Effects of tetracycline on the secondary metabolites and nutritional value of oilseed rape (Brassica napus L.)

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Abstract

Secondary metabolism, which helps a plant cope with external stress, is sensitive to environmental changes and plays a prominent role in maintaining plant health. However, few studies of the effects of tetracycline on the relationships between secondary metabolism and plant stress responses have been performed. Here, secondary metabolism, nutritional value, and oxidative stress responses in oilseed rape (Brassica napus L.) exposed to tetracycline for 14 days were investigated. Tetracycline inhibited growth and biomass accumulation and decreased the chlorophyll content. The sinapine, phenol, and flavonoid contents were 118.46%, 99.67%, and 93.07% higher, respectively, but the carotenoid content was 76.47% lower in plants exposed to 8 mg/L tetracycline than the control plants. Tetracycline affected the nutritional value of oilseed rape. Tetracycline decreased the dietary fiber, soluble sugar contents, and microelement (Fe, Mn, and Zn) contents. The antioxidant system also responded strongly to tetracycline. The catalase and peroxidase activities were increased and the superoxide dismutase activity was decreased by tetracycline. Tetracycline caused oxidative damage and secondary metabolite disturbances and adversely affected oilseed rape growth and quality. The results provide a new perspective on the effects of tetracycline on plants in relation to secondary metabolites and improve our understanding involved in the toxicity of tetracycline.

Keywords Oilseed rape · Tetracycline · Antibiotics · Secondary metabolism · Nutritional value

Introduction

Monitoring studies increasingly show prevalent contamination of water and soil with antibiotics, and the contamination is linked to antibiotic use (Chen et al. 2018; Li et al. 2011; Oberoi et al. 2019; Van Boeckel et al. 2015; Wang et al. 2021; Zhao et al. 2018). Global annual consumption of veterinary antibiotics in aquaculture and animal husbandry is currently > 63,000 t and is expected to reach 106,000 t by 2030. Larger amounts of tetracycline antibiotics than other antibiotics are produced and used (Van Boeckel et al. 2015; Xie et al. 2010). Between 30 and 90% of an antibiotic consumed by an animal will be excreted in feces and urine as the parent compound or metabolites (Watanabe et al. 2010). As untreated or composted livestock manure is widely used as a fertilizer, a large number of antibiotic residues are found in agricultural soil (Li et al. 2011; Zhao et al. 2018). Long-term exposure to low doses of antibiotics can directly cause toxic effects in plants. Antibiotics are usually absorbed by the roots and transferred to various tissues through transpiration and diffusion, meaning antibiotics can accumulate in crops (Madikizela et al. 2018).

Under antibiotic stress, plants produce excessive reactive oxygen species (ROS) (Riaz et al. 2017). In the meantime, plants have developed complex systems to protect against oxidative damage (Farooq et al. 2013). One such protection mechanism is the enzymatic antioxidant system, which is composed of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and other enzymes (Hegedus et al. 2001). The production of secondary metabolites is another protective mechanism. Secondary metabolites play...
important roles in decreasing the amount of oxidation that occurs and protecting against pathogens and insects by regulating complex signaling pathways (Erb and Kliewenstein 2020; Hiltpold and Turlings 2012). Many secondary metabolites, such as phenolic acids and flavonoids, play important roles in resistance to oxidative stress (Meraj et al. 2020). Flavonoids, sinapines, and other phenolic compounds in oilseed rape plants can protect the plants from ultraviolet B stress (Schiebold et al. 2011). Opris et al. found that exposing wheat plants to nine antibiotics, including tetracycline, markedly increased the amount of monoterpane (a secondary metabolite in wheat) that was produced (Opris et al. 2013). These results indicate that plants counteract the negative effects of environmental stress by undergoing specific metabolic changes.

On the other hand, the nutritional value of plants is known to play important role in biological systems. Dietary fiber can decrease the risk of a human developing several chronic diseases, including cardiovascular disease and some cancers (Dahl and Stewart 2015). Sugar molecules act as nutrients and also regulate metabolism, growth, stress responses, and development (Rolland and Sheen 2005). Plant growth is also controlled by essential trace elements. A deficiency or surplus of a trace element can cause an imbalance between nutrients and may lead to toxic effects (Chen et al. 2015; Jordi et al. 2012). Previous studies have shown that the Ca, Mg, K, and N contents in bean plants were decreased on exposure to antibiotics (Batchelder 1982). However, data on the responses of secondary metabolism and nutrients under environmental pressure are limited.

*Brassica napus* L. is the third most important oil crop in the world and the most important oil crop in China. To better understand how tetracycline stresses plants, we conducted a series of experiments on rapeseed. Morphological characteristics, antioxidant mechanism, ROS, and concentration of different mineral nutrients and secondary metabolites were considered during the vegetative growth phase. The results were expected to improve our understanding of the environmental and ecological effects of antibiotics and allow health risk assessments to be performed and antibiotic safety management systems to be developed.

**Materials and methods**

**Materials**

Oilseed rape (*Brassica napus* L.) plants were obtained from the Zhejiang Academy of Agricultural Sciences. Tetracycline hydrochloride, rutin, and gallic acid were purchased from Shanghai Yuanye Bio-Technology (Shanghai, China). All other reagents were of analytical grade.

**Plant cultivation**

Oilseed rape seeds were soaked in 3% H₂O₂ for 10 min and then rinsed six times with ultrapure water. The seeds were then scattered evenly in seven plastic boxes (each 8 cm × 14 cm). Each box was covered with gauze and then 20 mL of a tetracycline solution was added. Tests were performed using tetracycline at concentrations of 0, 0.25, 0.5, 1, 2, 4, and 8 mg/L. Each box was placed in an incubator at 25 °C in the dark. After 72 h of germination, young seedlings of the same size were selected and each seedling was placed in a 50-mL flat-bottomed centrifuge tube. Each centrifuge tube contained Hoagland nutrient solution containing tetracycline at a concentration of 0, 0.25, 0.5, 1, 2, 4, or 8 mg/L. The centrifuge tubes were placed in an incubator at 23 ± 2 °C and 60% humidity with a 14-h light/10-h dark cycle (light intensity 4500 lx). The nutrient solution (containing the appropriate amount of tetracycline for the test) was changed twice each week. The oilseed rape seedlings were harvested for analysis after 14 days.

**Determining secondary metabolites**

**Determining the total phenolic acid and total flavonoid contents**

The total phenolic acid contents were determined using the Folin–Ciocalteu reagent using a previously published method with some modifications (Ibrahim and Jaafar 2011). First, 0.5 g of an oilseed rape tissue sample was ground with 4 mL of 60% v/v ethanol, then the mixture was placed in a thermostat-controlled water bath at 70 °C for 50 min. The mixture was then centrifuged at 6000 g for 20 min. A 0.5-mL aliquot of the supernatant, 1 mL of Folin–Ciocalteu reagent, and 2 mL of 12% NaCO₃ were added to a container, then the mixture was allowed to stand for 2 h. The absorbance of the solution at 765 nm was then determined using a spectrophotometer (Agilent Technology, USA). Gallic acid was used as a standard to establish a calibration curve. The flavonoid contents were determined using an aluminum chloride colorimetric method (Toor and Savage 2005). A 1-g aliquot of a tissue sample was homogenized with 10 mL of 70% v/v ethanol, then the mixture was centrifuged at 6000 g for 20 min. A 2-mL aliquot of the supernatant, 0.5 mL of 5% NaNO₂, 0.3 mL of 10% AlCl₃, and 2 mL of 4% NaOH were added to a container and then diluted to 10 mL with ethanol. The absorbance of the solution at 510 nm was determined after 12 min. Rutin was used as a standard to establish a calibration curve.
Determining the sinapine contents

A 500-mg aliquot of a plant tissue sample was ground, then 5 mL of 95% ethanol was added. The mixture was ultrasonicated at 50 °C for 30 min, then the mixture was centrifuged at 6000 g for 20 min. The supernatant was passed through a 0.22-μm membrane filter. The extracts were analyzed by high-performance liquid chromatography (Agilent Technology, USA) with ultraviolet detection (Agilent Technology, USA). Separation was achieved using a Hypersil ODS C18 column (Ekite, China). The flow rate, injection volume, and column temperature were 1.0 mL/min, 10 μL, and 35 °C, respectively. Acetonitrile-3% glacial acetic acid (volume ratio 15:85) was the mobile phase. The detection wavelength was 326 nm (Gao et al. 2022).

Determining chlorophyll and carotenoids

Chlorophyll and carotenoids were extracted from 0.1 g of leaf tissue using 10 mL of a 95:5 v/v mixture of ethanol and water. Absorbance at 470, 663, and 645 nm was determined, and the absorbance values were used to calculate the chlorophyll and carotenoid contents of the leaf tissue (Chen et al. 2020).

Phenylalanine-ammonia-lyase activity

The phenylalanine-ammonia-lyase (PAL) activity was determined using a previously published method with slight modifications (Wang et al. 2019). First, 0.5 g of a tissue sample was ground with 2 mL of 0.1 mol/L boric acid buffer (pH 8.7) containing 0.1 g of polyvinylpyrrolidone (to remove phenolic substances that would interfere with the colorimetric determination), then the mixture was centrifuged at 6000 g for 20 min at 4 °C. A 0.5-mL aliquot of the supernatant and 1 mL of L-phenylalanine were then reacted at 30 °C for 60 min. The absorbance at 290 nm was then determined using a spectrophotometer. One unit of PAL activity was defined as the amount of enzyme that caused an increase of 0.01 absorbance units at 290 nm per minute.

Determining the nutritional value

Determining dietary fiber

Insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were determined using an enzymatic gravimetric method described by Bao et al. (Bao et al. 2021). First, 1 g of a sample was mixed with 40 mL of 0.05 mol/L MES–Tris (mixture of 2-morpholinoethanesulfonic acid and trometamol) buffer. Heat-stable α-amylase, protease, and amylglucosidase were added while the mixture was continually agitated. After vacuum filtration, the mixture was washed with 95% ethanol and dried at 105 °C to a constant weight. The obtained product was IDF. Mix the filtrate with 95% ethanol and then dried at 105 °C. Duplicate samples were ashed for 5 h at 525 °C. The isometric crucible method was used to determine the ash content. Non-digestible protein was determined using the Kjeldahl method.

Determining soluble sugar

The soluble sugar contents were determined using the sulfuric acid anthrone method (Chen et al. 2016). First, 0.5 g of a sample was mixed with 5 mL of distilled water in a test tube. The mixture was placed in a water bath at 100 °C for 10 min, then the supernatant was collected. A 0.1-mL aliquot of the supernatant was transferred to a clean centrifuge tube, then 5 mL of anthrone reagent (1 g anthrone dissolved in 1000 mL of 80% concentrated sulfuric acid) was added and the mixture was placed in a thermostat-controlled water bath at 100 °C for 10 min. The mixture was cooled to room temperature, then absorbance at 620 nm was determined.

Quantifying trace elements

The Fe, Mn, and Zn contents of the leaf samples were determined. Each sample was dried at 80 °C for 48 h, then a 0.05-g aliquot of the sample was ground and digested in a mixture of HNO3 and HClO4 at 140 °C. The trace element contents (in mg/kg) were then determined by atomic absorption spectrophotometry (TAS-990, Persee, China) (Zaid and Mohammad 2018).

Measuring ROS production

The ROS level of leaves that had been exposed to tetracycline for 14 d was determined using the 2′,7′-dichlorodihydrofluorescein diacetate probe method (Jin et al. 2020). A 0.5-g aliquot of a sample was ground in 5-mL icy phosphate buffer solution in an ice bath. Then, the mixture was centrifuged at 8000 g for 20 min at 4 °C. A 2.7 mL aliquot of the ROS extract and 0.1 mM 2′,7′-dichlorodihydrofluorescein diacetate (pH 7.6) were mixed and diluted to 3 mL, then the mixture was kept at 37 °C in a water bath for 30 min. The fluorescence was determined using a SpectraMax M5 multilabel microplate reader (Molecular Devices, USA) with excitation and emission filters of 485 and 530 nm, respectively. ROS was expressed as the ratio between the fluorescence intensities for the drug exposure group and the control group.
Antioxidant system response analysis

A 0.5-g aliquot of a leaf tissue sample was ground with 4.5 mL of cold phosphate buffer, then the mixture was centrifuged at 15,000 g for 10 min at 4 °C. The supernatant was used to determine the enzyme activities. The SOD, CAT, and POD activities were determined using kits provided by Nanjing Jiancheng (Nanjing, China). The MDA content was determined using a previously published method with slight modifications (Han et al. 2019). A 0.5-g aliquot of an oilseed rape tissue sample was ground with 5 mL of 10% v/v trichloroacetic acid, then the mixture was centrifuged at 12,000 g for 15 min. A 2-mL aliquot of the supernatant was mixed with 2 mL of 0.6% thiobarbituric acid, then the mixture was heated to 100 °C for 15 min. The mixture was cooled rapidly, then the absorbance at 450, 532, and 600 nm was measured, and the absorbance values were used to calculate the MDA content.

Statistical analysis

Each experiment was performed in triplicate, and each result is presented as the mean ± standard deviation. Analyses of variance were performed to identify statistically significant differences between variables at $P < 0.05$. The LSD test was applied after the analysis of variance using Statistics 25 software to identify interactive effects between treatment factors on the growth and physiological variables. GraphPad Prism software was used to plot graphs.

Results and discussion

Effect of plant growth

The morphological characteristics of the oilseed rape plants exposed to tetracycline were assessed. Tetracycline inhibited oilseed rape plant growth in a dose-dependent manner. In this study, the fresh weights of plants exposed to tetracycline at concentrations of 0, 0.25, 0.50, 1, 2, 4, and 8 mg/L were 1.09, 1.28, 1.03, 0.96, 0.64, 0.45, and 0.32 g, respectively (Fig. 1). Tetracycline at a concentration of 0.25 mg/L clearly promoted growth. These results show that tetracycline can promote growth at low concentrations. But as the concentration increases, it would inhibit the growth of oilseed rape.

The chlorophyll contents of plants are often measured to assess the effects of environmental stress (Ibrahim et al. 2017b). The effects of tetracycline on the chlorophyll a, chlorophyll b, and total chlorophyll (chlorophyll a + chlorophyll b) contents and the chlorophyll a/chlorophyll b ratios for oilseed rape plants exposed to tetracycline for 14 days are shown in Table 1. The total chlorophyll contents of the plants exposed to tetracycline were 33–96% lower than the total chlorophyll contents of the control plants. The chlorophyll a/chlorophyll b ratio was also lower for plants exposed to tetracycline than for the control group plants. This agrees with an analogous reduction in chlorophyll contents in ryegrass plants treated with tetracycline (Han et al. 2019).

Changes in secondary metabolite contents

Secondary metabolism in plants is key to mediating responses to environmental stress. Phenolic acid and flavonoids are very important specialized metabolites that are involved in plant signaling and defense (Jiang et al. 2016); therefore, we first tested the effect of tetracycline on the total phenolic acid flavonoid content in plants. The total phenolic acid and flavonoid contents gradually increased as the tetracycline concentration increased (Fig. 2A, B). For example, the phenol contents (1.13 mg/g) and flavonoid contents (1.19 mg/g) were 118.46% and 99.67% higher, respectively, in the plants exposed to tetracycline at a concentration of 8 mg/L than in the control group plants. Phenolic acids act as antioxidative compounds by acting as reducing agents,
hydrogen donors, singlet oxygen quenchers, and superoxide radical scavengers (Heleno et al. 2015; Riaz et al. 2017). Flavonoids can decrease oxidative damage caused by ROS (Dong and Lin 2021) and therefore protect a plant from oxidative stress.

Besides, sinapine is an important secondary metabolite in cruciferous plants (Fang et al. 2012). Sinapine provides sinapic acid and choline. Coincidentally, sinapic acid is the precursor for the biosynthesis of phenolic compounds such as flavonoids (Milkowski and Strack 2010). As shown in Fig. 2C, all of the treatments stimulated sinapine production. The sinapine contents under 0.25, 0.5, 1, 2, 4, and 8 mg/L tetracycline treatment were 163.91, 176.00, 185.73, 197.73, 213.36, and 300.78 µg/g, respectively, which was 5.22%, 12.98%, 19.23%, 26.93%, 36.96%, and 93.08% higher than the control group (155.78 µg/g). The phenolic group in sinapine has marked antioxidant properties and is effective at scavenging hydroxyl radicals (Thiyam et al. 2006).

The photosynthesis pigment content is a recommended indicator of environmental contamination (Rydzynski et al. 2019). Carotenoids are important lipid-soluble antioxidants. Carotenoids are also integral parts of pigment-binding complexes and are involved in light harvesting and quenching excess energy (Opris et al. 2013). In addition, carotenoids can buffer the number of ROS in plants (Rogers and Munne-Bosch 2016). Exposure to tetracycline for 14 days significantly decreased the carotenoid content (Fig. 2D). The carotenoid contents were markedly higher in the control group plants than in the plants exposed to tetracycline. The carotenoid contents of plants treated with 0.25, 0.5, 1, 2, 4, and 8 mg/L tetracycline were 0.1893, 0.1498, 0.1159, 0.1017, 0.0763, and 0.0664 mg/g, respectively, which were 33.01%, 46.99%, 58.99%, 64.01%, 73.00%, and 76.50% lower than those of the control group 0.2826 mg/g. This was consistent with the results of a study performed by Han et al. (Han et al. 2019). The responses of carotenoids to tetracycline in this paper suggested that they are involved in oxidative stress.

In addition, we quantified the PAL activity in the oilseed rape plants to improve our understanding of how tetracycline increases the secondary metabolite contents. We found that tetracycline significantly induced PAL activity (Fig. 3). Maximum PAL activity (89.98 U/g) was found at a tetracycline concentration of 8 mg/L. PAL is generally recognized as a marker of environmental stress (He et al. 2020). In addition, the PAL activity followed a similar trend to the flavonoid and total phenolic acid contents. The results indicated that tetracycline can increase the phenolic acid and flavonoid contents of oilseed rape plants by inducing PAL enzyme activity. Other researchers also found that the activity of PAL enzyme activity in peach fruit treated with 2,4-epibrassinolide and glycine betaine corresponded to the contents of phenols and flavonoids (Liu et al. 2014; Wang et al. 2019). PAL activity was also induced under exposure to Cu and Cd and increased flavonoid and phenolic acid contents (Ibrahim et al. 2017a). This suggests that PAL is a key enzyme involved in the production of flavonoids and phenols.

### Changes in nutritional value

The effects of tetracycline on the dietary fiber, soluble sugar, and trace element contents are shown in Figs. 4 and 5. Soluble fiber decreases cholesterol concentrations in the blood through several mechanisms. Insoluble fiber causes rapid gastric emptying, so may decrease the intestine transit time and promote digestive regularity (Soliman 2019). The SDF and IDF contents both decreased as the tetracycline concentration increased (Fig. 4). The SDF contents (0.1866 g/100 g) and IDF contents (0.46 g/100 g) were 15.14% and 9.22% lower, respectively, in plants exposed to tetracycline at a concentration of 8 mg/L than in the control group plants. Dietary fiber can influence the bioavailabilities of secondary metabolites by interfering with lipids (Palafox-Carlos et al. 2011). The decrease in DF contents in this experiment may reflect the decrease in the transport of

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### Table 1 The effect of tetracycline on Chl a, Chl b, Chl a+b, and Chl a/b of oilseed rape

| Treatment concentration (mg/L) | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Chlorophyll a+b (mg/g) | Chlorophyll a/b |
|-------------------------------|----------------------|----------------------|------------------------|----------------|
| 0                             | 1.139 ± 0.009a       | 0.262 ± 0.009a       | 1.401 ± 0.017a         | 4.351 ± 0.113a |
| 0.25                          | 0.753 ± 0.012b       | 0.185 ± 0.014ab      | 0.938 ± 0.024b         | 4.074 ± 0.250a |
| 0.5                           | 0.589 ± 0.040c       | 0.156 ± 0.027ab      | 0.712 ± 0.015c         | 3.816 ± 0.368a |
| 1                             | 0.456 ± 0.081d       | 0.156 ± 0.059ab      | 0.646 ± 0.023d         | 3.162 ± 1.014ab|
| 2                             | 0.409 ± 0.075d       | 0.134 ± 0.064b       | 0.542 ± 0.015e         | 3.317 ± 0.815ab|
| 4                             | 0.453 ± 0.041d       | 0.145 ± 0.057ab      | 0.460 ± 0.026f         | 3.331 ± 0.840ab|
| 8                             | 0.253 ± 0.084e       | 0.143 ± 0.120ab      | 0.276 ± 0.021g         | 2.258 ± 0.897b|

Data are the means of three replicates (± SE). Different letters indicate remarkable differences at P < 0.05.
Fat-soluble secondary metabolites, such as carotenoids, as a response to the stress caused by tetracycline.

Soluble sugars can help protect plants before or during oxidative stress (Xiang et al. 2011) because they can directly or indirectly trigger the production of ROS scavengers and/or repair enzymes (Van den Ende and Valluru 2009). In addition, sugar acts as a respiratory substrate to provide energy for a variety of physiological activities in plants (Wang et al. 2019). The soluble sugar contents of leaves were higher in plants exposed to tetracycline at low concentrations than in the control group plants (Fig. 5A). However, the soluble sugar contents of leaves decreased as the tetracycline concentration increased. Both decreases and increases in sugar contents can disturb respiratory metabolism and increase ROS production (Keunen et al. 2013). Zhou et al. (Zhou et al. 2021) found that the sugar content of pakchoi decreased as the Cd and sulfamethazine concentrations increased. The changes in soluble sugar contents were primarily caused by tetracycline affecting the activities of enzymes involved in sugar synthesis, conversion, and metabolism (Sil et al. 2019). Moreover, we hypothesized that the decrease of soluble

Fig. 2 Disturbance of secondary metabolism on oilseed rape by tetracycline. A Total phenolic contents. B Total flavonoid contents. C Sinapine contents. D Carotenoid contents. Data are the means of three replicates (± SE). Different letters indicate remarkable differences at $P < 0.05$

Fig. 3 The effect of tetracycline on the PAL enzyme. Data are the means of three replicates (± SE). Different letters indicate remarkable differences at $P < 0.05$. 

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sugars could provide the substance or energy for phenolic accumulation, which attributed to the phenylpropanoid metabolism pathway and played a critical role in antioxidant capacity.

The Fe, Mn, and Zn contents were significantly lower in the plants exposed to tetracycline than in the control group plants (Figure 5B, C, D). For example, when tetracycline concentrations were 0.25, 0.5, 1, 2, 4, and 8 mg/L, the Zn contents in plants were 7.35, 6.74, 5.59, 4.40, 3.86, and 3.19 mg/kg, respectively, which were 11.87%, 19.18%, 32.97%, 47.24%, 53.71%, and 61.75% lower than those in the control group (8.34 mg/kg), respectively. Fe
deficiency can impair electron transport and induce the production of ROS, leading to oxidative stress (Graziano and Lamattina 2005). Moreover, the leaves of plants turn yellow which is also a typical sign of iron deficiency in plants (Ahammed et al. 2020). This was consistent with our results that plants continue to turn yellow as their iron content decreases. In a previous study, the herbicide imazethapyr decreased trace elements of Arabidopsis thaliana and negatively affected the distributions of various nutrients in the plants (Chen et al. 2015). The concentration of nutrient elements may provide valuable information regarding the health and disease status. The decrease of trace elements indicates that oilseed rape is under stress under the interference of tetracycline. These results suggested that tetracycline reduced the nutritional value of oilseed rape.

**Changes in oxidative stress and antioxidant responses**

Total phenols and flavonoids act as non-enzyme participants in ROS-scavenging mechanisms and share the roles of antioxidant enzymes (Ma et al. 2019). The phenolic group in sinapine has marked antioxidant properties and effectively scavenges hydroxyl radicals (Thiyam et al. 2006). The ROS contents of leaves can be buffered by carotenoids (Rogers and Munne-Bosch 2016). Arab also found that secondary metabolites can contribute to ROS scavenging in plants exposed to O3 (Arab et al. 2022). Therefore, the effects of tetracycline on the ROS level, MDA content, and antioxidant enzyme activities are investigated.

As shown in Fig. 6A, the ROS level increased as the antibiotic concentration increased. The MDA content was higher in plants exposed to tetracycline at concentrations in the range of 0.25–4 mg/L than in the control group plants. The highest MDA content was 9.53 μmol/g when the concentration of tetracycline was 4 mg/L. The MDA content was somewhat lower in plants exposed to tetracycline at a concentration of 8 mg/L (the maximum concentration) than in plants exposed to tetracycline at a concentration of 4 mg/L but was still significantly higher than the MDA content of the control group plants (Fig. 6B). The remarkable changes in the MDA content of the plants exposed to tetracycline were indicative of extensive oxidative damage. If the amount of ROS produced exceeds the scavenging capacity of the antioxidant system, ROS will accumulate in the plant cells and MDA production will be induced. This can damage the cell membranes and organelles and even cause apoptosis (Amjad et al. 2015).

The CAT activity increased significantly as the tetracycline concentration increased. The CAT activity was 2.51 times higher for the plants exposed to tetracycline at a concentration of 8 mg/L than for the control group plants (Fig. 7A). The POD activity followed a similar pattern (Fig. 7B). Similar results were found in a previous study (Han et al. 2021). The increases in the POD and CAT activities indicated that both enzymes effectively scavenged H2O2. POD and CAT can directly catalyze the conversion of H2O2 to H2O and O2 (Gill and Tuteja 2010, Mittler 2002). The SOD activity decreased as the tetracycline concentration increased and reached a minimum at a tetracycline concentration of 8 mg/L (Fig. 7C). The SOD activity may have decreased because of increased consumption or inactivation of the detoxifying enzymes (Mates 2001). SOD is a special enzyme and the main scavenger of O2−(Holley et al. 2014; Peng et al. 2018). The synergistic actions of SOD, POD, and CAT can maintain relatively low free radical concentrations in a plant and therefore decrease the damage caused by ROS to plant cell membranes. With the increase in treatment dose, changes in antioxidant enzymes showed that oilseed rape underwent oxidant stress.

![Graphs showing ROS level and MDA content in tetracycline treatments](image-url)
Conclusion

In conclusion, the disturbance of secondary metabolism, nutritional value, and enzyme system helped elucidate the stress effect of tetracycline on oilseed rape. Tetracycline treatment considerably increased the contents of phenolic acids, flavonoids, and sinapine but decreased the contents of carotenoids. Moreover, the nutritional value of oilseed rape was also affected; specifically, the content of trace elements and dietary fiber decreased, while the contents of soluble sugar initially increased but then decreased. The decrease in DF contents may reflect the decrease in the transport of fat-soluble secondary metabolites, such as carotenoids. The decrease in soluble sugars may provide the substance or energy for phenolic accumulation. Plants respond to negative environmental impacts by regulating limited material resources. The antioxidant system also responded strongly to tetracycline. Specifically, the activities of CAT and POD, and MDA contents were all significantly induced, and the SOD decreased. Antioxidant enzymes and secondary metabolites work together to remove reactive oxygen species. These results provide a perspective on the effects of tetracycline on plants about secondary metabolites and improve our understanding of the mechanisms involved in the toxicity of tetracycline.
Author contribution Mengting Zhao: Conceptualization, methodology, supervision, writing—original draft preparation. Jun Li: Methodology, visualization. Shanshan Zhou: Investigation. Guive Rao: Software, formal analysis. Dongmei Xu: Project administration, writing—reviewing and editing.

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Data availability The data that support the findings of this study are available and should be requested by e-mail.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication All authors have read and approved this manuscript.

Competing interests The authors declare no competing interests.

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