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Effects of Humic Acid on the Germination Traits of Pumpkin Seeds under Cadmium Stress

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Abstract

The study tackled the effect of humic acid and cadmium concentrations on the pumpkin seed germination characteristics throughout were studied. Treatments were cadmium concentrations on three levels: 0, 100 and 200 ppm and humic acid concentration of 0, 100, 200, 300 and 400 mg lit^{-1}. Results showed that interaction of humic acid and cadmium was not significant on germination traits, but there was a significant effect on seedling growth indexes. Radicle and plumule length increased by 86 and 192% in comparison with control, of the mixture of 200 ppm cadmium and 300 mg lit^{-1} of humic acid. Cadmium had stimulatory effect on radicle and cotyledon dry weight and the highest values obtained with 200 ppm in mixture with 200 mg lit^{-1} of humic acid. Also, maximum plumule dry weight was recorded in 200 ppm cadmium and 300 mg lit^{-1} of humic acid. The highest of indexes were observed of 200 ppm cadmium and 400 mg lit^{-1} humic acid. In conclusion, the humic acid had detoxifying effect on cadmium stress in the culture and responded antagonistically against cadmium, but it seems that these concentrations of cadmium are low for the pumpkin seed and can be increased in order to reach the toxicity level.

Key words: cadmium, germination, humic acid, pumpkin, seedling growth

Introduction

Soil contamination with heavy metals is a major problem in agricultural lands that affects the fauna and flora of soils, resulting in the reduction of the quality of products. Heavy metals can be accumulated via the erosion of rocks and soil colloids, but the main source is the human activities using chemical fertilizers, industrial sewage, and agrochemicals. Cadmium (cd) is one of the heavy metals which has high mobility in the soil, while its absorbance by plants leads to toxicity and environmental hazards for humans and animals (Mojahedi *et al.*, 2011).

Pumpkin (*Cucurbita pepo* L.) is a valuable medicinal plant due to its pharmaceutical characteristics and its oil extract is used in pharmacy. Pumpkin grain oil content is about 35-55% (Tyler *et al.*, 1988). The most important oil composition is linoleic and oleic acids, sterols, micro nutrient elements, vitamins and carotenoids. Fatty acids like palmitic, stearic, oleic and linoleic acids are present in the pumpkin grain oil, which linoleic (44-55%) and oleic (27-38%) acids are present in a higher percentage. Sterols free of glycosides contain about 0.1% of total extractable oil. Micro nutrient elements consist of zinc, selenium and potassium, while zinc and selenium have great importance from the point of view of human nutrition. Vitamins are 0.03% of the oil, and the major one is y -tocopherol (vitamin E). Essential amino acids and cucurbitin are the other parts of grain constituents (Lashkari and Seokabarghi, 1998). Vitamin E is an antioxidant and prevents vitamin A degradation.

Mohammadzadeh *et al.* (2011) reported that lead (Pb) and Cd, had the lowest respectively highest negative effect on the germination traits of annual alfalfa and the sensitivity of roots to heavy metals was greater than that of aerial parts. Al-Rumaih *et al.* (2001) showed that $CdCl_2$ caused the reduction in germination percentage and rate in cowpea, but increased the mean of germination time. These responses were dose-related and under high concentrations of $CdCl_2$ more reduction was observed in germination traits. There is a linear relation between seed germination and Cd concentration (Cheng and Zhou, 2002). Eghareba and Omoregie (2010) demonstrated that Cd decreased the germination percentage and plant height in *Vigna unguiculata*. In *Pisum sativum*, 40 ppm of Cd has a vital effect on 50% of seeds and can reduce germination, embryo growth, NADPH, H+ concentration, and number of mitochondria and peroxisomes (Smiri, 2010). Cd usually accumulates in root cells and slightly moves to leaves and causes to decrease in dry and fresh weight and leaf area (Soltani *et al.*, 2007). Humus contains humic acid (HA) and folic acid which constitutes about of 70-75% of soil organic matter (Turkman *et al.*, 2005). Organic mate-

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rials have hormone-like properties and cause the increase in germination percentage, root development and rapid growth of aerial parts of plants (Tan, 2003).

Humic acid (HA) increased lettuce seedlings dry and fresh weight significantly due to cell elongation and efficient water uptake, but tomato seedlings were not different in dry weight in response to HA (Piccolo *et al.*, 1993). Priming of sunflower seeds with HA had no significant effect on radicle and plumule dry weight, while Cd severely affected these traits (Mojahedi *et al.*, 2011). Jafari *et al.* (2011) reported that biologic fertilizers like HA and biophosphorous increased radicle and plumule length of two wheat cultivars. Since soil contamination with heavy metals is one of the major problems for seed germination, the objective of this study was to determine the HA potential for Cd detoxifying in pumpkin-cultivation soils.

Materials and methods

In order to determine the effect of humic acid and cadmium on the germination traits of pumpkin seeds, a factorial experiment was conducted based on completely randomized design with three replications. Treatments involved different concentrations of cadmium solution including 0, 100 and 200 ppm and humic acid as 0, 100, 200, 300, and 400 mg lit^{-1}. Pumpkin seeds were sterilized with sodium hypochlorite 3% for 3 min and planted in 14 cm diameter Petri dishes. A 10 ml of each solution was added to each petridish and transferred to a germinator at 25 °C. Germinated seed counting was done daily and final counting was on the 8^{th} day. At the end of the experiment, five seedlings from each plot randomly selected and radicle and plumule length and dry weight were recorded. Germination rate (GR) was calculated according to Ellis and Roberts (1981):

$$GR - \frac{\Sigma n}{\Sigma(Dn)}$$

Where is the number of germinated seeds and Dn is the counting day.

Mean of daily germination (MDG) calculated by the formula below (Hunter *et al.*, 1984): MDG=FGP/DGS

That FGP is the final germination percentage and DGS is daily germination speed.

Daily germination rate was calculated according to Maguir (1962): RDG=1/MDG

Primary and secondary seedling vigor was calculated from formula below (Abdul-Baki and Andersom, 1973):

SV1= seedling dry weight (gr) germination percentage

SV2= seedling length (cm) germination percentage

Data analysis was performed using SAS 9.1 software after normal testing and comparison of means was performed using Duncan's multiple range test at 0.01 % probability level.

Results

The effects of HA and Cd and their interaction were not significant for GP, GR, MGT and MDG, but the interaction was significant on radicle and plumule length, dry and fresh weight (Tab. 1).

Tab. 1. Analysis of variance table for the effect of cadmium and humic acid on the germination traits of pumpkin seeds

SOV	df	MS					
		GP	GR	MTG	MDG	PL	RL
Cadmium (Cd)	2	21.69	0.001	0.063	0.34	21.69**	**0.001
Humic Acid (HA)	4	113.78	0.0002	0.031	1.8	**113.78	**0.0002
Cd*HA	8	65.24	0.0017	0.161	5.3	**65.24	**0.0017
Error	30	60.8	0.001	0.124	3.4	60.8	0.001
CV (%)		8.9	10.8	11.56	30.5	8.9	10.8

**significant effect at 1% probability level. GP = germination percent, GR = germination rate, MGT = Mean of germination time, MDG = Mean of daily germination, PL = plumule length, RL = radicle length.

Tab. 1. Continue

SOV	df	MS							
		RFW	PFW	CFW	RDW	PDW	CDW	SV_2	SV_1
Cadmium (Cd)	2	0.063**	0.34**	0.003	0.003**	0.0021**	0.045	20170.1**	0.39**
Humic Acid (HA)	4	0.031**	1.8**	0.131	0.0005**	0.0016**	0.012	5076.6**	0.24**
Cd*HA	8	0.161**	5.3**	0.133	0.0019**	0.0023**	0.027	21811.3**	0.106**
Error	30	0.124	3.4	0.05	0.000044	0.000071	0.007	437.9	0.003
CV (%)		11.56	30.5	12.53	10.27	15.45	12.01	13.48	16.29

**significant effect at 1% probability level. RFW = radicle fresh weight, PFW = plumule fresh weight, CFW = cotyledon fresh weight, RDW = radicle dry weight, PDW = plumule dry weight, CDW = cotyledon dry weight, SV1 = seedling weight index, SV2 = seedling length index

The longest radicle observed for 200 ppm Cd and 300 mg lit^{-1} HA (86% increase over control) and the shortest one was related to 100 ppm Cd and 400 mg lit^{-1} HA (56% decrease in comparison with control) (Fig. 1). The longest plumule was observed in 200 ppm Cd and 400 mg lit^{-1} HA (192% increase over control) and the shortest one was related to 400 mg lit^{-1} HA without Cd (44% decrease over control)(Fig 2).

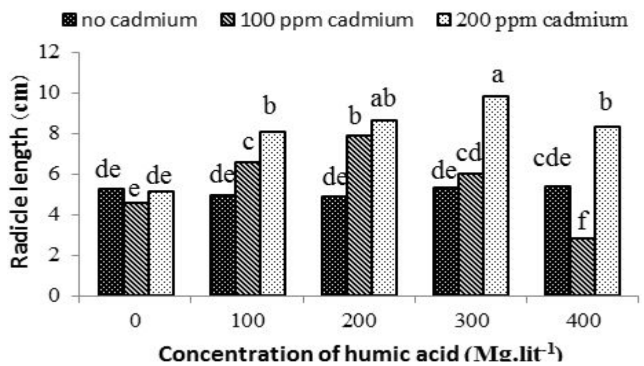

Fig. 1. Effect of Humic acid and cadmium concentration on pumpkin seedling radicle length

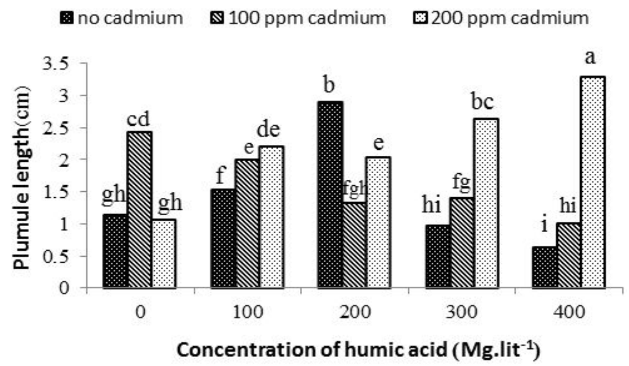

Fig. 2. Effect of humic acid and cadmium concentration on pumpkin seedling plumule length

Application of 200 ppm Cd with 200 mg lit-1 HA and 200 ppm Cd with 300 mg lit^{-1} HA, respectively produced the highest radicle and plumule fresh weight (respectively, 361% and 331% increase in comparison with control) (Fig. 3 and 4).

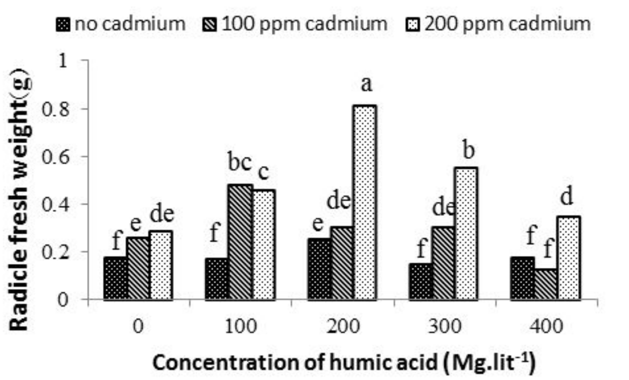

Fig. 3. Effect of humic acid and cadmium concentration on the radicle fresh weight of pumpkin seedling

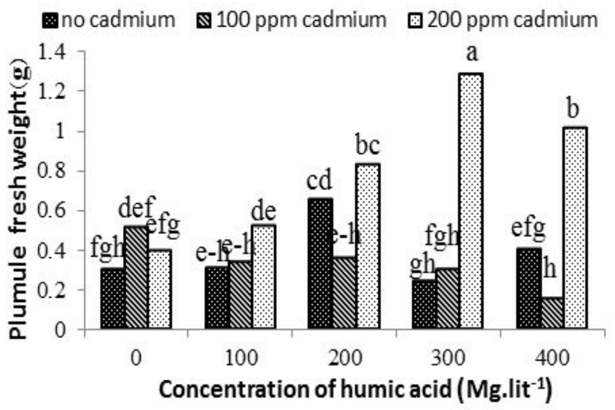

Fig. 4. Effect of humic acid and cadmium concentration on the plumule fresh weight of pumpkin seedling

The highest dry weight of radicle and plumule respectively, were observed in 200 ppm Cd with 400 mg lit^{-1} HA and 200 ppm Cd with 300 mg lit^{-1} HA (respectively, 152 and 109% increase over control) (Fig. 5 and 6).

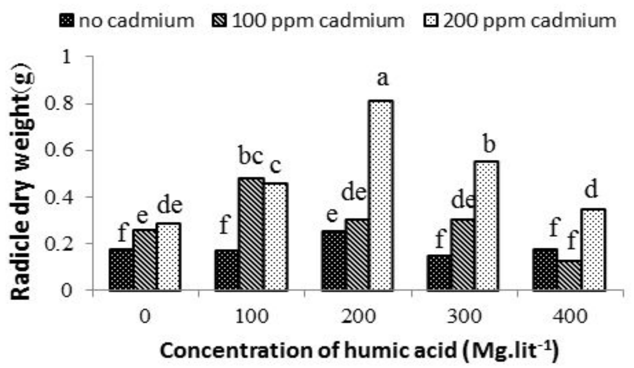

Fig. 5. Effect of humic acid and cadmium concentration on the radicle dry weight of pumpkin seedling

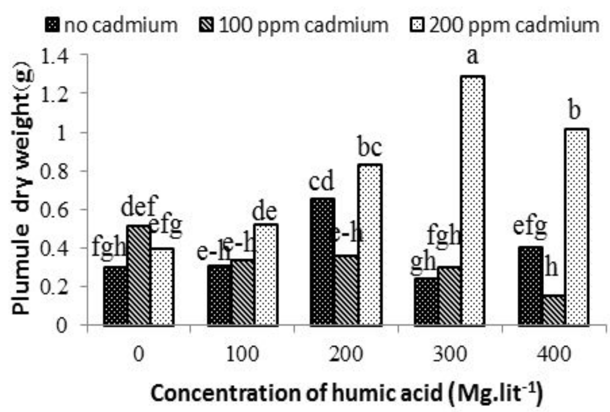

Fig. 6. Effect of humic acid and cadmium concentration on the plumule dry weight of pumpkin seedling

The highest fresh and dry weight of cotyledon were observed in 100 ppm Cd with 200 mg lit^{-1} HA and 100 mg lit^{-1} HA without Cd, respectively (20 and 14% increase over control, respectively) (Fig. 7 and 8).

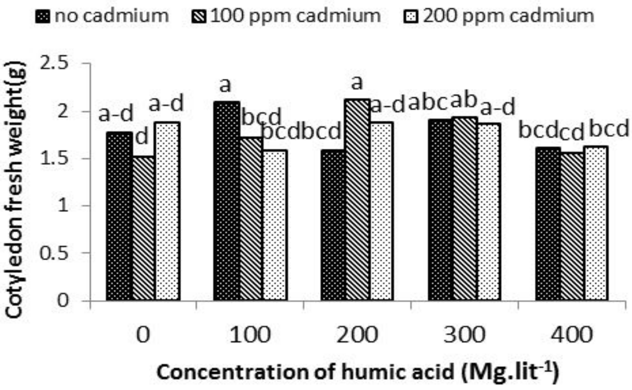

Fig. 7. Effect of humic acid and cadmium concentration on the cotyledon fresh weight of pumpkin seeds

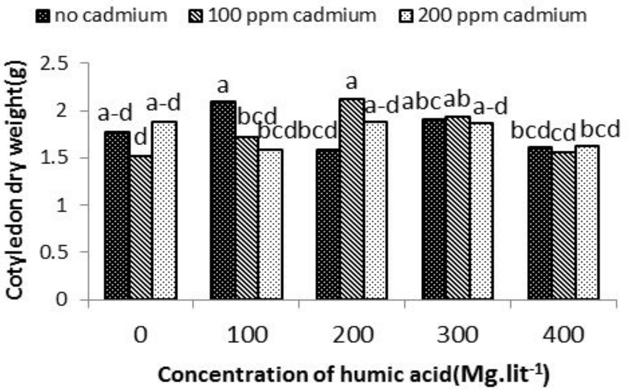

Fig. 8. Effect of humic acid and cadmium concentration on the cotyledon dry weight of pumpkin seeds

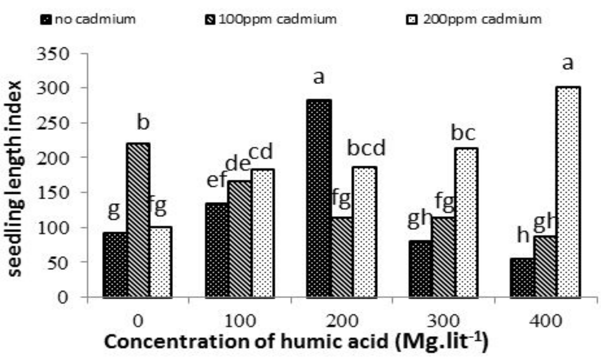

Fig. 9. Effect of humic acid and cadmium concentration on the length index of pumpkin seedlings

The highest seedling length index (301) was related to 400 mg lit^{-1} HA and 200 ppm Cd (Fig. 9), and the highest seedling weight index (1) was also observed in the case of this treatment (Fig. 10).

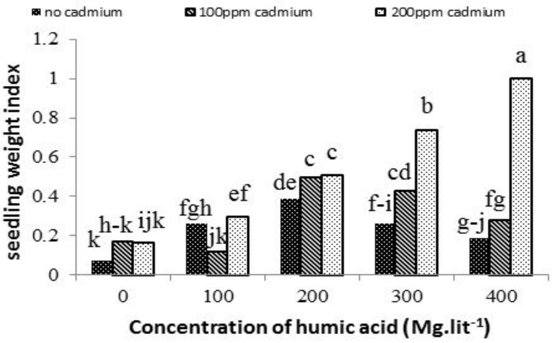

Fig. 10. Effect of humic acid and cadmium concentration on the weight index of pumpkin seedlings

Discussion

GP, MGT and GR were not affected by HA and Cd. It can be conculded that there is an antagonistic effect between HA and Cd. Amirjani (2012) reported that Cd can decrease GP of wheat seeds. GP and GR in wheat seeds (Saremi-Rad *et al.*, 2011) and cowpea (AI-Rumaih *et al.*, 2001) decreased in presence of Cd. Priming with HA and Cd increased GP and GR and decreased MGT in lettuce seeds (Mojahedi *et al.*, 2011) and tomato (Piccolo *et al.*, 1993). Jafari *et al.* (2011) reported an increase in radicle and plumule length of wheat seedlings in response to HA and Cd. Kouser and Azam (1985) found that application of 54 mg lit^{-1} HA increases 50% in radicle length and 22% in dry matter of wheat. HA in the concentration of 50 mg lit^{-1} increased cell elongation in pea root cells and higher concentrations had not significant effect (Vagan, 1974). Soltani *et al.* (2007) demonstrated that Cd decreases seedling dry and fresh weight and leaf area in rapeseed. HA increased root dry weight in bent grass and caused to increase in the activity of enzymes responsible for root respiration (Liu and Cooper, 2000). HA had an inhibitory effect on the activity of phosphatase in root cells and caused a decrease in root growth. Seed vigor also increased by applying HA and Cd (Vagan, 1974). These results are not in consistence with the results reported by Poschenreider *et al.* (1989). HA affected nitrate uptake and ATP-ase activity in the plasma membrane and caused cell elongation (Pinton *et al.*, 1999).

Conclusion

Applying humic acid caused pumpkin seeds to be more tolerant to environmental stresses like heavy metals, especially Cd. In this experiment, a concentration of 300 mg lit^{-1} of HA had the betst results in detoxifying of Cd in high concentration (200 ppm).

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