Effects of alginic acid on radish growth and osmotic adjustment substance content under cadmium stress

Huafeng Wang¹, Li Wang²*, Mao Yong¹, Shibin Wang¹, Fang Cheng¹, Qing Li¹, Jinxin Hu¹

¹Institute of Olericulture, Tibet Academy of Agricultural and Animal Husbandry Sciences, Lhasa, Tibet, 850000, China
²College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China

*E-mail: 510830161@qq.com

Abstract. To study the effects of alginic acid on the growth of vegetables, a pot experiment was conducted to study the effects of spraying alginic acid at different dilution folds on the growth and osmotic adjustment substance content of radish under cadmium stress condition. The results showed that the biomass, leaf length and leaf width of radish with 600, 900, 1200 and 1500 dilution folds alginic acid were higher than those of the control, while the soluble protein content, relative conductivity and malondialdehyde content of the radish were lower than those of the control. When the dilution factor of alginic acid is 600 dilution folds, the biomass, leaf length and leaf width of radish's roots, root tubers, leaves and edible parts all reach the maximum. Increased by 19.39%, 35.27%, 19.74%, 29.44%, 22.11% and 14.01% compared with the control, respectively; the soluble protein content, relative conductivity and MDA content of radish reached the lowest, which were 38.76%, 29.86% and 35.61% lower than the control, respectively. Sprayed diluted alginic acid can promote the growth of radish, and the best dilution factor is 600 dilution folds.

1. Introduction

With the rapid development of industry, the content of heavy metals in farmland has increased greatly, leading to agricultural products being polluted by heavy metals, with cadmium (Cd) being the most prominent [1]. Cadmium pollution of agricultural products such as rice and vegetables directly threatens human health [2]. Therefore, through some measures to reduce the content of Cd in the soil or reduce the accumulation of Cd by crops, it has become a hot and difficult point in future research on agricultural safety production [3]. There are some agronomic measures such as hybridisation, grafting and plant hormones can promote plant growth and decrease the Cd accumulation of plants. The hybridisation can increase the biomass and decrease the Cd content of Solanum Photeinocarpum [4]. The grafting also can increase the biomass and decrease or increase the Cd content of Cyphomandra Betacea [5], Nasturtium officinale [6] and Solanum Photeinocarpum [7], and these effects can transfer to the post generations of Galinsoga Parviflora [8,9] and Cyphomandra Betacea [10].

Alginic acid is a natural polysaccharide found in the cell wall of brown algae, also known as alginate, alginic acid, etc. In recent years, many studies have shown that alginic acid has significant effects on plant growth and yield. Studies show that the application of alginic acid compound fertilizer can
significantly increase corn yield and above-ground biomass and promote corn nutrient absorption [11]. Alginic acid foliar fertilizer can reduce the height of cucumber, shorten internodes, increase the number of cucumbers, and balance the vegetative and reproductive growth of cucumbers [12, 13]. Alginic acid 200 folds solution can not only premature maturity of loquat but also increase the content of soluble solids in loquat [14]. Spraying brown algae extract to radish can improve the growth, yield and nutrient content of radish [15]. In addition to the effects on plant growth and development, alginate also plays an important role in improving plant stress resistance. In low temperature adverse environment, alginate can delay the early flowering of tobacco caused by low temperature and increase the yield of tobacco [16]. In salt-stressed environments, treatment of black fruit wolfberry seedlings with seaweed liquid fertilizer can reduce its relative conductivity and malondialdehyde content [17].

Radish (Raphanus sativus L.), a cruciferous plant, is an important vegetable native to China. Radish tastes crispy and juicy, has high nutritional value, and is rich in vitamins, dietary fiber, and phenolics [18, 19]. At present, there have been some studies at home and abroad on improving radish yield and commodity value [20-22], but these studies are mostly limited to ordinary fertilizers or plant growth regulators, and less research on alginic acid. Based on this, this experiment investigated the effects of exogenous algic acid on the growth of radish and osmotic adjustment substances by spraying algic acid at different dilution folds on the radish surface. Therefore, the optimal dilution factor of spraying algic acid was determined, which provided a theoretical basis for algic acid fertilization on radish.

2. Materials and methods
2.1. Experiment materials
The radish variety was "red-skinned radish", an early-maturing variety that was purchased on the market. Alginic acid was purchased on the market. The soil was taken from the farmland around Gongping Town, Wenjiang District, Chengdu, Sichuan Province, China. It was a fluvo-aquic soil. Its basic physical and chemical properties were pH 7.58, organic matter 28.02 g/kg, total nitrogen 1.44 g/kg, total phosphorus 0.43 g/kg, and total potassium 24.55 g/kg, alkaline nitrogen 58.68 mg/kg, fast-acting phosphorus 19.23 mg/kg, fast-acting potassium 58.33 mg/kg, and available cadmium content was not detected.

2.2. Experiment design
The soil was air-dried and pulverized. After passing through a 6.72 mm sieve, it was placed in a plastic circular flower pot of 15 cm × 18 cm (height × diameter). Each pot was filled with 3.0 kg of soil, and CdCl₂·2.5H₂O Add to the soil and mix to make the soil cadmium concentration 5 mg/kg. After that, keep the soil moist so that its water content is about 80% of the soil water capacity in the field. Leave it for 1 month and mix it from time to time to ensure that the soil is fully mixed. In September 2019, the radish seeds were directly sown in pots filled with the prepared cadmium contamination, and 10 seeds were evenly spread in each pot. When the radish seedlings emerged and grew to two true leaves, reduced the number of seedlings in the pot, and kept 4 healthy radish seedlings with consistent growth in each pot. Then spray the algic acid solution with different concentrations on the leaves of the radish seedlings, the spraying amount shall be based on the dripping of the leaves. The algic acid concentration was diluted 0, 600, 900, 1200, and 1500 dilution folds according to its original solution, and each treatment was repeated 3 times (3 pots). During the entire growth process of radish, water is often used to keep the soil in the pot moist. At the same time, the position of the pot and pot is exchanged irregularly to reduce the marginal effect, and weeds are removed in time to prevent and treat pests and diseases. 40 days after spraying algic acid, harvested the plant samples were collected for determination of related indicators.

2.3. Determination of items and methods
Measure the leaf length and leaf width (widest part) of the longest leaf of each radish with a ruler. The mature radish leaves (the fifth leaf from the top) were used to determine the content of osmotic adjustment substance: soluble protein, relative conductivity, malondialdehyde (MDA). The soluble
protein content was measured by coomassie brilliant blue method, relative conductivity was measured by conductivity meter, and MDA content was measured by barbituric acid method [23]. Then, harvest the entire radish plant, rinse it with tap water first, and then rinse it with deionized water 3 times. After separating the plant roots, root tubers and leaves, absorb the moisture on the surface with absorbent paper and weigh to determine the radish root freshness. Weight the fresh roots and fresh leaves. Edible part biomass = root tuber biomass + leaf biomass.

2.4. Data processing method
The data were analyzed by variance using SPSS 20.0 (Duncan's new complex range method for multiple comparisons).

3. Results and Discussion
3.1. Effects of alginic acid with different dilution folds on radish biomass
Alginic acid diluted 600, 900, 1200 and 1500 folds can increase the biomass of radish roots, root tubers, leaves and edible parts compared undiluted alginic acid. When the dilution ratio of alginic acid was 600 folds, the biomass of radish root, root tubers, leaf and edible part reached the maximum, increasing by 19.39% (p < 0.05), 35.27% (p < 0.05), 19.74% (p < 0.05) and 29.44% (p < 0.05), respectively, compared with the control group. When the alginic acid was diluted by 1200 and 1500 folds, the biomass of radish roots, root tubers, leaves and edible parts were not significantly different from the control.

| Alginic acid (dilution fold) | Roots (g/plant FW) | root tubers (g/plant FW) | Leaves (g/plant FW) | Edible portion (g/plant FW) |
|-----------------------------|-------------------|-------------------------|---------------------|---------------------------|
| 0                           | 0.825±0.020c      | 25.63±1.80c             | 15.45±0.30c         | 41.07±2.11c               |
| 600                         | 0.985±0.016a      | 34.67±1.18a             | 18.50±0.39a         | 53.16±1.57a               |
| 900                         | 0.932±0.025ab     | 31.12±1.48ab            | 16.61±0.37b         | 47.72±1.85b               |
| 1200                        | 0.862±0.047bc     | 29.06±1.64bc            | 16.28±0.31bc        | 45.34±1.95bc              |
| 1500                        | 0.835±0.035c      | 27.01±1.05c             | 15.64±0.35c         | 42.65±1.40c               |

Values are means (±SE) of three replicate pots. Different lowercase letters within a column indicate significant differences based on one-way analysis of variance in SPSS 20.0 followed by the least significant differences at the 5% confidence level. Edible part biomass = root tuber biomass + leaf biomass. FW = fresh weight.

3.2. Effect of alginic acid with different dilution folds on osmotic adjustment substance of radish.
After spraying alginic acid at different dilution folds to radish, with the increase of alginic acid dilution, the soluble protein content, relative conductivity and MDA content of radish showed a trend of first decrease and then increase, but all were lower than the control. When the dilution factor of alginic acid was 600 folds, the soluble protein content, relative conductivity and MDA content of radish reached the lowest values, which were 38.76% (p < 0.05), 29.86% (p < 0.05), and 35.61% (p < 0.05) lower than the control, respectively. When the dilution factor of alginic acid was 1200 folds and 1500 folds, the MDA content of radish was not significantly different from the control. It can be seen that spraying alginic acid with a proper dilution factor on radish can reduce the permeability of cell membranes, reduce the content of MDA, and reduce the damage caused to plants by MDA.

3.3. Effects of alginic acid at different dilution folds on leaf length of radish
After spraying alginic acid at different dilution folds to radish, the leaf length of radish increased. When the dilution factor of alginic acid was 600 folds, the leaf length of radish increased the most, increasing by 22.11% (p < 0.05); When the dilution factors of alginic acid were 900 folds and 1200 folds, the length of radish leaves increased by 5.70% (p < 0.05) and 3.93% (p < 0.05); When the
alginic acid dilution factor was 1500 folds, the radish leaf length increased by 2.68% ($p > 0.05$), and there was no significant difference from the control. It can be seen that spraying diluted alginic acid on radish can promote the increase of radish leaf length and help the growth of radish.

Table 2. Effect of alginic acid with different dilution folds on osmotic adjustment substance of radish.

| Alginic acid (dilution fold) | Soluble protein content (mg/g FW) | Relative conductivity (%) | MDA content (μmol/kg FW) |
|-----------------------------|---------------------------------|--------------------------|--------------------------|
| 0                           | 73.99±0.72a                     | 17.58±0.53a              | 9.66±0.52a               |
| 600                         | 45.31±0.32d                     | 12.33±0.42c              | 6.22±0.36b               |
| 900                         | 51.43±0.89c                     | 12.63±0.37c              | 6.85±0.02b               |
| 1200                        | 51.48±0.65c                     | 13.38±0.98c              | 8.90±0.26a               |
| 1500                        | 68.34±0.79b                     | 15.52±0.16b              | 9.11±0.58a               |

Values are means (±SE) of three replicate pots. Different lowercase letters within a column indicate significant differences based on one-way analysis of variance in SPSS 20.0 followed by the least significant differences at the 5% confidence level. FW = fresh weight.

Figure 1. Effect of alginic acid at different dilution folds on leaf length of radish. Values are means (±SE) of three replicate pots. Different lowercase letters within a column indicate significant differences based on one-way analysis of variance in SPSS 20.0 followed by the least significant differences at the 5% confidence level.

3.4 Effects of alginic acid with different dilution folds on leaf width of radish

After treating radishes with different dilution folds of alginic acid, the leaf width of radishes increased (Figure 2). The increase of radish leaf width is 600 folds > 900 folds > 1200 folds > 1500 folds > 0 folds. When the dilution factor of alginic acid was 600 folds, the leaf width of radish increased by 14.01% compared with the control ($p < 0.05$). When the dilution factors were 900, 1200, and 1500 folds, although the width of radish leaves increased, they were not significantly different from the control.

4. Conclusions
Spraying alginic acid with different fold dilutions on radish can promote the increase of radish biomass, leaf length and leaf width, and reduce its soluble protein content, relative conductivity and MDA content. With the increase of the alginic acid dilution folds, the biomass, leaf length and leaf width of radish increased first and then decreased, and the soluble protein content, relative conductivity and MDA content decreased first and then increased. When the alginic acid dilution factor was 600 folds, the biomass, leaf length and leaf width of radish reached the maximum, the soluble protein content, the relative conductivity and the MDA content reached the minimum. This test believes that spraying diluted alginic acid on radish can promote the growth of radish, and that alginic acid has the best dilution effect at a 600-fold dilution.

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