

Effects of Uniconazole on Growth and Cadmium Accumulation of Accumulator Plant *Stellaria media*

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Abstract: A pot experiment was carried out to investigate the effects of applying uniconazole on growth and cadmium (Cd) accumulation of accumulator plant *Stellaria media*. The results showed that application of uniconazole inhibited the growth of *S. media*, and promoted the absorption and transport of Cd from soil. The biomass and soluble sugar content of *S. media* decreased with the increase of the concentration of uniconazole, whereas the activities of SOD, POD and CAT, the contents of photosynthetic pigments and soluble protein improved. With the increase of concentration of uniconazole, the Cd content and Cd accumulation in the shoots of *S. media* increased first and then decreased, and reached the maximum up to 67.24 mg/kg and 190.42 µg/plant respectively when the dose of uniconazole was 40 mg/L, which increased by 39.10% and 10.47% compared with the control, respectively. Therefore, application of uniconazole could enhance phytoremediation ability of *S. media* at the dose of 20-40 mg/L.

Introduction

Uniconazole (S-3307) is a triazole derivative, which can promote plant photosynthesis, enhance absorption capacity of roots, protect plants from various environmental stresses, and has a broad-spectrum fungitoxic activity [1-2]. The biological activity of uniconazole is higher than that of paclobutrazol, and uniconazole is lower cost, lower toxicity, less pollution and specific capability to help to confer resistance in plants to low temperature stress as compared with paclobutrazol, making it as an ideal candidate for assisting remediation of heavy metal contaminated soil, especially in winter [3-6]. However, there are very few studies on cadmium (Cd) phytoremediation by application of uniconazole [7-9]. *Stellaria media* is a widely distributed annual herb of Caryophyllaceae, and also a Cd-accumulator plant [10]. In this study, we used the different concentrations of uniconazole to treat *S. media*, and studied the effects of uniconazole on growth and Cd accumulation of *S. media*. The aim of the study was to screen the best uniconazole concentration which could enhance the phytoremediation ability of *S. media*, and provided a reference for applying the plant hormones on other hyperaccumulators or accumulators for improving phytoremediation ability.

Materials and Methods

Materials. The soil samples used in the experiment were inceptisol soil, which were collected from the Ya'an campus farm of Sichuan Agricultural University (29° 59' N, 102° 59' E) in August 2014. The *S. media* seedlings with height of 10 cm were collected from the Ya'an campus farm in October 2014.

Experimental Design. The soil samples were air-dried and passed through a 5-mm sieve. Four kilograms of the air-dried soil was weighed into each polyethylene pot (18 cm high, 21 cm in diameter). Cd was added to soils as CdCl₂•2.5H₂O at 25 mg/kg in August 2014, and the soil moisture was

maintained at 80% of field capacity for 2 months. Four uniform *S. media* seedlings were transplanted into each pot in October 2014, and watered every day to keep the soil moisture content maintaining at 80% of field capacity. When *S. media* seedlings grow one month in Cd-contaminated soil, 5 concentrations (0, 10, 20, 40 and 80 mg/L) of uniconazole with 3 replicates were sprayed on the leaves of plants for each pot, respectively. The amount of each pot was 25 ml of uniconazole solution. After uniconazole treatment one month, the upper mature leaves of *S. media* were collected to determine the photosynthetic pigment (chlorophyll a, chlorophyll b, total chlorophyll and carotenoid) contents [11]. The upper young shoots (2 cm in length) were collected to determine the superoxide dismutase (SOD) activity, peroxidase (POD) activity, catalase (CAT) activity and soluble protein content [11]. Then, the whole plants were then gently removed from the soil. The treatments of plants are described in the reference of Lin et al. (2014) [12]. The Cd concentrations in roots, stems and leaves were determined using an iCAP 6300 ICP spectrometer (Thermo Scientific, Waltham, MA, USA) [13]. The soluble sugar contents in shoots of *S. media* were determined by anthrone colorimetry with dry weight plant samples [11].

Experimental Design. Statistical analyses were conducted using SPSS 13.0 statistical software (IBM, Chicago, IL, USA). Data were analyzed by one-way analysis of variance with least significant difference (LSD) at the $p = 0.05$ confidence level.

Results and Discussion

Biomass. Compared with the control, spraying uniconazole decreased the root and shoot biomass of *S. media* with the increase of uniconazole concentration (Table 1). When the dose of uniconazole was 10, 20, 40 and 80 mg/L, the root biomass decreased by 1.68% ($P > 0.05$), 2.29% ($P > 0.05$), 8.87% ($P < 0.05$) and 14.83% ($P < 0.05$) respectively compared with the control, and the shoot biomass decreased by 8.95% ($P < 0.05$), 11.83% ($P < 0.05$), 20.58% ($P < 0.05$) and 24.09% ($P < 0.05$) respectively. With the increase of uniconazole concentration, the root/shoot ratio of *S. media* increased at first and then reduced (Table 1).

Table 1 Biomass of *S. media*

Treatments (mg/L)	Root biomass (g/plant)	Shoot biomass (g/plant)	Total biomass (g/plant)	Root/shoot ratio
0	0.654±0.011a	3.566±0.093a	4.220±0.105a	0.183
10	0.643±0.014a	3.247±0.066b	3.890±0.081b	0.198
20	0.639±0.010a	3.144±0.062b	3.783±0.072b	0.203
40	0.596±0.008b	2.832±0.045c	3.428±0.054c	0.210
80	0.557±0.013c	2.707±0.151c	3.264±0.164c	0.206

Photosynthetic pigment content. The chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents of the *S. media* increased first and then decreased with the increase of the concentration of uniconazole (Table 2). The contents of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid reached the maximum when the dose of uniconazole was 40 mg/L. When the dose of uniconazole was 10, 20, 40 and 80 mg/L, the total chlorophyll content increased by 2.12% ($P > 0.05$), 2.59% ($P > 0.05$), 5.58% ($P < 0.05$) and 4.58% ($P > 0.05$) respectively compared with the control, and the content of carotenoid increased by 0.71% ($P > 0.05$), 1.42% ($P > 0.05$), 7.47% ($P < 0.05$) and 6.05% ($P > 0.05$) respectively. The chlorophyll a/b of *S. media* had a tendency to increase first and then decline with the increase of concentration of uniconazole, which reached the maximum value at 2.917 when the dose of uniconazole was 10 mg/L (Table 2).

Contents of carbon-nitrogen metabolites and antioxidant enzyme activity. The application of uniconazole increased soluble protein content, but decreased the soluble sugar content of *S. media* (Table 3). Uniconazole improved the activities of SOD, POD and CAT (Table 3). When the dose of uniconazole was 40 mg/L, SOD, POD and CAT activities got the maximum, respectively. When the dose of uniconazole was 10, 20, 40 and 80 mg/L, the activity of SOD increased by 1.59% ($P > 0.05$), 4.49%

($P > 0.05$), 71.25% ($P < 0.05$) and 19.05% ($P < 0.05$) respectively compared with the control, the activity of POD increased by 6.28% ($P > 0.05$), 11.27% ($P > 0.05$), 46.65% ($P < 0.05$) and 29.43% ($P < 0.05$) respectively, and the activity of CAT increased by 8.27% ($P > 0.05$), 8.79% ($P > 0.05$), 60.42% ($P < 0.05$) and 43.79% ($P < 0.05$) respectively.

Table 2 Photosynthetic pigment content of *S. media*

Treatments (mg/L)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Chlorophyll a/b	Carotenoid (mg/g)
0	1.264±0.035b	0.438±0.017b	1.702±0.051b	2.886	0.281±0.009b
10	1.294±0.036ab	0.444±0.016ab	1.738±0.052ab	2.917	0.283±0.014b
20	1.297±0.040ab	0.449±0.019ab	1.746±0.059ab	2.886	0.285±0.010ab
40	1.327±0.011a	0.470±0.015a	1.797±0.026a	2.825	0.302±0.010a
80	1.313±0.014ab	0.466±0.006ab	1.780±0.010ab	2.816	0.298±0.004ab

Table 3 Contents of carbon-nitrogen metabolites and antioxidant enzyme activities of *S. media*

Treatments (mg/L)	Soluble protein content (mg/g)	Soluble sugar content (%)	SOD activity (U/g)	POD activity (U/g)	CAT activity [mg/(g·min)]
0	15.63±0.91c	9.78±0.54a	107.86±1.94c	909.21±3.75c	16.15±0.54c
10	16.18±0.88c	8.86±0.16ab	109.57±3.37c	966.34±84.01c	17.49±0.56c
20	20.12±0.17b	8.495±0.36bc	112.70±1.62c	1011.69±85.98bc	17.58±0.48c
40	26.40±1.41a	7.79±0.18c	184.70±4.73a	1333.33±59.73a	25.92±0.73a
80	22.36±1.13b	8.01±0.44bc	128.41±1.19b	1176.83±54.61ab	23.23±0.71b

Cd Accumulation. With the increase of the concentration of uniconazole, the contents of Cd in roots of *S. media* decreased, while the Cd content in shoots of *S. media* increased first and then decreased (Table 4). The maximum of Cd content in shoots was at 40 mg/L uniconazole. When the dose of uniconazole was 10, 20, 40 and 80 mg/L, Cd content in the shoots increased by 6.87% ($P > 0.05$), 21.00% ($P < 0.05$), 39.10% ($P < 0.05$) and 30.58% ($P < 0.05$) respectively compared with the control. Uniconazole decreased Cd amount in roots of *S. media*, and had the decreasing trend with the increase of the concentration of uniconazole (Table 4). However, when the dose of uniconazole was 20 and 40 mg/L, Cd amount in shoots of *S. media* increased, which increased by 6.68% ($P < 0.05$) and 10.47% ($P < 0.05$) respectively compared with the control. When the dose of uniconazole was 10 and 80 mg/L, Cd amount in shoots of *S. media* decreased. The same as the Cd amount in shoots, only 20 and 40 mg/L uniconazole increased Cd amount in whole plants of *S. media*, and 10 and 80 mg/L uniconazole decreased that.

Table 4 Cd accumulation of *S. media*

Treatments (mg/L)	Cd content in roots (mg/kg)	Cd content in shoots (mg/kg)	Cd amount in roots (µg/plant)	Cd amount in shoots (µg/plant)	Cd amount in whole plant (µg/plant)
0	139.88±4.07a	48.34±1.90c	91.48±1.08a	172.38±2.25b	263.86±3.33ab
10	139.24±3.87a	51.66±2.35c	89.53±0.52ab	167.74±4.19b	257.27±4.71b
20	136.28±2.09a	58.49±2.11b	87.08±0.01b	183.89±2.99a	270.98±2.97a
40	134.27±6.04a	67.24±1.75a	80.02±2.46c	190.42±1.92a	270.45±4.38a
80	128.27±4.62a	63.12±1.58ab	71.45±0.94d	170.87±5.26b	242.31±4.32c

Conclusions

The application of uniconazole inhibited the growth of *S. media*, and promoted the absorption and transport of Cd from soil. The biomass and soluble sugar content of *S. media* decreased with the increase of the concentration of uniconazole, whereas the activities of SOD, POD and CAT, the contents of photosynthetic pigments and soluble protein improved. With the increase of concentration

of uniconazole, the Cd content and Cd accumulation in the shoots of *S. media* increased first and then decreased, and reached the maximum up to 67.24 mg/kg and 190.42 µg/plant respectively when the dose of uniconazole was 40 mg/L, which increased by 39.10% and 10.47% compared with the control, respectively. Therefore, application of uniconazole could enhance phytoremediation ability of *S. media* at the dose of 20-40 mg/L.

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References

- [1] D. Wang, H. Yang, F. Zhang and D. Cao: *Agricultural Research in the Arid Areas* Vol. 34, (2016), p. 94.
- [2] R.A. Fletcher and G. Hofstra: *Journal of Plant Growth Regulation* Vol. 9 (1990), p. 207.
- [3] X. Liu and X. Liu: *Acta Prataculturae Sinica* Vol. 15 (2006), p. 48.
- [4] B. Xie, Q. Wang, H. Zhang, A. Li, F. Hou, B. Wang, S. Dong and L. Zhang: *Acta Agriculturae Boreali-Sinica* Vol. 31 (2016), p. 155.
- [5] X. Wang, M. Yu and L. Tao: *Crops* Vol. 2 (1993), p. 33.
- [6] K. Izumi, I. Yamaguchi, A. Wada, H. Oshio and N. Takahashi: *Plant & Cell Physiology* Vol. 25 (1984), p. 611.
- [7] R.M. Thomas and V.P. Singh: *Photosynthetica* Vol. 32 (1996), p. 145.
- [8] S. Purohit and V.P. Singh: *Photosynthetica*. Vol. 36 (2000), p. 597.
- [9] V.P. Singh: *Journal of Plant Growth Regulation* Vol. 12 (1993), p. 1.
- [10] L. Lin, B. Ning, M. Liao, H. Lan and H. Liang: *Ecology and Environmental Sciences* Vol. 23 (2014), p. 673.
- [11] Z.B. Hao, J. Chang and Z. Xu: *Plant Physiology Experiment* (Harbin Institute of Technology Press, China 2004).
- [12] L. Lin, M. Liao, L. Mei, J. Cheng, J. Liu, L. Luo and Y. Liu: *Environmental Progress & Sustainable Energy* Vol. 33 (2014), p. 1251.
- [13] A. Tessier, P.G.C. Campbell and M. Bisson: *Analytical Chemistry* Vol. 51 (1979), p. 844.