Annals of the University of Craiova
The Chemistry Series
Volume 51, No. 1 (2025) 67-75
homepage: chimie.ucv.ro/anale/
10.52846/AUCCHEM.2025.1.09

Doxycycline toxicity in Brassicaceae plants☆

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Received: 25.10.2025 / Accepted: 30.10.2025 / Published: 06.11.2025

Abstract:
Extensive use and poor waste management of antimicrobials have led to environmental contamination, emergence and spread of resistance, and the compromise of the effectiveness of some antimicrobials. Seeds of two plant species from the *Brassicaceae* family were sown on solidified nutrient solutions supplemented with doxycycline, at concentrations ranging from 5 to 100 μmol/L. Growth parameters and certain biochemical indexes of the plants were monitored for 7 days. Dose-dependent inhibition of seedlings growth, decrease of leaf pigments concentration and increase of peroxidase and catalase activity were observed in the seedling exposed to doxycycline, compared to unexposed ones.

Keywords: doxycycline, plants, leaf pigments, antioxidant response, antibiotic resistance.

☆Paper presented at the XVIIth Edition of the National Chemistry Symposium, Craiova, November 7, 2025

1. INTRODUCTION

Doxycycline is a broad-spectrum antibiotic from the second generation of tetracyclines, whose bacteriostatic activity targets protein synthesys in bacterial cells [1].

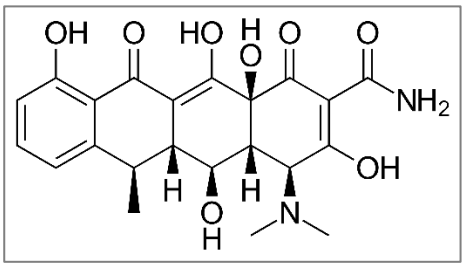

Figure 1. Doxycycline structure [1].

As many other antibiotics, it is used not only to treat infections with sensitive germs, but also to prevent illness and improve feeding in animal husbandry complexes [2]. About 70% of the doses of tetracycline, chlortetracycline, oxytetracycline and doxycycline administered are excreted unchanged by the kidneys [3].

Extended uses of these antibiotics led to environmental contamination and spread of antimicrobial resistance, posing a real threat to antibiotherapy [4, 5].

The experimental study the results of which will be presented below, aimed to investigate some biochemical and growth responses of two plant species from *Brssicaceae* family that grew on doxycycline containing media.

2. MATERIALS AND METHODS

2.1. Biological material and experimental conditions

Seeds of cress (*Lepidium sativum,* Fam *Brassicaceae*) and rocket (*Eruca sativa* sp., Fam. *Brassicaceae*) were purchased from a local store. These plants, whose leaves contain isothyocyanates that give them not

only a distinctive aroma but also health benefits [6], are consumed raw, used for salad or as spices.

The seeds were sown in ϕ 90 mm Petri dishes containing 50 mL of Hoagland nutrient solution solidified with 0.5% agar, 20 seeds per treatment. Part of the nutrient solutions were supplemented with appropriate volumes of a stock solution of 5 mmol/L of doxycycline (DOX) as hyclate ($C_{22}H_{24}N_2O_8 \cdot HCl \cdot 0.5H_2O \cdot 0.5C_2H_6O$, MW=512.94), to achieve the concentrations presented in Table 1.

Plants grown on nutrient media without antibiotic were considered as controls. The Petri dishes were placed under natural lighting conditions, at 21°C in the laboratory.

Table 1. Significance of the experimental variants

Experimental variant	C_{DOX} µmol/L	Final volume/ mL	DOX quantity	
			µg/probe	µg/plant
1 (CONTROL)	-	50	0	0
2	5	50	111.11	5.555
3	10	50	222.22	11.111
4	50	50	1111.1	55.5
5	100	50	2222.2	111.11

2.2. Analysis methods

7 days after planting, the seedlings were harvested, their growth parameters were recorded, and samples were collected for biochemical tests.

Leaf samples weighing about 0.03 g were ground in a mortar with 4 mL of 95% ethanol; two hours thereafter, the samples were centrifuged at 10,000 rpm and 4°C for 10 min, in a Sigma 2-16 K refrigerated centrifuge. Absorption spectra of the obtained extracts were recorded in the range 350-750 nm; the absorbance values were converted into concentrations using Lichtentaler's equations [7].

The obtained results were reported to the mass of leaf tissue used for analysis, and expressed as µg/g F.W.

Protein extracts were also prepared from the plants. Leaf samples weighing about 0.08 g were ground with quartz sand and homogenized with 4 mL of Tris-HCl buffer solution, pH 7.2. After 2 h, the homogenates were centrifuged similarly to the ethanolic extracts. The obtained supernatants were used to assays catalase [8] and peroxidase activity [9], and the concentration of soluble proteins by the biuret method [10].

3. RESULTS AND DISCUSSION

3.1. Growth parameters of the seedlings

The biometric data of the seedlings, presented in Figure 1a and b, showed that, at doxycycline concentration of 5 and 10 μmol/L, stem growth was not inhibited compared to control, while marked inhibition occurred at higher concentrations.

The effective concentration(s) of doxycycline, required for a 50% inhibition of the analyzed parameters compared to control seedlings (EC_{50}) are gathered in Table 2. Sigmoidal regression of the data on growth inhibition of the seedlings pointed out that rocket was more prone to growth cessation than cress, in the given conditions.

Table 2. EC_{50} for doxycycline on growth inhibition of *Brassicaceae* seedlings

ROCKET	lg EC_{50}	EC_{50} μmol/L	CRESS	lg EC_{50}	EC_{50} μmol/L^{-}
root	0.8	6.31	root	1.2	15.85
shoot	1.47	29.51	shoot	1.72	52.84

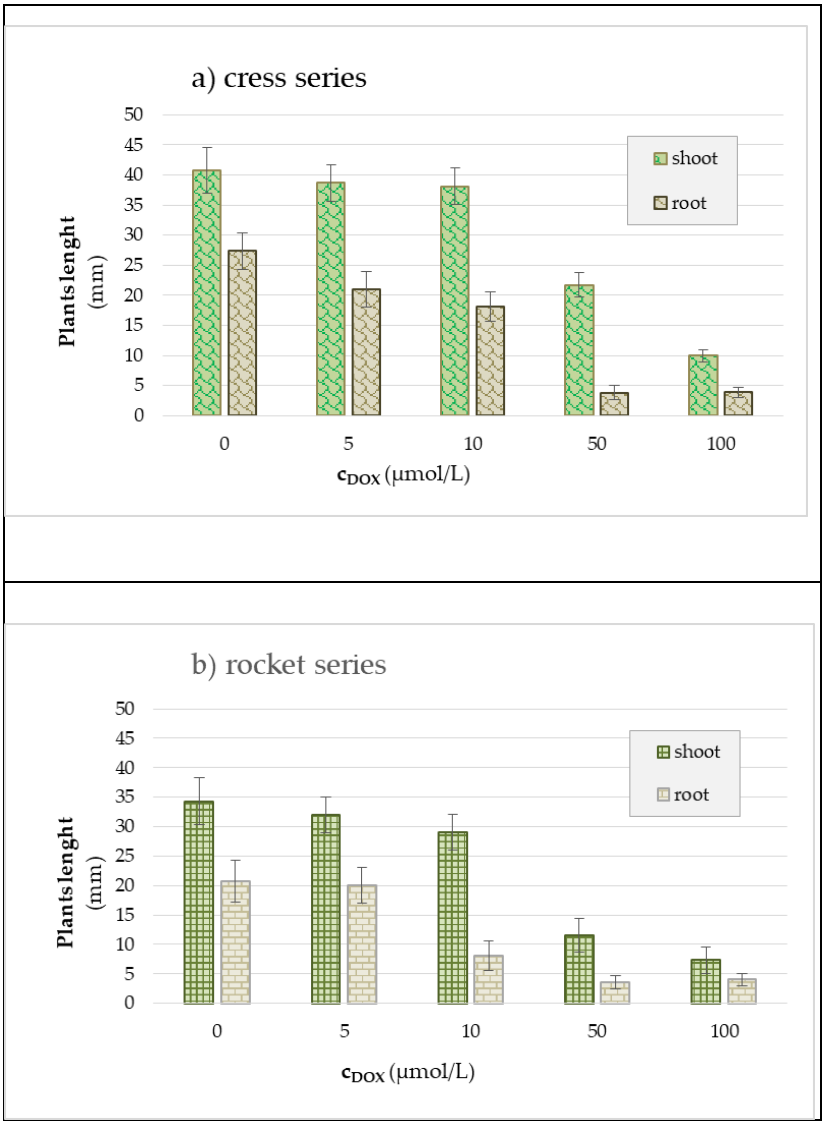

Figure 1. Growth parameters of the *Brassicaceae* seedlings exposed to doxycycline for 7 days.

3.2. Photosynthetic pigments content

The exposure to doxycycline resulted in a dose-dependent decrease of the pigments' concentration in rocket leaves, while in cress the effect was moderate (Figure 2).

Low chlorophyll concentrations were correlated with marked growth inhibition. Reduced height, small leaves and short roots were observed in seedlings exposed to high concentrations of doxycycline, suggesting that structural damage and chloroplast dysfunction occurred.

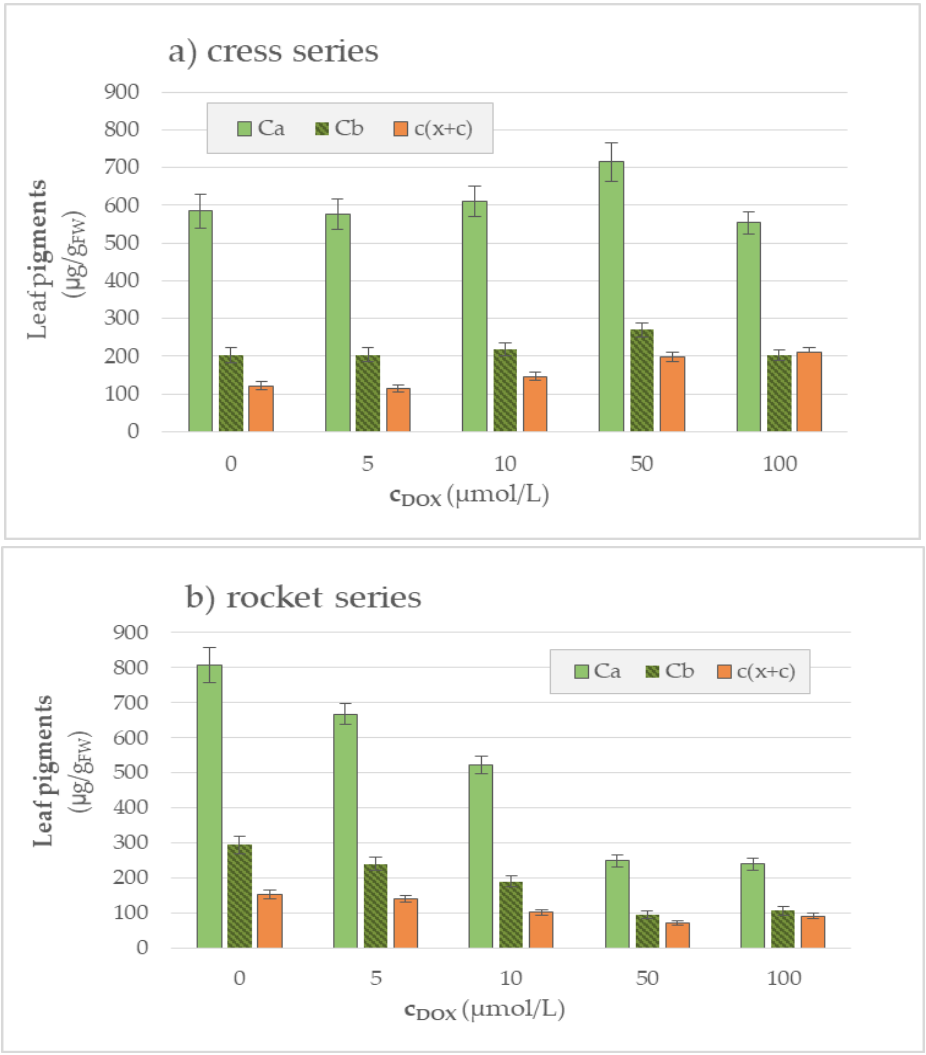

Figure 2. Leaf pigments concentration in the seedlings of two species from *Brassicaceae* family, that germinated and grew for 7 day on nutrient solutions containing doxycycline; (a) cress (*Lepidium sativum* L.); (b) rocket (*Rucola sativa* L.)

3.3. Antioxidant responses

It is known that, at high concentrations, tetracycline antibiotics, as inhibitors of protein synthesis, can also affect mitochondrial function, generating oxidative stress to which plants respond with antioxidants.

The seedlings exposed to DOX at 10 µmol/L showed a moderate increase of catalase activity compared to the unexposed ones (Figure 3).

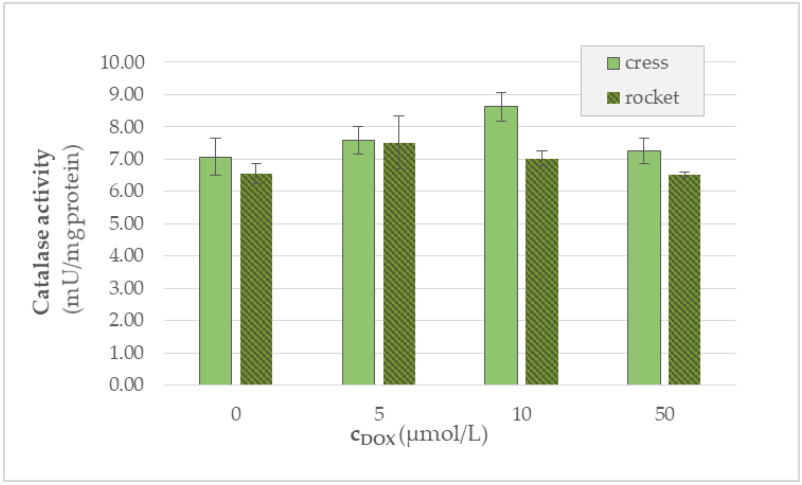

Figure 3. Catalase activity in the leaves of the plants that were exposed to doxycycline.

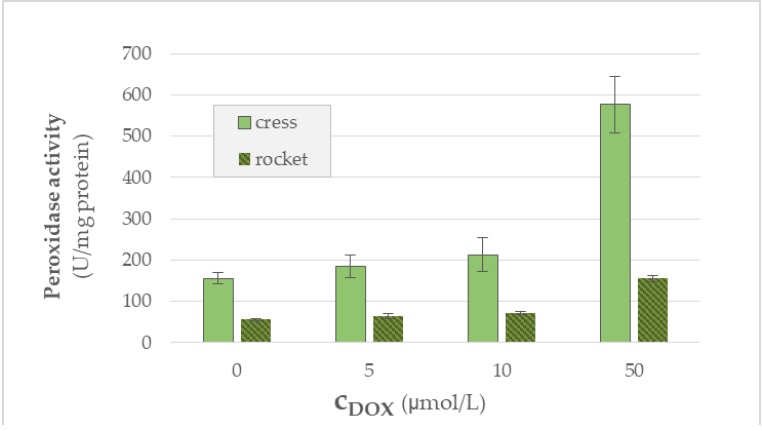

Figure 4 Peroxidase activity in the leaves of the plants that were exposed to doxycycline.

A 22% increase of catalase activity was observed in the leaves of cress plants at 10 μM DOX, compared to control. In rocket plants, approx. 15% increase *vs*. control occurred at 5 μM DOX.

In the absence of doxycycline, peroxidase activity was three fold higher in cress than in rocket seedlings. In both species tested, leaf peroxidase activity increased alongside the concentration of doxycycline (Figure 4).

4. CONCLUSION

Some effects associated with doxycycline exposure on the growth and metabolic status of two plant species in the *Brassicaceae* genus: (garden) cress and arugula (rocket) were observed.

Compared to the plants that weren't exposed to the antibiotic, those grown for 7 days on media supplemented with doxycycline showed a dose-dependent growth inhibition. Exposure to doxycycline was followed by dose-related decreases of the concentration of photosynthetic pigments.

Leaf catalase and peroxidase activities increased after the exposure to doxycycline, indicating an activation of antioxidant defense processes. The obtained results showed that the responses of plants to doxycycline depend on both the level of exposure (*i.e*. doxycycline concentration), and the plant species involved.

The bacteriostatic action of tetracyclines occurs by inhibition of protein synthesis in bacteria by binding to the 30S subunit of the bacterial ribosomes. A similar mechanism might explain doxycycline phytotoxicity. Plants' mitochondria and chloroplasts have their own ribosomes, similar to prokaryotic ones, whose function might be blocked by doxycycline through a similar mechanism as in bacterial cells.

Data on growth and biochemical parameters of the plants in our experiments, clearly highlighted the phytotoxic effects of doxycycline. Contamination of soils and waters with antibiotics and their inevitable entry into food chains can lead to the development and spread of antibiotic resistance and to the disruption of ecological balances.

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