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Vanadium Toxicity Induced Changes in Growth, Antioxidant Profiling, and Vanadium Uptake in Pepper (*Capsicum annum* L.) Seedlings

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Abstract: Heavy metal contamination is one of the current serious environmental and agricultural soil issues, and it is mainly due to anthropogenic activities. Vanadium (V) is found in low concentrations in a wide range of plants and is widely distributed in soils. The current study aimed to determine how pepper seedlings responded to various V concentrations, as well as the detrimental effects of V on growth, root morphological traits, photosynthetic performance, reactive oxygen species (ROS), osmolytes production, antioxidant enzyme activities, and V uptake. Pepper seedlings (5 weeks old) were grown in hydroponic culture with six V levels (0, 10, 20, 30, 40, and 50 mg L^{-1} NH_4VO_3). After two weeks of V treatment, low level of V (10, 20 mg L^{-1}) enhanced the growth status, conversely higher V (30, 40, and 50 mg L^{-1}) level reduced the growth. The leaf gas exchange elements, pigments molecules, and root growth characteristics are also affected by higher V concentrations. Moreover, V uptake was higher in roots than in the shoot of pepper seedlings. Similarly, osmolytes content, ROS production, and antioxidant enzymes activities were significantly improved under V stress. Concluding, lower V (10, 20 mg L^{-1}) concentration positively affected pepper growth, and higher V (30, 40, and 50 mg L^{-1}) concentration had a detrimental effect on pepper physiological and biochemical mechanisms.

Keywords: pepper; vanadium; enzymes root growth; osmolytes; photosynthesis

1. Introduction

Heavy metal pollution in agricultural soil has received worldwide attention in recent years due to its detrimental impacts on environmental health, crop quality, and food security [1]. Vanadium (V) is a widely dispersed and naturally occurring trace metal present in the atmosphere and soil rhizosphere. V occurs naturally in soils in various mineral forms, including vanadinite, chileite, patronite, and carnotite, with varying distribution ranges (3–310 mg kg^{-1}) [2]. However, out of total V, very minute amounts (less than 1%) are leachable as well as extractable with water with limited mobility in soil [3,4]. Previous studies suggest that V was reported to be a very harmful element for plants [5], which significantly affects growth and development of crops. It was only in the 1950s when Bertrand [6] found that a lesser amount of V (10 ng V g^{-1} soil) was able to affect plant growth significantly. Numerous studies found that V toxicity and bioavailability are highly dependent on its oxidation state of V: −1, 0, +2, +3, +4, and +5 [7]. In the environment, V is often present in tetravalent (V^{+4}) and pentavalent (V^{+5}) oxidation states, whereas, V^{+5} compounds are usually more toxic than V^{+4} in plants [8]. Vanadate is the most harmful of all V chemical compounds, and its greater concentration impeded plant growth by inhibiting physiological, morphological, and biochemical processes [9].

Plants readily absorb V from the soil, but its impact on plants is proportional to its concentration. Plant growth is regulated by a lower V concentration, which promotes nitrogen fixation, chlorophyll production, and potassium consumption [8]. García-Jiménez et al. [10] reported that application of V (5 μM) causes significant an increase in pepper growth by enhancing amino acid, total sugar, mineral nutrient uptake, and floral bud development. Moreover, reports suggest that the higher V (40 mg/L) concentration noticeably decreased morphological traits of tomato [11]. Similarly, V (25 mg/L) application significantly reduced plant biomass production and root and shoot length of chickpea [12].

Photosynthesis is a key metabolic process in plants that may improve carbon absorption and boost crop yield [13]. Photosynthetic efficiency is severely hampered by metal stress [14]. Previous findings have shown that metals cause a substantial reduction in pigment molecules and gas exchange elements in tomato, watermelon, and potato [15–17]. Furthermore, cadmium (150 mg/L) application considerably affected chlorophyll fluorescence parameters, chlorophyll content, and photosynthetic pigments of tomato [18]. Heavy metals primarily impair plant photosynthesis by interfering with electron transport and damaging the chloroplasts membrane integrity.

Roots are the most essential organ in plants for metal and non-metal nutrient absorption. As a result, roots are the first organs to be harmed by metals in the soil [19]. Roots not only provide structural support to the plant but are also the first-line defense against metal toxicity. Roots may protect themselves against metal-induced injury by reducing unnecessary metal absorption [19]. Nawaz et al. [15] reported that V application dramatically decreased root traits of watermelon seedlings. Similarly, our previous study revealed that V-treated tomato seedlings impaired root architecture and mineral nutrient uptake [20]. Moreover, in chickpea, higher V concentration considerably decreased root morphological traits, such as root length, volume, surface area, forks, tips, and crossings [21]. Due to differential responses to various V concentrations, it is clear that further research is required to determine the optimal doses of this element as a bio-stimulant in various genotypes of cultivated crops.

In the solanaceous family, pepper (*Capsicum annum* L.) is an important horticultural crop due to its economic significance. Due to its nutritional and economic importance, it is considered a valuable cash crop over the globe. Pepper fruit is an excellent source of antioxidants, vitamins, proteins, carbohydrates, fats, and phenolic compounds [22]. Recent studies revealed that the usage of beneficial elements might help boost the productivity of horticultural crops. Previously, García-Jiménez et al. [10] reported that V (5 μM) stimulates pepper growth and development by enhancing biomass production and yield. V negatively affects plant growth in many plant species by hampering the antioxidant mechanisms under its toxicity [15,20]. The reports on the physiological and biochemical response under V toxicity of the pepper plant are still low in number, which warrants further investigation. Therefore, the function of V in pepper plants must be understood, particularly in determining at which level V becomes toxic to pepper. The present study is designed to study and assess the impact of various V applications on pepper, and determine at what concentration V begins to suppress pepper growth, as well as to examine the impact of V on pepper growth, root architecture, gas exchange parameters, and photosynthetic pigments.

2. Material and Methods

2.1. Pepper Seed, Reagent, and Growth Conditions

Pepper seeds (*Capsicum annum* L. Var. Ca-59) were collected from Horticulture Research Station, Haikou, China. Vanadium (Ammonium metavanadate (NH_4VO_3)) was purchased from a scientific store in Haikou. The experiment was performed in a plant growth room under specific conditions (25 °C $\pm$ 5 temperature; 16/8 h dark/light period; and 55–90% relative humidity).

2.2. Seedling Growth and Treatment

Healthy pepper seeds were sown in 40-cell seedling trays, filled with nutrient-rich peat soil. After 5 weeks of sowing, pepper seedlings were transferred into 4-litre plastic containers, filled with Hoagland's nutrient solution (pH 5.5 ± 1) for hydroponic conditions. To provide a fresh supply of nutrients, the nutrient solution was replaced every fifth day. After a five-day adaptation period, seedlings were divided into five groups. Each group received a different V concentration *viz.* 0, 10, 20, 30, 40, 50 mg L^{-1}. After two weeks of V stress, plant samples were collected for further analysis.

2.3. Growth Variables

After applying the treatment, plant height (PH), fresh shoot weight (FSW), fresh root weight (FRW), dry shoot weight (DSW), dry root weight (DRW), and leaf area (LA) were determined. The top leaves from each plant were used to determine LA.

Plant height was measured with the help of a ruler. LA was recorded with a portable laser LA meter (CI-202). For quantifying the fresh weights, shoots and roots of the plants were separated and weighed individually. After that, for the measurement of dry weights, plant samples were put in an oven at 80 °C for 3 days [23]. Growth tolerance index (GTI; in %) was formulated for roots and shoots separately by using dry weight and following the formula described by Wiszniewska et al. [24].

$$\mathrm{GTI_S} = \frac{\text{average dry weight of Cd} - \text{treated shoot}}{\text{average dry weight of Cd} - \text{untreated shoot}} \times 100 \tag{1}$$

$$\mathrm{GTI_R} = \frac{\text{average dry weight of Cd} - \text{treated root}}{\text{average dry weight of Cd} - \text{untreated root}} \times 100 \tag{2}$$

2.4. Root Morphology

Roots of five similar plants (from each replication) were taken and rinsed with running tap water for root measurement. The root was scanned using an Imagery Scan Screen (Epson Expression 11000XL, Regent Instruments, Chemin Sainte-Foy, QC, Canada), and the root traits were determined using WinRHIZO 2003a software [25].

2.5. Gas Exchange Parameters and SPAD Index

A portable photosynthesis system (CIRAS-3, Hansatech Co., Amesbury, MA, USA) was employed to check for the gas exchange elements. Fully developed leaves were used to calculate gas exchange parameters [26]. A SPAD-502 Chlorophyll Meter (Minolta Camera Co., Ltd., Osaka, Japan) was used to determine the leaves' relative chlorophyll content (the third leaf from the top).

2.6. Photosynthetic Pigments

The pigments' content of leaves was determined using Lichtenhaler and Wellburn's [27] method. One gram of fresh plant samples was crushed and homogenized with 80% acetone. Ten mL of this solution was placed in test tubes and centrifuged for 15 min at 3000 rpm. The value of absorption was recorded at 662, 645, nm, and 470 nm.

2.7. Malonaldehyde (MDA) and Hydrogen Peroxide (H$_2$O$_2$), Catalase (CAT), and Superoxide Dismutase (SOD) Measurement

After 14 days of V treatment, leaf samples were collected for analysis of H$_2$O$_2$ and MDA level, as well as SOD and CAT activities. Three plants (from each replicate) were taken and their leaves were collected and frozen using liquid nitrogen. Then, samples were kept at 80 °C for a short time before being used for further analysis.

To determine the MDA level, H$_2$O$_2$ content, and antioxidant enzyme (CAT, SOD) activity, frozen leaf samples (0.5 g) were crushed using liquid nitrogen. As prescribed in the kit, the ground leaf samples were homogenized in 900 μL of 100 mM phosphate buffer

(pH 7.4) [28]. Each homogenized sample was centrifuged at $12,000 \times g$ for 15 min at $4\,^\circ\text{C}$, followed by the addition of the supernatant to another falcon tube for further analysis.

The activities of CAT and SOD, and the levels of MDA and H_2O_2 were calculated using the instructions included in kits (A007-1, A001-1, A003-3, and A064) purchased from Nanjing Jiancheng Bioengineering Institute in Nanjing, China. The activities of CAT and SOD, and the levels of MDA and H_2O_2 were measured at 405 nm, 550 nm, 550 nm, and 530 nm, respectively.

2.8. Proline and Soluble Sugars Content

The proline content was determined using the technique described by Bates et al. [29]. A leaf sample (0.5 g) was homogenized in 5 mL sulfosalicylic acid (3%, *m/v*), and centrifuged at $12,000 \times g$ for 20 min at $4\,^\circ\text{C}$. Following that, an equal amount (2 mL) of supernatant, glacial acetic acid, and acid ninhydrin was added, followed by 1 h of boiling at $100\,^\circ\text{C}$, and 20 min in ice. The mixture was then rapidly vortexed with 4 mL toluene. The upper toluene layer was removed and the proline concentration was determined at 520 nm.

The phenol-sulphuric acid technique was used to determine soluble sugars [30]. Then, 0.1 g of dried leaves were homogenized in deionized water, then filtered and the extract was treated with 2% (*w/v*) phenol and 98% sulphuric acid. After 1 h of incubation at room temperature, the absorbance at 490 nm was determined using a spectrophotometer.

2.9. V Determination, Uptake and Translocation

Six plants from each replication were taken and rinsed with deionized water to determine the V level in the leaves and root. The dried plant tissues were crushed and digested (*v/v*, 4:1) with HNO_3 and $HClO_4$. The V concentration was measured using an atomic absorption spectrophotometer (Modal AA-7000). V uptake [31] and root to shoot translocation [32] were expressed as:

$$\text{V uptake (mg)} = \text{tissues V concentration} \ \times \ \text{tissues dry mass} \tag{3}$$

$$\text{Root to shoot V translocation} = \frac{\text{concentration of V in shoot}}{\text{concentration of V in root}} \tag{4}$$

2.10. Statistical Analysis

SPSS software was used to conduct the statistical analysis. A significant difference in V concentrations ($p \leq 0.05$) is shown by different letters. In the figures, the values are always shown as the means $\pm$ SE of four independent replicates. The differences between the V concentrations were analyzed using Fisher's least significant difference (LSD) test ($p \leq 0.05$).

3. Results

3.1. Plant Growth and Growth Tolerance Index

After two weeks of V treatment, we observed that low level of V (10 and 20 mg L^{-1} V) is beneficial for pepper growth, conversely, a higher level of V (30, 40, and 50 mg L^{-1} V) restricted pepper growth (Figure 1). The outcomes showed that growth attributes (FSW, FRW, DSW, DRW) increased considerably after two weeks of V (10 mg L^{-1} V) treatment as compared to control (0 mg L^{-1} V) treatment (Table 1). On the other hand, V application (20, 30, 40, and 50 mg L^{-1} V) significantly ($p < 0.05$) reduced these growth attributes (Table 1). Similarly, in case of PH and LA, these growth attributes increased significantly at low levels of V (10 and 20 mg L^{-1} V) (Figure 2). Furthermore, the plant biomass reduction was more noticeable at 40 and 50 mg L^{-1} V, when compared with 30 mg L^{-1} V (Table 1, Figures 1 and 2).

The calculation for GTI, by using Equations (1) and (2), was shown in Table 1. GTI_S enhanced significantly 1.16-folds in 10 mg L^{-1} V. Conversely, GTI_S was reduced considerably by 85% in 20 mg L^{-1} V, 56% in 30 mg L^{-1} V, 34% in 40 mg L^{-1} V, and 23% in 50 mg L^{-1} V (Table 1). The root growth was markedly enhanced at 10 mg L^{-1} V. GTI_R

increased 1.07-folds in 10 mg L^{-1} V, while GTI$_R$ was decreased 72% in 20 mg L^{-1} V, 48% in 30 mg L^{-1} V, 34% in 40 mg L^{-1} V, and 22% in 50 mg L^{-1} V (Table 1).

Table 1. Effect of vanadium on fresh and dry biomass of pepper seedlings.

Vanadium (mg L^{-1})	Biomass Yield Per Plant (g)				Growth Tolerance Index (%)	
	Fresh		Dry		Shoot	Root
	Shoot	Root	Shoot	Root		
0	5.310 ± 0.217 b	0.728 ± 0.015 b	1.483 ± 0.054 b	0.106 ± 0.001 b	100	100
10	6.381 ± 0.125 a	0.791 ± 0.007 a	1.722 ± 0.066 a	0.114 ± 0.001 a	116	107
20	4.793 ± 0.229 c	0.586 ± 0.015 c	1.266 ± 0.058 c	0.077 ± 0.003 c	85	72
30	2.843 ± 0.138 d	0.389 ± 0.012 d	0.845 ± 0.056 d	0.051 ± 0.002 d	56	48
40	1.473 ± 0.153 e	0.193 ± 0.010 e	0.510 ± 0.052 e	0.036 ± 0.001 e	34	34
50	0.929 ± 0.111 f	0.135 ± 0.007 f	0.351 ± 0.041 f	0.024 ± 0.001 f	23	22

Mean ± SE of four replicate. Lowercase letters in each column show significant difference from each other according to the Fisher's LSD test at $p \leq 0.05$.
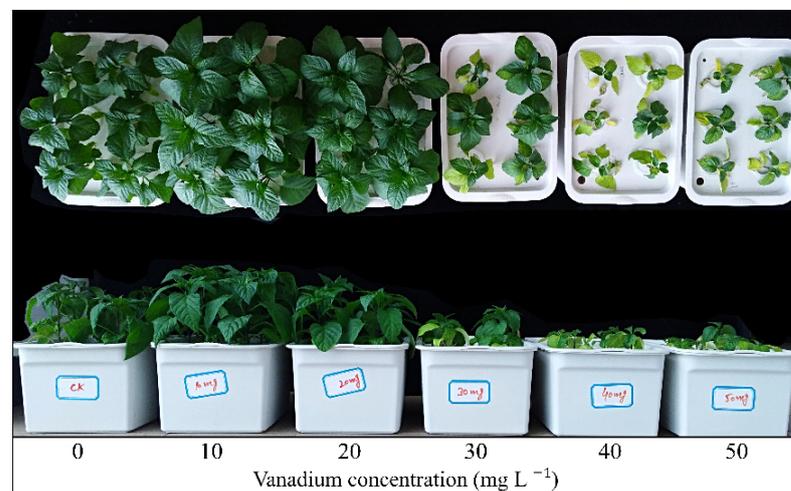
**Figure 1.** Visual demonstration of pepper seedlings under vanadium toxicity.
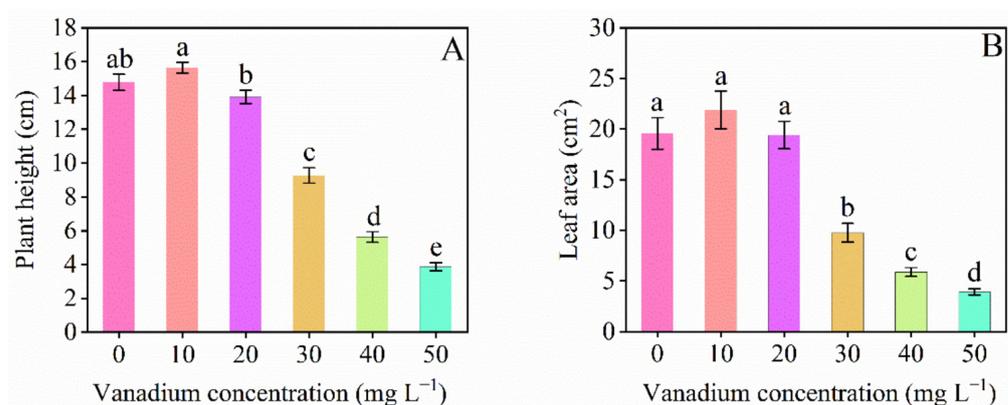
Figure 2. Effect of vanadium on plant height (**A**) and leaf area (**B**) in pepper seedlings. Means ± SE, n = 4, significant differences are exhibited by lowercase letters (at $p < 0.05$), according to Fisher's least significant difference test.

3.2. Root Morphology

In our study, we observed that the root growth of pepper seedlings was enhanced at 10 mg L^{-1} V concentration. The pepper seedlings that received 20 to 50 mg L^{-1} V showed

significant decline in root growth characteristics (Figures 3 and 4). The root length, root volume, surface area, average diameter, root tips, root forks, root crossing, and projected area were increased by 2.76%, 1.58%, 0.76%, 4.89%, 4.40%, 7.21%, 2.88%, and 3.07%, respectively, after the supplementation of 10 mg L^{-1} V as compared to control (Figure 3). Ten mg L^{-1} V maintains traits of roots on the control plant level. Significant changes were observed after 20–50 mg L^{-1} V, and show reduction root length from 7.58 to 70.86% (Figure 3A), root volume 11.58 to 86.43% (Figure 3B), surface area 10.72 to 79.54% (Figure 3C), average diameter 11.19 to 59.11% (Figure 3D), root tips 9.74 to 66.66% (Figure 3E), root forks 6.97 to 80.47% (Figure 3F), root crossing 10.46 to 83.39% (Figure 3G), and projected area 11.52 to 83.24% (Figure 3H).
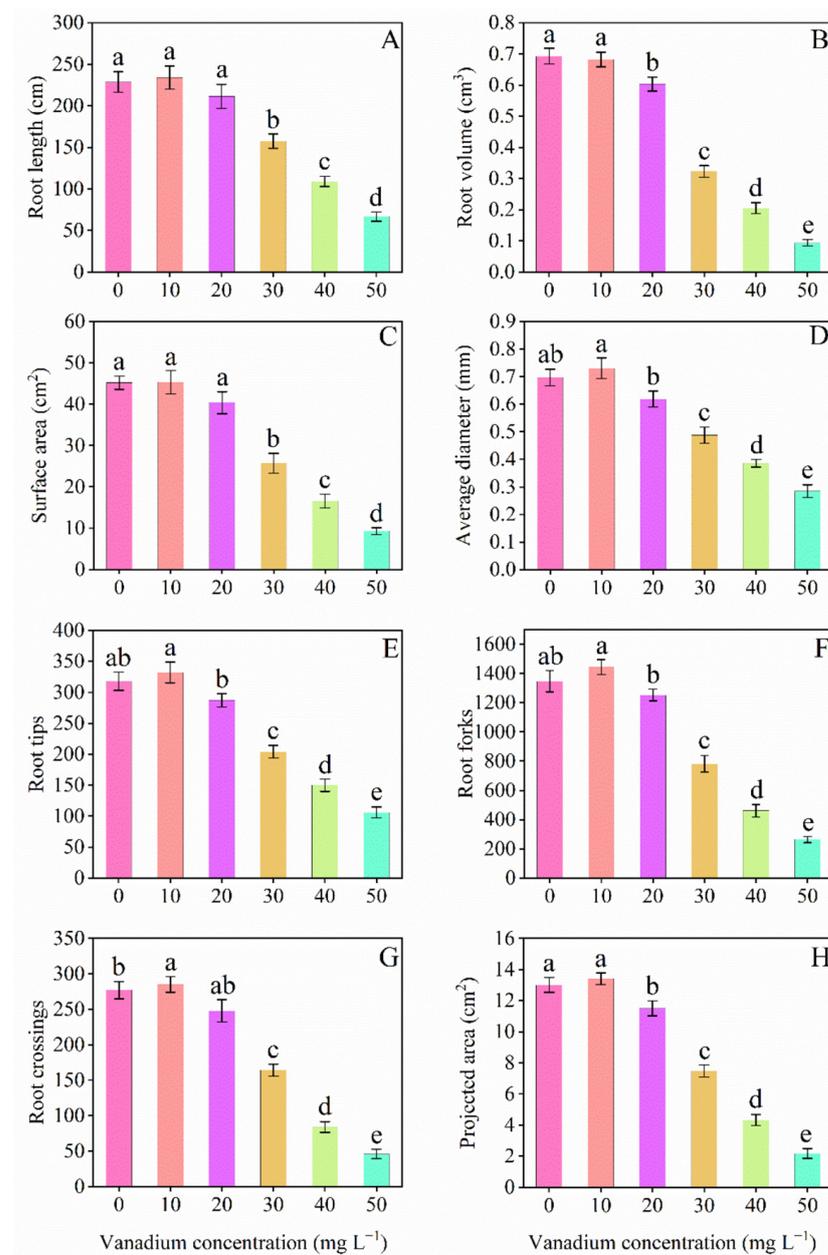
**Figure 3.** Effect of vanadium on root morphology [Root length (**A**), root volume (**B**), surface area (**C**), average diameter (**D**), root tips (**E**), root forks (**F**), root crossings (**G**), and projected area (**H**)] in pepper seedlings. Means ± SE, n = 4, significant differences are exhibited by lowercase letters (at $p < 0.05$), according to Fisher's least significant difference test.
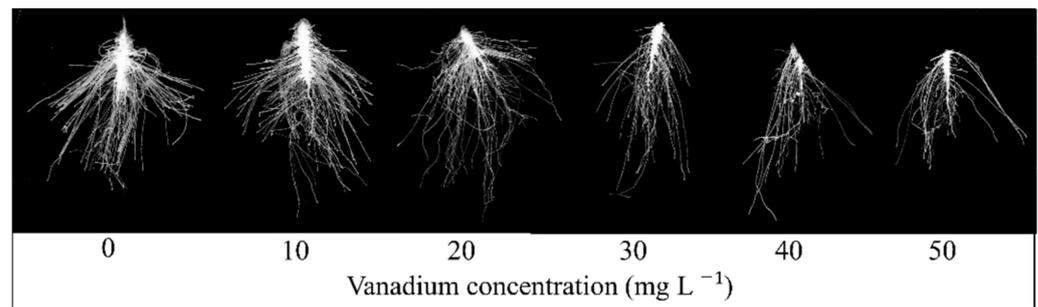
Figure 4. Root architecture system of pepper seedlings grown under V toxicity.

3.3. Leaf Gas Exchange Elements

Our findings revealed that higher V (30, 40, and 50 mg L^{-1} V) concentrations had a significant impact on the photosynthetic activities of pepper seedlings (Figure 5). When compared to control, the photosynthetic assimilation (Pn) was reduced by 32.45%, 49.29%, and 63.91%, and stomatal conductance (Gs) rate was reduced by 35.48%, 53.98%, and 69.99%, respectively, when pepper plants were exposed to 30, 40, and 50 mg L^{-1} V, respectively. (Figure 5A,B). The intercellular CO_2 (Ci) and transpiration rate (Tr) were dramatically reduced by 34.69%, 50.53%, and 58.94%, and 28.16%, 41.54%, and 54.22%, respectively, following treatment with 30, 40, and 50 mg L^{-1} V (Figure 5C,D). The leaf gas exchange elements of pepper seedlings receiving 10 and 20 mg L^{-1} V were found statistically similar with the elements of pepper seedlings receiving 0 mg L^{-1} V (Figure 5).
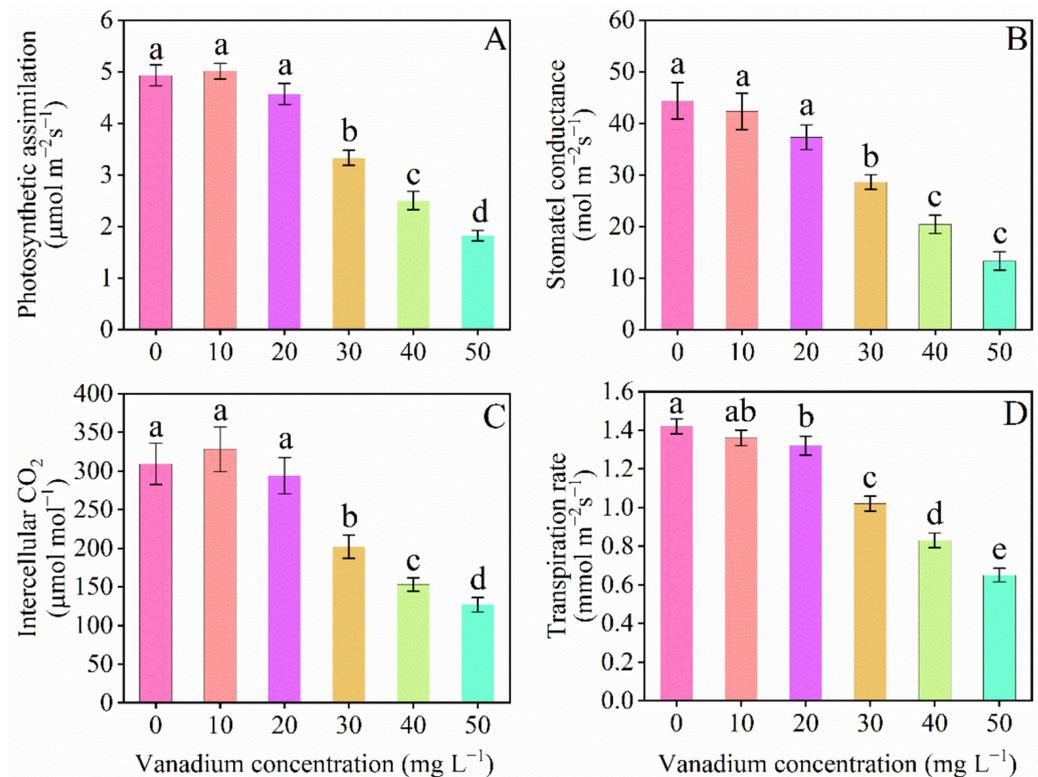
Figure 5. Effect of vanadium on leaf gas exchange elements [Photosynthetic assimilation (**A**), stomatal conductance (**B**), Intercellular CO_2 (**C**), and transpiration rate (**D**)] in pepper seedlings. Means ± SE, n = 4, significant differences are exhibited by lowercase letters (at $p < 0.05$), according to Fisher's least significant difference test.

3.4. SPAD Index and Pigments Content

The findings showed that increasing V concentrations from 30 to 50 mg L^{-1} substantially lowered SPAD index and photosynthetic pigments compared to CK seedling (Figure 6). The SPAD (relative chlorophyll content) were reduced up to 31.18%, 52.57%, and 67.48% when seedlings were supplied with 30, 40, and 50 mg L^{-1} V, respectively, when compared with CK plants (Figure 6D). The addition of 30, 40, and 50 mg L^{-1} V to pepper seedlings decreased the chlorophyll a and chlorophyll b by 20.27%, 35.81%, and 50.01% and 27.58%, 54.31%, and 74.71%, respectively (Figure 6A,B). Carotenoids in pepper plant leaves were reduced by 33.33%, 58.02%, and 75.30%, respectively, following treatment 30, 40, and 50 mg L^{-1} V compared to control plants (Figure 6C). The concentration of pigment molecules observed in the leaves were similar in pepper seedling treated with 0, 10, and 20 mg L^{-1} V (Figure 6A–C).
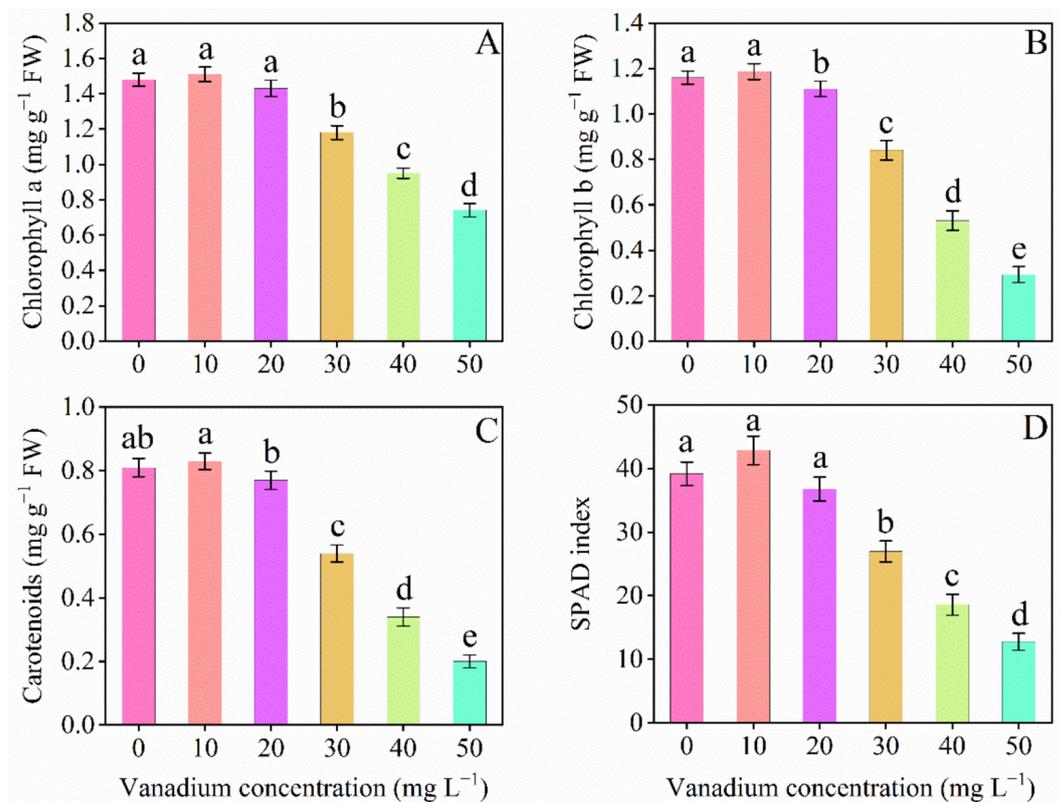
Figure 6. Effect of vanadium on photosynthetic pigments [Chlorophyll a (**A**), chlorophyll b (**B**), carotenoids (**C**), and SPAD index (**D**)] in pepper seedlings. Means $\pm$ SE, n = 4, significant differences are exhibited by lowercase letters (at $p < 0.05$), according to Fisher's least significant difference test.

3.5. Proline and Soluble Sugar Content

When compared to control plants, the presence of V resulted in a considerable increase in proline and soluble sugar content of the leaves (Figure 7). The proline content was significantly increased up to 1.01-, 2.54-, 4.20-, 5.88-, and 8.04-fold, respectively, following treatment with 10, 20, 30, 40, and 50 mg L^{-1} V (Figure 7A). Similarly, when pepper plants were subjected to 10, 20, 30, 40, and 50 mg L^{-1} V, the soluble sugar content were enhanced by 0.76-, 1.51-, 2.32-, 3.21-, and 4.01-fold, respectively, when compared with control seedlings (Figure 7B).
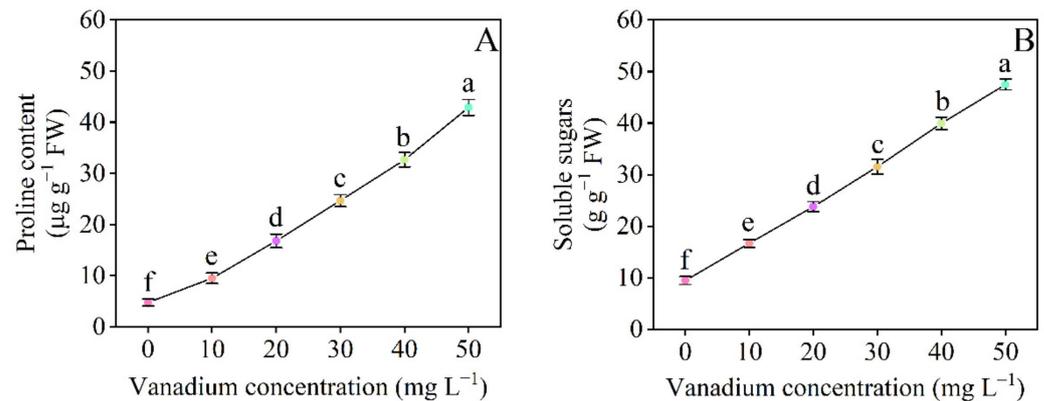
Figure 7. Effect of vanadium on proline (**A**) and soluble sugars (**B**) content in pepper seedlings. Means $\pm$ SE, n = 4, significant differences are exhibited by lowercase letters (at $p < 0.05$), according to Fisher's least significant difference test.
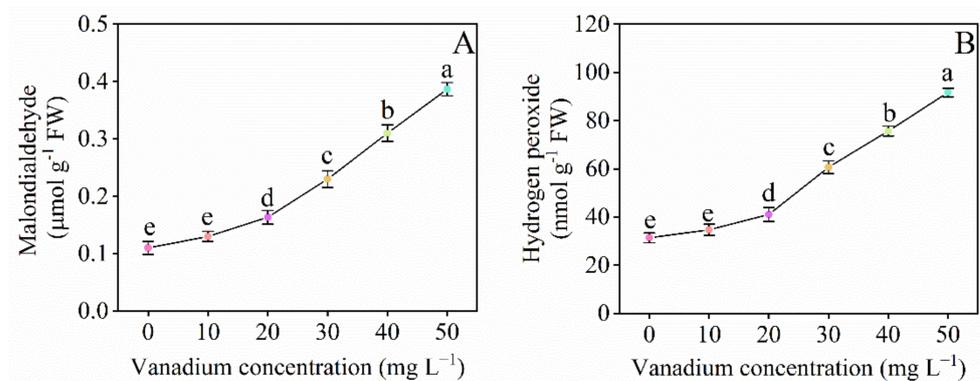
3.6. Malonaldehyde and Hydrogen Peroxide Content

The pepper plants were subjected to various V concentrations, resulting in the highest increase in MDA and H_2O_2 content of pepper leaves when compared to control seedlings (Figure 8). The current results revealed that a lower level of V (10 mg L^{-1}) maintained the H_2O_2 and MDA levels (Figure 8A,B). Further, MDA levels were increased by enhancing the concentration of V beyond 10 mg L^{-1}. When pepper seedlings were exposed to 20, 30, 40, and 50 mg L^{-1} V, the MDA level were increased 0.48-, 1.09-, 1.81-, and 2.51-fold, respectively, when compared with control plants (Figure 8A). Similarly, V supplementation significantly enhanced the H_2O_2 production of pepper seedling. H_2O_2 production in pepper leaves was enhanced 0.30-, 0.93-, 1.41-, and 1.92-fold, respectively, following treatment of 20, 30, 40, and 50 mg L^{-1} V compared to control seedlings (Figure 8B).
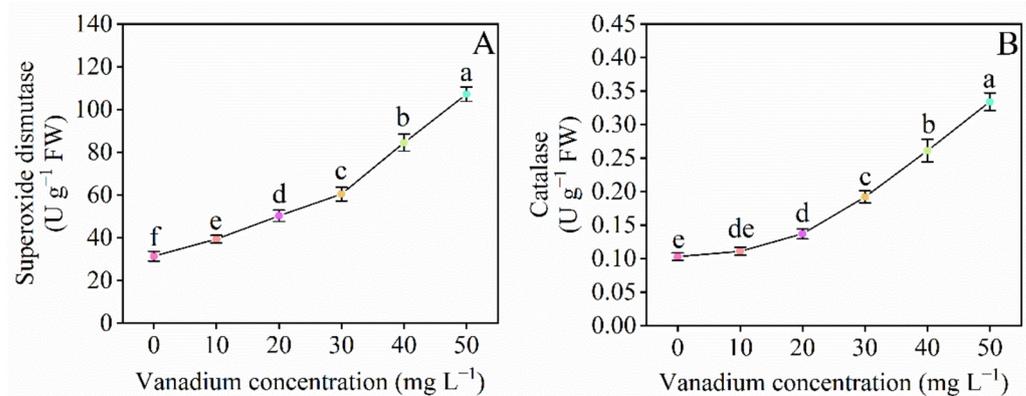

Figure 8. Effect of vanadium on Malonaldehyde (**A**) and hydrogen peroxide (**B**) content in pepper seedlings. Means $\pm$ SE, n = 4, significant differences are exhibited by lowercase letters (at $p < 0.05$), according to Fisher's least significant difference test.

3.7. Catalase and Superoxide Dismutase

When pepper seedlings were subjected to varying V concentrations, there were substantial changes in antioxidant enzyme activities; a positive relationship was established between V addition and antioxidant enzyme activities (Figure 9). Our results indicated that CAT and SOD levels remained similar at minor level of V (10 mg L^{-1}) when compared with the control (Figure 9A,B). The activities of antioxidant enzymes (CAT and SOD) increased consistently as V concentrations increased from 20 to 50 mg L^{-1} V, with the greatest increase in antioxidant enzyme activities occurring at 40 and 50 mg L^{-1} V. The CAT activity in pepper seedlings were increased by 0.33-, 0.86-, 1.53-, and 2.24-fold with the supplementation of 20, 30, 40, and 50 mg L^{-1} V, respectively, as compared with control

(Figure 9A). Similarly, an increase of 0.60-, 0.92-, 1.69-, and 2.41-fold was noticed in SOD activity with 20, 30, 40, and 50 mg L^{-1} V, respectively (Figure 9B).

Figure 9. Effect of vanadium on Superoxide dismutase (**A**) and Catalase (**B**) in pepper seedlings. Means $\pm$ SE, n = 4, significant differences are exhibited by lowercase letters (at $p < 0.05$), according to Fisher's least significant difference test.

3.8. Plant Tissues Vanadium Concentration, Uptake, and Root to Shoot Translocation

The findings revealed that when the V levels increased, the V concentration in the tissues (root and shoot) increased dramatically. As compared to the control, the V addition resulted in considerable accumulation in pepper roots and shoots (Table 2). The maximum V 51.8 mg kg^{-1} DW was accumulated in root and 29.4 mg kg^{-1} DW in shoots as compared with the control when treated with 50 mg L^{-1} V. The results show that V was accumulated more in roots than in shoots (Table 2). The absorption of V by shoots and roots increased significantly as V levels increased. Similarly, root to shoot V translocation also increased by increasing V concentration (Table 2).

Table 2. Effects of vanadium on vanadium translocation (root to shoot), vanadium concentrations and uptake (in roots and shoot; mg kg^{-1} DW) in pepper seedlings.

Vanadium (mg L^{-1})	V Concentration		V Uptake		V Translocation (Root to Shoot)
	Root	Shoot	Root	Shoot	
0	4.851 $\pm$ 0.351 f	1.530 $\pm$ 0.202 f	0.513 $\pm$ 0.043 c	2.261 $\pm$ 0.284 c	0.316 $\pm$ 0.037 d
10	12.543 $\pm$ 0.967 e	4.573 $\pm$ 0.661 e	1.428 $\pm$ 0.095 ab	7.903 $\pm$ 1.251 b	0.361 $\pm$ 0.025 cd
20	19.697 $\pm$ 1.380 d	8.376 $\pm$ 0.481 d	1.515 $\pm$ 0.051 a	10.665 $\pm$ 1.074 a	0.426 $\pm$ 0.007 c
30	31.011 $\pm$ 1.035 c	13.963 $\pm$ 0.739 c	1.555 $\pm$ 0.039 a	11.743 $\pm$ 0.508 a	0.449 $\pm$ 0.009 bc
40	39.577 $\pm$ 1.523 b	20.806 $\pm$ 1.122 b	1.434 $\pm$ 0.003 ab	10.537 $\pm$ 0.771 a	0.529 $\pm$ 0.049 ab
50	51.876 $\pm$ 1.735 a	29.409 $\pm$ 1.213 a	1.266 $\pm$ 0.118 b	10.221 $\pm$ 1.006 ab	0.569 $\pm$ 0.042 a

Mean $\pm$ SE of four replicate. Lowercase letters in each column show significant difference from each other according to the LSD test at $p \leq 0.05$.

3.9. Pearson's Correlation Coefficient

The Pearson's correlation coefficient analysis was performed in different traits in the Pepper plant under V toxicity conditions. Under V toxicity condition, there was a significant reduction in the plant morphological condition, which is revealed by the negative correlation between the plant morphological parameters (FSW, DSW, FRW, DRW, PH, LA, RL, RV, RSA, and ARD and antioxidant enzyme profile (SOD and CAT) (Figure 10). Similarly, VCR and VCS showed to have a negative correlation with plant morphological parameters, which revealed the detrimental effect of V toxicity on plant growth and development. The photosynthetic parameter (SAPD, Chl a, Chl b, Caro, Pn, Gs, Ci and Tr) in pepper plants under V toxicity showed a stronger negative correlation with V toxicity parameters and antioxidant enzymes. However, VUR and VUS do not affect these parameters significantly, as shown in Pearson's correlation analysis (Figure 10). Per contrary, the

morphological parameter showed to have a positive correlation with the photosynthetic parameters, which was obvious, as a higher photosynthetic rate leads to higher biomass and better plant growth and development.
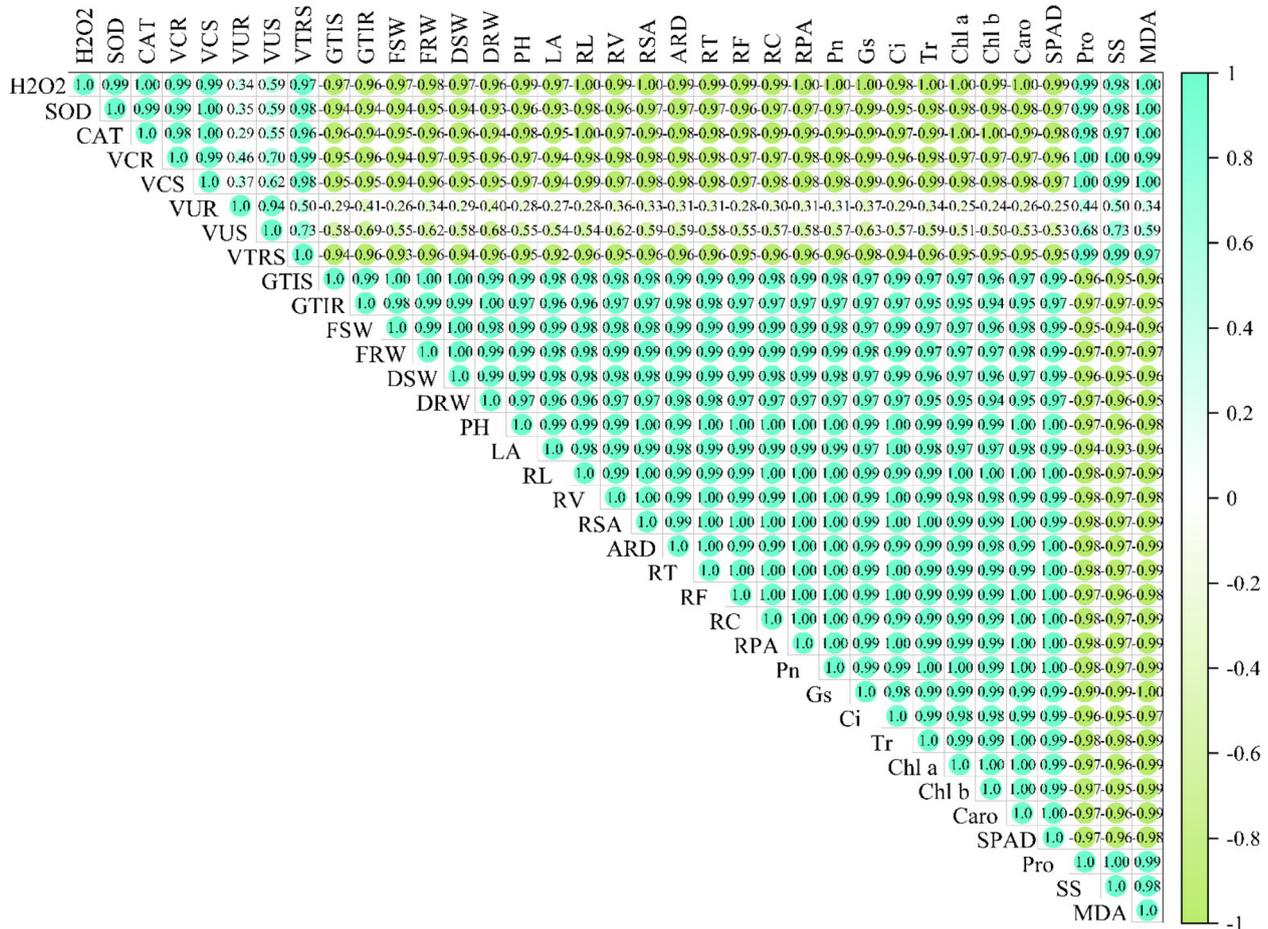
Figure 10. Correlation analysis ($p < 0.05$) between various measured attributes of pepper. The abbreviations are as follows: FSW (fresh shoot weight), DSW (dry shoot weight), FRW (fresh root weight), DRW (dry root weight), PH (plant height), LA (leaf area), RL (root length), RV (root volume), RSA (root surface area), ARD (average root diameter), RT (root tips), RF (root forks), RC (root crossings), RPA (root projected area), Pn (photosynthetic assimilation), Gs (stomatal conductance), Ci (Intercellular CO_2), Tr (transpiration rate), Chl a (chlorophyll a), Chl b (Chlorophyll b), Caro (carotenoids), SPAD (relative chlorophyll content), Pro (proline). SS (soluble sugars), MDA (Malonaldehyde), H_2O_2 (hydrogen peroxide), SOD (superoxide dismutase), CAT (catalase), VCR (vanadium concentration in root), VCS (vanadium concentration in shoot), VUR (vanadium uptake in root), VUS (vanadium uptake in shoot), GTIS (growth tolerance index in root), GTIR (growth tolerance index in root), VTRS (vanadium translocation from root to shoot).

4. Discussion

Agricultural soils are being contaminated by a wide variety of chemical contaminants by anthropogenic activities throughout the globe. According to researchers, heavy metals such as V, cadmium, nickel, and lead enter the soil as a major source of agricultural soil pollution. V has received attention in recent decades as a result of its excessive industrial usage and deposition in the environment, including the atmosphere, water, and agricultural land [14]. Therefore, we examined the physiological, morphological, and biochemical changes in the leaves and roots of pepper seedlings grown hydroponically to determine the influence of V stress on plant growth and development.

Even though beneficial elements are not considered essential, they have the potential to effectively affect plant growth, development, and yield [33]. Each beneficial element has its own particular role, and the effects vary based on its chemical form, dosage, frequency of treatment, and genotype. Beneficial elements stimulate growth at low concentrations, and pose inhibitory or toxic effect at high concentrations [34]. In this study, we found that a low level of V (10 mg) enhanced plant growth as compared to control. Conversely, high levels of V (20, 30, 40 and 50 mg) restricted the growth of pepper seedlings (Figure 1). Heavy metals produce toxicity and considerable reductions in plant growth and development such as FSW, FRW, DSW, DRW, and PH of plants [35]. This kind of decrease normally occurs differently in various parts of the plants [12]. Minor levels increase the growth status of the plants [36]. The results of present study revealed that V (20, 30, 40, and 50 mg) supplementation significantly reduced the growth attributes of pepper seedlings (Table 1, Figure 2). In our recent study, Altaf et al. [20] observed that V (40 mg) application effectively decreased the growth status (FSW, FRW, DSW, DRW) of tomato seedlings. In another work, higher V concentrations negatively affected the growth of rice and chickpea [12,21]. Higher V levels caused a nutritional imbalance and disrupted their involvement in anabolic pathways, interrupting normal developmental processes.

Modifying root structure and root size may improve nutrient absorption and use efficiency, resulting in improved plant development [37]. In our study, minor V (10 mg L^{-1}) level enhanced root morphological traits (root length, root volume, surface area, root tips, root, crossing, and root forks), and higher V level reduced these root characteristics (Figure 4). Similarly, Nawaz et al. [15] reported that V (50 mg) markedly decreased root morphology (root length, root volume, surface area, root tips, root, crossing, and root forks) of watermelon seedlings. In another study, nickel treated tomato seedlings showed a significant reduction in root length, volume, surface area, forks, tips, and crossing [38]. The decrease in root length may be caused by mitotic cell division, which may limit root tip growth [39]. These results are similar to those reported previously by Cai et al. [40] in tomato, Cao et al. [41] in cucumber, and Imtiaz et al. [12] in chickpea.

The Pn represents a plant's capacity to extract CO_2 and is involved in a variety of metabolic activities [42]. Heavy metal toxicity has a detrimental impact on photosynthesis, which is an important part of the carbohydrate synthesis process. The present results revealed that leaf gas exchange elements such as Pn, Gs, Ci, and Tr were considerably reduced in pepper plants by enhancing the V (20, 30, 40, and 50 mg) stress level (Figure 5). Similarly, a previous study by Hasan et al. [43] revealed that Cd high concentration decreased the Pn of tomato seedlings. Higher concentrations of V have a negative impact on the Pn, Tr, and other crucial physiological processes involved in the production of energy, matter, and matter translocation, resulting in decreased crop development and biomass production [44]. V concentrations may cause damage to chloroplast and ultra-structures in plants, as well as disruptions in electron transport mechanisms and photosystem I electron diversions, which may result in a reduction in the photosynthetic activities of plants. The present study' results are consistent with previous investigations that measured plant growth in response to different metal stressors, e.g., pepper under boron toxicity [45] and tomato under Lanthanum, Nickel, and Sulphur toxicity [17,46,47].

Plants can produce an enormous variety of pigment molecules, which are involved in a variety of biochemical mechanisms in chloroplast and mitochondria. The basic pigment molecules for photosynthesis are chlorophyll a, chlorophyll b, and carotenoids. In this research, higher V (20, 30, 40, and 50 mg) concentrations significantly decreased the levels of chlorophyll a, chlorophyll b, and carotenoids in tomato seedlings (Figure 6A–C). In our previous study, V treated tomato seedlings showed a significant decline in pigments content [20]. Similarly, in another study, Sarafi et al. [45] reported that boron (100 µM) supplementation remarkably reduced chlorophyll a, chlorophyll b and carotenoids content of pepper seedlings. Under V stress, photosynthesis pigments were reduced, which might be linked to changes in membrane permeability and component degradation caused by oxidative stress [48]. The production of reactive oxygen species (ROS) is a key element in

the lowering of leaf chlorophyll content [49]. Similar findings were indicated in tomato [17], fenugreeks [50], rice [21], and chickpea [51] under metal stress. In this study, the SPAD value (relative chlorophyll content) also decreased with the increasing level of V (Figure 6D). Similar results are also in line with Nawaz et al. [15] in watermelon under V stress.

A decrease in water content of leaf tissues was linked to a greater accumulation of the osmo-protectant molecules, proline, and soluble sugars, which might lead to cellular dehydration and osmotic stress in pepper seedlings (Figure 7) This hypothesis was confirmed by Jahan et al. [17], who affirmed that under nickel stress, plants exhibited reciprocal behavior by accumulating proline in response to increased cellular dehydration. The concentration of proline and soluble sugars were found to be higher in a stress environment [52]. Additionally, soluble carbohydrates might act as a regular osmo-protectant, protecting cellular membranes and regulating turgor pressure in plant, thereby preventing the detrimental effect of V toxicity.

ROS are produced in plants as a result of an interaction between fatty acids and heavy metals [53]. H_2O_2 is a major element of ROS and is generated in excess when plants come into contact with heavy metals. Our results stated that V application significantly increased the level of H_2O_2 in pepper seedlings (Figure 8A). In a recent study, Altaf et al. [20] found that V supplementation remarkably enhanced the production of H_2O_2 in tomato seedling. In another study, under V stress, the level of H_2O_2 was increased in watermelon seedlings [15]. MDA is a byproduct of lipid peroxidation that is produced mainly under oxidative stress [54]. The present results revealed that the V application markedly enhanced the MDA level of pepper seedlings (Figure 8B). Similarly, Jahan et al. [17] reported that nickel-treated tomato seedlings showed the maximum level of MDA. Similar results were described in rice [21], chickpea [51], watermelon [15], and tomato [20] under V stress.

In plants, the antioxidant defense system and redox homeostasis play a critical role in controlling ROS overproduction and lowering oxidative stress under various environmental conditions. In this work, we observed that the activity of antioxidant enzymes (CAT and SOD) was gradually increased with increasing levels of V (Figure 9). Similarly, when watermelon seedlings were subjected to V stress, antioxidant enzymes' (CAT and SOD) activity was also increased [15]. This increase in enzymatic activity might be related to both direct and indirect effects of heavy metal ions on the generation of free oxygen radicals [55]. In another study, the activity of antioxidant enzymes was increased in cucumber under high iron stress [56]. The findings in this research are consistent with recent findings in oilseed crop under selenium toxicity by Ulhassan et al. [57], tomato under nickel toxicity by Jahan et al. [17], and red cabbage under copper toxicity by Posmyk et al. [58]. The antioxidant enzymes are effective scavengers of ROS and other free radicals, maintaining the stability and integrity of plant cellular membranes under stress.

In our results, with increasing V concentrations, the absorption and accumulation of vanadium in pepper roots and shoots increased. The content of V in the shoot was greater than in the root tissues (Table 2). In Chinese green mustard, Vachirapatama et al. [11] found that the roots absorb more V than the leaves. In our previous study, we observed that V concentration was more in tomato roots than in leaves [20]. In another study, Nawaz et al. [15] found that V content was higher in roots than leaves of watermelon. Similarly, maximum V uptake was observed in the roots for many plant species [21,51,54,59].

5. Conclusions

The obtained results stated that 10 mg L^{-1} V enhanced the growth status of pepper seedlings. Per contra, 20, 30, 40, and 50 mg L^{-1} V significantly restricted the growth by changing the physiological, morphological, and biochemical parameters. The finding revealed that a higher V (20, 30, 40, and 50 mg L^{-1} V) level considerably decreased the leaf gas exchange elements and pigment content, which ultimately caused a decline in pepper growth. The poor root growth was noticed because of V supplementation, and it was also observed that V maximum uptake occurred in roots than the shoot of pepper seedlings. The current results also stated that V application enhanced osmolytes content,

ROS production, and antioxidant enzymes activity. In the future, further research is needed to explore the mechanism and gene expression involved in cell death caused by V toxicity in pepper plants.

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