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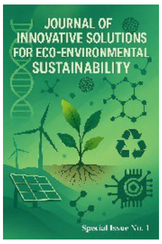

Research Article

Combined Approaches of Biochar and ZnO Nanoparticle-Based Nanopriming for Enhancement of Wheat (*Triticum aestivum* L.) Seed Germination Indices Under Salinity Stress

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ABSTRACT

Soil salinization is a significant ecological issue that reduces soil fertility, inhibits plant growth, and decreases crop productivity. The impact of biochar and ZnO nanoparticle (ZnO-NP) based nanopriming on early seedling growth in wheat under salinity stress was investigated. Experimental trials were conducted in (i) a soil-based bioassay under greenhouse conditions with 1.3% (w/w) biochar, (ii) a greenhouse seed bioassay to assess the effects of ZnO-NPs, and (iii) at different concentrations of biochar and ZnO-NPs (50 mg/L and 100 mg/L), either alone or in combination, to determine which treatment was most effective under varying salinity levels (low, medium, and high) for wheat genotypes (V1: Gohar and V2: Van). Results showed that germination rate (GR), germination percentage (GP), mean daily germination (MDG), germination vigour index (GVI), stress tolerance indices including PI and GSTI, as well as seed content, were significantly reduced by increasing salinity levels. The V1 (Gohar) genotype showed that the combined application of 1.3% biochar and 50 mg/L ZnO-NPs was most beneficial for seed germination; in contrast, the V2 ("Van") genotype responded best to individual treatments of either 1.3% biochar or ZnO-NPs at 50 or 100 mg/L, depending on the salinity level. Overall, the V2 genotype exhibited the highest salinity stress tolerance. These findings demonstrate that biochar and ZnO nanoparticles, applied separately or together, have great potential for improving wheat seedling establishment in saline environments. The research highlights the emerging role of biochar and pre-sowing nanopriming in the agro-industry for soil enhancement, stress management, and crop productivity.

1. Introduction

Climate change affects ecosystem processes, increasing biotic and abiotic stress. Salinity is one of the main abiotic stresses that dramatically interferes with the agroeconomy [1]. Soil salinity is becoming increasingly alarming in food production globally via its influence on the physiochemical processes such as photosynthesis, antioxidants activity and ion homeostasis [1]. The salinity elevates the levels of sodium and chloride ions that accumulate in plants to disrupt the physiological processes and affect overaccumulation of sodium ion (Na^+) that decreases the nitrogen (N) level and potassium ion (K^+) concentration in plant tissues by suppression of proper mineral ion carrier proteins associated with their absorptions [2]. Salt negatively affects plant growth and under extreme conditions can cause plants to die by nutrient imbalances, membrane wounding and enzyme inactivation by the combined effects of ionic and osmotic stress [3]. Germination is one of the most important processes which ensures plants establishment in agriculture, it is also essential for crop quality [4–6]. The rapid growth of seedlings allows for the quick expansion of leaves and roots, which may result from a process involving nutrient uptake, translocation within the transpiration stream, and biomass production [4,7,8]. Slow germination can cause young seedlings, which are among the most vulnerable stages of plant growth, to be exposed to a wide range of stress conditions or pathogens in the environment, leading to a decline in vigour and yield, which results in economic losses for farmers [9].

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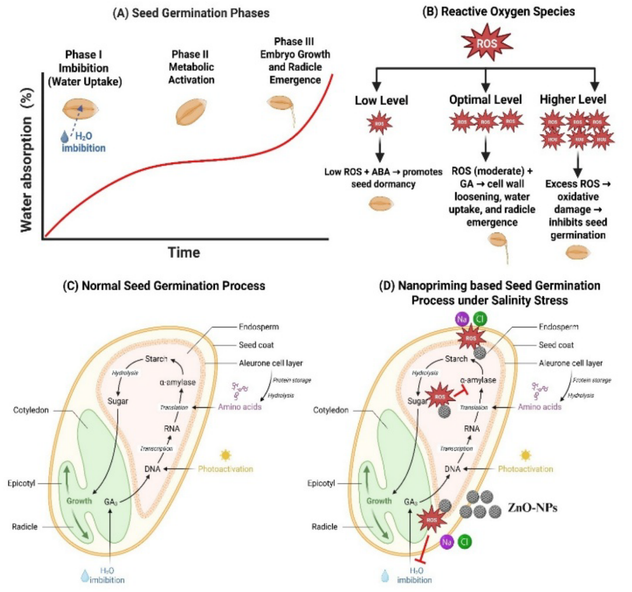

Fig. 1. This figure show that (A) seed germination phases, the role of (B) ROS in seed germination, the process, and the role of (C-D) ZnO-NPs in seed priming to alleviate salinity stress and promote seed germination.

The germination process of the seed was generally divided into three stages as described in Figure 1A [10]. It starts with imbibition (phase I), during which the rapid water imbibition leads to basal metabolism of seeds, such as the transcription, protein synthesis and mitochondrial functioning. In phase II (activation or lag phase), limited water uptake occurs with hyperactivity of metabolism; enzymes involved in the mobilization of reserves and embryo development, such as amylases, endoxylanase and phytase are synthesized (Fig 1 C). Phase III: seeds show once more fast water uptake, and embryo growth ends with radicle protrusion [10,11]. The seed germination is precisely controlled by signaling molecules, for example reactive oxygens species (ROS) and phytohormones (Fig 1B) [12–14]. Apoplastic ROS production is closely linked to cell wall relaxation, permitting water influx and subsequent cell expansion [12,14]. The abscisic acid (ABA) and gibberellins (GA) act antagonistically to establish or break seed germination whereas auxins might also have a role in maintenance of seed dormancy [12]. The ROS-induced process control gene expression and phytohormone signalling, and abscisic acid, gibberellins, auxins, and ethylene homeostasis, which modulate cellular processes associated with seed germination [12,13]. But oxidative damage due to excessive production of ROS has negative effects on seed germination [12]. To ensure that germination is completed, ROS levels must be controlled both spatially and temporally within the defined oxidative window [12].

Wheat (*Triticum aestivum* L.) is a major staple food in many areas of the world, especially in developing countries [15]. It fulfills 40% of the world food requirements and contains high protein and carbohydrate content [16,17]. Approximately 80% of the global crops are winter wheat grown in the autumn when weather conditions range between 0 and 5 °C, since seedlings need a chilling period at vegetative stage [18]. In South Asia or North Africa, spring wheat is planted in the spring and harvested late summer or fall [18]. The harvest of 2025 winter crops in Armenia including mainly wheat start in the Ararat Valley from July and the 2025 spring crop including barley also began to be harvested since July [19]. Near-average precipitation levels and temperatures since May, together with sufficient availability of irrigation water, make a favorable impact on vegetation conditions throughout Armenia, specifically in parts of the western Armavir and Aragatsotn provinces where some 20% of annual wheat output is harvested [19]. As salinity stress also a serious problem in Armenia which affected the cultivation of wheat crop that created food security problem in future [20].

Seed priming is a water-based process that hydrates seeds in solution. The seed is then allowed to be hydrated for a particular period of time under defined environmental conditions and subsequently dried after such process, so that germination starts, but while the seed is leaning towards germination [11,21]. The priming agents can stimulate the metabolism that usually occurs in the early stages of germination (‘pre-germinative metabolism’), which then speeds up germination and emergence [21]. Pre-germination seeds also show environmental stress tolerance [22]. Among these hydropriming (water), osmopriming (ethylene glycol and inorganic salts), hormonal priming and nutrients priming are some of the most frequently utilized techniques [23,24].

Nanotechnology has revolutionized agriculture through the application of NPs, including Zn, Ag, Ti, Si, Cu, and Fe which have unique properties at nanoscale (1–100 nm) from their respective bulk counterparts. These NPs have been proposed as nanofertilizer and nanopesticide to increase the nutrient uptake of crops, and crop productivity sustainably [21]. The idea of seed nano-priming, a new approach based on the use of NPs for treatment with seeds, has also been proposed as a method (Fig. 1C- D) [22]. Nanotechnology-mediated seed priming is a relatively new field that is insignificant initially (Fig 1 C-D) [23]. Zinc (Zn) is recognized as a vital micronutrient for plant and animal [24]. Zn is taken up by higher plants predominantly in the form of Zn2+. In addition, this metal is very vital for plant’s enzyme system, which works as cofactors, metal components and other regulatory factors of many enzymes [25]. Zn is a key macronutrient required for enzymes activation and protein synthesis as well as maintenance of normal metabolism [26]. Sustainable and cost-effective seed priming techniques are now being developed which utilize the small size and large surface area of ZnO-NPs for efficient nutrient uptake, as well as, energy effective quick plant growth with low environmental impacts [27]. Previous various experiments show that application of ZnO-NPs as priming agents for maize [28], pea [29], tomato [30,31], bean [32], rice [33,34], coffee [35] wheat [36] can alleviate salt stress (Fig 1 D).

Salinization of soil can be minimized in many ways, including organic matter addition. Biochar, an organic matter representative of carbon-rich charcoal, is produced from any type of biomass waste, for example, poultry litter, animal and crop residues. Biochar, generated in heat under anoxia or low oxic conditions at high temperature (pyrolysis) [37]. Plants might even grow better in biochar. Biochar can contribute to the growth of plants under salinity in two ways.

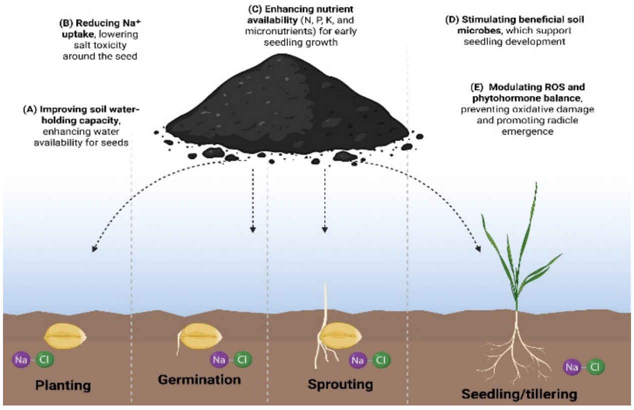

Fig. 2. Mechanism of biochar in enhancing salinity tolerance and promoting seed germination.

Table 1. Classification of soil salinity degree based on ECe (dS/m).

Salinity Level	ECe (dS/m)	Description
Control / non-saline	< 2	No negative effect on plant growth
Light (Low) salinity	2 – 4	Slight restriction for salt-sensitive crops
Medium (Moderate) salinity	4 – 8	Yield reduction in most crops
High (Severe) salinity	8 – 16	Only salt-tolerant crops can grow

The direct-action mechanism of biochar under application is growth promotion due to supplying mineral nutrients (Mg, Ca, K, S and P) to plants. However, indirect mode of action stimulates the plant growth through improved soil physiochemical and biological properties [38]. In addition, physiochemical properties of the soil such as water holding capacity, pH, CEC (cation exchange capacity), soil structure and surface area also influence [39,40]. Biochar application is important for enhancing the efficiency of plant biological processes under natural conditions, as it increases germination traits in many plants. Biochar is highly recalcitrant owing to its high aromaticity, and its properties (ash content, pH, nutrient content, and total carbon) are based on the pyrolysis temperature and feedstock for biochar production. Under the fluctuation of low and high temperature input on biochar pyrolysis, the pH value (alkaline, acidic) of biochar will change. Alkaline biochar is rich in pH, alkalinity (CaCO3), and EC, which can enhance the EC and pH of soil. Other studies further indicated that pH 7.0 (neutral) biochar increased electrical conductivity (EC) and soil pH more than acidic biochar [41]. Various studies show that the application of biochar improved the seed germination of wheat [37,42], maize [41,43] barley [44] under salinity stress.

To assess the impact of biochar and ZnO-NP–based nanopriming on early seedling growth, we conducted the analysis in three ways: (i) a soil-based bioassay was utilized to evaluate wheat seed germination under greenhouse conditions in the presence of 1.3% (w/w) biochar; (ii) a greenhouse-based nanopriming seed bioassay was performed to investigate the variability in wheat seed germination following ZnO-NP priming; and (iii) different concentrations of biochar and ZnO-NPs (50 and 100 mg/L), applied separately or together, were tested to determine the most effective treatments for both soil and seed germination responses under salinity stress. Wheat was chosen as the test crop because it is the main cereal cultivated in Armenia.

2. Materials and Methods

2.1 Seed Collections

The study focused on the seeds of the "Gohar" and "Van" genotypes of T. aestivum (soft wheat), which were obtained from the "Gyumri city selection station" in the Shirak region and the "Scientific Center of Agriculture" in Etchmiadzin. Wheat (*T. aestivum*) is of significant agricultural importance, being one of the most widely cultivated cereal crops worldwide. It belongs to the Poaceae family and is mainly used for flour production. Wheat is sensitive to various abiotic stresses, including salinity stress, which hampers its growth and yield. This trait makes it a suitable subject for study under saline conditions, particularly during the application of ZnO-NPs and biochar. Soil salinity is often classified based on the electrical conductivity of saturated paste extract (ECe, dS/m), as defined by the U.S. Department of Agriculture. Non-saline soils have ECe < 2 dS/m and usually do not pose a problem for plant growth. When ECe ranges between 2 and 4 dS/m, salinity is considered light, with sensitive plants beginning to show mild stress symptoms. Moderate salinity (4–8 dS/m) typically results in noticeable yield reductions in many crop plants. Saline soils (ECe of 8-16 dS/m) have a strong capacity to support plant growth but are limited by salt-tolerant species. Soils with ECe above 16 dS/m are highly saline, rendering most plants unable to grow. This classification system is widely used and has been scientifically supported by USDA Handbook No. 60 (Table 1) [45].

To study the effect of ZnO-NPs and biochar on the germination of wheat seeds under salinity stress conditions, we used three levels of salinity: control, low salinity, medium salinity, and high salinity.

2.2 Experimental setup

For laboratory studies, we used seeds from the "Gohar" (V1) and "Van" (V2) genotypes of soft wheat. A total of 150 seeds were collected from each genotype and subjected to treatment.

For treatment, 24 experimental variants were developed to assess the effect of biochar and ZnO-NPs on wheat seed germination under different salinity conditions (Table 2). In our treatment, we used ZnO-NPs solutions (40 nm) at concentrations of 50 mg/L and 100 mg/L to prime the seeds, while wheat husk was employed to produce biochar, which was applied at a rate of 1.3% (w/w in soil) in small containers (Fig 3) [37,44,46].

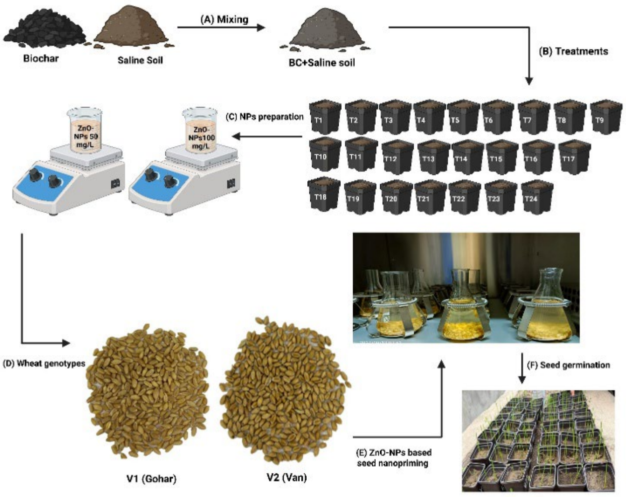

Fig. 3. Experiment treatments, layout, and set-up.

For priming, the seeds were placed in flasks with NPs solutions and then kept in a shaker-incubator (Orbital Shaker-Incubator ES-20/60) for 12 hours at 25°C. After 12 hours of incubation, five seeds were sown in each container, and all experiments continued under greenhouse conditions. Three days after sowing, seed germination was monitored and recorded over one week.

Table 2. Treatment information for seed germination experiments.

No	Description
T1	Control
T2	Light salinity
T3	Medium salinity
T4	Higher salinity
T5	BCs 1.3%
T6	BCs + Light salinity
T7	BCs + Medium salinity
T8	BCs + Higher salinity
T9	50 mg/L ZnO-NPs
T10	100 mg/L ZnO-NPs
T11	Light salinity + 50 mg/L ZnO-NPs
T12	Medium salinity + 50 mg/L ZnO-NPs
T13	Higher salinity + 50 mg/L ZnO-NPs
T14	Light salinity + 100 mg/L ZnO-NPs
T15	Medium salinity + 100 mg/L ZnO-NPs
T16	Higher salinity + 100 mg/L ZnO-NPs
T17	BCs + 50 mg/L ZnO-NPs
T18	BCs + 100 mg/L ZnO-NPs
T19	Light salinity + BCs + 50 mg/L ZnO-NPs
T20	Medium salinity + BCs + 50 mg/L ZnO-NPs
T21	Higher salinity + BCs + 50 mg/L ZnO-NPs
T22	Light salinity + BCs + 100 mg/L ZnO-NPs
T23	Medium salinity + BCs + 100 mg/L ZnO-NPs
T24	Higher salinity + BCs + 100 mg/L ZnO-NPs

2.3 Seed Germination Parameters

Within the framework of the study, germination indicators were calculated, including the germination rate (GR), germination percentage (GP), germination vigour index (GVI), mean daily germination (MDG), and germination energy (GE). Additionally, germination stress tolerance indicators under stress conditions (PI and GSTI) were also assessed.

2.3.1 Germination Indices

Seeds were regarded as germinated if a healthy green sprout was visible during the initial count. These counts were conducted every 24 hours and continued for 8 days. The potential for seed germination (GR, GP, GVI, MDG, and GE) was assessed after 8 days. Germination indices, including all seed germination indices, were calculated according to the following formula [47–52]:

(1) GR (Germination rate):

$$\mathrm{GR} = \sum_i \frac{G_i}{T_i}, \tag{1}$$

where: i – number of days from starting of germination,
G_i – number of seeds germinated,
T_i – day of scoring (Total days of experiment).

(2) Germination percentage (GP%):

$$(\mathrm{GP\%}) = \frac{\text{Total seed germinated}}{\text{Total seed planted}} \times 100. \tag{2}$$

(3) Germination Vigour Index (GVI):

$$\mathrm{GVI} = \sum_i^K \frac{n_i}{t_i}, \tag{3}$$

where: n_i – % of seeds germinated on nth days,
t_i – number of days counted from starting of experiment,
K – last day of experiment germinated seeds.

(4) Mean Daily Germination (MDG)

$$\mathrm{MDG} = \frac{\text{Final Germination percentage (GP\%)}}{\text{No of days to final germination}}. \tag{4}$$

(5) Germination Energy (GE)

$$\mathrm{GE} = \frac{\text{\% of germinated seeds on 4th day}}{\text{Total No of tested seeds}}. \tag{5}$$

2.3.2 Germination Stress Tolerance Indices

Stress conditions, such as salinity stress, induce evidence of plant response, and data on germination along with stress tolerance indices are beneficial for the evaluation of plants at the seedling level. Germination speed is denoted by the PI values, and the ability to germinate under stress is indicated by the GSTI. GSTI indices were computed according to the following equations [53–55]:

(1) PI (Promptness index)

$$\mathrm{PI} = nd_1(1.00) + nd_2(0.75) + nd_3(0.50) + nd_4(0.25), \tag{6}$$

where nd_1, nd_2, nd_3 and nd_4 are number of seeds germinated on first, second, third and fourth day.

(2) GSTI (Germination Stress Tolerance Index)

$$\mathrm{GSTI} = \frac{\text{PI of stresses seeds}}{\text{PI of control seeds}} \times 100. \tag{7}$$

2.4 Statistical Analysis

The calculation of indicators, data processing, statistical analysis, and construction of graphs were performed using Microsoft Excel 2021.

3. Results and Discussion

3.1. Effects of BCs and ZnO-NPs on Seed Germination Parameters Under Salt Stress

The combined effect of salinity levels, biochar amendment, and ZnO-NPs (priming) on germination traits is shown in Figure 4-8. The results indicated a strong tolerance response between salinity stress, biochar, and ZnO-NP treatments on seed germination tolerance indices (Table 3). However, the interactions between salinity stress, biochar, and ZnO-NPs had significant effects. The rate and percentage of seed germination varied between treatments and salinity levels.

Figure 4 shows the changes in germination rate (GR) of two soft wheat genotypes under different salinity levels and treatment conditions. As seen in figure 4 for the seeds of genotype V1, the highest GR in the non-saline environment was observed in the control sample (10.77). With increasing salinity levels, the germination rate decreased. The lowest germination rate was recorded under high salinity conditions compared to the control sample (0.78). Under greater salinity stress, the GR decreased by approximately 93% relative to the control sample in V1 genotype of wheat (Fig 4).

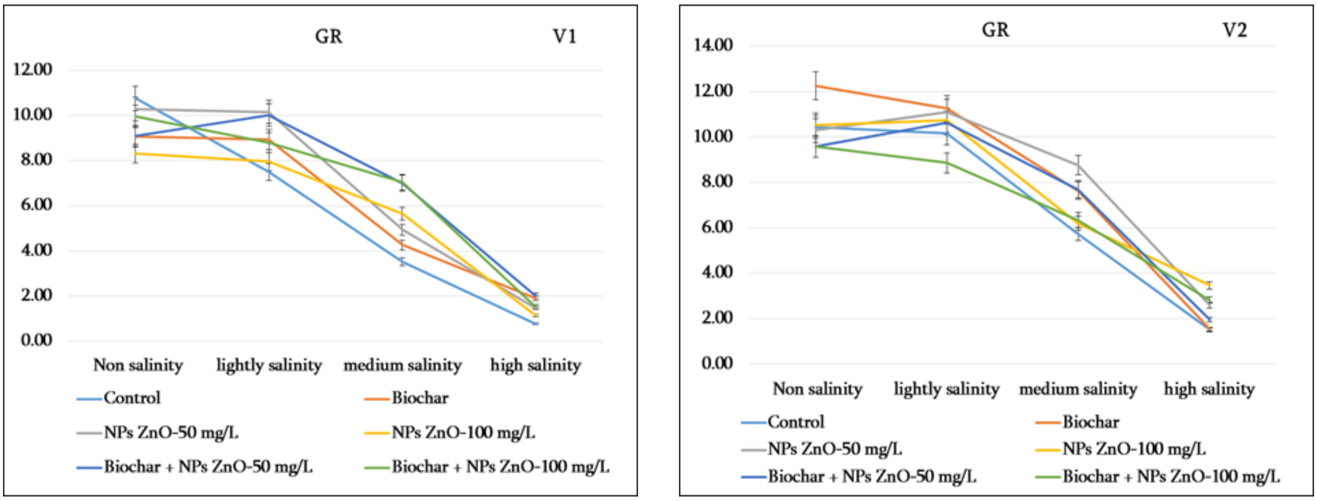

Fig. 4. Interactive effects of different combined treatments of biochar (1.3% w/w), ZnO-NPs (50-100 mg/L), and salinity levels (non, light, medium, and high) on GR of two wheat genotypes (V1; Gohar, V2; Van).

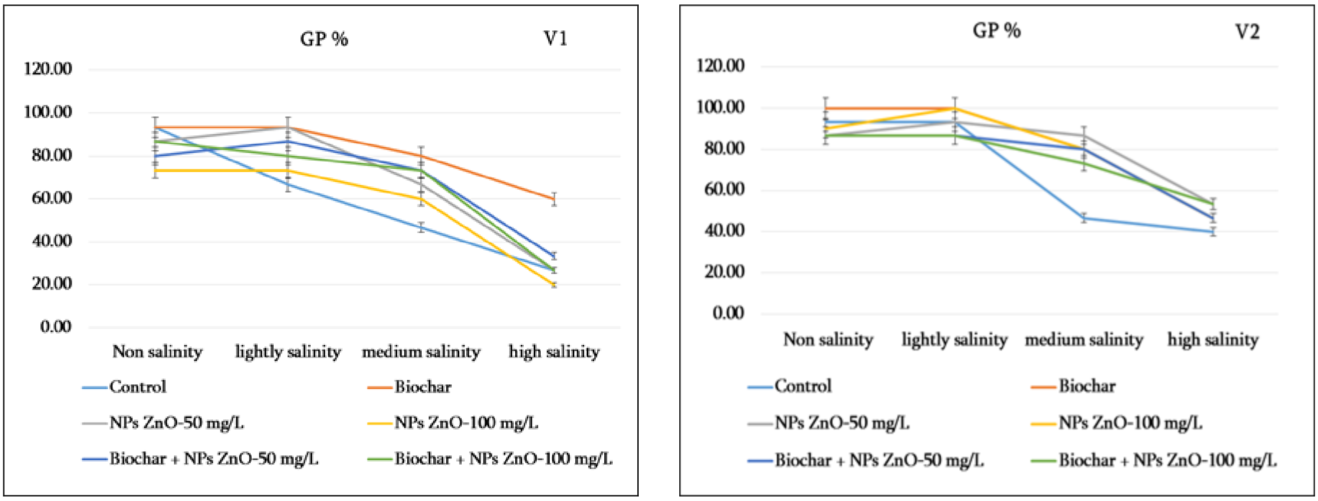

Fig. 5. The combined interactive effects of biochar (1.3% w/w), ZnO-NPs (50-100 mg/L), and different salinity levels (non, light, medium, and high) on the germination percentage (GP%) of two wheat genotypes (V1; Gohar, V2; Van) were studied.

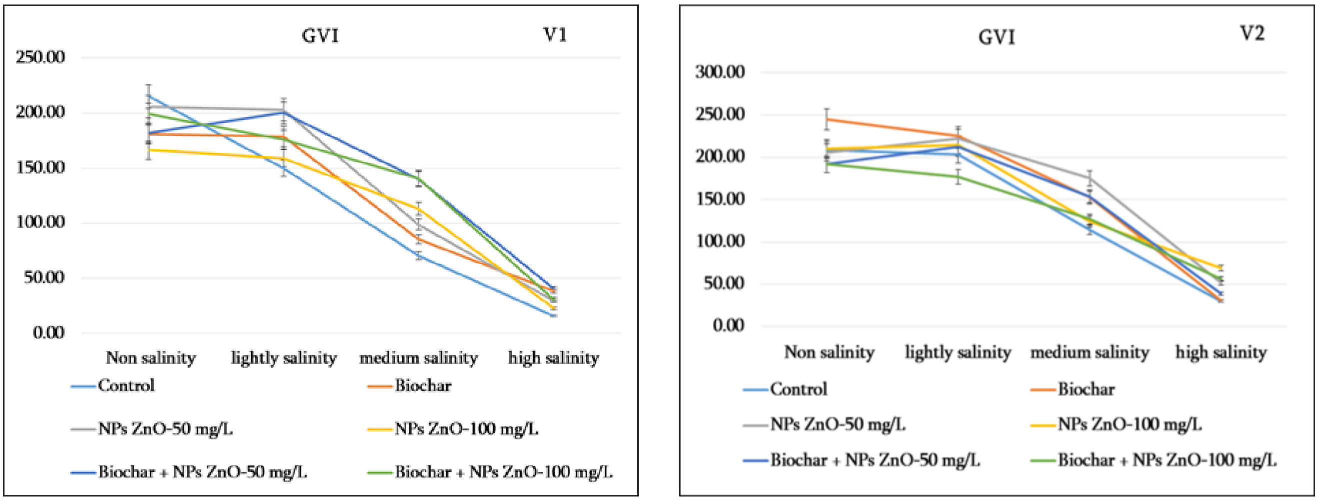

Fig. 6. The study examined the interactive effects of biochar (1.3% w/w), ZnO-NPs (50-100 mg/L), and different salinity levels (non, light, medium, and high) on the GVI of two wheat genotypes (V1; Gohar, V2; Van).

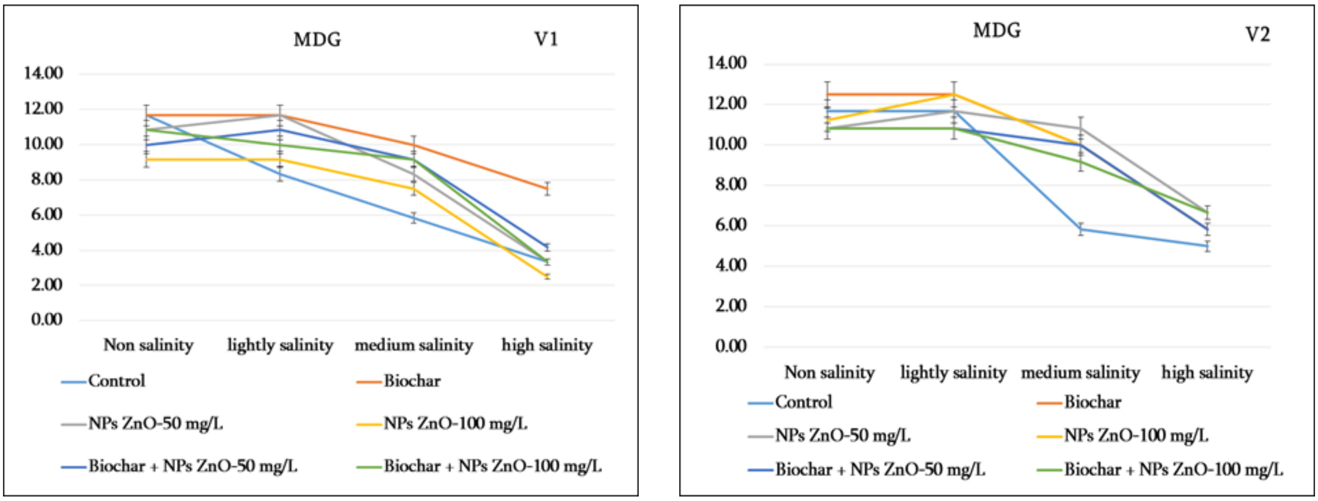

Fig. 7. The research explored biochar (1.3% w/w), ZnO-NPs (50-100 mg/L), and different salinity levels (non, light, medium, and high) interactively influenced the MDG of two wheat genotypes (V1; Gohar, V2; Van).

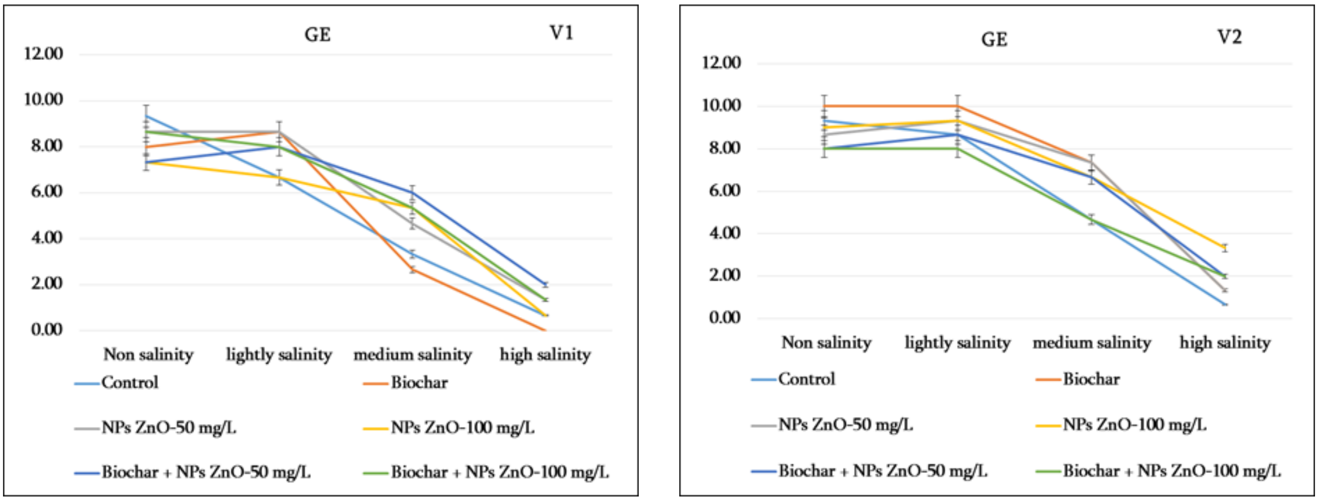

Fig. 8. The research investigated the combined effects of biochar (1.3% w/w), ZnO-NPs (50-100 mg/L), and different salinity levels (non, light, medium, and high) on the GE of two wheat genotypes, namely V1 (Gohar) and V2 (Van).

In the case of seeds belonging to genotype V2, the highest germination rate was observed under non-saline conditions with biochar treatment (12.25). The lowest germination rate was recorded under high salinity conditions compared to the control (1.50). Overall, under higher salinity, the germination rate decreased by approximately 85.6% compared to the control. According to this indicator, the most significant positive effect on the germination rate of V1 was observed with the combined application of 1.3% biochar and 50 mg/L ZnO-NPs. In the case of V2, the results varied depending on the level of salinity; the combined application of 1.3% biochar and 50-100 mg/L ZnO-NPs showed different levels of effectiveness (Fig 4).

Figure 5 shows the changes in germination percentage (GP%) of two soft wheat genotypes (V1 and V2) under varying salinity levels and treatment conditions. As seen in Figure 5, for genotype V1, the highest GP% was found in the non-saline control and with 1.3% biochar application (93.3%), while the lowest germination percentage occurred under high salinity with 100 mg/L ZnO nanoparticle treatment (20.0%). For genotype V2, the highest GP% was observed in non-saline conditions with 1.3% biochar (100%), and the lowest in the high-salinity control (40.0%). Overall, compared to the non-saline control, the GP% of seeds in the highly saline control decreased by about 71% for V1 and 57% for V2. According to figure 5, the most beneficial effect on germination (GP%) of V1 seeds was with 1.3% biochar, while for V2, the effect varied depending on salinity levels. The most positive results, depending on salinity, were recorded with 1.3% biochar and 50 or 100 mg/L ZnO-NPs.

Figure 6 shows the changes in germination vigor index (GVI) of two wheat genotypes (V1, V2) under different salinity levels and treatment conditions. As can be seen from figure 6, in the case of genotype V1, the highest germination GVI was observed in the control sample under non-saline conditions, while the lowest was recorded in the control under high salinity levels. For genotype V2, the highest GVI was observed under non-saline conditions with biochar treatment, and the lowest was recorded in the control sample under high-salinity conditions. According to figure 6, the most positive effect on the germination vigor index of genotype V1 seeds was observed with the combined application of 1.3% biochar and 50 mg/L ZnO nanoparticles. In the case of genotype V2, the results varied depending on the level of salinity: under low salinity, the most positive effect was observed with 1.3% biochar application; under moderate salinity, with 50 mg/L ZnO nanoparticle treatment; and under high salinity, with 100 mg/L ZnO nanoparticle treatment.

Figure 7 illustrates the changes in the mean daily germination (MDG) index of two wheat genotypes (V1 and V2) under various salinity levels and treatment conditions. As shown in Figure 4, for genotype V1, the highest MDG value (11.67) was recorded in the non-saline environment with both the control sample and biochar treatment, as well as in the low-salinity environment with the application of biochar and 50 mg/L ZnO nanoparticles. In the case of genotype V2, the highest MDG (12.50) was observed with biochar treatment under non-saline and low-salinity conditions and with 100 mg/L ZnO nanoparticles under low-salinity conditions. According to figure 7, the most positive effect on the mean daily germination index of genotype V1 seeds was observed with biochar application. For genotype V2, the results varied depending on the salinity level, with the most positive effects seen from individual applications or controls of 1.3% biochar, 50 mg/L, and 100 mg/L ZnO-NP treatments.

Figure 8 illustrates the changes in germination energy (GE) for two wheat genotypes under varying salinity levels and treatment conditions. From the figure, it is clear that for genotype V1, the highest germination energy (GE) was observed in the non-saline control sample (9.33). The lowest value was recorded under high salinity conditions with biochar treatment. For genotype V2, the highest germination energy (GE) appeared in non-saline and low-salinity environments with biochar treatment, reaching 10.00. The lowest value was seen under high-salinity conditions in the control sample (0.67). Additionally, Figure 6 demonstrates that for seeds of genotype V1, under low salinity conditions, the most significant positive effects on germination energy were achieved with the individual applications of 1.3% biochar and 50 mg/L ZnO nanoparticles. Under moderate and high salinity levels, the combined application of 1.3% biochar and 50 mg/L ZnO nanoparticles yielded the greatest positive outcomes. For genotype V2, depending on the salinity level, the most beneficial effects were observed with the individual applications of 1.3% biochar, 50 mg/L, and 100 mg/L ZnO nanoparticles.

Seed germination and early seedling establishment are important developmental stages in plants, as these enhance resistance to both biotic and abiotic stresses [56]. Seed priming is an applied technique to improved seed germination and seedling emergence by upregulation of metabolic activities that ensure rapid and even seedling establishment under field conditions [57]. This approach has been reported to enhance nutrient uptake, water use efficiency, and plant performance which help in increase in crop productivity and yield under salinity stress [58]. Nowadays researchers much more focus on ZnO-NPs due to the capacity to enhance the seed germination and physio-biochemical features in numerous crops under salinity stress conditions.

The results of this study showed that priming seeds with 50 and 100 mg/L ZnO-NPs significantly improved the germination rate (GR), percentage of germination (GP), germination vigor index (GVI), mean daily germination (MDG), and germination energy (GE) compared to non-primed and hydro-primed seeds. These enhancements are thought to be due to the importance of Zn in the biochemical processes that initiate germination. These changes are associated with dormancy loss, degradation of inhibitors, maximal ROS production, promotion of imbibition, and activation of germination-mediating enzymes. Similar experiment with the application of ZnO-NPs nanopriming solution of 50 and 100 mg L^{-1} on seed germination performance improvement of rapeseed (*Brassica napus* L.) under salinity stress [59]. Sharma et al. [60] found that ZnO nanoprimed 20 and 40 ppm of rice seeds showed early germination and radicle emergence than hydro-primed treatment. Comparable increases in germination and seedling vigour were also reported in wheat and maize after the application of ZnO-NPs due to higher amylase activity [61]. Kumar et al. [62] reported that 250 ppm ZnO-NPs were regarded as optimum for better germination in chickpea

Higher emergence uniformity (CUE) in the control and beneficial effects of wheat straw biochar (WSB) at moderate salinity levels suggested that WSB can reduce certain salt-mediated stresses during germination. Biochar has been used to promote seed germination under stress conditions through enhanced soil aeration and water holding capacity [63]. It was recorded that, under NaCl stress WBC improved seed germination through improved soil conditions which resulted in decreased EC and soluble Na^+ and Cl^- in the soils under salt stress. Moreover, the application of biochar increased soil cation exchange capacity, soil organic matter, humic acid, total nitrogen, and total phosphorus contents of the soil these all showed that with following mechanism biochar have improved the soil nutrient under saline condition [64]. Soil physical properties and nutrient availability being improved that also leads to improve the emergence and seedling growth under salinity stress [65]. Meanwhile, the germination rate difference of BC treatments and salinity levels is the same as that reported by Zhang et al. [66] which also found the positive effect of BC, whose physical and chemical properties can stimulate seed germination under stressful environmental conditions. The positively effects of WSB for seed germination under salt stress in various salinity levels may be related to factors that modified pH of the soil that increased nutrient availability and reducing toxic ion contents [67]. In the present study, the germination rate (GR), percentage of germination (GP), germination vigour index (GVI) mean daily germination (MDG), and germination energy (GE) increased under the influence of biochar treatments at 1.3% concentration of wheat (Fig 4-8). Similar experiment suggested that application of agricultural woody residues-based biochar at different concentrations (0% (control), 0.1%, 0.2%, 0.5%, 1%, and 2%) can improved the germination percentage (GP%), germination rate index (GRI), germination energy (GE%), fresh and dry weight (mg) of seedlings, and radicle length (mm) of cornflower (*Centaurea cyanus* L., Asteraceae) [68]. Biochar derived from corn core (CCB) was mixed with soil at varying levels (0.5, 1, 1.5, 2, 2.5, and 3% w/w) prior to sowing the seeds. Results showed that the increasing CCB application rate has neutral to beneficial effects on seed germination and seedling growth of maize [69]. In the saline soil, biochar rates (0%, 2.5%, 5% and 10%) improved seedling emergence percentage on sorghum seedling growth under salinity stress [70]. In general, addition of biochar as organic amendment in soil and seed priming with ZnO-NPs have beneficial effects on the growth of wheat seed germinations traits under salinity stress [31,59,65,66,68–73].

3.2 Effects of BCs and ZnO-NPs on Seed Germination Stress Tolerance Indices Under Salt Stress

Table 3 presents the changes in germination tolerance indices of two wheat genotypes (V1: Gohar, V2: Van) under stress conditions. As shown in the table, the GSTI values (100%) for seeds belonging to genotypes V1 and V2 were the same in variant T1, which was the control sample. In variant T5 of genotype V2, the highest GSTI value (117.19%) was observed, which was even higher than that of the control sample. This indicates that the 1.3% biochar applied in variant T5 had a positive effect on germination tolerance indices. High GSTI values were also observed in variants T6, T11, T14, and T19, where 1.3% biochar, 50 mg/L ZnO-NPs, 100 mg/L ZnO-NPs, and the combined application of biochar and 50 mg/L ZnO-NPs were applied, respectively. For genotype V1, the lowest PI and GSTI values were recorded for variant T4. For genotype V2, the lowest indices were observed for variants T4 and T8, with PI of 1.08 and GSTI of 10.16.

In many treatments the GSTI values (T5, T6, T11, T14) for genotype V2 seeds are slightly higher than those of genotype V1 seeds. At the same time, in some treatments (T18, T19), nearly similar values are observed between the two genotypes. The combined application of biochar and 50 mg/L ZnO-NPs had the most positive effect on the PI and GSTI indices of genotype V1.

Previous various studies are shows that PIs and stress tolerances index both a key parameter for analysis of application of different amendments e.g., biochar and ZnO-NPs help to mitigated the effects of various abiotic stresses [74–84].

Table 3. Changes in germination tolerance indices of two wheat genotypes (V1: Gohar, V2: Van) under stress conditions.

Treatment	V1		V2	
	PI	GSTI	PI	GSTI
T1	11,00	100,00	10,67	100,00
T2	7,67	69,70	10,25	96,09
T3	3,42	31,06	5,83	54,69
T4	0,50	4,55	1,08	10,16
T5	9,08	82,58	12,50	117,19
T6	9,08	82,58	11,50	107,81
T7	3,67	33,33	7,75	72,66
T8	1,00	9,09	1,08	10,16
T9	10,50	95,45	10,50	98,44
T10	8,50	77,27	10,75	100,78
T11	10,25	93,18	11,33	106,25
T12	4,75	43,18	8,75	82,03
T13	1,33	12,12	2,00	18,75
T14	8,00	72,73	10,92	102,34
T15	5,67	51,52	6,25	58,59
T16	0,92	8,33	3,42	32,03
T17	9,17	83,33	9,67	90,63
T18	10,17	92,42	9,67	90,63
T19	10,08	91,67	10,83	101,56
T20	6,92	62,88	7,67	71,88
T21	1,83	16,67	1,58	14,84
T22	9,00	81,82	9,00	84,38
T23	6,92	62,88	6,08	57,03
T24	1,33	12,12	2,25	21,09

5. Conclusions

Summarizing the literature data and the results of our research, it can be stated that soil salinization is a widespread and serious ecological problem. It negatively affects the physical and chemical properties of the soil and reduces soil fertility by impacting plant growth and development, which in turn leads to a decrease in agricultural yield. Currently, this issue poses a significant threat to soil degradation and crop productivity and requires considerable attention.

Based on the results of this study, the following conclusions can be drawn:

1. With the increase in salinity levels, there was a significant decrease in the germination rate (GR), germination percentage (GP), germination vigor index (GVI), mean daily germination (MDG), and germination energy (GE) of both wheat genotypes (V1 Gohar and V2 Van), as well as a reduction in stress-related germination tolerance indices (PI and GSTI).
2. The most positive effect on the germination of V1 (Gohar) genotype seeds was observed with the combined application of 1.3% biochar and 50 mg/L ZnO nanoparticles.
3. For the V2 (Van) genotype, the most effective treatments for improving seed germination were the individual applications of 1.3% biochar, 50 mg/L, and 100 mg/L ZnO nanoparticles, depending on salinity levels.
4. The results of the study indicated that the V2 (Van) genotype exhibited higher tolerance to salt stress compared to the V1 (Gohar) genotype.
5. In future it can be suggested that more emphasis should be given on the improvement of V1 (Gohar) genotype under long-term pot experiments to enhance this genotype for crop improvement. Moreover, the V1 and V2 genotypes should be assessed under combined treatment e.g., organic amendments with biochar to improve tolerance at both vegetative and reproductive stages. Such integrated strategies will provide a comprehensive understanding of salinity tolerance mechanisms and support the development of more resilient wheat cultivars.

Overall, the obtained results indicate that the combined or separate application of biochar and ZnO nanoparticles significantly improved the germination capacity of wheat seeds under salt-stress conditions. Therefore, the use of nanoparticles and biochar in agriculture is advisable to enhance crop productivity, improve the physical, chemical, and biological properties of the soil, and promote effective management of agricultural waste.

Declarations

Ethics approval and consent to participate

Not applicable.

Author Contributions

Conceptualization, K.G., A.S.; investigation, A.C., N.D.,K.G., A.S.; resources, A.C., N.D.,K.G., A.S.; writing-original draft preparation, A.C., N.D.,K.G., A.S.; writing-review and editing, A.C., N.D.,K.G., A.S.; visualization, A.C., N.D.,K.G., A.S.; . All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability Statement

The datasets generated during the current study are available from the corresponding author on reasonable request.

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