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EFFECTS OF CIPROFLOXACIN-OXYTETRACYCLINE COMPOUNDS ON TOBACCO SEEDLING GROWTH IN HYDROPONICES

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ABSTRACT

Tobacco leaf quality matter its value but is easily influenced by environmental factors, such as antibiotics, which reside in soil by utilization of manure. Experiments were conducted by adding ciprofloxacin (CIP) and oxytetracycline (OTC) compounds to hydroponic tobacco seedlings with three varieties, Honghua Dajinyuan (HD), Yunyan87 (Y87) and K326, and their growth was observed. Results showed that concentration of chlorophyll a decreased by 83.33%, 88.36% and 87.59% in HD, Y87 and K326 at 60 mg L^{-1} CIP+60 mg L^{-1} OTC treatment. Aboveground fresh weight decreased from 1.24 g to 0.2 g in HD, from 0.53 g to 0.25 g in Y87 and from 0.83 g to 0.38 g in K326 at 60 mg L^{-1} CIP+60 mg L^{-1} OTC treatment. With increasing antibiotic concentrations, CIP was accumulated from 1.33 to 1.56 µg g^{-1} in Y87 and from 4.66 to 7.98 µg g^{-1} in K326, and OTC was accumulated from 0.54 to 0.84 µg g^{-1} in Y87 and from 3.30 to 5.53 µg g^{-1} in HD. With increasing antibiotic concentrations, chlorophyll and carotenoid contents, root activity, enzyme activity and fresh weight decreased significantly, and more antibiotics accumulated from 0.54-7.98 µg g^{-1} differently in all three varieties of tobacco. Comparing experiment results it suggests that chlorophyll and carotenoid contents and aboveground fresh weight decreased less in Y87, and it accumulated less antibiotics as well. Y87 could grow better and accumulate less antibiotic compared with the other two varieties.

Keywords: antibiotic contamination, ciprofloxacin, hydroponics, oxytetracycline, tobacco.

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INTRODUCTION

Tobacco is an important economic crop in China, and its economic benefits are based on the grade, which depends on the quality of tobacco leaves. The quality of tobacco leaves is affected by many factors, such as variety, cultivation technology and planting environment. Among the environmental factors that affect the growth and quality of tobacco leaves, organic fertilizer is one of the most crucial factors. In recent years, a large number of studies have shown that antibiotic-contaminated organic fertilizer utilization is a negative factor to tobacco cultivation (Ni, *et al.*, 2015; Pan *et al.*, 2009).

Antibiotics are chemical compounds produced by microorganisms or animals that can affect physiological and biochemical reactions in living organisms. In the 1940s, antibiotics were introduced into livestock and poultry production. In addition to acting as drugs, they were also used as subtherapeutic additives in animal feed to stimulate animal growth and increase production. At present, antibiotics play an important role in livestock and poultry breeding and are mostly used in the treatment of bacterial diseases and feed (Timothy *et al.*, 2007; Yakhkeshi *et al.*, 2012). Commonly used antibiotics are tetracyclines, quinolones, sulfas, macrolides and β-lactams (Wang *et al.*, 2020, 2021a).

Antibiotic pollution is caused by antibiotics that are not completely absorbed by humans or animals and are residual in the environment, causing damage to ecology (Roggo *et al.*, 2013; Kümmerer *et al.*, 2009). Antibiotic pollution can cause antibiotic resistance genes and add antibiotic treatment dosages when treating several kinds of disease, which adds difficulties in fighting diseases caused by viruses and other microorganisms and brings potential risks to humans (Kümmerer, 2004; Lapworth *et al.*, 2012). Antibiotic pollution can also damage other aspects of biology, such as plants (Wang *et al.*, 2021b, 2022).

Many research on antibiotic contamination damage has been studied, but little is known about the effects of antibiotics on the growth of plants. In recent years, tetracyclines have been found to be the highest among 14 kinds of antibiotics found in feces, and oxytetracycline (OTC) content is the highest in water bodies and feces. Quinolone is the second largest antibiotic used in medicine (Zhang *et al.*, 2005; Wang *et*

al., 2006a). The widespread utilization and incomplete metabolism of antibiotics in animal husbandry can be frequently detected in river water, sewage, drinking water and soil, and concentrations can reach mg L^{-1} in pharmaceutical wastewater. Quinolone residues in the aquatic environment can seriously threaten water safety and human health (Tang *et al.*, 2015; Zhang *et al.*, 2015; Bengtsson *et al.*, 2016). Antibiotics can be detected in milk, eggs, meat and plants, in addition to water and feces, indicating that antibiotics can indirectly affect animals and plants (Angela *et al.*, 2006; Richard *et al.*, 2010; Cristina *et al.*, 2007). Zhao analyzed the content of antibiotics in livestock and poultry feces from eight provinces and cities in China. Among them, ciprofloxacin (CIP) content in pig manure and cow manure was between 33.98 and 29.59 mg Kg^{-1}, and OTC content was between 59.06 and 59.59 mg kg^{-1}(Zhao *et al.*, 2009). In conclusion, CIP and OTC had high residual content in feces, pharmaceutical wastewater and water bodies. Therefore, CIP and OTC were selected as antibiotics to test.

In this study, four combined antibiotic concentrations of CIP and OTC treatments were introduced in the tobacco seedling stage and the effects of combined antibiotic pollution on the growth of tobacco were measured. Seedling quality indexes, root activity, stress-resistant enzyme activity, chlorophyll content, and dry matter accumulation and antibiotic accumulation were measured. The effects of different concentrations of mixed antibiotic pollution on the growth of tobacco plants were analyzed to provide references for growing high-quality tobacco. Antibiotic accumulation during the growth of tobacco and its influence on the growth and development of tobacco was further clarified to provide theoretical references and technical support for reducing the impact of residual antibiotics in organic fertilizer in the environment to the production of high-quality products of oriental tobacco.

MATERIALS AND METHODS

Hydroponic cultivation experiments were performed between November to December 2019 in a greenhouse at Yunnan Agricultural University, and measurements were conducted between January to March 2020 in the laboratory of the College of Tobacco Science.

Materials: The varieties of tobacco used were Honghua Dajinyuan (HD), Yunyan87 (Y87) and K326, which were produced by Yuxi Zhongyan Seed Limited Company. OTC hydrochloride (95%) and CIP hydrochloride (88.5%) were analytically pure.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. Seedling substrate was a mixture of peat, vermiculite and perlite in a certain proportion of 3:1:1. Experimental water was obtained from laboratory-purified deionized water at Yunnan Agriculture University.

Methods: The experiment was conducted during the seedling stage, when plants were treated with combined antibiotic pollution. As can be seen in Table 1, the experiment was set up with four antibiotic compound concentrations, which were mixed with concentrations of 15, 30, 45 and 60 mg/L (indicated as A1, A2, A3 and A4, respectively) which preliminary experiments have been done and suggested that from 0 to 60 mg/L is the suit concentrations to conduct. The size of seedling floating trays was 162 wells, with three trays for each treatment as three replicates. The floating seedling nutrient solution was formulated with Afodonine nutrient solution, and each plate of nutrient solution was maintained at 14 L. A0 was a control without antibiotic treatment.

Table 1. Concentrations of antibiotic treatments.

Treatment	Contents
A0	0 mg/L CIP + 0 mg/L OTC
A1	15 mg/L CIP + 15 mg/L OTC
A2	30 mg/L CIP + 30 mg/L OTC
A3	45 mg/L CIP + 45 mg/L OTC
A4	60 mg/L CIP + 60 mg/L OTC

Methods of determination for indexes and agronomic characters: Fresh weight, dry weight, maximum leaf length and width of aboveground and underground parts were measured. Determination methods were followed by Investigating and Measuring Methods of Agronomic Characters of Tobacco (YCT142-2010, 2010).

Determination of chlorophyll content: Leaves from tobacco seedlings were removed from the midrib and tip of plants after treatment. 0.5 g of leaves were cut into pieces, placed in a mortar with a small amount of quartz sand, ground and extracted with 96% ethanol, then measured with spectrophotometry. Chlorophyll a, chlorophyll b and carotenoids were measured at 470, 649 and 665 nm, respectively (Wang *et al.*, 2006b).

Measurement of root activity: Root activity was measured according to Zhang, and root activity was expressed as % active absorption area (Zhang *et al.*, 2005).

Determination of antistress enzyme activity: Leaves were collected and placed on ice for preparation in a mortar to determine catalase (CAT) activity by spectrophotometry at 240 nm (Gutteridge and Halliwell, 1990; Bergmeyer, 1983). The Guaiacol method was used for peroxidase (POD) activity (Xiao and Wang, 2005). Superoxide dismutase (SOD) activity was determined by

nitrogen blue tetrazole (NBT) (Wang, 2006). The content of malondialdehyde (MDA) was determined by thiobarbituric acid (TBA) colorimetry (Lin, 2004).

Determination of antibiotic content and accumulation: The Shimadzu 20AT high performance liquid chromatography (HPLC) system at Zhejiang University, the Zhida N2000 chromatographic working station and the Agilent ODSC18 column (5μm, 250 mm × 4.6 mm) were used to measure antibiotic content and accumulation in tobacco. When determinate OTC, acetonitrile:0.05M citric acid = 15:85 (v/v) was set as mobile phase and flow rate is 1.0 mL/min, with 40℃ column temperature and 280 nm UV detection wavelength. When determinate CIP, methanol:1% acetic acid = 20:80 (v/v) was set as mobile phase and flow rate is 1.0 mL/min, with 35℃ column temperature and 277 nm UV detection wavelength. The external standard method was used for quantitative determination in this experiment (Nian *et al.*, 2019).

For standard curve preparation, 1.0 mg of CIP and OTC standards were accurately weighed and dissolved in chromatographic ethyl alcohol for ultrasonic dissolution as the reserve solution. Chromatographic methanol was used to dilute the reserve solution into a series of 100.0, 50.0, 10.0, 5.0, 1.0 and 0.5 μg/mL standard solutions, and samples were injected for analysis. The peak area (Y) of CIP and OTC and their concentration (X) were taken to calculated the standard curve equation.

For precision tests, low, medium and high concentrations of standard samples, 0.5, 5.0 and 50.0 μg/mL, respectively, within the above standard curve, were prepared as quality control (QC) samples. Samples were injected and analyzed according to the above sample treatment method and were injected five times in the same day. The coefficient of variation of the peak area (RSD) of HPLC at three concentrations was calculated. Similarly, each concentration sample was injected five times at different times within one week, and the daytime precision of the method was calculated. In the replicate experiment, the same batch of young tobacco leaves was collected to prepare five samples as test solutions, and samples were injected according to the established method. The value and standard deviation of CIP and OTC peak areas from samples were calculated, and the repeatability of the detection method was calculated.

For stability tests, the same samples were collected with five preparations as test solutions at 1, 12 and 24 h, according to the sample analysis method. Samples were separately measured for CIP and OTC peak areas and the stability test samples within 24 h according to the peak area change of samples was calculated.

For recovery tests, 0.02 mL of CIP and 0.02 mL of OTC standard 100 μg/mL solutions were extracted and dried in a 45ºC water bath with nitrogen flow. 0.1 mL of the prepared tobacco leaf sample solution was added to the solution. After ultrasonic mixing, the standard sample containing CIP and OTC was prepared. According to the established method, average recovery rate and deviation of CIP and OTC were calculated by the ratio of their contents.

For sample determination, according to chromatographic conditions determined in this experiment, sample solutions were prepared according to the above method, and 20 μL of each sample was injected to determine the contents of CIP and OTC (%), with three replicates for each sample.

Data analysis was performed with SPSS 24.0 and Excel 2019 software.

SPSS software was used to compare the mean value and conduct homogeneity test of variance. One-way ANOVA Technique was used to analyze the significant difference of chlorophyll and carotenoid contents, root activity, stress-resistant enzymes, growth, antibiotic accumulation and were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Effects of antibiotics on chlorophyll and carotenoid contents in tobacco: Table 2 shows that chlorophyll a, chlorophyll b and carotenoids decreased in HD with increasing antibiotic concentrations. All three indexes decreased significantly at A4, and compared with A0, chlorophyll a, chlorophyll b and carotenoids decreased by 83.51%, 83.67% and 83.33%, respectively, at A4.

Table 2. Effects of compound antibiotics on chlorophyll contents (mg/g FW) in HD.

Treatment	chlorophyll a	chlorophyll b	carotenoids
A0	1.94 ± 0.15a	0.49 ± 0.04a	0.12 ± 0.01a
A1	1.20 ± 0.27b	0.30 ± 0.07b	0.08 ± 0.02b
A2	0.72 ± 0.09c	0.18 ± 0.03c	0.05 ± 0.01c
A3	0.43 ± 0.01d	0.11 ± 0.01d	0.03 ± 0.00d
A4	0.32 ± 0.03d	0.08 ± 0.01d	0.02 ± 0.00d

Note: different lowercase letters indicate significant differences between treatments ($P < 0.05$).

Table 3. Effects of compound antibiotics on chlorophyll contents (mg/g FW) in Y87.

Treatment	chlorophyll a	chlorophyll b	carotenoids
A0	1.46 ± 0.10a	0.36 ± 0.025a	0.09 ± 0.09a
A1	1.41 ± 0.06ab	0.35 ± 0.02b	0.09 ± 0.09ab
A2	1.32 ± 0.07b	0.33 ± 0.02b	0.08 ± 0.08b
A3	0.10 ± 0.05c	0.02 ± 0.02c	0.01 ± 0.01c
A4	0.17 ± 0.01c	0.04 ± 0.01c	0.01 ± 0.01c

Note: different lowercase letters indicate significant differences between treatments (P < 0.05).

Table 3 shows that chlorophyll a, chlorophyll b and carotenoids decreased in Y87 with increasing antibiotic concentrations. Compared with A0, chlorophyll a, chlorophyll b and carotenoids decreased by 88.36%, 88.89% and 88.89%, respectively, at A4.

Table 4 shows that chlorophyll a, chlorophyll b and carotenoids decreased in K326. Compared to A0, chlorophyll a, chlorophyll b and carotenoids decreased by 87.59%, 95.31% and 93.75%, respectively, at A4.

Figure 1 shows that chlorophyll a, chlorophyll b and carotenoids decreased in tobacco with increasing antibiotic concentrations. These pigments decreased less significantly in Y87 than in HD and K326, which indicated that Y87 received less damage to chlorophyll and carotenoids from antibiotics.

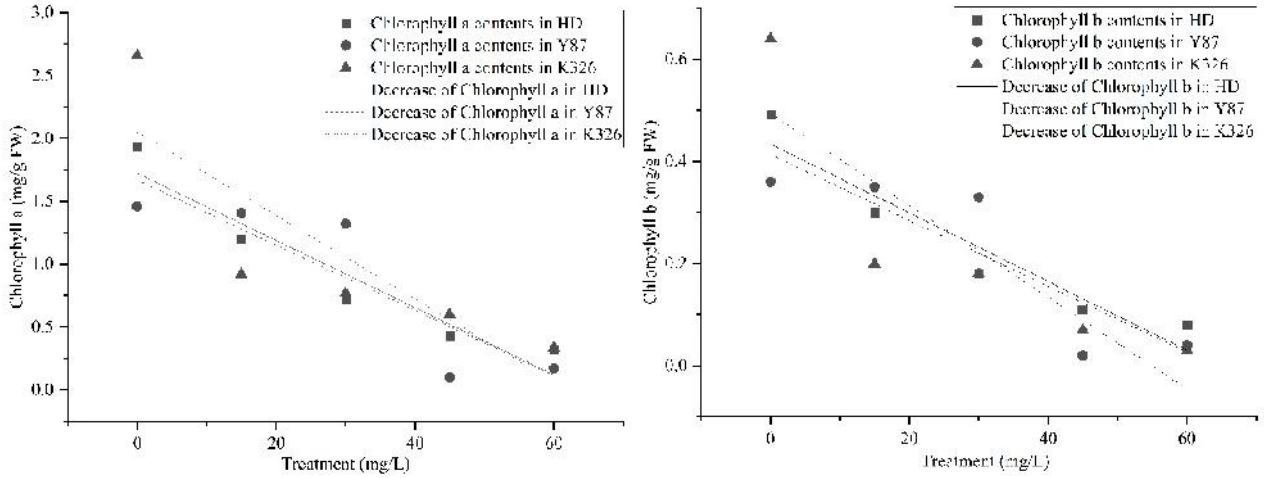
As can be seen from Table 5, with increasing antibiotic concentrations, root activity in Y87 and HD decreased. Root activity decreased by 81.82% in Y87 at A3, by 60% in K326 at A2 and by 66.67% in HD at A4. Meanwhile, compared to A0, the three varieties decreased by 72.73%, 0% and 66.67%, respectively, at A4. Based on the above analysis, the sensitivity of roots to antibiotics was different among the three varieties of tobacco seedlings. The inhibition degree of the three varieties was K326 > Y87 > HD, which was consistent with the sensitivity degree of chlorophyll to mixed antibiotics among the three varieties in Tables 2-4.

Figure 2 shows that root activity in tobacco decreased with increasing antibiotic concentrations. Root activity decreased less significantly in K326 than in HD and Y87, which indicated that K326 received less damage to root activity from antibiotics. This is opposite to chlorophyll and carotenoid trends in the three varieties.

Table 4. Effects of compound antibiotics on chlorophyll contents (mg/g FW) in K326.

Treatment	chlorophyll a	chlorophyll b	carotenoids
A0	2.66 ± 0.15a	0.64 ± 0.08a	0.16 ± 0.02a
A1	0.92 ± 0.06b	0.20 ± 0.01b	0.05 ± 0.00b
A2	0.77 ± 0.02c	0.18 ± 0.03b	0.04 ± 0.01b
A3	0.60 ± 0.04d	0.07 ± 0.03c	0.02 ± 0.01c
A4	0.33 ± 0.03e	0.03 ± 0.02c	0.01 ± 0.01c

Note: different lowercase letters indicate significant differences between treatments (P < 0.05).



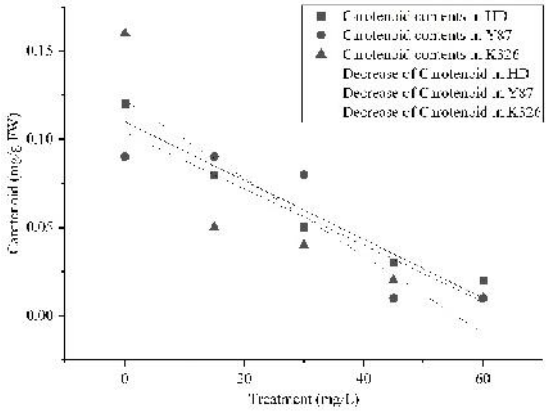


Figure 1. Decreased chlorophyll and carotenoid contents in three varieties of tobacco.

Effects of antibiotics on root activity

Table 5. Effects of compound antibiotics on root activity (m^2) in different tobacco seedlings.

Treatment	HD	Y87	K326
A0	0.12 ± 0.04a	0.11 ± 0.01a	0.05 ± 0.02a
A1	0.06 ± 0.01b	0.06 ± 0.01b	0.02 ± 0.02a
A2	0.05 ± 0.02b	0.05 ± 0.02b	0.03 ± 0.02a
A3	0.06 ± 0.02b	0.02 ± 0.01d	0.03 ± 0.02a
A4	0.04 ± 0.01b	0.03 ± 0.01c	0.05 ± 0.03a

Note: different lowercase letters indicate significant differences between treatments ($P < 0.05$).

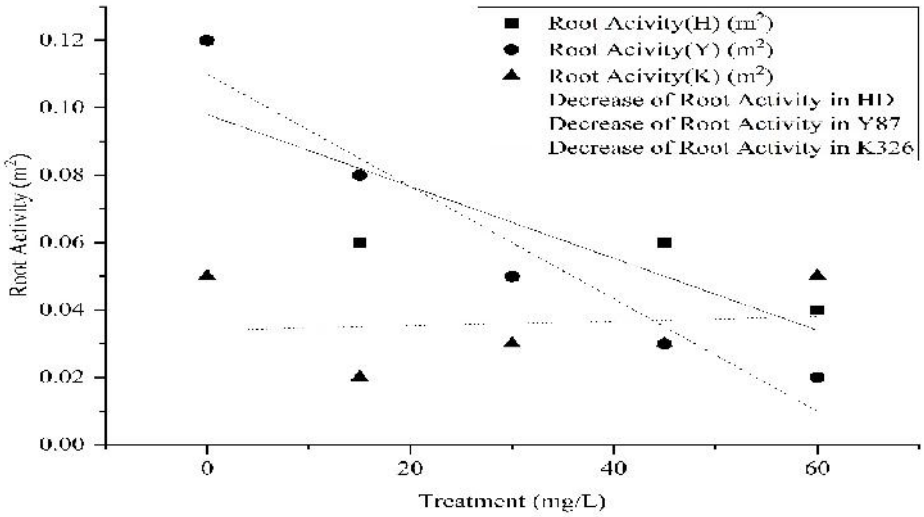


Figure 2. Decreased root activity in three varieties of tobacco.

Effects of combined antibiotics on stress-resistant enzymes in tobacco seedlings: Table 6 shows that, with increasing antibiotic concentrations, the antistress enzymes POD, SOD, CAT and MDA decreased in HD. Compared to A0, these enzymes decreased by 53.13%, 95.17%, 97.30% and 82.38%, respectively, at A4. The antistress enzymes were inhibited at different degrees under different treatments. SOD and CAT increased by 15.14% and 46.76%, respectively, at A1. It can be seen from above that SOD and CAT were more sensitive to antibiotics than MDA and POD in HD. SOD and CAT can clear free radicals at low concentrations. However, the accumulation of reactive oxygen species exceeded the scavenging range of antioxidant enzymes, and enzyme activity was inhibited.

Table 6. Effects of compound antibiotics on stress-resistant enzymes in HD tobacco seedlings.

Treatment	POD (μg^{-1} min^{-1})	SOD (μg^{-1} FW^{-1})	CAT (μmol min^{-1} g^{-1})	MDA (nmol g^{-1})
A0	1128.00 ± 8.00a	794.03 ± 6.15b	1005.55 ± 5.22b	5.22 ± 0.30a
A1	768.00 ± 2.00c	914.21 ± 14.06a	1475.71 ± 3.12a	3.12 ± 0.20b
A2	467.33 ± 17.01e	250.41 ± 2.56c	786.56 ± 1.11c	1.11 ± 0.10d
A3	831.00 ± 1.00b	174.13 ± 15.02d	789.73 ± 1.73c	1.73 ± 0.11c
A4	528.67 ± 37.11d	38.33 ± 2.96e	27.20 ± 0.92d	0.92 ± 0.03d

Note: different lowercase letters indicate significant differences between treatments ($P < 0.05$).

Table 7 shows that, with increasing antibiotic concentrations, the activities of POD, SOD, CAT and MDA decreased in Y87. There were different turning points and decreasing proportions in different stress-resistant enzymes under the mixed antibiotics treatments in the same variety. Compared to A0, POD significantly decreased by 79.47% at A2; SOD significantly decreased by 82.06% at A4; CAT significantly decreased by 91.33% at A1; and MDA significantly decreased by 75.70% at A2. According to the above data analysis, CAT had a better ability to resist antibiotic stress than other enzymes in Y87, followed by SOD, POD and MDA. Compared to A0, POD, SOD, CAT and MDA decreased by 76.18%, 82.06%, 81.36% and 68.54%, respectively, at A4. According to the comparison between A4 and A0, the activities of stress-resistant enzymes in tobacco seedlings exceeded the tolerance range of enzymes, and the ability to resist external stress was reduced.

Table 7. Effects of compound antibiotics on stress-resistant enzymes in Y87 tobacco seedlings.

Treatment	POD (μg^{-1} min^{-1})	SOD (μg^{-1} FW^{-1})	CAT (μmol min^{-1} g^{-1})	MDA (nmol g^{-1})
A0	1091.33 ± 3.06a	486.38 ± 6.39a	1461.08 ± 2.01a	3.91 ± 0.50a
A1	396.66 ± 7.02b	560.51 ± 9.51a	126.71 ± 7.83e	2.57 ± 1.08b
A2	245.33 ± 10.07d	171.15 ± 139.57bc	229.61 ± 4.36d	0.95 ± 0.82c
A3	224.00 ± 4.00e	272.37 ± 5.51b	902.66 ± 1.50b	1.59 ± 0.10bc
A4	260.00 ± 10.00c	87.27 ± 17.37c	268.41 ± 2.11c	1.23 ± 0.12c

Note: different lowercase letters indicate significant differences between treatments ($P < 0.05$).

Table 8 shows that POD, SOD and MDA in K326 had different sensitivities to antibiotics with increasing antibiotic concentrations. POD decreased by 73.95% at A4. SOD significantly decreased by 32.75% at A2. CAT decreased by 52.07% at A2, and MDA decreased by 79.32% at A4. Meanwhile, SOD and CAT showed an increasing trend at A1 and A3. In conclusion, the sensitivity of SOD and CAT was higher in K326 than that in the other two varieties. At the same time, the increase in SOD and CAT to different degrees indicated that SOD and CAT can improve the activities of stress-resistant enzymes to eliminate free radicals under low antibiotic stress. However, if the accumulation of reactive oxygen species exceeds the scavenging range of antioxidant enzymes, enzyme activity will be inhibited.

Table 8. Effects of compound antibiotics on stress-resistant enzymes in K326 tobacco seedlings.

Treatment	POD (μg^{-1} min^{-1})	SOD (μg^{-1} FW^{-1})	CAT (μmol min^{-1} g^{-1})	MDA (nmol g^{-1})
A0	1336.00 ± 4.00a	2283.90 ± 8.05c	1152.25 ± 29.98b	4.40 ± 1.20a
A1	1276.00 ± 6.00b	2334.97 ± 38.00b	1090.70 ± 0.40c	2.55 ± 1.42b
A2	888.00 ± 13.00c	1536.02 ± 7.12e	551.99 ± 1.54d	3.11 ± 0.20ab
A3	496.00 ± 7.00d	4005.32 ± 4.13a	1243.04 ± 61.39a	1.83 ± 0.30bc
A4	348.00 ± 44.14e	1980.50 ± 2.31c	1247.36 ± 14.70a	0.91 ± 0.10c

Note: different lowercase letters indicate significant differences between treatments ($P < 0.05$).

Tables 6,7 and 8 show that, under the same treatment conditions, different varieties of tobacco showed different activities of stress-resistant enzymes. The activities of stress-resistant enzymes were inhibited in Y87. SOD and CAT were highly sensitive to antibiotics in HD and K326, which could improve activity and remove harmful substances at low concentrations of antibiotics.

Enzyme measurements showed that antibiotics caused stress-resistant enzymes to increase at low concentrations but decrease at high concentrations in tobacco.

Influence on growth: As can be seen from Table 9, the fresh weight of underground and aboveground parts showed a decreasing trend in the three varieties. Compared to A0, the underground fresh weight in Y87, K326 and HD decreased by 52.83%, 41.00% and 84.00%, respectively, at A4. Compared to A0, the underground fresh weight in Y87, K326 and HD decreased by 65.90%, 48.20% and 72.14%, respectively, at A4. The aboveground and underground parts increased at low antibiotic concentrations in Y87 and K326. The aboveground fresh weight in Y87 increased by 11.96% at A1. The underground fresh weight in K326 increased by 4.82% at A2. The fresh weight in HD did not increase in the four treatments. These results indicated that the combined antibiotics inhibited the growth of tobacco. The inhibition degree was HD > Y87 > K326, which suggested that stress resistance was higher in K326 than in the other two varieties.

Table 9. Effects of compound antibiotics on fresh seedling weight (g) of different tobacco varieties.

Treatment	HD		Y87		K326	
	Aboveground	Underground	Aboveground	Underground	Aboveground	Underground
A0	1.24 ± 0.23a	10.66 ± 1.79a	0.53 ± 0.12a	5.60 ± 1.85a	0.83 ± 0.40a	6.37 ± 1.45a
A1	0.69 ± 0.18b	6.91 ± 1.13b	0.50 ± 0.10a	6.27 ± 3.00a	0.53 ± 0.32a	4.53 ± 0.55b
A2	1.01 ± 0.35a	8.09 ± 2.94ab	0.43 ± 0.06a	5.63 ± 1.27a	0.87 ± 0.55a	4.37 ± 0.90b
A3	0.61 ± 0.19b	7.36 ± 1.13b	0.53 ± 0.06a	4.93 ± 1.42ab	0.49 ± 0.17a	4.45 ± 0.99b
A4	0.20 ± 0.09c	2.97 ± 0.69c	0.25 ± 0.03b	1.91 ± 0.51b	0.38 ± 0.07a	3.30 ± 0.10b

Note: different lowercase letters indicate significant differences between treatments (P < 0.05).

Figure 3 shows that fresh weight of tobacco decreased with increasing antibiotic concentrations. The underground fresh weight decreased less significantly in K326 than in Y87 and HD, which indicated that K326 received less damage to underground fresh weight from antibiotics. This is similar to the root activity trends in the three varieties. Aboveground fresh weight decreased less significantly in Y87 than in K326 and HD, which indicated that Y87 received less damage to aboveground fresh weight from antibiotics. This is similar to the chlorophyll and carotenoid trends in the three varieties.

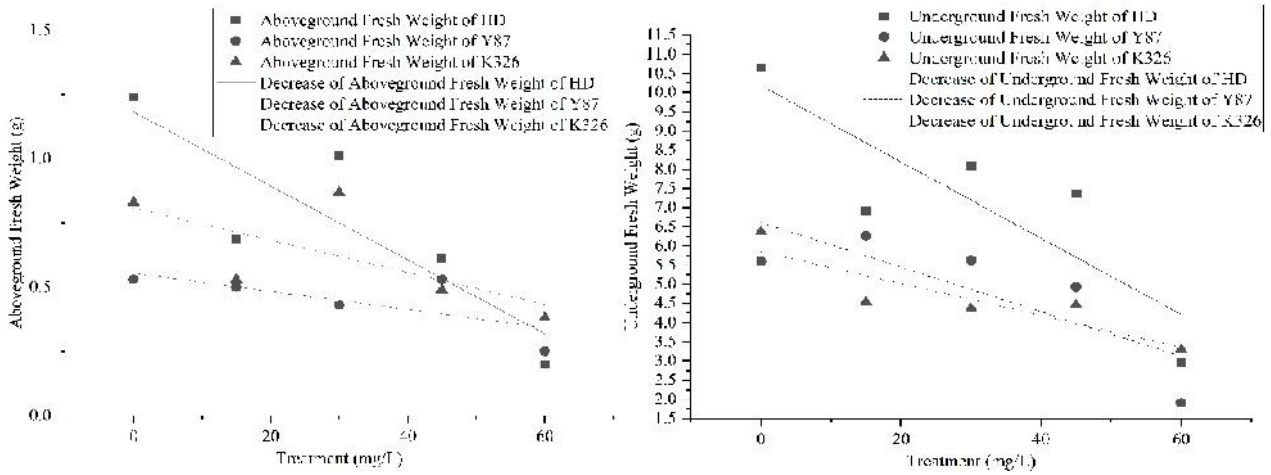


Figure 3. Decreased fresh weight in three varieties of tobacco.

Antibiotic accumulation

Table10. CIP accumulation (μg/g).

Treatment	HD	Y87	K326
A1	2.69 ± 0.01c	1.33 ± 0.13b	4.66 ± 0.00d
A2	2.71 ± 0.01c	1.35 ± 0.06b	5.85 ± 0.08c
A3	3.62 ± 0.37b	1.49 ± 0.03ab	6.17 ± 0.03b
A4	4.35 ± 0.02a	1.56 ± 0.02a	7.98 ± 0.02a

Note: different lowercase letters indicate significant differences between treatments (P < 0.05).

As can be seen from Table 10, CIP accumulation increased with increasing antibiotic concentrations in the three varieties. Compared to A1, the accumulation of CIP increased by 17.29%, 71.24% and 61.71% in Y87, K326 and HD, respectively, at A4. Accumulation in Y87 was relatively stable across concentrations. CIP significantly increased in K326 compared to HD and Y87. However, the physiological and biochemical changes in Y87 were similar to those in the other two varieties, based on the previous physiological and biochemical tests.

As can be seen from Table 11, OTC accumulation in seedlings increased with increasing antibiotic concentration in the three varieties. Compared to A1, OTC increased by 55.56%, 77.66% and 67.58% in Y87, K326 and HD, respectively, at A4. Compared to A1, OTC content increased at a higher rate in Y87 and K326 at A4, but the overall accumulation of OTC was not as high as that of CIP (Table 10).

Figure 4 shows that both CIP and OTC accumulation in Y87 were much lower than in K326 and HD, which indicated that Y87 accumulated less antibiotics compared to the other two varieties at the same antibiotic concentrations. This indicated that Y87 get less potential risk compared with the other two varieties in the same antibiotic-contaminated environments.

Table 11. OTC accumulation (µg/g).

Treatment	HD	Y87	K326
A1	3.30 ± 0.50b	0.54 ± 0.06a	0.94 ± 0.44a
A2	5.50 ± 0.08a	0.56 ± 0.25a	0.78 ± 0.56a
A3	5.49 ± 0.08a	0.54 ± 0.03a	0.89 ± 0.41a
A4	5.53 ± 0.10a	0.84 ± 0.03a	1.67 ± 0.03a

Note: different lowercase letters indicate significant differences between treatments ($P < 0.05$).

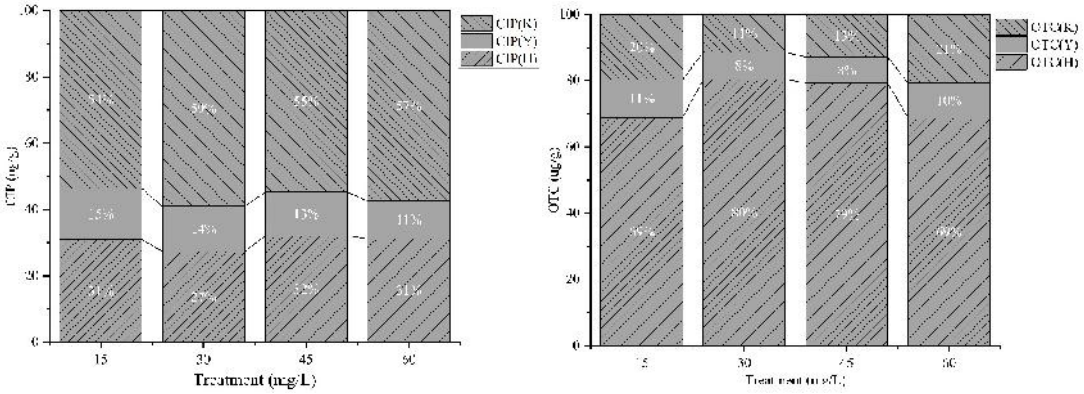


Figure 4. Accumulation of CIP and OTC in three varieties of tobacco.

These results showed that CIP and OTC can inhibit chlorophyll and carotenoid contents, and the inhibitory effect became more obvious with increased mixed antibiotic concentrations. These results are consistent with a previous study, in which OTC had a more significant inhibitory effect on chlorophyll a, chlorophyll b and carotenoids with increasing concentrations in pakchoi (Pan, 2017). We found that high antibiotic concentrations could inhibit the quantity of plastid pigments and bioaccumulation, which was consistent with the study by Zhang *et al.,* (2017) where tetracycline promoted growth of tobacco at low concentrations and inhibited photosynthesis at high concentrations. After treatment with CIP and OTC, the root activity of tobacco decreased. Low concentrations of quinolones and tetracycline had toxic effects on the roots of lettuce, tomato, carrot and cucumber and also affected the germination of herbs and gramineous plants (Pan *et al.*, 2016; Vanessa *et al.*, 2017). When talking about these effects and inhibition caused by CIP and OTC and their transport mechanism at molecular and cellular levels, some researcher consider that some antibiotics could be dissolved by water and chelate with metal positive irons and be absorbed. What consist this theory with this experiment is that CIP and OTC may chelate with some metal positive irons including Mg^{2+}, which is important to chlorophyll synthesis. And this chelation caused lower chlorophyll contents, weaken the photosynthesis and affect plant growth furthermore. But more research should be study to make it clear.

Comparing this decrease in three varieties, we found that with increasing antibiotic concentrations, the activities of stress-resistant enzymes decreased, which is consistent with most studies that show free radicals in the body will gradually increase when plants are subjected to environmental stress. SOD, POD and CAT can eliminate

anionic free radicals, hydrogen peroxide and hydroxyl free radicals to reduce damage to the cell membrane from reactive oxygen species and reduce membrane peroxidation, but this activity will decrease if it is beyond the scavenging range (Fazeli, 2007; Selote, 2006). This is in accordance with some previous research results that different varieties tobacco have different ability to resist the bad environment (Li *et al.*, 2020).

Comparing the experimental statistics from the three varieties, we found that the influence of antibiotics was less significant on leaves and aboveground fresh weight but more significant on root activity and underground fresh weight in Y87, and there was less accumulation of these two antibiotics, which indicates that Y87 could grow better than the other two varieties in compound antibiotic contamination.

Conclusions: Hydroponic growth was inhibited with CIP and OTC compound contamination in all three varieties of tobacco seedlings and was inhibited more significantly with increasing antibiotic concentrations.

Y87 could grow better and accumulate less antibiotics in antibiotic-contaminated environments compared to the two other varieties, which suggests that selection of Y87 for culture could improve yield and quality in antibiotic-contaminated tobacco plantings.

Authors' Contributions: Fuzhao Nian, Leifeng Zhao and Fei Wang planned and designed the experiment. Qiang Xie, Xiao Lei, Jinchao Zhao, Gai Zhang, Mingjin Zhang, Jiangwen Nian and Yaojun Wang took part in the measurements and sampling. Fuzhao Nian, Jiangwen Nian and Yaojun Wang processed the growth data and conducted the statistical analyses. Yaojun Wang and Fuzhao Nian wrote, revised and submitted the manuscript. Percentage contributions are Y.J. Wang: 30%, J.W. Nian: 5%, F. Wang: 5%, Q. Xie: 5%, X. Lei: 5%, J.C. Zhao: 5%, G. Zhang: 5%, M.J. Zhang: 5%, L.F. Zhao: 5% and F.Z. Nian: 30%. All authors read the manuscript and approved the final version.

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