003-2701-0551

Introduction

The changing climate scenario, declining agricultural lands, skyrocketing human population and decreasing farm income are necessitating multiplying food crops yield in order to ensure food security (Qin and Liu, 2010; Choudhary et al., 2021). Globally, vegetable oil demand is mounting with each passing year, while competition with staple crops is putting oil seed crops on disadvantageous side in farmer's perspectives (Abbas et al., 2015; Iqbal, 2015a). Many countries are importing edible oil which has jeopardized food and nutritional security owing to disrupted supply chain in the wake of disasters like COVID-19 (Iqbal, 2015b; Vital et al., 2017) and Türkiye is no exception to it (Elicin et al., 2022). Thus, this scenario demands more concrete efforts domestically in order to increase vegetable oil production to reduce the import bill and impart sustainability to edible oil supply chain. Among oil seed crops, sunflower (*Helianthus annuus* L.) constitutes vital position for being the rich source of comparatively healthier edible oil (Chowdhury et al., 2010; Farzanian et al., 2010; Faisal et al., 2020). It belongs to the Compositae (Asteraceae) family while its genus id helianthus (Cechen et al., 2015; Vital et al., 2017). It contains abundant oil content in the range of 40-47% along with being rich in protein content (20-27%) (Ahmad and Jabeen, 2009; Andrew et al., 2013). In addition, sunflower oil is also composed of other vital constituents such as calcium and numerous vitamins including A, D and E (Diovisalvi et al., 2018; Primo et al., 2018). Interestingly, sunflower has been cultivated around the world and hold potential to be incorporated in diversified cropping systems (rainfed and irrigated) in Türkiye. However, its yield has remained far abject than the genetic potential of elite genotypes under cultivation in various part of Türkiye. The prime reason of meagre sunflower oil production is suboptimal achene yield caused by low germination and restricted growth and development of crop plants (Iqbal et al., 2015; Li et al., 2017). There is dire need to boost germination and seedling growth of sunflower plants that can result in higher achene yield and one of the biologically viable ways could chemo-priming with micro-nutrients especially boron (B) and zinc (Zn). The quality of seed especially seed viability and vigour impart profound influences on the germination, seedling establishment, achene yield and seed oil content of sunflower. In addition, healthy plants having robust root systems tend to mobilize limiting concentration of nutrients in the soil and a vigorous early seedling establishment was found to be associated with significantly higher yield of crops (Harris et al., 2000). Seed priming (seed soaking in water and drying) effectively induces numerous biochemical alterations to initiate germination process by breaking seed dormancy, hydrolysis of inhibitors, seed imbibition (seed swelling after moisture absorption) and activation of various types of enzymes (Harris et al., 2000; Afzal et al., 2013; Iqbal et al., 2015b; Ebrahim Pour Mokhtari and Emeklier 2018a; Ebrahim Pour Mokhtari and Kızılgeçi, 2021). In addition, primed seeds could rapidly attain imbibition which leads to seed metabolism revival, and ultimately germination rate increased through reduction in the inherent physiological heterogeneity (Rowse, 1995; Ebrahim Pour Mokhtari and Emeklier, 2018b). Moreover, seed priming holds potential to improve seedling establishment that enables plants to effectively endure drought tolerance, incidence of pest, owing to rapid development of root network (Mussa et al., 1999; Harris et al., 2000).

The micronutrients like Zn and B tend to trigger photosynthesis along with contributing to enzymatic systems activation for biosynthesis of protein, fats etc. (Tahir et al., 2014; Iqbal et al., 2019). The B application in minute concentration tends to stimulate germination, pollen tubes development that promoted fertilization and seed set in sunflower (Sher et al., 2015). In addition, water relations maintenance, sugars translocation and pollen viability along with cation-anion balancing are prominent roles of B in crop plants (Asad et al., 2003). Debbarma et al. (2017) inferred significant increment in seed yield of sunflower (1.4 t ha^{-1}) owing to B triggered vegetative growth and assimilates partitioning towards head which promoted head weight (Anjum et al., 2015). Similarly, zinc (Zn) applied as seed priming agent or through foliage feeding improved vegetative growth and achene yield and oil content (Babaeian et al., 2011; Brunetto et al., 2018).

To the best of our study, research findings are scant pertaining to the influence of varying doses of Zn and B chemo-priming on sunflower germination, root growth and seedling establishment. Thus, in order to bridge this research gap, this study was undertaken with hypothesis that a certain dose (s) of Zn and/or B applied as seed priming agents can perform superiorly over other in terms of germination, root and shoot growth of sunflower. The prime aim was to sort out and optimize the dose of Zn and B to be used as priming agent to boost germination and seedling establishment of low-vigour sunflower seeds.

Material and Methods

A laboratory experiment was conducted at the Variety Registration and Seed Certification Centre, Ankara, Türkiye to investigate the effect of boron and zinc micronutrients on germination and seedling establishment of sunflower. The trial was arranged in a completely randomized design with three replications. Sunflower (cv. Pioneer 64LL62) seed were obtained from Seed Gen Bank Centre, Ankara, Türkiye. Sunflower seeds were primed with six levels of boron (0 as control treatment, 0.2, 0.4, 0.8, 1.6 and 3.2 ppm) and six levels of zinc (0 as control treatment for comparison purpose along with 4, 8, 12, 16 and 20 mm) for the experiment, while deionised water was used as the control treatment. H_3BO_3 (B 17%) was used as a source of boron. Zinc ($ZnSO_4 .7H_2O$) was used as a source of zinc. Seeds surface were sterilized with 10% hydrogen peroxide for 3 min, and rinsed with deionized water for 5 min. After sterilization process, seed were soaked in the priming solution for 24 h. Then seeds were rinsed 3times with distilled water to remove excess salts from the seed coat. Then primed seeds were left in room temperature for 24 h to re-dry to their original moisture level. For each replicate, 50 primed seeds were placed in the sterile petri dish containing Whatman filter paper (90 mm-diameter) moistened with 3 mL deionised water. Petri dishes were left in a germinator at 25 ± 1°C for a germination test. The filter paper in the petri dishes were changed every 24 h to prevent inhibitor effect of leaching micronutrient as seed coat. According to ISTA rules (ISTA, 2011), germination was checked once a day for 7 days. At the end of 7-day, germination rate, germination percentage, coleoptile length, root length, seedling length, root fresh weight, root dry weight, seedling fresh weight and seedling dry weight were investigated (ISTA, 2011). In the second part of the experiment, primed seeds (four replicates of 50 seeds/lot) were sown 3 cm deep in sand in plastic seedling boxes (40×40×10 cm). Seedling boxes were placed in an incubator at 25 ± 1°C and 70% humidity. Each box was irrigated with a 30 ml of sterile water, every 2 days. The experiment was terminated 12 days after sowing. According to ISTA rules (ISTA, 2011) the number of emerged seedlings was counted at 7 and 12 days, and normal seedling percentages were calculated.

Statistical Analyses

The recorded data were arranged and subjected to analysis of variance (ANOVA) using JMP 10th statistical package program to determine overall significance, while least significant difference (LSD) test was performed for estimation of significance among treatment means at 5% level of probability. The graphs were prepared using Microsoft's Excel program while manually putting significance symbols as per analyses results.

Result and Discussion

Germination Rate and Vigour

The varying doses of B had significant impact on germination parameters of sunflower. The higher doses of B imparted adverse effects on germination and as the maximum germination was recorded for control treatment (Figure 1).

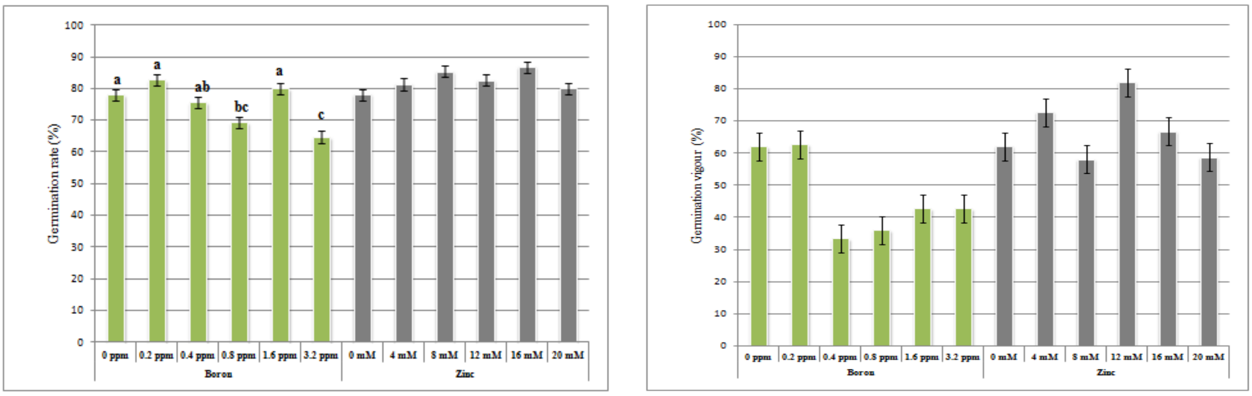

Figure 1. Effect of varying doses of B and Zn chemo-priming on seed germination and vigour of sunflower.

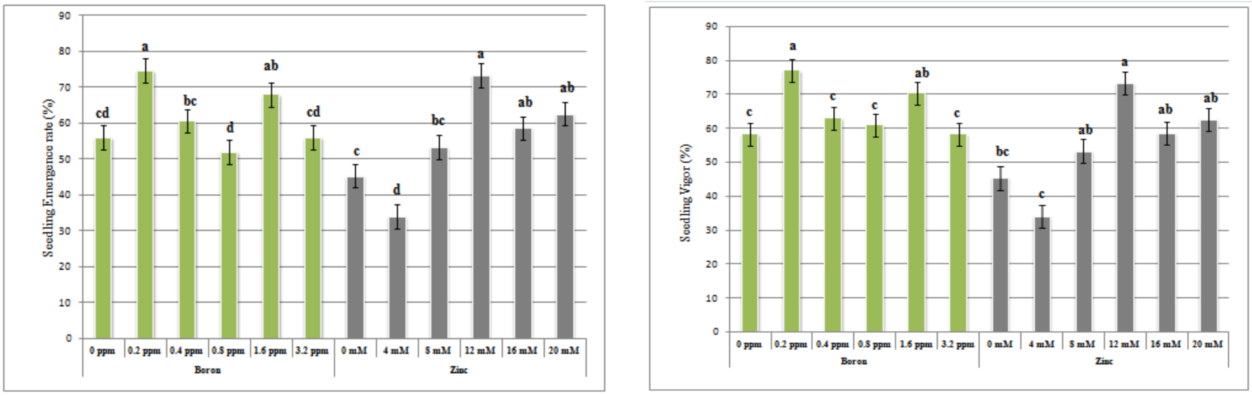

Figure 2. Effect of varying doses of B and Zn chemo-priming on seedling emergence and vigour of sunflower.

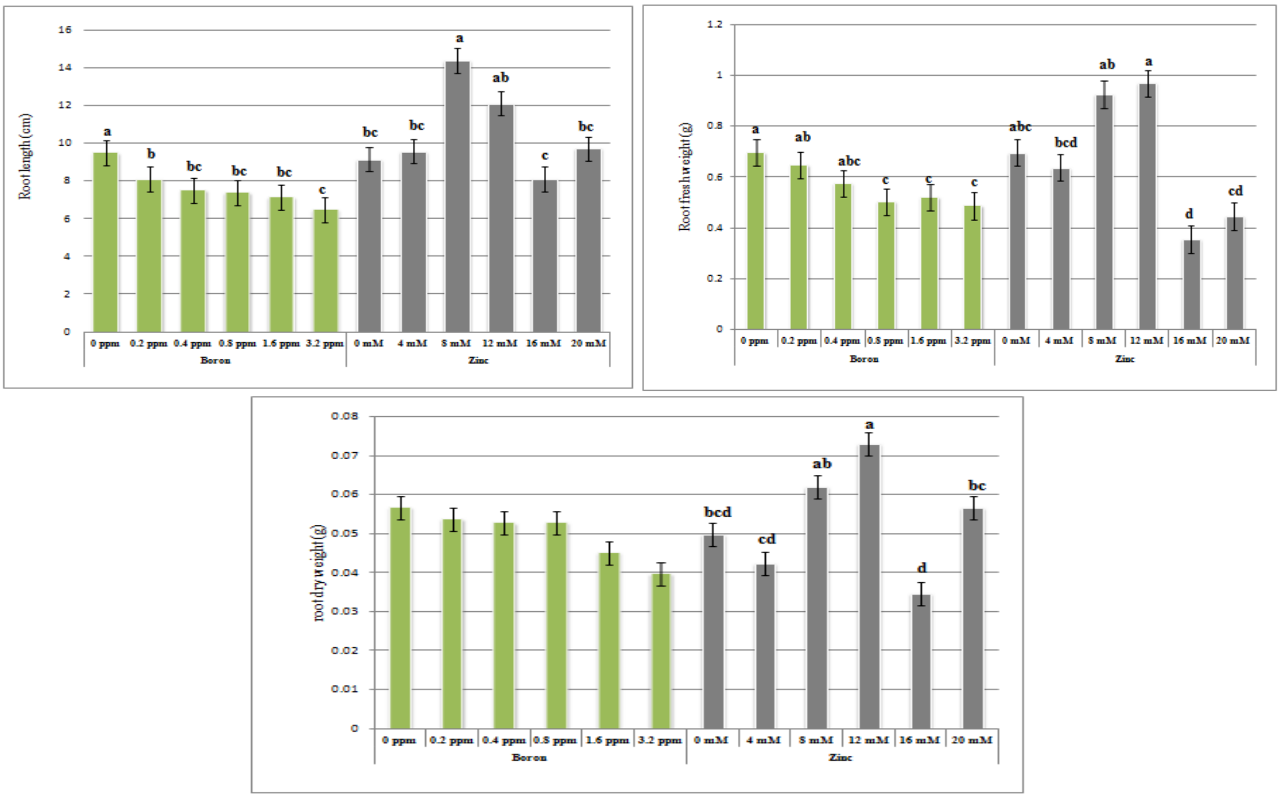

Figure 3. Effect of varying doses of B and Zn chemo-priming on root length, fresh and dry weights of sunflower.

In contrast, all doses of Zn remained ineffective in imparting any positive or negative influence on germination rate of sunflower. Likewise, all treatments entailing varying doses of B and Zn could not exert any impact germination vigour of low-vigour seers of sunflower. Our findings are in contradiction with previous reports whereby un-primed seeds resulted in poor crop stands that left hefty gaps among the plant canopies, that were abruptly filled by robustly and vigorously growing weeds especially at the onset of rainy season.

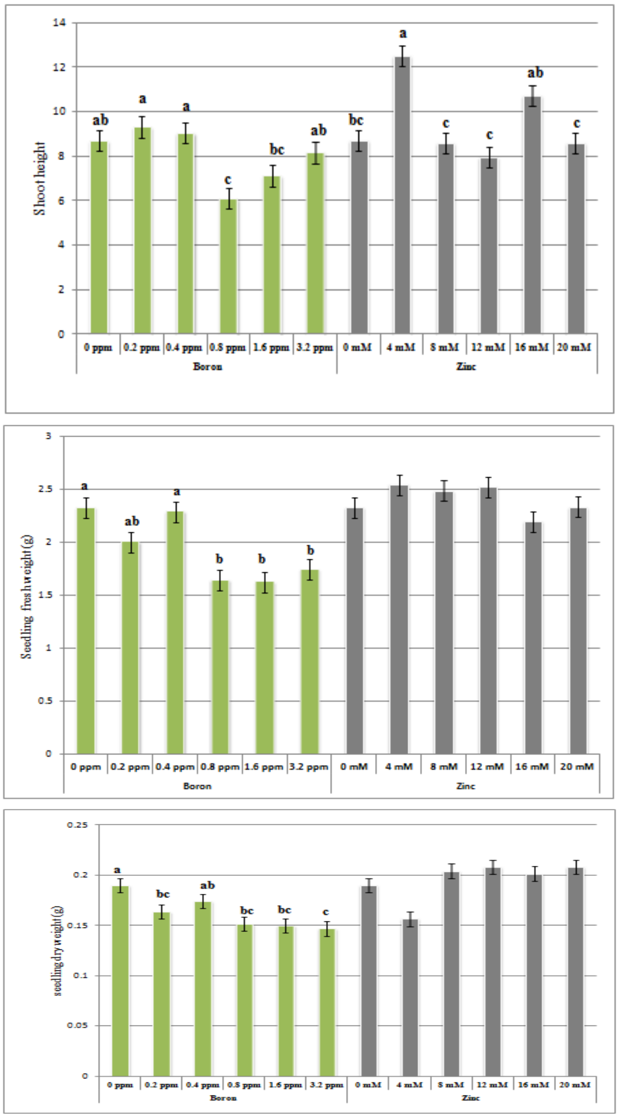

Figure 4. Effect of varying doses of B and Zn chemo-priming on shoot/seedling length/height, fresh and dry weights of sunflower.

Thus, sowing of un-primed seeds recorded intense crop-weed competition for agro-environmental resources such as moisture, light, and limiting (Kropff and van Laar, 1993). It was also suggested that micronutrients especially Zn and B hold potential to increase germination rate through acceleration of germination process that led to optimal crop establishment in different crops such as rice and maize and impact of micronutrients was even more pronounced in Zn deficient soils (Harris et al., 1999; Slaton et al., 2001; Ajouri et al., 2004). However, in our study, it is perhaps smaller duration of priming that could be attributed to non-significant impact of Zn and B on seed germination rate and vigour of sunflower.

Seedling Emergence Rate and Vigour

All the treatments recorded significant influences on seedling parameters (seedling emergence rate and seedling vigour). The maximum seedling emergence rate was recorded for B dose of 0.2 ppm that was statistically at par to 1.6 ppm dose (Figure 2). Among Zn doses, the maximum performance was recorded for 12 mM that performed comparatively at par to higher doses of 16 and 20 mM. Interestingly, lowest dose of Zn could not match the seedling emergence rate recorded by the control treatment. Likewise for seedling vigour, 0.2 ppm and 12 mM doses of B and Zn respectively remained unmatched compared to rest of treatments. In accordance with findings of our trial, Bort et al. (1998) also inferred that varying effects of micronutrient could be recorded for crop establishment and robust early growth of barley in the eastern Mediterranean region. It was also reported that exogenous application of micronutrients in safer limits through foliar feeding and chemo-priming could boost seedling emergence and their vigour that ultimately put crop plant on advantageous side in competitive environment with various exotic and indigenous weeds through higher use efficiency of farm inputs like primary nutrients and applied irrigation water (Matar et al., 1992). Previously, it has been established that Zn promotes protein synthesis and facilitates gene expression that might be attributed for improved seedling emergence and vigour of sunflower (Cakmak, 2000; Broadley et al., 2007). In addition, over 10% of plant proteins in all biological systems direly need Zn in order to maintain their structural as well as functional integrity (Andreini et al., 2006). During seed germination of sunflower, reactive oxygen species (ROS) were synthesized (Bailly et al., 2002; Qin and Liu, 2010) while Zn remained instrumental in detoxifying the ROS in plant cells (Cakmak, 2000; Broadley et al., 2007; Bilen et al., 2011; Jabeen and Ahmad, 2011).

Root Length, Fresh and Dry Weights

The results revealed that chemo-priming treatments entailing varying doses of Zn and B had significant impact on sunflower roots length and their fresh and dry weights (Figure 3). As far as root length and fresh weight were concerned, antagonistic effect of all doses of B was recorded as significant decline in both length and fresh weight with respect to increasing doses of B was observed. The control treatment remained superior compared to chemo-priming with B. However, Zn chemo-priming using the dose of 8 mM surpassed rest of treatments in terms of sunflower root length, while 12 mM dose remained statistically at par to it. Interestingly, higher doses of Zn had adverse effect on root length of sunflower. In addition, seed priming with 12mM dose of Zn recorded the maximum fresh weight that was statistically comparable to dose of 8 mM, while higher doses (16 and 20 mM) of Zn remained contra-productive for fresh weight of sunflower. All doses of B remained insignificant for root dry weight, on the other hand, Zn doses of 8 and 12 mM remained effective in boosting the root dry weight of sunflower. We interpret these findings as micronutrients especially Zn contributes in triggering the activation of in cell division that resulted in higher root length along with maximum fresh and dry weights of sunflower roots. These findings corroborate with those of Iqbal et al. (2015) who inferred that micronutrients tend to trigger enzymes activation which boosted root length and their weight. It was also suggested that Zn-priming significantly enhanced Zn content in seed coat and husk and thus could be more effective strategy to rectify Zn deficiency compared to foliage or soil application of Zn, B and other micronutrients (Gitte et al., 2005).

Shoot Length, Fresh and Dry Weights

The recorded findings explored that chemo-priming with different doses of Zn and B significantly affected the shoot attributes of sunflower. The lower doses of B recorded higher shoot length and shoot fresh weight that were statistically at par to control treatment (Figure 4). The higher doses of B especially 1.6 and 3.2 ppm imparted adverse influence on shoot traits. In contrast, Zn doses of 8 and 12 mM remained instrumental in recoding the maximum shoot length and fresh weight, while Zn dose increment resulted in proportionate decrease of shoot attributes. Regarding shoot dry weight, all doses of B especially 1.6 and 3.2 ppm caused significant decline compared to control treatment. In contrast, Zn treatment in varying doses remained ineffective in imparting any positive or negative effect on shoot dry weight of sunflower. These findings are in concurrence with a number of previous researchers whereby micronutrients seed dressing or coating recorded significant effects on shoot length and its weight under micronutrient deficient soils. It was also recommended that chemo-priming with Zn and B could serve as highly economical approach as well as convenient strategy for attaining robust shoot growth of field crops (Adhikari and Rattan, 2000; Kapila and Shivay, 2008).

Conclusions

The findings of this trial remained in accordance with the postulated research hypothesis as varying doses of Zn and B had significant influence on germination and seedling parameters of sunflower. To conclude with, Zn remained superıor as a priming agent especially the dose of 5 mM which resulted in significantly higher root and shoot lengths along with their fresh and dry weights, while higher doses of Zn imparted adverse effects on root and shoot traits under study. All treatments could not affect germination rate and seed vigour, however B (0.2 ppm) and Zn (12 mM) boosted seedling emergence rate and vigour. It is recommended to use these findings as baseline to conduct further in-depth field studies entailing varying doses of Zn and B and thereafter chemo-priming with these micro-nutrients might be recommended to sunflower growers for general adoption in Türkiye and areas having similar agro-climatic conditions worldwide.

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