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Effect of Titanium and Vanadium on Antioxidants Content and Productivity of Red Cabbage

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Abstract: The present work studied the effect of foliar spray of different concentrations of titanium (Ti, applied as titanium dioxide) and vanadium (V, applied as vanadium pentoxide) on growth, chemical composition, antioxidant contents, antioxidant enzymes, antioxidant capacity, yield and quality criteria of red cabbage plants. For this purpose, 2.0, 4.0 and 6.0 mg L^{-1} of Ti and V were used to treat red cabbage plants. The control plants were treated with tap water. Our results showed that plants treated with 4.0 mg L^{-1} of Ti recorded the highest values of plant growth and bioactive compounds, while antioxidant capacity was decreased compared to the other treatments. In addition, plants treated with Ti and V at 2.0 and 4.0 mg L^{-1}, respectively, showed higher values of all of the growth, yield, non-enzymatic antioxidants and antioxidants enzymes' parameters compared to the untreated plants. Based on the obtained results, it could be concluded that the low concentrations of both Ti and V (2.0 and 4.0 mg L^{-1}) were able to enhance red cabbage growth and yield, as well as the antioxidant contents, enzymes and capacity.

Keywords: antioxidant enzymes; non-enzymatic antioxidants; red cabbage; titanium; vanadium

1. Introduction

Red Cabbage (*Brassica oleraceae* L.), which belongs to Cruciferae family, is one of the most important dietary leafy vegetables, due to its high content of bioactive compounds. It contains antioxidants, polyphenols, vitamin C, vitamin E and carotenoids, that can scavenge for free radicals in humans [1].

Titanium (Ti) is categorized as a beneficial element for plants that promotes their growth and development. Ti affects the uptake of nutrients and the activity of several enzymes [2–4]. Ti can be found in high concentrations in the soil. However, the majority of

Ti cannot be absorbed by the plants, due to its mineral forms (such as TiO_2 and $FeTiO_3$) that are insoluble in water [5]. Previous studies showed the beneficial effect of Ti application to increase the yield, vitamin C in fruits and to enhance the uptake of elements in tomato plants [3]. According to the Food and Drug Administration (FDA, Title 21, Volume 1, CITE: 21CFR73.575; https://www.fda.gov/), Ti dioxide is considered to be safe for use if the dose not exceed 1% by weight of the food.

Vanadium (V) is a trace element that is important for plant growth and some biochemical processes [6]. It was reported that V, in low concentration, enhanced plant growth and has an important role in the fixation of nitrogen in plants [7]. During the growth of some plant species, V is considered fundamental for chlorophyll contents and porphyrin biosynthesis. However, application at high rates of V can reduce plant productivity and retard growth [8,9]. García-Jiménez et al. [10] reported that V can regulate the growth and development of pepper plants, even though contrasting effects were found between species and handling circumstances. Another well-known role of V is its participation as a cofactor of the enzyme nitrogenase [11]. According to Imtiaz et al. [12], the V level is approximately 0.025 mg/day, which is found in several multi-vitamin/mineral dietary supplements. The safe level of V for human intake is not known and more research is required. Below 4500 μg/day intake, the toxicity was not recorded [12]. At the moment, regardless of the benefits of V for plant enhancement, V is still classified as a toxic heavy metal and more studies are required to evaluate the negative effects of V on human health [7]. Thus, the present study aims to investigate the effects of Ti and V on yield, content of antioxidant compounds and enzymes and the antioxidant capacity of the red cabbage.

2. Materials and Methods

The trial was conducted for two successive years, 2019 and 2020, at the experimental farm of Mansoura University (Egypt). Red cabbage (*Brassica oleracea* L. var. *capitata*) cv. Nadine 42 days old seedlings were transplanted on the 27th (first experiment) and 29th (second experiment) of October, in 2019 and 2020, respectively. The distance between plants was 50 cm. The experimental plot was 10.5 m^2, consisting of 3 ridges, 0.70 cm wide and 5.0 m long. Titanium dioxide (TiO_2) and vanadium pentoxide (V_2O_5) were used as the sources for Ti and V, respectively. Seven treatments were tested, three concentrations of Ti or V, i.e., 2.0, 4.0 and 6.0 mg L^{-1}, and the control treatment, i.e., foliar application with tap water. The experiment was carried out as a complete randomized block design with three replicates for each treatment. Foliar applications were performed 20, 30, 40, 50 and 60 days after transplanting. Each plant sprayed with 140 mL of Ti and V solutions.

Titanium as titanium dioxide (TiO_2) and vanadium as vanadium pentoxide (V_2O_5) were obtained from Loba Chemie for laboratory reagents and fine chemicals, India.

Five plants were randomly selected from each treatment, after 100 days from planting, to evaluate the following morphological and chemical parameters.

2.1. Vegetative Growth, Yield and Chlorophyll Content

Weight and number of outer leaves.

Total yield was measured as ton/ha. Ten heads were selected randomly to measure the head length (cm) and diameter (cm).

Total chlorophyll content (mg g^{-1} FW) in the outer leaves was determined, according to Sadasivam and Manickam [13]. In brief, fresh samples of red cabbage leaves were extracted in 80% acetone. Then, the extract was filtered by Whatman filter paper 1. Finally, the samples were measured by the spectrophotometer at 663 nm and 645 nm.

2.2. Titanium and Vanadium Contents

Ti and V content in red cabbage leaves were measured, according to the method of Enders and Lehmann [14]. Briefly, fresh samples (5 mg) were digested with a mixture of concentrated H_2O_4, HF and HNO_3 acids, and then 0.10 g of the remaining powder was used to measure Ti and V by an inductively coupled plasma optical emission spectrometer method. The results were recorded as mg/kg^{-1} DM.

2.3. Non-Enzymatic Antioxidants

Vitamin C (Vit. C) (mg 100^{-1} g FW): was estimated by using 2.6-dicholorophenol dye solution (0.2 g L^{-1} water) for titration, according to the method described by Mazumdar and Majumder [15].

Vitamin E (Vit. E) (mg/kg): was determined by spectrophotometric method, according to Prieto et al. [16]. Briefly, fresh samples were extracted with hexane monophasic. The extracted was measured at 695 nm versus the blank.

Carotenoids (mg g^{-1} FW): were determined spectrophotometrically by the procedure reported by Lichtenthaler [17]. Briefly, 50 mg of ground leaves were extracted with 25 mL acetone and left for 24 h. Then, the samples were centrifuged at 14,000 rpm and measured by spectrophotometer at 537, 647, and 663 nm

Total phenols (mg/100 g FW) were estimated by Folin–Ciocalteu's reagent method, as described previously by Awad et al. [18]. Briefly, 2 g of the samples were extracted with methanol for 30 min. Then, a spectrophotometer was used to determine the content of phenolic compounds at 765 nm. Results were expressed as g gallic acid equivalent.

Anthocyanin (mg 100 g^{-1}) was measured, as described by Darwish et al. [19]. Briefly, the samples were extracted with 40 mL of ethanol to quantitatively evaluate anthocyanin content, then centrifuged for 20 min at 6000 rpm. The supernatant was then filtered and was used to determine anthocyanin content by different pH buffers (pH 1.0 and pH 4.5).

2.4. Antioxidants Enzymes

Fresh leaves (0.2 g) were ground in 4 mL of 0.1M ice-cold sodium phosphate buffer (pH 7.0) containing 1% (w:v) polyvinylpyrrolidone (PVP) and 0.1mM EDTA, centrifuged at 10,000× g at 4 °C for 20 min. The supernatant was used for the enzyme activity assays. To calculate the specific activity of different enzymes, total soluble protein was also determined in the supernatant, according to Bradford [20].

Ascorbate peroxidase (APX) (unit mg^{-1} protein) was determined, as described by Zhu et al. [21]. The total volume of the reaction mixture was 2 mL consisting of 25 mM (pH 7.0) sodium phosphate buffer, 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2 and 100 µL enzymes extract. H_2O_2-dependent oxidation of ascorbate was followed by a lowering in the absorbance at 290 nm.

Peroxidase (POX) (unit mg^{-1} protein): was determined, according to Tarchoune et al. [22]. Briefly, samples of leaf were ground and extracted in 50 mM potassium phosphate (pH7). Peroxidase activity (EC 1.11.1.7) was determined as guaiacol-induced peroxidation of H_2O_2. The activities of antioxidant enzymes were calculated as mg^{-1} protein (µmolmg^{-1} protein min^{-1}).

2.5. Antioxidant Capacity

The free radical scavenging procedure DPPH (%) was determined, according to Nuengchamnong and Ingkaninan [23]. Briefly, 100 µL of each extracted sample was mixed with 2.9 mL of 0.05 mM methanolic DPPH solution (2.4 mg DPPH in 100 mL 100% methanol), and absorbance was determined by a spectrophotometer at 517 nm.

2.6. Statistical Analysis

Data were statistically analyzed by SPSS software program (SPSS for Windows, SPSS Inc., Chicago, IL, USA). The data were submitted for ANOVA test. The treatment means were compared using the Duncan test at a $p < 0.05$ level of probability.

3. Results

3.1. Vegetative Growth and Total Chlorophyll

According to the data presented in Figure 1, the application of Ti or V significantly improved Chl a, Chl b, total chlorophyll, the weight and number of the outer leaves compared to the untreated plants. Generally, the highest values were obtained by the treatment of Ti2 (4.0 mg L^{-1}) in Chl a, total chlorophyll, weight and number of outer leaves compared to the other treatments. This increase can be evaluated as 32% in weight, 20.9% in number and 42.5% in total chlorophylls of the outer leaves, respectively, compared to the untreated plants. On the other hand, the treatment of V2 (4.0 mg L^{-1}) showed the maximum Chl b values compared to the other treatments.

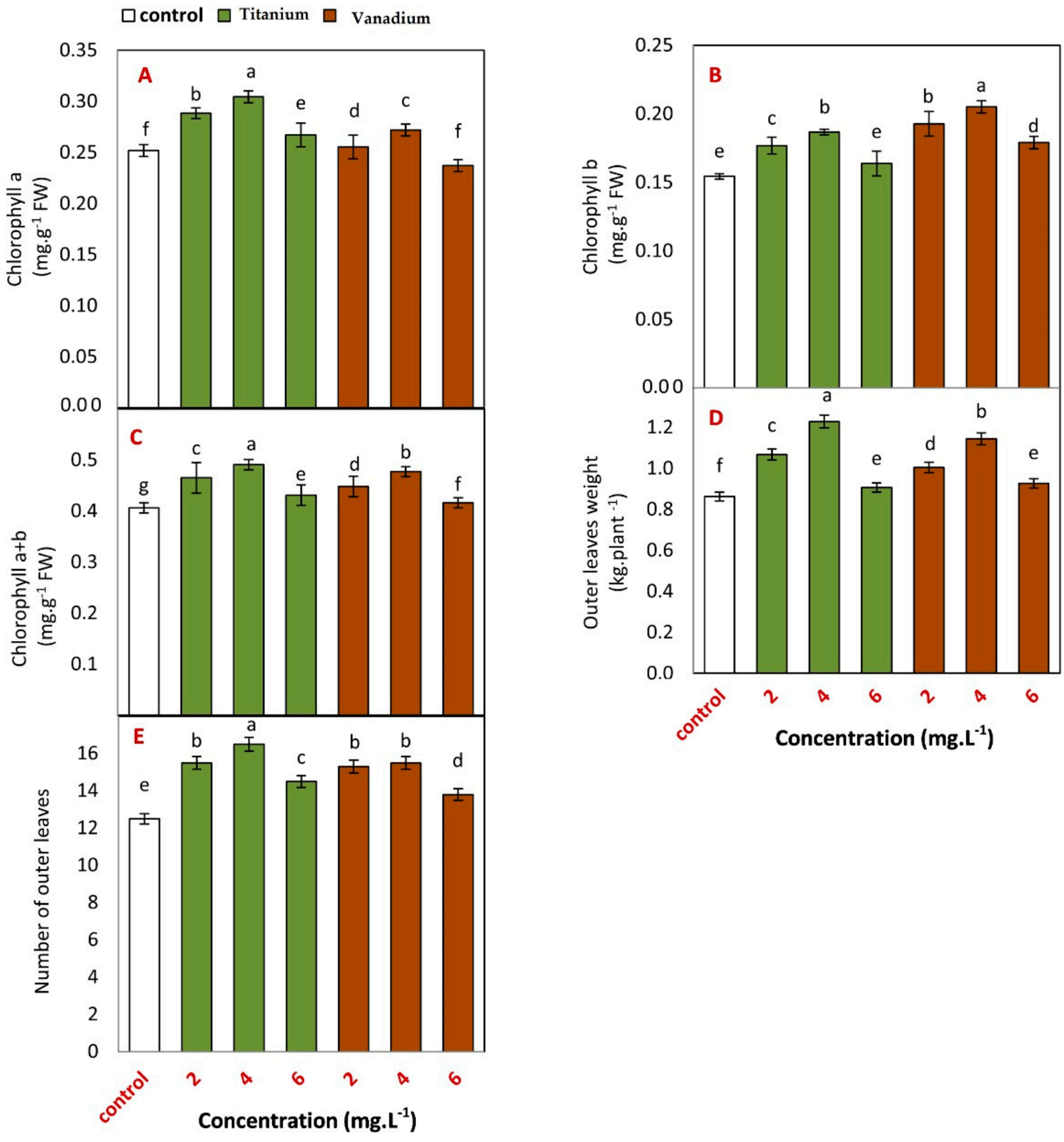

Figure 1. Chlorophyll a (**A**); chlorophyll b (**B**); total chlorophyll (**C**); outer leaves weight (**D**); and number of outer leaves (**E**) of red cabbage plants treated with Ti and V. Data are presented as means of three replicates. Different letters refer to significant difference at $p < 0.05$ according to the Duncan test ($n = 3$).

3.2. Titanium and Vanadium Concentrations

Our results in Figure 2 indicated that the Ti and V contents in red cabbage leaves pronouncedly increased with an increase in the concentration of Ti and V.

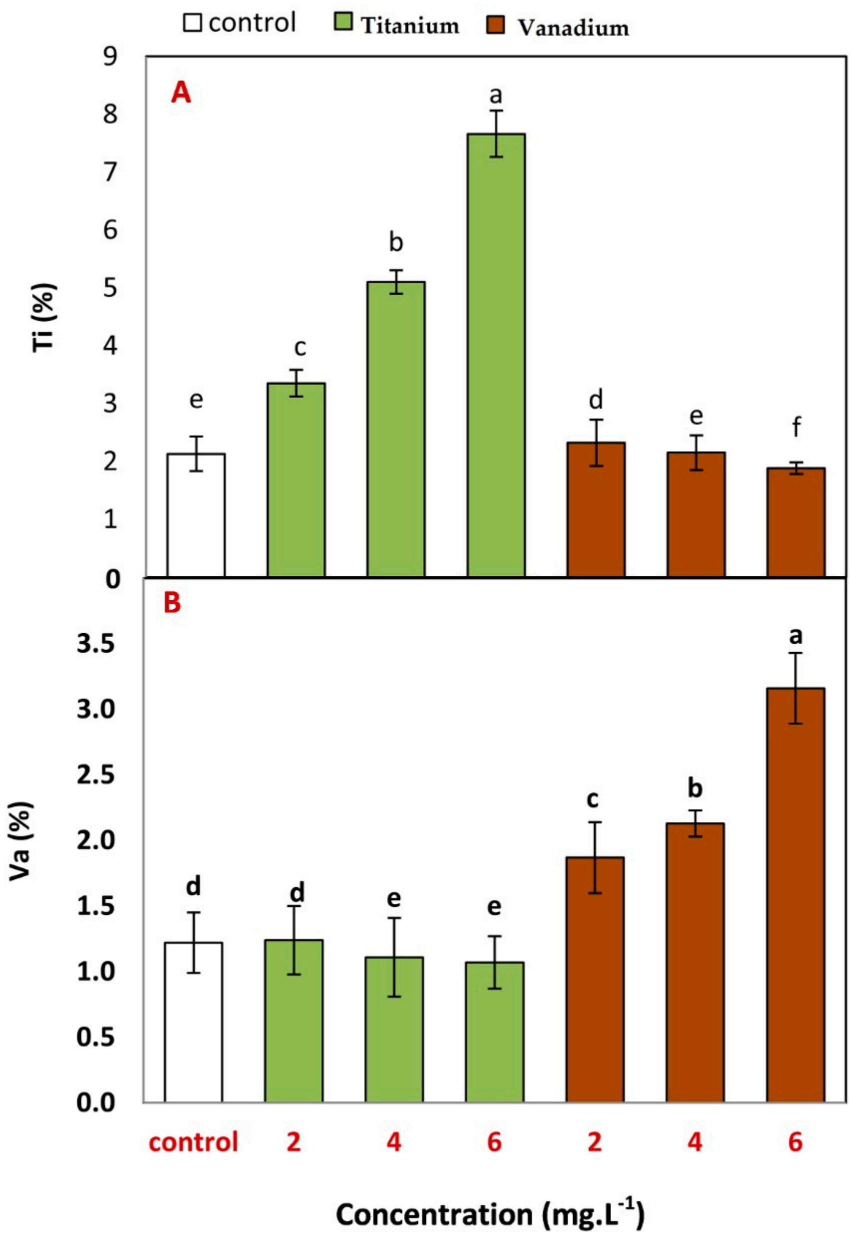

Figure 2. Ti (**A**) and V (**B**) (mg kg^{-1} DM) in red cabbage as affected by foliar applications with Ti and V. Data are presented as means of three replicates. Different letters refer to significant difference at $p < 0.05$ according to the Duncan test ($n = 3$).

3.3. Non-Enzymatic Antioxidants

Results of Figure 3 show the influence of Ti and V applications on the non-enzymatic antioxidants (vitamin C, vitamin E, carotenoids, anthocyanin and total phenols) of red cabbage leaves. Foliar application with Ti2 (4.0 mg L^{-1}) resulted in maximum values of all of the previous parameters compared with the control. In addition, the control treatment showed the lowest values compared to the other treatments. Ti application was more effective than V application for enhancing vitamin C, vitamin E, carotenoids, anthocyanin and total phenols.

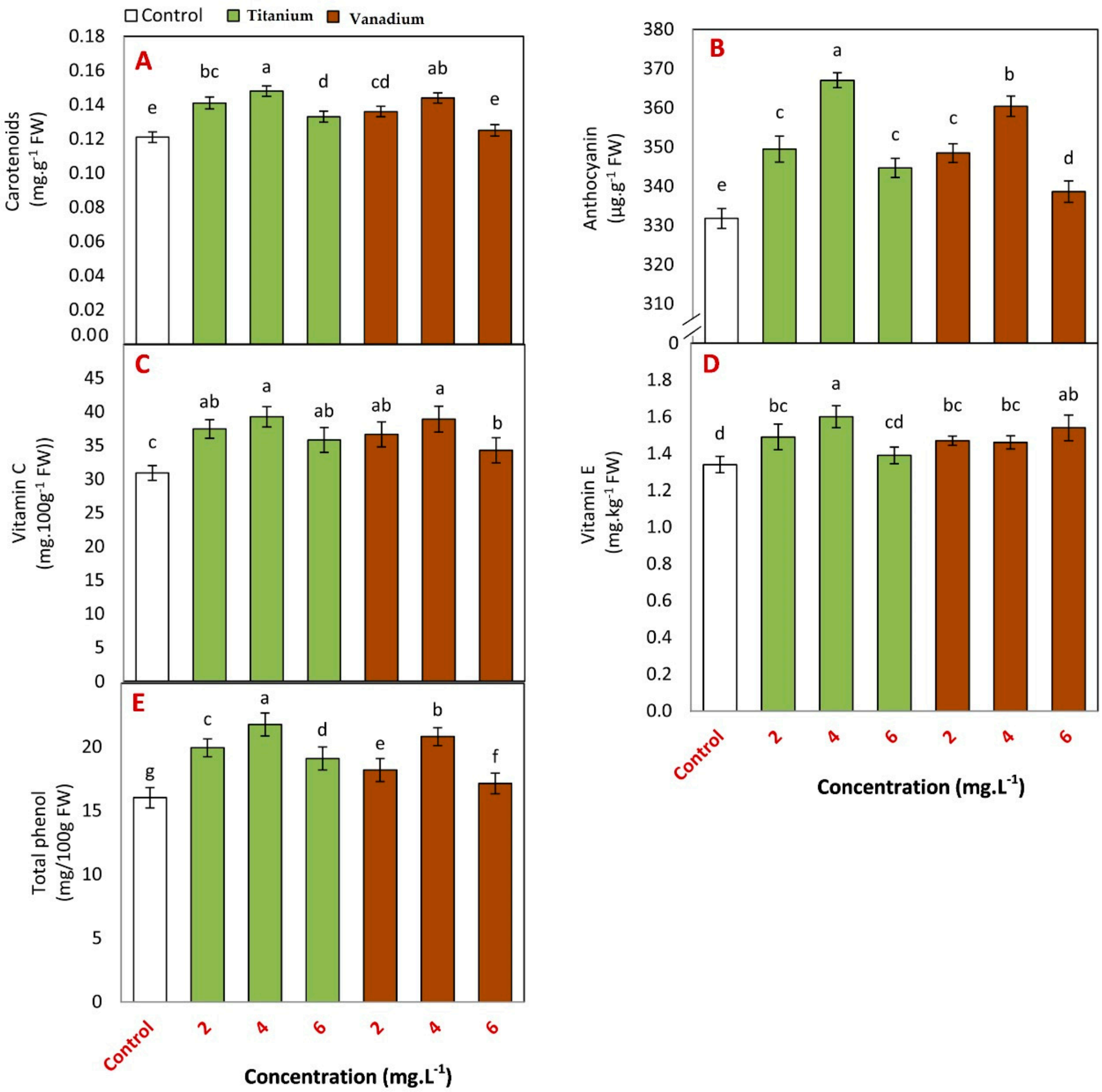

Figure 3. Contents of carotenoids (**A**); anthocyanin (**B**); Vitamin C (**C**); vitamin E (**D**); and total phenols (**E**) in red cabbage leaves as affected by foliar applications with Ti and V. The mean values followed by different letter are significantly different at $p < 0.05$ according to the Duncan test ($n = 3$).

3.4. Antioxidant Enzymes

The anti-oxidant enzyme activities of red cabbage leaves are presented in Figure 4. Ti2 treatment recorded the highest activities of antioxidant enzymes (POX and APX), compared to the other six treatments. Moreover, the values of POX and APX significantly increased as the Ti levels increased up to 4.0 mg L^{-1}, then decreased at Ti 6.0 mg L^{-1}, while the values of APX significantly increased with increasing V levels.

3.5. Antioxidant Capacity

The free radical scavenging procedure DPPH of red cabbage is presented in Figure 5. The maximum value of scavenging activity DPPH obtained from Ti2 followed by V3 plants. Foliar application with Ti2 (4.0 mg L^{-1}) enhanced antioxidant capacity by 13.1% over control treatment.

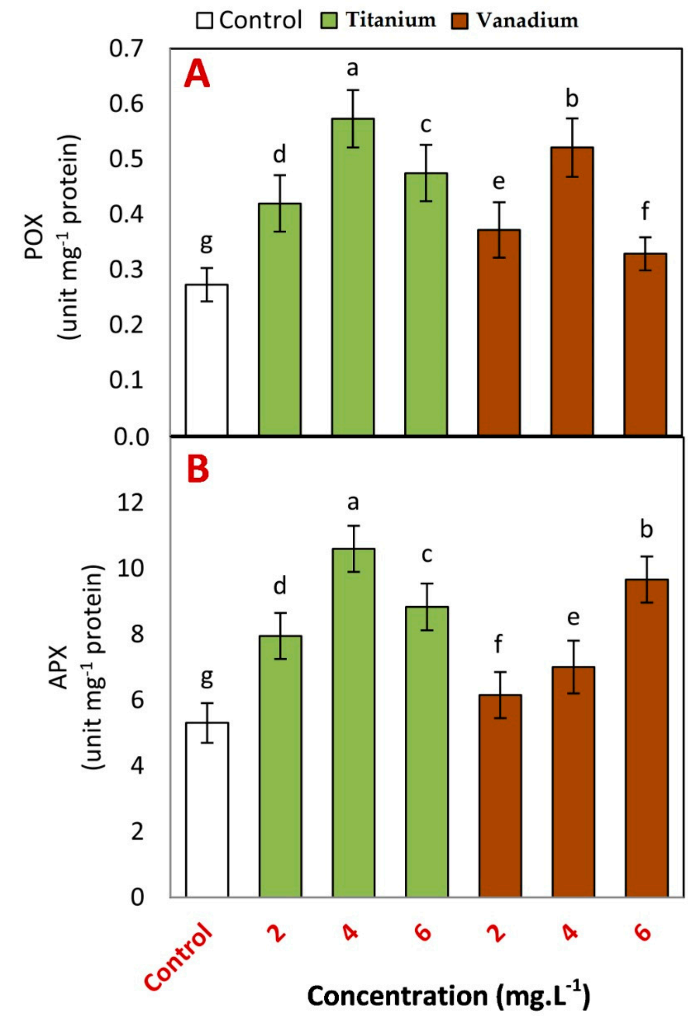

Figure 4. Effect of Ti and V foliar applications on peroxidase (**A**) and ascorbate peroxidase (**B**) activities in red cabbage leaves. The mean values followed by different letter are significantly different at $p < 0.05$ according to the Duncan test ($n = 3$).

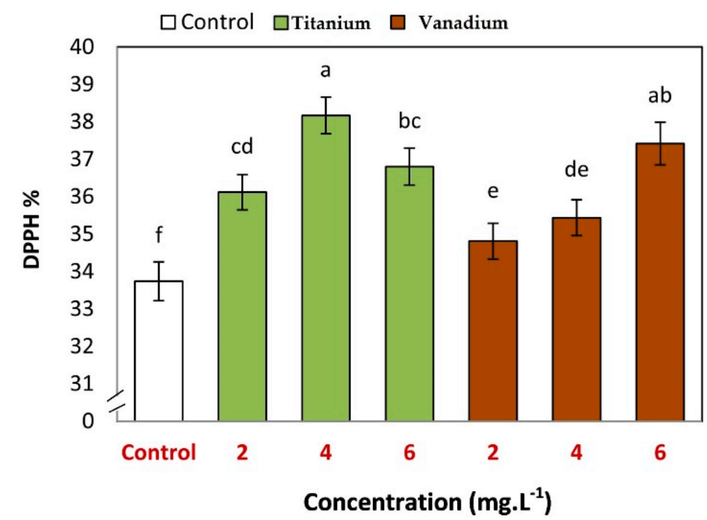

Figure 5. DPPH-scavenging activity of red cabbage leaves treated with different concentration of Ti and V. The mean values followed by a different letter are significantly different at $p < 0.05$ according to the Duncan test ($n = 3$).

3.6. Yield and Head Physical Quality

Figure 6 showed that total yield and physical quality of the head (diameter, length and weight) of red cabbage were significantly improved by the foliar application with Ti and V, compared to the control. The highest values of yield and physical quality of the head were obtained from foliar spraying with Ti at 4.0 mg L^{-1}, while the next treatment was foliar spraying with V at 4.0 mg L^{-1}.

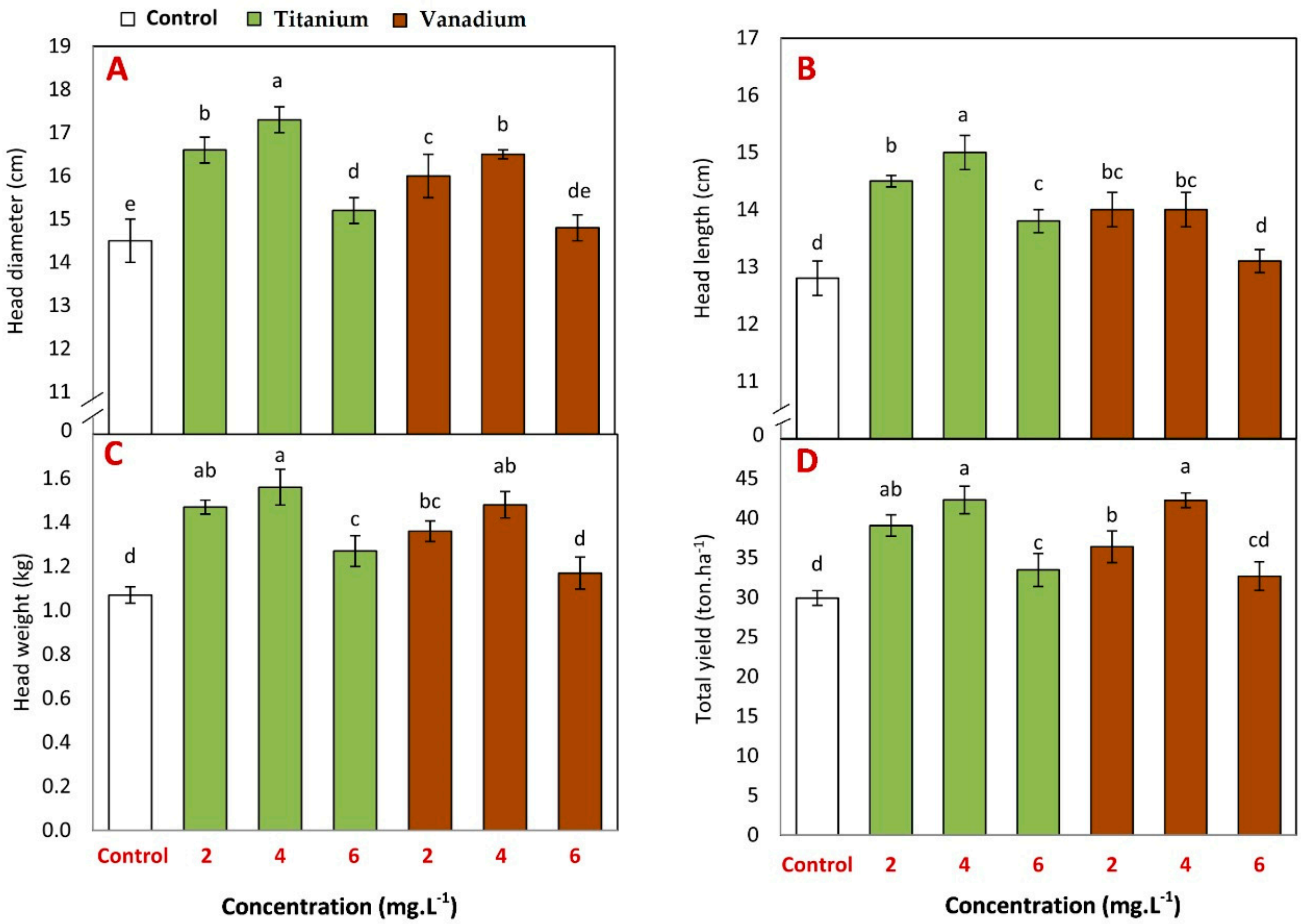

Figure 6. Head diameter (**A**); head length (**B**); head weight (**C**); and total yield (**D**) of red cabbage as affected by foliar applications with Ti and V. The mean values followed by a different letter are significantly different at $p < 0.05$ according to the Duncan test ($n = 3$).

4. Discussion

V is considered a beneficial element at a low concentration for cell growth and development via its conferring better flexibility to the tissues, providing greater water volume that would be related to cell expansion and plant growth [24]. In addition, it helps in accumulation biomass, particularly the formation of new leaves via improved chlorophyll synthesis, amino acids, sugars and other metabolites, especially the enzymatic and non-enzymatic antioxidant system [25]. V application increased the chlorophyll concentration by improving the photosynthetic activity of chloroplasts and improving plant growth [7]. On the contrary, enhancing the V concentration leads to an increase in the bioaccumulation of V in plant tissues and the seriousness of negative symptoms [26] due to overproduction of ROS, which leads to oxidative stress [27]. The reduction in leaf growth with high levels of V may be related to the activity reduction of the enzymes, nitrate reductase and transaminase, involved in the synthesis of amino acids [28]. In addition, a decline in the photosynthetic reactions when V is in high concentration may be due to a disorder of the thylakoid membranes and photosystems in the chloroplasts [29], and nutritional imbalance, as well as disrupting their participation in the anabolic path, disturbing the normal developmental processes [30]. V participates in physiological systems including the normalization of sugar levels and participation in different enzyme systems as an inhibitor and cofactor of amine oxidase. During plant growth of some plants species, V is considered essential for chlorophyll contents and porphyrin biosynthesis. These results are supported by the findings of previous reports [25,26,31]. V is beneficial for plants at low concentrations (2.0 and 4.0 mg L^{-1}), while its toxicity may appear at high levels (6.0 mg L^{-1}).

The positive effect of Ti on chlorophyll content under low concentrations may be due to promoting multi-metal cofactor synthesis, as well as several metal-binding storage proteins, such as phyto-ferritins or to enhance the activities of Fe, Cu, and Zn in leaf cell chloroplasts and cytoplasm [32–34]. This enhancement of photosynthesis consequently enhanced vegetative growth and yield. Raliya et al. [35] found that chlorophyll content was increased in tomato plants by Ti fertilizer. In addition, the improvement of plant growth by Ti application at a low rate could be due to the enhancement of nutrient uptake by Ti application [36].

Our results in Figure 2 indicated that the V content in red cabbage plants was increased by V foliar application, while Ti levels remained constant compared with control. The same trend was observed in Ti content. These results are in agreement with Ram et al. [37] and Pais [38], who found that Ti content was increased by Ti application in the leaves of beans and maize plants, respectively.

In this study, Ti application enhanced the bioactive compounds (vitamin C, vitamin E, carotenoids, anthocyanin and total phenols). In accordance with our results, Biacs et al. [39] found that Ti application increased the carotenoids of red pepper. In addition, vitamin C in tomato and strawberries was increased by Ti application [3,40].

Enhancing antioxidant enzymes' activity in red cabbage by Ti and V application could be correlated with high levels of vitamin C and the total phenols which protect plants from oxidative damage. In addition, APX is unsettled by the loss of ascorbic acid [41–43]. The different results of DPPH under Ti and V application may be due to the reaction of total phenols with free radicals of different chemical structures [1]. Additionally, carotenoids may participate in enhanced free radical scavenging [44].

In the present study, increased Ti levels increased the Ti concentration in leaves. This is incompatible with the results of El-Ghamry et al. [45], in contrast with the present result, Ti application had no influence on the Ti concentration in tomato leaves [33]. This contrariness may be due to the different genotype and concentrations of Ti, which were high in the present study (2, 4, 6 mg L^{-1}) compared to the previous study (1, 2 mg L^{-1}).

In this study, Ti application improved total yield and the red cabbage head characteristics such as weight, diameter and length. Our results are in accordance with Raliya et al. [34], who found that Ti treatment significantly increased the total yield of tomatoes.

Generally, Ti and V increased the chlorophyll content and photosynthesis, resulting in increased plant growth, yield, and antioxidant compounds of red cabbage plants. Higher V concentration retarded the plant growth and reduced the quality. This could be due to the toxicity of V and the damage of the plant roots, which affects their function efficiently [45–47].

5. Conclusions

The results of this study showed that Ti and V application had a positive effect on growth, yield and antioxidant contents of red cabbage plants at a concentration of 2.0 and 4.0 (mg L^{-1}), as shown in Figure 7. However, both of them had a negative effect at the high rate. According to the present results, Ti and V enhance positively the growth and chemical compounds of red cabbage plants at a low concentration. However, more studies are required to investigate the effect of their accumulation on human health.

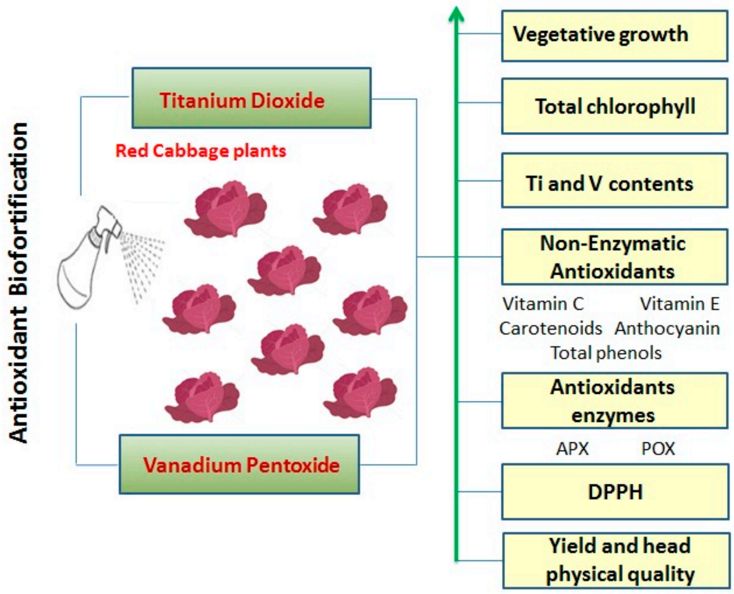

Figure 7. Simplified conclusion for the suggested effect of Ti and V application as a foliar spray on the growth and bioactive compounds of red cabbage plants grown under field conditions.

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