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Original Research

The Responses of Invasive *Wedelia trilobata* and Native *Wedelia chinensis* to Levofloxacin Hydrochloride: Implication for Biological Invasion

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Abstract

Invasion of alien plants has become a global problem, and antibiotics, a new pollutant, have received widespread attention. We hypothesized that different concentration levels of Levofloxacin hydrochloride facilitate different physiological, growth and antioxidant system responses in *W. trilobata* and *W. chinensis*. The toxicity of Levofloxacin hydrochloride was assessed in *W. trilobata* and *W. chinensis* through physiological, chlorophyll, and antioxidant system. Compared with single species, the two plants were more susceptible to the influence of levofloxacin hydrochloride when mixed; in sand culture, levofloxacin hydrochloride affected physiological parameters (leave size, number of leaves, plant height, stem length, and dry weight biomass). The exogenous addition of levofloxacin significantly restricted the root development of plants, especially in the case of hydroponics. Both plants showed prominent oxidative stress characteristics that leaves yellowed and withered, as well as showed on photosynthesis and antioxidant system. SOD presents different trends under different experiment units. CAT activity showed a significant decrease in all treatment groups. No significant difference observed in POD activity between both plants under sand culture conditions, and it increased after Levofloxacin hydrochloride application. Overall,our results revealed that invasive *W. trilobata* adapted well against Levofloxacin hydrochloride with better growth and antioxidant system, which strengthens the biological invasion process.

Keywords: levofloxacin hydrochloride, invasive and native plant, sand culture, hydroponics, physiological responses, single species, mixed species

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Introduction

Rapid globalization and development have led to human society's expansion and worldwide environmental pollution and biological destruction [1]. The whole ecological substances are presented with a persistent intensifying hazard of being contaminated with evolving, constant organic chemicals [2]. The more significant threat is that infections can easily withstand traditional treatment technologies and further can translocate painlessly in all environments [3, 4]. Antibiotics are one such class due to their continual nature and indeterminate metabolic fate [5]. The existence of antibiotics in the atmosphere may promote antibiotic resistance and negatively impact different plants [4, 6]. Generally, antibiotics hand-me-down in the hospital includes extracts from bacteriological culture fluids and chemically manufactured [7]. Antibiotics are widely used by humans and in various scenarios. Due to improper treatment, they flow into the environment in large quantities, causing much harm to the ecosystem. They may also flow into the human body through the food chain, pretense a menace to human health [8, 9]. Levofloxacin hydrochloride (L.H.) is a broad-spectrum antibacterial antibiotic that significantly impacts ecosystems and human health [10]. L.H. can potentially harm marine animals' life, i.e., superfluous levofloxacin contemporary in water, affecting them [11]. LH-encompassing effluent, if used for agriculture, could also affect plant functioning and development [12].

More than 500 invasive plants in China impact ecosystem functions and socioeconomic development due to their vast geographic range and increasing human activities across regions [13]. The invasive plants of the Compositae family account for the most significant proportion, and the invasion range is the widest, causing a wide range of ecological disasters [14, 15]. Among invasive plants, *Wedelia trilobata*, a sneaking herb with broad malleability, is considered a native plant to different parts of Central America and conquered semitropical territories [16]. In late of the twentieth century, it was familiarised to another part of China as a decorative plant; nevertheless, after a certain period, it was distinguished as an invasive [17] and, in recent days, extensively disseminated in different provinces of China, including Fujian, Guangdong, and Guangxi. Invasive species showed durable reactions under other environmental and climate circumstances, and obtained reserves to decrease the development of their native contender [18]. Some scientists also reported that invasive groups showed improved functional traits and stimulated their growth under different environmental conditions because of higher phenotypical plasticity and improved rivalry strength compared to their natives [19, 20].

Excessive reactive oxygen species (ROS) accumulation in different plants under different abiotic stresses can cause and source different oxidative in other plants' cellular organelles [21]. Disproportionate accretion of ROS causes oxidative damage to the electron transport chain and mitochondria, deactivates enzyme activity, proteins, and nucleic acids, and eventually reduces photosynthesis and the development and yield of plants [22, 23]. The antioxidant enzymes (Ascorbate peroxidase (APX), catalase (CAT), dehydroascorbate reductase (DHAR), Glutathione reductase (G.R.), glutathione *S*-transferase (GST), peroxidase (POD), and superoxide dismutase (SOD) decrease the ROS growth and accumulation, thereby complementary the ROS synthesis and gesticulating to provide increase different environmental fluctuations stress tolerance in other plant parts [24]. ROS rummaging boiling stable antioxidant enzymes were studied in three different most widespread varieties of Lantana: pink, red, and violet of invasive weeds under various abiotic stresses [25, 26].

There are many explanations for the successful invasion mechanism of invasive plants. The resource competition hypothesis and the successful invasion of invasive plants may also be related to higher resistance to adverse environments [27, 28]. Invasive plants can grow and reproduce in harsher environments than native plants because they have fewer resources to counteract the hostile environment or are more tolerant of adverse environmental factors [29, 30]. Regarding environmental factors, antibiotics are still scarce in the research on invasive plants' invasion process, and antibiotics' effect on invasive plants' invasion process in different growth environments is still unclear [31]. This study focused on the impact of different L.H. concentrations on invasive plants' invasiveness in different growth environments and compared them with their natives. We used different concentrations of L.H. in hydroponics and sand cultivation to determine the physiological, chlorophyll content, and antioxidant system of invasive *W. trilobata* and native *W. chinensis*

Materials and Methods

W. trilobata and *W. chinensis* were collected from Nanning City, Guangxi Province, and the greenhouse of the School of Environment and safety Engineering, Jiangsu University, respectively. The greenhouse experiment was conducted at the School of School of Environment and safety Engineering, Jiangsu, University, Zhenjiang, (32.20°N, 119.45°E), China, where the temperature of 25±5ºC during the day, 18±2ºC during the night, and 70% relative humidity were maintained throughout the experimental period. The regenerated stem with healthy- and constant-growth (same length and size) was designated, and two nodes were engaged for each stem section. Cuttings were cultured in 90×60×80 mm round plastic pots where two cuttings of each plant were maintained and planted in each pot. Early, we ensured no particular nutrients in the substrate as far as possible and the air penetrability of plant roots, washed, dried, and sterilized with green zeolite (sand: green zeolite = 5:1). The selected stem cuttings were cultured straight up in the same position in each pot, rinsed with special deionized water. The levofloxacin solution was applied 4 to 5 days after the new shoots emerged. In the hydroponic treatment, plant cuttings were placed in a hydroponic bottle containing 200 mL of the solution and then wrapped with tin foil for light-proof treatment. The levofloxacin hydrochloride (Jiangsu Aosaikang Pharmaceutical Co., Ltd.) solution in the test was purchased from the market. All treatment reagents in the experiment were prepared with pure water. In the hydroponic treatment, the levofloxacin hydrochloride solution was prepared with 0.1 times the Hoagland solution into three treatment groups 0 mg/L (0.9% NaCl), 1 mg/L,3 mg/L, and the 0.1 times Hoagland solution was used as the control; in the sand cultivation treatment, with 0.1 times of Hoagland solution The levofloxacin hydrochloride solution was configured into three treatment groups 0 (0.9% NaCl) mg/kg, 1 mg/kg, 3 mg/kg, and the 0.1 times Hoagland solution was the control. They were all cultivated in the Environmental Plant Cultivation Room of Jiangsu University. Hydroponics was supplemented with 10 mL of treatment solution every week to replenish lost water, and sand was cultured with 50 mL of treatment solution every week. After six weeks of treatment, they were allowed to grow freely for a week (treated with tap water). The leaf characteristics, plant height, biomass, root development, and enzyme activity of the plants were measured after seven weeks of culture. Each indicator was repeated three times.

Phenotypic Growth Indicators

Plants were harvested when they reached the seventh week of growth, and the plants were harvested and measured. Growth indicators (number of leaves, leaf area, number of lateral roots, root length, plant height, root-shoot ratio and biomass) were measured in five replicates, and the results were averaged.

Chlorophyll Content and Antioxidant Enzymes Activities

A chlorophyll meter was used for measuring chlorophyll contents. To regulate the antioxidant activities (CAT and POD), 0.1 g of plant leaves were evaluated and weighed up, ground with liquid nitrogen, after adding 2.5 ml phosphate buffer (pH = 7.8), the solution was centrifuged at four ºC for 15 minutes at 10000 rpm. Later the ultraviolet spectrophotometry and guaiacol method was measured for further activities. CAT activity was evaluated in a mixed reaction encompassing 0.1 ml of enzyme extract, 1 ml of H_2O_2 (0.3%), and 1.9 ml of deionized water. The reaction system recorded photographic density at 240 nm for

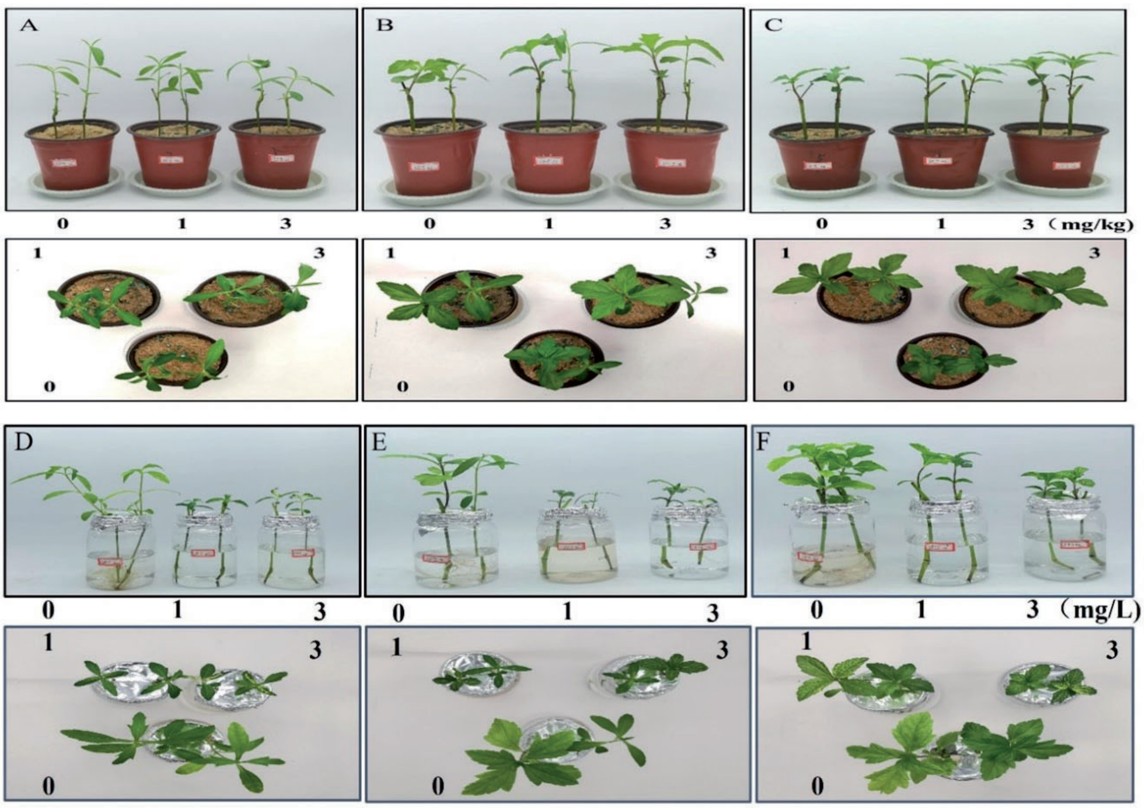

Fig. 1. Effect of different L.H. concentration levels on growth of *W. trilobata* and *W. Chinensis.*

3 min, and enzyme CAT activity (U/g FW·min) was articulated as the enzyme-catalyzed the breakdown of H_2O_2 over time. SOD activity was by the NBT photoreduction method.

Data Analysis

The software Origin 8.1 was used for graphing the statistical analysis of data. A significance level of 0.05 for all biomarkers ($P<0.05$). SPSS (25.0) software was used for variance analysis and correlation analysis.

Results and Discussion

Enhancement of soil quality by fertilization with compost is the most crucial cause of the pollution of agricultural land with antibiotics [32]. According to the effect of different culture media on *W. trilobata* and *W. chinensis*, we found that under the condition of sand culture, both plants increased their plant height, leaf number, leaf area, stem height, root development, and root shoot ratio. We found that under sand culture, both plants increased their plant height; however, under hybrid, levofloxacin treatment had no significant

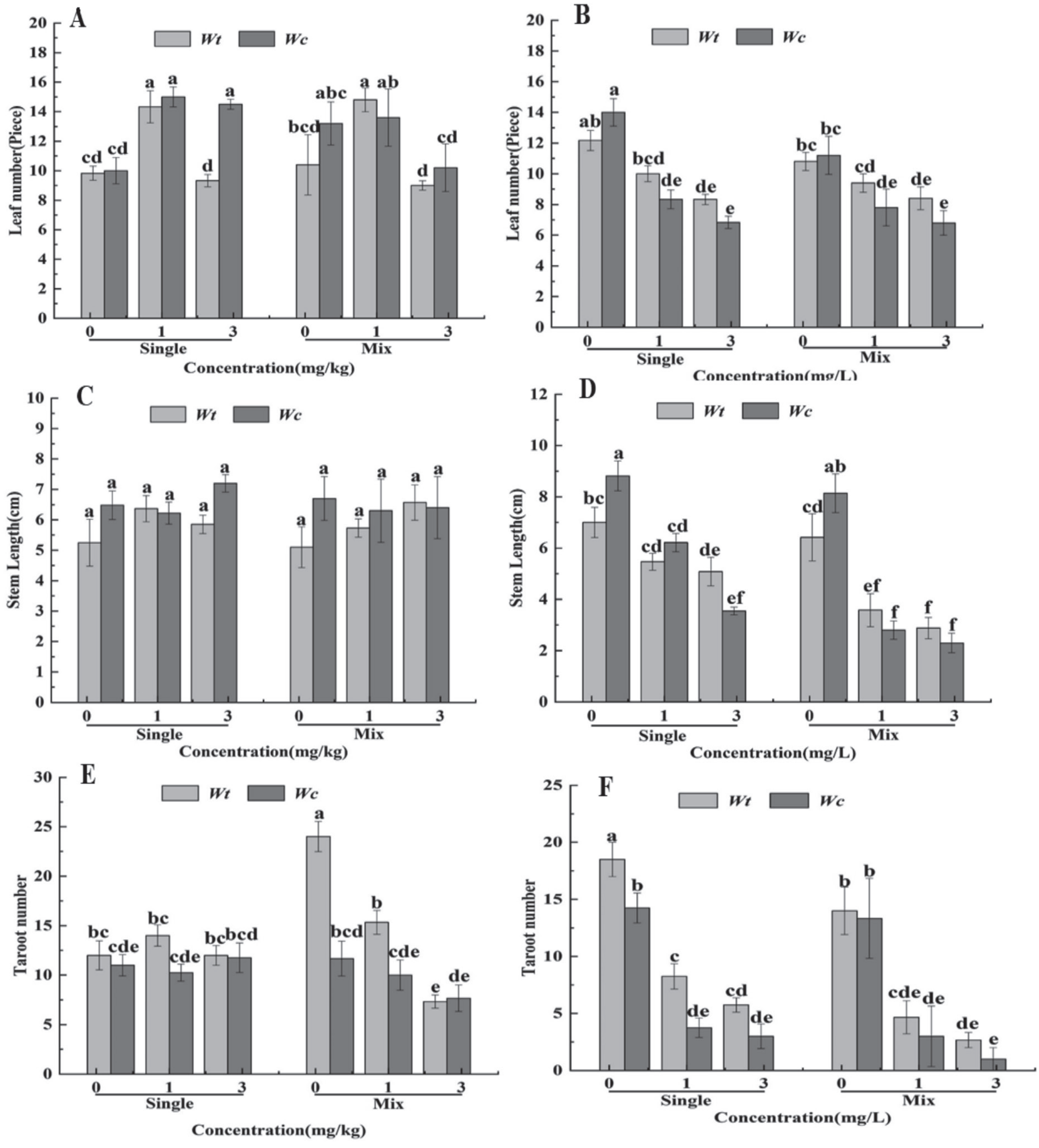

Fig. 2. Effect of different L.H. concentration levels on different physiological growth parameters(leaf number, stem length and taroot numb) of *W. trilobata* and *W. Chinensis*. A, C, and E are under sand culture and B, D and F are under hydroponics with single and mixed species. Graphs indicate the significance between different treatments (P<0.05).

consequence on *W. trilobata* and *W. chinensis*. When both plants were exposed to levofloxacin in hydroponics, they showed prominent oxidative stress characteristics, that is, part of the leaves yellowed and withered, indicating that levofloxacin treatment may have a more significant impact on the photosynthesis of plant leaves (Fig. 1). The number of leaves of both plants increased when the concentration was lower (1 mg/kg) and decreased with a higher concentration (3 mg/kg). Plants cultivated in soil infected with antibiotics take them up and collect them in different tissues [33].

Compared with the control group (0.9% NaCl), after the application of 1 mg/kg of antibiotics, the number of leaves of native *W. chinensis* increased significantly by 46.46% and 67.84%, respectively (P<0.05) (Fig. 2a). When the antibiotic concentration was three mg/L, the number of leaves in each group decreased significantly (P<0.05), and both native and invasive species decreased by 21.34% and 51.85%, respectively (Fig. 2). High antibiotics concentration were reported in leaves, observed by shoots and roots in various plants [34, 35]. Both species' leaf areas increased under the exogenous addition of levofloxacin compared to the control group. When the antibiotic concentration was 3 mg/L, the leaf area of each group decreased most significantly. (P<0.05), the leaf area of native and invasive plants

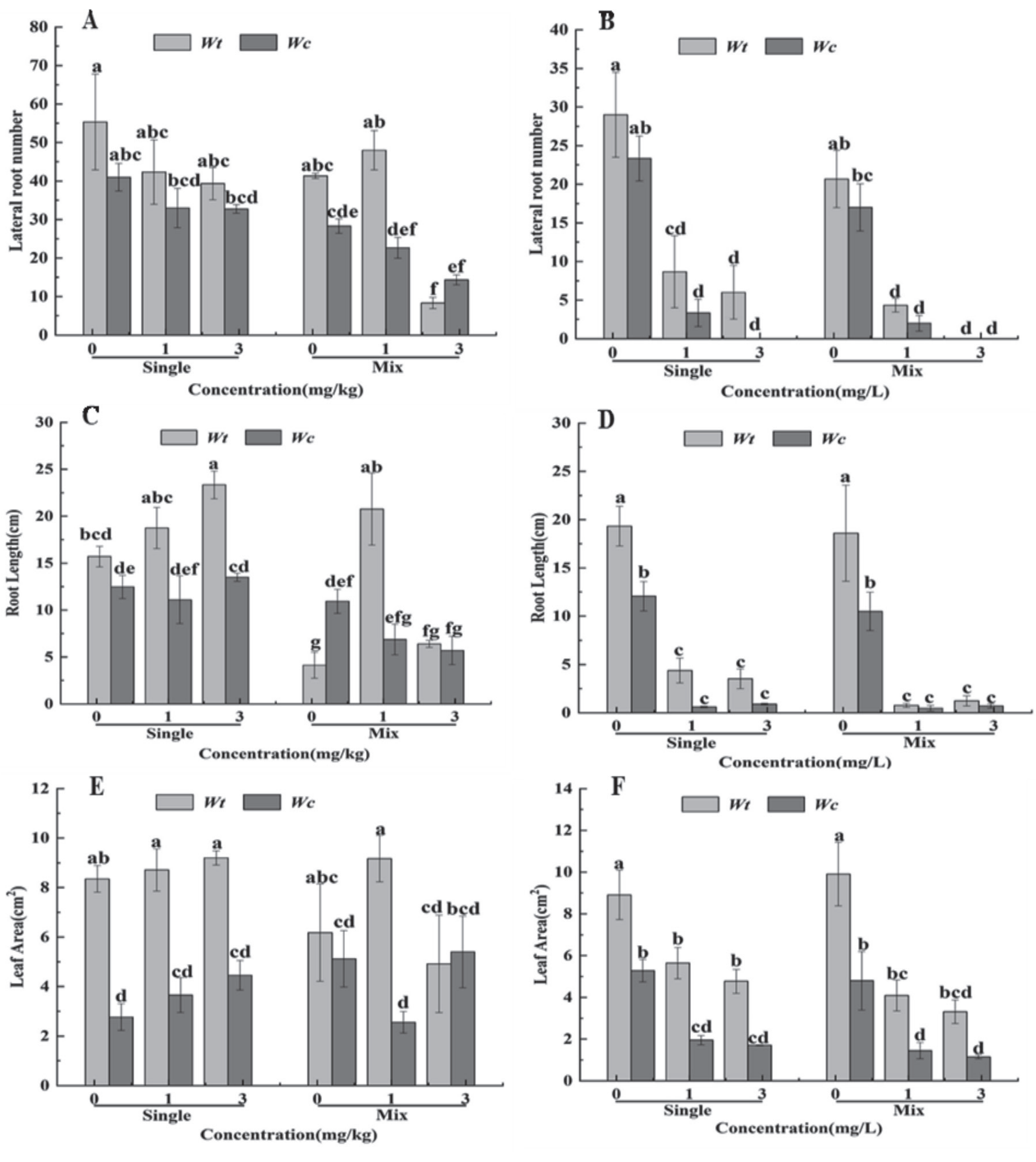

Fig. 3. Effect of different L.H. concentration levels on different Phenotypic growth parameters (lateral root number, root length and leaf area) of *W. trilobata* and *W. Chinensis*. A, C, and E are under sand culture and B, D and F are under hydroponics with single and mixed species. Figures indicate the significance between different treatments (P<0.05).

decreased by 49.3% and 66.85%, respectively (Fig. 3). This antibiotic concentration affects the growth and development of different plants and is distributed in plant tissues [36].

Under the sand culture environment, the stem length of both plants increased after the exogenous addition of levofloxacin. Compared with the control group, the stem lengths of the single species of *W. trilobata* and the native species increased by 48.72% and 25.06%, respectively. Stem lengths of *W. trilobata* and *W. chinensis* increased by 13.65% and 23.05%, respectively. Compared with the control group, the stem lengths of single and native species were reduced by 18.41% and 60.06%, respectively. The stem lengths of the native and native *W. chinensis* decreased by 55.14% and 71.74%, respectively. Other antibiotics absorbed in plant tissues may obstruct photosynthesis and physiological growth [37]. In the present work, we demonstrated that the L.H. range harmed the physiological. Photosynthesis and

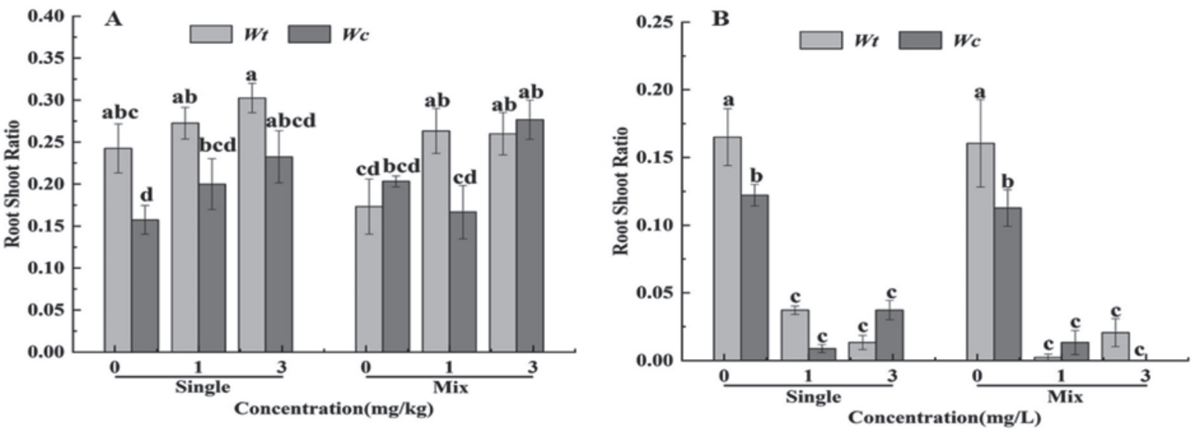

Fig. 4. Effects of different L.H. concentration levels on root shoot ratio of *W. trilobata* and native *W. chinensis*, A showed sand culture and B showed hydroponics with single and mixed species. Graphs indicate the significance between different treatments (P<0.05).

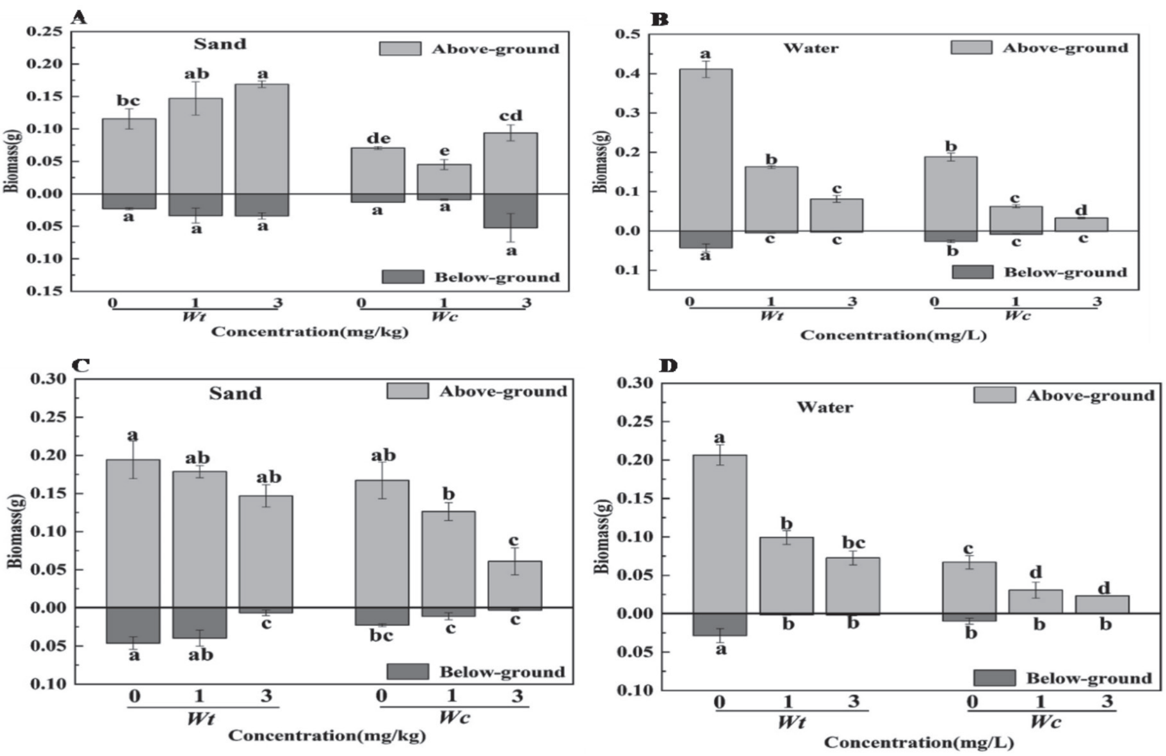

Fig. 5. Effect of different L.H. concentration levels on biomass of *W. trilobata* and *W. chinensis*. A and C showed under sand culture and B and D showed under hydroponics, with single and mixed species. Graphs indicate the significance between different treatments (P<0.05).

antioxidant system of both plants. Both plants showed protruding oxidative stress during L.H. application, which affects leaf character size, indicating its damage and slow photosynthesis. L.H. application increases the proportion of PSIIb and stressed photosynthesis process in the different organisms [38].

Culture environment, after the exogenous accumulation of levofloxacin, the stem length of both plants increased. Compared with the control group, the stem lengths of *W. trilobata* and native *W. chinensis* increased by 48.72% and 25.06%, respectively. Stem lengths of *W. trilobata* and native *W. chinensis* increased by 13.65% and 23.05%, respectively. We found that no matter what culture media, planting methods, and treatment methods, the root lengths of the same group were significantly larger than those of the native plant. In the hydroponic environment, after the exogenous addition of levofloxacin, the root growth of both plants was seriously affected, and the root length was significantly reduced ($P<0.05$). Compared with the control group, after applying one mg/L levofloxacin, the root length of *W. trilobata* and *W. chinensis* decreased by 77.36% and 95.88%, respectively. The root lengths were reduced by 96.69% and 94.92% (Fig. 5). We also observe and compare the biomass allocation of both plants. In the hydroponic environment, after the application of the highest concentration (3 mg/L) of levofloxacin, the biomass of the two plants showed the most significant reduction. The aboveground biomass of the single species of *W. trilobata* and the native *W. chinensis* decreased by 57.64% (Fig. 6) and 76.41%, respectively (Fig. 5). Under different antibiotic treatments, plant shoot length dry weight biomass is remarkably reduced in all treated plants [39, 40], which leads to plant growth, including native and invasive weeds [41, 42]. The previous study also reported that antibiotic stress, including *L.H.,* abridged plant growth, i.e., height and dry weight biomass [19, 43], reported that tetracycline and norfloxacin have adverse effects on the development of the *Brassica rapa.* High levels of antibiotics, including L.H., altered soil and microbial activity, affecting plant growth [15]. We also found that *L.H.* concentration in different plant parts of both plants increased after the exogenous addition of levofloxacin in the stem and roots, reducing stem and root length and directly impacting plant growth as compared with the control group.

To effect of antibiotics on chlorophyll content disturbs the functioning of the early chlorophyll biosynthesis stage [44]. According to the effects of levofloxacin on the chlorophyll of both plants under different culture substrates, we found that exogenous addition of levofloxacin, the chlorophyll content of all plants in all treatment groups showed a moderate decreasing trend, and the chlorophyll

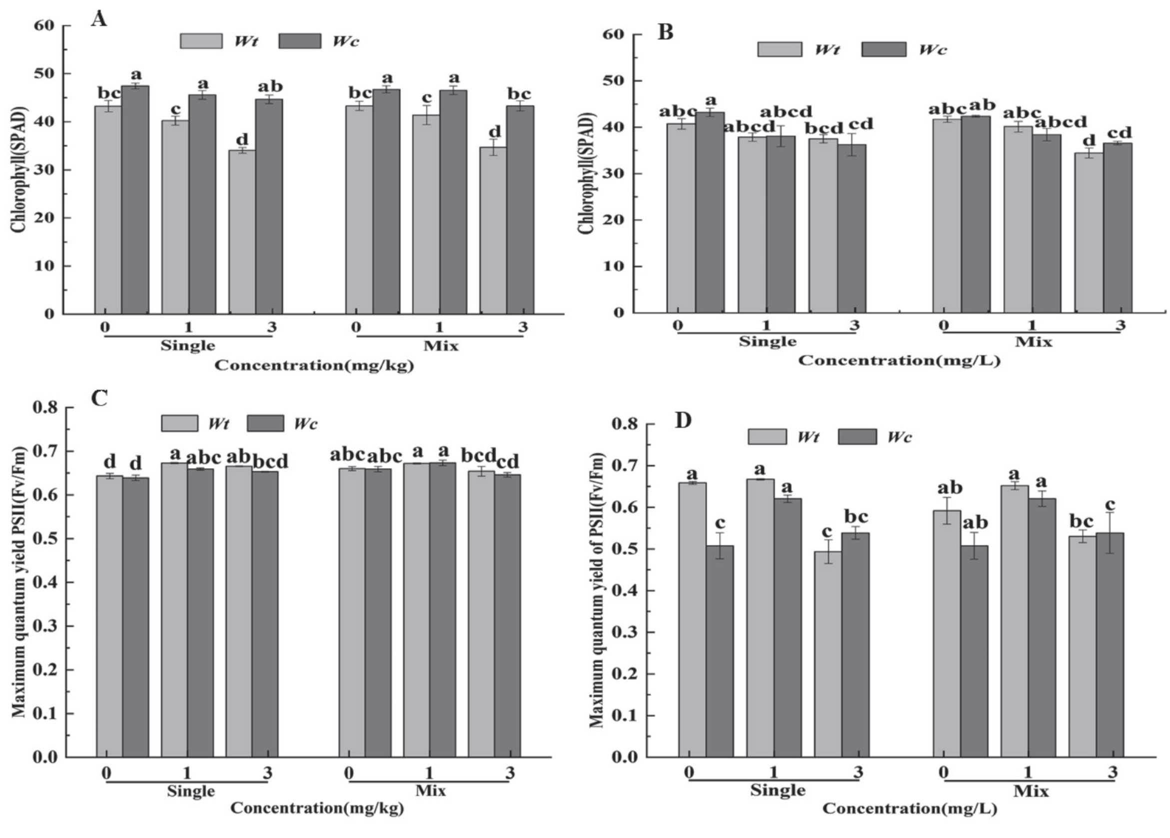

Fig. 6. Effect of different L.H. concentration levels on chlorophyll content (A and B) and maximum quantum yield of PII(Fv/Fm) (C and D) in *W. trilobata* and native *W. chinensis*, with single and mixed species. A and C showed under sand culture and B and D showed under hydroponics Graphs indicate the significance between different treatments ($P<0.05$).

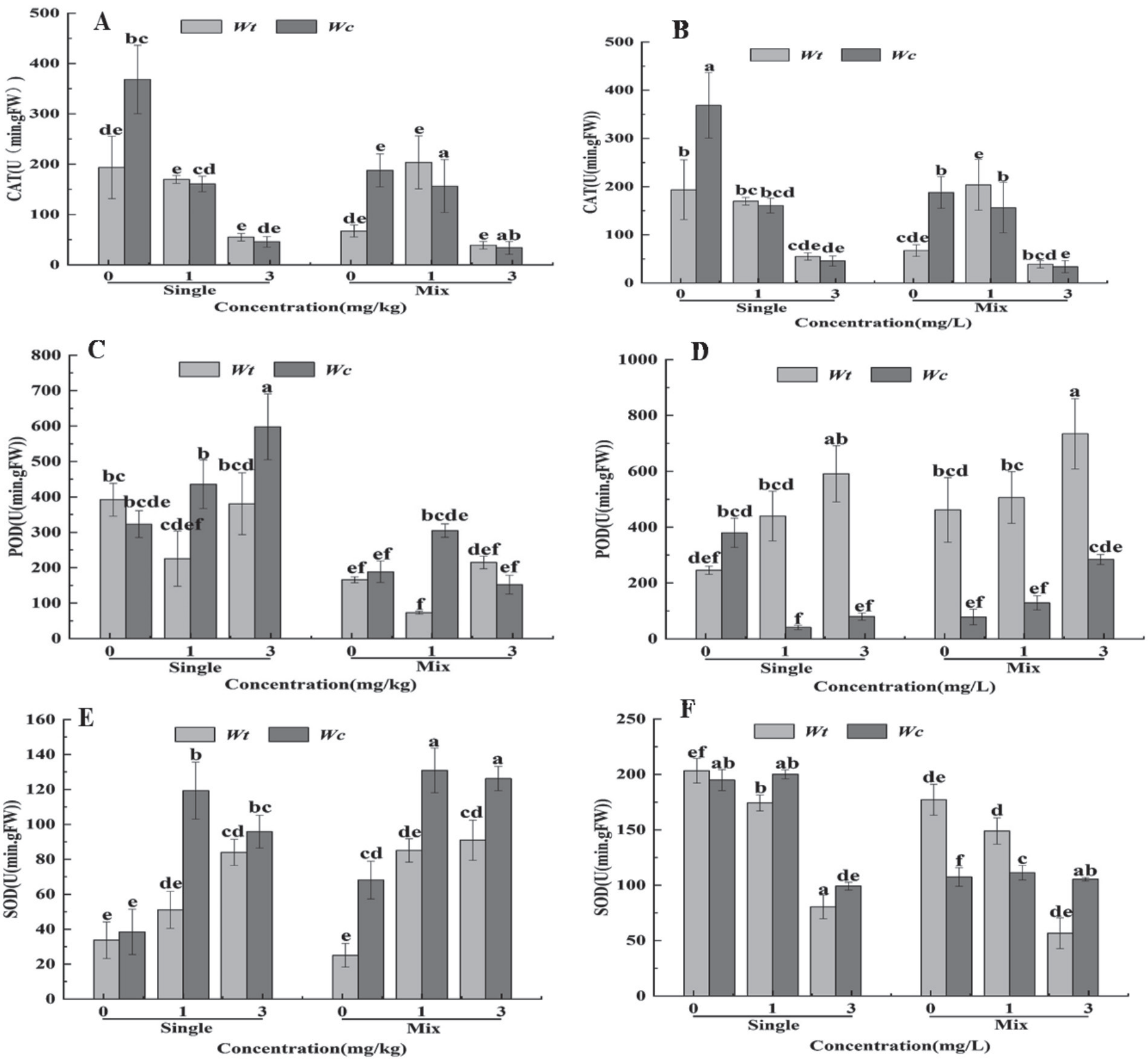

Fig. 7. Effect of different L.H. concentration levels on antioxidant system of *W. trilobata* and native *W. chinensis*, with single and mixed species. A and C showed under sand culture and B and D showed under hydroponics. Graphs indicate the significance between different treatments (P<0.05)

content of plants increased, When the concentration of antibiotics increased than its decreased gradually. Most studies claim antibiotic effects on photosynthesis are conjectural; however, this action leads to hydrogen peroxide accumulation in chloroplasts and a damaged photosystem [45]. Previous results show that low-content antibiotics indorse chlorophyll biosynthesis by distressing nucleic acid and protein in cells and delaying the chlorophyll degradation process in plants [46, 47]. In exogenous effects of L.H., *W. trilobata,* and *W. chinensis,* chlorophyll content moderately decreased regardless of culture medium and planting method, but this was the apparent effect on both plants. In *Spinacia oleracea*, chlorophyll content degradation under tetracycline is noticeable and less decreased in leaves [48]. It shows that a low concentration of antibiotics supports chlorophyll synthesis by affecting chloroplast protein and nucleic acid, reducing the activity of chlorophyllase, and stopping the degradation of chlorophyll [45]. In *Spinacia oleracea*, chlorophyll content degradation under tetracycline is noticeable and less decreased in leaves [48]. Regardless of the culture medium and planting method, the application of levofloxacin had no noticeable effect on the chlorophyll content of both plants (Fig. 6). Previous studies reported that the effect of chlorophyll content depends on the concentration of antibiotic concentration [49].

In the antioxidant system, SOD presents different trends; in the sand culture environment, when the two plants were grown alone, compared with the control group, the SOD activities of 3 mg/kg levofloxacin-treated *W. trilobata* and native plant increased by 98.41 and 149.27%, respectively. In contrast, in the hydroponic environment, compared with the control group, 3mg/kg levofloxacin treated, the SOD activity of both plants officinalis showed a significant decrease, decreased by 60.4% and 49.1%, respectively. Antibiotic applications in different organisms, including plants, reported less production of the defense-related system [50]. In the present research, SOD showed a distinct trend in the sand and hydroponic culture compared to the control group, but under applying *L.H.* in both plants, SOD concentration decreased. SOD concertation also showed different trends in plants under control compared with different antibiotic concentrations [51]. All treatment groups showed a considerable reduction in CAT activity relative to the control group. In the sand culture environment, whether a single or mixed species, the CAT activity of the invasive plant was lower than that of the native *W. chinensis*. In contrast, in the hydroponic environment, In the sand culture environment, regardless of single species or mixed species, the reduction of CAT of the invasive plant was much more significant than of the native plant, which decreased by 88.52%, 87.98%, and 49.46%, 24.97%, respectively, compared with the control group (Fig. 7).

There was no significant difference in POD activity between the two plants under sand culture conditions, whether a single or mixed species. Under hydroponic conditions, the POD activity of invasive plants increased, and the activity of native plants decreased when single-planted. In contrast, the POD activity of both plants increased when mixed planted. Under the hydroponic condition associated with the control group, the POD activity of *W. trilobata* increased by 70.58% and 59%, respectively. The disproportion between the detoxification rate and H_2O_2 production mains to the increase of H_2O_2, further inhibiting SOD activity in cells [52]. It also reported that SOD concentration fluctuation in *Ceratozamia Mexicana* increased significantly when exposed to ciprofloxacin [53]. On the other hand, POD and CAT activities reported a high concentration of antibiotic-treated plants as compared with their control group [54, 55]. POD activity decreases and weak defense systems in plants under different antibiotic applications [56]. CAT concentration differed and fluctuated in other cultures, but under different concentration levels, L.H. showed fewer values than the control group. CAT activity fluctuation or increased hydrogen peroxide at the cellular level promotes cell death in the plant body [57].

Conclusions

Our result demonstrated that *W. trilobata* and *W. chinensis* were too low and showed fluctuation compared to the control group. During comparison between *W. trilobata* and *W. chinensis* L.H concentration were reported in leaves and in different parts and effects its growth-related parameters. Both plants showed protruding oxidative stress during L.H. application, affects its leaf character size, promotes its damage, and reduce photosynthesis process. While during antioxidant system (POD, CAT and SOD) no significant difference was observed in both plants and perform better in different culture mediums. Both native and invasive plants respond differently in an antioxidative system, which shows that invasive plants are more robust than their native plants. Overall, *W. trilobata* adapted well against L.H with better growth and an antioxidant defense system, which strengthens its biological invasion process.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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