

Tylosin in Hydroponic Systems: Assessing Growth and Antibiotic Residue in Lettuce Cultivation

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Abstract. Antibiotic persistence in the environment, including water sources, is a significant concern because of the increasing prevalence of antibiotic-resistant bacteria. Tylosin is a common macrolide antibiotic used as a growth promoter in cattle, with 71% of feedlots administering it. Antibiotics such as tylosin can persist as residual contaminants in surface water, groundwater, and wastewater. This poses a risk when these contaminated waters are used for irrigation, leading to uptake of antibiotics by plants and potentially contributing to antibiotic resistance in both humans and other organisms. To determine antibiotic uptake and its effect on crops, we conducted two experiments to test the same two concentrations of tylosin (5 and 10 mg·L⁻¹) in a nutrient film technique horizontal (Expt. 1) and vertical (Expt. 2) hydroponic system by using lettuce (*Lactuca sativa*) as the model crop. A reverse-osmosis water control was used as the third treatment in each experiment. In each experiment, we measured aerial weight, head diameter, plant height, root weight, and root length after 4 weeks of exposure to the experimental treatments. Tylosin treatments reduced root weight and length significantly, by 42% and 33%, respectively, in the horizontal system. The 10 mg·L⁻¹ tylosin treatment in the vertical system increased significantly the head diameters by 13% and the root length by 15%, compared with the other treatments. Tylosin concentrations in lettuce leaf tissue were 19 times greater than the water-only control in the horizontal experiment only. Multivariate correlation analysis revealed negative correlations between tylosin concentration and all growth parameters. These findings highlight the negative impact of tylosin bioaccumulation on hydroponically grown lettuce, raising important considerations for using recycled or alternative water sources in hydroponic agriculture, particularly in terms of food safety and crop productivity.

Climate change is increasing water scarcity worldwide (Intergovernmental Panel on Climate Change 2023). This challenge is compounded by significant population growth during the past 25 years, with the global population increasing by 33%, adding nearly 2 billion people, and the United States growing by nearly 23% (Ritchie et al. 2023). In addition, freshwater distribution is not uniform across the globe, further exacerbating water scarcity in agriculture

(du Plessis 2017). One alternative to address this problem is to use alternative water sources derived from treated wastewater facilities in municipal or agricultural sources. This practice can expose the environment, humans, and plants to potential pathogens (Mishra et al. 2023), as well as contaminants of emerging concern (CECs) (Kolpin et al. 2002; Petrie et al. 2015). Although wastewater treatment plants have made technological advancements that enhance the removal efficiency of pollutants from influent water (Loganathan et al. 2023), complete removal of these CECs has not yet been achieved (Golovko et al. 2021; Zhang et al. 2021). As a result, such contaminants can be present in untreated and treated effluents. Furthermore, agricultural sources from animal feeding operations and/or waste lagoons typically only undergo precipitation or flocculation to separate solid and liquid wastes

(Pruden 2009). They often rely on the temporal and environmental breakdown of compounds found in animal waste, which could result in greater concentrations of compounds if this waste or water is used in agricultural production (Bradford et al. 2008).

Various chemical groups can be found within these CECs, including pesticides, industrial chemicals, personal care products, microplastics, and pharmaceuticals (Nwokiediegwu et al. 2024). Antibiotics are widely used in veterinary and human medicine, as well as in aquaculture and agriculture (Kovalakova et al. 2020). Frequently detected antibiotics in environmental samples include beta-lactams, macrolides, fluoroquinolones, tetracyclines, sulfonamides, and diaminopyridines (Yang et al. 2021). These antibiotics can be found in concentrations ranging from nanograms per liter to micrograms per liter (Yang et al. 2021), with greater concentrations observed in concentrated animal feed operation lagoons (Pruden 2009). Tylosin is a veterinary antibiotic in the macrolide group (Blondeau 2022). This group also includes human-use macrolides such as azithromycin, clarithromycin, and erythromycin (Patel and Hashmi 2023). Tylosin is often used in food animals as a growth promoter, particularly in cattle and pigs (Pyörälä et al. 2014). In the United States, a 2011 report indicated that 71% of cattle in feedlots were administered this antibiotic (US Department of Agriculture 2013). Environmental samples have shown that the concentration of tylosin can range from 0.001 to 72 µg·L⁻¹ (García-Sánchez et al. 2013; Watkinson et al. 2009; Yang et al. 2006), although higher concentrations in the microgram-per-liter range have also been reported (El Gemayel 2018). The uptake and accumulation of antibiotics is possible and has been recorded in several studies, with the greatest accumulation most often found in the roots, with lower translocation to fruit (Geng et al. 2022). In addition, human health risks associated with bioaccumulated antibiotics have been estimated to be moderate, meaning the accumulation of antibiotics could pose a risk (Mohy-u-Din et al. 2023). There is also a concern that the uptake of trace amounts of antibiotics by humans could reduce the effectiveness of antibiotics over time and lead to antibiotic resistance (Williams-Nguyen et al. 2016). Moreover, the metabolites formed from the parent compounds during metabolism could be a major risk for humans, but research has been limited on this topic (Geng et al. 2022).

Research has been conducted on the effects of contaminants on plant growth (Aristilde et al. 2010; Azanu et al. 2016; Christou et al. 2019; Piotrowicz-Cieślak et al. 2010; Wen et al. 2012; Xie et al. 2011). For example, spinach plants exposed to ciprofloxacin showed reduced root length and impaired photosynthesis (Aristilde et al. 2010). When tomato plants were treated with a mixture of sulfamethoxazole, trimethoprim, and diclofenac, there were noticeable changes in the soluble solids and sugar content of their fruit (Christou et al. 2019). Greater concentrations of sulfamethazine

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resulted in increased electroconductivity and dry mass, indicating potential harm to legume seedlings (Piotrowicz-Cieslak et al. 2010). In addition, maize plants experienced negative effects on both shoot and root growth when exposed to high concentrations of chlortetracycline (Wen et al. 2012). Other studies using concentrations in the milligram-per-liter range have shown detrimental effects on plant growth as a result of amoxicillin and tetracycline (Azanu et al. 2016). Interestingly, some research on tetracycline indicated that it could stimulate the growth of wheat seedlings (Xie et al. 2011). Overall, research suggests that although low concentrations of antibiotics can enhance plant growth, high concentrations may negatively affect plant development, and this effect can vary by species (Yang et al. 2021).

Lettuce production worldwide reached 28 million t in 2023, with China leading as the top producer, valued at \$16.2 billion (Food and Agriculture Organization of the United Nations 2023). The United States followed as the second-largest producer, contributing 2.2 million t with a value of \$1.5 billion (US Department of Agriculture–National Agricultural Statistics Service 2023). Hydroponics has become a widely used production system for growing lettuce in the leafy vegetable industry as a result of its higher water efficiency and shorter periods from sowing to harvest (Sharma et al. 2018). Among the various hydroponic systems, the nutrient film technique (NFT) allows for closed circulation, enabling the nutrient solution to be reused multiple times. Therefore, as a result of the nature of closed NFT systems, one potential downside is the risk of continuous exposure to contaminants from alternative water sources in the nutrient solution, which could lead to bioaccumulation effects.

When using alternative water sources for food production, it is crucial to evaluate their chemical composition to ensure safety. Although extensive research has been conducted on various antibiotics, limited information is available about specific substances, such as tylosin. This is of particular concern because of its pervasive use in the veterinary industry, and the potential for crop exposure if animal wastewater or water sources adjacent to animal production facilities are used for irrigation. This gap in data makes it challenging to understand tylosin's effects on plants, especially those grown in closed recirculating systems such as hydroponics. Therefore, in our study, we assessed the growth of lettuce plants when tylosin is added to the water reservoir in NFT-based horizontal and vertical hydroponics systems. In addition, we determined the concentration of tylosin present in lettuce leaves and the nutrient solution.

Material and Methods

Experimental design. Two experimental systems were used to test the study question. The vertical system was used during Fall 2022, from November to December (temperature minimum, 18.9 °C; maximum, 31 °C; average, 24.4 °C) in a three-dimensional-printed vertical NFT-based hydroponic system. Each vertical tower consisted of four

levels, with each level containing three plant holders. Thus, 12 plants in a single tower were assigned one of the three tylosin treatments, bringing the total number of plants for the first experiment to 36 (Fig. 1A). The horizontal experiment was conducted during Spring 2023, from April to May (temperature minimum, 20.6 °C; maximum, 34.2 °C; average, 26 °C) in a horizontal NFT-based hydroponic system. Aluminum frame towers with two levels for each tower were used, and each level contained four channels. Each horizontal channel could hold six plants, resulting in 24 plants per tylosin treatment fed from the same nutrient reservoir, and 72 total plants in the experiment (Fig. 1B).

Both experiments included two treatments of tylosin at concentrations of 5 and 10 mg·L⁻¹, with reverse-osmosis water as the control. Lettuce (*Lactuca sativa*) seeds were germinated in rockwool, watered every 2 to 3 d to ensure proper moisture, and fertilized once with 50 ppm N. When the second true leaf was completely developed, they were transferred to the hydroponic system. The nutrient reservoir was filled with 18 L of water during the experiments. For the vertical experiment, a 110 ppm N solution was used throughout the entire experiment. Water consumption was monitored weekly, and water, nutrients, and tylosin were replenished as necessary to the reservoir. Therefore, a bioaccumulation effect was obtained. For the horizontal experiment, an initial fertilizer concentration of 100 ppm N was used, with an increase to 150 ppm N after 2 weeks. A single water replenishment was conducted 7 d after measuring water consumption. During weeks 3 and 4, water changes were conducted, increasing N levels to 200 and 225 ppm, respectively. Supplemental lighting was provided for the duration of the experiments, with a minimum-target daily light integral of 12 mol·m⁻²·d⁻¹. During both experiments, water samples were taken

for tylosin concentration analysis at every water adjustment or change. After collection at the experiment site, the samples were frozen and stored at -80 °C until analysis. After 35 d, both experiments were harvested, and aerial weight, head diameter, aerial height, root weight, and root length were measured. Plant tissue samples were flash-frozen in liquid N and stored at -80 °C for later analysis of tylosin concentration.

Tylosin analysis. For tylosin concentration analysis, frozen tissue was freeze-dried using a vacuum, ground to a fine powder with a pestle and mortar, and 250 mg of the tissue was weighed and transferred into 5 mL centrifuge tubes for extraction purposes. Weighed tissue samples were spiked with 1 mL tylosin solution at a concentration of 10 µg·mL⁻¹, using high-performance liquid chromatography (HPLC)-grade methanol as the solvent. Spiked tissues were combined with a 1:1 ratio of phosphate buffer (8.35 g K₂HPO₄ + 0.25 g KH₂PO₄ dissolved in 500 mL with diH₂O) and HPLC-grade methanol (1.25 mL buffer and 1.25 mL methanol; 2.5 mL in total). Tubes were then sonicated for 5 min and shaken for 30 min in an orbital shaker. The supernatant was then filtered through a 25 mm syringe filter disk with a 0.45 µm nylon membrane filter, and 1 mL was transferred to a vial for HPLC analysis. For our water samples, the cleanup protocol involved using 10 mL water, which was filtered through a 25 mm syringe filter disk with a 0.45 µm nylon membrane. A volume of 1.5 mL was then transferred to a vial for analysis. HPLC analysis for plant tissue and water samples was performed at the US Arid Land Agricultural Research Center in Maricopa, AZ, USA, using a Thermo Orbitrap Exploris™ 240 Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical analysis. Data from each experiment were analyzed using analysis of variance to assess the effects of tylosin concentration in the nutrient solution on plant performance

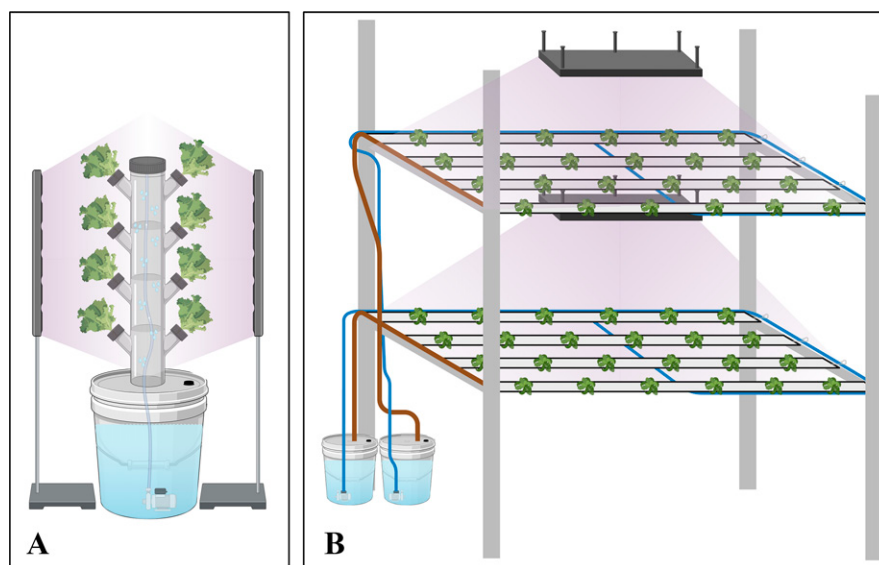


Fig. 1. Visual representation of the vertical (A) and horizontal (B) nutrient film technique system used during the experiments. Image created using BioRender. Note that in panel A, only two of three plants at each level are shown in the illustration.

metrics, including aerial biomass, root biomass, aerial height, maximum root length, head diameter, and tylosin accumulation in leaf tissue. To account for potential correlations among response variables, residual maximum likelihood methods were used to examine multivariate relationships when appropriate. All analyses were performed using JMP Pro v. 18 (SAS Institute Inc, Cary, NC, USA), and a significance level of $\alpha = 0.05$ was used to determine statistical differences.

Results

The impact of tylosin antibiotics on lettuce plants grown in a hydroponic system was investigated through two experiments. During the harvest of both experiments, we measured various biomass variables, including aerial weight, root weight, head diameter, plant height, and maximum root length. For the vertical NFT, aerial measurements were not significantly different between tylosin treatment concentrations; however, the control-treated plants were numerically larger, wider, and taller than the treated plants. At the root level, the weight and length were affected significantly and negatively by the application of tylosin in the nutrient reservoir, as shown in Table 1 ($P < 0.05$). More specifically, roots were largest in the control treatments. In the horizontal experiment, the biomass weights were not statistically different among treatments, but the 5 mg·L⁻¹ tylosin treatment showed the greatest weights ($P > 0.05$). For head diameter and maximum root length, plants exposed to the highest tylosin concentration (10 mg·L⁻¹) were significantly wider and had longer roots ($P < 0.05$ and $P < 0.001$, respectively) (Table 1).

It is important to note that at the time of harvest, the concentration of tylosin in the lettuce leaf tissue was significantly greater in both treatments than in the control group ($P < 0.05$) for the vertical NFT. However, no significant difference was observed between the 5 and 10 mg·L⁻¹ tylosin treatments (Table 2). Interestingly, for the horizontal NFT, the tylosin concentration in lettuce leaves was not significantly greater when tylosin was applied to the nutrient reservoir throughout the experiment ($P < 0.05$). Still, tylosin increased significantly with an increase in tylosin treatments ($P < 0.1$) (Table 2).

Water samples were collected throughout the experiment in both experiments to identify the tylosin concentrations in the nutrient solution for each treatment. Figure 2A illustrates the concentrations at three time stamps during each experiment. Although three water adjustments were made during the vertical experiment, a constant decrease in tylosin concentration is evident. For the horizontal experiment, a decrease in tylosin was seen closer to the harvest day. Moreover, the 10 mg·L⁻¹ treatment did not change much after 3 weeks (Fig. 2B). In addition, it is worth noting that, as expected, no tylosin was detected in any of the control treatments (Fig. 2).

A multivariate correlation analysis was conducted to explore the relationships among biomass measurements collected at harvest

Table 1. Effect of antibiotic tylosin on lettuce growth in two nutrient film technique systems.

Treatment	Aerial wt (g) ⁱ	Root wt (g) ⁱⁱ	Head diam (cm) ⁱⁱⁱ	Plant ht (cm) ^{iv}	Max root length (cm) ^v
Vertical experiment					
Control	26.05	6.17 a ^{vi}	20.64	4.28	19.75 a
5 mg·L ⁻¹	24.08	3.57 b	17.73	3.74	14.12 b
10 mg·L ⁻¹	14.02	4.99 ab	17.33	3.66	13.32 b
<i>P</i> value	0.245 NS	0.003**	0.316 NS	0.58 NS	0.005**
Horizontal experiment					
Control	8.39	15.94	10.15 b	3.89 b	22.87 c
5 mg·L ⁻¹	10.04	16.14	10.96 ab	4.43 a	25.58 b
10 mg·L ⁻¹	9.95	15.53	11.45 a	4.30 a	29.30 a
<i>P</i> value	0.2662 NS	0.669 NS	0.013*	0.026*	<0.0001***

ⁱ Aerial weight is total head weight.

ⁱⁱ Root weight is belowground tissue.

ⁱⁱⁱ Head diameter was measured as the distance between the two widest points of the head.

^{iv} Plant height was measured from the base of the plant to the highest center point.

^v Maximum root length was measured from the base of the plant to the longest root tip.

^{vi} Mean separation in columns by Student's *t* test at $P \leq 0.05$.

Different letters indicate the significance level.

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

and tylosin concentration in leaf tissue. As expected, among all biomass variables, there was a very high positive correlation in both experiments ($P < 0.001$), except for maximum root length (Tables 3 and 4). The tylosin concentration in the tissue for the vertical NFT correlated negatively with height, diameter, and root weight ($P < 0.001$) and aerial weight ($P < 0.01$). When the treatments were analyzed individually, correlations between aerial weight and tylosin concentration were not related significantly (Supplemental Tables 1-3). However, only the tylosin treatments showed a negative relationship (Supplemental Tables 1-3).

For the horizontal NFT, the tylosin concentration in the tissue correlated negatively ($P < 0.05$) with all measured variables except head diameter (Table 4). However, the greatest significant negative correlation was reported for root weight ($P < 0.001$), which follows results similar to those of the vertical NFT (Table 4). At the treatment level, the multivariate correlations between aerial weight and tylosin concentration in the leaf tissue remained not significant, but only the 10 mg·L⁻¹ treatment showed a negative relationship (Supplemental Tables 4-6).

Discussion

The presence of antibiotics in alternative water sources, such as wastewater and animal farm

lagoons, could significantly challenge crop production as a result of contamination and the potential for plant uptake (Cifuentes-Torres et al. 2021). This issue becomes more critical in closed hydroponic systems, where bioaccumulation effects are more likely (Mai et al. 2023). Although previous studies have examined the uptake of pharmaceutical “cocktails” in hydroponics (Herklotz et al. 2010; Liu et al. 2013; Srichamnong et al. 2021; Yu et al. 2022). Limited attention has been given to tylosin specifically. Our study evaluated tylosin uptake and its effects on lettuce growth in two NFT systems—vertical and horizontal—by applying tylosin directly to the nutrient reservoir.

Although individual experiments used NFT principles, their configurations and environmental conditions differed significantly. The vertical NFT system allowed nutrient solutions to cascade down through stacked channels, creating a film over the roots. However, this design introduced variability in light exposure and nutrient delivery, with plants at the top receiving more light and potential nutrients than those at the bottom. This gradient was reflected in plant size, with top-tier plants outperforming those at lower levels, consistent with findings by Toulatos et al. (2016), who reported yield reductions from top to bottom in vertical systems and demonstrated a main disadvantage of these vertical towers.

Table 2. Tylosin concentration in lettuce leaf tissue at harvest after 35 d grown in two nutrient film technique systems.

Treatment	Tylosin content (mg·g ⁻¹ FW)	Total tylosin taken up by plants (mg/plant)
Vertical experiment		
Control (0 mg) ⁱ	0.0015 b ⁱⁱ	0.0425 b
5 mg·L ⁻¹ (225 mg)	0.0285 a	0.4270 a
10 mg·L ⁻¹ (450 mg)	0.0252 a	0.2085 ab
<i>P</i> value	0.0204*	0.0100**
Horizontal experiment		
Control (0 mg)	0.0019	0.0167
5 mg·L ⁻¹ (378 mg)	0.0028	0.0350
10 mg·L ⁻¹ (759 mg)	0.0091	0.0699
<i>P</i> value	0.0996NS	0.0538NS

ⁱ Values in parentheses represent the accumulated amount of tylosin added for each treatment.

ⁱⁱ Mean separation in columns by Student's *t* test at $P \leq 0.05$.

NS, *, ** Nonsignificant or significant at $P \leq 0.05$, or 0.01, respectively. FW = fresh weight.

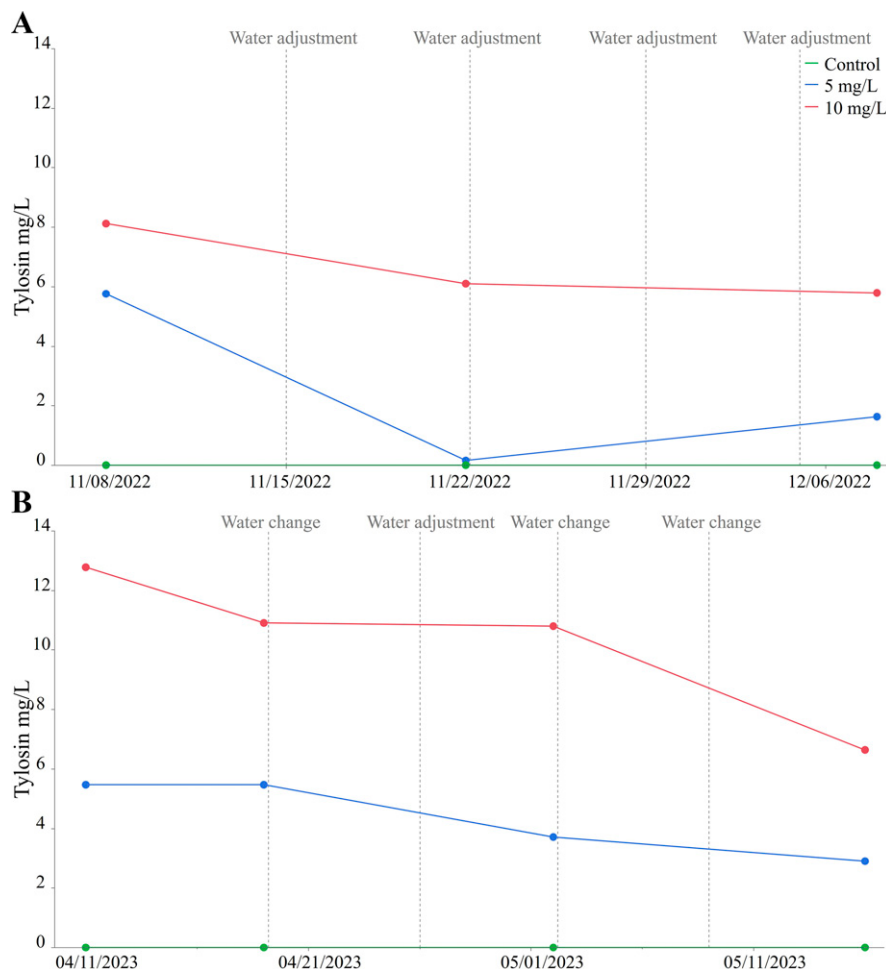


Fig. 2. Tylosin concentration in the nutrient solution reservoir during the vertical (A) and horizontal (B) nutrient film technique experiments.

In contrast, the horizontal NFT system provided more uniform light exposure, but was conducted under warmer temperature conditions (20.6–34.2 °C). The average, minimum, and maximum temperatures were higher than in the vertical NFT, which was conducted in the fall. Lettuce, a cool-weather crop, thrives between 19 and 24 °C (Brechtner et al. 1996), and the elevated temperatures in the horizontal NFT likely contributed to reduced growth and biomass. Previous research has documented that air temperature can affect plant growth (Thompson et al. 1998).

The fertilizer brand also played a role in the observed differences. The vertical NFT used a more effective nutrient formulation, whereas the horizontal NFT used a fertilizer higher in NH_4 , which underperformed. To compensate, more frequent and complete water changes were

made in the horizontal system. Despite this, biomass remained low, suggesting that fertilizer inefficiency and environmental stress outweighed any benefits from increased nutrient concentration. Therefore, plant growth was markedly better in the vertical NFT system, with larger shoot biomass and more vigorous development (Supplemental Fig. 1). In contrast, the horizontal NFT system exhibited stunted growth across all treatments (Supplemental Fig. 2). Interestingly, root systems in the horizontal NFT were larger, indicating a possible shift in energy allocation from shoot to root development under stressful conditions (Franco et al. 2011). Unfortunately, root tissue mass was insufficient for tylosin analysis, preventing a full comparison of translocation patterns.

Roots serve as the primary entry point for contaminants, with uptake influenced by root

traits and compound properties (Keerthan et al. 2021; Miller et al. 2016). Once inside root cells, compounds move via apoplastic, symplastic, or transmembrane pathways. Uptake and translocation are governed by physicochemical factors such as hydrophobicity ($\log K_{ow}$), ionization, and molecular weight, where $\log K_{ow}$ refers to n-octanol-water partition coefficient. Compounds with $\log K_{ow} < 4$ and $\log D_{ow}$ between 0.5 and 3 are more mobile, whereas neutral molecules such as caffeine tend to accumulate more readily (Malchi et al. 2014; Wei et al. 2023; Wu et al. 2015). Although a high molecular (>400 $\text{g}\cdot\text{mol}^{-1}$) weight often limits movement, prolonged exposure in hydroponic systems can enable translocation to aboveground tissues (Chuang et al. 2019; Li et al. 2019). For instance, tylosin has a molecular weight of 916.1 $\text{g}\cdot\text{mol}^{-1}$ and a $\log K_{ow}$ of 1.63.

Tylosin concentrations in plant tissues varied between systems. In the vertical NFT, tylosin was added incrementally with water replenishment, leading to a gradual accumulation in the reservoir. Therefore, total tylosin accumulated to 225 and 450 mg for the 5 and 10 $\text{mg}\cdot\text{L}^{-1}$ treatments, respectively. Consequently, tissue concentrations were significantly greater than those reported by El Gemayel et al. (2020)—up to 70 times greater, which suggests that the vertical system's conditions favored the uptake and translocation of tylosin. In contrast, the horizontal NFT received greater total tylosin inputs as a result of more frequent complete water changes, as shown in Table 2. The concentration of tylosin added in the horizontal NFT was 68% higher than in the vertical NFT, yet tissue concentrations were less (0.0019–0.0091 $\text{mg}\cdot\text{g}^{-1}$ fresh weight). In previous experiments, Dodgen et al. (2015) found that temperature and relative humidity affected the uptake and accumulation of compounds in lettuce. They reported that a hotter and drier environment leads to greater translocation of compounds toward the leaves, whereas cooler and humid conditions produce less translocation (Dodgen et al. 2015). Therefore, it is implied that greater plant transpiration could lead to greater translocation of compounds (Pérez et al. 2023). However, this result was not observed in our study, and the discrepancy may be attributed to limited plant growth or sorption of tylosin to system surfaces. For instance, the horizontal system was larger and circulated the nutrient solution through a longer route than the vertical NFT. Therefore, the tubing, channels, and other surfaces could contain more sites for compound sorption than the other system (Guo et al. 2018). Other explanations for the differences could

Table 3. Restricted maximum likelihood correlation analysis of variables measured at harvest from the vertical nutrient film technique of lettuce treated with tylosin.

Variable	Height (cm)	Diam (cm)	Aerial wt (g)	Root wt (g)	Max root length (cm)	Tylosin ($\text{mg}\cdot\text{g}^{-1}$ FW)
Height (cm)	1	—	—	—	—	—
Diameter (cm)	0.790***	1	—	—	—	—
Aerial weight (g)	0.823***	0.794***	1	—	—	—
Root weight (g)	0.571***	0.548***	0.576***	1	—	—
Max root length (cm)	0.073	0.067	−0.036	0.208	1	—
Tylosin ($\text{mg}\cdot\text{g}^{-1}$ FW)	−0.651***	−0.688***	−0.563**	−0.712***	−0.332	1

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. FW = fresh weight.

Table 4. Restricted maximum likelihood correlation analysis of variables measured at harvest from the horizontal nutrient film technique of lettuce treated with tylosin.

Variable	Height (cm)	Diam (cm)	Aerial wt (g)	Root wt (g)	Max root length (cm)	Tylosin (mg·g ⁻¹ FW)
Height (cm)	1	—	—	—	—	—
Diameter (cm)	0.883***	1	—	—	—	—
Aerial weight (g)	0.951***	0.870***	1	—	—	—
Root weight (g)	0.707***	0.597*	0.725***	1	—	—
Max root length (cm)	0.700**	0.579*	0.738***	0.701**	1	—
Tylosin (mg·g ⁻¹ FW)	-0.646*	-0.507	-0.611*	-0.951***	-0.606*	1

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively. FW = fresh weight.

be attributed to pH, O₂ concentrations, or transpiration. Although these factors were not measured in our study, future research should explore these components to determine how they interact with tylosin accumulation and uptake. Nonetheless, a slight bioaccumulation effect was still observed, as the concentration found in the leaves was greater than that reported in other studies (El Gemayel et al. 2020; Youssef et al. 2020). However, it was less than other studies, even when the concentration applied in our study was greater (Bhalsod et al. 2018; Shen et al. 2021). These findings align with other similar studies of CEC uptake, where the concentrations and effects of these compounds in plants varied by environment (Dodgen et al. 2015), species (Chang et al. 2024; He et al. 2017; Zheng et al. 2014), system (Chang et al. 2024), and compounds used (Herklotz et al. 2010; Zheng et al. 2014).

Tylosin, a relatively large molecule, is generally expected to have limited translocation to shoots (Bhalsod et al. 2018; Chuang et al. 2019; Kumar et al. 2005). However, extended exposure and bioaccumulation in closed systems can facilitate its movement into above-ground tissues (Chuang et al. 2019). This was evident in both NFT systems, where tylosin was detected in leaf tissues despite differences in system design and environmental conditions.

These results underscore the importance of system design, environmental control, and nutrient management in hydroponic production. The vertical NFT system, despite its internal variability, supported better plant growth and tylosin uptake. Conversely, the horizontal NFT system, although more uniform in design, suffered from environmental stress and nutrient limitations that hindered both growth and compound uptake.

Conclusion

Our study demonstrates that tylosin in hydroponic nutrient solutions can affect lettuce root development adversely, although its effects on aerial growth parameters remain largely unaffected. The significant accumulation of tylosin in lettuce leaves raises concerns about the safety of using treated wastewater in hydroponic systems, particularly regarding the potential for antibiotic bioaccumulation. The variation in tylosin accumulation between the two experiments suggests that specific water management practices—particularly the frequency of water changes—influence significantly the extent of tylosin buildup in the system. These findings emphasize the importance of monitoring and managing CECs in alternative water sources to safeguard plant health and food safety. Future research

should also include measuring tylosin concentration in root tissue, given its molecular weight, which makes it a relatively heavy compound. In addition, a focus on developing strategies to reduce antibiotic contamination in hydroponic systems and exploring the long-term effects of CECs on plant growth and human health are needed.

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