J. SHANXI AGRIC, UNIV. (*Natural Science Edition*)
2016, 36(8):572 03435

Effects of Humic Acid on Germination and Antioxidant System of Tomato Seeds under Plumbum Stress

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Abstract: [Objective] To reveal the effects of humic acid on germination and antioxidant system of tomato seeds under plumbum stress. [Method] Under 255mg·L^{-1} plumbum stress, “Yuanshuai No. 1” tomato seeds were soaked in different concentrations of Highly Active Humic Acid to determine the effects on their germination. [Results] The results showed that the germination percentage, germination index, vigor index, fresh weight, superoxide dismutase (SOD) and per-oxidase (POD) activity, radicle and hypocotyl of the tomato seeds increased gradually, while the content of malondialdehyde (MDA) decreased gradually, as the diluted concentration of Highly Active Humic Acid increased; soaking in the high concentration (70 times) of Highly Active Humic Acid liquid inhibited the germination of the tomato seeds. [Conclusion] Thus, soaking in an appropriate concentration of Highly Active Humic Acid had different mitigation effects on the germination of tomato seeds under plumbum stress, and the optimal effect was observed when the Highly Active Humic Acid liquid was diluted 470 times.

Keywords: Humic acid; Tomato; Plumbum stress

Chinese Library Classification No.: S641.2 **Document Code**: A **Article No.**: 1671-8151(2016)08-0572-04

With the advancement of industrial production, the contamination of soil, groundwater, and crop yield and quality caused by heavy metals has become increasingly prominent. The farmland surrounding urban areas is particularly affected by such pollution. One of the main heavy metal pollutants is plumbum, which is emitted through automobile exhaust and industrial wastewater[1]. Plumbum causes significant harm to soil, groundwater, and crops. The absorption of plumbum fixed and accumulated in the soil not only affects seed germination and seedling growth, but also leads to poisoning, which further affects crop yield and quality and jeopardizes human health[2-6]. While there have been abundant reports on the effects of plumbum stress mitigation on seed germination, physiology and biochemistry of plants[7,8], research on the effects of humic acid on tomato seeds under plumbum stress is scarce. Humic acid is a natural macromolecular organic substance primarily derived from the microbial decomposition and transformation of plant residues through a series of chemical processes[9]. It has the ability to form complexes with heavy metal cations, impeding the movement of heavy metal ions and mitigating heavy metal stress[10-12]. With tomatoes as the material, this test focuses on investigating the effects of humic acid on the germination characteristics, radicle and hypocotyl lengths, superoxide dismutase (SOD) and per-oxidase (POD) activity, and malondialdehyde (MDA) contents of tomato seeds under plumbum stress. It will also discuss the mechanism by which humic acid mitigates the poisoning caused to plants by plumbum stress, thereby providing a theoretical basis and practical guidance for enhancing vegetable yield and promoting agricultural environmental protection.

1. Material and Method

1.1 Test Material

The test material was “Yuanshuai No. 1” tomatoes (Lycopersicon esculentum Mill.); the Highly Active Humic Acid liquid was supplied by Xinyi Sumeng Fertilizer Co., Ltd. in Jiangsu; the chemical reagent was analytical pure lead acetate.

Date of Reception: April 12, 2016 **Date of Revision:** May 7, 2016
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Fund Project: Shanxi Provincial Project for Science and Technology Development (20130311008-4); Doctoral Research Start-up Fund Project of Shanxi Agricultural University for Talent Recruitment (2013YJ23); Shanxi Provincial Demonstration Action Project of Agricultural Technology Promotion (SXNKTG11)

1.2 Test Design

The test involved 6 treatments in total, as detailed in Table 1.

Table 1 Test Design

Treatment	Methods
CK	Soaking in deionized water, 0mg·L^{-1} plumbum treatment
T1	Soaking in deionized water, 255mg·L^{-1} plumbum treatment
T2	Soaking in Highly Active Humic Acid liquid diluted 70 times, 255mg·L^{-1} plumbum treatment
T3	Soaking in Highly Active Humic Acid liquid diluted 270 times, 255mg·L^{-1} plumbum treatment
T4	Soaking in Highly Active Humic Acid liquid diluted 470 times, 255mg·L^{-1} plumbum treatment
T5	Soaking in Highly Active Humic Acid liquid diluted 670 times, 255mg·L^{-1} plumbum treatment

Seeds of uniform size were chosen and subjected to sterilization using a 0.1% potassium permanganate solution. They were then rinsed with deionized water and soaked in Highly Active Humic Acid liquids with different dilution factors (70, 270, 470 and 670 times) and deionized water for 6h (27℃). In a culture dish lined with double layers of sterile filter paper as the germination bed, 8mL 255mg•L^{-1} plumbum solution was added in each layer (8mL deionized water was added to CK) to moisten the filter paper, and 40 tomato seeds were placed in each culture dish for each treatment. The same process was replicated 5 times. The seeds were incubated in a dark environment provided by artificial intelligence climate incubators (27℃). During the test process, 1-2mL deionized water and plumbum solution were alternately added every other day. The germinated seeds were counted from Day 2, with a root length of 2mm as the criterion for germination[13]. The test was concluded on Day 7, when the germination percentage, germination index, vigor index, hypocotyl length, radicle length, seedling fresh weight, superoxide dismutase (SOD) and per-oxidase (POD) activity, and malondialdehyde (MDA) contents were calculated.

1.3 Measurement Indicators

Germination Percentage (*GP*) = Number of germinated seeds on Day 7/total number of tested seeds × 100%;

Germination Index (*GI*) = $\sum Gt/Dt$ (*Gt* represents the number of germinated seeds at *t*, and *Dt* represents the number of days when the seeds are germinated);

Vigor Index (*VI*) = *GI* × *S* (*GI* represents germination index, and *S* represents the fresh weight of seedlings).

Fifteen seedlings were chosen at random from each treatment. A ruler was used to measure their radicle lengths and hypocotyl lengths. An analytical balance was then used to measure their fresh weight, and an ultraviolet spectrophotometer was used to measure their antioxidant enzyme activity. The SOD activity was measured with the nitroblue tetrazolium (NBT) reduction method[14] and expressed as U•mg^{-1}FW; the POD activity was measured with the guaiacol method[14] and expressed as ΔOD_{470nm}•g^{-1}FW•min^{-1}; the MDA contents in the tomato seedlings were measured with the thiobarbituric acid method[14] and expressed as μmol•g^{-1}FW.

SAS and Microsoft Office Excel 2013 were used for statistics and variance analysis of the test results.

2. Result and Analysis

2.1 Effect of Humic Acid on Germination of Tomato Seeds under Plumbum Stress

As shown in Table 2, under 255mg•L^{-1} plumbum stress, the germination percentage, germination index and vigor index of the tomato seeds were significantly lower than those in CK ($P<0.05$). This indicated that the plumbum stress inhibited the germination of tomato seeds. After being treated with Highly Active Humic Acid liquid diluted 270-670 times, the germination indicators of the tomato seeds under plumbum stress increased to varying degrees. Moreover, as the dilution factor of the Highly Active Humic Acid liquid increased, the germination percentage, germination index and vigor index all first increased and then decreased, which indicated that the Highly Active Humic Acid liquid could, to some extent, mitigate the inhibitory effect of the heavy metal plumbum on the germination of tomato seeds, and the optimal mitigation effect was observed when the Highly Active Humic Acid liquid was diluted 470 times, with germination percentage, germination index and vigor index increasing by 28.23%, 33.87% and 77.55%, respectively, when compared with those at T1. The differences were significant. However, when the Highly Active Humic Acid was diluted 70 times, the germination percentage was lower than that at T1. It was clear that an excessive concentration of Highly Active Humic Acid was not able to facilitate the germination of tomato seeds.

2.2 Effect of Humic Acid Treatment on Radicle and Hypocotyl Lengths of Tomato Seeds under Plumbum Stress

As shown in Table 2, compared with CK, T1 was found to show radicle and hypocotyl growth of tomatoes significantly inhibited by plumbum, with radicle and hypocotyl lengths equal to about 1/3 and 1/2 of those in CK. Under plumbum stress, soaking in 3 different concentrations of Highly Active Humic Acid liquid (except for 70 times) could mitigate the radicle and hypocotyl growth of tomatoes under plumbum stress.

Table 2 Effect of Humic Acid Treatment on Germination Characteristics of Tomato Seeds under Plumbum Stress

Treatment	Germination Percentage/%	Germination Index	Vigor Index	Radical Length/cm	Hypocotyls Length/cm	Fresh Weight/g
CK	95.50a	31.37a	1.35a	8.03a	4.73a	0.043a
T1	62.00d	17.89d	0.49d	2.91cd	2.56cd	0.027c
T2	46.00e	15.11e	0.32e	2.09d	2.35d	0.021d
T3	68.50c	20.65c	0.64c	3.63c	3.48b	0.031c
T4	79.50b	23.95b	0.87b	4.95b	4.26a	0.036b
T5	65.50cd	18.11d	0.51d	3.61c	3.25be	0.028c

Note: Different lowercase letters in the same column indicate significant difference ($P<0.05$), the same below.

Specifically, the Highly Active Humic Acid liquid diluted 470 times had the most significant effect, with the radicle and hypocotyl lengths increased by about 0.7 times more than those at T1, although they did not recover to the normal levels.

2.3 Effect of Humic Acid Treatment on SOD Activity in Tomato Seeds under Plumbum Stress

As shown in Figure 1, plumbum stress could significantly decrease the SOD activity in the tomato seeds. Soaking in an appropriate concentration of Highly Active Humic Acid liquid could effectively increase the SOD activity in the tomato seeds, and the difference was significant. On the whole, the activity of the protective enzyme SOD in the tomato seeds first increased and then decreased as the dilution factor of the Highly Active Humic Acid liquid increased. The highest SOD activity was observed when the Highly Active Humic Acid liquid diluted 470 times was added, increasing by 22.38% when compared with that at T1, which indicated that the concentration of 470 times had the optimal effect on mitigating plumbum stress.

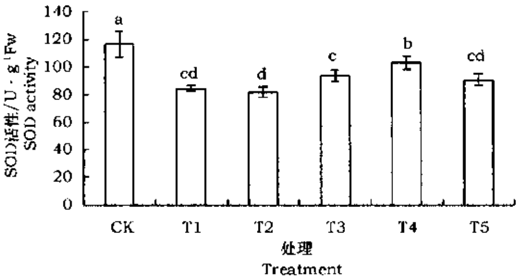

Figure 1 Effect of Humic Acid on SOD Activity in Tomato Seeds under Plumbum Stress

2.4 Effect of Humic Acid Treatment on POD Activity in Tomato Seeds under Plumbum Stress

As shown in Figure 2, T1 could significantly inhibit the POD activity in the tomato seeds. Under plumbum stress, the POD activity in the tomato seeds soaked in the Highly Active Humic

Acid liquid diluted 470 times was significantly increased when compared with that at T1, which indicated that an appropriate concentration of Highly Active Humic Acid liquid could effectively mitigate the effect of plumbum stress on the germination of tomato seeds.

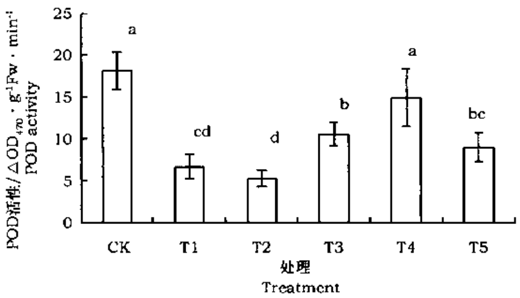

Figure 2 Effect of Humic Acid on POD Activity in Tomato Seeds under Plumbum Stress

2.5 Effect of Humic Acid Treatment on MDA Contents in Tomato Seeds under Plumbum Stress

As shown in Figure 3, the MDA contents in the tomato seeds under 255mg•L^{-1} plumbum stress were significantly increased when compared with that in CK. It was clear that plumbum stress hindered the germination of the tomato seeds by causing peroxidation of plasma membranes. It was found that the MDA contents in the tomato seeds treated with Highly Active Humic Acid liquids diluted 270, 470 and 670 times were lower than those at T1. The lowest MDA content was observed when the Highly Active Humic Acid liquid diluted 470 times was used to treat the tomato seeds, and the difference was significant. This indicated that soaking in an appropriate concentration of Highly Active Humic Acid solution could mitigate the peroxidation of the plasma membranes of the tomato seeds, thereby decelerating the inhibitory effect of plumbum stress on the germination of tomato seeds.

3. Discussion and Conclusion

Plants are frequently exposed to stress under unfavorable circumstances such as drought, salinity, heavy metal contamination, extreme temperatures, and air pollution in the natural environment. Such stress will induce numerous reactive oxygen species (ROS) within plants, which in turn cause considerable damage to the cellular membrane systems, proteins, nucleic acids, and other macromolecules of plants[15].

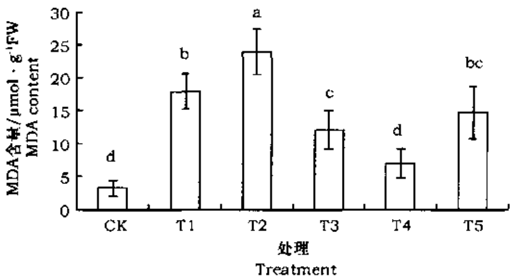

Figure 3 Effect of Humic Acid on MDA Content in Tomato Seeds under Plumbum Stress

It is believed that the membrane protection system in plants can eliminate surplus ROS under unfavorable circumstances, and this protective enzyme system is actually an antioxidant system, comprised of key antioxidant enzymes such as SOD and POD. These enzymes collaborate to eliminate detrimental ROS within the organism, safeguard plant cells from the harm caused by ROS, and consequently protect the membrane system of the plants[16].

Under unfavorable circumstances, plants experience membrane lipid peroxidation, resulting in some important products including MDA. Consequently, the measurement of MDA content in plants serves as an indicator for assessing the extent of membrane lipid peroxidation under unfavorable circumstances. The study conducted by Zhang Ying et al.[17] showed that heavy metals affect seed germination and seedling growth by compromising membrane permeability. The study conducted by Wang You Bao et al.[18] showed that heavy metals inhibit enzyme activity and hinder the growth and division of plant cells, as evidenced by a decline in germination percentage and germination potential.

The results in this paper showed that the germination percentage, germination index, vigor index, radicle length and hypocotyl length of the tomato seeds were significantly lower than those in CK under 255mg•L^{-1} plumbum stress; the reduced activity of antioxidant enzymes SOD and POD and the increased contents of MDA in the tomato seeds and the affected system that could have eliminated ROS in the cells of the tomato seeds seriously inhibited the germination. This indicated that the cell membrane of the tomato seeds seriously suffered from the poisoning caused by the plumbum ions. When the tomato seeds were treated with different concentrations of humic acid, low concentrations of humic acid could mitigate the inhibitory effects of plumbum stress on the tomato seeds to varying degrees. Appropriate concentrations of Highly Active Humic Acid could increase the SOD and POD activity and reduce the MDA contents in the tomato seeds to protect membrane integrity under plumbum stress, thereby mitigating the poisoning of the tomato seedlings caused by the heavy metal plumbum. Specifically, the optimal effect was observed when the Highly Active Humic Acid was diluted 470 times. However, an excessive concentration of Highly Active Humic Acid could inhibit the germination of the seeds. Therefore, soaking in an appropriate concentration of Highly Active Humic Acid could effectively mitigate the poisoning of tomatoes caused by plumbum and shows promise for application in green vegetable production. However, further research is needed to investigate other physiological metabolism mechanisms by which humic acid enhances the germination of tomato seeds.

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(Editor: Wu Yingyao)