RESEARCH PAPER

Effect of Humic Acid on Tolerance indexes of Barley plant to Cadmium Toxicity
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A B S T R A C T:
This study was carried out to evaluate the effect of different levels of Humic acid (0, 400, 800 ppm) on tolerance index of barley plant to different levels (0, 25, 50 and 100 ppm) of Cadmium. The experiment was designed according factorial completely randomized design with three replications. The results revealed that the combination effect of Humic acid and Cadmium significantly increased the total dry weight, number of spikes and dry weight of straw, as well as in total dry weight of plant. Cadmium tolerance was found by treatments HA0CD50, HA0CD10, HA400CD0, HA400CD25, HA400CD100, HA800CD50, HA800CD100. Cadmium significantly decreased the relative chlorophyll production rate (RCPR) of Barley plant.

Key worlds: Humic acid; Cadmium; Tolerance index; Barley plant.

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1. INTRODUCTION:

Barley (Hordeum vulgaris L.) is annual grass belong to gramineae family, which is a monocotyledonous herb, it is a short season, early found in varying environment (Gene technology regulator, 2017). It is an important cereal crop in the world after maize, wheat and rice. It is one of the earliest domesticated food crops since the start of civilization. Barley is cultivated in a wide range of environmental conditions (Wali et al., 2018), its used as animal food, and also for human, as well as used in industrial purposes for producing starch (Mantilla, 2015). Humic acid (HA) is natural organic compounds that can be used to increased nutrient availability, increase growth and yields. Humic acid produced commercially which is produced in decomposition of organic compounds.

It contains nutrients that increased fertility of soil, availability of water and nutrients (Hamad and Tantawy, 2018). HA has indirectly many physiological roles in plants such as cell respiration, photosynthesis process, synthesis of proteins, the activity of enzymes which lead to growth and development of plants (Wali et al., 2018), (Gomes Junior et al., 2019) reported that HA increased uptake of nutrients such as phosphorus, magnesium, potassium, sodium in wheat plant, was particularly important for transportation and availability of micro nutrients under different environmental condition. Humic acid are well known as stimulators of plant germination and growth, which have anti-stress effects under abiotic stress condition such as unfavourable temperature, salinity, pH, etc. (Abdellatif et al., 2017) mentioned that HA significantly increased plant height, fresh weight, number of flower clusters, number of flowers per plant and yield components such as fruit number per plant, fruit weight, total yield in tomato plant.

Heavy metals make a main source of environmental pollution as a result of human
activates such as energy and fuel production, power transmission, smelting, sludge damping, intensive agriculture (Jali et al., 2016). Cadmium is one of the non-essential toxic elements to plants that transported from soil to plants by their roots, and accumulated in the roots, shoot, fruits and seeds. Accumulation of cadmium in plants cause phytotoxicity, inhibit growth and development of the plant, as well as effect on various physiological, morphological, biochemical activities of plant species like chlorosis, leaf rolls, necrosis, imbalance of mineral nutrients, variation of enzymes activity, photosynthetic rate, chlorophyll content (Liu et al., 2014). The adverse effect of cadmium on growth of plant, exchange of gases and chlorophyll fluorescence decreased by application of humic acid in (Orthosiphon stamineus) plant (Ibrahim et al., 2017). (Chaab et al., 2016) found that humic acid has a great role in decrease the negative effect of cadmium on maize plant. (Konakci et al., 2018) mentioned that Humic acid induced the toxicity of cadmium stress by changing photosynthetic apparatus and antioxidant activity in wheat leaves.

High tolerance of plants to heavy metals toxicity related to the ability of roots to tolerate Cd entrance as the toxic metal to the roots first, which make the plants to have a potential mechanism to metal tolerance such as cadmium stress (Azevedo et al., 2012). (Venkataramaiah et al. 2011) showed that rice plant has several strategies to improve tolerance against heavy metal pollutant especially cadmium.

Rapid increase of industrialization and population growth at 15 last decade in Kurdistan region led to increase the environmental pollution particularly the soil pollution by heavy metals like Cadmium, thus the aim of this work was to study the assessment effect of foliar spray application of Humic acid on tolerance index of Barley plant to cadmium toxicity.

1. MATERIALS AND METHODS

1.1. Experimental design

A Pot experiment was conducted in glass house of biology department in the college of science- Salahaddin University-Ebil to study the effect of Humic acid on tolerance index of Barley plant cadmium toxicity. The experiment was involved 36 plastic pots with 24 cm diameter and 21 cm lenght, each pot was filled with 7kg of dried sandy loam soil collected from Aski kalak area, the soil sieved through 2mm pore size sieves. In each pot, five seeds were sown and then thinned to three plants later. Factorial experiment consisted of 12 treatment combinations of both Humic acid at does (0, 400, 800) ppm and soil irrigation containing (0, 25, 50 and 100 )ppm cadmium replicated three times in completely randomized design (CRD) . Diammonium phosphate (DAP) Fertilizers containing 18% N and 64% P, were added to each pots.

At harvest the plants were cut at soil surface from each pots, placing them in weighted bag and oven dried at (70) °C for (48) hrs and after that the total dry weight, of thousand grain weight, straw weight, spike number, grain number, Relative growth rate (RGR), Relative chlorophyll rate (RCPR) were obtained.

The area of flag leaf (cm²) was measured according by adopting stickler’s linear measurement method (Stickler et al., 1961). Leaf area cm²=L*B* 0.747

L= Length of leaf, B=width of leaf

Chlorophyll content in leaves was estimated by chlorophyll meter by clipping the sensor on different locations on leaf surface except the veins (Padilla et al., 2015).

2.2. Statistical analysis

Analysis of variance was conducted to the obtained data and the treatment means were compared according to Duncans multiple range test at 0.05 and 0.01 level tests for greenhouse and laboratory parameters (Al-Rawi and Khalafulla, 1980). The statistical analysis was done by using Statistical Package for Social Sciences (SPSS version 26 software). For drawing graph, Excel 2010 software was used.

2. RESULTS AND DISCUSSION

Table (1) shows that HA800CD50 is superior significantly to some other treatment combinations with respect to total dry weight of Barley plants which indicates that higher concentration of HA causes the decreases of negative effect of Cadmium, these results agreed with those obtained by (Ibrahim et al., 2017), who found that the humic acid application decrease the negative effect of cadmium on total biomass in Orthosiphon stamineus plant. HA
stimulate plant growth by increasing the chlorophyll content, uptake of nutrients as well as the adverse the effect of cadmium in Radish plant (Farouk et al., 2011). These combinations also have significant effect of total dry weight at treatment (HA 800 CD50) as compared to control.

The results in table (2) show that combination effect of Humic acid and cadmium significantly increased the number of spike at treatment (HA 800 CD 50), as well as dry weight of straw at treatment( HA400 CD100) as compared to controls. Application of organic substance like humic acid enhance plant growth by increasing cell membrane permeability, respiration, oxygen and phosphorus uptake and increasing the growth of root cells (Chaab et al., 2016). Figure (2) show that cadmium significantly decreased the RCPR in barley plant at treatment (CD100) as compared to the control These results agree with those obtained by (Yang et al., 2020), who found that the content of chlorophyll b and total chlorophyll decreased significantly with different concentration of cadmium in Davidia inolucrata plant. Chlorophyll content influenced by higher concentration of Cd. Cd might be accumulated by the plant and inhibit plant growth and other crops, nearly 40% of Cd may be absorbed and transported to the upper parts of the plant which directly affect the plants.(Abedi and Mojiri, 2020).

Fig (3) indicates that plants treated with (HA0CD25, HA400CD50, HA800CD0,HA800CD25)are non-tolerant (sensitive) plants to Cd toxicity as their tolerance indexes are negative according to the means of total dry weight compared to the control (HAOCDO), while the rest of the plants of positive tolerance indexes considered tolerant to the toxicity. On the other hand, control plants are moderate. (Yasmin khan, et al., 2017) revealed that HA enhanced shoot dry biomass as well as decreased the effect of Cd concentration in (Brassica rapa) plant. Foliar application of HA alleviating the symptoms of cd toxicity by increasing the content of photosynthetic pigments, root, shoot and leaf biomass in Hybrid Pennisetum (Song et al., 2020). HA successfully decreased the harmful effect of Cd stress by increasing total dry weight, number of spike and dry weight of straw.

**Table 1: Combine effect of Humic acid and cadmium on some vegetative growth of Barley plant:**

| Treatments   | Chlorophyll 1 | Chlorophyll 2 | First plant height(cm/plant) | Second plant height(cm/plant) | Flag leaf area (cm²) | Total dry weight (gm) |
|--------------|--------------|--------------|-----------------------------|-------------------------------|----------------------|-----------------------|
| HA 0 CD 0    | 53.96 ab     | 39.93 a      | 36.66 a                     | 62.00 a                       | 5.35 a               | 6.29 b                |
| HA0 CD25     | 57.200 ab    | 49.36 a      | 41.16 a                     | 63.00 a                       | 7.27 a               | 3.69 b                |
| HA 0CD 50    | 54.20 ab     | 47.36 a      | 43.00 a                     | 74.33 a                       | 5.47 a               | 8.79 ab               |
| HA 0CD100    | 54.16 ab     | 48.73 a      | 39.66 a                     | 70.00 a                       | 9.99 a               | 8.30 ab               |
| HA400CD0     | 56.16 ab     | 47.63 a      | 45.03 a                     | 72.00 a                       | 4.02 a               | 8.23 ab               |
| HA400CD25    | 56.30 ab     | 51.43 a      | 44.33 a                     | 67.66 a                       | 5.94 a               | 7.043 ab              |
| HA400CD50    | 56.56 ab     | 51.6 a       | 45.20 a                     | 64.33 a                       | 7.59 a               | 6.96 b                |
| HA400CD100   | 50.70 b      | 53.43 a      | 39.53 a                     | 62.33 a                       | 4.14 a               | 8.53 ab               |
| HA800CD0     | 53.66 ab     | 41.10 a      | 45.83 a                     | 65.33 a                       | 3.52 a               | 6.78 b                |
| HA800CD25    | 56.20 ab     | 42.23 a      | 33.33 a                     | 61.33 a                       | 6.58 a               | 6.21 b                |
| HA800CD50    | 59.00 a      | 53.66 a      | 43.66 a                     | 65.66 a                       | 8.78 a               | 12.26 a               |
| HA800CD100   | 54.93 ab     | 48.23 a      | 53.83 a                     | 69.66 a                       | 6.31 a               | 8.18 ab               |
Table 2: Combine effect of Humic acid and cadmium on some yield characteristics of Barley plant:

| Treatments | RCPR | Spike number /plant | RGR (g/g/days) | Dry weight of grains (gm) | Dry weight of straw(gm) | Thousand GW(gm) |
|------------|------|---------------------|----------------|---------------------------|------------------------|-----------------|
| HA 0 CD 0  | 0.0065 a | 1.66 c | 0.103 a | 2.64 abc | 0.59 cde | 138.08 abc |
| HA0 CD25   | 0.0032 a | 2.33 bc | 0.0094 a | 1.55 bc | 0.18 e | 88.07 bc |
| HA 0CD 50  | 0.0028 a | 4.00 abc | 0.0114 a | 2.82 abc | 0.64 bcde | 147.50 abc |
| HA 0CD100  | 0.0021 a | 2.66 bc | 0.0123 a | 3.29 ab | 0.50 de | 171.79 abc |
| HA400CD0   | 0.0033 a | 3.00 bc | 0.0095 a | 2.96 abc | 0.63 bcde | 166.96 abc |
| HA400CD25  | 0.0018 a | 2.33 ab | 0.0094 a | 3.61 ab | 0.54 de | 196.43 abc |
| HA400CD50  | 0.0020 a | 2.66 bc | 0.0084 a | 2.45 abc | 0.86 abcd | 133.89 abc |
| HA400CD100 | 0.0011 a | 4.66 ab | 0.0092 a | 4.33 a | 1.18 a | 270.41 a |
| HA800CD0   | 0.0070 a | 3.00 bc | 0.0079 a | 4.00 a | 1.13 abc | 251.53 a |
| HA800CD25  | 0.0073 a | 2.66 bc | 0.0133 a | 3.36 ab | 0.66 bcde | 218.54 ab |
| HA800CD50  | 0.0020 a | 5.66 a | 0.0094 a | 1.15 c | 1.16 ab | 72.22 c |
| HA800CD100 | 0.0026 a | 4.00 abc | 0.0054 a | 3.27 ab | 0.86 abcd | 212.35 ab |

Fig 1: Combined effect of Humic acid and Cadmium on cadmium content of leaves in Barley plant.
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**Fig 2: Effect of Cadmium on relative chlorophyll production rate in Barley plant.**

**Fig 3: Combine effect of Humic acid and Cadmium on Tolerance index of total dry weight in Barley plant.**

**Conclusion**
By the present study, the combine effect of Humic acid and Cadmium significantly increased the total dry weight, number of spikes and dry weight of straw, total dry weight of plant have tolerance. Although Cadmium significantly decreased the relative chlorophyll production rate (RCPR) of Barley plant. Humic acid has positive effect to decrease the cadmium effect in barley plant, thus suggested that Humic acid able to decrease the harmful effect of more heavy metals which has negative effect on plants and environment.

**3. REFERENCES**
Abedi, T. ans A. Mojiri. (2020). Cadmium Uptake by Wheat (Triticum aestivum L.): An Overview. Journal of Plants.1-14.
Al-Rawi, K.M. and A.A.M. Khalafulla. (1980). Design and Analysis of Agriculture Experiments. Univ. Of Mousl. Ministry of Higher Education and
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Scientific Research. Mousl. Iraq. Pp488. (In Arabic)

Azevedo, R.A; P.L. Gratao; C.C. Monteiro and R. F. Carvalho. (2012). Cadmium induced stress in plants. Journal of food and energy security. Pp, 1-8.

Farouq, S.; A.A. Mosa; A.A. Taha; H. M. Ibrahim and A.M. A. El-Gahmery. (2011). Protective Effect of Humic acid and Chitosan on Radish (Raphanus sativus, L. var. sativus) Plants Subjected to Cadmium Stress. Journal of Stress Physiology & Biochemistry, Vol 7(2), pp. 99-116.

Chaab, A.; A. Moezzi; G.olamabas Sayyad and M. Chorom. (2016). Alleviation of Cadmium Toxicity to Maize by the Application of Humic acid and Compost. Life Science Journal, ;13(12), 56-63.

Gomes Júnior, G. A.; R. A. Pereira; G A. Sodré and E. Gross. (2019). Humic acids from vermicompost positively influence the nutrient uptake in mangosteen seedlings. Pesq. Agropec. Trop., Goiânia,pp1-8.

Hamad, M. M. and M. F. A. Tantawy. (2018). Effect of different Humic Acids Sources on the Plant Growth, Calcium and Iron Utilization by Sorghum. Egypt. J. Soil. Sci. Vol. 58, No. 3, pp. 291-307.

Ibrahim, M. H.;A. Ismail; H. Omar and N.A. Mohd zain. (2017). Application Effects of Cadmium and Humic Acid on the Growth, Chlorophyll Fluorescence, Leaf Gas Exchange and Secondary Metabolites in Misai Kucing (Orthosiphon stamineus) Benth. Annual Research and review Biology, Vol. 18 (3).

Jali, P.; C. Pradhan and A. Bandhu Das. (2016). Effects of Cadmium Toxicity in Plants: A Review Article. Scholars Academic Journal of Biosciences (SAJB), 4(12):1074-1081.

Konakci, C. O.; E.Yildiztugay; M. Bahtiyar and M. Kucukoduk. (2018). The humic acid- induced changes in the water status, chlorophyll fluorescence and antioxidant defense systems of wheat leaves with cadmium stress. Ecotoxicology and environmental safety. Vol,155, 66-75.

Liu, L.; H. Sun; J. Chen; Y. Zhang; D. Li; C. Li. (2014). Effects of cadmium (Cd) on seedling growth traits and photosynthesis parameters in cotton (Gossypium hirsutum L.). Plant Omics Journal, 7(4):284-290 (2014).

Mantilla, U. H. (2015). Composition and structure of barley (Hordeum vulgare L.) grain in relation to end uses. VTT Science 78, Ph desert, Finland.

Office of the gene technology and regulator. (2017). The biology of Barely (Hordum vulgaris L.) plant. Austrian Government department healthm version 2.

Padilla, F. M., Peña-Fleitas, M. T., Gallardo, M., and Thompson, R. B. (2015). Threshold values of canopy reflectance indices and chlorophyll meter readings for optimal nitrogen nutrition of tomato. Ann. Appl. Biol. 166, 271–285.

Song, X.; M. Chen; W. Chen; H. Jiang and X. Yue. (2020). Foliar application of humic acid decreased hazard of cadmium toxicity on the growth of Hybrid Pennisetum. Acta Physiol Plant 42, 129.

Venkataramaiah, N., S. V. Ramakrishna, and R. Sreevaths.a.(2011) . Overexpression of phytochelatin synthase (AtPCS) inrice for tolerance to cadmium stress. Biologia 66:1060–1073.

Wali, A. M.; A. Shamseldin; F. I. Radwan; E. M. Abd El Lateef and N. M.(2018). ZakiResponse of Barley (Hordeum vulgare) Cultivars to Humic Acid, Mineral and Biofertilization under Calcareous Soil Conditions.Vole 7 (1), p 71-82.

Yang, Y.; L. Zhang; X. Huang; Y. Zhou; Q. Quan ; Y. Li and X. Zhu. (2020). Response of photosynthesis to different concentrations of heavy metals in Davidia involucrate. Journal of Pone.1-16.

Yasmin Khan, K; B. Ali; X. Cui Y. Feng; P. J. Stoffella; L. Tang and X. Yang. (2017). Effect of humic acid amendment on cadmium bioavailability and accumulation by pak choi (Brassica rapa ssp. chinensis L.) to alleviate dietary toxicity risk. Agronomy and soil science. Vol 63(10), 1431-1442.