

Study on quinolone accumulation and growth inhibition induced by quinolone levels of hydroponics

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Abstract. Residual antibiotics can enter soil and water bodies through organic fertilizers with food safety risk via plants absorption, while how do plant growth and quinolone accumulation respond to residual antibiotics levels in soil or water is not clear. Hydroponic experiment in greenhouse was conducted with floating seedlings of tobacco as model plant to investigate the responses of quinolone antibiotics accumulation and plant growth to different levels of ciprofloxacin (CIP) and norfloxacin (NOR). Results showed that CIP and NOR inhibited the growth of tobacco seedlings. The plant height, stem circumference, maximum leaf width, and maximum leaf area of tobacco seedlings were significantly decreased. So as to the plant biomass of leaves, stems, and roots. Accumulation of CIP in the tobacco seedlings in the T3 was 1.1 times that of the tobacco seedlings in the T1, NOR in the T4 was 1.2 times that of the tobacco seedlings in the T1. And the higher the concentration, the more significant the inhibitory effect. Both antibiotics can be absorbed and accumulated by tobacco seedlings. Additionally, the inhibitory effect of CIP was greater than that of NOR.

1 Introduction

Antibiotics are widely used in medicine for its inhibition to microorganisms or virus, which could prevent and treat animal diseases in veterinary industry and control infection caused by bacterium and fungus in agriculture. Antibiotics can't be absorbed entirely almost all application in any field and a large portion will reside in water body, soil and organisms through ecology cycle. With increasing importance of environmental protection worldwide more and more research on antibiotic pollution detected quinolones in streams of Hubei Province of China, Fujian Province of China and Watershed in the valley of Grat, Switzerland [1]. Quinolones are widely used in humans and animals, such as ciprofloxacin and norfloxacin are two main products [2,3]. Residual antibiotics can enter soil and water bodies through organic fertilizers derived from human and animal excreta, with food safety risk via plants absorption. It was reported that there were 0.465-0.715 ug/kg of norfloxacin residuals in soil-vegetable systems [4], residuals of four quinolone antibiotics were detected in more than 80% vegetables grown in Dongguan, China, which highest content was more than 100 ug/kg (dry weight) [5]. Researchers conducted experiment by adding antibiotics in different combination which including CIP, NOR and tetracycline (TET) to Chinese cabbage roots to study antibiotics toxicity, and results

showed that no matter single antibiotic or antibiotics combination could inhibited the root growth of Chinese cabbage and combinations caused larger toxicity [6]. Finding a kind of plant to remediate antibiotic pollution is necessary. Tobacco, a kind of finical plant, is widely cultured in southeast area of China. There are research found that tobacco could absorb and accumulate pollutions in soil like heavy metals, which could be used to soil pollution remediation [7,8,9]. Therefore, tobacco was used as model crop in this research to study the responses of quinolone antibiotics accumulation and plant growth to different quinolone levels of hydroponics with tobacco seedlings, aiming at finding novel antibiotic utilization on tobacco culture and environmental remediation of antibiotic pollution. Section 2 outlines the materials and methods related to the research. Section 3 surveys our results and discussions. In Section 4 the conclusion of the research is presented.

2 Materials and methods

2.1 Selected tobacco variety and antibiotics

The experiment was carried out with a variety of flue-cured tobacco and two antibiotics. Yunyan97, a new flue-cured tobacco variety, was produced by hybrid

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breeding of Yunyan85 and CV87 from Yunnan institute of tobacco agricultural science. In recent years, yunyan97 is one of the flue-cured tobacco varieties widely cultivated in Yunnan province. Additionally, ciprofloxacin (CIP) and norfloxacin (NOR) are two commonly used quinolones antibiotics. According to local planting practices and common standard of quinolone antibiotics, the flue-cured tobacco variety used was Yunyan97. CIP and NOR were purchased from BoMei biotechnology co., LTD., Hefei, China, with purities $\geq 99\%$ were used.

2.2 Experimental design

Flue-cured tobacco were treated with 25 (T1), 50 (T2), 75 (T3), and 100 (T4) mg antibiotic/L for CIP and NOR, respectively, by adding antibiotics to distilled water. In addition, we applied one control treatment 0 (CK) (distilled water).

The experiments were performed in the solar greenhouse of Yunnan Agricultural University. Floating seedlings were used, distilled water was irregularly added, and the volume of the nutrient solution was maintained at 14 L. Twenty days after sowing, after the tobacco seedlings had grown 4 true leaves, the antibiotic treatment was started. Samples were taken at 30 d, 40 d, and 50 d after antibiotic treatment (i.e., 50 d, 60 d, and 70 d after sowing). Fifteen tobacco seedlings were randomly collected for each treatment, and every five plants were repeated once. The roots, stems, and leaves were separated and weighed, and these values were taken as the fresh weights.

2.3 Measurement indicators and methods

The method used by Nian et al. [10] was used to determine the antibiotic contents in the seedlings and to calculate the cumulative amount of antibiotics per plant. The method outlined in the "YCT142-2010 investigation and measurement methods of tobacco agronomic traits" [11] was used to determine the agronomic traits of the tobacco seedlings 50 days after antibiotic treatment. And then, data analysis was performed with SPSS 2.0 and Microsoft Excel 2013 software.

3 Results and Discussions

3.1 Responses of tobacco seedlings growth to different levels of ciprofloxacin and norfloxacin

Plant growth indexes of height, stem circumference, and maximum leaf length, width, area, were all decreased continuously with increasing antibiotic concentration in nutrient solution (table 1). Particularly, the plant height, stem circumference, maximum leaf width, and maximum leaf area of tobacco seedlings were significantly decreased 16.9%, 7.5%, 10.4%, and 13.1% by 100mg/L CIP, and 11.3%, 10.1%, 12.6%, and 16.7% by 100mg/L NOR orderly. It indicated that the plant growth

especially plant height and leaf area of tobacco seedlings were inhibited by increased antibiotic levels.

Plant biomass of leaves, stems, and roots were all decreased continuously with increasing antibiotic concentration in nutrient solution (table 2). Particularly, the plant biomass of leaves, stems, and roots of tobacco seedlings were significantly decreased 25.6%, 12.7%, 28.0% by 100mg/L CIP, and 11.6%, 10.3%, 9.2% by 100mg/L NOR orderly. It suggested that the plant biomass especially the leaves biomass of tobacco seedlings were declined by increased antibiotic levels, with higher rate decreased by CIP than NOR.

Table 1. Growth traits of tobacco seedlings after 50 days treated with CIP and NOR levels.

Treatment	mg/L	0	25	50	75	100
Plant height (cm)	CIP	12.67a	12.17ab	11.56bc	11.33bc	10.53c
	NOR	13.30a	12.40b	12.10b	11.80b	11.80b
Stem circumference (cm)	CIP	2.13a	2.07ab	2.04ab	2.03ab	1.97b
	NOR	1.98a	1.88ab	1.86ab	1.80b	1.78b
Maximum leaf length (cm)	CIP	12.10a	12.02a	11.97a	11.83a	11.73a
	NOR	13.10a	12.90a	12.60a	12.40a	12.30a
Maximum leaf width (cm)	CIP	8.00a	7.83ab	7.67bc	7.54bc	7.17c
	NOR	8.70a	8.60a	8.60a	7.90b	7.60b
Maximum leaf area (cm ²)	CIP	61.42a	59.71b	58.25c	56.60d	53.36e
	NOR	71.21a	67.95b	67.66c	65.66d	59.31e

Note: different letters in the same column of data represent significant differences ($P < 0.05$).

Table 2. Plant biomass (FW, g/plant) of tobacco seedlings leaves, stems, and roots after 50 days treated with different CIP and NOR levels

Treatment	mg/L	0	25	50	75	100
CIP	Leaves	6.53a	6.27a	5.94b	5.72b	5.60c
	stems	2.75a	2.66a	2.49b	2.47b	2.40b
	roots	0.72a	0.68b	0.68b	0.63c	0.59d
NOR	Leaves	7.38a	6.92b	6.94b	6.68b	6.52c
	stems	2.91a	2.84a	2.69b	2.62b	2.61b
	roots	0.87a	0.83a	0.78b	0.83a	0.79b

Note: different letters in the same column of data represent significant differences ($P < 0.05$).

3.2 Responses of ciprofloxacin and norfloxacin concentrations in tobacco seedlings to different levels of ciprofloxacin and norfloxacin

Contents of CIP and NOR in tobacco seedlings leaves all increased continuously with increasing antibiotic

concentration in nutrient solution and time (table 3). At 30, 40, and 50 days Contents of CIP increased in the leaves of tobacco seedlings 85%, 99.1%, 115.9% by 100mg/L CIP, the CIP contents of the T1, T2, T3, and T4 groups of tobacco seedlings at 50 days of treatment increased by 42%, 48%, 51%, and 36%. Contents of NOR increased in the leaves of tobacco seedlings 84%, 95.7%, 115.6% by 100mg/L NOR, the NOR contents of the T1, T2, T3, and T4 groups of tobacco seedlings at 50 days of treatment increased by 39%, 42%, 36%, and 38%. It indicated that the quinolone antibiotics treated with different concentrations did not disappear in tobacco seedlings and the content increased with time.

Table 3. CIP and NOR contents in tobacco seedlings (ug/g FW) treated with different CIP and NOR levels

Antibiotic	Treatment days	0	25	50	75	100
CIP	30	0	72.4c	77.2c	80.8c	85.0c
	40	0	96.7b	106.3b	97.0b	99.1b
	50	0	102.6a	113.9a	121.7a	115.9a
NOR	30	0	64.3c	66.2c	77.4c	84.0c
	40	0	72.1b	76.0b	89.1b	95.7b
	50	0	89.3a	93.8a	104.9a	115.6a

Note: different letters in the same column of data represent significant differences ($P < 0.05$).

3.3 Responses of ciprofloxacin and norfloxacin accumulation in tobacco seedlings to different levels of ciprofloxacin and norfloxacin

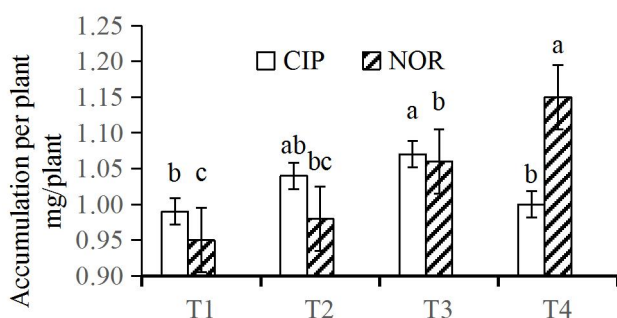


Figure 1. Antibiotic accumulation of tobacco seedlings after 50 days treated with CIP and NOR levels.

After 50 days, accumulation of CIP in tobacco seedlings reached the maximum in the T3 treatment group and accumulation of NOR in tobacco seedlings leaves increased continuously with increasing antibiotic concentration in nutrient solution (figure 1). Accumulation of CIP in the tobacco seedlings in the T3 was 1.1 times that of the tobacco seedlings in the T1, accumulation of NOR in the tobacco seedlings in the T4 was 1.2 times that of the tobacco seedlings in the T1. When the CIP concentration in the nutrient solution

reached a certain value, the cumulative amount of CIP in the tobacco seedlings reached a maximum.

4 Conclusion

With the increase of quinolone antibiotic concentration, the growth of tobacco seedlings was inhibited with the increase of treatment concentration. This is consistent with the results of a previous study, which found that the growth of corn seedlings was inhibited when the concentration of norfloxacin exceeded 1 mL/L. [12] It was also found that the maximum leaf area of the tobacco seedlings was significantly inhibited, which is consistent with the results of a study by Qin et al. on the effects of ciprofloxacin on the agronomic traits of corn. [13] From the above we know that antibiotics can not only be absorbed and accumulated in plants, but also enter the human body through food, It brings potential risks to human health and ecological environment. With the increase of the concentration of two quinolones, the agronomic characters of tobacco seedlings became worse, plant height and biomass decreased, but the content and accumulation of antibiotics increased. So the hypothesis, quinolone antibiotics may affect the root activity of tobacco seedlings or the enzyme activity and chlorophyll in the body, so they have an inhibitory effect on the growth of tobacco seedlings, the future research direction can be extend from the biochemical direction of tobacco seedlings.

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