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Research Article

Influence of Selenium on Growth, Physiology, and Antioxidant Responses in Maize Varies in a Dose-Dependent Manner

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There is a very narrow margin in selenium deficiency and toxicity although it is an important element for humans, animals, and plants. Effects of selenium (Se) on the growth and physiomorphological parameters in maize were studied grown in soil spiked with sodium selenate (Na_2SeO_4) in 5 different concentrations (i.e., 0. 2.5, 5.0, 10.0, and 20.0 mg kg^{-1}). The growth of plants was affected by high Se concentration. However, maximum increases in plant height and root length were observed at low Se (2.5 mg kg^{-1}) which were 17.89 and 23.17%, respectively. At higher Se concentrations (20 mg kg^{-1}), a considerable reduction was observed in dry matter, root length, antioxidant enzymes, and other physiological parameters. The dry matter of plants was also analyzed for nutrient (Fe and Zn) concentrations. Results indicated that Se stress inhibits plant growth. Gas exchange parameters were also found to be decreased under stress conditions, but at a lower Se level (2.5 mg kg^{-1}), improvement in transpiration rate (63.46%), photosynthetic rate (47.47%), and stomatal conductance (54.55%) was observed. The reduction in growth attributes may be due to the high accumulation of Se in roots and the disturbance in gas exchange parameters. However, the principal component analysis revealed that higher Se levels were more hazardous for maize growth and physiological responses as compared to low Se levels.

1. Introduction

The problem of soil contamination with heavy metals and metalloids has risen from the continuous use of wastewater for irrigation, excessive fertilizer applications, increased industrialization, and other anthropogenic activities [1, 2]. Given the industrial revolution since the 19th century, there is an increased pressure on water resources and the overall environment that ultimately leads to soil pollution with heavy metals [3, 4]. Some of these metals are important for plant growth as micronutrients such as copper (Cu) [5]. But, the excessive concentration of these metals negatively affects the plant's morphological, physiological, and biochemical attributes [6].

For human beings and animals, selenium (Se) is set among basic nutrients [7, 8]. It is also useful at low concentrations, but at higher concentrations, it causes damage in humans, animals, and plants [9]. There is a slight gap in Se toxicity and essentiality which have both health and environmental impacts; that is why many researchers characterized the Se as a "double-edged sword" [10].

Selenium enters the environment through natural and anthropogenic sources like weathering of rocks and volcanoes [11] while the anthropogenic sources and agricultural practices that raise selenium stacking in soil comprise irrigation and utilization of organic fertilizers like manures [12].

Previous studies describe that the low Se concentration improves plant growth, but at higher concentration, reduction in growth was also observed [13]. As plants show varied responses toward Se application and its uptake, so it is revealed that toxicity of Se changes among various crops [14]. As Se plays a dual role, thus in plants, it acts as an antioxidant at smaller concentrations whereas under Se stress plants can produce reactive oxygen species (ROS) that results in deterioration of several cellular processes [14].

Maize (*Zea mays* L.) is considered an important crop worldwide [15, 16]. The annual yield of maize in Pakistan is about 3.13 million tons [17]. It is a nutritious crop as it contains carbohydrates, starch, fiber, protein, vitamins, and minerals [18]. Maize can be used as the main ingredient in different dietary products. The maize crop has also been shown to accrue various metals, for example, cadmium [19]. Maize is also considered a metal tolerant crop [20]. So, it can also be used in heavy metal phytoremediation processes, due to the extensive biomass production, metal bioaccumulation, and translocation to the aerial part as has been shown previously [21, 22].

Selenium is considered to be an important element for many plants at lower concentrations but it causes toxicity at higher concentrations [15]. Presently, the research is concentrating on revealing the particular physiological and biochemical mechanisms that cause the positive or negative effects of Se in plants. However, the effects of Se on plant growth, physiology, and antioxidant machinery under varying concentration levels remained largely unknown. In addition, an adequate supply of micronutrients is required for the proper functioning of the plant due to their importance in the plant's metabolism Therefore, it is important to explore the effects of Se on the uptake of these elements. Taking this background into account, we hypothesize that Se supplementation may cause variable effects on maize growth and physiology by altering antioxidant responses; however, its effects may vary depending on the dose of Se applied. The specific objectives of this study were to (i) evaluate the effects of increasing Se concentration on growth, physiological attributes, and antioxidant enzymes and (ii) assess the uptake of Fe and Zn in maize plants grown under hydroponic conditions.

2. Materials and Methods

2.1. Experimental Design and Sample Preparation. The experiment was performed in the wirehouse of the Institute of Soil and Environmental Science (ISES), University of Agriculture, Faisalabad (UAF). The soil was sampled from the soil science farm of UAF and then air-dried, ground, sieved, and homogenized before sowing of the crop. A sodium selenate (Na_2SeO_4) was utilized as a Se source with five treatments; i.e., 0, 2.5, 5, 10, and 20 mg kg^{-1}, 2.39 mg kg^{-1} salt was calculated for 1 mg kg^{-1} Se and further calculation was done accordingly. The analysis of soil was also done by adopting a standard procedure to check its various physic-chemical properties (Table 1).

The experiment was set in a complete randomized design (CRD) with three repeats. Twelve kg soil was filled in plastic bags and transferred in the pots. In each pot, five seeds of maize were planted, and prescribed doses of NPK (250 : 120 : 125 kg ha^{-1}) were applied; nitrogen was applied as urea in three splits while the phosphorus (diammonium phosphate) and potassium (sulfate of potash) were applied in a single dose. After 10 days of germination, three plants per pot were established.

2.2. Measurement of Plant Growth Parameters. Plants were harvested after 60 days of sowing. Shoots and roots were isolated carefully. The fresh weights of roots and shoots were recorded at the spot by using portable electric balance, and the length of shoots and roots was also determined by a meter rod. Plant roots and shoots were washed with distilled water and sun-dried and then kept in an oven at 70°C until plants were dried completely. The dried samples were ground and preserve for further analysis of micronutrients (Fe and Zn) and Se. Before grinding, dry weights of shoots and roots were also determined.

2.3. Determination of Physiological and Gas Exchange Attributes. Before harvesting, chlorophyll contents were recorded with a chlorophyll meter (Minolta SPAD-502 Meter). Stomatal conductance, rate of photosynthesis, and rate of transpiration were monitored and recorded by an infrared gas analyzer (IRGA, LCA-4, Hoddesdon, UK) before harvesting on a fully expanded younger leaf.

Physiological parameters, i.e., relative water contents (RWC) and membrane stability index (MSI) were also recorded by collecting the fresh leaves before final harvesting [22]. In order to determine the RWC, firstly, the fresh weights of leaves were taken and for twelve hours plunged in deionized water; then, the weight of fully turgid leaf (TW) was calculated. The leaves were then kept in the oven at 67°C for 48 hours to take the dry weight (DW). To measure MSI, a 0.1 g fresh leaf sample was retained in 10 ml of distilled water in two sets. One set was placed for half an hour at 40°C, C1 (electrical conductivity) was measured, and C2 of the other set was also calculated by placing it at 100°C in a boiling water bath for 15 minutes.

The following equations were used to calculate RWC and MSI:

Table 1: Physicochemical properties of the tested soil.

Parameter	Value
Textural class	Sandy clay loam
Sand (%)	48.31
Silt (%)	23.45
Clay (%)	26.64
pH_s	8.16
ECe (dS m^{-1})	3.48
CO_3^{2-} ($mmol_c$ L^{-1})	—
HCO_3^- ($mmol_c$ L^{-1})	4.70
Cl^- ($mmol_c$ L^{-1})	12.78
$Ca^{2+} + Mg^{2+}$ ($mmol_c$ L^{-1})	4.87
Na^+ ($mmol_c$ L^{-1})	24.84
SAR ($mmol_c$ L^{-1})$^{1/2}$	15.27
OM (%)	0.81

$$\mathrm{RWC} = \left[\frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})}\right], \tag{1}$$

$$\mathrm{MSI} = \left[1 - \left(\frac{C_1}{C_2}\right)\right] \times 100. \tag{2}$$

2.4. Determination of Antioxidant Activity. The activities of antioxidant enzymes were determined by taking a fresh leaf (0.5 g). Leaf sample was ground in five ml of fifty mM cold phosphate buffer (pH 7.8) kept in an ice bath. The subsequent homogenate was centrifuged at $15000 \times g$ for 20 minutes at 4°C temperature. Antioxidant enzymes were determined by utilizing the supernatant. SOD activity was measured by its capacity to hinder the photoreduction of nitro blue tetrazolium [23]. Peroxidase and catalase were also measured by spectrophotometer using the method given by Aebi [24].

2.5. Chemical Analysis of Plant Samples. Dried plant samples were crushed to make powder by using a mechanical grinder. Then, these samples were processed with HNO_3 : $HClO_4$ with 2 : 1 proportion (method 54a of U.S. Salinity Lab staff 1954) [25]. The samples were retained overnight after adding 1g of plant sample and 10 ml di-acid mix in a conical flask. In order to get transparent material, flasks were warmed on a hot plate [26]. Afterward, samples were cooled, and 25 mL volume was made by using distilled water and kept in airtight bottles. The prepared samples were run on atomic absorption spectrophotometer (AAS) for the detection of selenium, iron, and zinc.

2.6. Statistical Analysis. Results presented in this experiment were an average of 3 replicates. The statistical software package "Statistics 8.1" was used for interpreting the results [27]. The LSD test was used to compare the treatment means at $P \leq 0.05$. Data variability was stated as the standard deviation (SD). Principal component analysis (PCA) was performed on XLSTAT (version 2018) computer based-software.

3. Results

3.1. Growth Parameters. Different concentrations of selenium affected the growth parameters of maize. Generally, it was observed that the growth rate was faster at low selenium treatments and slowed down with increasing selenium concentration. The maximum increase in shoot length (17.89%) was noted where the Se was applied at the rate of 2.5 mg kg^{-1} as compared to control treatment. However, the Se stress negatively affected the shoot length, and the minimum shoot length (35.27%) was recorded where Se was applied at the rate of 20 mg kg^{-1} as compared to control plants (Table 2). The same results were also noted in fresh and dry biomass of shoot and root. Low levels of Se improve the shoot fresh weight up to 17.30% of maize plants as compared to control whereas the high level of selenium showed the lowest shoot fresh weight which was 28.24% as compared to control plants. Shoot dry weight was also observed maximum (20.37%) at low levels of selenium as compared to control and Se stress reduced the SDW 29.39% as compared to control plants.

3.2. Gas Exchange Parameters. A significant reduction in gas exchange parameters was observed as Se concentration increases and the maximum reduction was observed where Se was applied at the rate of 20 mg kg^{-1} (Table 3). However, the application of 2.5 mg kg^{-1} Se improved the transpiration rate, photosynthetic rate, and stomatal conductance which were 63.46%, 47.47%, and 54.55%, respectively, as compared to control plants.

3.3. Physiological Parameters. Maximum chlorophyll contents (27.19%) were observed in maize plants where the Se was applied at the rate of 2.5 mg kg^{-1} as compared to control plants while the minimum chlorophyll contents were observed where a high dose of Se is applied which is 20 mg kg^{-1} (Table 3). Se stress reduced chlorophyll contents (13.89%) as compared to control plants. As the concentration of Se increased, chlorophyll contents were decreased. Minimum relative water content (53.33%) was observed where a high dose of Se is applied which is 20 mg kg^{-1} whereas the highest (85.43%) was noticed at lower selenium levels. Similarly, the maximum membrane stability index (74.33) was observed where the Se was applied at the rate of 2.5 mg kg^{-1} while the minimum (39) was observed where a high dose of Se is applied which is 20 mg kg^{-1}. As the concentration of Se increased, the membrane stability index was decreased (Table 3).

3.4. Antioxidant Activity. Various Se concentrations affected the activities of these enzymes ($P < 0.05$). It was noticed that the activities of superoxide dismutase (SOD) were considerably enhanced at lower levels of Se (2.5 mg kg^{-1}) by 14.59%, as compared to control plants, but at high Se level (20 mg kg^{-1}), these activities were decreased. Activities of peroxidase (POD) and catalase (CAT) also showed improvement by 47.13% and 38.81% in plants where Se was

Table 2: Effect of different selenium levels on plant growth parameter of maize.

Se treatments (mg kg^{-1})	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)
0	65.33 ± 2.19^{bc}	13.50 ± 0.06^{c}	11.67 ± 1.00^{cd}	3.21 ± 0.12^{cd}	93.33 ± 1.45^{d}	21.00 ± 0.58^{c}
2.5	79.00 ± 1.53^{a}	16.95 ± 0.03^{a}	24.00 ± 0.53^{a}	5.10 ± 0.13^{a}	113.67 ± 1.86^{a}	27.33 ± 1.20^{a}
5.0	69.67 ± 2.03^{b}	14.48 ± 0.27^{b}	17.00 ± 1.00^{b}	4.11 ± 0.17^{b}	103.33 ± 0.88^{b}	23.50 ± 0.76^{b}
10.0	64.33 ± 0.88^{c}	12.69 ± 0.33^{d}	14.00 ± 0.73^{bc}	3.66 ± 0.15^{bc}	98.67 ± 0.88^{c}	21.00 ± 0.58^{c}
20.0	52.33 ± 0.88^{d}	10.43 ± 0.15^{e}	9.67 ± 1.20^{d}	2.45 ± 0.13^{d}	69.00 ± 1.15^{e}	18.00 ± 0.58^{d}

Each value is an average of three replications ± SE. Various letters (a–d) showed that the significant difference between treatments was determined by the LSD test ($P < 0.05$).

Table 3: Effect of different selenium levels on transpiration rate, stomatal conductance, photosynthetic rate, chlorophyll contents, relative water contents (%), and membrane stability index.

Se treatments (mg kg^{-1})	Transpiration rate	Stomatal conductance	Photosynthetic rate	Chlorophyll contents (SPAD value)	Relative water contents (%)	Membrane stability index
0	0.77 ± 0.01^{d}	0.15 ± 0.01^{c}	2.80 ± 0.06^{d}	22.13 ± 0.36^{c}	69.28 ± 1.58^{b}	55.00 ± 1.73^{bc}
2.5	2.12 ± 0.01^{a}	0.33 ± 0.02^{a}	5.33 ± 0.03^{a}	30.40 ± 0.44^{a}	85.43 ± 1.84^{a}	74.33 ± 1.76^{a}
5.0	1.65 ± 0.02^{b}	0.23 ± 0.01^{b}	4.52 ± 0.04^{b}	26.00 ± 0.26^{b}	66.56 ± 0.63^{bc}	57.33 ± 2.33^{b}
10.0	1.22 ± 0.08^{c}	0.16 ± 0.01^{c}	3.46 ± 0.06^{c}	23.13 ± 0.75^{c}	61.86 ± 4.29^{c}	49.33 ± 2.03^{c}
20.0	0.64 ± 0.02^{d}	0.13 ± 0.01^{d}	2.33 ± 0.06^{e}	19.43 ± 0.24^{d}	53.33 ± 1.16^{d}	39.00 ± 1.73^{d}

Each value is an average of three replications ± SE. Various letters (a–d) showed that the significant difference between treatments was determined by the LSD test ($P < 0.05$).

applied at lower levels (2.5 mg kg^{-1}), as compared to control plant (Figure 1, antioxidant activity).

3.5. Selenium Concentration in Maize Plants. The Se treatments affected the shoot and root Se concentration in maize plants in a dose-dependent manner. It is noticed that as the Se concentration increases in the medium, Se concentration was also found higher in maize plants. Results revealed that the maximum Se concentration was found in roots and shoots of maize where Se was applied at the rate of 20 mg kg^{-1} (Figure 2(a), Se concentration). The Highest Se concentrations recorded in roots and shoots were 7.03 mg kg^{-1} and 6.30 mg kg^{-1}, respectively. However, more Se was found in roots of maize compared to shoots.

3.6. Effect of Selenium on Iron and Zinc Concentration in Maize Plants. The concentration of iron (Fe) was decreased as the concentration of Se increases (Figure 2(b), Fe concentration). Under Se stress conditions, plants show a reduction in Fe uptake but the application of Se at low levels may help plants in Fe uptake. Results of this experiment revealed that more concentration of Fe was noted in plants where Se was applied at the rate of 2.5 mg kg^{-1} and a considerable reduction was noticed in Fe concentration where Se was applied at the rate of 20 mg kg^{-1}. However, more Fe concentration was found in roots (2.13 mg kg^{-1}) than shoots (0.94 mg kg^{-1}) of maize.

The Se treatments also affected the shoot and root Zn concentration ($P < 0.05$). Zn concentration was also increased in maize plants as the concentration of Se increases in the medium (Figure 2(c), Zn concentration). In this study, the application of higher Se treatments causes an increase in Zn concentration in shoots and roots. The highest Zn concentration was recorded where Se was applied at the rate of 20 mg kg^{-1}, i.e., 0.60 mg kg^{-1} in shoots and 0.77 mg kg^{-1} in roots of the maize plant. The lower rate of Zn translocation from root to shoot may be the cause of lower levels of Zn in shoots.

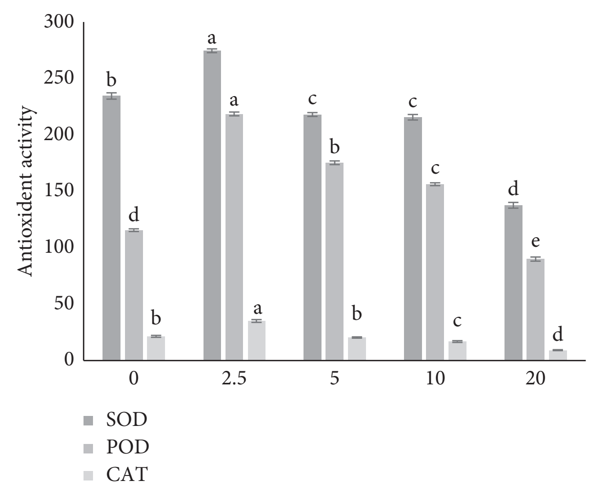

Figure 1: Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) of maize grown under different treatments of Se. Error bars represent the SE for the mean of three replications.

3.7. Results from Principal Component Analysis. The loading plots and scores of some significant studied parameters of maize under Se stress are shown in Figure 3. It can be seen that the first two components (PC1) and (PC2) of the principal component analysis showed maximum contribution and accounted for 97.71% fluctuations in the studied data. The individual contribution of PC1 and PC2 was 79.29 and 18.42%, respectively. All of the studied parameters and

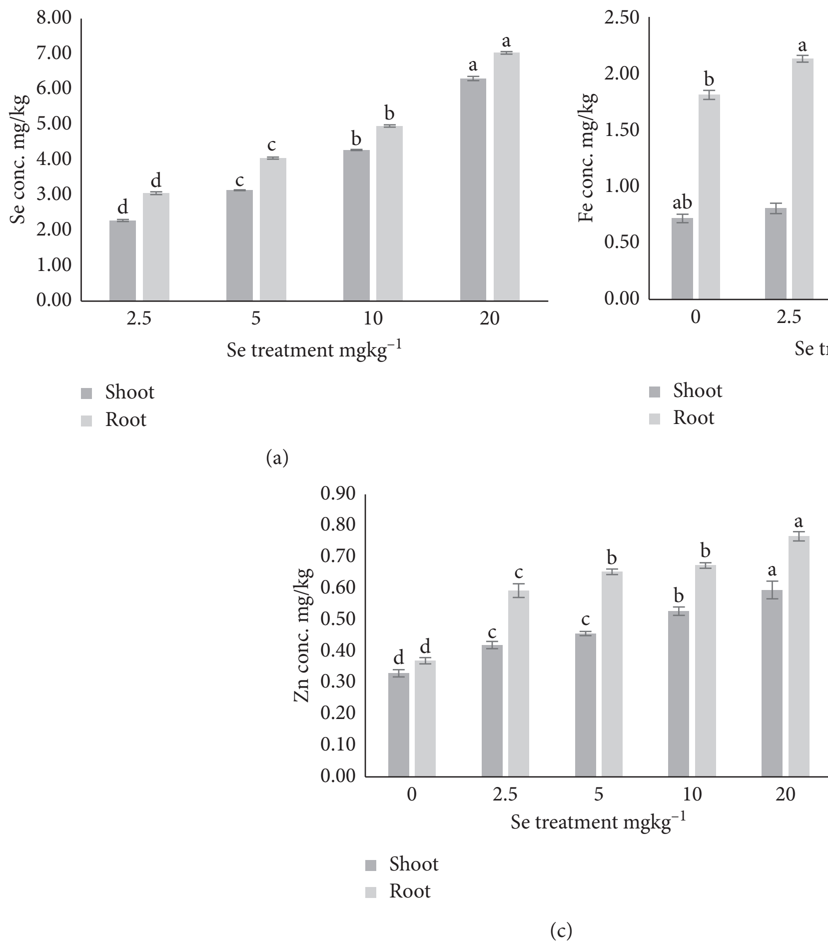

Figure 2: Effect of different doses of Se application on (a) selenium (Se), (b) iron (Fe), and (c) zinc (Zn) concentration in maize shoot and root. Error bars represent the SE for the mean of three replications.

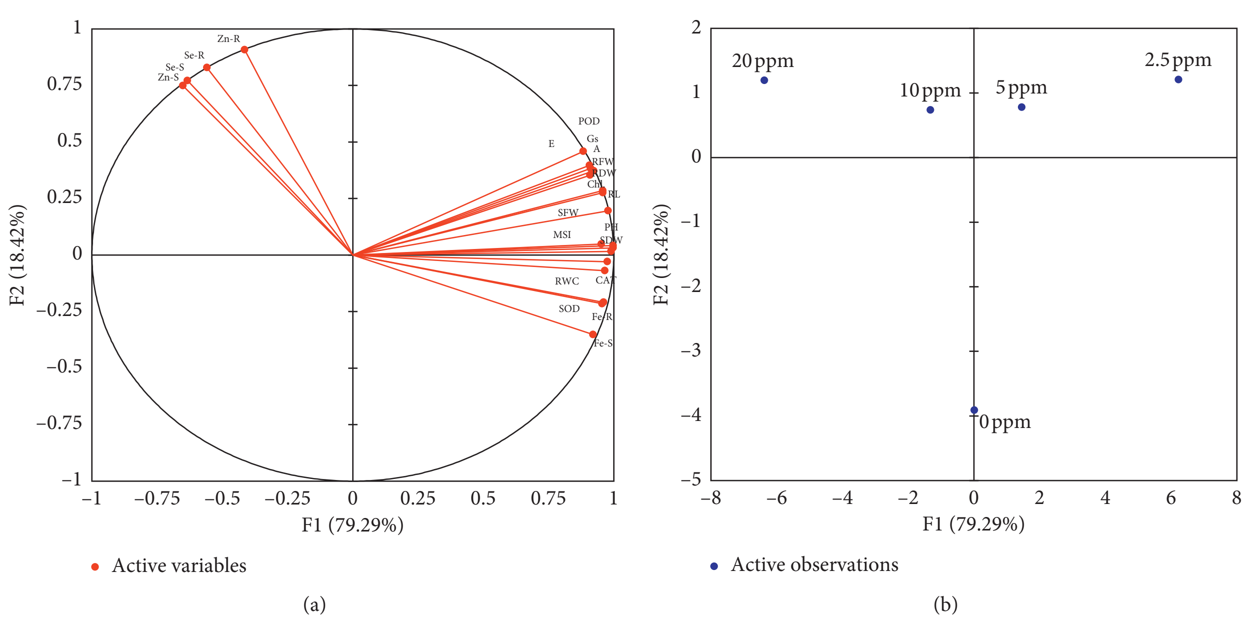

Figure 3: Principal component analysis of variables (parameters observed A) and observations (treatments applied B). PH: plant height, SFW; shoot fresh weight, SDW: shoot dry weight, RDW: root dry weight, RFW: root fresh weight, E: transpiration rate, A: photosynthetic rate, Gs: stomatal conductance, Chl.: chlorophyll (SPAD) contents, RWC: relative water contents, MSI: membrane stability index, Se-R: selenium in roots, Se-S: selenium in a shoot, SOD: sodium dismutase, CAT: catalase, POD: peroxidase, Zn-S: Zn in a shoot, Zn-R: Zn in roots, Fe-S: Fe in a shoot, Fe-R: Fe in roots. (a) Variable PCA. (b) Observation PCA.

applied treatments were found to be displaced successfully, giving an indication that the Se significantly affected the growth, physiological, chemical, and antioxidant responses of maize under varying Se levels (Figures 3(a) and 3(b)). The highest level of Se, i.e., 20 mg kg^{-1}, was more displaced from other treatments indicating hazardous effects of higher Se levels on maize attributes. The parameters falling in PC1 were positively correlated with each other while a strongly negative relationship existed between parameters PC1 and PC2 (Figures 3(a) and 3(b)).

4. Discussion

Results show that the application of selenium affected biomass production, and maximum biomass was observed at low levels of Se (Table 2). But under Se stress reduction in these traits was also observed. Heavy metal stress differentially affects the growth and physiology of maize [28]. Earlier research work also revealed that Se has a double effect on plant growth; at a lower concentration, it improves the growth of flue curd tobacco but its higher concentration reduced the growth [29]. In another study, Jiang et al. [17] also found the same trend in maize plants where the application of high Se dose considerably reduces the root and shoot growth of maize plant. Hawrylak-Nowak et al. [9] also observed the same trend in cucumber plants where higher selenium concentration caused a reduction in biomass production. Toxic effects of high Se application like reduction in plant growth and dry biomass were also found in tomato [30]. Similar findings were also presented in several earlier works [31]. Reduction in plant growth parameters may be because of the reduction in photosynthetic activity and chlorophyll content [12]. This was further confirmed in the present study by PCA where Se in roots and shoots was negatively correlated with a shoot and root fresh and dry biomass and plant height (Figure 3).

Various levels of Se also affect the photosynthetic and water relations of maize. These parameters play a significant role in plant development and disturbance in these mechanisms because metal stress may cause a diminution in plant growth and productivity [32–34]. The results of this study indicated that Se stress also causes a reduction in chlorophyll contents and photosynthetic parameters (Table 2). However, improvement in these parameters, which are mentioned above, was also noticed at lower Se levels (2.5 mg kg^{-1}). Low levels of Se may help in protecting chlorophyll enzymes which then improve the chlorophyll content [35]. Higher selenium concentration reduces net photosynthetic rate (A), which ultimately leads to a reduction in biomass production. Application of a higher concentration of selenium significantly reduces the transpiration rate (E) in maize genotypes and that was also confirmed in earlier findings in different crop species, e.g., sunflower [36], safflower [37], and maize [38]. Selenium stress significantly reduces the stomatal conductance (gs) in maize plants, and similar results were found in previous findings in different crops [39, 40]. The effect of Se on plants largely depends on its concentration in the growth medium. In this regard, Se was found toxic when 10 or above 10 mg kg^{-1} was applied in growth medium for tomato plants [41, 42]. Furthermore, [43] also found that the effect of low Se concentration on the stomatal and photosynthetic cells of garlic was progressive. So, the increase in photosynthetic activities was due to the positive effect of Se on photosynthesis. Moreover, PCA revealed that high selenium level (20 mg kg^{-1}) was more displaced from control suggesting that higher levels of Se caused a maximum reduction in growth, physiology, and chemical attributes of the maize in the present study (Figure 3).

Membrane stability index and relative water contents also decrease as the Se level increases, but at lower levels (Se at the rate of 2.5 mg kg^{-1}), an improvement in both of these parameters was observed (Table 2). The reason behind this improvement might be the improvement in membrane stability [44]. Similar to these results, [45] observed the highest value of relative water content in *Hordeum vulgare* plants where Se was applied at low levels and reduction was observed at higher levels.

In this experiment, it is observed that under low Se concentration the accumulation of Se in shoots of maize was low. However, when the concentration of Se increases in growth media, selenium accumulation was greater. In our study, more Se was found in roots as compared to shoots. These results correspond to some earlier studies on tobacco [46] and rice [47] in which the Se concentration was found less in shoots compared to roots. These results come to an agreement with earlier findings, which describe that higher Se dose in growth media can cause a considerable improvement of Se concentration in plants [48]. Micronutrients like Fe and Zn are essential for plant growth as they are the cofactors of many enzymes and also take part in many plant activities [49]. However, the presence of selenium in high concentration competes with these micronutrients in plants and disrupts their uptake [50]. It is also noted that high Se also favors the Zn content in maize plants; however, Fe shows the opposite trend (Figures 2(b) and 2(c)). Iron concentration in roots was only increased at the lowest concentration of Se [51]. However, it is also reported in earlier studies that Se affects the uptake of various constituents [52]. Improvement in the enzyme activities in maize plants exposed to low Se level was observed in this experiment. But Se stress causes a reduction in the antioxidant activities, and the lowest values of these activities were observed at higher Se level (20 mg kg^{-1}) as shown in Figure 1. Destruction in antioxidant activity might be due to the production of reactive oxygen species (ROS) under Se stress conditions [53]. These ROS adversely affect the antioxidant activity, water relation, and gas exchange parameters, which are important for normal plant growth [54]. The formation of ROS severely damages the proteins and DNA and could interfere with natural cellular functioning in plants, resulting in cellular death. Some earlier studies described that the exogenous application of selenium at lower levels can improve the antioxidant activity and the tolerance of plants [31]. Similar results of some other researches also describe that low Se doses can enhance the antioxidant activities whereas higher Se causes a reduction [15, 50].

5. Conclusions

The findings of this research illustrate that the effects of Se application on physiomorphological parameters of maize depend on Se level in growth media. All growth, physiological, water relation, and gas exchange parameters have shown improvements at low Se level but higher Se level causes reduction in all these parameters, which ultimately reduce the growth of maize plants. This reduction in growth attributes may be due to the high accumulation of Se in roots and the disturbance in gas exchange parameters. It was also noticed that the presence of Se affects the uptake of Zn and Fe in maize grown under hydroponic conditions. Future work should include investigating the underlying molecular mechanisms of Se in plants to further understand and enhance its functional role.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Conceptualization was done by M. A. H., X. W., M. A. E.-E., and A. M.; methodology, M. A. H. and X. W.; formal analysis, H. S., H. A. M., M. A. E.-E., and N. F.; software, M. A., A. M., and M. A. E.-E.; original draft preparations, M. N.; reviewing and editing, M. N., M. A. E.-E., and A. M.

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