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ACCUMULATION AND DISTRIBUTION OF OXYTETRACYCLINE AND CHLORTETRACYCLINE IN TOBACCO SEEDLINGS AND THEIR EFFECTS ON GROWTH

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ABSTRACT

The accumulation and distribution of oxytetracycline (OTC) and chlortetracycline (CTC) in tobacco seedlings, and their effects on tobacco seedling growth, were studied using the floating seedling method in greenhouse conditions. Results showed that both OTC and CTC could inhibit the growth of tobacco seedlings, and inhibition effects were greater at higher concentrations. Compared with controlling tobacco seedlings treated with the highest concentration T4 (100 mg/L), the root length and root fresh weight in OTC treatment group decreased by 39% and 53%. The length and fresh weight of stem decreased by 34% and 39%. The maximum leaf area and fresh leaf weight decreased by 34% and 46%. The root length and fresh weight in CTC treatment group decreased by 37% and 54%. The length and fresh weight of stem decreased by 53% and 62%. The maximum leaf area and fresh leaf weight were reduced by 59% and 70%. The higher the concentration of antibiotics in the nutrient solution, the higher the accumulation of antibiotics in each tissue of the tobacco seedlings, and accumulation increased significantly from 2 to 80 µg/g over time (7-20 days). And accumulation in leaves (5 to 60, 5 to 80 µg/g respectively) was greater than that in roots (3 to 30, 3 to 15 µg/g respectively) and stems (2 to 25, 2 to 25 µg/g respectively), and CTC inhibited tobacco seedlings more than OTC. The above mentioned two kinds of antibiotic have an effect on tobacco seedling.

Keywords: accumulation, chlortetracycline, growth, oxytetracycline, seedling, tobacco.

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INTRODUCTION

Antibiotics are widely used in agriculture, medicine, and animal husbandry, but once residual antibiotics enter the environment, they can cause environmental harm (Isabel, *et al.*, 2016; Sun, 2017; Liu, 2017). TCs is one of the most widely used in so many kinds of antibiotics, which including tetracycline, OTC, CTC, doxycycline, tigecycline and some other Semi-synthetic derivative of minocycline. Abuse of TCs in medicine, agriculture and animal husbandry could cause huge portion of residual and cycle in environment, which would accumulate in plants, animals and human beings though food chain and bring huge potential risk. Research have found that TCs could inhibit root elongation of cabbage significantly when their contents are lower than 5 mg/L and CTC effected obviously mostly and OTC followed (Zhu, *et al.*, 2018). OTC could affect root length of corn easily and inhibit their net photosynthetic rete, stomatal conductance and transpiration (Xiong *et al.*, 2018). Two kinds of sensitive and insensitive wheats were chosen to study effects of OTC on wheat by testing their soil urease, phosphatase, sucrase, catalase and microbial biomass result showed that these effects are different from genes, which insensitive less obviously than sensitive (Yao, *et al.*, 2010). A previous study showed that the detection rate of tetracycline antibiotics in soil was 69-92%, and the average total content of organic vegetable base soil was higher than that of green vegetable base soil (Tai, *et al.*, 2014). In another study, when antibiotic load was 100 mg/m^2, the highest cumulative concentration range of four tetracycline antibiotics in stems and leaves of pakchoi was 17-52 µg/kg (He, *et al.*, 2014). The antibiotics remaining in soil can accumulate in plants. Previous studies have shown that a low concentration of tetracycline antibiotics (<4 mg/L) can promote elongation of roots and buds of pakchoi, whereas high concentrations of oxytetracycline can inhibit elongation (Zhu, *et al.*, 2018, Wang, *et al.*, 2017). How to minimize effects of antibiotics on plant production is critical. Tobacco, for instance, can absorb a large number of heavy metals and other harmful substances. Studies have shown that the roots and leaves of tobacco can absorb heavy metals from the soil, which is easy to absorb and enrich heavy metals. Even after the tobacco is roasted, the contents of As, Cd, Cr and Pb in each cigarette can still reach 0.17,0.86,2.35 and 0.44 µg/g (Zhang, *et al.*, 2012, Zhao, *et al.*, 2018). This paper uses tobacco as the focal plant to study accumulation and

distribution of tetracycline antibiotics and its effects on seedlings, providing a foundation for further study of the effects of antibiotic pollution on plants.

MATERIALS AND METHODS

Flue-cured tobacco cultivar was Yunyan 87. Oxytetracycline hydrochloride standard product (Oxytetracycline•HCl, OTC) and chlortetracycline hydrochloride standard product (Chlortetracycline•HCl, CTC) were purchased from the National Institutes for Food and Drug Control, China.

Nutrient solution （ Hoagland's formula ）: $CaN_2O_6 \cdot 4(H_2O)$: 945 mg/L, KNO_3:506 mg/L, $(NH_4)_3PO_4$:80 mg/L, KH_2PO_4:136 mg/L, $MgSO_4$:493 mg/L, ferric salt solution:2.5 ml/L, trace element: 5ml/L（ KI:0.83 mg/L, H_3BO_3:6.2 mg/L, $MnSO_4$: 22.3 mg/L, $ZnSO_4$:8.6 mg/L, Na_2MoO_4: 0.25 mg/L, $CuSO_4$:0.025 mg/L, $CoCl_2$:0.025 mg/L）, pH=6.0. Five concentrations (mg/L nutrient solution) were set for OTC and CTC, respectively: 0 (C), 25 (T1), 50 (T2), 75 (T3) and 100 (T4).

Experiments were performed in a solar greenhouse of Yunnan Agricultural University. Floating seedlings were used, distilled water was irregularly added, and the volume of nutrient solution was kept at 14 L. Twenty days after sowing, when tobacco seedlings had 4 true leaves, the antibiotic treatments were started. Every week, 15 seedlings were taken from each treatment, 5 of which were repeated once and 3 times for each treatment. Roots, stems, and leaves were separated, antibiotic content of each was measured, and accumulation was calculated. The experimental period was 4 weeks. After 28 days, agronomic traits (root length, stem length, maximum leaf area, fresh weight) (YCT142-2010, 2010), root activity (Zhang, *et al.*, 2009), chlorophyll content in leaves (Shanghai Society of plant physiology., 1999), antibiotic content and accumulation (Nian, *et al.*, 2019) were measured.

In this study, the contents of two kinds of antibiotics in tobacco were measured by ultra-performance liquid chromatography (UPLC). Shimadzu 20AT UPLC system, Zhejiang University Zhida N2000 chromatographic workstation, Agilent ODSC18 column (5 m, 250 mm×4.6 mm) were utilized. The determination method of OTC was as follows: mobile phase was acetonitrile-0.05m citric acid = 15:85 (V/V), flow rate was 1.0 mL/min, column temperature was 40℃, and UV detection wavelength was 280nm. The quantification method was used in this experiment. The detection method of CTC was as follows: mobile phase was methanol-0.01M sodium dihydrogen phosphate solution (pH=2.7) = 35:65 (V/V), flow rate was 1.0 mL/min, column temperature was 35℃, and UV detection wavelength was 355nm. The external standard method was used for quantification in this experiment. Methanol and acetonitrile were chromatographic pure, citric acid and n-hexane were analytical pure, all of which were purchased from Langbao Biotechnology Service Department, Hangzhou Economic and Technological Development Zone. Ultra-pure water was self-made in the laboratory. McIlvaine Buffer Solution: Mix 625 mL 0.2mol/L disodium hydrogen phosphate solution with 1000mL 0.1mol /L citric acid solution and adjust with NaOH or HCl to pH = 4.0±0.5 0.1mol /L. Na_2EDTA-McIlvaine Buffer Solution: Weighed 60.50 g EDTA-McIlvaine Disodium into 1625mL McIlvaine Buffer Solution, dissolved it, and shook well. A standard solution is prepared and a standard curve is drawn, then samples were prepared and their concentration was calculated.

Data analysis was performed with SPSS 17.0 and Excel 2013 software. SPSS software was used to compare the mean value and conduct homogeneity test of variance. One-way ANOVA method was used to analyze the significant difference of the data.

RESULTS AND DISCUSSION

With increased antibiotic concentration, root length, stem length, and the maximum leaf area of tobacco seedlings decreased. The three indicators of tobacco seedlings treated with OTC showed no significant difference with C for T1 and T2 treatments, but significant difference with C for T3 and T4 treatments. However, the three indicators of CTC treatment were significantly different with C at T1 treatment. At the same antibiotic concentration, effects of CTC on tobacco seedlings were greater than OTC (Table 1).

With increased antibiotic concentrations, the fresh weight of roots, stems, and leaves of tobacco seedlings decreased. There were significant differences between the roots, stems, and leaves of tobacco seedlings treated with OTC and the C for the T2、T3 and T4 treatments, and there were significant differences for the traits with CTC and the C at T1 treatment. Effects of CTC on fresh weight of tobacco seedlings was greater than for OTC（Table 2）.

Active absorption significantly area of flue-cured tobacco seedlings roots decreased significantly, which from 0.085 to 0.05 and 0.084 to 0.003 m^2/plant with the effects of OTC and CTC respectively. With an increase in antibiotic concentration, there is a significant difference between the two antibiotic-treated tobacco seedlings for all treatments compared to the control group. In the five bar charts, only the control group was no significant difference between OTC and CTC. CTC value of other treatments were all lower than that of OTC, especially in the T4 treatment. This shows effects of CTC on the root activity of seedlings was greater than that of OTC at the same concentration (Fig. 1).

Table 1. Effects of OTC and CTC on agronomic traits of tobacco seedlings.

Treatment	Root length（cm）		Stem length（cm）		Maximum leaf area (cm^2)	
	OTC	CTC	OTC	CTC	OTC	CTC
C	16.83 ^{a}	17.17 ^{a}	10.29 ^{a}	9.96 ^{a}	91.98 ^{a}	90.31 ^{a}
T1	15.33 ^{ab}	11.50 ^{b}	8.92 ^{ab}	6.69 ^{b}	90.00 ^{a}	67.50 ^{b}
T2	13.83 ^{ab}	10.38 ^{b}	8.42 ^{ab}	6.31 ^{b}	77.73 ^{ab}	58.30 ^{bc}
T3	12.50 ^{ab}	9.38 ^{b}	7.25 ^{b}	5.44 ^{b}	54.38 ^{bc}	40.78 ^{cd}
T4	10.33 ^{b}	7.75 ^{b}	6.83 ^{b}	5.13 ^{b}	50.42 ^{c}	37.81 ^{d}

Note: that different lowercase letters represent significant differences ($P < 0.05$) for a given response variable (each column).

Table 2. Effects of OTC and CTC on fresh weight per plant of tobacco seedlings (g/plant)

Treatment	Root		Stem		Leaf	
	OTC	CTC	OTC	CTC	OTC	CTC
C	1.66 ^{a}	1.65 ^{a}	5.09 ^{a}	4.76 ^{a}	10.45 ^{a}	10.78 ^{a}
T1	1.66 ^{a}	1.25 ^{b}	3.28 ^{ab}	2.46 ^{b}	10.19 ^{a}	7.64 ^{b}
T2	1.57 ^{a}	1.17 ^{b}	2.73 ^{b}	2.05 ^{b}	9.90 ^{a}	7.42 ^{b}
T3	1.19 ^{ab}	0.89 ^{bc}	2.62 ^{b}	1.96 ^{b}	8.29 ^{a}	6.22 ^{b}
T4	1.02 ^{b}	0.76 ^{c}	2.38 ^{b}	1.79 ^{b}	4.32 ^{b}	3.24 ^{c}

Note: Different lowercase letters represent significant differences ($P < 0.05$) for a given response variable (each column).

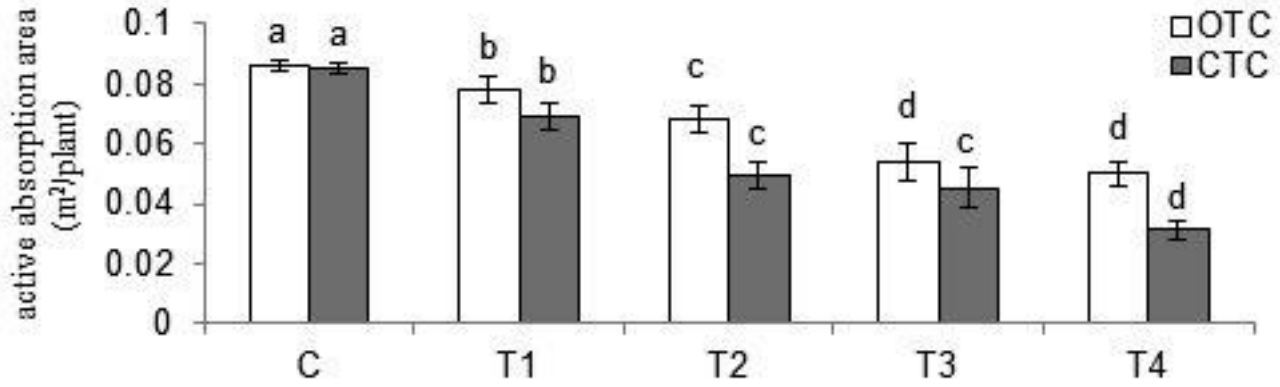

Fig.1 Effects of OTC and CTC on active absorption area of flue-cured tobacco seedlings roots. That different
Note: Lowercase letters represent significant differences ($P < 0.05$) for a given response variable (Same columnar color).

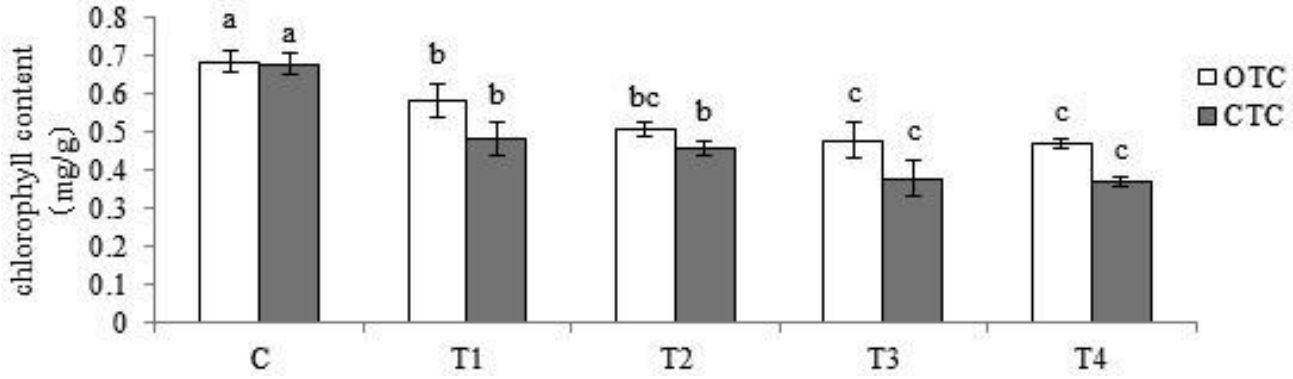

Fig.2 Effects of OTC and CTC on chlorophyll content of tobacco seedling leaves. That different lowercase letters represent significant differences ($P < 0.05$) for a given response variable (Same columnar color).

Chlorophyll content of tobacco seedling leaves decreased significantly, which from 0.68 to 0.49 and 0.67 to 0.42 mg/g with the effects of OTC and CTC respectively. There were significant differences between the control group and the tobacco seedlings treated with CTC and OTC—with an increasing concentration,

chlorophyll decreased significantly. At the same concentration level, although there was no significant difference between CTC and OTC, the former tended to be lower than the latter（Fig. 2）.

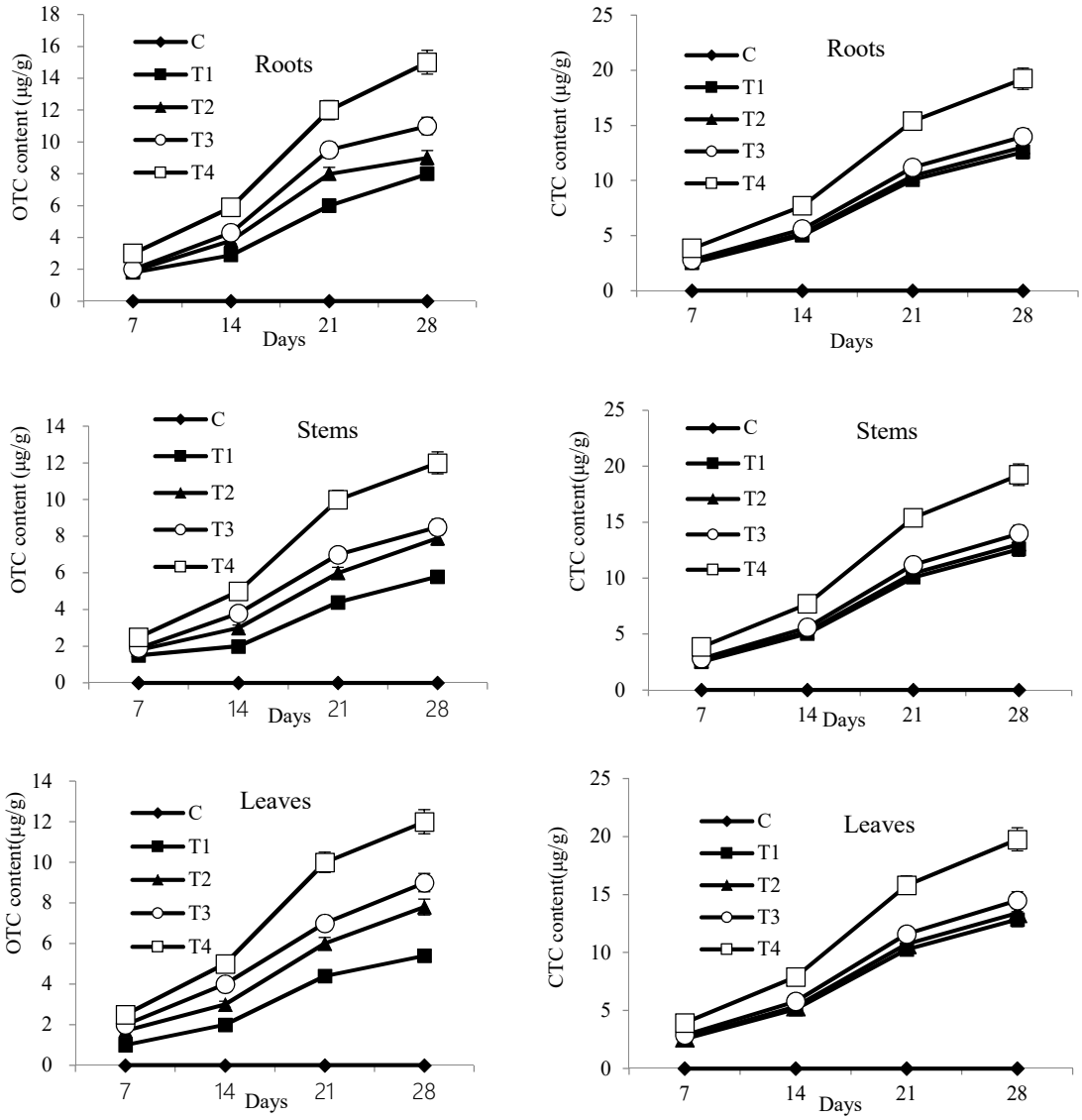

Fig.3 OTC contents and CTC contents in tobacco seedling roots, stems, and leaves

OTC and CTC contents in tobacco seedling roots increased from 2 to 15, 2 to 20 μg/g respectively over time (7 to 28 days), from 2 to 12, 2 to 20 μg/g in stems, and from 1 to 12, 2 to 20 μg/g in leaves. The higher the concentration of antibiotics in the nutrient solution, the higher the content of antibiotics in each tissue of the tobacco seedlings, and the content of antibiotics increased significantly over time. Content of CTC in each seedling tissue was higher than that of OTC at the same number of days and concentrations, and the content of CTC in the roots, stems, and leaves of tobacco seedlings at T4 treatment was 1.4、1.7 and 1.7 times higher, respectively, than that for OTC (Fig. 3).

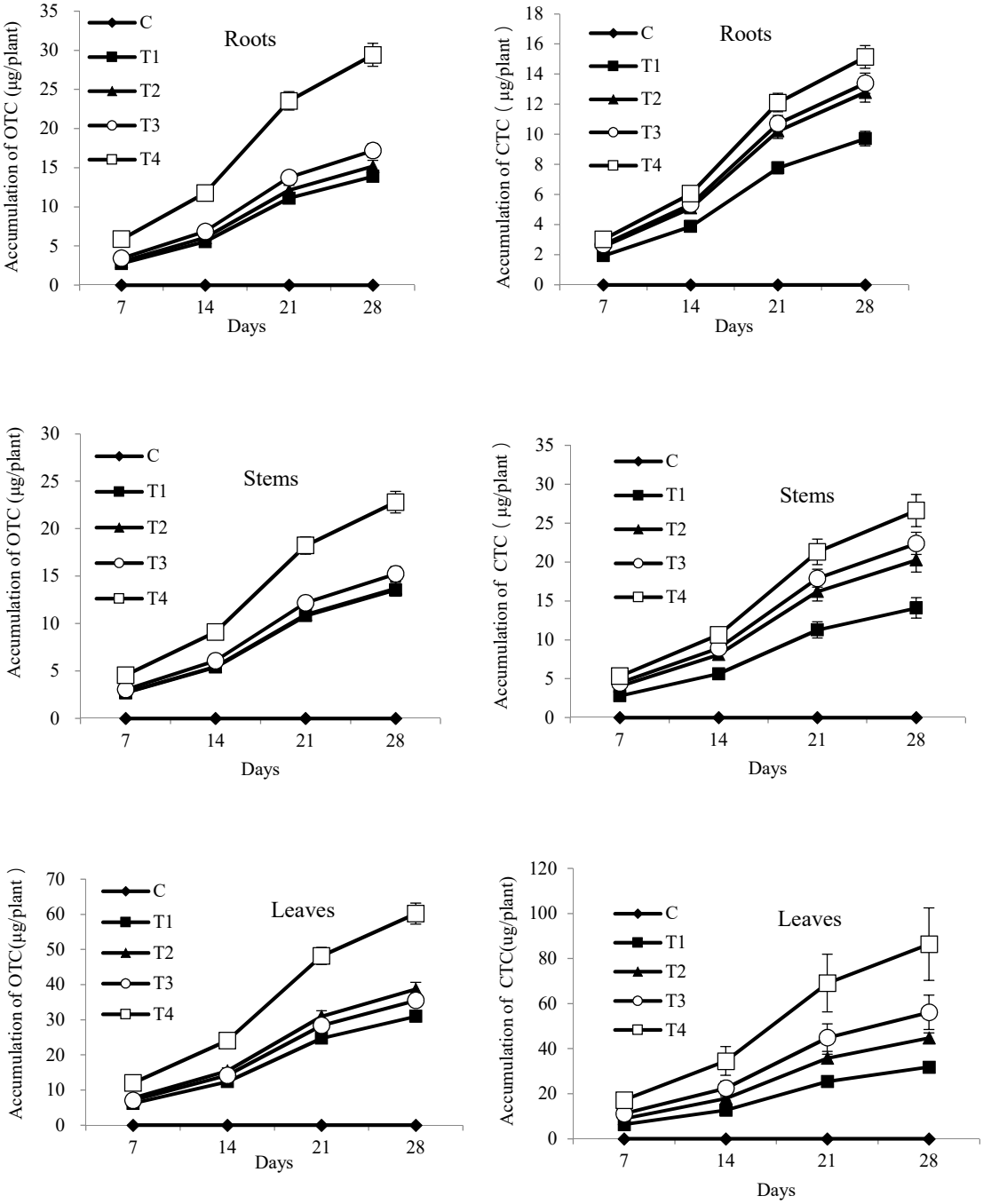

Fig.4 Accumulation of OTC and CTC in roots, stems, and leaves of flue-cured tobacco

OTC and CTC accumulation in flue-cured tobacco roots increased from 2 to 30, 2 to 15 μg/plant respectively over time (7 to 28 days), from 2 to 24, 2 to 27 μg/plant in stems, and from 6 to 60, 5 to 85 μg/plant in leaves. The higher the concentration of antibiotics in the nutrient solution, the higher the accumulation of antibiotics in each tissue of the tobacco seedlings, and accumulation increased significantly over time. Accumulation of OTC in leaves in the T4 treatment was 3 times that in roots and stems, and that for CTC in leaves in the T4 treatment was 4 times of that in roots and stems. Accumulation in stems and leaves for CTC in the T4 treatment was 1.3 and 1.5 times that of OTC（Fig. 4）.

Results showed that both OTC and CTC could be absorbed and accumulated by tobacco seedlings. With an increase in concentration of OTC and CTC in solution, accumulation in tobacco seedlings increased significantly, with accumulation in leaves greater than in roots and stems. Maximum leaf area and fresh weight of tobacco seedlings decreased, and growth was affected. This is consistent with the conclusion by Zhang *et al.*, (2017). That high tetracycline addition has certain ecotoxic effects on tobacco growth, development, and photosynthesis. In this paper, even low concentrations of OTC and CTC inhibited growth of tobacco seedlings, different from the study of Zhang *et al.*, (2017), which may be caused by the different medium of tobacco seedling (this study used water culture, but Zhang *et al.*, (2017). used soil culture). One study has added CTC and OTC in nutrition liquid and soil of culturing red lentils to study their effects in different cultivation mediums, and results found that their induction to red lentils reduced in soil because of CTC and OTC were chelated by divalent metal ion in soil which caused their contents decreased (Batchelder, 1982 and Ma *et al.*, 2010). Results of this study suggest that antibiotics differ with respect to their effects on plants in water and soil. At the same antibiotic concentrations and culture times, accumulation of CTC in tobacco seedlings was significantly greater than that of OTC, and the growth of tobacco seedlings was more inhibited. Although both OTC and CTC are tetracycline antibiotics, there are significant differences on tobacco seedlings. To deeper mechanism, TCTs may affect several kinds of enzymatic activity and metabolism of amino acids, organic acids, nitrogen and carbon. Kong's research found that TCTs could accumulate in root of alfalfa and their leaves became yellow with increasing contents of TCTs, and result showed that synthesis of chlorophyll was inhibited since structure of TCTs are similar to Chlorophyll synthetase (Kong, *et al.*, 2007). In another study, tetracycline resulted in decreased concentrations of carotene and chlorophyll in ryegrass seedlings, increased production of root species of active oxygen species (ROS) and increased cell permeability, thus leading to potential loss of mitochondrial membrane (Ao, *et al.*, 2021). It also affected the biosynthesis of amino acyl-tRNA and nitrogen metabolism in ryegrass roots. The results suggested that tetracycline may affect the root growth of ryegrass by regulating the synthesis or degradation of these metabolites (Han, *et al.*, 2019). No matter from the macro or micro perspective, many studies have shown that tetracycline antibiotics can produce stress on plants. The influence mechanism may be related to the molecular structure of antibiotics, which warrants further study.

Authors' Contribution: Fuzhao Nian, Leifeng Zhao, Yunsheng Xia planned and designed the experiment. Shuwu Liu, Yonghui Zhang, Fei Wang, Lifang Wang, Ruirui Liu, Xiaolin Liao, Chun Xia and Xiaojuan Wang took part in the measurements and sampling. Xiaojuan Wang, Jiangwen Nian, Yaojun Wang processed the growth data and conducted the statistical analyses. Xiaojuan Wang, Fuzhao Nian and Yaojun Wang wrote, revised and submitted the manuscript. All authors read the manuscript and approved the final version.

REFERENCES

Ao, M.M., J. Wei, Z.L. Chen, L. Liu and Y.H. Song (2021). Research progress on environmental behavior and ecological toxicity of tetracycline antibiotics. Chin. J. Environ. Eng. Technol., (02): 314-324.

Batchelder, A.R. (1982). Chlortetracycline and oxytetracycline effects on plant growth and development in soil systems. J. Environ. Qual. 11(4): 515-518.

Han, T., Y.P. Liang, Z.N. Wu, L. Zhang, Z.W. Liu, Q.F. Li, X.J. Chen, W.L. Guo, L.N. Jiang, F.F. Pan, S.D. Ge, Z.R. Mi, Z.C. Liu, H. Huang, X.Z. Li, J.G. Zhou, Li Yang, J.L. Wang, Z. Zhang, Y.Y. Tang, L.R. Yang and M.D. Wu (2019). Effects of tetracycline on growth, oxidative stress response, and metabolite pattern of ryegrass. J. Hazard. Mater., 380.

He, D.C., G.Y. Wu, Z.C. Xu, J.D. Fang and S.K. Zhang (2014). Absorption and accumulation of tetracycline antibiotics in Chinese cabbage and white radish. J. Agro-Environ. Sci. (06):1095-1099.

Isabel, T.C. and S. Lúcia (2016). Antibiotics in the aquatic environments. Environ. Int., 417-434.

Kong, W.D., Y.G. Zhu, Y.C. Liang, J. Zhang, F.A. Smith and M. Yang (2007). Uptake of oxytetracycline and its phytotoxicity to alfalfa (Medicago sativa L.). Environ. Pollut., 147(1): 187-193.

Liu, S.W. (2017). Distribution and accumulation of oxytetracycline and chlortetracycline in tobacco seedlings and their effects on their growth. thesis (unpublished). Coll. of Tob. Sci., Yunnan Agri. Univ., Kunming.

Ma, Y., Y.X. Sun, J.J. Chen and Z.L. Chen (2010). Research progress on the effect of veterinary drug residues on ecological environment. Chin. J. Veterinary Sci., 40 (6): 650-654.

Nian, F.Z., Y.B. Sun and Y. Gu (2019). Determination of oxytetracycline in tobacco seedlings. Govt. China., Beijing.

Shanghai Institute of plant physiology, Chinese Academy of Sciences, Shanghai Society of plant physiology (1999). Experimental guide of modern plant physiology. Science Press; Shanghai (China).

Sun, Y.B. (2017) Establishment and cumulative distribution of two methods of antibiotic analysis

for quinolones in flue-cured tobacco seedlings. thesis (unpublished). Coll. of Tob. Sci., Yunnan Agri. Univ., Kunming.

Tai, Y.P., C.H. Mo, Y.W. Li, X.L. Wu, X.P. Huang, L. Xiang (2014). Concentrations and Distributions of Tetracycline Antibiotics in Soils of Green and Organic Vegetable Fields in Guangzhou, China. J. Agro-environ. Sci., 33(9): 1743-1748.

Wang, P.L., H. Zhang, R.F. Wang, Q.X. Yang, F.L. Gong and F.Y. Chang (2017). Effects of antibiotic exposure on seedling growth and endophytic bacteria of Chinese cabbage. J. Amo-Environ. Sci., (09): 1734-1740.

Xiong, H.Y., J.M. Qin and H.T. Ma (2018). Effects of soil containing oxytetracycline on physiological characteristics of maize. J. Soil Water Conserv., 32 (2): 283-289.

Yao, J.H., D.K. Niu, Z.J. Li, Y.C. Liang and S.Q. Zhang (2010). Effects of the antibiotic oxytetracycline on enzyme activities and microbial biomass in wheat rhizosphere soil. Chin. J. Agri. Sci., (04): 721-728.

YCT142-2010 (2010). Investigation and measurement methods of tobacco agronomic traits[S]. Tobacco industry standard of the people's Republic of China.

Zhang, J.X., G.M. Shen, F.Y. Kong, Z.F. Zhang, Y.C. Dai, J.Y. Zheng and J.G. Zhang (2017). Effects of tetracycline on growth and photosynthesis of flue-cured tobacco. J. Amo-Environ. Sci., (01):48-56.

Zhang, Y.T., X.P. Yang, L. Li, J.S. Cheng and G.F. Ao (2012). Growth and accumulation rules of Pb, Cr and Cd in tobacco. J. South. Agri., 43(11): 1679-1702.

Zhang, Z.L., W.J. Zhai and X.F. Li (2009). Experimental guidance of Plant Physiology (The Fourth Edition) [M]. Higher Education Press; Beijing (China).

Zhao, M.X. and Bao Z.J. Zhang (2018) Distribution, Influence and Control Measures of Heavy Metals in Tobacco. J. Anhui Agri. Sci., 46(7): 30-32, 102.

Zhu, X.H., D. Ding, S.L. Ru, X. Tan, Q.L. Xie, C.J. Deng and Q.Y. Zeng (2018). Effects of Ca (2+) and tetracycline antibiotics monomer, compound solution and soil desorption liquid on seed germination and root shoot elongation of Chinese cabbage. Jiangsu Agri. Sci., 46(17):141-144.