Grass Research

ARTICLE

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https://doi.org/10.48130/grares-0024-0026

Grass Research **2024**, 4: e028

The evaluation of herbicide tolerance on *Lolium multiflorum*

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Abstract

Lolium multiflorum is widely used to remediate pollutant residues in the environment. The tolerance of plants to pollutants is a key factor affecting the phytoremediation effect and limiting plant application. In this study, 13 herbicides including alachlor, butachlor, metolachlor, imazamox, atrazine, prometryn, fomesafen, quinclorac, flumetsulam, clomazone, isoxaflutole, pendimethalin, and 2,4-D with long residual time and serious environmental toxicity were used for evaluation of the tolerance ability of *L. multiflorum*. The seed germination and plant growth, as well as physiological characteristics of *L. multiflorum* after exposure to different herbicides concentrations were explored. The membership function value method was used to analyze the tolerance of *L. multiflorum* to different herbicides. Results revealed that low-concentration herbicides promoted the seed germination and plant growth of *L. multiflorum*, while high-concentration herbicides performed an inhibitory effect. The antioxidant enzyme activities in plants were increased under herbicide treatment. *L. multiflorum* had strong tolerance to quinclorac and weak tolerance to imazamox, atrazine, clomazone, and isoxaflutole.

Citation: Ma C, Chen J, Wang Y, Jia F, Li Y, et al. 2024. The evaluation of herbicide tolerance on *Lolium multiflorum*. *Grass Research* 4: e028 https://doi.org/10.48130/grares-0024-0026

Introduction

Herbicides are agrochemicals used worldwide for crop protection against weeds, to increase the yields of cultivated fields[1]. Due to their biomagnification and persistence, extensive and frequent use leads to herbicide residues in the soil environment. The migration and diffusion of herbicides may pollute entire ecosystems directly or indirectly through the media of soil, water, and air, causing serious harm to living organisms[2]. Studies showed that the average residue of atrazine in cropland soil of northeast China was 11 μg·kg^{-1}[3,4]. The residues of imidazolinone herbicides like imazapic and imazapyr in the paddy fields of Malaysia were 0.03–0.58 μg·mL^{-1} and 0.03–1.96 μg·mL^{-1}[5]. Fomesafen could be detected in cropland soil, and the residues were about 3.34–67.84 μg·kg^{-1} in soybean fields of Heilongjiang Province, China[6], and 11.20–55.40 μg·kg^{-1} in cropland soil of Jiangsu Province, China[7]. Herbicides not only cause residue in soil but also affect the water environment. Ten triazine herbicide compounds including atrazine and prometryn were detected from 64 stations in the Bohai Sea and the Yellow Sea, with concentrations ranging from 0.27–6.61 nmol·L^{-1}[8]. Different concentrations of ametryn, prometon, prometryn, tebuconazole, and atrazine were detected in the aquatic environments of Shandong Province, China, and ranged from 0.11–8.5 μg·L^{-1}[9].

The removal of the contaminated residues of herbicides has become an important aspect of environmental protection. Phytoremediation is a cost-effective and environmentally friendly technique that uses plants to remediate contaminated soil and water[10]. Phytoremediation technology has been widely used in the remediation of herbicide contamination residues due to its advantages of environmental protection, economic efficiency, and low implementation difficulty[11,12].

Plants' abilities to decontaminate polluted environments rely on their capability to overcome the presence of contaminants. The stronger the tolerance of plants to pollutant stress, the less adverse impact of accumulation pollutants in plant tissues. Therefore, screening out plants that are tolerant to pollutants is important. Turfgrass has strong vitality, reproduction, and regeneration capabilities, and excellent stress resistance[13]. In addition, turfgrass does not enter the food chain and threaten human health, and it can be mowed multiple times, which can better deal with environmental pollution than other plants such as crops[14].

L. multiflorum has an extensive root system and a large biomass, with the ability to withstand multiple mowing, making it a good pollutant remediation species[15,16]. *L. multiflorum* can remove up to 40% of the triazine herbicide terbuthylazine (TBA) in aqueous solutions[15] and can remove 91.5% to 99.5% of sulfonamides (SAs) in piggery wastewater, including sulfadiazine, sulfamethazine, and sulfamethoxazole[16]. *L. multiflorum* has also been found to effectively absorb the dinitroaniline herbicide trifluralin, which binds and metabolizes in plants[17]. Existing studies focus on degradation abilities, metabolic pathways, and remediation mechanisms of *L. multiflorum*[18]. However, the evaluation of the tolerance ability of *L. multiflorum* to herbicides needs further exploration.

In this study, 13 herbicides with long residual time and serious environmental toxicity were selected to apply to *L. multiflorum*. The tolerance of *L. multiflorum* to different herbicides was analyzed, and the types of herbicides with relatively strong and weak tolerance of *L. multiflorum* were obtained. The results not only provide data support and a theoretical basis for using *L. multiflorum* to remede herbicide residues, but also lay clues for research on the remediation effect of turfgrass on herbicides.

Materials and methods

Materials

The *L. multiflorum* material used in this experiment was sourced from The Forage Seed Laboratory, China Agricultural University (Beijing, China). The names, purity, and sources of herbicides used in this study are shown in Table 1. The action mechanisms, application places, and target plant categories of different herbicides are shown in Supplementary Table S1.

Seed pretreatment and germination experiment

The seeds of *L. multiflorum* were soaked in 10% sodium hypochlorite solution (NaClO) for 15 min. After soaking, the seeds were rinsed with distilled water until the cleaning solution is no longer turbid, and then air dried naturally.

Top of Paper Method (TPM) was chosen for the germination test. The agents were added to the petri dish and the filter paper was wet. The concentration settings were as follows:

Single-agent exposure treatment: 13 herbicides were added separately with concentrations of 0 (CK), 10, 30, 50, 100, 200, 600, 1,200, and 2,400 μg·L^{-1}.

Combined dose exposure treatment: Added 13 herbicides at 1, 5, and 10 μg·L^{-1} respectively for mixing, for a total of three combined dose concentrations.

The sterilized *L. multiflorum* seeds were placed evenly in a petri dish with a diameter of 9 cm, 50 seeds in each dish. The amount of herbicide added to the petri dish was 8 mL, and the petri dishes were sealed with sealing film to reduce the evaporation of the agent. Each treatment was four replicates. The petri dishes were placed in an intelligent low-temperature light incubator (DWGZ-500E2, Hefei Youke, Hefei, Anhui Province, China) controlled at 27 mmol·m^{-2}·s^{-1} photosynthetically active radiation at temperatures of 25/15°C (day/night) with 70%–80% relative humidity in an 8-h photoperiod for germination.

Table 1. Purity and source of tested herbicides.

Herbicide name	Standard purity	Source
Alachlor	98.7%	Shanghai Pesticide Research Institute Co., Ltd
Butachlor	98.4%	Shanghai Pesticide Research Institute Co., Ltd
Metolachlor	99.3%	Shanghai Pesticide Research Institute Co., Ltd
Imazamox	98.3%	Shenyang Shenhua Institute Testing Technology Co., Ltd
Atrazine	97.1%	Sigma-Aldrich (Shanghai) Trading Co., Ltd.
Prometryn	99.4%	Shanghai Pesticide Research Institute Co., Ltd
Fomesafen	99.0%	Shanghai Pesticide Research Institute Co., Ltd
Quinclorac	99.0%	Shanghai Pesticide Research Institute Co., Ltd
Flumetsulam	98.0%	Sigma-Aldrich (Shanghai) Trading Co., Ltd.
Clomazone	98.4%	Shanghai Pesticide Research Institute Co., Ltd
Isoxaflutole	98.6%	Sigma-Aldrich (Shanghai) Trading Co., Ltd.
Pendimethalin	98.2%	Shanghai Pesticide Research Institute Co., Ltd
2,4-Dichlorophenoxyacetic acid	99.1%	Shenyang Shenhua Institute Testing Technology Co., Ltd

The radicle breakthrough seed coat of 1 mm was used as the seed germination standard[19]. Seed germination parameters were recorded every day until the 7th day.

$$\text{Germination rate (\%)} = \frac{\text{No. of germinated seeds within 7 d}}{\text{Total no. of tested seeds}} \times 100\%$$

$$\text{Germination potential (\%)} = \frac{\text{No. of germinated seeds within 3 d}}{\text{Total no. of tested seeds}} \times 100\%$$

$$\text{Germination index} = \sum G_t/D_t$$

$$\text{Average germination speed (d)} = \sum(G_t \times D_t)/\sum G_t$$

where, G_t is the number of seeds germinated on the day corresponding to D_t; D_t is the number of germination days, d.

Hydroponic exposure treatment

The *L. multiflorum* seeds were sown in a square plastic flowerpot with a bottom area of 10 cm × 10 cm. The culture medium was vermiculite. 1/4 Hoagland nutrient solution was used for cultivation and watered once every 3 d, and the concentration of the nutrient solution was gradually increased until it reached 100%. After the seedlings grew steadily and the tillers were uniform after 30–40 d, the roots were rinsed with water until there was no vermiculite impurities present. The seedlings with consistent growth were selected and transplanted into a hydroponic container. Seedlings were wrapped at the base part of the tillers using a foam cube, inserted in a polystyrene sheet which was placed over the Hoagland's nutrient solution in a container (12.5 cm × 8.5 cm × 12 cm). There were 12 seedlings in each container and three containers each treatment. A total of 129 containers of seedlings were used in this experiment. The hydroponic container after transplanting was transferred to an intelligent artificial climate box (RXZ-430c, Ningbo Jiangnan, Ningbo, Zhejiang Province, China). The incubator was set at 750 μmol·m^{-2}·s^{-1} at temperatures of 25/15°C (day/night) and cultured for 7–10 d for adaptation.

The plants to be treated were uniformly cut to a height of 10 cm, and herbicides were added to the hydroponic container according to different concentrations.

Single-agent exposure treatment: 13 herbicides were added separately, and three single-agent concentrations of 0 (CK), 300, 600, and 1,200 μg·L^{-1} were set.

Combined dose exposure treatment: 13 herbicides were added with 10, 50, and 100 μg·L^{-1} respectively for mixing, for a total of three combined dose concentrations.

Each treatment was repeated three times. Nutrient solution was added to the hydroponic container every 1 d to the same content as that at day 0. The exposure experiment was treated for 5 d.

Physiological measurements

Twenty plants were randomly selected from each treatment, and the length from the upper part of the crown to the tip of the leaf of *L. multiflorum* was measured with a ruler, and the data were recorded as plant height, and the average values were calculated[20].

The aluminum box was placed in the oven at 100-105 °C for 2 h to a constant weight, then weighed (m_0). Ten plants were randomly selected from each treatment, washed with deionized water, cut into pieces, and weighed in an aluminum box (m_1). The aluminum box containing the plant was placed in the oven at 50–60 °C for 3–4 h (ventilation), 100–105 °C oven for 3–4 h (no ventilation), repeated three times, and weighed after

cooling (m_2). Then the leaf dry matter content (LDMC) was calculated according to the equation:

$$LDMC = \frac{m_2 - m_0}{m_1 - m_0} \times 100\%$$

Leaf chlorophyll was extracted by soaking fresh leaf tissues in 9 mL 95% ethanol. After full extraction, the absorbance of the leaf extract was measured at 665, 649, and 470 nm using a UH5300 UV spectrophotometer (HITACHI, Japan). The content of chlorophyll was calculated using the following equations[21]:

$$C_a\ (mg \cdot L^{-1}) = 13.95A_{665} - 6.88A_{649}$$

$$\text{Chlorophyll a content}\ (mg \cdot g^{-1}) = C_a \times V/W$$

$$C_b\ (mg \cdot L^{-1}) = 24.96A_{649} - 7.32A_{665}$$

$$\text{Chlorophyll b content}\ (mg \cdot g^{-1}) = C_b \times V/W$$

$$C_{chlorophyll}\ (mg \cdot L^{-1}) = C_a + C_b = 6.63A_{665} + 18.08A_{649}$$

$$\text{Chlorophyll content}\ (mg \cdot g^{-1}) = C_{chlorophyll} \times V/W$$

$$C_{carotenoid}\ (mg \cdot L^{-1}) = (1{,}000A_{470} - 2.05C_a - 114.8C_b)/245$$

$$\text{Carotenoid content}\ (mg \cdot g^{-1}) = C_{carotenoid} \times V/W$$

where, C_a: chlorophyll a concentration; C_b: chlorophyll b concentration; $C_{chlorophyll}$: chlorophyll concentration; $C_{carotenoid}$: carotenoid concentration; V: extraction liquid volume (L); W: weighed sample mass (g).

The minimum fluorescence intensity (*Fo*), the maximum fluorescence intensity (*Fm*), the potential photochemical activity (*Fv/Fo*) and the actual photochemical efficiency (*Fv/Fm*) of PS II were determined to estimate leaf photochemical efficiency using a OS30p + handheld chlorophyll fluorometer (OPTI-SCIENCES, USA). Leaf clips were used to adapt leaves in darkness for 30 min prior to the measurement with the fluorescence meter.

Superoxide dismutase (SOD) activity was measured by the nitroblue tetrazolium method (NBT)[21]. The amount of enzyme that inhibited 50% of the NBT photoreduction within a unit time was taken as 1 enzyme activity unit (U). Peroxidase (POD) activity was measured using the guaiacol method[21]. Taking a change of A_{470} by 0.01 per minute as 1 enzyme activity unit (U). Catalase (CAT) activity was measured by ultraviolet spectrophotometry[21]. The absorbance change of 0.001 per minute per gram of fresh weight (FW) sample was taken as one CAT activity unit (U). Ascorbate peroxidase (APX) activity was measured using the method of Chen[22]: 0.5 g of the material was added to the pre-cooled extract (50 mmol·L^{-1} K_2HPO_4-KH_2PO_4 buffer, pH 7.0 containing 2 mmol·L^{-1} AsA and 0.1 mmol·L^{-1} EDTA-Na_2) at a ratio of 1:5. After grinding, the extract was centrifuged at 10,000 g for 10 min, and the supernatant was the crude enzyme extract. PBK (pH 7.0) 1.8 mL, AsA 100 μL, extract 100 μL, and H_2O_2 1 mL were added to form a reaction system. The change in OD value within 90 s was measured at 290 nm immediately, and the enzyme activity was calculated.

Statistical analysis

SPSS 27 (IBM, USA) and Excel 2019 (Microsoft, USA) were used for statistical analyses. ANOVA and Duncan's multiple range tests were used to analyze significant differences at a probability level of 0.05.

Results

Effect of herbicides on seed germination ability of *L. multiflorum*

The seed germination index can reflect the germination ability and germination rate comprehensively. Except for the most treatment concentrations of butachlor, quinclorac, and flumetsulam, which had no significant effect on the germination index of *L. multiflorum* seeds, many single-agent treatments of herbicides showed a promoting effect at low concentrations (10, 30, 50 μg·L^{-1}) and medium concentrations (100, 200 μg·L^{-1}), and an inhibitory effect at high concentrations (600, 1,200, and 2,400 μg·L^{-1}) as shown in Table 2. The highest germination index reached 65.57 (10 μg·L^{-1}, 2,4-D), which was 20.24% higher than the control. The lowest was only 9.08 (2,400 μg·L^{-1}, alachlor), which was 83.35% lower than the control. Overall, the germination index of *L. multiflorum* seeds was more sensitive to alachlor and metolachlor, but better tolerance to quinclorac. The mixed agent treatment of three concentrations all had a positive effect on the seed germination index compared to the control with average seed germination index of 65.9, 67.2, and 60.2 at the mixed agent levels of 1, 5, and 10 μg·L^{-1}, separately.

Germination ability refers to the percentage of normally germinated seeds in the total number of tested seeds in the initial stage of the germination test, which can measure the level of seed vigor. The single-agent herbicide treatments showed an overall inhibitory affect on the germination ability of *L. multiflorum* as shown in Table 3. The higher the concentration, the more obvious the inhibitory effect. The highest germination energy reached 95.00% (50 μg·L^{-1}, pendimethalin), which was 3.83% higher than the control. The lowest was only 3.5% (2,400 μg·L^{-1}, alachlor), which is 96.17% lower than the control. At the same time, it was also significantly lower than other concentration treatments and other herbicide treatments. In addition, high concentrations of alachlor, metolachlor, clomazone, and isoxaflutole significantly inhibited effects on germination, and the mixed agent treatment of three concentrations had an inhibitory effect on seed germination ability compared to the control with average seed germination ability of 89.00, 85.50, and 78.00 at the mixed agent levels of 1, 5, and 10 μg·L^{-1}, separately.

The average germination speed is a negative indicator of seed tolerance during the germination period. The higher the average germination speed, the worse the seed germination condition. Overall, low and medium concentration herbicide treatments shortened the average germination speed of *L. multiflorum*, and high concentration herbicide treatments prolonged its average germination speed, while imazamox had no significant effect (Table 4). The results showed that lowest average germination speed was 4.74 (10 μg·L^{-1}, 2,4-D), which was 4.82% lower than the control. The highest reached 5.50 (1,200 μg·L^{-1}, metolachlor), which was 10.45% higher than the control. The seed germination speed after being treated with high concentrations of alachlor, metolachlor, clomazone, and isoxaflutole was significantly higher than other concentration treatments and other herbicide treatments. The mixed agent treatment of three concentrations all had an inhibitory effect on the seed germination speed compared to the control with average seed germination speed of 4.72, 4.69, and 4.75 at the mixed agent levels of 1, 5, and 10 μg·L^{-1}, separately.

Table 2. Effects of different herbicides on germination index of *L. multiflorum*.

Herbicide	Concentration (μg·L^{-1})								
	0	10	30	50	100	200	600	1,200	2,400
Alachlor	54.53 ± 0.96a	60.03 ± 1.19aB	60.82 ± 2.09aB	58.27 ± 2.03aBC	57.66 ± 4.22aABC	57.36 ± 4.42aAB	30.07 ± 9.88bD	12.51 ± 4.52cE	9.08 ± 1.21cE
Butachlor	54.53 ± 0.96b	58.91 ± 1.13aCD	54.26 ± 2.96bCD	53.51 ± 1.73bC	56.04 ± 5.10abC	51.17 ± 2.75bcB	58.53 ± 3.73abB	52.05 ± 4.17bAB	43.03 ± 4.31cBCD
Metolachlor	54.53 ± 0.96ab	57.28 ± 1.85aD	58.85 ± 1.35aC	58.23 ± 1.27aBC	55.72 ± 3.87aABC	51.37 ± 1.04bB	51.30 ± 4.07bBC	27.25 ± 2.14cD	17.72 ± 4.93dE
Imazamox	54.53 ± 0.96ab	56.87 ± 2.11abD	56.86 ± 2.26abCD	58.88 ± 2.87aAB	57.76 ± 3.48abABC	50.40 ± 0.94bcB	55.66 ± 5.23abBC	53.49 ± 10.60abABC	43.66 ± 7.11cBCD
Atrazine	54.53 ± 0.96cd	60.90 ± 2.49abB	64.65 ± 0.80aA	58.11 ± 1.73bcBC	57.41 ± 0.69bcABC	56.68 ± 1.69bcAB	52.08 ± 5.00dBC	51.80 ± 4.85dAB	41.46 ± 4.00eCD
Prometryn	54.53 ± 0.96cd	64.11 ± 2.33aAB	63.18 ± 2.11aAB	61.09 ± 2.27abAB	60.30 ± 4.22abAB	59.86 ± 3.06abA	56.65 ± 3.80bcB	50.40 ± 2.13dB	41.95 ± 5.57eBCD
Fomesafen	54.53 ± 0.96c	63.12 ± 1.66aABC	58.45 ± 2.13bcCD	62.72 ± 2.27aAB	60.03 ± 2.37abABC	55.64 ± 1.43cAB	49.12 ± 3.83dC	45.94 ± 3.23dC	39.84 ± 4.47eCD
Quinclorac	54.53 ± 0.96b	57.92 ± 0.72bD	58.27 ± 1.89bCD	57.85 ± 5.26bBC	55.70 ± 2.50bABC	56.73 ± 1.67bAB	62.57 ± 3.65aA	57.96 ± 2.78bA	57.24 ± 3.66bA
Flumetsulam	54.53 ± 0.96b	59.05 ± 3.03bB	58.97 ± 1.51bC	63.90 ± 1.23aA	58.88 ± 4.21bABC	58.36 ± 3.48bAB	56.46 ± 3.89bB	56.20 ± 2.47bA	48.13 ± 4.20cB
Clomazone	54.53 ± 0.96b	59.06 ± 8.76abAB	63.22 ± 2.07aAB	56.57 ± 4.36abBC	61.05 ± 3.93aA	53.75 ± 3.96bB	47.38 ± 1.39cC	43.38 ± 2.90cdC	36.97 ± 3.15dCD
Isoxaflutole	54.53 ± 0.96b	56.89 ± 1.30abD	55.82 ± 0.86bD	60.35 ± 2.51aB	60.76 ± 6.40aABC	53.24 ± 1.60bcB	49.42 ± 1.78cC	43.44 ± 1.52dC	36.15 ± 4.15eD
Pendimethalin	54.53 ± 0.96ab	58.40 ± 3.44aCD	56.21 ± 1.83abD	58.02 ± 1.32aBC	56.03 ± 2.60abABC	54.76 ± 2.10abAB	51.55 ± 5.90bcBC	48.60 ± 7.90cABC	42.53 ± 3.09dBC
2,4-Dichlorophenoxyacetic acid	54.53 ± 0.96e	65.57 ± 1.97aA	58.74 ± 0.67cdCD	61.89 ± 3.12bAB	60.01 ± 2.60bcABC	56.61 ± 1.71deAB	49.06 ± 2.55fC	43.92 ± 1.89gC	44.07 ± 1.29gB

Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

Table 3. Effects of different herbicides on germination ability of *L. multiflorum*.

Herbicide	Concentration (μg·L^{-1})								
	0	10	30	50	100	200	600	1,200	2,400
Alachlor	91.50 ± 3.42a	88.00 ± 3.65aABC	94.00 ± 2.83aA	90.50 ± 5.00aAB	90.50 ± 7.72aAB	92.00 ± 5.89aA	30.00 ± 12.00bE	9.50 ± 9.57cD	3.50 ± 4.44cE
Butachlor	91.50 ± 3.42a	90.50 ± 1.92abBC	89.00 ± 7.39abcAB	87.00 ± 4.16abB	84.50 ± 5.26bcABC	80.00 ± 2.83cdBC	77.50 ± 12.48bcdAB	76.50 ± 4.12cdA	60.00 ± 11.89eB
Metolachlor	91.50 ± 3.42ab	94.50 ± 1.00aA	93.00 ± 2.58aAB	91.50 ± 4.12abAB	90.00 ± 8.00abAB	81.00 ± 5.29BcABC	72.00 ± 15.92cABC	4.00 ± 2.31eD	15.00 ± 8.41dE
Imazamox	91.50 ± 3.42a	87.50 ± 3.42abBC	80.00 ± 5.89bcC	78.00 ± 3.27bcD	79.00 ± 5.29bcC	71.00 ± 5.03cD	76.50 ± 7.72cB	74.00 ± 4.32cA	58.50 ± 12.79dB
Atrazine	91.50 ± 3.42a	86.50 ± 4.44aC	86.50 ± 3.00aBC	87.00 ± 3.83aAB	87.00 ± 3.83aABC	87.50 ± 3.79aA	69.50 ± 7.72bBC	72.50 ± 5.75bA	48.00 ± 7.12cBC
Prometryn	91.50 ± 3.42a	87.00 ± 5.77abC	86.00 ± 4.32abBC	78.50 ± 3.42abD	88.50 ± 3.42aAB	78.50 ± 7.72abBC	84.00 ± 10.95abA	72.50 ± 7.72bA	54.00 ± 20.46cB
Fomesafen	91.50 ± 3.42a	87.50 ± 5.51abABC	90.50 ± 4.73aAB	90.00 ± 1.63aAB	81.00 ± 4.76abBC	76.50 ± 2.52bC	47.50 ± 17.69cCDE	38.00 ± 7.12cdC	33.50 ± 7.00dD
Quinclorac	91.50 ± 3.42a	84.00 ± 5.42abBC	89.00 ± 2.58aAB	86.00 ± 8.17abABC	85.00 ± 5.77abABC	84.50 ± 4.44abAB	90.50 ± 3.42aA	85.00 ± 11.83abA	77.00 ± 6.22bA
Flumetsulam	91.50 ± 3.42a	89.50 ± 8.06aABC	88.50 ± 2.52abB	90.00 ± 3.27aAB	86.00 ± 5.42abABC	84.50 ± 7.19abABC	80.00 ± 12.11abA	76.00 ± 8.49bA	59.00 ± 13.52cB
Clomazone	91.50 ± 3.42a	80.50 ± 11.36bAB	87.50 ± 6.61abB	83.00 ± 5.77abD	88.50 ± 5.75abABC	82.00 ± 5.42abABC	45.00 ± 7.75cDE	28.00 ± 1.63dC	15.00 ± 4.76eE
Isoxaflutole	91.50 ± 3.42a	90.50 ± 1.92aBC	87.50 ± 3.42aBC	91.50 ± 4.12aAB	86.00 ± 8.17aABC	85.50 ± 4.73aAB	54.00 ± 2.83bCD	26.00 ± 8.17cC	12.00 ± 2.83dE
Pendimethalin	91.50 ± 3.42a	89.50 ± 1.92aABC	90.00 ± 5.89aAB	95.00 ± 2.58aA	90.00 ± 5.89aAB	87.00 ± 2.58aA	67.00 ± 20.69bABC	54.00 ± 32.54bB	38.00 ± 6.53cCD
2,4-Dichlorophenoxyacetic acid	91.50 ± 3.42a	92.00 ± 3.65aAB	87.50 ± 5.26aBC	87.50 ± 4.44aABC	85.00 ± 3.83aABC	83.00 ± 5.29aABC	50.50 ± 9.15bDE	35.50 ± 5.75cC	30.50 ± 9.85cD

Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

The seed germination rate refers to the percentage of the number of germinated seeds in the total number of tested seeds, which characterizes the final germination condition of the seeds. Except that quinclorac, clomazone, and 2,4-D had no significant inhibitory effect on the germination rate, the other herbicides generally showed an inhibitory effect on the germination rate of *L. multiflorum*, and as the concentration increases, the inhibitory effect was significantly enhanced as shown in Table 5. The highest seed germination rate reached 100.00% (50 μg·L^{-1}, pendimethalin), and the lowest was only 23.00 (2,400 μg·L^{-1}, alachlor), which was 76.88% lower than the control value, it was also significantly lower than other concentration treatments and other herbicide treatments. High concentrations of alachlor and metolachlor had an obvious inhibitory effect on the germination rate of *L. multiflorum* seeds, and the mixed agent treatment of three concentrations inhibited the seed germination rate compared to the control with average seed germination rate of 98.00, 98.00, and 91.50 at the mixed agent levels of 1, 5, and 10 μg·L^{-1}, separately.

With the exception that the seedling morphology under quinclorac and 2,4-D treatments were not changed significantly, the other herbicides all had varying degrees of impact on seed germination and seedling growth of *L. multiflorum* (Fig. 1). In general, herbicides severely inhibited the growth of *L. multiflorum* seedlings and caused curling, deformity, and chlorosis. The higher the concentration, the more significant the inhibitory effect. Under the treatment of high-concentrations of alachlor (2,400 μg·L^{-1}) and metolachlor (1,200, 2,400 μg·L^{-1}), the grass seeds did not germinate. In addition, the seedlings of *L. multiflorum* treated with high-concentration clomazone (above 600 μg·L^{-1}) showed purple symptoms from the stem to the tip of the leaf, the seedlings were curled, the root system was weak, and the growth was severely inhibited, and some seeds had died. The seedlings of *L. multiflorum* treated with high-concentration isoxaflutole (above 600 μg·L^{-1}) were albino, and a small number of seedlings were purple, the roots became thinner and slightly purple, the seed growth was inhibited, and some seeds died. Under the treatment of high-concentration pendimethalin (above 600 μg·L^{-1}), the root system was partially purple, there were few fibrous roots, and some root tips were rod-shaped.

Overall, the seeds of *L. multiflorum* still had relatively normal germination conditions under the exposure to many of the tested herbicides. Among them, quinclorac, 2,4-D, atrazine, prometryn, fomesafen, flumetsulam, and pendimethalin had little effect on the seed germination of *L. multiflorum*. Among them, quinclorac had the least effect on the seed germination of *L. multiflorum*, while the seeds treated with alachlor, metolachlor, clomazone, or isoxaflutole had poor germination conditions.

Effect of herbicides on growth characteristics of *L. multiflorum*

The impact of these 13 herbicides varied on the growth performance of *L. multiflorum* (Fig. 2). In general, they can all cause different degrees of growth retardation and dwarfing of *L. multiflorum*, and also cause deformity, curling, and twisting of leaves. High concentrations of atrazine, prometryn, fomesafen, and pendimethalin obviously led to dwarfing of plants. Metolachlor and clomazone can cause an obvious reduction in tillering and sparse plants. High concentrations of atrazine, prometryn, and fomesafen caused obvious curling and shrinking of *L. multiflorum* leaves. High concentrations of atrazine, prometryn, fomesafen, quinclorac, clomazone, and pendimethalin caused obvious yellowing of *L. multiflorum* leaves. High concentrations of clomazone and isoxaflutole caused severe whitening of *L. multiflorum* leaves. The herbicides which had a greater impact on the performance of *L. multiflorum* include atrazine, prometryn, fomesafen, and clomazone.

As the concentration of herbicides added increases, the height of *L. multiflorum* generally showed a downward trend (Fig. 3). The height of CK (26.78 cm) was significantly higher than that of the 13 herbicides and the mixed agent treatment ($p < 0.05$). Except for clomazone, the plant heights of the other 12 herbicides treated with 300 μg·L^{-1} were significantly higher than those treated with 1,200 μg·L^{-1}. The treatment with the highest plant height was quinclorac at 300 μg·L^{-1} (24.82 cm), followed by butachlor at 300 μg·L^{-1} (23.23 cm), and there was no significant difference between the two treatments ($p > 0.05$), and the two herbicides had weak inhibitory effects on *L. multiflorum*. The herbicide with the lowest plant height under 300 μg·L^{-1} treatment was imazamox (15.81 cm), and the lowest plant height under 1,200 μg·L^{-1} treatment was atrazine (9.49 cm), which was also the lowest plant height among all treatments. The results showed that atrazine was a herbicide with strong inhibitory effect on the plant height of *L. multiflorum*.

The LDMC of CK (8.26%) was significantly lower than that treated with 13 single-agent herbicides and mixed agents (Fig. 4). With the increase in concentration, the dry matter content showed an obvious gradient effect in three herbicides: atrazine, prometryn, and fomesafen. Among all treatments, the highest leaf dry matter content was treated with 1,200 μg·L^{-1} atrazine (26.77%). Treatment with 1,200 μg·L^{-1} prometryn and fomesafen also significantly increased the leaf dry matter content of *L. multiflorum*, which reached 19.44% and 18.57% respectively. Under three different concentrations, 2,4-D, flumetsulam and clomazone treatment had little effect on the LDMC of *L. multiflorum* leaves.

Effect of herbicides on physiological characteristics of *L. multiflorum*

The treatments of metolachlor, atrazine, fomesafen, and flumetsulam showed a trend of decreasing total Chl and Car contents with increasing concentration (Fig. 5). This might because the higher the herbicide concentration, the stronger the inhibitory effect on photosynthesis. Due to the damage of herbicide treatment to the leaves of *L. multiflorum*, the establishment of the protection mechanism of *L. multiflorum* was promoted. With the increase in treatment concentration, alachlor treatment leads to an increase of total Chl and Car contents with increasing concentration.

Imazamox, atrazine, and the mixture of 50 and 100 μg·L^{-1} all caused extremely significant decreases in Chl and Car contents. Overall, Chla, Chlb, total Chl, and Car showed a similar trend. Alachlor had little effect on the photosynthetic pigment content of *L. multiflorum*, while imazamox, atrazine, clomazone, and isoxaflutole have a greater effect.

The *Fv/Fo* and *Fv/Fm* of *L. multiflorum* treated with three concentrations of atrazine, prometryn, clomazone, and isoxaflutole were significantly lower than those of CK ($p < 0.05$) (Fig. 6). Among them, atrazine, prometryn, and clomazone had more obvious inhibitory effects on the fluorescence values, and the inhibitory effects of atrazine on *Fv/Fo* and *Fv/Fm* showed an obvious gradient effect of 1,200 μg·L^{-1} > 600 μg·L^{-1} > 300 μg·L^{-1}.

Table 4. Effects of different herbicides on mean germination speed of *L. multiflorum*.

Herbicide	Concentration (μg·L^{-1})								
	0	10	30	50	100	200	600	1,200	2,400
Alachlor	4.98 ± 0.03b	4.83 ± 0.02bBC	4.85 ± 0.05bC	4.87 ± 0.06bAB	4.90 ± 0.07bAB	4.91 ± 0.10bBCD	5.33 ± 0.19aA	5.35 ± 0.23aABC	5.40 ± 0.31aAB
Butachlor	4.98 ± 0.03ab	4.88 ± 0.02dAB	4.97 ± 0.03bcA	4.96 ± 0.06bcA	4.90 ± 0.11bcdAB	5.04 ± 0.06abA	4.84 ± 0.08dCD	4.94 ± 0.07bcdDE	5.12 ± 0.09aBCD
Metolachlor	4.98 ± 0.03b	4.91 ± 0.03bAB	4.88 ± 0.05bB	4.89 ± 0.03bAB	4.93 ± 0.08bABC	5.05 ± 0.02bAB	4.95 ± 0.11bBCD	5.50 ± 0.02aA	5.46 ± 0.26aA
Imazamox	4.98 ± 0.03a	4.87 ± 0.04aAB	4.88 ± 0.05aB	4.83 ± 0.07aABC	4.89 ± 0.10aABC	4.97 ± 0.08aAB	4.84 ± 0.11aCD	4.86 ± 0.23aCDE	4.95 ± 0.05aDE
Atrazine	4.98 ± 0.03b	4.81 ± 0.07dBC	4.75 ± 0.02dD	4.89 ± 0.06cAB	4.90 ± 0.03bcABC	4.91 ± 0.03bcBC	4.95 ± 0.10bcBC	4.92 ± 0.04bcDE	5.16 ± 0.04aBC
Prometryn	4.98 ± 0.03bc	4.77 ± 0.09eBCD	4.76 ± 0.04eD	4.80 ± 0.05deBC	4.81 ± 0.07deCD	4.83 ± 0.07deD	4.91 ± 0.08cdCD	5.04 ± 0.06bD	5.17 ± 0.15aBCD
Fomesafen	4.98 ± 0.03c	4.76 ± 0.08eBCD	4.86 ± 0.05dBC	4.77 ± 0.03eCD	4.84 ± 0.03deCD	4.91 ± 0.04cdBCD	5.08 ± 0.10bAB	5.17 ± 0.07aBC	5.26 ± 0.09aBC
Quinclorac	4.98 ± 0.03a	4.85 ± 0.07bcAB	4.86 ± 0.04bcBC	4.86 ± 0.04bcB	4.92 ± 0.03abAB	4.91 ± 0.07abBCD	4.80 ± 0.07cD	4.87 ± 0.07bcDE	4.91 ± 0.08abE
Flumetsulam	4.98 ± 0.03ab	4.87 ± 0.06cAB	4.87 ± 0.04cB	4.76 ± 0.05dCD	4.84 ± 0.06cdCD	4.88 ± 0.10cCD	4.91 ± 0.06bcCD	4.84 ± 0.03cdE	5.04 ± 0.11aCD
Clomazone	4.98 ± 0.03d	4.74 ± 0.06gCD	4.78 ± 0.07fgD	4.86 ± 0.03efB	4.83 ± 0.06fBC	4.94 ± 0.05deBC	5.16 ± 0.04cAB	5.27 ± 0.06bB	5.41 ± 0.06aAB
Isoxaflutole	4.98 ± 0.03d	4.93 ± 0.03dA	4.93 ± 0.03dAB	4.82 ± 0.07eBC	4.82 ± 0.11eBC	5.01 ± 0.05dABC	5.11 ± 0.04cAB	5.28 ± 0.05bB	5.46 ± 0.07aA
Pendimethalin	4.98 ± 0.03bc	4.89 ± 0.08cAB	4.94 ± 0.03bcA	4.92 ± 0.03cAB	4.95 ± 0.06bcAB	4.98 ± 0.05bcAB	5.02 ± 0.17bcABC	5.10 ± 0.20abBCDE	5.20 ± 0.05aBC
2,4-Dichlorophenoxyacetic acid	4.98 ± 0.03c	4.74 ± 0.03fD	4.87 ± 0.04deB	4.82 ± 0.07eABC	4.82 ± 0.06eBC	4.92 ± 0.04cdBCD	5.10 ± 0.04bAB	5.24 ± 0.04aB	5.24 ± 0.03aBCD

Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

Table 5. Effects of different herbicides on germination rate of *L. multiflorum*.

Herbicide	Concentration (μg·L^{-1})								
	0	10	30	50	100	200	600	1,200	2,400
Alachlor	99.50 ± 1.00a	97.00 ± 1.16aAB	99.50 ± 1.00aAB	97.00 ± 3.46aABC	98.00 ± 2.31aA	98.50 ± 1.00aAB	70.00 ± 5.89bE	30.50 ± 5.51cE	23.00 ± 5.29dF
Butachlor	99.50 ± 1.00aA	98.50 ± 1.92aA	97.50 ± 3.79aAB	95.50 ± 2.52abBC	95.50 ± 1.92abAB	97.00 ± 2.00aAB	96.00 ± 0.00aB	92.00 ± 3.27bBC	88.00 ± 3.65cCD
Metolachlor	99.50 ± 1.00aA	98.50 ± 1.92aAB	98.50 ± 1.92aAB	98.00 ± 0.00aABC	96.50 ± 1.00abA	98.50 ± 1.92aAB	92.00 ± 5.89bD	74.00 ± 6.33cD	47.00 ± 6.00dE
Imazamox	99.50 ± 1.00aA	95.00 ± 1.16aB	95.50 ± 1.92aB	95.50 ± 1.92abC	97.00 ± 1.16abA	92.00 ± 5.89abAB	91.00 ± 1.16bD	87.50 ± 3.42bC	80.00 ± 5.89cD
Atrazine	99.50 ± 1.00aA	97.00 ± 2.58abAB	98.50 ± 1.92aAB	98.50 ± 1.92aAB	98.00 ± 1.63aA	97.00 ± 2.00abAB	93.50 ± 5.26abCD	92.00 ± 6.33bcBC	87.50 ± 5.51cCD
Prometryn	99.50 ± 1.00aA	98.50 ± 3.00aAB	97.00 ± 2.58aAB	96.50 ± 1.92aBC	96.00 ± 3.27aA	97.00 ± 2.00aAB	97.50 ± 1.92aAB	97.00 ± 2.00aAB	89.00 ± 3.83bCD
Fomesafen	99.50 ± 1.00aA	96.50 ± 4.73aAB	97.00 ± 2.00aAB	96.50 ± 1.92aBC	97.50 ± 2.52aA	95.50 ± 1.92aB	95.00 ± 1.16aBC	96.00 ± 1.63aAB	89.50 ± 5.26bBC
Quinclorac	99.50 ± 1.00aA	95.00 ± 4.16aAB	96.50 ± 1.92aB	96.00 ± 6.73aABC	96.00 ± 4.32aA	97.00 ± 3.46aAB	98.50 ± 1.00aA	96.50 ± 1.00aAB	98.00 ± 1.63aA
Flumetsulam	99.50 ± 1.00aA	98.50 ± 1.00aA	98.50 ± 1.00aAB	97.50 ± 3.79aABC	96.00 ± 4.32aA	97.00 ± 3.46aAB	96.50 ± 3.00aAB	91.50 ± 2.52bBC	91.50 ± 1.00bABC
Clomazone	99.50 ± 1.00aA	90.00 ± 8.64bAB	98.50 ± 1.92aAB	93.50 ± 8.06abABC	98.50 ± 1.92aA	94.00 ± 4.32abAB	98.50 ± 1.92aA	97.00 ± 2.00abAB	92.00 ± 6.73abABC
Isoxaflutole	99.50 ± 1.00aA	99.00 ± 1.16abA	97.50 ± 1.00abAB	97.00 ± 2.58abBC	97.00 ± 4.76abA	98.50 ± 1.00abAB	99.00 ± 1.16abA	98.00 ± 0.00abA	94.50 ± 5.75bABC
Pendimethalin	99.50 ± 1.00aA	98.50 ± 1.00abA	99.50 ± 1.00aA	100.00 ± 0.00aA	99.50 ± 1.00aA	99.50 ± 1.00aA	95.50 ± 2.52bcBC	95.00 ± 2.58cAB	90.50 ± 4.73dABCD
2,4-Dichlorophenoxyacetic acid	99.50 ± 1.00aA	99.00 ± 1.16aA	98.00 ± 2.83aAB	99.00 ± 1.16aABC	96.50 ± 1.92aA	97.50 ± 1.92aAB	97.00 ± 2.58aAB	96.50 ± 1.92aAB	96.50 ± 1.00aAB

Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

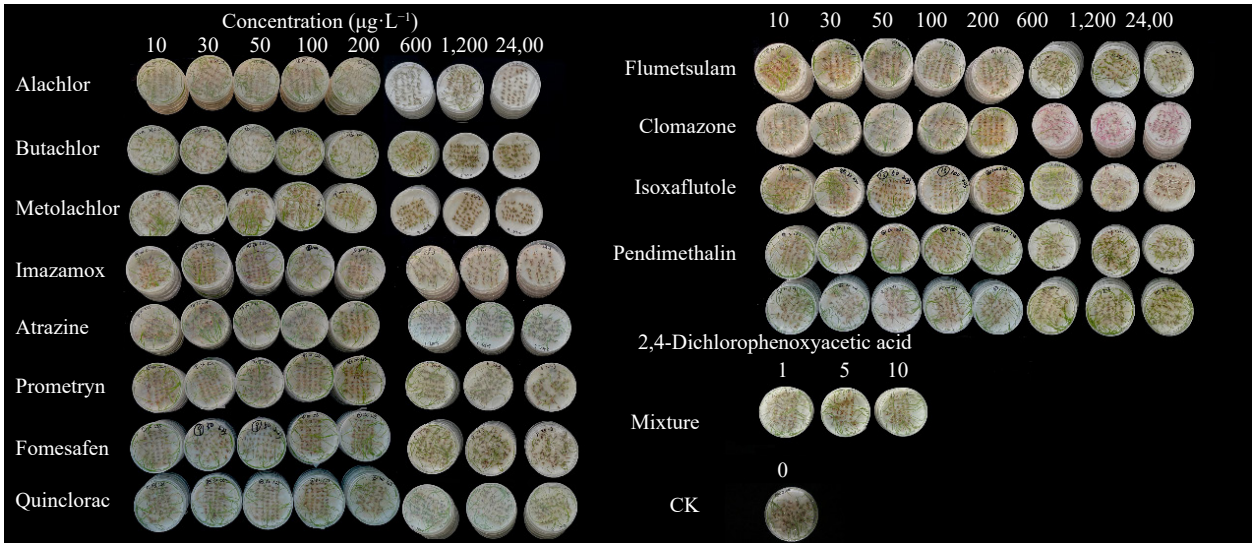

Fig. 1 Germination of *L. multiflorum* under different herbicide treatments (7 d).

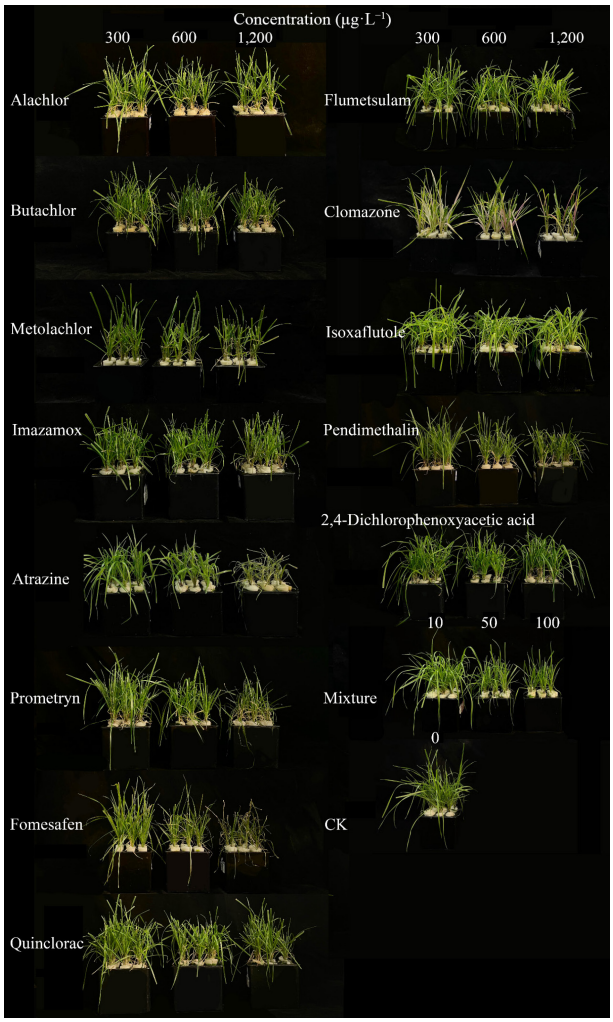

Fig. 2 Growth of *L. multiflorum* plants under different herbicide treatments (5 d).

The results showed that different concentrations of atrazine, prometryn, fomesafen, quinclorac, flumetsulam, and clomazone significantly increase the SOD activity of *L. multiflorum* leaves (Fig. 7). Among them, *L. multiflorum* treated with 300 μg·L^{-1} flumetsulam had the highest SOD activity (294.73 U·g^{-1}). The mixed agent treatment of 50 μg·L^{-1} and 100 μg·L^{-1} were also increased the SOD activity of *L. multiflorum* leaves. The activity of SOD increased with the increase treatment concentration of pendimethalin and the mixture.

Different concentrations of atrazine, prometryn, fomesafen, quinclorac, and flumetsulam significantly increased the POD activity of *L. multiflorum* leaves (Fig. 7). *L. multiflorum* treated with 300 μg·L^{-1} pendimethalin had the highest POD activity (3.81 × 104 U·g^{-1}), while the treatment of 1,200 μg·L^{-1} pendimethalin had an inhibitory effect (1.52 × 104 U·g^{-1}). The activity of POD increased with the increased treatment concentration of alachlor, atrazine, and 2,4-D, while it decreased with the increased treatment concentration of butachlor, clomazone, isoxaflutole, and pendimethalin.

Different concentrations of atrazine, prometryn, fomesafen, quinclorac, flumetsulam, clomazone, isoxaflutole, and pendimethalin significantly increase the CAT activity of *L. multiflorum* leaves (Fig. 7). Among them, the highest CAT activity of *L. multiflorum* was treated with 600 μg·L^{-1} pendimethalin (914.34 U·g^{-1}). The mixed agent treatment at different concentrations will also increase the CAT activity. The activity of CAT was increased with the increased treatment concentration of imazamox, atrazine, and isoxaflutole, while it was decreased with the increased treatment concentration of alachlor, metolachlor, quinclorac, flumetsulam, and clomazone.

The results showed that different concentrations of herbicide treatments significantly reduced the activity of APX except 1,200 μg·L^{-1} fomesafen (1.72 U·g^{-1}), and 600 μg·L^{-1} clomazone (2.00 U·g^{-1}) (Fig. 7). Metolachlor at 600 μg·L^{-1} had the strongest inhibitory effect on APX activity (0.20 U·g^{-1}), followed by alachlor at 300 μg·L^{-1} (0.25 U·g^{-1}). The activity of APX increased with the increased treatment concentration of alachlor, prometryn, flumetsulam, fomesafen, and the mixture, while it decreased with the increase of treatment concentrations of atrazine.

Correlation between variables

Based on the above experimental results, the membership function value method was used to comprehensively evaluate

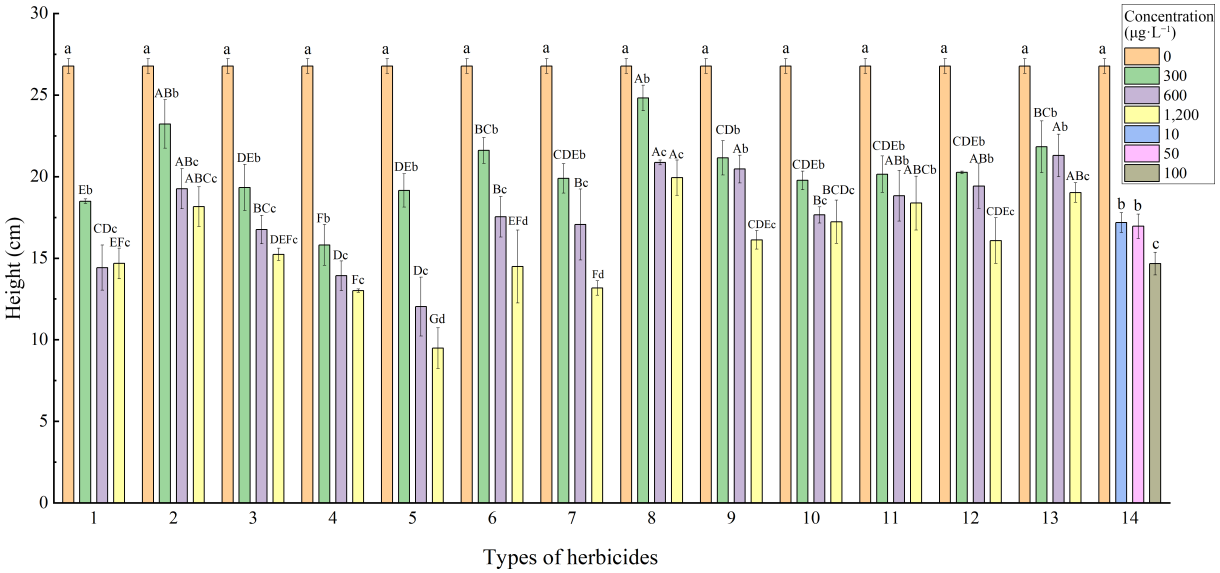

Fig. 3 Effects of different herbicides on plant height of *L. multiflorum*. Types of herbicides - 1: Alachlor; 2: Butachlor; 3: Metolachlor; 4: Imazamox; 5: Atrazine; 6: Prometryn; 7: Flumetsulam; 8: Fomesafen; 9: Quinclorac; 10: Clomazone; 11: Isoxaflutole; 12: Pendimethalin; 13: 2,4-Dichlorophenoxyacetic acid; 14: Mixture (the above 13 mixed). Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

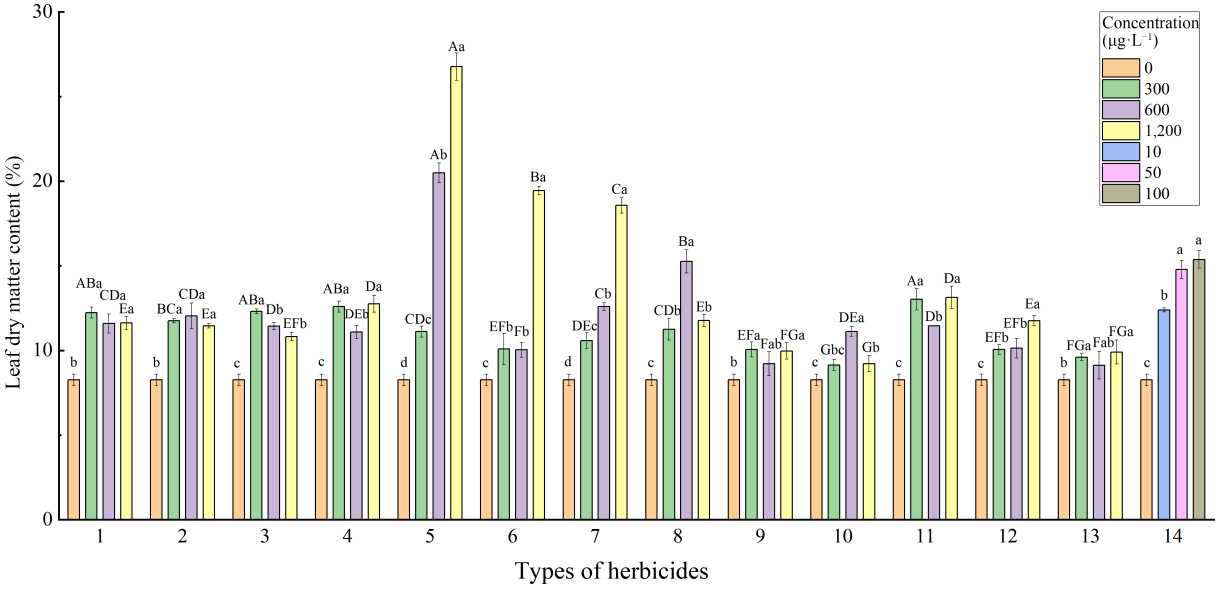

Fig. 4 Effects of different herbicides on leaf dry matter content of *L. multiflorum*. Types of herbicides - 1: Alachlor; 2: Butachlor; 3: Metolachlor; 4: Imazamox; 5: Atrazine; 6: Prometryn; 7: Flumetsulam; 8: Fomesafen; 9: Quinclorac; 10: Clomazone; 11: Isoxaflutole; 12: Pendimethalin; 13: 2,4-Dichlorophenoxyacetic acid; 14: Mixture (the above 13 mixed). Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

the four germination indexes and 13 growth and physiological indexes of *L. multiflorum*, so as to evaluate the tolerance of *L. multiflorum* to different herbicides and their different concentrations.

The average value method of membership function was used to standardize the indicators. The calculation equations are as follows:

$$\mu(Xi) = \frac{Xi - Xmin}{Xmax - Xmin}$$

$$\mu(Xj) = \left(1 - \frac{Xi - Xmin}{Xmax - Xmin}\right), \quad j = 1, 2, 3 \cdots n$$

In the above two equations, *Xi* is the measured value of the indicator; *Xmin*, *Xmax* are respectively the minimum and maximum values of a certain indicator of all tested materials. The first equation indicates that the indicator is positively correlated with tolerance ability, and the second indicates that the indicator is negatively correlated with tolerance ability.

According to the ranking of membership function values of germination indicators of *L. multiflorum* seeds under different

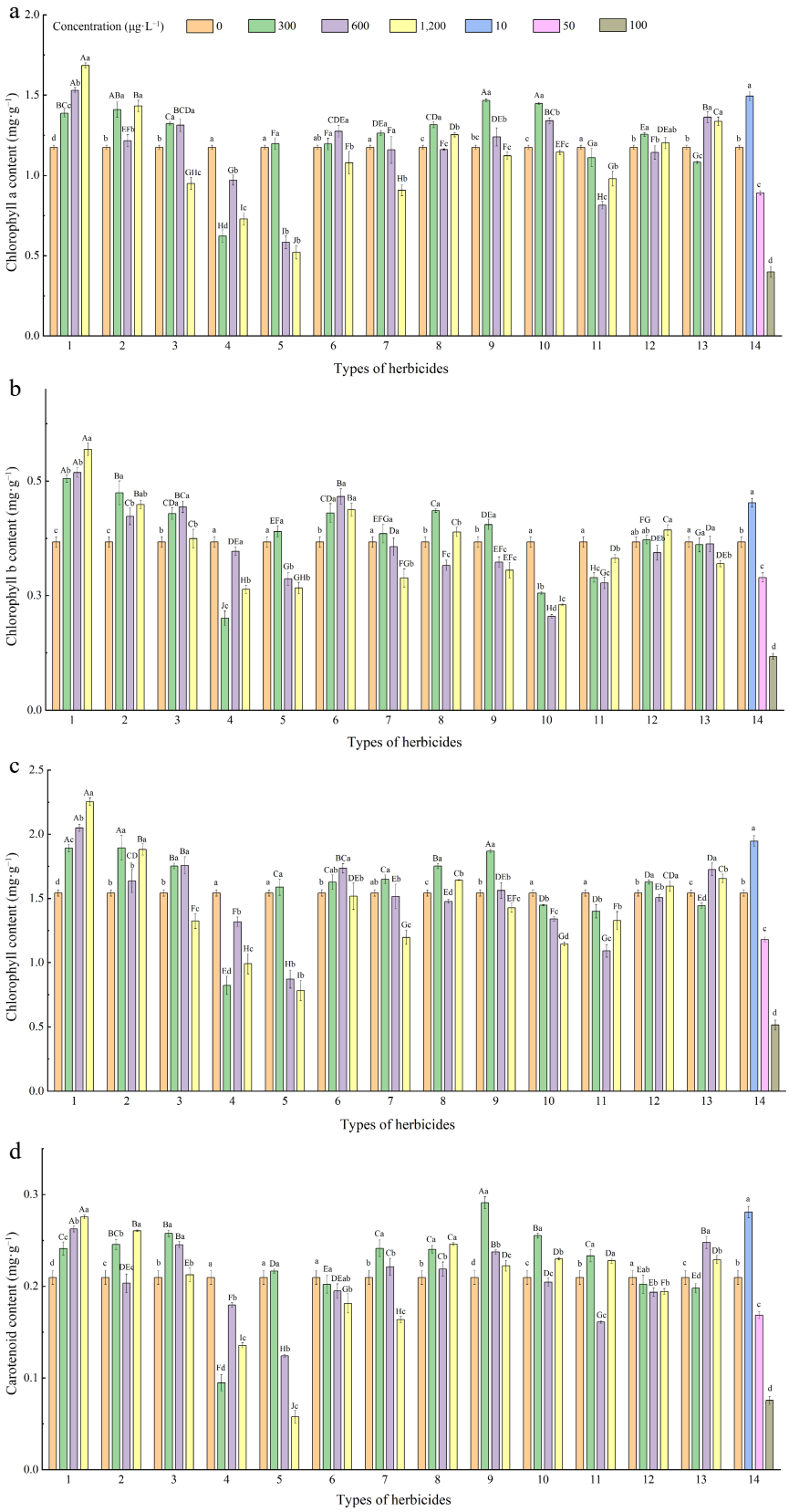

Fig. 5 Effects of different herbicides on photosynthetic pigment content of *L. multiflorum*. Types of herbicides - 1: Alachlor; 2: Butachlor; 3: Metolachlor; 4: Imazamox; 5: Atrazine; 6: Prometryn; 7: Flumetsulam; 8: Fomesafen; 9: Quinclorac; 10: Clomazone; 11: Isoxaflutole; 12: Pendimethalin; 13: 2,4-Dichlorophenoxyacetic acid; 14: Mixture (the above 13 mixed). Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

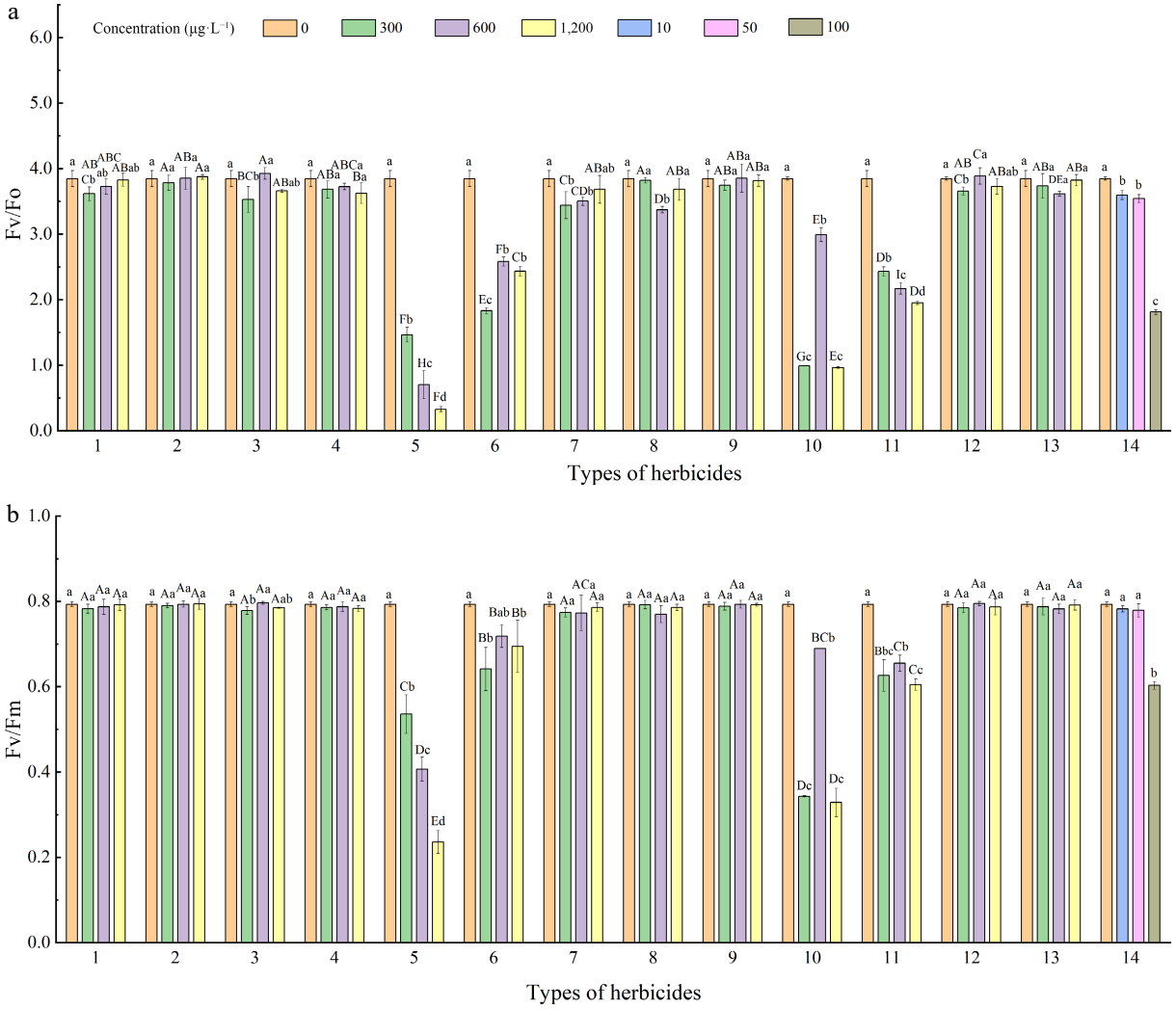

Fig. 6 Effects of different herbicides on chlorophyll fluorescence parameters of *L. multiflorum*. Types of herbicides – 1: Alachlor; 2: Butachlor; 3: Metolachlor; 4: Imazamox; 5: Atrazine; 6: Prometryn; 7: Flumetsulam; 8: Fomesafen; 9: Quinclorac; 10: Clomazone; 11: Isoxaflutole; 12: Pendimethalin; 13: 2,4-Dichlorophenoxyacetic acid; 14: Mixture (the above 13 mixed). Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

herbicide treatments (Supplementary Table S2), it was concluded that the top five herbicides in descending order for the tolerance of *L. multiflorum* seeds to single herbicides were: 10 μg·L^{-1} 2,4-D, 50 μg·L^{-1} flumetsulam, 30 μg·L^{-1} atrazine, 10 μg·L^{-1} prometryn, and 50 μg·L^{-1} fomesafen. Overall, *L. multiflorum* seeds had strong tolerance to atrazine, prometryn, quinclorac, and flumetsulam, but not tolerant to butachlor, metolachlor, imazamox, clomazone, and isoxaflutole. The tolerance to concentration in descending order was low concentration > medium concentration > high concentration.

According to the ranking of membership function values of growth and physiological indicators of *L. multiflorum* plants under different herbicide treatments (Supplementary Table S3), we concluded that the top five herbicides in descending order of tolerance of *L. multiflorum* plants to single herbicides were: 300 μg·L^{-1} flumetsulam, 1,200 μg·L^{-1} alachlor, 300 μg·L^{-1} quinclorac, 300 μg·L^{-1} fomesafen, and 1,200 μg·L^{-1} quinclorac. Overall, *L. multiflorum* plants had strong tolerance to quinclorac and weak tolerance to imazamox, atrazine, clomazone and isoxaflutole. The tolerance to concentration was in descending order of low concentration > medium concentration > high concentration.

Discussion

Seed germination and seedling growth of *L. multiflorum* in response to herbicides

The germination rate of seeds reflects the overall germination ability of seeds, and the germination index, germination ability, and average germination speed reflect the germination speed, uniformity, and germination quality of seeds[23]. The herbicides have different degrees of impact on the seed germination process. Zhou found that when the concentration of pendimethalin is 330 mg·L^{-1}, it had a promoting effect on the germination rate, germination ability, and germination index of seeds of five crops including wheat, corn, soybean, mung bean, and peanut and there was an inhibitory effect when exceeded this concentration[24]. Han & Zhang found that 2,4-D at a

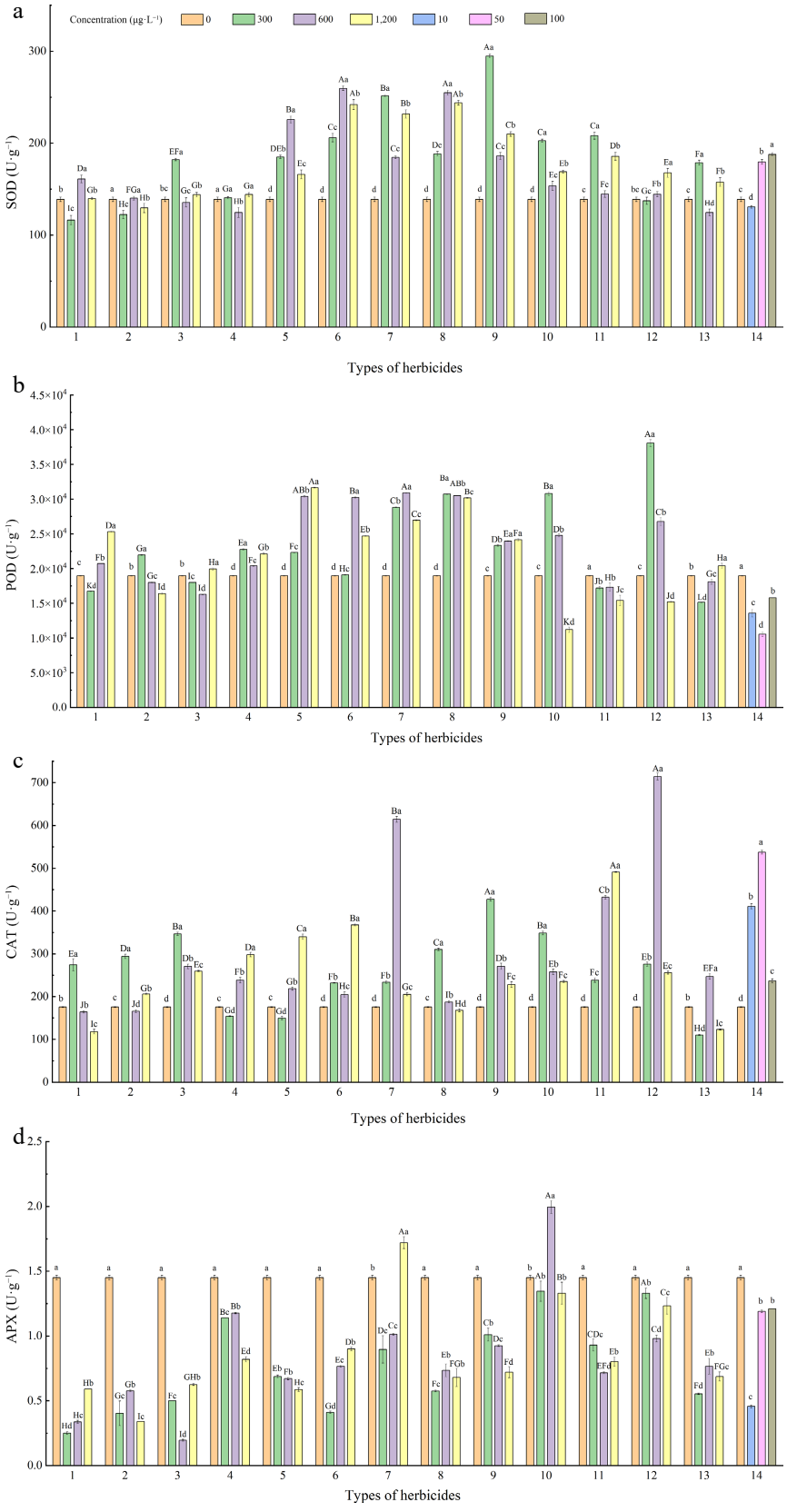

Fig. 7 Effects of different herbicides on leaf antioxidant enzymes of *L. multiflorum*. Types of herbicides - 1: Alachlor; 2: Butachlor; 3: Metolachlor; 4: Imazamox; 5: Atrazine; 6: Prometryn; 7: Flumetsulam; 8: Fomesafen; 9: Quinclorac; 10: Clomazone; 11: Isoxaflutole; 12: Pendimethalin; 13: 2,4-Dichlorophenoxyacetic acid; 14: Mixture (the above 13 mixed). Different lower-case letters indicate significant differences ($p < 0.05$) between treatments of different concentrations of the same herbicide, different upper-case letters indicate significant differences ($p < 0.05$) between treatments of different herbicides at the same concentration.

concentration of 72 mg·L^{-1} can promote the germination of wheat seeds, and exceeded this concentration range, it showed an inhibitory effect[25]. In this study, pendimethalin and 2,4-D had little effect on the seed germination of *L. multiflorum* and low concentrations (10, 30, 50, 100) of 2,4-D can promote the germination index. In general, the herbicides that had a greater impact on the germination ability of *L. multiflorum* seeds included alachlor, metolachlor, imazamox, clomazone, and isoxaflutole, and a lot of herbicides showed promotion at low concentrations and inhibited at high concentrations.

It was found that under the treatment of high-concentration herbicides, the seedlings produced corresponding damage symptoms. Among them, the effect of amide herbicides alachlor, butachlor, metolachlor, and the organic heterocyclic clomazone and isoxaflutole were more obvious. Studies have shown that amide herbicides can cause damage to plant root tip cells, inhibit mitosis of root tip cells and root growth, resulting in short and soft roots[26]. In the study on wheat, it was found that amide herbicides mainly inhibited the growth of roots and young buds, causing dwarfing and deformity of seedlings and young buds and leaves cannot be fully unfolded[27]. Chloracetamide herbicide varieties usually inhibited seed germination and the growth of young buds, causing severe dwarfing of young buds and death[28]. The amide herbicide metolachlor is absorbed by young buds and then conducts upward, inhibiting seed protein synthesis, affecting the infiltration of choline into phospholipids and interfering with the formation of lecithin, and ultimately destroying the growth of young buds and roots[29]. In addition, some studies have found that clomazone inhibited the biosynthesis of chlorophyll and carotenoids in sensitive plants by inhibited deoxy-D-xylulose phosphate synthase (DXS), resulting in plant whitening, yellowing, or chlorosis[30]. In addition, isoxaflutole is a hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitor, by inhibiting HPPD activity leads to a decrease in plastid quinones which is a necessary synergistic factor for phytoene desaturase to complete its normal function. This indirectly inhibits the biosynthesis of carotenoids[31]. Therefore, this explains the corresponding changes of *L. multiflorum* under the treatment of clomazone and isoxaflutole.

The growth and physiology of *L. multiflorum* plants in response to herbicides

After 5 d of hydroponic exposure treatment, typical symptoms of herbicide poisoning appeared in *L. multiflorum*. The symptoms under the treatment of atrazine, prometryn, fomesafen, clomazone, and isoxaflutole were more obvious. Imazamox, atrazine, clomazone, and isoxaflutole significantly inhibited the production of Chla, Chlb, Chl, and Car in *L. multiflorum*. In conclusion, there are different mechanisms and damage symptoms with different types of herbicides.

Imazamox is a biosynthesis inhibitor that can inhibit the first reaction in the biosynthesis of branched-chain amino acids required to catalyze plant growth and development by acetolactate synthase (ALS), causing plant metabolic disorders, leading to a decrease in the photosynthetic capacity of *L. multiflorum*, affecting the synthesis of chlorophyll, and a decrease in Chla, Chlb, and Chl, which make plants turn yellow and grow slowly[32]. Existing studies have proven that gramineous plants have a low tolerance to ALS inhibitor herbicides[33], which explains the high sensitivity of *L. multiflorum* to imazamox.

Atrazine, prometryn, and fomesafen are all photosynthesis inhibitors. Dang & Gao[34] found that atrazine did not affect the germination of *Secale cereale* seeds but affected the development after emergence, and it was similar to the results of this study. Therefore, it was supposed that the early development phenomenon of *L. multiflorum* seedlings and the growth status of plants may be related to atrazine affecting the photosynthesis of seedlings. Yang et al.[35] found that atrazine had a significant inhibitory effect on the chlorophyll fluorescence parameters of four vegetables, and the Fv/Fm decreased by 10%, which was similar to the results of this study. The toxic reaction of plants to atrazine is closely related to its absorption and accumulation[36]. Sánche et al.[37] evaluated the ability of tall fescue, barley, ryegrass, and corn to degrade atrazine in soil, the results showed that the four plants could absorb and detoxify atrazine to a certain extent, but when the initial dose of atrazine exceeds 2 mg·kg^{-1}, plant toxicity occurred, biomass decreases, and chlorosis, stunted growth, and even leaf death occurred under the treatment of a concentration of 10 mg·kg^{-1}, which is consistent with the results of this study. Therefore, it was supposed that the effect of atrazine on *L. multiflorum* is related to its large accumulation in tissues.

Clomazone is a plant growth inhibitor. The roots and young buds of plants can effectively absorb clomazone. It is transported upward from the roots through the stem with transpiration and reaches the leaves through the xylem by diffusing, inhibiting the synthesis of chlorophyll and carotene in plants[38,39]. *L. multiflorum* is a clomazone-sensitive plant, after absorbing clomazone, the plant conductivity deteriorates sharply, because photosynthesis is blocked, leaves cannot synthesize chlorophyll and carotene. In this study, the synthesis of Chlb in *L. multiflorum* was severely inhibited when treated with clomazone for 5 d, and the Chl content was significantly reduced. Therefore, *L. multiflorum* turns albino in a short time, chlorosis with purple color and then dies.

Isoxaflutole is an HPPD inhibitor. HPPD is an important enzyme in the tyrosine metabolism process in organisms. Tyrosine generates 4-hydroxyphenylpyruvic acid under the action of tyrosine aminotransferase, and then HPPD catalyzes the conversion of p-hydroxyphenylpyruvic acid into homogentisic acid under the participation of O_2, which is converted into plastid quinones and tocopherol in plants[40]. When the activity of HPPD is inhibited, the normal metabolism of tyrosine in plants will be blocked, resulting in the lack of carotenoids and the weakening of chlorophyll photooxidation, affecting photosynthesis, and thus causing yellowing and slight albinism of *L. multiflorum* plants.

Leaf dry matter content (LDMC) is a functional trait of plants and reflects the ability of plants to obtain and maintain resources such as light, water, and nutrients[41]. Plant leaves with lower LDMC have a stronger production capacity and the greater the LDMC content, the stronger the resistance to biotic and abiotic stresses[42]. In this study, after treatment with different herbicides, the LDMC of *L. multiflorum* was increased, indicating that *L. multiflorum* responded to heterogeneous environments by increasing LDMC[43]. As LDMC increases, the tolerance of plants to herbicide stress increases. The promoting effect of different herbicides on LDMC is different, which is related to the mechanism and molecular target of herbicides. In this study, the herbicides with strong promoting effects on LDMC were atrazine, fomesafen, and quinclorac, indicating that

the leaves of *L. multiflorum* treated with atrazine, fomesafen, and quinclorac had obvious resistance to herbicide stress, which improved the plant tolerance.

Antioxidant enzymes have an affinity for various types of xenobiotics, which enables them to participate in many detoxification processes[44]. In this study, after treatment with different concentrations of herbicides, the activities of SOD, CAT, and POD in *L. multiflorum* increased overall, indicating that the ROS scavenging system was stimulated after herbicides entered *L. multiflorum* and increased activities of SOD, CAT, and POD enzymes to protect plants from herbicides. Liu et al.[45] found that abiotic stress can induce an increase in the contents of SOD, POD, and CAT in perennial ryegrass, and reduce the oxidative damage to the plant, which is consistent with this study. In this study, the activity of APX decreased, and only increased under the treatment of clomazone. Tan et al.[46] also found that under the stress of herbicides such as acetochlor, fluoroglycofen-ethyl, and paraquat, the activity of APX was significantly reduced in grape leaves. This may be because the antioxidant mechanism of APX is different from that of SOD, CAT, and POD, it mainly catalyzes ascorbic acid and H_2O_2 and promotes the metabolism and conversion of H_2O_2 in plants, and it is a key enzyme to clear H_2O_2 in chloroplasts.

In addition, some studies have found that the SOD activity of plant leaves is positively correlated with plant dwarfing. The higher the activity, the more dwarfed the tree. This is similar to the results of this study in that the plant heights of *L. multiflorum* treated with atrazine, prometryn, and fomesafen were all inhibited, and the SOD activity was relatively high. It was supposed that the reason was that the high-activity SOD and CAT in *L. multiflorum* eliminates a large amount of reactive oxygen species, resulting in blocked cell wall elongation and thus dwarfing of plants. In addition, SOD activity also increased with the increase of dwarfing degree. Therefore, this is an adaptive regulatory mechanism of *L. multiflorum* in response to the increase of O^{2-}, H_2O_2, etc. and the accumulation of reactive oxygen species in plants under herbicide stress conditions, so as to reduce cell damage caused by the increase of O^{2-}[47].

Conclusions

A lot of the research on the relationships between plants and herbicides focuses on tolerance mechanisms and other aspects, while there are relatively few studies on the tolerance of plants to herbicides. In terms of turfgrass, there are relatively few studies on the tolerance and tolerance mechanism of turfgrass to herbicides. This study investigated the effects of 13 different herbicides on the seed germination ability, seedling growth, and physiological metabolism of *L. multiflorum*. The research results showed that *L. multiflorum* had strong tolerance to quinclorac and 2,4-D, and weak tolerance to imazamox, atrazine, clomazone, and isoxaflutole. This study provides data support and a theoretical basis for the remediation of herbicide residues in the environment by *L. multiflorum*, and provides a reference for research on the remediation effect of turfgrass on herbicides.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Sun Y, Jia F; data collection: Chen J, Li Y, Ma C; analysis and interpretation of results: Chen J, Wang Y, Ma C; draft manuscript preparation: Hu Q, Ma C. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Acknowledgments

This research was funded by the Turf Producers China of Chinese Turfgrass Society.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information accompanies this paper at (https://www.maxapress.com/article/doi/10.48130/grares-0024-0026)

Dates

Received 3 November 2024; Revised 29 November 2024; Accepted 11 December 2024; Published online 26 December 2024

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