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Original Research

Physiological and Biochemical Changes Induced by Tetracycline in Wheat

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Abstract

This paper is devoted to the study of the peculiarities of the 48-hour effect of different concentrations of tetracycline on the physiological and biochemical parameters of wheat seeds and shoots. As a result of the research, it was established that the increase in the frequency of seed germination, elongation of shoot height (up to 3.5-4.5 cm) and root length (up to 3.8 cm) occurred when the concentrations of tetracycline were 1-10 mg/L. The above parameters were suppressed by tetracycline applied in the concentrations ranging from 50 to 300 mg/L. When tetracycline was applied at lower concentrations (1-10 mg/L), the specific activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) slightly increased by 0.2-0.4 units. At the concentrations of 50-200 mg/L, the specific activities of SOD, CAT and POD were characterized by irregular fluctuations linked to the dose increase of tetracycline. However, all variables increased as compared to the control values obtained after 48 hours of tetracycline exposure. The study results indicate that wheat bioassay can be used as an effective test for tetracycline toxicity and environmental pollution monitoring. The subsequent works should be aimed at the use of the maximum allowable soil concentrations of antibiotics, which stimulate the physiological and biochemical processes in plants. It will foster the adaptation of the technology for growing industrial crops in antibiotic-contaminated soils.

Keywords: antibiotics, antioxidative system enzymes, physiological parameters of wheat, seed germination, tetracycline effects

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Introduction

Each year, the problem of environmental pollution becomes more and more pressing on a global scale. This is due to the emissions of pollutants among which heavy metals and herbicides are common [1]. Naturally, pollutants are not isolated in the technosphere and they migrate into aquatic systems and soils. As estimated by Van Boeckel et al. [2], more than two hundred thousand tons of antibiotics are produced worldwide. In Europe, tetracycline has been the most used antibacterial drug during the last two decades [3]. Tetracycline has clear advantages, such as activity against a wide range of bacteria and relatively low toxicity. Moreover, production of this antibacterial drug is usually associated with low costs [4]. Nevertheless, the use of tetracycline has significant disadvantages as confirmed by the contamination of soil and agricultural products. This is due to the fact that most of tetracycline does not undergo metabolic modifications in the digestive tract of animals and is excreted in their feces and urine, eventually contributing to soil pollution [5]. In addition, there are gaps in tetracycline removal from wastewater, as this antibiotic has a long half-life of up to 120 days [6].

Apart from being harmful to animals, antibiotics may have a negative impact on the growth and development of various types of cultivated plants. This evidence is presented by other authors in their previous papers [2, 7]. Multiple defense mechanisms are present in plants to protect the cells against external environmental factors, for example, higher plants have a defense system that includes antioxidant enzymes and non-enzymatic defense compounds. In particular, the antioxidant enzymatic link includes superoxide dismutase, catalase, and enzymes of the ascorbate-glutathione (As-GSH) cycle. They act in a coordinated manner to protect the cell from oxidative stress [8]. Non-enzymatic protective compounds include biologically active substances that can affect plant immunity. Most of the enzymes involved in the detoxification of harmful substances in plants are present not only as several enzymes of one or another type, but also as a variety of isoforms [9]. Among these enzymes, catalase and peroxidase are of special interest, which are components of the antioxidative system. The key function of the antioxidant system is to ensure the adaptability of plants to the influence of any environmental stressors. Enzymes of the antioxidant system play an important role in regulating the content and balance in cells of reactive oxygen species (ROS), which perform the functions of mediators and signaling molecules in response to the influence of various environmental factors.

The effects of some antibiotics on the activities of catalase and peroxidase have been previously demonstrated in the legume model [9, 10]. In addition to enzymes protecting plants from oxidative stress, there are enzymes that help plants to survive thermal stress, dehydration, and interaction with halophilic microorganisms [11]. Some studies devoted to the investigation of plant responses to antibiotics accumulated in different media (including soil) have been conducted [6, 12-14]. Based on the information presented above, the purpose of this paper is to explore the effects of different concentrations of tetracycline, which is regarded as one of the most common antibiotics, on some physiological and biochemical parameters of plants. The objective of the study is to determine the optimal concentrations of tetracycline for the stimulation of wheat growth. The latter defines the novelty of the present study.

Literature Review

It is a known fact that modern animal husbandry widely uses compounds having antimicrobial properties. Although antibiotics are primarily used to prevent and treat disease, up to 90% of the administered doses are excreted in animal feces and urine [5]. When manure and slurry from treated animals are used as fertilizer in agricultural fields, residues of various classes of antibiotics are released into the environment, where they have been abundant during the past 20 years [12]. It has been observed that antibiotics released into agroecosystems can contribute to the emergence of antibiotic-resistant bacteria in the environment [4]. As to human health, the available experimental data suggesting the exchange of multidrug resistance genes between soil bacteria and human pathogens are of concern. Finally, the potential risks of adverse impact on human health and environment associated with the presence of antibiotic residues were assessed [13].

Wheat is one of the most important type of cereals along with maize and rice [15]. Wheat makes up a significant portion of the daily food and feed intake for a large number of people and animals. Thus, antibiotic contamination of wheat can lead to significant human exposure. Until now, the presence of antibiotics in wheat has been analyzed mainly in simulation experiments, with a very rare sampling from agricultural fields [7]. In view of the above, tetracycline is an important antibacterial compound. Currently, tetracycline is found in high and varying residual concentrations in soils, sediments, surface, and ground water [6]. For example, a recent study conducted by fellows showed that the concentration of tetracycline in some agricultural soils in Spain was up to 150 mg/kg as a result of irrigation with domestic sewage and application of animal feces and sewage sludge [14]. To date, environmental risk assessments of tetracycline have been widely conducted using the data obtained for aquatic organisms [16].

Along with aquatic ecosystems, higher plants function as one of the dominant terrestrial ecosystems producers and play an important role in maintaining the integrity of terrestrial ecosystems. However, the transfer of various pollutants into the system can lead to abnormal physiological and biochemical metabolism and trigger changes in plant genetic parameters [17].

Problem Statement

Tetracycline is known as an important antibacterial agent and is widely used in animal husbandry. At present, tetracycline is found in high and varying residual concentrations in soils, sediments, surface, and ground waters, where it is delivered in organic matter of decayed plants and dead animals. However, little research has been done on the adverse biological effects of tetracycline on crops. Similarly, the physiological and biochemical responses of plants to tetracycline-induced toxicity remain to be compared and elucidated. There is a lack of information regarding the limiting concentrations of antibiotics in soil, which will not only inhibit the physiological and biochemical responses of plants, but also allow better adaptation of the technology for growing industrial crops in antibiotic-contaminated soils.

Considering the above, the purpose of this paper is analyzing the effects of different concentrations of tetracycline, which is regarded as one of the most common antibiotics, on some physiological and biochemical parameters of plants using the wheat model. To achieve this purpose, the researchers must accomplish a series of objectives. Firstly, they must choose different concentrations of tetracycline for experimental studies using germinating wheat seeds. Secondly, the study intends to explore the effects of tetracycline concentrations on seed germination, having regard to the growth stimulation and inhibition, with the determination of the optimal dose of the antibiotic. Next, the study aims to assess the main physiological parameters of the growth of wheat seeds and shoots exposed to different concentrations of tetracycline. The last objective is to evaluate the main biochemical parameters of the antioxidative defense system of roots of germinated wheat shoots exposed to different concentrations of tetracycline.

Experimental

Preparation of Solutions

Tetracycline was diluted in distilled water and used at concentrations of 1, 10, 50, 100, 200, and 300 mg/L. All reagents used in the experiment were of analytical grade.

Germination of Wheat Seeds

At the preparatory stage, dry wheat seeds were soaked in distilled water for 14 hours and allowed to germinate on wet filter paper for subsequent use in the experiments. Thereafter, 14% of the wheat seeds in each experimental group were exposed to various concentrations of tetracycline as indicated above. Meanwhile, wheat seeds in the control group (14% or 70 pieces) were also soaked in distilled water. The germination rate of the total number of examined seeds (500 seeds) was measured after 48 hours of tetracycline exposure [18]. To measure the length of the root and buds, other wheat seeds were soaked in distilled water until the seedlings reached a root length of 1.5 mm. Twenty-five wheat seedlings from the seeds soaked in distilled water were treated by tetracycline for 48 hours. The length of the seminal root, buds and shoots were measured after each exposure. Plant height was measured as the shortest distance between the upper part of the main tissues involved in photosynthesis (excluding inflorescences) and the ground level, expressed in centimeters. Root length was measured by contact using photosensitive paper prints with subsequent use of a curvimeter [19]. All experimental samples were kept in a thermostat at 28ºC.

Assessment of Biochemical Parameters

Enzyme solutions were extracted by homogenizing the fresh roots with mortar and pestle in liquid nitrogen after 48 hours of growth. Fresh roots (2 g) were homogenized in 20 mL of an extraction buffer, pH 7.5 [20]. After filtration, the homogenate was centrifuged at 15,000 g for 10 minutes. All manipulations were performed at 4ºC using a chiller. The supernatant was used to analyze enzyme activity. SOD activity was determined based on its ability to inhibit the photochemical reduction of nitro blue tetrazolium [21] and expressed in units per gram fresh weight. CAT activity was measured at 240 nm after H_2O_2 hydrolysis [22]. POD activity was determined spectrophotometrically at 470 nm [23] and expressed in units per minute per gram fresh weight.

Statistical Analysis

The obtained results were tested for reliability using multivariate analysis of variance (MANOVA) and documented using Microsoft Excel software and the Statistica 10 software package [24]. Differences in the results obtained were considered significant at $p \leq 0.05$ according to the Student's t-test.

Results and Discussion

The effects of different concentrations of tetracycline on the germination of seeds, root length and shoot height were heterogeneous. Application of different amounts of tetracycline resulted in either growth inhibition or stimulation (Fig. 1).

As demonstrated above, the concentrations of tetracycline causing the most pronounced decrease in the proportion of germinated seeds ranged from 50 to 300 mg/L. Remarkably, the application of higher concentrations of tetracycline caused further inhibition of the seed germination rate. At the same time,

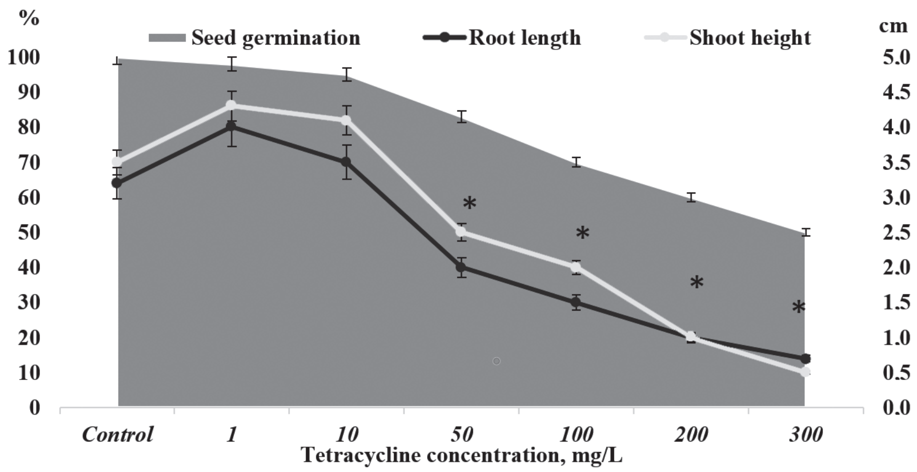

Fig. 1. Physiological parameters of wheat (seed germination (%), root length (cm) and shoot height (cm)) exposed to different concentrations of tetracycline.
Note: *values are statistically significant at p≤0.05.

the proportion of germinated seeds was stable at the concentration of tetracycline amounting to 10 mg/L. The percentage of germinated wheat seeds slightly increased at 1 mg/L.

Tetracycline application at the dose of 1 mg/L had a positive effect on the growth of wheat roots (the root length was 3.8 cm in the experimental group and 3 cm in the control group). Application of higher concentrations of the antibiotic inhibited the growth of wheat roots (Fig. 1). The growth of wheat shoots was the most intensive at the concentrations of tetracycline equal to 1-10 mg/L (Fig. 1). The inhibition of the height of wheat shoots was apparent when tetracycline was applied at the concentration range of 50-300 mg/L, with the most pronounced effect induced by the concentrations of 200 mg/L and higher. Based on the results obtained, it can be assumed that exposure to high concentrations of tetracycline may have a cytotoxic effect and affect the process of cell division. The above information is indicative of the fact that application of higher doses of tetracycline is primarily associated with the root length reduction (Fig. 1). The shoot height and proportion of germinated seeds are inhibited to a lesser degree.

To understand the biochemical mechanism of wheat resistance to tetracycline, it makes sense to measure the activities of enzymes that protect the plant. Thus, the activities of SOD, CAT, and POD in wheat roots were measured (Fig. 2).

High concentrations of tetracycline caused high levels of SOD, POD, and CAT activities in the roots of experimental plants as compared to the control ones. The reason for this phenomenon is unclear. However, more active protection against active oxygen species may indicate a consistently higher level of their formation in the experimental plants exposed to tetracycline. Thus, application of high doses of tetracycline (50-300 mg/L)

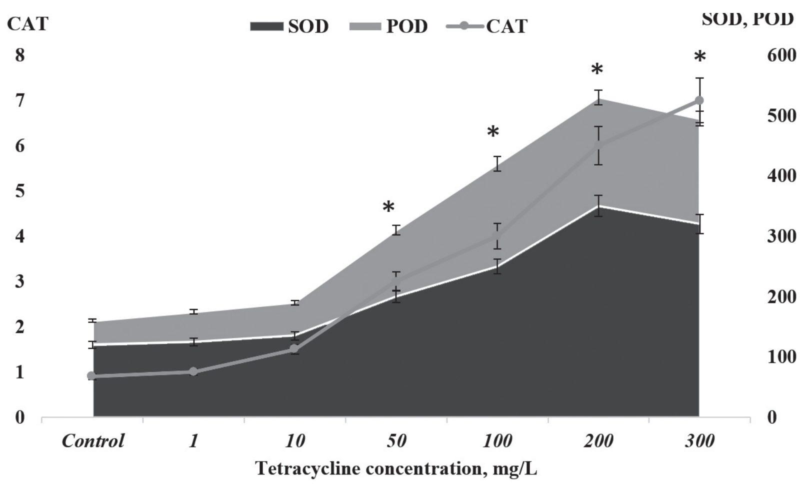

Fig. 2. Biochemical parameters of wheat (the activities of SOD (units/g), CAT (units/min × g) and POD (units/min × g)) exposed to different concentrations of tetracycline.
Note: *values are statistically significant at p≤0.05.

stimulated the synthesis and activities of SOD, CAT, and POD. The activities of these antioxidant enzymes were minimal at the lowest concentrations (1-10 mg/L) of the studied antibiotic.

As shown in Fig. 2, there were three levels of the activity of wheat antioxidative defense system based on the dose of tetracycline applied. At the lowest concentrations of tetracycline (1-10 mg/L), the activities of SOD, POD, and CAT enzymes were quite stable or increased insignificantly. When the investigated antibacterial drug was applied at moderate doses, the activities of the considered enzymes increased after 48 hours of exposure as compared to the control values, and the average increments were 68%, 125% and 154%, respectively, as compared to the control values. At the concentration of 300 mg/L, the increase in the activities of SOD, CAT and POD was significantly fluctuating, and the average increments were 162%, 408% and 326%, respectively, as compared to the control values.

This suggests the enhanced production of reactive oxygen species in the roots of the experimental plants. The study demonstrated the dual effect of antibiotics on the growth of cereal grains using the wheat model. The researchers observed stimulation of germination, growth of wheat shoots and roots at low concentrations of tetracycline. This may be the result of the antiseptic effect of the studied antibiotic, which enables increased water uptake into the seeds and in some cases induces the production of α-amylase [25]. At the same time, the researchers noted inhibition of the wheat growth parameters in question at high doses of the antibiotic ranging from 50 to 300 mg/L. This is indicative of the cytotoxic effect of tetracycline translated into prevention of cell division [26]. Enzyme activity is inhibited, in particular, cyclin-dependent kinase 1 (Cdk1), which is one of the Cdk enzymes that control the cell cycle, which leads to disruption of cell division processes in wheat roots. In addition, tetracycline can provoke the dysfunction of cellular organelles, thanks to a wide spectrum of activity, they penetrate well into eukaryotic cells, which causes most of the manifestations of their toxicity due to blocking the work of mitochondrial ribosomes [27].

Cells may undergo structural changes in the form of plasmolysis and deposition of starch grains, thylakoid lamellae in chloroplasts may be deformed, and vacuoles may increase in size. Also, high doses of tetracycline cause disturbances in plants at different levels:

- damage to chlorophylls a and b,
- a shift in the quantitative ratio of these pigments,
- violation of the path of chlorophyll biosynthesis,
- damage to other photosystem components (in particular, PSII),
- ROS generation,
- violation of electron transfer between both photosystems,
- inhibition and destruction of RuBisCo,
- changes in the number, size, and structure of chloroplasts (inhibition of chloroplast division, formation of macrochloroplasts, reduction of the internal membrane system, disappearance of chloroplast ribosomes),
- in some cases, inhibition of chloroplast DNA,
- replication, transcription and translation, regulation of nuclear genes related to chloroplasts.

Some of these effects are not easily predicted from data on the effects of antibiotics on microorganisms (for example, gyrase inhibitors in some studies affected not only plastid DNA replication but also electron flow to the cytochrome b6/f complex). Failure of the mentioned organelles' functionality leads to a violation of proteins' biosynthesis from mRNA, osmoregulation, storage of reserve substances, the activity of photosynthetic activity of plants, respiration, growth processes, as well as possible delays in plant development [28].

It has been previously shown that higher concentrations of tetracycline can also induce gene mutations and thus affect cell growth and differentiation [16].

It is a known fact that SOD, POD, and CAT are important enzymes of the antioxidative defense system. They are produced in both higher plants and algae for protection from reactive oxygen species and pathogens [29]. In addition, these enzymes, along with other compounds, promote adaptation of wheat to osmotic stress [30]. It has been revealed that the ability of plants (in particular, wheat) to produce significant amounts of SOD, POD, and CAT has been also used in medicine [31]. Species exposed to oxidative stress often form a toxic form of superoxide, which is a central component of the signal transduction mechanism that causes activation of enzymes or enhancement of the expression of SOD-encoding genes [17]. The analysis of the obtained results confirmed the ability of wheat seedlings to adapt to a certain range of tetracycline concentrations by increasing the activity of POD, which is a component of the antioxidative system. Earlier, the effect of oxidative stress on the plant was evaluated depending on the increase in the activity of CAT [32]. CAT was considered in the light of the H_2O_2 removal, with the concentration of H_2O_2 linearly regulating the activity of the mentioned enzyme in a number of cases [33]. High levels of CAT activity are indicative of stress induced by exposure to tetracycline. It is related to the removal of H_2O_2, which causes oxidative damage to wheat cells. For effective neutralization of H_2O_2, which is perceived by cells as a toxic metabolite, POD is activated in the first place, and its insufficient effect is enhanced by CAT, if needed [33, 34].

The ability of wheat to stimulate the antioxidative defense system while adapting to tetracycline-induced stress is confirmed by an increase in the activities of CAT, POD, and SOD [35]. The experiment exhibited that low concentrations of tetracycline (1-10 mg/L) did not significantly affect the metabolic processes in wheat, since low doses of the antibiotic do not promote the production of reactive oxygen species and activation of the antioxidative defense system. Other enzymatic or non-enzymatic mechanisms of plant resistance to antibiotics cannot be excluded [36]. Thus, it is known that exogenous ascorbic acid plays a central role in the non-enzymatic antioxidative defense mechanism. It forms the front line of defense against reactive oxygen species and destructs H_2O_2 [37].

According to the experiment described in this work, the growth of wheat seedlings is inhibited at the concentrations of tetracycline ranging from 50 to 300 mg/L. These data are consistent with the increase in the activities of the antioxidative defense system enzymes under investigation (SOD, CAT, and POD), which prevent oxidative stress in plant [9]. Nevertheless, the researchers assume that this system is not effective enough to prevent the inhibition of wheat cell growth by lipid peroxidation of cell membranes.

Conclusions

This paper describes the effects of different concentrations of tetracycline on the physiological and biochemical parameters of wheat seeds and shoots. In addition, the authors of this article determine the optimal concentrations of tetracycline that can be used to stimulate the growth of wheat. It has been revealed that physiological indicators (including the percentage of germination, the length of roots and shoots) and antioxidant biochemical parameters of wheat (including the activities of SOD, CAT, and POD) changed significantly as tetracycline concentration increased. An increase in seed germination, wheat shoot height and root length occurred when the concentrations of tetracycline were in the range 1-10 mg/L. These parameters were suppressed by tetracycline applied at the concentrations of 50-300 mg/L. In particular, a significant level of inhibition was observed when the concentration of tetracycline was 200 mg/L or higher. At the concentration range of 50-300 mg/L, the inhibition of wheat growth increased in a concentration-dependent manner. Additionally, as the concentration of tetracycline increased, the root length reduction became more pronounced as compared to shoot height and germination frequency. The degree of inhibition followed the order: root length>shoot height >germination frequency.

The study results indicate that wheat bioassay can be used as an effective test for tetracycline toxicity and environmental pollution monitor. The subsequent works should be aimed at the use of the maximum allowable soil concentrations of antibiotics, which stimulate the physiological and biochemical processes in plants. It will foster adaptation of the technology for growing industrial crops in antibiotic-contaminated soils.

Conflict of Interest

The authors declare no conflict of interest.

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