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Effects of tetracycline and oxytetracycline on growth and chlorophyll fluorescence in lettuce seedlings

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Abstract. At present, the problem of antibiotic pollution in soil is becoming more and more serious, and antibiotics entering the soil can be absorbed by plants, causing normal physiological metabolism inhibition. In this experiment, the effects of tetracycline (TC) and oxytetracycline (OTC) on the growth of lettuce were studied. Matrix culture experiments were used to investigate the effects of different levels (0, 2, 10, 50, and 250 mg/kg) of TC and OTC on the growth of lettuce, chlorophyll fluorescence parameters, and antioxidant enzyme activities. The results showed that TC and OTC caused different degrees of inhibition on the growth of lettuce due to the different concentrations. Under antibiotic's treatment, F_v / F_m , F_v' / F_m' and $Y(II)$ of lettuce also showed a downward trend under tetracycline (TCs) treatment, however, the non-photochemical quenching (NPQ) of absorbed light energy showed an increasing trend. TCs also inhibited the antioxidant enzyme activity of lettuce, SOD activity was reduced by 3.27 (light concentration) and 52.68% (high concentration), and POD activity was decreased by 6.27 (light concentration) to 55.86% (high concentration) compared to control. The results showed that TCs could cause oxidative stress on plant growth, which should be concerned.

1. Introduction

Antibiotics are widely used in livestock and poultry farming and aquaculture, they are discharged into water and soil in large quantities and have potentially harmful to the ecological environment. China is a major producer and consumer of TCs, with an annual export volume of 1.34×10^7 kg. Human and animals can not fully digest such large amounts of antibiotics, 80% to 90% of which are discharged into the environment as feces. Soil is the ultimate destination of antibiotics. In recent years, antibiotics, especially TCs [1], have been found in vegetable soil in many areas of China, with the same content as abroad and a higher detection rate [2]. TCs can be converted into their metabolites when they accumulate in soil. Over time, they gradually destroy the soil ecosystem through a series of environmental behaviors [3], posing a potential threat to the quality and safety of agricultural products. Its mass concentration ranges from a few $\mu\text{g/kg}$ to thousands of $\mu\text{g/kg}$, and the consumption of some vegetables exceeds the ecological toxicity trigger value of antibiotics prescribed by the European Union [4]. OTC and TC are widely used among tetracycline antibiotics, plant uptake of OTC and TC and their phytotoxicity are rarely studied. As a vegetable that people eat daily, lettuce has the characteristics of short growth cycle, easy cultivation and sensitivity to pollutants. It is often used as an experimental plant to evaluate the absorption and toxicity effect of plants to pollutants.

Therefore, based on the previous studies, we are going to study the effects of tetracycline antibiotics on physiological characteristics and photosynthesis of lettuce, which is expected to provide



theoretical basis for the blocking of antibiotic pollution in the environment and the safety of vegetable products.

2. Material and methods

2.1 Experimental materials

The test crop was Italian lettuce (*Lactuca sativa* L. var. *Ramosa* Hort.)

2.2 Experimental design

In order to avoid soil and organic fertilizers containing tetracycline antibiotics, this experiment was used a 2: 1 mixed matrix of perlite and vermiculite, and the test materials were planted in a greenhouse in the campus of Sichuan Agricultural University. When the lettuce were 5 true leaves, the seedlings were planted in nutrient bowls (10 cm × 10cm). The experiment was designed with 5 levels, which were 0, 2, 10, 50, and 250 mg/kg, each bowl was filled with 150 g of mixed substrate and 10 ml of antibiotics of different concentrations, and there was a plate under each bowl. If there is a leak, turn the water back to the bowl. 0mg/kg was considered as control. Samples were taken after 20 days of antibiotic treatment, samples were stored in -80 °C ultra-low temperature refrigerator for measuring physiological indicators.

2.3 Experimental methods

Chlorophyll fluorescence parameters were measured using a chlorophyll fluorescence meter (PAM-2500). After dark adaptation for 30 min, the parameters of F_o , F_m , F_v / F_m , $Y (II)$, F_v' / F_m' , and NPQ were measured. SOD activity was measured by nitrogen blue tetrazole photochemical reduction method[5]. The peroxidase POD activity was measured by guaiacol colorimetry, methods[6].

2.4 Analysis of data

Data were processed using one-way analysis of variance. According to Duncan's multiple range test, there were significant differences at $p < 0.05$.

3. Results and discussion

3.1 Effect of OTC and TC on the growth index of lettuce seedlings

As is shown in Figure 1, OTC and TC generally showed inhibition in the growth of lettuce. Under OTC's treatment, the fresh weight of the shoots and roots of lettuce were decreased significantly among different concentrations, with the fresh weight of the shoot reduced by 10.14% to 21.84% when compared to CK, and the roots reduced by 11.41% to 23.41%. However, there was an increase in the concentration of 2 mg/kg, but it was not significant (only by 6.13%). With TC's treatment, there was also a trend of inhibition in shoots and roots, decreased by 4.61% to 27.02%, and 5.84% to 25.88%, respectively. Pan compared the effects of different types of antibiotics on the growth of lettuce, tomatoes, carrots and cucumbers and found that lettuce was more sensitive to antibiotics [7]. In this experiment, the growth of lettuce was inhibited at high levels of TC, which was similar to previous studies[8]. In addition, there was a slight increase in the fresh weight of roots under low level of OTC(2 mg/kg), which to some extent reflected the bi-directional effect of antibiotic on plant growth, i.e. low concentrations promoted growth and high concentrations inhibited growth[9-10]. Studies have suggested that low concentration of antibiotic stress will increase the vitality of plant root system, which may be the reason that the plant root system resist the adversity and alleviate the harm of antibiotic stress by improving metabolism, low concentration of antibiotics can promote the synthesis of intracellular nucleic acids and proteins[11].

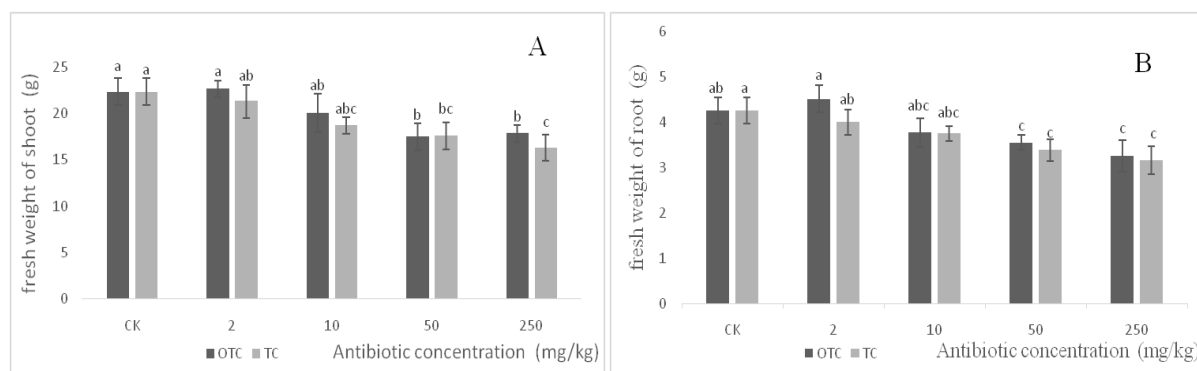


Figure 1 Effects of different concentrations of OTC and TC on the fresh weight of shoot and root of lettuce

3.2 Effect of OTC and TC on chlorophyll fluorescence

From Table 1, there was a trend of reduction on Y(II) and Fv/Fm with the increasing concentration of under OTC's treatment, reflecting the inhibition of photosynthesis electron transport. Y(II) were decreased by 15.17% after adding of 2 mg/kg and 40.77% under 250 mg/kg. While Y(II) were only reduced significantly by TC under 250 mg/kg. Fv/Fm were decreased significantly under the highest concentration (250 mg/kg) of both OTC and TC, indicating the irreversible damage to PSII under this level. Effective photochemical quantum yield (Fv'/Fm') and (NPQ) could reflect PSII photoenergy capture efficiency and heat dissipation, respectively. The value of Fv'/Fm' decreased significantly under antibiotic treatment. The value of NPQ increased significantly under antibiotic treatment. NPQ showed a rise trend followed by the increasing concentration of antibiotic treatment. It may help to dissipate excess excitation energy to protect photosynthesis mechanism, while considered as stress reaction [12].

Table 1. Effect of OTC and TC treatment on chlorophyll fluorescence parameters at different concentrations

Treatments(mg/kg)		Fv/Fm	Y(II)	Fv'/Fm'	NPQ
TC	CK	0.761±0.005a	0.327±0.025a	0.514±0.013a	1.060±0.066e
	2	0.765±0.007a	0.301±0.023a	0.506±0.011a	1.359±0.076d
	10	0.752±0.009a	0.256±0.02ab	0.487±0.01bc	1.624±0.049c
	50	0.760±0.005a	0.259±0.03ab	0.457±0.009c	1.965±0.098b
	250	0.705±0.015b	0.228±0.018b	0.391±0.012d	2.359±0.130a
OTC	CK	0.761±0.005a	0.327±0.025	0.514±0.013a	1.060±0.066c
	2	0.751±0.007a	0.277±0.031	0.512±0.013a	1.406±0.095b
	10	0.758±0.003a	0.238±0.016	0.475±0.011b	1.883±0.094a
	50	0.754±0.007a	0.234±0.023	0.460±0.006b	1.912±0.069a
	250	0.730±0.009b	0.193±0.023	0.453±0.005b	1.973±0.134a

3.3 Effect of OTC and TC on antioxidant enzyme activities of lettuce seedlings

As is shown in Table 2, under TC and OTC's treatments, the SOD and POD activities of lettuce showed a general downward trend in both shoots and roots. Under the 2 mg/kg and 250 mg/kg of OTC's treatments in leaves, SOD activity was reduced by 3.27% and 23.86%, respectively, and it reduced by 5.52% and 29.80% under TC treatment, respectively. There was similar result in root under the OTC and TC's treatments in SOD activity. This is consistent with the conclusions of study by Liu et al. [13], which may be due to antibiotics that impair metabolic processes such as normal electron flow in the respiratory electron delivery chain, and also induces excessive H₂O₂ to cause plant cell damage, resulting in reduced activity of antioxidant enzymes [14]. However, the speed of reduction of the POD activity by TC's treatment was slower than that of OTC's treatment in leaves while faster

in roots. This is contrary to the findings of the Riaz et al[15]. It could be the reason that plants are sensitive to antibiotics and the result vary from antibiotic types, plant types, concentrations, and culture medium, and the effects of plant antioxidant systems have certain limits. Thus, TCs will cause oxidation stress to plant growth, but its mechanism of action and influencing factors need further study.

Table 2. Effects of OTC and TC treatment on antioxidant enzyme activity in the above and underground parts of lettuce at different concentrations

Treatments (mg/kg)	SOD of shoot (U/g · Fw)	SOD of root (U/g · Fw)	POD of shoot (U/g · Fw)	POD of root (U/g · Fw)
TC-CK	125.960±0.650a	68.900±4.220a	113.324±2.124a	184.845±1.255a
TC-2	119.005±0.555ab	64.090±3.010a	106.200±2.000ab	108.120±4.860b
TC-10	113.765±4.095b	47.530±3.360b	101.404±4.911b	88.215±4.085c
TC-50	102.070±1.090c	36.840±0.520bc	84.115±2.115c	88.230±2.230c
TC-250	88.425±1.515d	38.295±0.855c	58.155±2.052d	81.595±2.995c
OTC-CK	125.960±0.650a	68.900±4.220a	113.324±2.124a	184.845±1.255a
OTC-2	121.845±2.135ab	65.410±2.630a	83.632±2.184b	152.270±1.300b
OTC-10	118.470±2.120b	58.805±2.725a	73.154±5.618bc	126.165±4.235c
OTC-50	98.275±2.375c	42.575±3.765b	73.000±6.902bc	120.095±1.615c
OTC-250	95.900±1.370c	32.575±2.455c	66.184±0.957c	92.565±4.415d

4. Conclusion

The results showed that OTC and TC inhibit the growth of lettuce in general. Moreover, two antibiotics have a certain inhibitory effect on the photosynthesis process and antioxidant enzyme activity of lettuce, and the inhibition effect is increasing with the increase of tetracycline addition. Combined with the results of the present experiment, tetracycline has an ecotoxic effect on lettuce growth and photosynthesis.

References

- [1] Li Y W, Wu X L, Mo C H, et al. (2011) Investigation of sulfonamide, tetracycline, and quinolone antibiotics in vegetable farmland soil in the pearl river delta area, southern China. *Journal of Agricultural and Food Chemistry*, 59(13): 7268-7276.
- [2] Li Y W, Mo C H, Zhao N, et al. (2011) Study on the pollution characteristics of sulfonamide and tetracycline antibiotics in vegetable soil. *environmental science* (6): 204-208.
- [3] Dong L, Gao J, Xie X, et al. (2012) DNA damage and biochemical toxicity of antibiotics in soil on the earthworm *Eisenia fetida*. *Chemosphere*, 89(1): 44-51.
- [4] Ahmed M B M, Rajapaksha A U, Lim J E, et al. (2015) Distribution and accumulative pattern of tetracyclines and sulfonamides in edible vegetables of cucumber, tomato, and lettuce. *Journal of Agricultural and Food Chemistry*, 63(2): 398-405.
- [5] Qu M, Qin L N, Liu Y J, et al. (2014) Comparison of two methods to detect the activity of SOD enzymes. *Journal of Food Safety Quality Inspection*, (10): 3318-3323.
- [6] Tang X Q, Wang K C, Chen X. (2011) Effect of flooding stress on the activity of ethanol dehydrogenase and peroxidase in the period of blue seedlings. *Journal of Jiangxi Agricultural*, 23(2): 70-73.
- [7] Pan M, Chu L M. (2016) Phytotoxicity of veterinary antibiotics to seed germination and root elongation of crops. *Ecotoxicology and Environmental Safety*, 126: 228-237.
- [8] Pan M, Chu L M. (2011) Fate of antibiotics in soil and their uptake by edible crops. *Science of the Total Environment*, 599-600: 500-512.

- [9] Migliore L, Cozzolino S, Fiori M. (2003) Phytotoxicity to and uptake of enrofloxacin in crop plants. *Chemosphere*, 52 (7): 1233-1244.
- [10] Liu L, Liu Y H, Liu C X, et al. (2013) Potential effect and accumulation of veterinary antibiotics in *Phragmites australis* under hydroponic conditions. *Ecological Engineering*, 53: 138-143.
- [11] Zhou W L, Qiao X T, Xiao H, et al. (2009) Effects of antibiotics on chlorophyll-a level in marine microalga. *Marine Environmental Science*, 3(28): 268 - 271.
- [12] Zhao L Y, Deng X P, Shan L. (2011) Effect of osmosis stress on chlorophyll fluorescence parameters of wheat seedlings . *chinese journal of applied ecology*, 16(7): 1261-1264.
- [13] Gomes M P, Goncalves C A, de Brito J C M, et al. (2017) Ciprofloxacin induces oxidative stress in duckweed (*Lemna minor* L.) : implications for energy metabolism and antibiotic-uptake ability. *Journal of Hazardous Materials*, 328: 140-149.
- [14] Sun S Q, Hem, Cao T, et al. (2011) Effects of Pb and Ni stress on antioxidant enzyme system of *Thuidium cymibifolium*. *Chinese Journal of Applied Ecology*, 20(4) : 937-942.
- [15] Riaz L, Mahmood T, Coyne M S, et al. (2011) Physiological and antioxidant response of wheat (*Triticum aestivum*) seedlings to fluoroquinolone antibiotics. *Chemosphere*, 177: 250-257.