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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.

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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, chrolophyll 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) ^{o}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 (cm^2) was measured according by adopting stickler's linear measurement method (Stickler *et al.,* 1961).

Leaf area cm^2=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 ($cm^{2)}$	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

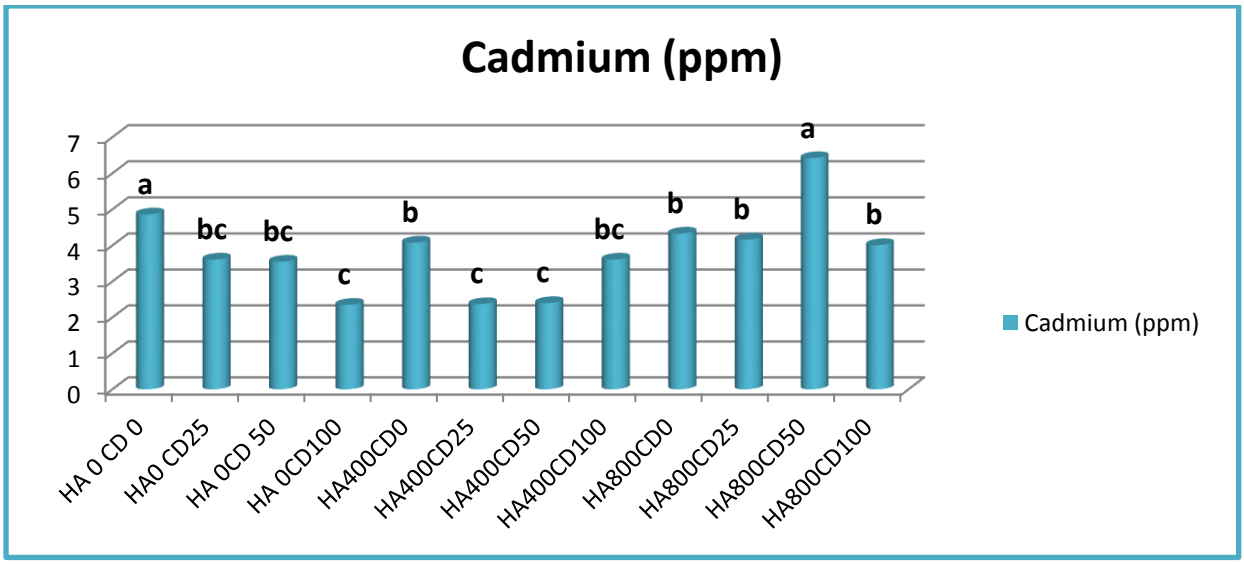

Fig 1: Combined effect of Humic acid and Cadmium on cadmium content of leaves in Barley plant.

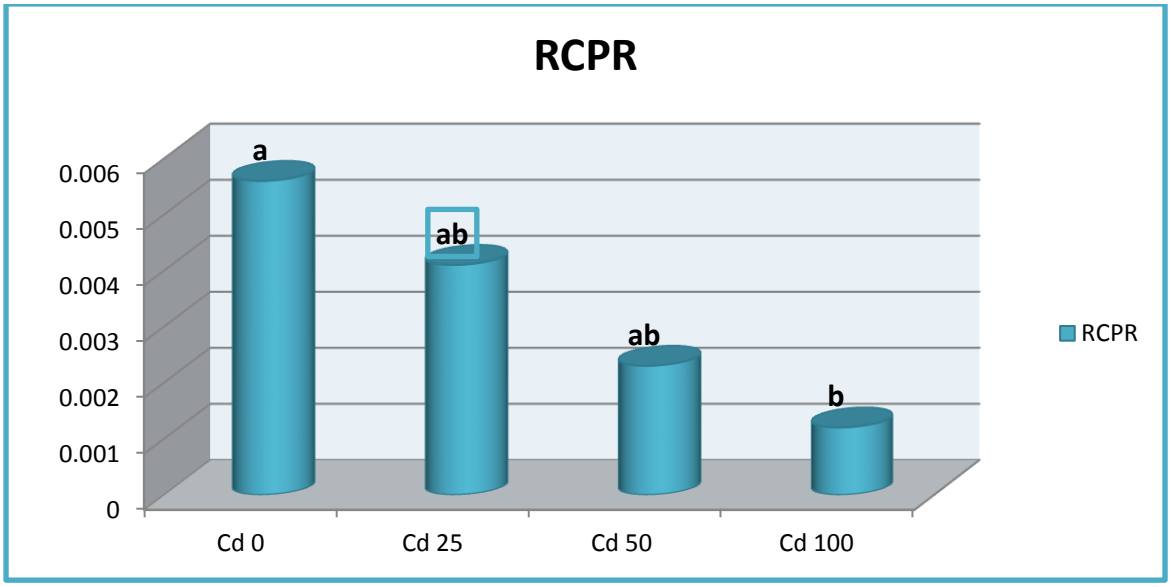

Fig 2: Effect of Cadmium on relative chlorophyll production rate in Barley plant.

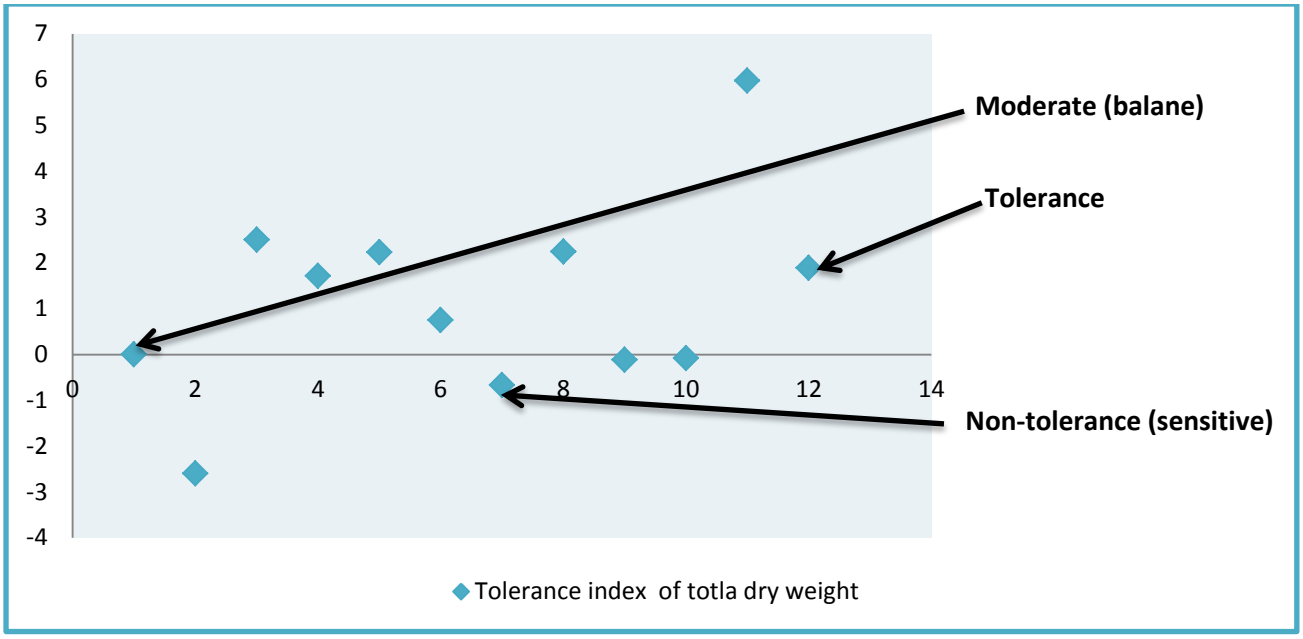

Fig 3: Combine effect of Humic acid and Cadmium on Tolerance index of total dry weight in Barley plant.

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 enviromrnt.

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