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**Research Article**

# Effect of different levels of spraying with ascorbic acid (Vitamin c) and application of super absorbent polymer on eco-physiological characteristics of plants in soils contaminated by Lead

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## ABSTRACT

In order to study the effects of application super absorbent polymer and sprayingof ascorbic acid in the soils contaminated by Lead on wheat plant, this experimentwas carried out in factorial form with two factors completely randomized design with 9 treatments and 3 replications in crop year 1393 in Varamin. For contaminating the soil, Lead chloride 200 mg per kg of soil applied for all treatments.The first factor is superabsorbent in three levels (0, 4 and 8 g per kg soil) and the second factoris ascorbic acidinthree levels (0, 75 and 150 ppm). The results of this experiment showed that increasing concentrations of ascorbic acid and superabsorbent inwheatIncreased the morphological traits such as plant height, spike and seeds, grain weight and total weight of shoot and root dry weight andthe weight of one thousand seedsas well as increased physiological traits such as protein and chlorophyll a, b and total wheat and superabsorbent and ascorbic acid concentration in the plant, such as a reduction in antioxidant enzyme superoxide dismutase, catalase, and the physiological traits such as proline and increasedthe relative water content leaves and reduce the amount of Lead in leaves and roots.So it can be concluded that it seems necessary that given thatIran located in the arid and semiarid and according to Iranian soil pollution with heavy metals such as ascorbic acid and superabsorbent, the use of effective treatments that can enhance the plant's water-holding capacity as well asreduce the toxic effects of these elements. Due to the long life and non-degradable heavy metals in soil, hydrophilic polymers insoluble in water with different percentages of carboxylic groups are used. The surface carboxylic groups of the polymer (SAP) due to exposure to environments, Ionize and a solid linkconstitute with the metals contaminating soil ,finally, form a gel and are separated from the soil.

**Key words:** polymers and superabsorbent, ascorbic acid, chloride, Lead, wheat

## INTRODUCTION

Soil pollution with heavy metals is as a result of human activities such as mining, metallurgical and fertilizers application, agricultural pesticides and fungicides etc…that endanger human health and the ecosystem. Today's pattern of industrial activities leads to environmental pollution, especially pollution with heavy metals.

In many acidic soils and approximately, half of the agricultural lands having the potential for food production the heavy metals are as a factor limiting plant roots. Lead has a relatively higher mobility than other heavy metals in soil and plants so it is easily absorbed by plants and showsits toxic effects(Shyam, 2001).

Lead asThe most dangerous heavy metal polluting the environment, effects on metabolic activity and physiological influences on living

organisms, so ithas a greater attention in recent yearsby researcher (Badot 2003).

**Specific goals and needs of research**

1. The role of the application of superabsorbent polymers by measuring the Lead accumulation in the seeds and shoots of wheat in the conditions with and without application of these polymers.
2. The studyof response of antioxidant enzymes, superoxide, catalase in wheat and their effects against Lead contamination.
3. The study of morphological and physiological characteristics of the plant under conditions of heavy metal contamination compared with the use of super absorbent polymer.
4. The role of spraying of ascorbic acid plant physiological traits such as chlorophyll and proline as well as the activity of antioxidant enzymes such as CAT and SOD.

**Materials of experiment**

Present researchwas carried out in a greenhouse experiment in Varamin University Pishva Educational Research Greenhouse in March 1393.

According to the long-term average rainfallprecipitation is annually170 mm. according to the long-term statistic of area have been reported that average minimum and maximum temperatures are 5/43 and 14 degrees Celsiusrespectively.

**Selection of the plant in question**

Genus Triticum was selected for refining vegetableLead figureSW ofplants from the Poaceae family,. In this study, tolerance of the above crop to heavy metal as well as the refinement power wasstudied.

**Geology of the Region**

Varamin plains generally consist of alluvial soil that has been formed by erosion of the southern slopes of the Alborz Mountains by the Jajrood river and there are few places that are derived from colluvium deposits and have formed from erosion of the hill and plain border.

Low unit that occurs inVaramin plain around the Charmshahr and the studied area is composed of soils Svlnchak that are loam and clay soils, clay and silt, with salt. In some areas due to the underlying clay soils and saline pusan is composed of salt, they have poor drainage about 23.9 percent of Varamin plain area consists of the soil type. Saline soils are mostly in the southern plains of Varamin and due to the high salinity, they are not now suitable for cultivation and there are also a lot of wind erosion that are an important factor in keeping the land unused.

**Soil sampling**

After preparing the soil for pots to evaluate the physical and chemical properties

Of soil, samples were taken from 5 regions and then the mixture was sent to the laboratory. Based on the results of soil analysis, testedsoil is salty-clay andhave 29 percent sand and 26 percent silt and 45 percent clay. Also Its pH was equal to 7.7 and Its EC was equal to 4.3 mmol/cm.

**Experiment Method**

Experimental design used in this study

The project is in factorial form in a completely randomized design with 9 treatments in 3 replications and includes two factors. Lead chlorideamount of 200 Mg per kg of soil applied to contaminate soilfor all treatments.This project was conducted in nursery education - Research University Rector Varamin. Some seeds were planted in each pot.

The first factor:ascorbic acid (F), the three levels that include:

$f_1$ : 0 ppm

$f_2$ : 75 ppm

$f_3$ : 150 ppm

The second factor: Super absorbent (S), the three levels that include:

$s_1$ : 0 g per kg of soil

$s_2$ : 4 grams per kilogram of soil

$s_3$ 8 g per kg of soil

**Measured traits**

1- Proline (Bates, 1973 )

2- Amount of relative water content **(RWC** )
(1981, **Turner** )
**3** - The amount of antioxidant enzyme superoxide dismutase **(SOD** ) (Yoshikawa (1979))
4 - The amount catalase **(CTA** ) Paglia (1997)
5 - The amount of Lead in root
6 - The amount of Lead in straw
7 - Lead levels in the grain

**Research findings**

**Proline**

Among the compatible solutes, Proline is probably the most common and most widely Osmolytes.(Kuznetsoy and Shevyakova, 1999).

Proline is an amino acidduringthestressaccumulation in all plant tissues and its accumulation place isin leaves (widespread andquickly).

This Osmolyteshas been investigated extensively as metabolites consistent in tension conditions.(Paleg et al., 1989)

In other words, increasing the concentration of proline,is the most common reactionas soon as rinsing due to the lack of water or osmotic effect, has been observed not only in plants but also in algae, bacteria, organic, marine invertebrates and protozoa.Although prolineaccumulates in mature plant tissues during stressbutthe fastest and most extensive accumulation is in leaves.

Proline accumulation in rootsis the lower expanding and delay with respect to the accumulation in the leaves. The study shows thatthe increasing of Proline is due to the transfer of the leaf stem.(Delauney and Verna, 1993)

Effect of super absorbent polymer in1 Percent level and the effect of ascorbic acid spraying with 1 percent level and the interaction in ascorbic acid spraying in five percent level on proline are significant.

It also can be seen in Table 4.5, according to a simple comparison of the effects of the use of superabsorbent, the highest level of proline occurs in the use of superabsorbent polymers 0 kg soil as well as the lowest proline is obtainedin conditionsthat superabsorbent polymer was used at a rate of 8 grams per kilogram of soil. It seems that the use of superabsorbent polymers preventsits absorption by plant roots by fixation the Lead in the soil and by reducing oxidative stress caused by heavy metals reduces the amount of proline activities under the conditions that superabsorbent polymers is used in the soil.

Also, it can be seen from the table of comparison of the effect of application of ascorbic acid spraying, the highest rate of Proline is in 0 and 75 ppm level as well as the lowest level of prolineactivityoccurs in the level that ascorbic acid was applied at the rate of 150ppm. It seems that by application of ascorbic acid, the plantprefersin order to cope withfree radicalsOxidative stress of heavy metals by ascorbic acidcope withradicals and in these conditions reducesthe amount of proline.

One of the tasks of Proline is to set out osmosis and keep the osmotic pressure within the plant. Free proline is usuallyvery low in theplants that are at an optimal levelandamount of this material increasesBy reducing water stress in the tissue. (Abbas-Zadeh et al., 1386)

The interaction between the use of superabsorbentandspraying with ascorbic acid have been observed, in situations where superabsorbent polymer did not use the highest level of proline in which the plants had been sprayed with a concentration of 0 ppm of ascorbic acid. However, at the level of 150 ppm of ascorbic acid the difference is not significant.

interactions between application of super absorbent and spraying with ascorbic acid have been observed, in situations where we did not use super absorbent polymer, the highest rate of proline occurs in the level at which the plants had been sprayed with a concentration of 0 ppm of ascorbic acid. However, at the level of 150 ppm of application ascorbic acid difference is not significant.

Also,in situations where amount of 4 and 8 grams of superabsorbent polymer were used, the highest plant height related to the of application 0ppm ascorbic acid. However, at the level of 8 grams of superabsorbent polymer and 75 ppm of application ascorbic acid, difference is not significant.

Similar resultshave reportedincreased proline on different plants, such as sunflowers and beans under stress(Zhang et al., 2000; Costa et al., 1994).

Stresses such as cadmium, Increase theproline by accelerating leaf aging. MadanEt al (1994)studiedthe effect of leaf aging on the increasing of proline inBrassica junceaunder the salt stress.As can be observed, with increasing the concentration of superabsorbent polymersdecreases heavy metalsuptaking in plants and with increasing concentrations of ascorbic acid, the plant prefers to copewith heavy metals by the antioxidant and reducesproline production and consumesenergy to produce these enzymes to improve its growth.

**The amount of Lead in the root**

The effect of super absorbent polymer at the levelone percent and the effect of spraying of ascorbic acid at the levelof one percent and the interaction between application of superabsorbent spraying with ascorbic acid at the levelof five percent on the amount of lead in root was significant.

Also according to the table of average comparison of the effects of the use of superabsorbent it can be observed thatthe highest rate of Lead at the rootoccurs in the use of superabsorbent polymers at the level of 0 kg soil.

Also according to the table of average comparison of the effects ofSpraying with ascorbic acidit can be observed that the highest amount of leadIn the rootIs at the level of 0 ppm.

The interaction between the use of superabsorbent and spraying with ascorbic acid can be insituation where we did not usesuperabsorbent polymer the highest rate of Lead inrootoccurs in the level that the plantswas sprayed with a concentration of 0 ppm ascorbic acid.

Also insituation where the superabsorbent polymerswere usedat the rate of 4 and 8 grams, the highest level of Lead in root was the use of 0 ppm ascorbic acid.

**Lead levels in leaves**

The effect of super absorbent polymer in onepercent and the effect of spraying with ascorbic acid at the level of five percent and the interaction between application of superabsorbent sprayingwith ascorbic acid at a level of one percent on the Leadwithin the leafis significant.

Also according to the table of average comparison of the effects of the use of superabsorbentthe highest rate of Lead in leaves occurs in the use of superabsorbent polymers 0 kg soil.

Also according to the table of comparison of the effect of spraying of ascorbic acid

The highest amount of lead in the leaves is at the level of 0 ppm.

At 0 ppm

The interaction between application of SAP and spraying with ascorbic acid it can be seen that in situation where we did not use superabsorbent polymer the highest amount of lead in the leaves occurs in the level where the plants had been sprayed with a concentration of 0 ppm of ascorbic acid.

Also in situation where weused superabsorbent polymers amount of 4 and 8 gramsthe highest rate of Lead in the leaf was related tothe use of 0 ppm ascorbic acid.

However, at the level of 4 and 8 grams of superabsorbent polymer and the use of 75 ppm ascorbic acid difference was not significant.

**Grains of Lead**

The effect of super absorbent polymer atonepercent level and the effect of application of spraying of ascorbic acid one percent level and

the interaction between superabsorbent in spraying with ascorbic acid at the level of five percent on the Lead in seed was significant.

Also according to the table of average comparison of the effects of the use of superabsorbent, the highest Lead levels in seeds occurs in the use of superabsorbent polymers 0 kg soil as well as the lowest amount of Lead in the seedwas obtained in situation where super absorbent polymers were used at a rate of 8 g per kg of soil. It seems that the use of superabsorbent polymers, by fixation Lead in the soil, prevents its absorption by plant rootsand by reducing oxidative stress caused by heavy metals reduces the Lead content in seedsunder the condition of the use of superabsorbent polymers.Also the use of superabsorbent polymers for water supply of plants can be an important factorin order to increase the resistance of plants to heavy metal stress and delays stress in plant.

Also according to the table of average comparison of the effects of the spraying of ascorbic acid is observed thatthe highest level of Leadgrainsoccurs inthe levels of 0 and 75 ppm as well as the lowest amount of Lead in the grainobtained in situation where ascorbic acid was applied at the rate of 150 ppm. It seems that the with use of ascorbic acid, plantprefers to cope with free radicals caused by oxidative stress of heavy metals cope with these radicalsby ascorbic acid and in these conditions the amount of Lead in the grain is reduced.

The interaction between the use of superabsorbent and spraying with ascorbic acid can be seen, in situation wherewe did not use super absorbent polymer, the highest level of Lead in the grainoccurs in the level that the plantssprayed with a concentration of 0 ppm ascorbic acid.

Also , in situation wherewe usedsuperabsorbent polymers at the levels 4 and 8 grams, the highest rate of Lead in the seed was related to the level the use of 0 ppm ascorbic acid. However, at the level of 4 grams of superabsorbent polymer and the use of 75 ppm ascorbic acid was not a significant difference.

As can be seen with the increasing of concentrations of superabsorbent polymers, the absorption of heavy metals in plants decreases and with increasing concentrations of ascorbic acid, the plant prefers to cope with heavy metals by this antioxidant and reducesLead production in the grainandthe energy needed for the production of these enzymes consumes to improve its growth and development.

**Relative water content(RWC**)

The effect of super absorbent polymer at level of one percent and the effect of spraying with ascorbic acid at level of one percent and the interaction spraying with ascorbic acid at the level of five percent on the relative water content is significant.

Also according to table of average comparison of the effects of the use of superabsorbent, the highest relative water content occurs in the use of superabsorbent polymer, at the level of 8 g per kg of soil. In the study by Haghighyet al (1393), super absorbent improves the growth parameters of tomatoesin stress conditionso that In the field capacity 50% superabsorbent 10 volume percent causes 14 percent increasing in relative water content of tissues compared to the treatment without superabsorbent applying. The superabsorbent 10volume percent, respectively, causes 28 and 53% increasing in relative water content of tissues in the field capacity 25% compared to the treatment without applying the superabsorbent in the 5% level.The use of super absorbent polymer canresult in normal plant growth in stress by the increasing traits such as increasing of water holding capacity of soil and root.

Also according tothe table of average comparison of the effect of spraying of ascorbic acid it can be observed that the highest relative water content is at level of s150 ppm.

The interaction betweenthe use of super absorbent and spraying with ascorbic acidcan be seen, in situation where we did not use super absorbent polymer the highest water content relativeoccurs in the level that the plantssprayed with a concentration of 150 ppm Ascorbic acid.

Also in condition of theuse of superabsorbent polymers the highest relative water content was related to the levels of the use of ascorbic acid 4 and 8 grams

**Biochemical traits**

Antioxidant enzyme activities

Superoxide dismutase

Superoxide dismutase (SOD) isthe metal enzymes that catalyze proportion of free radicals, superoxide to hydrogen peroxide and oxygen and it seems that they have an important role in protecting cells from the indirect harmful effects caused bythese radicals (Luis *et al*., 1983).

According to the table 1of analysis of variance we observe that the effect of super absorbent polymer atonepercent level and the effect of spraying with ascorbic acid at one percent level and the interaction between application of superabsorbent and spraying with ascorbic acid at one percent level on superoxide dismutase is significant.

Also according to the table 2, the average comparison of the effects of the use of superabsorbent we observe that the highest levels of superoxide dismutase occurs in the use of superabsorbent polymers 0 kg in soil and the lowest values of superoxide dismutase activity were obtained in the situation wheresuper absorbent polymer was used at the rate of 8 g per kg of soil. It seems that the use of superabsorbent polymers, by fixation of Lead in the soil,prevents its absorption by plant rootsandreduces the activity of this enzyme by reducing oxidative stress caused by heavy metals under the condition of the use of superabsorbent polymers in soiland so prevents disruption of the flow of electrons in photosynthesis, reduces the rate of photosynthesis and plant dry matter production.

Also according to the table of comparison of the effect of spraying with ascorbic acid,it is observed that the highest amount ofsuperoxide dismutase is at 0 ppm as well as the least amount of superoxide dismutase activity occurs in the level where it was used at the rate of 150 ppm of ascorbic acid. It seems that the use of ascorbic acid, the plantprefers by ascorbic acid cope with the free radicals caused by oxidative stress caused by heavy metals,andin these conditions, the production of superoxide dismutase activity reduce. It should be considered that the plant, for the production of these enzymes, always consumes some energy from food to produce these enzymes. In other words the amount of energy that mustbe consumed in normal conditions for growth and development, consumes to produce these enzymes so external sprayingwith ascorbic acid, decreases the production of these enzymes and the energy that should be consumed to produce these enzymes,causes plant growth. As we can see in the table (3), the interaction between the use of superabsorbent and spraying with ascorbic acid when we did not use super absorbent polymer, the highest levels of superoxide dismutase occurs in the level that the plant is sprayed with a concentration of 0 ppm Ascorbic acid

Also under the condition of the use of superabsorbent polymers at levels 4 and 8 grams, the highest amount of superoxide dismutase occurs in the level of use 0 ppm ascorbic acid.

As can be seen, absorption of heavy metals decreased with increasing concentrations of superabsorbent polymers and withthe increasing of ascorbic acidconcentration, the plant prefers to cope with heavy metals by the antioxidant and reduces the production of superoxide dismutase and consume energy to produce these enzymes to improve their growth and development.

Stress causes that reactive oxygen species (ROS) in plant isincreased. in these circumstances plants use different mechanisms to remove and destroy the reactive oxygen species, it seems that the activation of superoxide dismutase are in response to the damaging effects of oxygen free radicals produced from heavy metal stress in these plants. Controlling of destructive oxygen level by these enzymes in steady-state conditions is an important protection mechanism against oxidative stress in cells, because these compounds acts as a trailblazer for more toxic or more active derivatives(Khatun et al., 2008).

**Catalase**

According to variance analysis table 1, it can be seen that the effect of super absorbent polymer at the level ofone percent and the effect of spraying of Ascorbic acidat the level ofone percent and the interaction between superabsorbent application and spraying with Ascorbic acidat the level ofone percent on catalase were significant.

Also according to table 2 of the average comparison ofthe effects of the use of superabsorbent, we observed that the highest rate of catalase occurs in the levelof application of 0 g kg superabsorbent polymer soil as well as the lowest rate of catalase activitywas obtained in situations where thesuperabsorbent polymer was used at a rate of 8 grams per kilogram of soil.It seems that the use of superabsorbent polymers, by fixation of Lead in soil, Prevents its absorption by plant roots and by reduces oxidative stress caused by heavy metals decreased enzyme activity under the conditions of use of superabsorbent polymers in the soil. So it prevents disruption of the transitionof electrons in photosynthesis and reduces photosynthesis and dry matter production in the plant. Also according to the table of comparison of the effects of spraying with ascorbic acid, it is observed that the highest catalase is at the level of 0 ppm as well as the lowest catalase activity occurs in the level where ascorbic acid was used at a rate of 150 ppm.It seems that with the use of ascorbic acid, the plant prefers thatby ascorbic acidcope with free radicals caused by oxidative stress resulted from heavy metalsand in these circumstancesamount of the production of catalase activity is decreases. It should be considered thatplant always consumessome of the energy derived from food to produce these enzymes. In other words, the amount of energy that should be consumed under normal conditions for growth and development uses to produce these enzymes so externalspraying with ascorbic acid decreased amount of production of these enzymes and the energy that should be consumedto the production of these enzymes is consumed for plant growth.

As shown in Table 3,interactions between application of super absorbent and sprayingwith ascorbic acid have been observed,in the situation where we did not use super absorbent polymer the highest rate of catalase occurs in the level where the plants had been sprayed with a concentration of 0 ppm Ascorbic acid.

Alsoin the situation where the superabsorbent polymer is used in amount of 4 and 8 grams the highest catalase was related to level 0 ppm of Ascorbic acid. As can be seen, concentrations of heavy metals in plant is decreased with the increasing ofsuperabsorbent polymers absorption and with increasing concentrations of ascorbic acid, the plant prefers that by this antioxidant copes with heavy metals and reduces the production of catalase and the energy required to produce these enzymes, uses to improve itsGrowth and development. This indicates that the use of superabsorbent polymers and spraying with ascorbic acid, plants will be in better condition and have been resistant against heavy metal stress.It is also possible that this result is due to activation of the immune system other than the antioxidant defense system to cope with free radical oxygen and sweep them like theincreasing of proline amino acid that plays the role of cleansing of oxygen free radical. (ROS Scavenger)

It has been reported that organic solute likethe proline play an important role in osmotic adjustment and alsothroughsweeping of ROS,Protect cells. (Reddy et al., 2004)

**Table 1.** Biochemical analysis of variance

| | d f(Degrees of freedom) | Superoxide dismutase | Catalase |
|---|---|---|---|
| Super absorbent (S) | **2** | **316 200/09**** | **49332/17**** |
| Spray a solution of ascorbic acid (F) | **2** | **13737/86**** | **6200/33**** |
| The interaction of super absorbent spraying (SF) | **4** | **179/73**** | **199/7006**** |
| Error | **18** | **104/51** | **39/48** |
| The total error | **26** | | |
| Coefficient of variation (CV) | | **1/85** | **3/96** |

**Table 2** comparisonof the average of the effects of spraying with ascorbic acid and superabsorbent polymers

| Catalase mg pro.min-1 | Superoxide dismutase mg pro.min-1 | the amount of | Treatment |
|---|---|---|---|
| 237 / 75 a | **729/20a** | **0g** | **The use of super absorbent polymer** |
| 147 / 05 b | **570/23b** | **4g** | |
| 91 / 04 c | **355/69c** | **8g** | |
| 182 / 81 a | **589/13a** | **0ppm** | **Spray a solution of ascorbic acid** |
| 162 / 32 b | **554/82b** | **75ppm** | |
| 130 / 71 c | **511/18c** | **150ppm** | |

**Table 3** comparison of the average of the interaction between spraying with ascorbic acid and superabsorbent polymers based on morphological traits and yield components of wheat

| The use of super absorbent polymer | The effect of spraying with ascorbic acid | Superoxide dismutase mg pro.min-1 | Catalase mg pro.min-1 |
|---|---|---|---|
| | **0** | **767/75a** | **264/27a** |
| 0 | **75** | **611/85d** | **177/91d** |
| | **150** | **387/80g** | **106/27f** |
| | **0** | **728/53b** | **240/92b** |
| 4 | **75** | **573/93e** | **151/30e** |
| | **150** | **361/99h** | **94/75g** |
| | **0** | **691/32c** | **208/08c** |
| 8 | **75** | **524/92f** | **111/95f** |
| | **150** | **317/30i** | **72/11h** |

## CONCLUSION

According to the obtained results, it can be concluded that:

1. heavy metals in the soil such as Leadprevent the longitudinal growth of the root meristem, absorption of water and nutrients are reduced. Amount of plant photosynthesis and assimilation is decreased and causes the decreasing of morphological traits such as plant height and ear number and grain, grain weight and total weight of shoot and root dry weight and thousand grain weight in wheat.

2. Lead in wheat also increasesthe biochemical characters and antioxidant enzymes such as superoxide dismutase, catalase, and the physiological traits such as proline, relative water content and the amount ofLead in the shoots and root.

3. the increasing of superabsorbent concentration byfixation of the Lead in the soil

Prevents its absorption by plant roots and by reduces oxidative stress caused by heavy metal, increasesthe morphological traits such as plant height and ear number and grain, grain weight and total weight of shoot and root dry weight and seed weight as well as an increases the physiological traits such as protein and chlorophyll a, b and total in wheat plant. The increasing of the superabsorbent in plant also reduces biochemical characters and antioxidant enzymes such as superoxide dismutase, catalase, as well as reduces physiological traits such as proline, EC, Lead levels in roots and leaves and seeds and increases the relative water content.

4. The increasing of ascorbic acid concentration is associated with the increasing of free radicalswhich results in the morphological traits such as plant height and ear number and grain, grain weight and total weight of shoot and root dry weight and seed weight as well as an increasing in physiological traits such as protein and chlorophyll a, b and total in wheat plant. Also the increasing of the ascorbic acid in the plant results in a reduction in biochemical characters and antioxidant enzymes such as superoxide dismutase, catalase, and also reducing the physiological characteristics of proline, an increase in relative water content and reducing the amount of lead in the shoots and roots. It should be considered that the plant uses some of the energy from its food, for the production of antioxidant enzymes such as superoxide dismutase and catalase. In other words some of its energy that should be used for the growth and development in the normal conditions consumes to produce these enzymes. So by external spraying with Ascorbic acid decreases amount of production of these enzymes and the energy that should be consumed for the production of these enzymes, applies for the growth and development of the plant.

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