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Research Article

Effect of different ascorbic acid levels (Vitamin C) on eco-physiological properties of barley in soils contaminated with lead

Kamdin Akhavan Samimi, Hamid Reza Tohidimoghadam

and Mohammad Nasri

Agroecology, Islamic Aazad University,

Varamin Branch, Iran.

ABSTRACT

This study was carried out to examine the effects of foliar application of ascorbic acid on barley in contaminated soil in a completely randomized factorial design with 2 factors, 9 treatments and 3 replications in Varamin in 1393.150 mg of lead nitrate per kg of soil were applied to infect the soil for all treatments. Superabsorbent was the first factor used in three levels (0, 3, 6 g per kg soil) and ascorbic acid as the second factor was also used in three levels (0, 50 and 100 ppm). The results of this experiment showed that increase in superabsorbent and ascorbic acid concentrations in barley improved the morphological traits such as plant height and spike and grain number, grain weight, total weight of shoot, root dry weight and thousand grain weight and also improved physiological traits such as protein content and chlorophyll a, b and total chlorophyll in barley, moreover, increase in ascorbic acid in the plant resulted in reduction in antioxidant enzymes content such as superoxide dismutase andcatalase, and physiological traits such as proline, increased relative water content and reduced lead content in leaves and roots.So it can be concluded that, given that the country is located in arid and semiarid regions and considering Iran's soils pollution with heavy metals,using effective treatments such as ascorbic acid can enhance crop water holding capacity and also reduce the effects of these elements toxicity. Therefore,the use of ascorbic acid seems essential.Due to the non-degradable and long life of heavy metals in soil, insoluble hydrophilic polymers with different amounts carboxylic groups are used. The surface carboxylic groups of the polymer (SAP) due to exposure to pH are ionized and make strong bonds with soil pollutant metals, and eventually form a gel and are separated from soil.

Keywords: Ascorbic acid, Lead nitrate, Barley.

INTRODUCTION

In this study the effects of different concentrations of lead were studied on some physiological and biochemical indices of barley.Metal toxicity in plants is very complex and depends on plant species, element type, concentration, pH of the soil and soil composition. Heavy metalspollution such as lead results indetrimental effects on plant growth and metabolism. When heavy metals are absorbed by plants and accumulated in tissues, then they cause toxicityin two ways:

1. Indirectly through competition with other essential nutrients and replacing them in pigments or enzymes structures and degradation of their performance 2. directly through damaging the cell structure, the presence of heavy metals causes oxidative stress and increased production of re active oxygen species (ROS), which in turn can cause various toxic effects on plants, such as reduced growth, reduced chlorophyll content, inhibit the activity of enzyme, damage to biomolecules such as

lipids, proteins and nucleic acids in particular DNA.

Heavy metals have irreversible effects on groundwater resources and ecosystem and their pollution is increasingly on the rise.Due to the non-degradable and long life of heavy metals in soil, insoluble hydrophilic polymers with different amounts carboxylic groups are used. The surface carboxylic groups of the polymer (SAP) due to exposure to pH are ionized and make strong bonds with soil pollutant metals, and eventually form a gel and are separated from soil. Acrylic polymers and acryl amide were significantly used to eliminate lead. Being simple and relatively economic are the advantages of removing soil contaminants by polymers.

Water and nutrients are directly absorbed by the hairs root grown inside superabsorbent gels.Super absorbent protects capillary flow of water to the root zone through liberalization of the water and based on moisture gradient. Super absorbent can release up to 95% of absorbed water and make it available to plants; therefore, soil moisture potential will be available to plants for a longer time.

Research specific objectives and necessities

1. Investigating the responses of antioxidant enzyme activities of superoxide dismutase, catalase and glutathioneperoxidase in barleyand their effects against lead

2. Examining the morphological and physiological characteristics of plants under conditions of heavy lead contamination

Material and Methods

This study was carried out in greenhouse condition in March 1393 in educational and research greenhouse atVaramin-Pishva,Varamin University. According to the long-term data, average precipitation is 170 mm per year and average minimum and maximum temperatures are 5/43 and -14 degrees Celsius respectively.

Plants selection

To examine plants against lead, *Hordeum Vulgare*cv. Hashiye Nosratwas selected. Tolerance against heavy metal and refinement potential of crop were assessedin this study.

Soil characteristics

Varamin Plain is generally composed of alluvial soil resulted from erosion in southern slopes of the Alborz Mountains by Jajroodriver and there are limited areas resulted from colluvium sediments and are formed from the border hill and plain erosion. Low plains of Varamin, which is located around Charmshahr and study area, is composed of solonjak soils including loam and clay loam, clay, clay and silt soils with salt and alkalin pH. In some areas, due to the underlying clay and salty soils, salt pusan are formed that have a poor drainage. This type of soil forms about 23.9 percent of the Varamin plain. Saline soils are located in the south of the plain and often due to high salinity are not suitable for cultivation and are subject to a lot of wind erosion is a major factor of these lands uselessness.

Test Method

This experiment was done in a completely randomized factorial design with 2 factors, 9 treatments and 3 replications.150 mg of lead nitrate per kg of soil were used for each treatment. This study was carried out in greenhouse condition in March 1393 in educational and research greenhouse at Varamin-Pishva Branch, Varamin University. Some seeds were planted in each pot.

The first factor ascorbic acid (F) was used in three levels:

f1: 0 ppm

f2: 50 ppm

f3: 100ppm

Measured characteristics

1-Proline content

2-Leaf relative water content (RWC)

3-Antioxidant enzyme of superoxide dismutase 3 (SOD), catalase levels (CTA) contents

4. Lead amount in root

5. Leadamount in straw

6- Leadamount in seeds

Data Analysis

The data were analyzed using SAS statistical software. The means were compared by Duncan's multiple range tests at the five percent level. Corresponding graphs were plotted using Excel software.

Proline

Among the compatible dissolved substances, proline is probably the most common and extensive osmolyte (Kuznetsoy and Shevyakova, 1999).

Proline, an amino acid that accumulates in all organs during stress and leaves are where it is widely accumulated. This Osmolytes has been widely studied as compatible metabolite in terms of stress (Paleg*et al*., 1989).

In other words, increasein the concentration of proline is the most common reaction caused by water scarcity or reduced osmotic potential, that have been observed not only in plants but also in algae, bacteria, marine invertebrates and protozoa. Although, proline accumulates in all plant organs during stress, but it has the fastest and widest accumulation in leaves. Proline accumulation in roots occurs slowerand with time delay compared to accumulation in leaves.A survey shows that the increase in root proline is resulted its transfer to leaves. (Delauney and Verna, 1993).

It is observed that the effect of super absorbent polymers at one percent level and effect of foliar application of ascorbic acid at the level of five percent and also the interaction of superabsorbent sprayed with ascorbic acid spraying at the level of five percent have been significant on the proline content.

The highest level of proline content was observed in the absence of superabsorbent polymers (0 g per kg of soil) and also the lowest proline content was obtained at 6 g superabsorbent polymer per kg of soil. It seems that the use of superabsorbent polymers and lead stabilization in soil prevents its absorption by plant roots and by reducing oxidative stress caused by heavy metals decreased proline activity with super absorbent polymers in soil.

Moreover, according to the mean comparison table of the effect of foliar application of ascorbic acid, the highest level of proline is at 0 ppm level of ascorbic acid. There was no significant difference at 50 ppm level and also proline showed the highest activity at 100 ppm of ascorbic acid. It seems that the application of ascorbic acid,Plants use ascorbic acid in order to deal with free radicals caused by oxidative stress resulting from heavy metals during which reduces the amount of proline.

Osmotic adjustment and maintenance of osmotic pressure within the plant is one of the roles of proline. In general, free proline content in plants in desirable conditions is very low andany reduction in tissue water content under stress increases the proline content in plants (Abbas-Zadeh et al., 1386).

The interactions of super absorbent and foliar application with ascorbic acid have been observed, as in the absence of super absorbent polymer, the highest level of prolinewas shown where no ascorbic acid (0 ppm) had been sprayed.

Furthermore, in the presence of superabsorbent polymer at the amount of 3 and 6 g, the highest level of proline was observed under 0 ppm of ascorbic acid application.

Similar results were reported on the increase in prolin content under stress in different plants such as sunflowers and beanson the increased stress proline (Zhang et al., 2000; Costa et al., 1994). Stresses such as cadmium increase proline content through accelerating leaf senescence. Madan et al. (1994) also studied the effect of leaf senescence on proline content in Brassica juncea under salt stress.

As it can be seen,the absorption of heavy metals in plants has been reduced by increasing the concentrations of superabsorbent polymers and by increasing the concentration of ascorbic acid, plant uses this antioxidant to confront heavy metals, reduce proline production and consume the energy required to produce these enzymes to improve its growth and development.

Lead level in root

Super absorbent polymer and foliar spraying with ascorbic acid and superabsorbent interaction with ascorbic acid showed significant effects (at 1% probability level) on the amount of lead in the roots.

Moreover, according to table 2-4 on the mean comparison of effects of superabsorbent polymers application, the highest level of lead in root was found at 0 g superabsorbent per kg of soil.

Also according to the mean comparison of the effect of ascorbic acid foliar application, the highest level of lead in root was observed under 0 ppm treatment.

The interaction of super absorbent and ascorbic acid foliar application have been observed, in the absence of superabsorbent polymer, the highest level of lead in root was found in plants received no ascorbic acid (0 ppm).

Furthermore, under 3 and 6 g superabsorbent polymer treatments, the highest level of lead in root was found in plants treated with 0 ppm ascorbic acid.

Lead level in leaf

The effects of super absorbent polymer and foliar application of ascorbic acid,and the interaction of superabsorbent and ascorbic acid foliar application were significant at 1% and 5% probability levels.

The highest level of lead in leaf was observed under at 0 g of superabsorbent polymers per kg of soil.

Furthermore, according to the mean comparison of the effect of foliar application of ascorbic acid, the highest level of lead in leaf was observed at 0 ppm.

As indicated in table 3-4 on theinteractionof ascorbic acid foliar applicationand superabsorbent application, in absence of superabsorbent polymer, the highest level of lead in leaf was found in plants received no ascorbic acid (0 ppm).

Moreover, under 3 and 6 g superabsorbent polymer treatments, the highest level of lead in leaf was found in plants treated with 0 ppm ascorbic acid.

Lead level in grain

The effect of super absorbent polymer was significant (1% probability),while the effect of ascorbic acid foliar application was not significant. And the interaction of superabsorbent and ascorbic acid foliar application on lead level in grain was significant (5% probability).

The highest and lowest levels of lead in grain were found in plants treated with 0 and 6 gper kg soil of super absorbent polymers, respectively. It seems that superabsorbent polymers prevents lead uptake by plant roots through stabilizing it, reduced heavy metals oxidative stresswhich resulted in less lead level in grains. Due to supplying needed water to plants, superabsorbent polymers can enhance plant tolerance to heavy metal stress and delay plant stress.

Furthermore, according to the mean comparison of ascorbic acidfoliar application effect, the highest and lowest lead level in seed belongto 0 and 100 ppm of ascorbic acid, respectively.It seems that plants use ascorbic acid to confront free radicals caused by oxidative stress resulting from heavy metals and reduce lead level in grains.

Also, 50 ppm foliar application of ascorbic acid showed no significant difference.

Super absorbent and ascorbic acid foliar application interactions showed thatthe highest lead level in grains was found in plants treated with neither super absorbent polymer nor ascorbic acid (0 ppm). In the presence of super absorbent polymer at 3 and 6 g, plants received 0 ppm ascorbic acid showed the highest lead level. However, there was no significant difference between plants treated with 3 g super absorbent polymer and 50 ppm ascorbic acid.

As it can be seen,by increasing the concentrations of superabsorbent polymers the absorption of heavy metals in plants has been reduced and with increasing the concentration of ascorbic acid, plant uses this antioxidant to confront heavy metals,reduce lead level in grains and consume the energy required to produce these enzymes to improve its growth and development.

Relative Water Content

Super absorbent polymer showed significant difference at 1% probability level, while ascorbic acidfoliar application showed significant difference at 1% probability level. Moreover, the interaction of superabsorbent and ascorbic acid foliar application showed significant effects (at 5% probability level) on relative water content.

Superabsorbent polymers at 6 g per kg of soil showed the highest relative water content. In Haghighiet al. (1393) study, superabsorbent improved tomato growth parameters under stress condition, as10% V superabsorbent at 50% field capacity increased relative water content of tissue by 14% compared to treatment with no superabsorbent. 10% V superabsorbent at 25% field capacity increased relative water content of tissue by 28% and 53% compared to those received to superabsorbent. Superabsorbent application results in usual plant growth under stress condition through enhancing features like water holding capacity in soil and roots.

Furthermore, according to the mean comparison of ascorbic acid foliar application effect, 100 ppm has the highest relative water content.

The interaction of super absorbent and ascorbic acid foliar application showed that in the absence of superabsorbent polymer, plants treated with 100 ppm ascorbic acid had the highest relative water content. Moreover, the combinations 3 and 6 g superabsorbent polymer and 100 ppm ascorbic acid showed the highest relative water content. However,no significant difference was observed under 3 g superabsorbent polymer and 100 ppm treatment.

Biochemical characteristics

Antioxidant enzymes activities

Superoxide dismutase

Super absorbent polymer, ascorbic acid foliar application and the interaction superabsorbent ascorbic acid foliar application showed significant differences ($P < 0.01$). Plants received 0 g per kg of soilsuperabsorbent polymers showed highest level of superoxide dismutase and the lowest level of superoxide dismutase activity wasfoundin plants treated with 6 g per kg of soil super absorbent polymer.It seems thatsuperabsorbent polymer reduces the activity of this enzyme through stabilizing lead and preventing its uptake by plant roots. Therefore, preventsthe reduction of electron flow in photosynthesis, photosynthesis rate and dry matter production in plant.

According to the comparison of ascorbic acid foliar application effect, the highest and lowest levels of superoxide dismutase were observed in plants treated with 0 and 100 ppm ascorbic acid. It seems that plants use ascorbic acid to confront free radicals caused by oxidative stress resulted from heavy metals and in such circumstances reduces the production of superoxide dismutase. It should be considered that plants consume some of food energy to produce these enzymes. Therefore, ascorbic acid spraying reduces the production of these enzymes and the energy is used for growth and development of plants.

Based on the interactions super absorbent and foliar application of ascorbic acid, the highest level of superoxide dismutase was observed in plants received neither super absorbent polymer nor ascorbic acid (0 ppm).

Under 3 and 6 g superabsorbent polymer treatment, the highest plant height was observed in plants received0 ppm ascorbic acid.Moreover,there was no significant difference at 3 g super absorbent polymer and 100 ppm ascorbic acid.

As you can see heavy metals absorption is reduced by increase in superabsorbent polymers level and with increase in the concentrations of ascorbic acid, plant uses this antioxidant to deal with heavy metals, reduces the production of superoxide dismutase and uses the energy required to produce these enzymes to improvegrowth and development.

In stress condition, the amount of reactive oxygen species (ROS) increases in plant, in such condition plants use different mechanisms to remove and eliminate reactive oxygen species. It seems that the activation of superoxide dismutase isa response to the damaging effects of free radical's oxygen production resulted from lead stress in this plants species. Controlling destructive oxygen levels by this enzyme in an important protection mechanism against oxidative stress in cell, as these compounds act as initiatives to more toxic or active derivatives (Khatun et al., 2008).

Catalase

Super absorbent polymer and ascorbic acid showed significant differences at 1% probability levels, and the interaction of superabsorbent and ascorbic acid on catalase was significant at 5% probability levels.

According to table 4.2 on the comparison of superabsorbent effects, the highest level of catalase was found at 0 g per kg of soil. The lowest level of catalase activity was observed in plants treated with 6 g super absorbent polymer. It seems that superabsorbent prevent lead abruption by plants roots through lead stabilization, and by reducing oxidative stress caused by heavy metals results in decrease in enzyme activity undersuperabsorbent application. Thus, avoids any disruption in electron flow pathway in photosynthesis, decreases photosynthesis rate and dry matter production in plant.

Also according to the comparison of foliar application of ascorbic acid effect, the highest level of catalase is observed in 0 ppmtreatment. The lowest level of catalase activity was found in plants received 100 ppm ascorbic acid.It seems that ascorbic acid is used by plants to deal with free radicals caused by oxidative stress resulting from heavy metals and decreases catalase production in these conditions.

It should be considered that these enzymes are produced through consuming some of the energy produced by plant food,in other words, some of its energy that should have normally been consumed for plant growth and development is used to produce these enzymes. Thus ascorbic acid spraying reduces the production of these enzymes and uses the energy for plant growth and development.

Based on the interaction ofsuper absorbent and foliar application of ascorbic acid results, the highest catalase level was observed in plants received neither super absorbent nor ascorbic acid (0 ppm).

Moreover, 3 and 6 g superabsorbent polymer resulted in the highest catalase under0 ppm ascorbic acid.

As it is obvious,increase in superabsorbent polymers level reduces heavy metals absorption and with increase in ascorbic acid level, plants use this antioxidant cope with heavy metals, reduce the production of catalase and energy required to produce these enzymes, and use the energy to improve growth and developmental.

This indicates that the use of superabsorbent polymers and ascorbic acid spraying provide a more favorable situationfor plants and makes them more resistant against heavy metal stress.

It also might be due to the activation of another the immune system rather than the antioxidant defense system to fight free radical oxygen and removethemlike proline which removes oxygen free radicals (ROS Scavenger).

It has been reported that osmolytes such as proline play an important role in osmotic adjustment and protect cell through removing ROS (Reddy et al., 2004).

Table 1. The analysis of variance

	Df	Superoxide dismutase	Catalase
SAP (S)	2	70.310,954 **	85.48768 **
Ascorbic acid spraying (F)	2	90.4046 **	17.1542 **
interaction of super absorbent and spraying (SF)	4	36.383 **	81.25 *
Error	18	93.70	03.24
total error	26		
coefficient of variation (CV)		70.1	11.3

Table 2. The comparisonof simple effects of foliar application of ascorbic acid and superabsorbent polymersaverages

Treatment	Amount	Superoxide dismutase mg pro.min-1	Catalase mg per.min-1
The use of super absorbent polymer	0 g	42.669 a	41.237 a
	3 g	35.522 b	141 b
	6 g	300 c	80.92 c
Ascorbic acid spraying	0 ppm	16.518 a	54.170 a
	50 ppm	03.398 b	45.156 b
	100 ppm	77.475 c	38.144 c

Table 3. The comparison of the interaction of foliar application of ascorbic acid and superabsorbent polymers averages on morphological traits and yield components of barley

The use of super absorbent polymer	The effect of foliar application of ascorbic acid	Superoxide dismutase mg pro.min-1	Catalase mg per.min-1
0	0	75.688 a	23.248 a
	50	84.553 d	155 c
	100	91.311 g	38.108 f
3	0	75.667 b	95.235 b
	50	08.526 e	22.141 d
	100	28.300gh	20.92 g
6	0	76.651 c	04.228 b
	50	14.487 f	30.127 e
	100	41.288 h	81.77 h

CONCLUSION

According to the results, we can conclude that:

1-Increasein lead nitrate concentration in plants resulted in reduction in morphological traits such as plant height,spike and grain number, grain weight and total weight of shoot, root dry weight and thousand grain weight and also reduction in physiological traits such as protein content and chlorophyll a, b and total chlorophyll in barley.

2-Moreover, increase in lead level in plants improved biochemical traits and antioxidant enzymes content such as superoxide dismutase andcatalase, and physiological traits such as proline, relative water content and lead content in shoot and root.

3-Increasein super absorbent concentration in plants improved morphological traits such as plant height,spike and grain number, grain weight and total weight of shoot, root dry weight and thousand grain weight and also improved physiological traits such as protein content and chlorophyll a, b and total chlorophyll in barley.

4-Furthermore,increase in super absorbent concentration in plantsreduced biochemical traits and increased antioxidant enzymes content such as superoxide dismutase andcatalase, and physiological traits such as proline, relative water content and lead content in shoot and root.

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