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Combinational Supplements of Organic Amendments Alleviate Copper-Induced Stress in *Withania coagulans* (Dunal)

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Abstract. The contamination with heavy metals in the whole biosphere, especially in soil, is accountable for most of the adverse consequences influencing the prosperity of fauna and flora. A pot experiment was constructed to investigate the effects of Copper (Cu) with and without combinational supplements of organic amendments (OAs) [cow dung, citric acid and amino acid (L-glutamine)] on various physiological and biochemical parameters of *Withania coagulans*. The peat moss soil was treated with two concentrations of Cu [TCu_1 and TCu_2 (10 mM, 15 mM)] alone and/or with organic amendments [cow dung (10 g, 15 g), citric acid (05 mM, 10 mM), and amino acid (05 mM, 10 mM)]. After four weeks, the results revealed that a higher concentration of Cu significantly reduced the plant agronomic traits by 31% (Number of leaves), 17% (Shoot length), 24% (Root length), 19% (Fresh weight), and 35% (Dry weight), whereas photosynthetic pigments by 34% carotenoids, 33% *chlorophyll a* (*chl a*), and 16% *chlorophyll b* (*chl b*) compared to control. Moreover, it was noticed that the combination of organic amendments alleviates the negative effects of a higher concentration of Cu on agronomic traits and photosynthetic pigments of a tested plant. To counteract the oxidative damage caused by the higher concentration of copper, the additional supplements of organic amendments improved the physiology of the plant and increased the activities of antioxidant enzymes. Our findings showed that higher doses of Cu had an impact on the agronomic and biochemical characteristics of *Withania coagulans*, whereas the addition of organic amendments alleviated that impact of Cu.

Keywords: Soil, Heavy metals, Phytoremediation, Organic amendments.

1. Introduction

Soil health is an essential requirement for agricultural sustainability. Presently, agricultural soil is subjected to maximum exploitation by various contaminants that have harmful effects on living organisms and ultimately on human health [1]. Soil contamination refers to the presence of chemical or harmful substances out of place or present at a higher concentration that unfavorably affects any living being [2]. Among the various pollutants, heavy metals (HMs) are maximally deteriorating soil health and are posing a serious threat due to their persistent nature in the environment, via flowing through the food chain and causing carcinogenicity to human beings [3].

Within heavy metals (HMs), copper (Cu) is one of the eight micronutrients that are essential to plant growth and are engaged in a variety of physiological and biochemical processes in plants. As a cofactor for several enzymes, such as laccase, cytochrome *c* oxidase, polyphenol oxidase, amino oxidase, copper/zinc superoxide dismutase (Cu/Zn-SOD), and phycocyanin, copper (Cu) plays an essential role in adverse settings [4]. Cu is also linked to signal modulation, protein trafficking, oxidative phosphorylation, and the metabolism of iron and lipids [5]. Therefore, Cu is a nutrient necessary for plants to have a proper metabolism. Plants grown in surroundings deficient in Cu display several abnormal characteristics, including stunted growth and reproductive development, twisted young leaves, and insufficient water transport [6]. Cu is necessary for laccase, polyphenol oxidase, Cu/Zn-SOD, ethylene receptor, and other multicopper oxidases [7-9]. A subset of the copper oxidase family, including cell wall-attaching amine oxidase enzymes, catalyses the oxidation of putrescine to produce hydrogen peroxide (H_2O_2), an essential compound for lignification, protein cross-linking in cell walls, and programmed cell death [10]. H_2O_2 is a signaling molecule involved in many physiological and biochemical processes. These processes include photosynthesis, senescence delay, cell wall strengthening, plant growth and development, stress tolerance and resistance, and stomatal movement [4].

Overly high concentrations of Cu in plants have an adverse effect on their growth, induce leaf chlorosis, and result in cytotoxicity [11]. The acceptable copper level in food crops is 30 mg kg^{-1}. Additionally, plants that contain too much copper experience oxidative stress due to the production of reactive oxygen species (ROS) that are detrimental to the plants [12]. Superoxide dismutase and peroxidase are essential for scavenging reactive oxygen species (ROS) from plants and lowering Cu toxicity in those plants [13]. Lipid peroxidation-induced oxidative stress damages vital macromolecules and disrupts multiple metabolic pathways [14]. Cu level and growth environment are the main determinants of Cu uptake and transport in plants [15]. However, their concentration in cells must be maintained low since elevated Cu induces modifications in DNA, photosynthesis, cell membrane integrity, enzyme activity, and respiration, which reduces growth and affects plant survival [16]. The most common sign of significant Cu stress in plants is a decrease in plant biomass [4].

Continuous attempts have been made to build technologies that are easy to use, sustainable, and economically viable to conserve good soil and water quality and are free from contamination [17]. Presently, phytoremediation has turned into a powerful, naturally effective, and technical solution used to extract metal contaminants [18]. Many soil factors affect the phytoavailability of metals; among these, organic matter has the greatest impact because it affects other physical and chemical aspects of the soil [19]. Furthermore, organic amendments enhance the physio-chemical characteristics of soil, promote plant development, and aid in the re-vegetation of contaminated soils [20]. Many plant species have been described and checked for their characteristics in the processing and accumulation of various heavy metals [21]. *Withania somnifera* can accumulate 764-944 mg of lindane per acre after 145-days of cultivation [22]. In this study, it was evaluated the negative effects of Cu on *Withania coagulans* under the combinational supplements of various organic amendments (cow dung, citric acid, and amino acid [L- glutamine]).

The medicinal plant *W. coagulans,* which is a member of the Solanaceae family, has demonstrated encouraging results in treating several disorders in models of pathological conditions in humans and animals. The phytochemicals found in *W. coagulans* seeds are important for medicine and have a big effect on a lot of different biological processes. One class of phytochemicals with antibacterial, anti-inflammatory, and anti-allergic qualities is flavonoids. It may also scavenge lipid peroxy radicals, hydroxyl radicals, and superoxide anions [23]. Like this, alkaloids are beneficial substances since they exhibit plant sensitivity to predators and parasites.

These also have the effect of reducing cholesterol. According to published reports, alkaloids can occasionally cause cancer [24]. Glycosides also exhibit hypoglycemia action. While tannins can scavenge free radicals and can exhibit spasmolytic effects in smooth muscle cells, glycosides are often hazardous because they lower heart rate, sympathetic activity, and systemic vascular resistance [25].

2. Materials and Methods

Withania coagulans Dunal, also known as *Puneeria coagulans* Stocks, was chosen as the test plant species for this study. *W. coagulans* seeds that were viable were imported from Pakistan's Khyber Pakhtunkhwa province and recognized by a taxonomist at King Abdulaziz University of Jeddah's Department of Biological Sciences, Faculty of Science. Before planting, the seeds underwent a three-minute immersion in 0.1% mercuric chloride ($HgCl_2$) and were thoroughly cleaned with double-distilled water (DDW). The pots were designed in a randomized manner. The pots were filled with 500 g (per pot) of peat moss soil. The viable seeds (3 seeds per pot) were sown in each pot and were irrigated with 30 ml (per pot) of DDW daily. The seeds germinated after one week of sowing. The young plants were irrigated with Hoagland's solution during the study period (28 days).

Copper was chosen as heavy metal in the form of copper sulfate pentahydrate ($CuSO_4.5H_2O$). $CuSO_4.5H_2O$ was dissolved in 1000 ml distilled water to make stock solutions and given in two concentrations 10 mM and 15 mM. While organic amendments (OAs) i.e., citric acid and L-glutamine were given in 05 mM and 10 mM solution, and cow dung was given in 10 g and 15 g concentrations. Following a 14-day treatment period, every plant in each treatment group was picked independently and packaged for additional analysis. To create a composite sample of each treatment, the matching triplicate of each was combined.

2.1 Assessment of the Agronomic Features

The number of leaves, root-shoot lengths, and fresh-dry weights of the plant are all examples of plant agronomic characteristics [26]. After using a simple electrical equilibrium machine to estimate plant new biomass, the plant samples were left to dry at 72 °C for two days to measure dry weights.

2.2 Estimation of Photosynthetic Pigments

After the fresh leaves were crushed, 0.5 g of the leaves from each treatment were obtained to determine the quantities of chlorophyll-*a*, *b*, and carotenoids. The test materials were properly crushed using a mortar and pestle. Ten milliliters of 80% acetone were mixed with the relevant samples, and the mixture was centrifuged at 5,000 g for ten minutes. The absorbance of the pertinent pigments was determined using a spectrophotometer (UV-1900) at 663, 645, and 470 nm [27].

2.3 Estimation of Proline Concentration

Using the following method, the proline content was calculated. Ten milliliters of liquid nitrogen containing three percent sulfosalicylic acid were used to shatter five grams of fresh leaf samples. For fifteen minutes, the sample was centrifuged at 11,500×g. Two milliliters of the filtered material were combined with two milliliters of glacial acetic acid and ninhydrin. Toluene (4 ml) was added after the mixture was incubated for 60 minutes at 100°C. 520 nm was the OD taken. Proline content was measured as µg/g FW based on a standard curve [28].

2.4 Estimation of Total Phenol Concentration (TPC)

The TPC was determined by applying the following methodology [29]. After blending 100 μl of Folin-Ciocalteu reagent with 850 μl of methanol, 50 μl of the methanolic residue was left to settle at the proper temperature for five minutes. Subsequently, 500 μl of 20% sodium carbonate was introduced, and the mixture was left to react for half an hour. The absorbance at 750 nm was computed. To determine the TPC, the ODs of known gallic acid concentrations were measured and utilized to create a standard curve. The TPC was measured in gallic acid equivalent (g kg FW).

2.5 Estimation of Total Flavonoids Concentration (TFC)

The following technique was employed to estimate TFC [30]. 250 μl of methanolic residue was mixed with 75 μl of $NaNO_2$ (5%) solution and 1.25 ml of deionized water. After being kept for six minutes, the liquid was mixed with 150 μl of a 10% aluminum chloride solution and 0.5 ml of a 1 M sodium hydroxide. Just five minutes later, the mixture was mixed with 275 μl of deionized water. The absorbance of the solution was measured at 510 nm. TFC was calculated using a standard curve with known catechin concentrations, and the flavonoid content was represented by g kg FW catechin equivalent.

2.6 Estimation of Antioxidant Enzymes in Leaves

2.6.1 Crude extract

To create a combination, two grams of leaf material that had been taken from each treatment were crushed using buffer for tris–HCl (20 mM, pH 7.2). The mixture was centrifuged at 10,000 rpm for ten minutes. 4ºC was set as the fixed temperature. For the antioxidant enzyme assay, the supernatant was maintained at -20 ºC.

2.6.2 Polyphenol oxidase activity (PPO)

The PPO was estimated using the method described [31]. The PPO activity was determined with catechol serving as the substrate. 0.2 ml of the extract was quickly added to 2.8 ml of the substrate (20 mM) solution that had been prepared in 0.01 M BPS (pH 6.8). The OD was measured at 400 nm and recorded for three minutes using a spectrophotometer. The enzyme activity was expressed as the rate at which the enzyme modifies the OD by 0.1 per minute.

2.6.3 Peroxidase activity (POD)

The POD activity was computed utilizing the following methods [32]. 0.08 ml of 0.5 M guaiacols, 0.25 ml of 0.2 M BPS (pH 5.5), 0.008 ml of 0.97 M H_2O_2, and the least amount of catalyst formed the reaction mixture. The spectrophotometer was used to measure the OD value fluctuation at 470 nm for one minute. The number of enzymes that altered the OD at a pace of 1.0 nm per minute under standard test conditions was used to describe the activity of the enzyme.

2.6.4 Catalase activity (CAT)

The methodology used to estimate CAT activity was followed [33]. A 2-milliliter substrate solution contained 0.5 milliliter of the plant extract and 25 millimeters of H_2O_2 in a 75-milliliter phosphate buffer solution (pH 7.0). A spectrophotometer was used to measure the OD for one minute at 240 nm. The enzyme activity for the other antioxidant enzymes was ascertained using prior references.

2.6.5 Statistical analysis

The experimental data was analyzed in a randomized design with three copies of each treatment using analysis of variance (ANOVA). The statistical programming tool SAS (SAS Institute Inc., 2000, Cary, NC, USA) was utilized for this. The Tukey test was applied to compare the mean values using one-way ANOVA, with a P value of $P < 5\%$.

3. Results

3.1 The Effect of Organic Amendments on the Agronomic Traits of W. Coagulans under Cu-Stress

Higher concentration of copper (TCu_2) significantly inhibited the morphological characteristics of *Withania coagulans* by 31% (Number of leaves), 17% (Shoot length), 24% (Root length), 19% (Fresh weight), and 35% (Dry weight) compared to control. On the other hand, the application of combinational supplements of organic amendments (OAs) reduced the adverse effects of TCu_2 on agronomic features and greatly enhanced the morphological characteristics of *Withania coagulans*. In contrast to TCu_2 alone, the inclusion of amendments along TCu_2 greatly increased the following metrics: 16% (Number of leaves), 9% (Shoot length), 18% (Root length), 50% (Fresh weight), and 59% (Dry weight). The impact of a lower concentration of copper (TCu_1) has improved the agronomic traits of the tested plant significantly as compared to the control: 32% (Number of leaves), 18% (Shoot length), 27% (Root length), 42% (Fresh weight), and 44% (Dry weight). Furthermore, TCu_1 with OAs compared to control has more significantly improved the morphological traits by 48-60% (Number of leaves), 35-53% (Shoot length), 48-66% (Root length), 53-61% (Fresh weight), and 80-95% (Dry weight) as shown in Fig.1.

3.2 The Effect of Organic Amendments on the Content of the Photosynthetic Pigment in Leaves of W. Coagulans under Cu-Stress

Comparing TCu_2 to control, photosynthetic pigments showed a significant reduction, followed by *Chl a* (33%), *Chl b* (16%), and carotenoids (34%). In contrast, TCu_1 showed a significant improvement in photosynthetic pigments, with *Chl a* (6%), *Chl b* (12%), and carotenoids (41%), showing an increase over control. The significant improvement of photosynthetic pigments under OAs with TCu_2 is as *Chl a* (6%), *Chl b* (15%), and Carotenoids (67%) compared to TCu_2 alone. Moreover, TCu_1 with OAs significantly improved *Chl a* 10-14%, *Chl b* 32-41%, and Carotenoids 153-171%. In our study, it was noted that the increase in the concentration of OAs resulted in the alleviation of copper-induced stress and a significant increase in the concentration of photosynthetic pigments (Fig. 2).

3.3 The Effect of Organic Amendments on the Proline Content of W. coagulans under Cu-Stress

TCu_2 significantly increased the concentration of proline in the tested plant by 209% as compared to the control, whereas there were no significant between TCu_1 alone and with amendments and control. The concentration of proline in the tested plant due to the negative impact of TCu_2 was alleviated by combinational supplements of OAs which were followed by 108-98% as compared to TCu_2 alone (Fig.3).

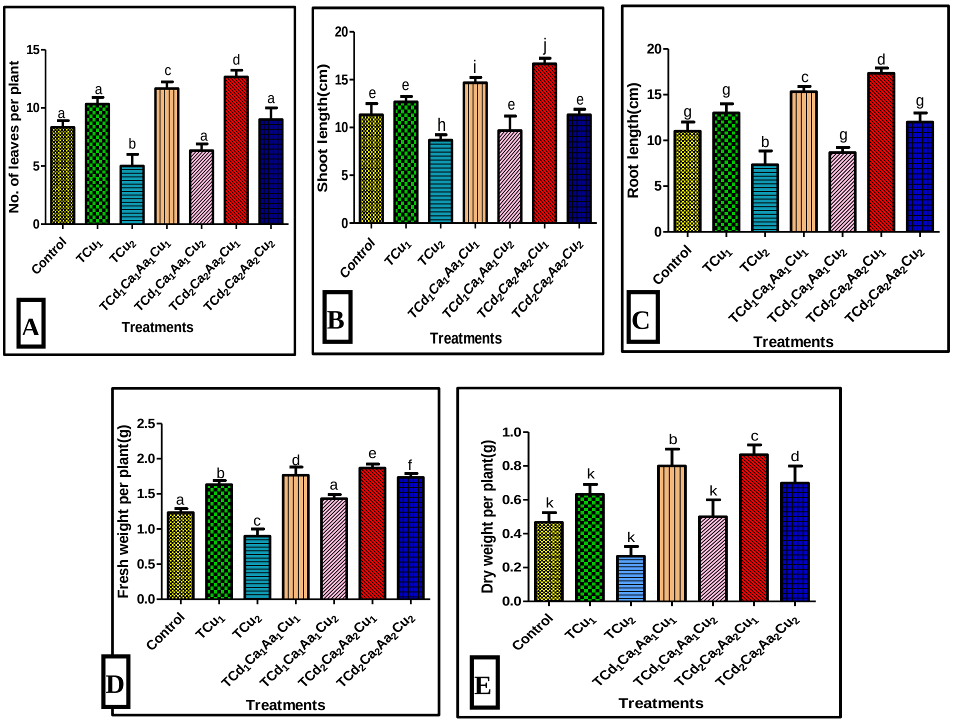

Fig. 1. The effect of organic amendments on the agronomic traits of *W. coagulans* under Cu-stress. (A) The number of leaves, (B) Shoot length, (C) Root length, (D) Fresh weight per plant, (E) Dry weight per plant. The Tukey test indicates that values with different letters are substantially different at P ≤ 0.05. Vertical bars show the ± SD of means for three replicates.

Keys; T: Treatment, Cu: Copper, Cd: Cow Dung, Ca: Citric Acid, Aa: Amino Acid, Cu_1: first conc. of copper (10 mM), Cu_2: second conc. of copper (15 mM), Cd_1: first conc. of cow dung (10 g) Cd_2: second conc. cow dung (15 g), Ca_1: first conc. of citric acid (05 mM), Ca_2: second conc. citric acid (10 mM), Aa_1: first conc. of amino acid (05 mM), Aa_2: second conc. of an amino acid (10 mM)

3.4 The Effect of Organic Amendments on the Antioxidant Content of Leaves of W. Coagulans under Cu-Stress

Total phenol content (TPC) in leaves was significantly higher in the tested plant grown in TCu_2 which was as follows: 168% as compared to the control. However, the application of OAs along TCu_2 resulted in relatively low values of TPC: 93%, indicating the alleviation of copper-induced stress (Fig.4). The total flavonoid content (TFC) in leaves was substantially higher (217%) in *Withania coagulans* grown in TCu_2 soil compared to the control (Fig.4). On the other hand, the addition of OAs mitigates the effect of TCu_2 and reduced significantly TFC by 110% compared to TCu_2. The increase in flavonoid content of plants grown in the contamination might be a defense mechanism for plants against abiotic stresses. There was no significant between TCu_1 alone, with OAs, and control (Fig.4).

3.5 The Effect of Organic Amendments on the Antioxidant Enzymes Activity of Leaves of W. Coagulans under Cu-Stress

In contrast, the addition of OAs with TCu_2 significantly reduced the activities of antioxidant enzymes by 35%, 158%, and 211%, compared to TCu_2 alone. This was observed in our study where the activities of antioxidant enzymes, namely polyphenol oxidase (PPO), peroxidase (POD), and catalase (CAT), were observed to be significantly increased under TCu_2, as compared to control: PPO; 77%, POD; 220%, and CAT; 267%. Furthermore, compared to control, the activities of antioxidant enzymes were considerably increased by 33-7%, 93-71%, and 155-123% with TCu_1 alone and in combination with OAs.

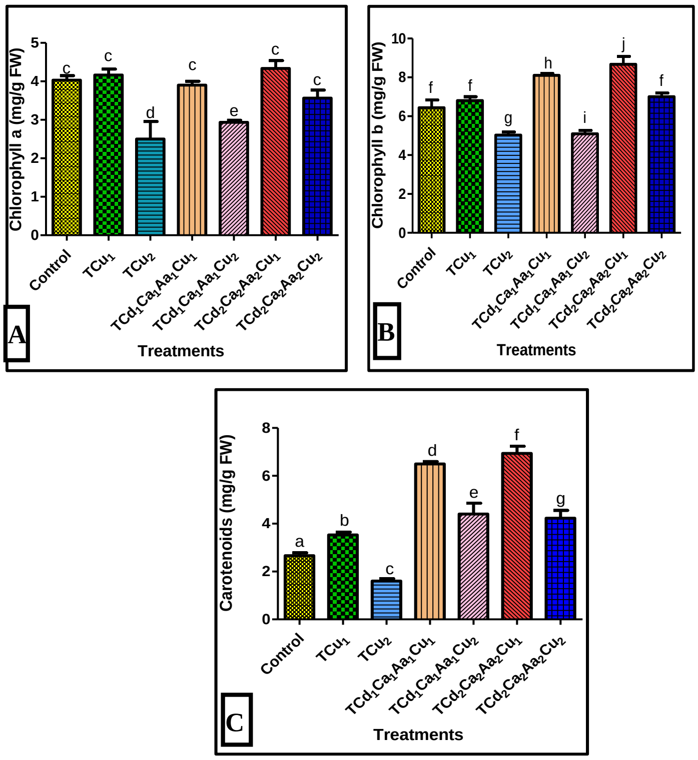

Fig. 2. The effect of organic amendments on the content of the photosynthetic pigment in leaves of *W. coagulans* under Cu-stress. (A) Chlorophyll a, (B) Chlorophyll b, (C) Carotenoids. The Tukey test indicates that values with different letters are substantially different at $P \leq 0.05$. Vertical bars show the ± SD of means for three replicates.

Keys; T: Treatment, Cu: Copper, Cd: Cow Dung, Ca: Citric Acid, Aa: Amino Acid, Cu_1: first conc. of copper (10 mM), Cu_2: second conc. of copper (15 mM), Cd_1: first conc. of cow dung (10 g) Cd_2: second conc. cow dung (15 g), Ca_1: first conc. of citric acid (05 mM), Ca_2: second conc. citric acid (10 mM), Aa_1: first conc. of amino acid (05 mM), Aa_2: second conc. of an amino acid (10 mM)

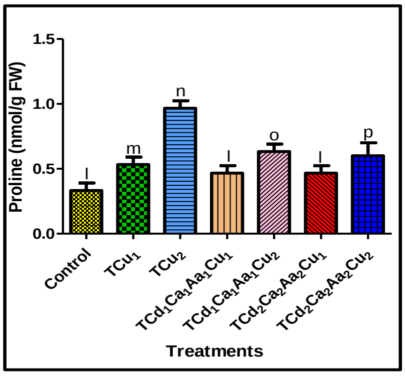

Fig. 3. The effect of organic amendments on the proline content of *W. coagulans* under Cu-stress. The Tukey test indicates that values with different letters are substantially different at $P \leq 0.05$. Vertical bars show the ± SD of means for three replicates.

Keys; T: Treatment, Cu: Copper, Cd: Cow Dung, Ca: Citric Acid, Aa: Amino Acid, Cu_1: first conc. of copper (10 mM), Cu_2: second conc. of copper (15 mM), Cd_1: first conc. of cow dung (10 g) Cd_2: second conc. cow dung (15 g), Ca_1: first conc. of citric acid (05 mM), Ca_2: second conc. citric acid (10 mM), Aa_1: first conc. of amino acid (05 mM), Aa_2: second conc. of an amino acid (10 mM)

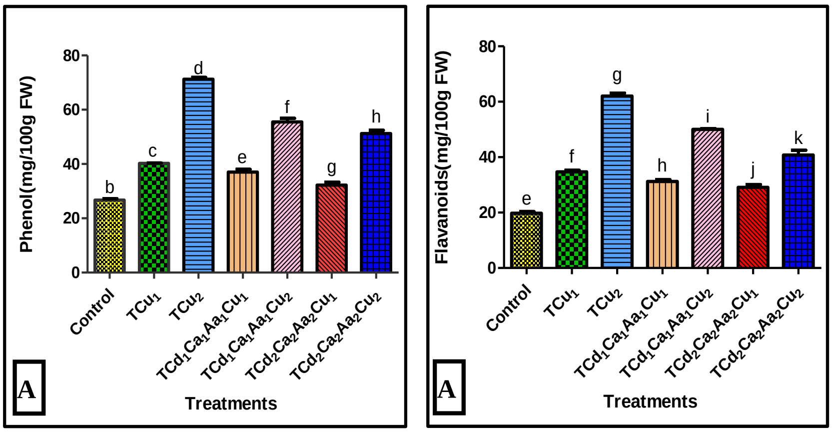

Fig. 4. The effect of organic amendments on the antioxidant content of leaves of *W. coagulans* under Cu-stress. (A) Phenol, (B) Flavanoids. The Tukey test indicates that values with different letters are substantially different at P ≤ 0.05. Vertical bars show the ± SD of means for three replicates.

Keys; T: Treatment, Cu: Copper, Cd: Cow Dung, Ca: Citric Acid, Aa: Amino Acid, Cu_1: first conc. of copper (10 mM), Cu_2: second conc. of copper (15 mM), Cd_1: first conc. of cow dung (10 g) Cd_2: second conc. cow dung (15 g), Ca_1: first conc. of citric acid (05 mM), Ca_2: second conc. citric acid (10 mM), Aa_1: first conc. of amino acid (05 mM), Aa_2: second conc. of an amino acid (10 mM)

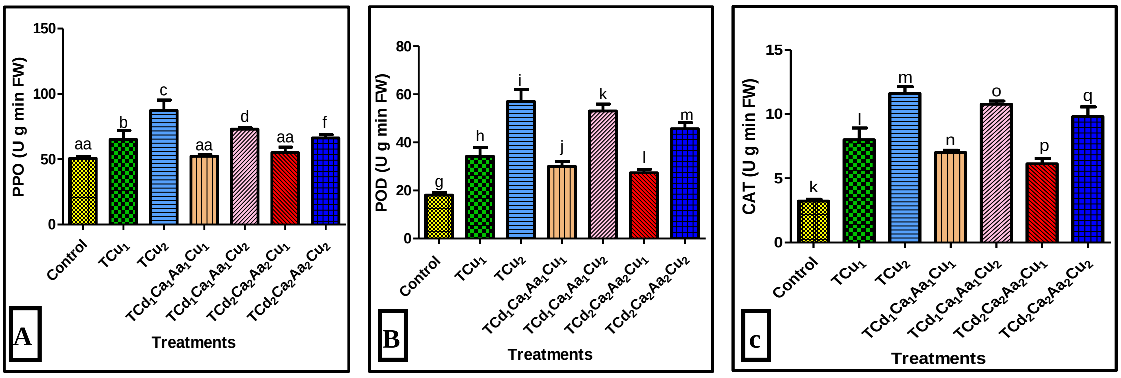

Fig. 5. The effect of organic amendments on the antioxidant enzymes activity of leaves of *W. coagulans* under Cu-stress. (A) PPO, (B) POD, (C) CAT. The Tukey test indicates that values with different letters are substantially different at P ≤ 0.05. Vertical bars show the ± SD of means for three replicates.

Keys; T: Treatment, Cu: Copper, Cd: Cow Dung, Ca: Citric Acid, Aa: Amino Acid, Cu_1: first conc. of copper (10 mM), Cu_2: second conc. of copper (15 mM), Cd_1: first conc. of cow dung (10 g) Cd_2: second conc. cow dung (15 g), Ca_1: first conc. of citric acid (05 mM), Ca_2 : second conc. citric acid (10 mM), Aa_1: first conc. of amino acid (05 mM), Aa_2: second conc. of an amino acid (10 mM)

4. Discussion

This study determines the efficacy of *Withania coagulans* in conjunction with organic amendments (OAs) and assesses the effects of higher copper (Cu) contents. Previous studies found that heavy metal intake inhibited plant growth by preventing food absorption and inhibiting all metabolic processes [34]. Our study revealed that a high concentration of Cu-induced stress had a significant effect on the growth of *Withania coagulans*. Still, the stress caused by Cu was reduced by the addition of OAs. Spinach exposed to OAs absorbs more essential nutrients and less heavy metals [35]. OAs cause spinach's cadmium to become immobile [36]. Citric acid (CA) applied as an organic amendment considerably lessened the chromium-induced inhibition of plant growth [37,38]. Other researchers have observed similar results in *Helianthus annus*, *Brassica napus*, *Oryza sativa*, and *Sorghum bicolor*, especially in tall fescue and Kentucky bluegrass, effectively attenuating the adverse effects of heavy metals

[39-44]. Previous research has shown that this boost in plant growth may be the result of plants' phyto-chelation (PC) of hazardous metals [45]. Numerous components of cells, including proline, can increase the activity of cellular enzymes and improve their capacity to remove heavy metals from the body. Additionally, it has been found that organic acids strengthen plants' defenses against stress from heavy metals [46].

Leaf chlorosis, which is brought on by damage to chlorophyll pigments, is one typical adverse consequence of heavy metal stress. The examined plant cultivated with lower levels of contamination had leaf contents that were noticeably higher than those of the plant grown with higher levels of contamination, according to our findings. The results of earlier research demonstrated that lower levels of contamination resulted in lower quantities of chlorophyll-*a* and chlorophyll-*b* in leaves are in line with these findings [47-50]. Moreover, studies have demonstrated that in contaminated environments, heavy metals decrease plants' photosynthetic efficiency, which causes the leaves of plants to lose their carotenoids and chlorophyll [51]. The amount of carotenoid in the leaves of a plant cultivated with a greater concentration of Cu was significantly lower. There was a discernible decrease in carotenoids. The carotenoid content of leaves was higher at 0.36 mg/g FW and significantly lower in contaminated Uniben Woods forest at 0.006 mg/g FW [52]. Similarly, as compared to contaminated areas, the non-polluted zone has a higher carotenoid concentration, according to studies by [47, 50]. In our experiment, we found that a rise in OAs concentration was associated with a decrease in stress caused by copper and an increase in the concentration of photosynthetic pigment (Fig. 2). The addition of biochar increased the concentration of photosynthetic pigments even in the presence of heavy metals [53]. Adding charcoal and OAs can lessen the stress brought on by metals [54].

The plant under examination has significantly more proline in its leaves due to growing in a higher proportion of soil contaminated with copper. These results are in line with those of other researchers [55,56] who discovered that proline concentrations in *Eucalyptus sp.* and *Mangifera indica* rose due to stress produced by metals. Furthermore, some plant species had noticeably higher proline concentrations in their leaves, suggesting that these plants have defense mechanisms in place when they are stressed by heavy metals [57]. OAs enhance plants' metabolic systems and reduce cadmium absorption [58].

Triticum aestivum exposed to heavy metal stress showed an increase in the concentration of non-enzymatic antioxidants when coupled with *Bacillus sp.* and citric acid (CA) chelate [59]. More Cu administered to the plant increased its cellular total phenol content (TPC), which is in line with research, that indicated test plants growing in metal-contaminated soil had noticeably higher TPC concentrations [60]. A plant cultivated in soil infused with copper showed a notably greater total flavonoid content (TFC) in its leaves when compared to the control (Fig. 4). Increasing TFC content in plants is a result of increased metal concentration in the growing medium [61]. The leaves of the *Erica andevalensis* species show a much higher TFC content when grown under cadmium stress [62]. Plants grown in polluted areas may increase the amount of flavonoids in their leaves as a means of defending against abiotic stresses [63].

In our study, we found that a plant growing with a greater concentration of Cu had noticeably more antioxidant enzymes. In the presence of heavy metal toxicity, plants' antioxidant defense mechanism is activated, and reactive oxygen species (ROS) are generated [64,65]. Heavy metals may trigger the expression of genes that code for stress-response proteins including metallothionein and phytochelatins, as well as the activity of antioxidant enzymes that scavenge reactive oxygen species [66]. The current work suggests that comparable tolerance mechanisms in *Withania coagulans* might also become activated in response to high Cu concentrations. Heavy metal stress affects PPO activity in meadow-fescue and Trifolium leaves [67]. Higher levels of cadmium and mercury stimulate the activity of antioxidant enzymes in the leaves of *Raphanus sp* [12]. According to scientific evidence, plants under heavy metal stress have much higher catalase activity levels [68].

5. Conclusion

High amounts of copper (Cu) significantly diminish the morpho-physiological and biochemical traits of *Withania coagulans*. The development of *Withania coagulans* is regulated by increasing the activity of antioxidant enzymes when organic amendments (OAs) and Cu are combined. Higher dosages of Cu had a more detrimental effect than the other treatments. Furthermore, as shown in Fig. 1, the morphological characteristics of *Withania coagulans* considerably enhanced with the addition of OAs. For instance, the addition of amendments along with a higher concentration of Cu significantly improved the morphology of *Withania coagulans,* as evidenced by measurements of 16% (number of leaves), 9% (shoot length), 18% (root length), 50% (fresh weight), and 59% (dry weight). Enhancing the agronomical and biochemical condition of this plant is one way that OAs help it adapt to Cu stress. Because OAs are widely accessible and reasonably priced, they provide a reasonable approach to mitigating the environmental risks associated with heavy metal-contaminated soils in situations when money is insufficient for effective remediation efforts. Our findings acknowledge the advantages of using OAs in the phyto-stabilization approach.

References

[1] Zaynab M, Al-Yahyai R, Ameen A, Sharif Y, Ali L, Fatima M, LiS (2022). Health and environmental effects of heavy metals. *Journal of King Saud University Science 34(1):* 101653.

[2] Sulaymon, Mei X, Yang S, Chen S, Zhang Y, Hopke PK (2020). PM 2.5 in Abuja,Nigeria: chemical characterization, source apportionment, temporal variations, transport pathways, and the health risks assessment. *AtmosRes 237*.

[3] Shams M, Tavakkoli Nezhad N, Dehghan A, AlidadiH, Paydar M, Mohammadi A A,Zarei A. (2022). Heavy metals exposure, carcinogenic and non-carcinogenic human health risks assessment of groundwater around mines in Joghatai, Iran. *International Journal of Environmental Analytical Chemistry 102(8):* 1884-1899.

[4] Nazir, F., Hussain, A., & Fariduddin, Q. (2019). Hydrogen peroxide modulate photosynthesis and antioxidant systems in tomato (Solanum lycopersicum L.) plants under copper stress. *Chemosphere*, *230*, 544-558.

[5] Leng, X., Mu, Q., Wang, X., Li, X., Zhu, X., Shangguan, L., & Fang, J. (2015). Transporters, chaperones, and P-type ATPases controlling grapevine copper homeostasis. *Functional & integrative genomics*, *15*, 673-684

[6] Zhang, D., Liu, X., Ma, J., Yang, H., Zhang, W., & Li, C. (2019). Genotypic differences and glutathione metabolism response in wheat exposed to copper. *Environmental and experimental botany*, *157*, 250-259

[7] Festa, R. A., & Thiele, D. J. (2011). Copper: an essential metal in biology. *Current Biology*, *21*(21), R877-R883.

[8] Rodrıguez, F. I., Esch, J. J., Hall, A. E., Binder, B. M., Schaller, G. E., & Bleecker, A. B. (1999). A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. *Science, 283*(5404), 996-998

[9] Choi, M., & Davidson, V. L. (2011). Cupredoxins—a study of how proteins may evolve to use metals for bioenergetic processes. *Metallomics*, *3*(2), 140-151.

[10] Møller, S. G., & McPherson, M. J. (1998). Developmental expression and biochemical analysis of the Arabidopsis atao1 gene encoding an H2O2-generating diamine oxidase. *The Plant Journal*, *13*(6), 781-791.

[11] Saleem, M. H., Kamran, M., Zhou, Y., Parveen, A., Rehman, M., Ahmar, S., Liu, L., (2020a). Appraising growth, oxidative stress and copper phytoextraction potential of flax (*Linum usitatissimum* L.) grown in soil differentially spiked with copper. *J. Environ. Manag.257*, 109994.

[12] Sharma N, HundalGS, Sharma I, BhardwajR (2012). Effect of 24-epibrassinolide on protein content and activities of glutathione-s-transferase and polyphenol oxidase in *Raphanus sativus* L. plants under cadmium and mercury metal stress. *Terrestrial Aquatic EnvironToxicol 6 (1):* 1-7.

[13] Huang, W.L., Wu, F.L., Huang, H.Y., Huang, W.T., Deng, C.L., Yang, L.T., Chen, L.S., 17 (2020). Excess Copper-Induced Alterations of Protein Profiles and Related Physiological 18 Parameters in Citrus Leaves. *Plants 9(3),* 291.

[14] Juang, K.W., Lo, Y.C., Chen, T.H., Chen, B.C., (2019). Effects of copper on root morphology, cations accumulation, and oxidative stress of grapevine seedlings. *Bull. Environ. Contam. Toxicol. 102(6),* 873-879.

[15] Saleem, M.H., Fahad, S., Khan, S.U., Din, M., Ullah, A., Sabagh, A.E., Liu, L., (2020b). Copper-induced oxidative stress, initiation of antioxidants and phytoremediation potential of flax (*Linum usitatissimum* L.) seedlings grown under the mixing of two different soils of China. *Environ. Sci. Pollut. Res. 27(5),* 5211-5221.

[16] Nair, P.M.G., Chung, I.M., (2015). Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (*Brassica juncea L.). Ecotoxicol. Environ. Saf. 113,* 302-313.

[17] Obaideen K, Shehata N, Sayed ET, Abdelkareem M A, Mahmoud MS, Olabi AG (2022). The role of wastewater treatment in achieving sustainable development goals (SDGs) and sustainability guideline. *Energy Nexus,* 100112.

[18] Rajendran S, Priya TAK, KhooK S, Hoang T K, NgHS, Munawaroh H S H, Show P L (2022). A critical review on various remediation approaches for heavy metal contaminants removal from contaminated soils. *Chemosphere 287:* 132369.

[19] Seregin, I., Kozhevnikova, A., (2006). The physiological role of nickel and its toxic effects on higher plants. *Russ. J. Plant Physiol. 53,* 257–277.

[20] Sabir, M., Ghafoor, A., Saifullah, M.Z.-u.-R., Murtaza, G., (2008). Effect of organic amendments and incubation time on the extractability of Ni and other metals from contaminated soils. *Pak. J.Agri. Sci.* 45, 1.

[21] Ghoma WEO, Sevik H, Isinkaralar K (2022). Using indoor plants as biomonitors for detection of toxic metals by tobacco smoke. *Air Quality Atmosphere & Health15(3):* 415-424.

[22] Nandita Singh, P. C. Abhilash (2009). *Withania somnifera* Dunal-mediated dissipation of lindane from simulated soil: Implications for rhizoremediation of contaminated soil. *Journal of Soils and Sediments 10(2):*272-282.

[23] Okwu, D.E. and M.E. Okwu, (2004). Chemical composition of spondia mombin plants. *J. Sustain. Agric. Environ, 6(2):* 140-147.

[24] Okwu, D.E. and C. Josiah, (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology, 5(4):* 357-361

[25] Koleva, I., T.A. van Beek, J.P. Linssen, A.D. Groot and L.N. Evstatieva, (2002). Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis, 13(1):* 8-17

[26] Yuniarti E, Dalmacio IF, CuevasVC, Raymundo AK, Paterno ES, Cadiz NM,Susilowati DN, Mulya K,Surono, Ikhwani (2022). Effects of Heavy Metal-Tolerant Microorganisms on the Growth of "Narra" Seedlings. *Sustainability14*: 9665.

[27] Lichtenthaler HK, Wellburn AR (1983). Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *BiochemSoc Trans 1:* 591-603.

[28] Bates LS, Waldren RP, Tear ID (1973). Rapid determination of proline for water-stress studies. *Plant Soil 39:* 205–207.

[29] Hoff JF, Singleton KI (1977). A method for determination of tannin in foods by means of immobilized enzymes. *J Food Sci 42*: 1566-1569.

[30] Zhishen J, Mengcheng T, JianmingW (1999). The determination of flavonoid contents in mulberries and their scavenging effects on superoxide radicals. *Food Chemistry 64:* 555-559.

[31] Jiang YM, Zhang ZQ, Joyce DC, Ketsa S (2002). Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). *Postharvest Biol Technol 26:* 241-252.

[32] Miranda MV, Lahor HMF, Cascone O (1995). Horseradish peroxidase extraction and purification by aqueous two-phase partition. *Appl Biochem Biotechnol* 53: 147-154.

[33] Bergmeyer HU, Gaweh K (1974). Methods of enzymatic analysis. *Verlag Chem 4*:23-32.

[34] Afshan, S., Ali, S., Bharwana, S.A., Rizwan, M., Farid, M., Abbas, F., Ibrahim, M., Mehmood, M.A., Abbasi, G.H., (2015). Citric acid enhances the phytoextraction of chromium, plant growth, and photosynthesis by alleviating the oxidative damages in *Brassica napus L. Environ. Sci. Pollut. Res. 22,* 11679e11689.

[35] Nobaharan K, Abtahi A, AsgariLajayer B, van Hullebusch ED (2022). Effects of biochar dose on cadmium accumulation in spinach and its fractionation in a calcareous soil. *Arabian Journal of Geosciences* 15(4): 1-14.

[36] Tanveer K, Ilyas N, Akhtar N, Yasmin H, Hefft DI, El-Sheikh MA, Ahmad P (2022). Role of biochar and compost in cadmium immobilization and on the growth of *Spinacia oleracea*. *PloS one17(5):* e0263289.

[37] Farid M, Ali S, Ishaque W, Shakoor MB, Niazi NK, Bibi I, Dawood M, Gill RA, Abbas F (2015). Exogenous application of ethylenediamine tetra acetic acid enhanced phytoremediation of cadmium by *Brassica napus* L. *Int J Environ Sci Technol 12:* 3981e3992.

[38] Farid M, Ali S, Rizwan M, Saeed R, Tauqeer HM, Sallah-Ud-Din R, Azam A, RazaN (2017). Microwave irradiation and citric acid assisted seed germination and phytoextraction of nickel (Ni) by *Brassica napus* L.: morpho-physiological and biochemical alterations under Ni stress. *Environ Sci Pollut Res 24:* 21050e21064.

[39] Bao Y, Guo A, Ma J, Pan C, HuL (2019). Citric acid and glycine reduce the uptake and accumulation of Fe_2O_3 nanoparticles and oxytetracycline in rice seedlings upon individual and combined exposure.

[40] Ehsan S, Ali S, Noureen S, Mahmood K, Farid M, Ishaque W, Shakoor MB, Rizwan M (2014). Citric acid assisted phytoremediation of cadmium by *Brassica napus* L. *Eco toxicol Environ Saf106*:164e172.

[41] Farid M, Ali S, Zubair M, Saeed R, Rizwan M, Sallah-Ud-Din R, Azam A, Ashraf R, Ashraf W (2018b). Glutamic acid assisted photo management of silver-contaminated soils through sunflower, physiological and biochemical response. *Environ Sci Pollut Res 25:* 25390e25400.

[42] Farid M, Ali S, Saeed R, Rizwan M, Bukhari SAH, Abbasi GH, Hussain A, Ali B, Zamir MSI, Ahmad I (2019). Combined application of citric acid and 5- aminolevulinic acid improved biomass, photosynthesis and gas exchange at- tributes of sunflower (*Helianthus annuus* L.) grown on chromium contaminated soil. *Int J Phytoremediation 21:* 760e767.

[43] Wang ST, Dong Q, Wang ZL (2017). Differential effects of citric acid on cadmium uptake and accumulation between tall fescue and Kentucky bluegrass. *Ecotoxicol EnvironSaf* 145.

[44] Mnasri M, Ghabriche R, FouratiE, Zaier H, SaballyK, Barrington S, LuttsS, AbdellyC, Ghnaya T (2015). Cd and Ni transport and accumulation in the halophyte *Sesuviumpor tulacastrum*: implication of organic acids in these processes. *Front Plant Sci 6:* 156.

[45] Muhammad D, Chen F, Zhao J, Zhang G, Wu F (2009). Comparison of EDTA- and citric acid enhanced phytoextraction of heavy metals in artificially metal contaminated soil by *Typha angustifolia*. *Int J Phytoremediation 11:* 558e574.

[46] Rasheed A, HassanMU, Fahad S, Aamer M, Batool M, Ilyas M, Li H (2021). Heavy metals stress and plants defense responses. In Sustainable Soil and Land Management and Climate Change: 57-82 CRC Press.

[47] Giri S, Shrivastava D, Deshmukh K, Dubey P (2013). Effect of Air Pollution on Chlorophyll Content of Leaves. *Curr Agric Res1(2):* 93-98.

[48] Iqbal MZ, Shafig M, Zaidi SQ, Athar M (2015). Effect of automobile pollution on chlorophyll content of roadside urban trees. *Glob J Environ SciManag 1(4):* 283-296.

[49] Leghari, SK, ZaidMA, Sarangzai AM, FaheemM, Shawani GR, Ali W (2013). Effect of roadside dust pollution on the growth and total chlorophyll contents in *Vitis vinifera* L (grape). *AfrJBiotechnol 13(11):*1237-1242.

[50] Pimple NS (2017). The adverse effect of air pollutants on the chlorophyll content in leaves from Pune, Maharashtra (India). *Int J Pharm Sci Rev Res 44(2):* 131-135.

[51] Chauhan A and Joshi PC (2008). Effect of ambient air pollution on photosynthetic pigments on some selected trees in the urban area. *Ecol Environ Conser 14:* 23–27.

[52] Ogboru RO, Okolie LPand Idibie CA (2016). Impact of air pollution on carotenoid and chlorophyll contents in three forest reserves in Edo State, *Nigeria. Int J Sci Res 5(1):* 616-622.

[53] Zeeshan M, Ahmad W, Hussain F, Ahamd W, Numan M, Shah M, Ahmad I (2020). Phytostabilization of the heavy metals in the soil with biochar applications, the impact on chlorophyll, carotene, soil fertility, and tomato crop yield. *Journal of Cleaner Production 255:* 120318.

[54] Liu Q, Huang L, Chen Z, Wen Z, Ma L, Xu S, Wu Y, Liu Y, Feng Y (2022). Biochar and its combination with the inorganic or organic amendment on growth, uptake, and accumulation of cadmium on lettuce. Journal of Cleaner Production.

[55] Assadi A, Pirbalouti AG, Malekpoor F, TeimoriN, Assadi L (2011). Impact of air pollution on physiological and morphological characteristics of *Eucalyptus camaldulensis*. *J. Food Agric Environ 9(2):* 676-679.

[56] Patidar S, Bafna A, BathamAR and Panwar K (2016). Impact of urban air pollution on photosynthetic pigment and proline content of plants growing along the A.B-road Indore City, India. *Int J CurrMicrobiol Appl Sci 5(3):* 107-113.

[57] Agbaire PO (2016). Impact of air pollution on proline and soluble sugar content of selected plant species. *Chem MatRes 8(5):* 72-76.

[58] Khosropour E, Weisany W, TahirNAR, Hakimi L (2022). Vermicompost, and biochar can alleviate cadmium stress by minimizing its uptake and optimizing biochemical properties in *Berberis integerrima* bunge. *Environmental Science and Pollution Research 29(12):* 17476-17486.

[59] Ilyas N, Akhtar N, Yasmin H, Sahreen S, HasnainZ, KaushikP, Ahmad P (2022). Efficacy of citric acid chelate and Bacillus sp. in amelioration of cadmium and chromium toxicity in wheat. *Chemosphere 290:* 133342.

[60] Radwan AM, ReyadNF, Donia ARM, GanaieMA (2018). Comparative studies on the effect of environmental pollution on secondary metabolite contents and genotoxicity of two plants in Asir area, Saudi Arabia. *TropJPharm Res 17(8):* 1599-1605.

[61] Mir AQ, Yazdani T, Ahmad S YunusM (2009). Total Flavonoids and Phenolicsin *Catharanthus roseus* L. and *Ocimum sanctum* L. as Biomarkers of Urban Auto Pollution. *Caspian J Environ Sci7(1):* 9-16.

[62] Marquez-GarciaB, Fernandez-Recamales MA, Cordoba F (2012). Effects of cadmium on phenolic composition and antioxidant activities of *Erica andevalensis*. *J Bot:* 1-6.

[63] Rezanejad F (2009). Air pollution effects on structure, proteins, and flavonoids in pollen grains of *Thuja orientalis* L. (Cupressaceae). *Grana 48:* 205–213.

[64] Anjum, S.A., Ashraf, U., Khan, I., Saleem, M.F., Wang, L.C., (2016). Chromium toxicity induced alterations in growth, photosynthesis, gas exchange attributes, and yield formation in maize. Pakistan J. Agric. Sci.

[65] Ashraf, A., Bibi, I., Niazi, N.K., Ok, Y.S., Murtaza, G., Shahid, M., Kunhikrishnan, A., Li, D., Mahmood, T., (2017). Chromium (VI) sorption efficiency of acid-activated banana peel over organo-montmorillonite in aqueous solutions. *Int. J. Phytoremediation 19*, 605e613.

[66] Hasan, M.K., Cheng, Y., Kanwar, M.K., Chu, X.Y., Ahammed, G.J., Qi, Z.Y., (2017). Responses of plant proteins to heavy metal stress da review. *Front. Plant Sci*.

[67] Polovnikova MG, Voskresenskaya OL (2008). Activities of antioxidant system components and polyphenol oxidase in the ontogeny of lawn grasses under Megapolis conditions. *Russian JPlant Physiol 55(5):* 699–705.

[68] Zaimoglu Z, KoksalN, BasciN, KesiciM, Gulen H, Budak F (2011). Antioxidative enzyme activities in *Brassica juncea* L. and *Brassica oleracea* L. plants under chromium stress. *J Food Agric Environ 9(1):* 676-679.

المكملات التشاركية للمواد العضوية تخفف من الإجهاد الناتج عن النحاس في ويثانيا كوجولانس (دُنال)

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المستخلص. ازدهار الفونا والفلورا. تم إجراء تجربة في أصص لدراسة تأثيرات النحاس (Cu) مع وبدون معدلات عضوية} روث البقر، حمض الستريك، والأحماض الأمينية (قلوتامين) {على مجموعة من المعايير الفسيولوجية والبيوكيميائية لنبات ويثانيا كوجولانس. تم معالجة تربة البتموس بتركزين من النحاس المعاجلة الأولى والمعالجة الثانية (١٠ مليمول، ١٥ مليمول) مع أو بدون المعدلات العضوية [روث البقر (١٠ غرام، ١٥ غرام)، حمض الستريك (٠٥ مليمول، ١٠ مليمول)، والحمض تأميني (٠٥ مليمول، ١٠ مليمول). بعد أربعة أسابيع، أظهرت النتائج أن التركيز الأعلى من النحاس قلل بشكل كبير من الخصائص الزراعية للنبات بنسبة ٣١٪ (عدد الأوراق)، ١٧٪ (طول الساق)، ٢٤٪ (طول الجذر)، ١٩٪ (الوزن الرطب)، و٣٥٪ (الوزن الجاف)، بينما تراجعت صبغات التمثيل الضوئي بنسبة ٣٤٪ للكاروتينات، ٣٣٪ للكلوروفيل أ، و١٦٪ للكلوروفيل ب مقارنةً بالعينة الضابطة. علاوة على ذلك، لوحظ أن الجمع بين المعدلات العضوية يخفف من الآثار السلبية للتركيز العالي من النحاس على الخصائص الزراعية وصبغات التمثيل الضوئي للنبات المعرضة للإجهاد. لمواجهة الضرر التأكسدي الناتج عن التركيز العالي من النحاس، أدت المكملات الإضافية من المعدلات العضوية إلى تحسين فسيولوجيا النبات وزيادة نشاط إنزيمات مضادات الأكسدة. أظهرت نتائجنا أن الجرعات العالية من النحاس كان لها تأثير على الخصائص الزراعية والبيوكيميائية لنبات ويثانيا كوجولانس، بينما خففت إضافة التعديلات العضوية من تأثير النحاس.

الكلمات المفتاحية: التربة، المعادن الثقيلة، المعالجة بالنباتات، التعديلات العضوية.