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Susceptibility of Cherry Tomato (*Lycopersicon Esculentum*) Plant to Simulated Foliar Cadmium and Nickel Exposures under Controlled Environment: Plant Health and Environmental Significances

Dahunsi B, Adebayo AJ and Oguntimehin I*

Chemical Science Department, Faculty of Science, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria

Abstract

The tolerance and responses of Cherry tomato to Cadmium and Nickel foliar exposures were examined in tomatoes planted and exposed to a simulated atmospheric levels (conc. of < 1mg/Kg) of Ni and Cd under 3 times/week mist spray treatments for 21d. Five (5) different sets of the tomatoes were used, one was sprayed with Ni solution, the second was sprayed with Cd solution, the third was sprayed with a mixture of both Ni and Cd solution, a fourth was treated with water treatment; being the same water used in preparing the metal solutions. Another set of tomato was used as Control; used without any foliar treatment. The Ni mist and Cd mist treated plants accumulated 0.23mg/Kg of Ni and 0.27mg/Kg of Cd independently but respectively in 21 days. Plants treated with the mixture of both Ni and Cd mists took up 0.19mg/Kg of Ni and 0.23mg/Kg of Cd together. The Control and Water treated tomatoes have similar metal contents and with apparently no symptoms of foliar damage. Foliar observations revealed that the solution of Ni + Cd mixture had the highest significant foliar damage on the plant, which shows a synergetic effects of the duo. The Bioconcentration factor directly correlated with the calculated Growth index factor. The relationships between BCF and GI vary inversely with the overall health status of tomato plant. Atmospheric concentrations of these heavy metals may pose health risk to plants health. Consequently, yield and economic losses impact may arose from poor plant health.

Keywords: Tomato; Toxicity; Nickel; Cadmium; Simulation and Environment

Introduction

Anthropogenic perturbations of the biosphere have resulted to broad global phenomena as a result of increased rate of industrialization and intensive agricultural practices which has not only wreaked havoc on the availability of natural resources but also causes widespread contamination of essential components of life on earth. Among the implications of human induced disturbance to the natural biogeochemical cycles, accumulation of heavy metals (HMs) is a problem of economic importance for ecological, nutritional and environmental reasons [1]. HMs have cytotoxic, genotoxic and mutagenic effects on living organism by influencing and tainting food chains, soil, irrigation or potable water and the surrounding atmosphere [2,3]. There are two kinds of metals found in the soils, one referred to as 'essential micronutrients', which are for normal plant growth (Fe, Mn, Zn, Cu, Mg, Mo and Ni) and the other 'nonessential elements' which have few or unknown biological and physiological function e.g. Cd, Sb, Cr, Pb, As, Co, Ag, Se and Hg [4,5]. Both underground and aboveground surfaces of plants are able to receive and absorb HMs [6]. Essential HMs plays a pivotal role in the structure of enzymes and proteins. Plants require them in minute quantities for their growth, metabolism and development. However, the concentration of both essential and nonessential metals is an important factor in the growing process of plants because their presence in excess can and often leads to reduction and inhibition of growth in plants. Heavy metals at toxic level have the capability to interact with several vital cellular biomolecules such as nuclear proteins and DNA, leading to excessive augmentation of reactive oxygen species (ROS). This could inflict serious morphological, metabolic and physiological anomalies on plants. Such ranges from chlorosis of shoot to lipid peroxidation and protein degradation [7]. Plants are equipped with a repertoire of mechanisms to counteract heavy metal (HM) toxicity, where these mechanisms are defective, the presence of HMs may result in distortion of growth and/or metabolic activities in the plant system

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***Correspondence:**
Oguntimehin Ilemobayo, Chemical Science Department, Faculty of Science, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria.
***E-mail:** oilemobayo@yahoo.com*

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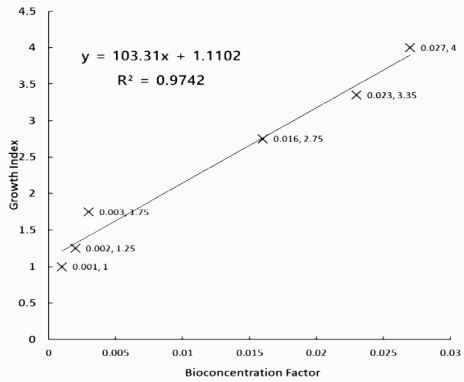

Figure 1: Bioconcentration factor vs Growth index for tomatoes treated with Cd mists in 21d.

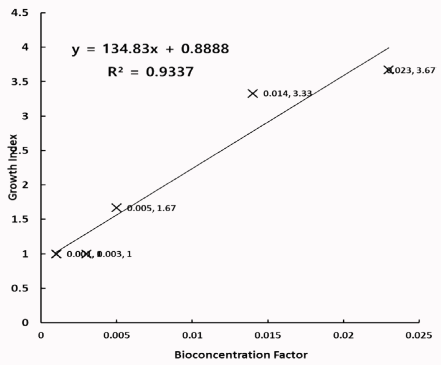

Figure 2: Bioconcentration factor vs Growth index for tomatoes treated with Ni mists in 21d.

and resultantly accompanied visible foliar symptoms [8].

Tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads and drinks. The body takes in 273 milligrams of potassium and just 5 milligrams of sodium per 100 grams of tomato [8]. Potassium ensures that the muscles and the nervous system function well and prevents high blood pressure. Tomatoes is therefore good for keeping blood pressure levels, Potassium is an important component of cell and body fluids that help in controlling heart rate, strengthen the muscles and control the nervous system. The antioxidants present in tomatoes are scientifically found to be protective against cancers, including colon, prostate, breast, endometrial, lung and pancreatic tumors [8].

This present study was designed to investigate the response of mini Caro tomato to Ni and Cd induced exposures using the response of growth, morphological and anatomical parameters of the plant to HM toxicity. It is imperative to determine the effect of Ni and Cd exposures and clarify their physiological stress on tomato growth as it is one of the most important vegetables in the world. Two major hypotheses were made in arriving at the mentioned objective. The first is the observable variation that may exist in the tolerance level and effect of Cadmium and Nickel on tomato while the second is that tomato could be able to tolerate trace amounts of Cadmium and Nickel with no sign of toxicity effect [7].

Materials and Methods

The samples (Cherry tomato; cultivar – 'Minicarol') were bought from Shoprite mall in Akure, Ondo state. The liquid fertilizer used was bought from Oja-Oba market in Akure, Ondo state and is composed of nitrogen 5.00%, potassium 0.50%, phosphorus 0.01%, manganese 0.10%, zinc 0.10%, copper 0.01%, iron 0.03% and other additives at pH of 5.0-8.0.

Tomato plant

About fifty healthy Cherry tomato seeds mixed with ashes were planted on the ground for two weeks before they were transplanted into the planting bags containing the loamy soil in a suitable environmental conditions ('OSUSTECH' green-house was used). The mean temperature and relative humidity measured inside and outside the greenhouse during the experimental period are comparable. 'Plantzyme' agricultural soluble fertilizer (N: P: K=5:5:5) was added to the soil medium after two weeks of growing. The seedlings were allowed to grow for 30 days. At the end of the 30 days, thirty (30) healthy tomatoes bags were divided into 5 sets (each set having six tomato plants) and separated by distance of about 4cm square. Each of the 5 sets was labeled as follow "control", "water treatment", "Ni solution", "Cd solution", "mixture of Ni + Cd solution" respectively.

Plant treatments

Plant treatments with solution mists started after one month has elapsed from the date of tomatoes planting. Each of the prepared solution was introduce to the plant's leaves of each of the corresponding labeled set of mini Caro tomato using a 2L capacity sprayer containing the appropriate solution every morning between 6.30HR and 7.00HR at 2 days' intervals. The base of the soil was covered before spraying each time to prevent direct addition of metal solution to soils. Set labeled with Cd was sprayed with Cd solution alone, set labeled with Ni was sprayed with Ni solution alone, set labeled with Cd + Ni mixtures was sprayed with mixture of Cd + Ni solution, set labeled with water was sprayed with water used in preparing the metal solutions, while the last set was used as control (not sprayed with either metal solution or water), the spaying was carry out every 2 to 3 days for 21d.

Sampling and digestion of plant materials

The sampling bags were well labeled corresponding to each plant's sets and day. Samples were labeled as day 0 (D0) i.e. taken before treatment began; day 1 (D1), day 3 (D3), day 7 (D7), day 14 (D14) and day 21 (D21), by cutting two portions of the leafs from the bottom (base) of each plant sets and kept inside the correctly labeled bag, the leafs where allowed to dry naturally in the screen/ green house. The procedure used in preparing the samples for AAS analysis was adapted from Manual of Chemical Methods of Food Analysis prepared by Food and Drug Administration and Laboratory Services, Federal Ministry of Health, Lagos [9], and Laboratory Procedure manual for heavy metals, Department of Environmental Services, University of Cincinnati (2004). Suitable amount of the sample was placed in a Kjeldahl digestion flask, 20ml of conc. Nitric acid was added and 20ml of water was also added. The solution was boiled until the volume reduces to about 20ml. The solution was cooled and 10ml of conc. sulfuric acid added. The solution was boiled again; small quantity of nitric acid was added again as the liquid begins to blacken. When the liquid no longer blacken the heating was continued until dense white fumes evolved. The solution was cooled and 10ml ammonium oxalate was added until dense white fumes evolved again-this is to facilitate the removal of colored nitro compound. The digest was then transferred into a 100ml volumetric flask and water was added to the mark point. The digest was then presented for AAS analysis.

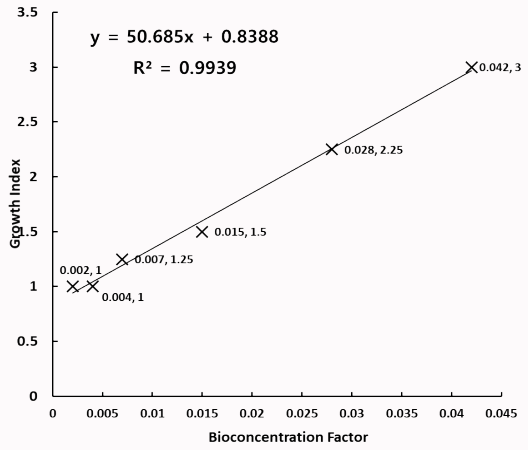

Figure 3: Bioconcentration factor vs Growth index for Cd and Ni in tomatoes treated with Cd + Ni mixture mists in 21d.

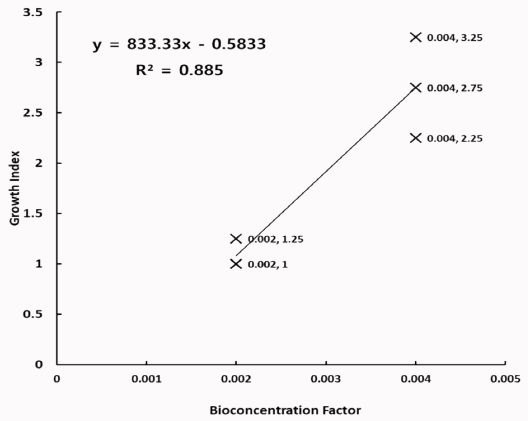

Figure 4: Bioconcentration factor vs Growth index for Ni and Cd HM in tomatoes treated with Water mists in 21d.

Calculation and statistics

The growth index of the plant for each sampling days was determined using the formula:

$$\text{Growth index} = \frac{\text{weight per sampling day}}{\text{weight at day 0}}$$

Bio-concentration Factor (BCF) of the plant for each sample was determined using the formula:

$$(\text{BCF}) = \frac{\text{measured conc. of metal in the plant}}{\text{actual conc. of metal in the solution}}$$

The percentage (%) dry content was determined using the formula:

$$\%\ \text{dry content} = \frac{\text{dry mass}}{\text{wet mass}} \times 100$$

The % wet content was determined using the formula:

% wet content = 100 - %dry content [10].

SPSS 13 (SPSS, USA) was used to analyze all measurements data. The results are averages of values from six tomatoes in each treatment group. Analysis of variance (ANOVA) was used to test the significance of the differences among the average values, after the verifying normality and homogeneity of the variance (one-way ANOVA, $p < 0.05$). Tukey posthoc test was used to compare means. Regression curves (Pearson correlation coefficient r, $p < 0.05$) of the various relationships between the eco-physiological parameters were

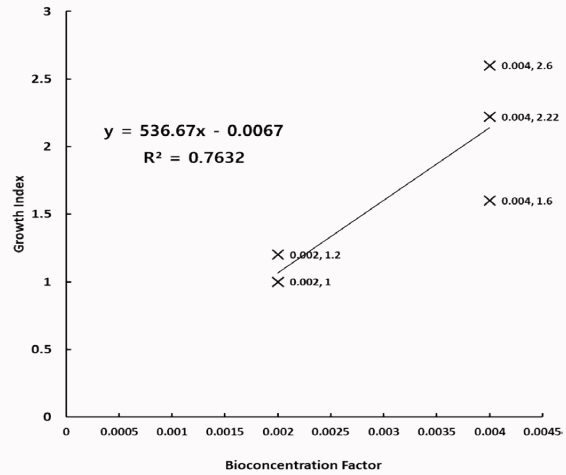

Figure 5: Bioconcentration factor vs Growth index for Ni and Cd HM in tomatoes treated as Control in 21d.

Table 1: Wet and dry mass analysis of tomato fruits treated under controlled environment in 21d.

	Control	Water (g)	Cd + Ni (g)	Ni (g)	Cd (g)
Wet mass	10	10	9.7	9.7	9.9
Dry mass	0.2	0.2	0.19	0.19	0.2
% of wet content	98	98	98.04	98.04	97.98
% of dry content	2	2	1.96	1.96	2.02

Table 2: Treatment types and the corresponding R^2 values from BCF and GI interactions.

Treatment Type	R^2 value of BCF vs GI interaction
Control	0.76
Water	0.89
Ni	0.93
Cd	0.97
Cd + Ni	0.99

constructed using MS Excel (2010). The coefficients of regression are displayed on each graph.

Results

The growth response of each treatment group is graphically represented by their relationships between their calculated growth indices and metal bioaccumulation factor. Each graph that is presented on the groups also reveals their R^2 value. This value obtained form their correlation coefficients signify how related the variables plotted against each other agrees. Higher values close to unity implies perfect relationships.

Table 1 shows the average wet and dry mass analysis on five samples of tomato fruit taken from each sets, the control has an average of 10.0g wet mass and dry mass of 0.2g (1.98%).

Those treated with water alone have same average weight with the control 10.0g wet mass and 0.2g dry mass. The average wet mass of tomatoes treated with Cd + Ni mixture mists was found to be 9.7g while the dry mass was 0.19g (1.96%).

Those treated with Ni mists alone have similar data to those treated with Ni mist alone. However, tomatoes treated with Cd mist alone had wet mass of 1.99g and dry mass of 0.2g (2.02%).

Table 3: Analysis of visible symptoms on tomato leaves after 21d period.

S/N	Treatment Type	Leaf Observation/Visible Symptoms	Comment	Overall Observation
1	Control	No visible symptom or malady	+	Healthy plant
		Green leaves, normal for tomatoes	+	
		Flowering at the end of treatment	+	
2	Water	No visible symptom or malady	+	Healthy plant
		Green leaves with little yellow streaks	+ -	
		Flowering at the end of treatment	+	
3	Nickel	Chlorosis covering nearly 50% of leaf surfaces	-	Sick plant
		Wilting of stems	-	
		Whitening of leaf and blight appearances	-	
		Delayed/No flowering	-	
4	Cadmium	Chlorosis covering nearly 80% of leaf surfaces	-	Sick and highly deficient plant
		Necrosis of leaves	-	
		Burning of leaf tips	-	
		Wilting of stems	-	
		Delayed/No flowering	-	
5	Mixed (Ni + Cd)	Stunted growth	-	Sick and almost dead plant
		Chlorosis of leaves covering >50% of leaf surfaces	-	
		Necrotic lesions and deadness of leaves	-	
		No flowering	-	

Figures 1 to Figure 5 are quite revealing of the dependence of the growth index parameter on the bioaccumulation factors of the HM in the tomatoes plants (Table 2). There seems to be the lowering trend in the Pearson correlation coefficients with the severity of foliar assessments carried out. As the control treatment with lowest GI vs. BCF correlation showed the highest health status of the tomatoes (Table 2, Table 3).

In Plate 1e, Ni + Cd mist treatments on tomatoes caused nearly complete withering and resultant leaf death of the tomato plant (Visible Chlorosis and necrosis). No such damage to the shoots was seen in the control plants as shown in plate 1a.

Discussion

Observable variation exists in the tolerance level and effect of Cd and Ni metalsl on Mini Caro tomato. This research indicated that Cd and Ni mists treatments exhibit different levels of negative health impact on the growth of mini Caro tomato. In this study, there were several reasons for the different in effects between Ni, Cd and Cd + Ni mixture. The first reason might be due to the different in the ability of the plants to accumulate these two metals and the second is that the ionic strength of cationic metal binding differs. The differences in the mode of interactions of some simulated pollutant mixtures inside different plants may be inherent in the physiological differences between them [11]. The symptoms observed in this study is similar to that of Oguntimehin et.al, [11] in their study on exposure of tomato plants to O_3 and O_3 + FLU which suffered chlorosis and necrosis. In their study, the biomass relationships of sprayed tomatoes did not significantly differ among treatments [12]. It shows that fluoranthene may affect the normal physical appearance of tomato plants. Since tomato fruits are important sources of antioxidants (including polyphenols, ascorbic acid, b-carotene, a-tocopherol) which are mainly coloured compounds [12]. As the accumulation of the metals in the plant increases, there are reduction in the plant leave and the plant height. Cd has the weakest accumulation ability by the plant and nearly the same dry mass value with the standard in the three sets of the HM treatments (Table 1 and 3 and plate1). Ni alone and Cd + Ni mixture have closely similar effects (higher reduction in leave size, stem and reduction in the plant height). Similarly, the accumulation potentials (plate1c & 1e) of Ni mist treatment alone and Ni + Cd mixture mists treatment are higher than that of Cd treatment alone. This is likely the reason for the slight variation observed in their fruits' dry masses.

Most of the cellular and molecular aspects of metal toxicity in plants are unknown, even though deleterious effects on crop production have long been recognized. Preliminary observations on putative metal genotoxic effects in plant are scarce. At the organ level, this symptom is common to numerous metals. Nickel accumulation in maize root apex reduces meristem mitotic activity, and this could be due to the lack of integrity of root meristems. Concentration- and time-dependant cadmium, copper and nickel clastogenic effects were observed in Helianthus annuus. Taken together, these observations suggest that genotoxic effects could be in part responsible for metal phytotoxicity, deserving more work to elucidate the underlying mechanisms.

The symptoms shown by Cd and Ni in the present study conform to previous study conducted on Cherry tomato [11]. Despite the constant low concentration used throughout, it was observed that after 3 weeks (21 days) of treatment, the effects seems significantly as possible accumulation of the metals by Mini Caro tomato plant increased, plants treated with Ni + Cd mists treatment grew with more stunted appearance than those treated with Cd alone. Plants treated with Cd mists alone appear to be least affected both in the growth reduction and the appearance of leaves symptoms, leaf spotting and chlorosis. This observation closely concur with earlier reports on Ni and Cd toxicity responses, describing inhibited growth and leaf lesions [13-15]. In one of their studies, the toxicity

Plate 1: The visible symptoms on tomatoes plants under various treatments.

symptoms of B. juncea plants exposed to soil Ni (in the range 0–100 µM NiCl) included chlorosis of young leaves and necrotic lesions on old leaves. Nickel may have both a direct and/or an indirect effect on photosynthesis, and can also adversely affect the accumulation of macro- and micronutrients in the roots. It appears that Ni inhibits cell elongation growth and photosynthesis, and reduces shoot water content due to a reduction of the transpiration rate [15]. Similar negative impact of Ni in the present study is establishing that both the root and foliar exposure routes are potential danger threats to plants, however, one may be greater than other.

Ni was positively associated with proteins inhibition germination and chlorophyll production [16]. The high concentration of Ni significantly decreased the chlorophyll content, stomatal conductance and a potential inhibitor of photosynthesis [17-19]. The number of leaves and chlorophyll contents decreased with 24 and 47%, respectively under the Ni concentration of 0.025mM. In the fresh leaves of maize, the concentration of chlorophyll content decreased with increased concentration of Ni from 20 to 100 µM. it was observed that chlorophyll-a decreased with 70% and chlorophyll-b decreased 50% under the Ni stress of 100µM in maize as compared to control plants. But there was no significant effect on 250 and 500 µM Ni concentration on the chlorophyll content in maize [20]. Accumulation of Ni in lower and upper parts of mungbeen's plants significantly decreased the chlorophyll content in the upper parts of plant [21]. The Ni stress in black gram (Vigna mungo) created a significant reduction in photosynthetic pigments [22].

In the past two decades, great number of studies have reported that the Ni toxicity is correlated with reduction or inhibition of photosynthesis in plants [23]. The Ni stress in sunflower reduced the stomatal conductance (gs) and photosynthetic activity [24]. Later, the study of Bazzaz et al. [24] was confirmed by Ouzounidou et al. in a study on wheat. The Ni stress of 200µM to Poplar (Populus nigra) plants significantly decreased the stomatal conductance (gs) especially in emerging leaves where the gs reduced from 0.40 to 0.03 $molm^{-2}s^{-1}$. This decline in gs resulted in direct decrease in photosynthesis [25]. Ni caused destruction of photosynthetic organs including the epidermal tissues and mesophyll cells [26]. Rauser and Dumbroff [27] stated that the Ni toxicity (200mM Ni for 24h) increased the stomatal resistance in P. vulgaris. In a study on Brassica juncea by Alam et al. [28], the Ni stress (100µM) decreased net photosynthetic rate and chlorophyll content. Photosynthetic rate in five test cultivars of *T. aestivum* significantly decreased under the Ni stress [29]. The Ni has toxic impact on both entire plant and on the chloroplast [30-33].

Cadmium toxicity may significantly alter the glycolytic pathway and the Cd-induced disorganization of the photosynthetic apparatus, and these effects may have an important impact on the plant's ability to withstand this type of stress. In the previous study, spot number 338 was identified as glyceraldehyde-3-phosphate dehydrogenase (GADPH). GADPH is known as an essential enzyme that catalyzes the sixth step of glycolysis, and it assists in breaking down glucose to obtain energy and carbon molecules. The key enzyme in the glycolysis process, GADPH has been observed to be increased in

abundance in poplar leaves. The GADPH levels were increased when A. thaliana plants were exposed to 10μM Cd exposure, and its level were also increased when A. thaliana cells were exposed to different concentrations of Cd. On the contrary, GADPH levels were decreased in the roots of two Cd-tolerant plants, poplar and *B. juncea*, after treatment with 20μM and 250μM Cd respectively. GADPH was observed to be increased following treatment with both low (10μM) and high (100μM) levels of Cd treatment compared to its level in controls in tomato plant roots [34]. However, previous studies of the alterations observed in carbohydrate metabolism-related proteins following exposure to Cd have demonstrated contradictory findings.

Previous studies also showed that the metabolic changes induced by Cd are tissue-specific. Consequently, GADPH is induced in leaf tissue but severely decreased its abundance in root tissues in poplar plants. GADPH was also induced in the leaves of rice and poplar plants [35] by treatment with various heavy metals. These results suggest that GADPH protein may play an active role in supplying energy to Cd-treated plants via the glycolytic pathway. Taken together, the previous reports together with the present study, indicate the changes in carbohydrate metabolism upon Cd exposure are dose dependent and plants elevate their energy consumption over energy production when it exposed to Cd stress.

Reactive oxygen species (ROS) continually produced as off-spins of different metabolic reactions that take place in different cellular parts of plants like mitochondria and chloroplast [36,37]. In plants, the mitochondria (energy factories) are the major responsible site for the production of ROS. Many abiotic and biotic stresses disturb the equilibrium between the cleaning and production of ROS like heavy metals, salinity, droughts, ultraviolet (UV)-radiation, air pollution, extremes of temperature, pathogens and herbicides [38]. The ROS are comparatively more reactive than O_2 and thus they have severe toxic impacts on living system. The toxicity of ROS can destroy the DNA structure; it can also stimulate the oxidation of lipids and proteins and degradation of chlorophyll pigments [5]. Heavy metals as well as Ni have capacity to create the OH by Haber/Fenton-Weiss reaction [39] but due to high reduction/oxidation capacity, Ni was not observed as a catalyst in this reaction [40]. It is known that the excessive amount of transition metals increased the production of ROS in plants. In normal condition, the ROS expeditiously cleaned by antioxidant system [22]. Hydroxyl radical ($OH^{\cdot-}$), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot-}$) are the main mediators for the peroxdative damage [22]. The cytotoxic protein damage and DNA disruption in plant tissues may be due to Ni [41,42]. Rao and Sresty [43] stated that the Ni toxicity increased the production of ROS and causing the peroxidative damage in membrane lipids. It was well documented that over accumulation of lipids peroxidation resulted in toxicity of heavy metals and oxidative damage. The H_2O_2 quantity significantly increased in leaf of wheat under the Ni stress [42]. In the roots of wheat, the ROS content increased under the Ni stress [44-49] and same type of result was observed by Boominathan and Doran in the hairy roots of Nicotiana tabacum and Alyssum bertolonii [50-52].

Conclusion

A low dosage of Cadmium and Nickel per plant used in the present study, resulted in considerable damage to tomato leaves in 21d. The difference in the observed foliar damage may be due to the difference in ease of Cd and Ni penetration abilities into the plants. The toxicity of Cd and Ni applied as mist to the foliage of tomato plants in our field experiment suggest that HMs deposition in mist, dew, frost, snow, and rain can negatively affect tomatoes' growth and quality. It is important to determine the potential effects of hazardous pollutants on major crop plants, such as tomatoes. This comparative study assess the overall health status of tomatoes treated with Cd alone, Ni alone and mixture of both metals on tomatoes further confirm negative health status they impacted on tomato growth leading to poor health status of the plant. The severity of damage by these metals increased in the following order: Ni + Cd mixture > Ni > Cd. Thus, the phytotoxicity of a metal does not depend only on its concentration but rather on the accumulation potential of the plant towards this metals and the metal stress in the plant's system. The mixture of Ni + Cd mixture have the highest significant damage on the plant.

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