

## ALLELOPATHIC EFFECTS OF THE AQUEOUS LEAF EXTRACT OF *QUERCUS MONGOLICA*

CHENGJUN YANG<sup>1</sup>, WEIZHE SUN<sup>1</sup>, LIU YANG<sup>2</sup> AND HUIYAN GU<sup>1\*</sup>

<sup>1</sup>School of Forestry, Northeast Forestry University, HarBin, 150040, P.R. China

<sup>2</sup>Liaoning Institute of Dryland Agriculture and Forestry, Chaoyang, 122000, P.R. China

\*Corresponding author's email: [ghuiyan@nefu.edu.cn](mailto:ghuiyan@nefu.edu.cn); [nxyycj@163.com](mailto:nxyycj@163.com)

### Abstract

The purpose of this study was to explore whether the scarcity of understory vegetation in *Quercus mongolica* is due to the allelopathy of *Quercus mongolica* leaves. *Quercus mongolica* leaves represented the donor plant, *Brassica pekinensis* and *Raphanus sativus* were the receptor plants, and the allelopathic influences of the aqueous extract of *Quercus mongolica* leaves on the growth and physiological responses of *Brassica pekinensis* and *Raphanus sativus* were investigated. Allelochemicals such as hydrocarbons, alcohols, esters and organic acids were detected by gas chromatography-mass spectrometry. In this research, we investigated the high-concentration aqueous extract (0.10 g/mL) had significant inhibitory effect on the seeds of receptor plants, that the extract had a significant concentration gradient, and that the extract had a bigger stimulatory influence in *Raphanus sativus* than on *Brassica pekinensis*. The malondialdehyde (MDA) content increased with increasing aqueous extract concentrations, except at 0.02 g/mL and 0.04 g/mL. The activity of antioxidant enzymes in recipient plants is greatly inhibited at high aqueous extract concentrations (0.06 g/mL, 0.08 g/mL and 0.10 g/mL) and significantly stimulated at low concentrations (0.02 g/mL and 0.04 g/mL). In summary, the leaves of *Quercus mongolica* have a certain inhibitory effect on its understory vegetation.

**Key words:** *Quercus mongolica*; Allelopathy; Physiological response; Leaf extract; Seedling growth.

### Introduction

Allelopathy is one of the chemical ecological phenomena which has specific chemical substances are released by an organism through various methods, such as leaching, root secretion, volatilization or plant decomposition, into the environment, directly or indirectly influencing the growth of the organism itself or of neighboring organisms (receptors) (Rice, 1984). Most allelochemicals are secondary plant metabolites, including alkaloids, terpenes, phenols, and glucosinolates (Muscolo *et al.*, 2001). Allelopathy is widespread in forest systems, such as among Gramineae, Leguminosae and Cruciferae; members of all these families have allelopathic effects (Blanco, 2007; Cummings *et al.*, 2012; Du *et al.*, 2012; Vladimir & Jacob, 2015). Allelopathy has an important impact on ecosystems. Aqueous extracts of plant organs are usually used to treat other plants to explore allelopathic effects and infer whether the extracts contain allelopathically active substances (Li *et al.*, 2008; Shi *et al.*, 2017).

*Quercus mongolica* is a deciduous tree of the genus *Quercus* in the Fagaceae family. This national second-level protected tree species is the main tree species component of the secondary deciduous broad-leaved forests in Northeast China (Yu *et al.*, 2001; Xu & Wang, 2002; Su *et al.*, 2016). In China, it is mainly distributed in the northeast, north and northwest. *Quercus mongolica* has strong environmental adaptability and has significant effects on soil and water conservation, fire prevention and wind prevention. Because of its hard material, it is a high-quality industrial timber forest resource (Zhang & Liu, 2018). Therefore, research on *Quercus mongolica* is very in-depth. *Quercus mongolica* has strong root tiller reproduction and adaptability, both of which can affect plant diversity. There are few other plants in and around the forests in which *Quercus mongolica* is found that can grow healthily. Research on *Quercus mongolica* has mainly focused on the characteristics of its ecological functioning and community structure, its

physiological characteristics, prediction models of its growth and harvesting, forest management and simulations, its habitat responses and interactions, the spatial patterns of its populations, (Guo *et al.*, 2015; Chen *et al.*, 2018; Liu *et al.*, 2020; Hu & Zhang, 2021). There are few studies on the biochemical characteristics of *Quercus mongolica*, and its allelopathy has not yet been reported. In previous studies, it was found that the aqueous extract of the leaf litter of Sawtooth oak inhibits the germination rate of seeds of the torch tree (Hou *et al.*, 2016). Yue Ming and others found that leaves are the most allelopathic component of *Castanea henryi* (Ming *et al.*, 2020). Li-wei Hou *et al.* found alkanes, alkenes, phenols and other volatile substances in the leaves of *Quercus mongolica* (Hou *et al.*, 2020). However, the allelopathic effects of the leaf extract of *Quercus mongolica* have yet to be reported. The purpose of this study was to investigate the allelopathic effects of the aqueous extract of *Quercus mongolica* leaves (CK, 0.02, 0.04, 0.06, 0.08 and 0.10 g/mL) on the seed germination rates, seedling growth rates and antioxidant defense systems of *Brassica pekinensis* and *Raphanus sativus*. Understanding the allelopathic relationships between *Quercus mongolica* and other plants is helpful to provide a theoretical basis for the resource management and rational utilization.

### Material and Methods

**Plant materials:** The test materials are leaves of 50 to 60 years old wild *Quercus mongolica* that are well-grown, disease-free, and approximately the same in growth. These were collected in August 2019. The receptor materials, *Brassica pekinensis* and *Raphanus sativus* were purchased from the Agricultural Seed Center (Haerbin City, Heilongjiang Province, China).

**Aqueous extract preparation:** First, 100 g of healthy *Quercus mongolica* leaves were weighed, and the leaves were crushed into a powder then placed in a 1500-mL

Erlenmeyer flask. Then, 1000 mL sterile water was added, the leaves were soaked at room temperature for 48 h (and shaken every 12 h for 5 min during this period). The resulting solution was filtered with 4 layers of gauze, the filtrate was collected in a centrifuge tube and after centrifugation, the supernatant was aspirated. Before the test, the extracts were prepared with sterile water to make treatment groups of 0.10, 0.08, 0.06, 0.04, and 0.02 g/mL concentrations.

**Seed germination and seedling growth:** Seeds were selected and sterilized. The seeds were placed evenly in a Petri dish; 5 mL of the *Quercus mongolica* leaf extract with different concentrations was added, and the addition of the extract with the same concentration was repeated 3 times. The seeds were placed in an incubator for the seed germination test. According to the humidity of the filter paper, the treatment solution with the corresponding concentration was replenished every 1 to 2 days to keep the filter paper moist. The standard metric was that the length of the radicle protruding from the seed coat was half the length of the seed. After seed germination became constant (no new seeds had germinated within 5 days), the total number of germinated seeds was counted. And the root length, shoot height and raw weight were measured. Five plants were randomly measured for each treatment, and the average values were obtained. Finally, the comprehensive allelopathic effect was analyzed, such as MDA, CAT, POD and SOD.

**Determination of MDA contents and antioxidant enzyme activities:** The material of newly germinated plant seedlings (0.3 g) was ground and extracted. The homogenate was centrifuged and the supernatant was collected.

The CAT activity was measured by Chen Jianjun method (Chen, 2000). The reaction mixture contained 50 mmol of phosphate buffer (pH 7.0), 100 mmol of hydrogen peroxide and 0.2 ml of enzyme solution. One unit of CAT activity was defined as a decrease of 0.1 in the absorption value per min at 240 nm.

The POD activity was measured by the guaiacol method. This reaction mixture included 2.9 mL phosphate buffer, 1 mL H<sub>2</sub>O<sub>2</sub>, 1 mL guaiacol, 0.1 mL enzyme solution. A change in  $\Delta A_{470}$  of 0.01 per min was considered as one unit of peroxidase activity.

The SOD was measured based on the inhibition of SOD on the enzyme in the photochemical reduction sample of nitrotetrazolium blue chloride (NBT) at 560nm.

The MDA content was measured with thiobarbituric acid. The reaction mixtures were examined at 450, 532 and 600 nm with a UV spectrophotometer.

**GC-MS analysis of leaf extracts:** *Quercus mongolica* leaf powder (2.5 g) was weighed, and 40 mL ethyl acetate was added to the extract. After being sonicated in a water bath for 24 h, the mixture was filtered, and an ethyl acetate extract was obtained after the extraction. The extract was concentrated to dryness at 4°C with a rotary evaporator and then dissolved in 1 mL methanol for the GC-MS detection. The testing instrument was Agilent 7890A-7000B. The chromatographic analysis conditions were as follows: the initial temperature of 40°C was maintained for 2 min; then, the temperature rose to 280°C (maintained for 2 min). Regarding the mass spectrometry analysis conditions, an

EI ionization source, interface temperature of 280°C, ion source temperature of 230°C, and scanning range of 30~500 u were used. NIST98 mass spectrometry database was used to analyze the mass spectrogram and determine the name of each component.

### Statistical analysis

SPSS and Origin software were used for statistical analysis and plotting of the data. Means were compared using the Duncan's test at  $p < 0.05$  level when significant differences were found (Slatni *et al.*, 2011). Williamson's allelopathy response sensitivity index (RI) was used to measure the allelopathy index (Williamson & Richardson, 1988). Among them, C is the control value, T is the treatment value, RI is the intensity of allelopathy, positive is the promoting effect, negative is the inhibition, and its absolute value reflects the strength of allelopathy. Allelopathy composite effect (SE) is the arithmetic mean of allelopathy indexes. The absolute value of the RI is consistent with the intensity of the allelopathic effect (Zhu *et al.*, 2022).

### Results

**Seed germination:** The aqueous extract of *Quercus mongolica* exhibited the same regular allelopathic influence on both *Brassica pekinensis* and *Raphanus sativus*, but *Raphanus sativus* was more sensitive to the extract (Table 1). The aqueous extract of *Quercus mongolica* had obvious and concentration-dependent inhibitory effects. The extract significantly inhibited the germination of *Brassica pekinensis* ( $p < 0.05$ ) at high concentrations (0.04-0.1 g/mL). The germination rate of *Brassica pekinensis* decreased by 34.4% at a high aqueous extract concentration (Fig. 1). The low-concentration (0.02 g/mL) aqueous extract showed a significant inhibitory effect on *Raphanus sativus* ( $p < 0.05$ ). At a high aqueous extract concentration (0.1 g/mL), compared with the control group, the germination rate of *Raphanus sativus* decreased by 70%. It is also worth noting that high aqueous extract concentrations (0.06-0.1 g/mL) completely inhibited *Raphanus sativus* germination. This may be the result of allelopathy affecting the germination and death of *Raphanus sativus*.

**Seedling growth:** The root lengths of the receptor plants were significantly inhibited with increasing aqueous extract concentrations ( $p < 0.05$ ) (Fig. 2). The root lengths of the control group of *Brassica pekinensis* and *Raphanus sativus* were  $9.04 \pm 0.88a$  cm and  $11.9 \pm 1.19a$  cm, respectively; the application of the aqueous extract with the highest concentration (0.1 g/mL) reduced the root lengths to  $0.74 \pm 0.18e$  cm and  $0.0 \pm 0.0$  cm, respectively (Table 2). The *Quercus mongolica* aqueous leaf extract inhibited the growth of *Brassica pekinensis* and *Raphanus sativus*, and *Raphanus sativus* was significantly inhibited at low concentration (0.02 g/mL). At the same time, the raw weight was proportional to the shoot height and root lengths. The raw weight of *Raphanus sativus* and *Brassica pekinensis* decreased continuously with increasing extract concentration. These results indicate that the extract of *Quercus mongolica* leaves inhibits the length of the root from being greater than the shoot height.

**Table 1. Effects of the aqueous extract of *Quercus mongolica* leaves on the germination of *Brassica pekinensis* and *Raphanus sativus* seeds.**

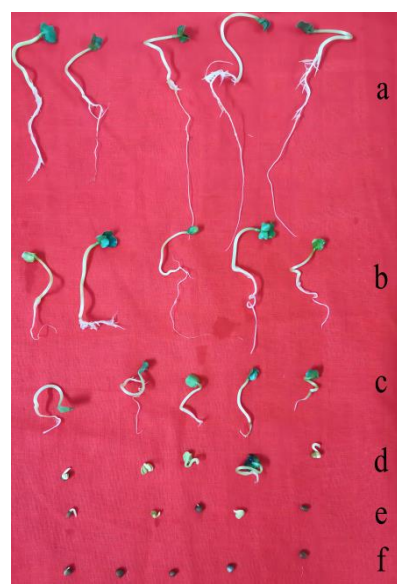
| Receiver plant             | Concentration (g/mL) | Germination percentage (%) | Germination energy (%) | Germination index |
|----------------------------|----------------------|----------------------------|------------------------|-------------------|
| <i>Brassica pekinensis</i> | CK                   | 98.9 ± 1.9a                | 94.4 ± 2.0a            | 50.13 ± 0.86a     |
|                            | 0.02                 | 96.7 ± 0.0ab               | 93.3 ± 3.4ab           | 49.48 ± 0.58a     |
|                            | 0.04                 | 93.3 ± 0.0b                | 88.9 ± 1.9ab           | 47.27 ± 0.58b     |
|                            | 0.06                 | 93.3 ± 0.0b                | 87.8 ± 3.9b            | 47.43 ± 0.29b     |
|                            | 0.08                 | 82.2 ± 5.1c                | 66.7 ± 3.4c            | 39.12 ± 0.71c     |
|                            | 0.1                  | 64.5 ± 3.9d                | 15.5 ± 3.9d            | 24.32 ± 1.21d     |
| <i>Raphanus sativus</i>    | CK                   | 71.1 ± 5.1a                | 52.2 ± 3.8a            | 26.0 ± 2.0a       |
|                            | 0.02                 | 24.4 ± 2.0b                | 8.9 ± 1.9b             | 6.3 ± 1.1b        |
|                            | 0.04                 | 15.5 ± 3.9c                | 2.2 ± 1.9c             | 2.4 ± 1.0c        |
|                            | 0.06                 | 5.6 ± 2.0d                 | 0.0 ± 0.0c             | 0.6 ± 0.3d        |
|                            | 0.08                 | 3.3 ± 0.0d                 | 0.0 ± 0.0c             | 0.3 ± 0.0d        |
|                            | 0.1                  | 1.1 ± 1.9d                 | 0.0 ± 0.0c             | 0.3 ± 0.5d        |

Note: Mean ± Standard deviation

**Table 2. Effects of the aqueous extract of *Quercus mongolica* leaves on the growth of *Brassica pekinensis* and *Raphanus sativus* seedlings.**

| Receiver plant             | Concentration (g/mL) | Root length (cm) | Shoot height (cm) | Raw weight (g)   |
|----------------------------|----------------------|------------------|-------------------|------------------|
| <i>Brassica pekinensis</i> | CK                   | 9.04 ± 0.88a     | 2.78 ± 0.33a      | 0.0322 ± 0.0057a |
|                            | 0.02                 | 5.74 ± 0.66b     | 2.32 ± 0.37ab     | 0.0304 ± 0.0041a |
|                            | 0.04                 | 2.44 ± 0.21c     | 2.42 ± 0.36bc     | 0.0292 ± 0.0033a |
|                            | 0.06                 | 2.02 ± 0.43cd    | 1.98 ± 0.41cd     | 0.0284 ± 0.0082a |
|                            | 0.08                 | 1.72 ± 0.38d     | 1.62 ± 0.19d      | 0.0273 ± 0.0043a |
|                            | 0.1                  | 0.74 ± 0.18e     | 1.00 ± 0.14e      | 0.0174 ± 0.0027b |
| <i>Raphanus sativus</i>    | CK                   | 11.9 ± 1.19a     | 4.44 ± 0.49a      | 0.1285 ± 0.0048a |
|                            | 0.02                 | 4.32 ± 0.96b     | 3.24 ± 0.35b      | 0.0779 ± 0.0200b |
|                            | 0.04                 | 2.12 ± 0.93c     | 2.14 ± 0.59c      | 0.0552 ± 0.0104c |
|                            | 0.06                 | 0.16 ± 0.26d     | 0.72 ± 0.67d      | 0.0204 ± 0.0196d |
|                            | 0.08                 | 0.00 ± 0.00d     | 0.00 ± 0.00e      | 0.0000 ± 0.0000e |
|                            | 0.1                  | 0.00 ± 0.00d     | 0.00 ± 0.00e      | 0.0000 ± 0.0000e |

Note: Mean ± Standar deviation

Fig. 1. Effects of the aqueous extract of *Quercus mongolica* leaves on the germination of *Brassica pekinensis*. a, b, c, d, e, and f represent ck, 0.02 g/mL, 0.04 g/mL, 0.06 g/mL, 0.08 g/mL, and 0.1 g/mL, respectively.Fig. 2. Effects of the aqueous extract of *Quercus mongolica* leaves on the growth of *Raphanus sativus* seedlings. a, b, c, d, e, and f represent ck, 0.02 g/mL, 0.04 g/mL, 0.06 g/mL, 0.08 g/mL, and 0.1 g/mL, respectively.

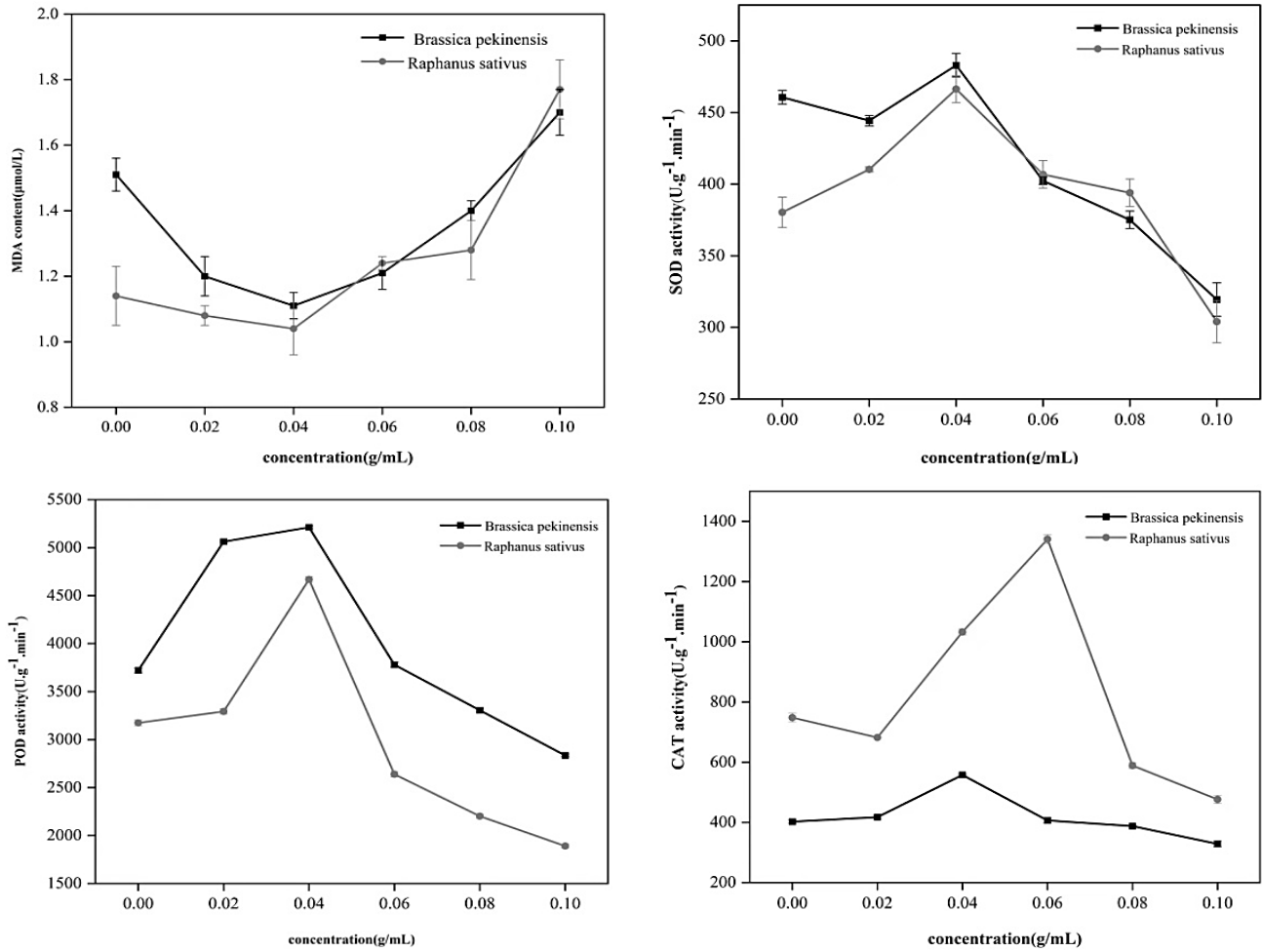


Fig. 3. Effects of the aqueous extract of *Quercus mongolica* leaves on the MDA contents and antioxidant enzyme activities in *Brassica pekinensis* and *Raphanus sativus*.

Malonaldehyde (MDA) content: Malondialdehyde (MDA) can circuitously reflect the intensity of membrane system damage and lipid peroxidation (Hermann *et al.*, 1991). Increases in MDA contents are also related to seed germination and seedling growth, which may be due to chemical damage to cell membranes. In this study, at aqueous extract concentrations of 0.02 g/mL and 0.04 g/mL, the malondialdehyde contents of *Brassica pekinensis* and *Raphanus sativus* seedlings decreased (Fig. 3). To prevent damage to the structures and functions of biofilm, reactive oxygen species were removed by a major antioxidant enzyme. At high aqueous extract concentrations, the MDA contents in seedlings of receptor plants increased with increasing concentrations of leaves (Fig. 3). At the *Quercus mongolica* leaf aqueous extract concentration of 0.1 g/mL, the MDA contents of *Brassica pekinensis* seedlings ( $1.70 \pm 0.07 \mu\text{mol/L}$ ) and *Raphanus sativus* seedlings ( $1.77 \pm 0.09 \mu\text{mol/L}$ ) were 12.58% and 55.26% higher, respectively, than those of the corresponding control seedlings. The malondialdehyde is necessary for antioxidant defense, and allelochemicals can increase plant malondialdehyde levels (Lara-Nuñez *et al.*, 2006; Eldarier, 2009; Song & Song, 2016; Al-Hawas & Azooz, 2018).

Antioxidant enzyme activities: In this study, high *Quercus mongolica* leaf aqueous extract concentrations

inhibited the activity in the studied receptor plants, and the activity of these enzymes was stimulated at lower concentrations, especially in *Raphanus sativus*. At low extract concentrations, the activity of protective enzymes in receptor plants was increased (0.02-0.04 g/mL) (Fig. 3). For example, at these concentration levels, the catalase activity of *Brassica pekinensis* and *Raphanus sativus* increased by 79.20% and 38.61%, respectively. Nevertheless, at the highest concentration, the catalase activity of *Raphanus sativus* and *Brassica pekinensis* decreased by 36.36% and 18.33%, respectively (Fig. 3).

Allelopathic effects: The analysis of the RI that incorporated the germination energy, germination rate, germination index, shoot height, root length, raw weight, antioxidant enzyme activity and MDA content was conducted to evaluate the allelopathic effect of the aqueous extract of *Quercus mongolica* leaves on receptor plants. The RI values of *Raphanus sativus* for the different extract concentrations were negative (-0.36 to -0.64), which obviously showed that different aqueous extracts of *Quercus mongolica* leaves showed inhibitory effects on *Raphanus sativus* (Table 3). At the same time, it also showed inhibitory effect on *Brassica pekinensis* (0.03 to -0.43), but was not sensitive to *Raphanus sativus*, indicating that the aqueous extract of *Quercus mongolica* leaves has different effects on the studied receptor species (Table 4).

**Table 3. Allelopathic influence of the *Quercus mongolica* leaf aqueous extract on the seed germination and seedling growth of *Raphanus sativus*.**

| Index                  | Response index (RI) Concentration (g/mL) |       |       |       |       |
|------------------------|--|-------|-------|-------|-------|
|                        | 0.02                                     | 0.04  | 0.06  | 0.08  | 0.1   |
| Germination percentage | -0.66                                    | -0.78 | -0.92 | -0.95 | -0.98 |
| Germination energy     | -0.83                                    | -0.96 | -1.00 | -1.00 | -1.00 |
| Germination index      | -0.76                                    | -0.91 | -0.98 | -0.99 | -0.99 |
| Root length            | -0.64                                    | -0.82 | -0.99 | -1.00 | -1.00 |
| Shoot height           | -0.27                                    | -0.52 | -0.84 | -1.00 | -1.00 |
| Raw weight             | -0.39                                    | -0.57 | -0.84 | -1.00 | -1.00 |
| MDA content            | -0.05                                    | -0.09 | 0.09  | 0.12  | 0.55  |
| CAT activity           | -0.09                                    | 0.28  | 0.44  | -0.21 | -0.36 |
| POD activity           | 0.04                                     | 0.32  | -0.17 | -0.31 | -0.40 |
| SOD activity           | 0.07                                     | 0.18  | 0.07  | 0.03  | -0.20 |
| Comprehensive effect   | -0.36                                    | -0.39 | -0.51 | -0.63 | -0.64 |

Note: Mean  $\pm$  Standard deviation**Table 4. Allelopathy of the *Quercus mongolica* leaf aqueous extract on the seed germination and seedling growth of *Brassica pekinensis*.**

| Index                  | Response index (RI) Concentration (g/mL) |       |       |       |       |
|------------------------|--|-------|-------|-------|-------|
|                        | 0.02                                     | 0.04  | 0.06  | 0.08  | 0.1   |
| Germination percentage | -0.02                                    | -0.06 | -0.06 | -0.17 | -0.35 |
| Germination energy     | -0.01                                    | -0.06 | -0.07 | -0.29 | -0.84 |
| Germination index      | -0.01                                    | -0.06 | -0.05 | -0.22 | -0.51 |
| Root length            | -0.37                                    | -0.73 | -0.78 | -0.81 | -0.92 |
| Shoot height           | 0.17                                     | 0.13  | 0.29  | 0.42  | -0.64 |
| Raw weight             | -0.06                                    | -0.09 | -0.12 | -0.15 | -0.46 |
| MDA content            | -0.21                                    | -0.26 | -0.20 | -0.07 | 0.11  |
| CAT activity           | 0.04                                     | 0.28  | 0.01  | -0.04 | -0.18 |
| POD activity           | 0.26                                     | 0.29  | 0.02  | -0.11 | -0.24 |
| SOD activity           | -0.04                                    | 0.05  | -0.13 | -0.19 | -0.31 |
| Comprehensive effect   | -0.03                                    | -0.05 | -0.11 | -0.16 | -0.43 |

Note: Mean  $\pm$  Standard deviation**Table 5. The contents of potential allelochemicals in *Quercus mongolica* leaf extract.**

| Retention time (min) | Chemical compound   | Molecular formula | Matching degree (%) | GC content |
|----------------------|---|-------------------|---------------------|------------|
| 3.89                 | 9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyloxy)-1-[[[(trimethylsilyloxy)methyl] ethyl ester, (Z,Z,Z)- | C27H52O4Si2       | 70.60               | 3.09       |
| 15.28                | Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester   | C16H30O4Si3       | 69.00               | 6.83       |
| 16.91                | Xylitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-   | C20H52O5Si7       | 90.00               | 5.34       |
| 18.49                | Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-   | C14H44O6Si7       | 72.00               | 3.61       |
| 18.81                | Tetracosane-2,6,14,18,22-pentaene-10,11-diol, 2,6,10,15,19,23-hexamethyl-                                   | C30H52O2          | 66.66               | 2.13       |
| 21.72                | Dibutyl phthalate Octasiloxane,   | C16H22O4          | 87.80               | 18.33      |
| 22.02                | 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-   | C16H50O7Si8       | 75.30               | 2.97       |
| 25.16                | Benzoic acid, 2, 5-bis (trimethylsiloxy)-, trimethylsilyl ester   | C16H30O4Si3       | 66.90               | 3.13       |
| 26.41                | Octadecane, 3-ethyl-5-(2-ethylbutyl)-   | C26H54            | 69.80               | 4.34       |
| 28.26                | Cyclononasiloxane, octadecamethyl-  | C18H54O9Si9       | 60.10               | 2.40       |
| 28.65                | Tetrapentacontane, 1,5,4-dibromo-   | C54H108Br2        | 69.20               | 8.28       |
| 31.52                | Tetracosane   | C24H50            | 70.10               | 10.44      |
| 35.31                | Pentacosane   | C25H52            | 70.90               | 12.20      |
| 40.36                | Tetracosane   | C24H50            | 67.60               | 9.46       |

Leaf extract analysis: From the results of the GC-MS analysis, it can be seen that there are multiple chemical components in the aqueous extract of *Quercus mongolica* leaves. Among them, there are more chemical components and higher relative content of the following 14 compounds

(Table 5). Including hydrocarbons, alcohols, esters and organic acids. The most abundant substances were Dibutyl phthalate, with a content of 18.33%. The allelochemicals of any single component may not show allelopathic activity but may increase the reaction with other allelochemicals.

## Discussion

Low concentrations of the extract markedly inhibited the two receptor plants used in this study. The observed delay in seed germination and growth may be caused by the entry of water-soluble allelochemicals into seeds (Reddy *et al.*, 2000). A similar decrease in seed germinations has been seen in other plants (Sodaiezadeh & Damme, 2009; Shang *et al.*, 2011; Mahmoud *et al.*, 2016). High concentrations of the extract markedly inhibited the two receptor plants used in this study, and the concentration and intensity are positively correlated. Huang *et al.* found that in low concentration donor extracts, the antioxidant enzyme activity of *Microcystis aeruginosa* increases, while their activity was inhibited when the extract concentration increased (Huang *et al.*, 2013). Liu *et al.* also found that the antioxidant enzyme activities in receptor plants were increased at low extract concentrations but significantly decreased at high extract concentrations. This may be due to the receptor plants are stimulated by the low concentrations of allelochemicals; additionally, antioxidant enzymes resist the stress response caused by oxidation, thereby generating reactive oxygen species and increasing enzyme activity (Bais *et al.*, 2003; Wang *et al.*, 2015). Nevertheless, higher extract concentrations may induce inhibit antioxidant enzymes, resulting in oxidative damage to the plant and ultimately cell death. This phenomenon be compatible with the physiological responses of *Brassica pekinensis* and *Raphanus sativus*. At high extract concentrations, excessive production and accumulation of reactive oxygen significantly inhibited the activity of antioxidant enzymes, and eventually leads to membrane system damage and lipid peroxidation. Therefore, at high extract concentrations the activity of antioxidant enzymes was markedly inhibited.

In our study, the detected substances are mainly hydrocarbons, alcohols, esters and organic acids, and these substances are allelochemicals that have been found in the research report. Yuan-Sen Hu *et al.*, used GC-MS analysis to identify allelochemicals from *Cucumis sativus* L., root exudates mainly benzoic acid, p-hydroxybenzoic acid, ferulic acid, phenylpropionic acid and other derivatives of benzoic acid, and found that these phenolic acid substances had an impact on *Cucumis sativus* L., seed radicle elongation and hyphal growth of *Fusarium oxysporum* (Hu *et al.*, 2007). Li-Mei Han *et al.*, extracted organic compounds such as organic acids, alcohols, esters, ketones, aldehydes, phenols, benzene and hydrocarbons from *Glycine max* (L.) Merrill root exudates, and soybean root exudates could inhibit *Glycine max* (L.) Merrill seed germination (Han *et al.*, 2000). Therefore, it can be inferred that the effect of *Quercus mongolica* leaves on *Brassica pekinensis* and *Raphanus sativus* may be concerned to these high levels of allelochemicals. The allelopathic mechanism of *Quercus mongolica* leaves is still unclear, and this study is expected to provide reference for future studies.

## Conclusions

The results indicated that the aqueous extract of *Quercus mongolica* leaves inhibited the growth of receptor plants. According to the study results of *Brassica pekinensis* and *Raphanus sativus* that the aqueous extract

of *Quercus mongolica* leaves has allelopathic effects and has various degrees of allelopathy. This is the first study on the allelopathy of *Quercus mongolica* leaves. Further utilization of *Quercus mongolica* is necessary to continue to study the characteristics of allelochemicals and the autotoxicity of this species.

## Acknowledgements

This work was supported by the Fundamental Research Funds for the Central Universities (2572020DR02) and Undergraduate Training Programs for Innovations by NEFU (202210225514).

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(Received for publication 5 April 2023)