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Litter autotoxicity limits natural regeneration of *Metasequoia glyptostroboides*

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Abstract

Widespread autotoxicity is an important obstacle to natural regeneration of many plants. The rare relict plant *Metasequoia glyptostroboides* is a difficult to natural regeneration and is affected by litter allelopathy. However, little is known about the potential influence of autotoxic substances on different regeneration stages of *M. glyptostroboides.* We identified multiple chemical compounds of aqueous extracts from fresh (recently accumulated) and natural litter (mixture of litter different phases of decomposition), to evaluate the autotoxic effects of the four most important detected compounds applied individually on seed germination and seedling growth of *M. glyptostroboides.* Results found that the 28 chemical compounds were identified in the aqueous extracts of *M. glyptostroboides* litter. The Jaccard similarity coefficient of chemical compounds in aqueous extracts of fresh and natural litter of *M. glyptostroboides* reached 50%. The number of chemical compounds in fresh litter was 5.56% more than that in natural litter. Catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol significantly affected seed germination rate, seed germination index, vigor index, shoot length and main root length of *M. glyptostroboides.* Specifically, benzoic acid had the strongest inhibitory effect, followed by catechol, trifluoroacetamide and D-(+)-arabitol. The autotoxic effect was concentration dependent, low concentrations were positive and neutral, high and extremely high concentrations were negative for all the chemical compounds. Moreover, catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol were autotoxic substances affecting the natural regeneration of *M. glyptostroboides,* as well as strongly inhibited at the shoot growth stage. This study confirms that natural regeneration of *M. glyptostroboides* is restricted by a large amount of litter coverage under the forest, highlighting how the chemical compounds responsible for the autotoxic characteristics of *M. glyptostroboides* affect the different regeneration stages of *M. glyptostroboides*.

Keywords *Metasequoia glyptostroboides* · Litter · Natural regeneration · Autotoxicity · Aqueous extract · Autotoxicity identification

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Introduction

Allelopathy refers to the inhibitory or stimulatory effect of one plant on another through the production of chemicals and their release into the environment (Rice 1984; Zhang et al. 2021). Allelochemicals are released into the environment through decomposition of plant residues (e.g. litter), leaching from plants by rainfall, rhizosphere secretion and volatilization, affecting individual performance and community structure (Kimura et al. 2015; Zhang et al. 2021). When the donor and receptor plants of the autotoxic compounds are of the same species, this is the special type of allelopathy called autotoxicity (Rice 1984; Singh et al. 1999; Lorenzo et al. 2012; Araniti et al. 2016). Autotoxicity is widespread in forest ecosystems and plays an important role in ecological processes such as natural plant regeneration, community succession and population restructuration (Francesco et al. 2004; Sinkkonen 2007). Litter is one of the important sources of autotoxic substances in trees (Sayer 2006; Wang et al. 2018a; Xu et al. 2021). Litter decomposition produces secondary metabolites that can affect plant regeneration by influencing the natural regeneration stages such as seed germination and seedling growth (Wang et al. 2018a). Previous studies have reported that litter autotoxicity severely inhibits natural regeneration of some plants (ie: *Pinus armandii*, *P. tabuliformis*, *P. rigida*, *Amygdalus pedunculata, Toona ciliata* var. *pubescens*), but most of these studies fail to distinguish the chemical compounds (Garnett et al. 2004; Alías et al. 2006; Wang et al. 2018b; Huo et al. 2019; Guo et al. 2019). Few studies have examined the autotoxic effects of autotoxic substances, let alone the comparative studies of autotoxic substances on different regeneration stages of plants.

The litter type plays an important role in seed germination and seedling growth stages (Facelli and Pickett 1991). Different litter types have different physical and chemical properties due to different decomposition rates, degradation chemicals and leaf traits, ultimately have different effects on plant growth (Facelli and Pickett 1991; Cornelissen 1996). For example, although *Picea abies* seedling emergence was significantly impaired by both *Fagus sylvatica* and *P. abies* litter, *F. sylvatica* had a stronger inhibitory effect because *F. sylvatica* litter had a lower red/far-red ratio (Asplund et al. 2017). The effect of *Schima superba* broadleaf litter on seedling emergence and survival of *Cunninghamia lanceolata* was greater than that of *C. lanceolata* needle litter (Zhao et al. 2019). Different decomposition stages of litter may have different secondary metabolites, and fresh litter may contain more allelochemicals than partially decomposed litter (Bokhari 1978). Ruprecht et al (2010) reported that *Stipa pulcherrima* fresh leaves had greater phytotoxicity than partially and mixed partially decomposed leaves. Different *M. glyptostroboides* litter types have different allelopathic effects (Xu et al. 2021), but the reason for this phenomenon is still unclear. Moreover, it is not clear how the autotoxic substances in fresh and natural litter of *M. glyptostroboides* differ.

Metasequoia glyptostroboides is listed as a first-class protected plant in the 'Chinese Plant Red Book' (Wang and Guo 2009) and as endangered plant in the IUCN list (IUCN 2021 https://www.iucnredlist. org/species/32317/2814244). *Metasequoia glyptostroboides* originated in the Mesozoic Cretaceous, and was widely distributed in the Northern Hemisphere during the Cenozoic Tertiary. It was almost completely extinct after the glacial period in the fourth century. Until the 1940s, the first surviving *M. glyptostroboides* was found in Lichuan, Hubei Province, which subsequently attracted more attention and research on this species (Lin et al. 2017; Wu et al. 2020). *Metasequoia glyptostroboides*, now has only 5 696 original mother trees (Xu et al. 2021), which mainly grow in the narrow triangle area among Lichuan in Hubei Province, Longshan in Hunan Province and

Shizhu in Chongqing Municipality (Fig. 1a). The natural regeneration of *M. glyptostroboides* is difficult, and only 1 ~ 2 seedlings are occasionally seen in the understory of *M. glyptostroboides* (Lin et al. 2017; Xu et al. 2021). Current studies on natural regeneration difficulties of *M. glyptostroboides* have focused on seed source differences (Wu et al. 2020), genetics and population structure (Liu 2020), anthropogenic disturbance (Lin et al. 2017) and survival conditions (Guo et al. 2018; Fan et al. 2020). However, natural regeneration failure is caused by complex natural environment, anthropogenic factors and the species' own characteristics. The factors and mechanisms that impede the natural regeneration of *M. glyptostroboides* have not been fully identified, because most studies have only explored the effects of a particular factor at a macro level.

A large amount of litter (average thickness up to about 2.5 cm) was found in the *M. glyptostroboides* mother forest (Xu et al. 2022). *Metasequoia glyptostroboides* litter presented allelopathic inhibition on seed germination, and this effect varied according to the litter type (Xu et al. 2021). However, there are still some unanswered questions: (1) what kind of autotoxic substances are present in *M. glyptostroboides* litter? (2) Are there any the differences of chemical compounds in different types of *M. glyptostroboides* litter? (3) How do these autotoxic substances affect the seed germination and early growth of *M. glyptostroboides,* thereby affecting the successful natural regeneration? To answer these questions, this study simulated the leaching process of natural rainfall on *M. glyptostroboides* litter, the chemical compounds presented in *M. glyptostroboides* litter were identified by gas chromatography–mass spectrometry technology, and the main detected autotoxic substances in litter were used to analyze the effects on seed germination and shoot growth of *M. glyptostroboides.*

Materials and methods

Sampling sites

Metasequoia glyptostroboides, deciduous arbor of the genus *Metasequoia* in the family Cupressaceae, is a fast-growing, monoecious, endangered and rare plant. In order to precisely analyze the effect of litter on the natural regeneration of original mother tree of *M.*

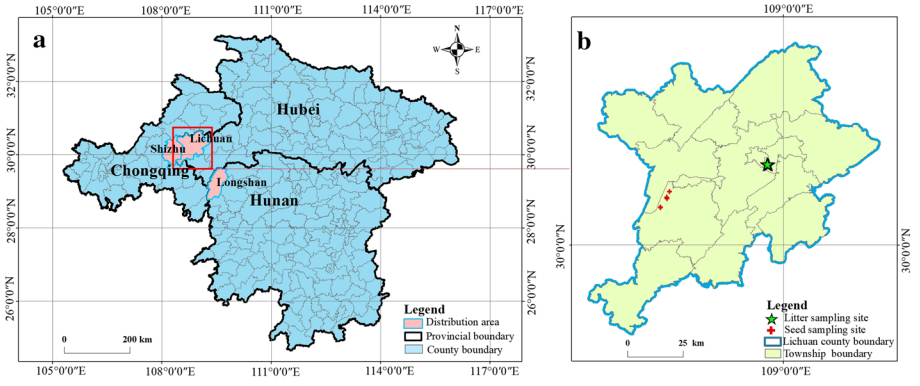

Fig. 1 Original mother tree distribution area of *M. glyptostroboides,* and *M. glyptostroboides* litter collection site and seed collection site

glyptostroboides, the original mother tree seeds of *M. glyptostroboides* were chosen for experiments in Lichuan, Hubei Province (Fig. 1b). However, we chose to collect *M. glyptostroboides* litter in the mother forest of *M. glyptostroboides* at the Institute of Forestry Science in Lichuan, Hubei Province (Fig. 1b) because of the complex and changeable natural environment. The litter collection site and seed collection site are not the same place, but both are located in Lichuan, Hubei Province, with the same overall climatic and geographical conditions. This area has an average altitude of 1 265.3 m, a subtropical continental monsoon climate, an annual sunshine duration of 1 518.9 h, an average annual temperature of 11.1 °C, an annual precipitation of 1 378 mm, an average relative humidity of 82% and a mountainous yellow–brown loam soil. The understorey shrubs mainly include *Robinia pseudoacacia*, *Euscaphis japonica*, *Hydrangea aspera*, *Euscaphis japonica* and *Paulownia fortunei*. While the understorey herbs mainly include *Houttuynia cordata*, *Farfugium japonicum*, *Elatostema involucratum*, *Rubus lambertianus, Miscanthus floridulus*, *Kalimeris indica*, *Kochia scoparia* and *Artemisia carvifolia*.

Litter collection

We randomly placed 30 litter collection frames (placed on the soil surface) under the mother forest of *M. glyptostroboide*s in Institute of Forestry Science in Lichuan, Hubei Province, to ensure the representativeness and uniqueness of *M. glyptostroboide*s litter collection (Appendix Fig. 5). Our investigation revealed that *M. glyptostroboide*s litter was mainly composed of leaves, branches, fruits and bark. At the end of October 2020, the current season's litter shed on *M. glyptostroboide*s mother trees was classified as fresh litter (Xu et al. 2021), and obtained through 15 litter collection frames. Fresh litter is the mixture that has not yet entered the decomposition process, and according to the observation of the actual composition of litter, the proportion of each component is set as: leaf: branch: peel = 11: 8: 1. After more than a month, the *M. glyptostroboide*s litter experienced a certain degree of natural decomposition in the forest. At the end of November 2020, all litter collected from the surface of the *M. glyptostroboide*s forest soil was identified as natural litter, which was obtained through 15 other litter collection frames. The natural litter consists of a mixture of branches, leaves and peels from each decomposition layer, where the ratio of undecomposed layer: semi-decomposed layer: fully decomposed layer = 1.2: 1.2: 1 (Xu et al. 2022). Both fresh and natural litter do not contain soil. The litter collected was air-dried at room temperature till constant weight and then treated accordingly.

Seed collection and selection

In November 2020, seeds were collected from five original open-pollinated *M. glyptostroboides* mother trees (No. 1 063, 1 076, 1 908, 1 947, 3 455) separately in Lichuan, Hubei Province (Fig. 1b, Appendix Fig. 6). After harvesting, seeds were air dried until seed moisture content decreased to less than 10%, and then stored in a refrigerator at 4 ℃.

For *M. glyptostroboides*, the existing original mother trees are open-pollinated under natural conditions (Cui 2011). To reduce individual differences in original mother trees, we used only seeds from one original mother tree of *M. glyptostroboides* (Wu et al. 2020). The tree is numbered 1076 (108°35'45"E, 30°07'20"N) and is 118 years old. The tree height and diameter at breast height reached 31 m and 87.6 cm, respectively. It has been proved by pre-experimants that this original mother tree has higher seed quality and germination rate

compared to other trees. The seed moisture content was 9.78 ± 1.13% and seed weight was 2.57 ± 0.17 g per 1 000 grains.

Separation and identification of litter chemical compounds

The air-dried fresh and natural litter of *M. glyptostroboides* were separately crushed (through a sieve with a aperture diameter of 150 μm), added distilled water at a ratio of 1:5 (5 mL of distilled water to 1 g of dry matter) (Xu et al. 2021), shaken at room temperature for 48 h, and then centrifuged at 10 000 r/min for 10 min. We finally obtained 200 $mg \cdot mL^{-1}$ supernatant for fresh and natural litter mother solution, respectively.

The litter aqueous extract was concentrated by rotary evaporation and freeze-dried to remove the water, then extracted and separated three times with ether, ethyl acetate and n-butanol in turn. The three extracts of the same solvent were combined and dried with N_2, and added 80 μL of pyridine and 80 μL of N,O-bis (trimethylsilyl) trifluoroacetamide with trimethylchlorosilane, covered and sealed, derivatized in 80 ℃ water bath for 1 h, and then blew dry with N_2. Then dissolved in 1 mL n-hexane, dehydrated with anhydrous sodium sulfate, and analyzed and identified by gas chromatography–mass spectrometry technology (GC–MS) (Wu et al. 1999; Pan et al. 2009).

The GC–MS instrument was Agilent 6 890N/5 975C, HP-5MS quartz capillary column (0.25 μm × 0.25 mm × 30 m), the carrier gas was helium with purity > 99.999%. The column temperature was initially held at 30 ℃ for 5 min, then programmed to 140 ℃ at a rate of 5 $℃ \cdot min^{-1}$, maintained for 15 min, from 140 ℃ to 250 ℃ at a rate of 10 $℃ \cdot min^{-1}$, final hold time of 5 min. The injection volume was 1 mL with the splitless mode, the injector and detector temperature were maintained at 260 ℃. The bombardment voltage of electron bombardment source was 70 eV, mass spectral scan time from *m/z* 30 to 550 was 1.0 s, and the whole process was scanned. The chemical compounds in the fresh and natural litter aqueous extracts were respectively searched by the computer in the standard mass spectrometry database NIST14 of GC–MS, and their relative percentage contents were respectively determined by peak area normalization method.

The ether, ethyl acetate, n-butanol, anhydrous sodium sulfate, benzoic acid, trifluoroacetamide, D-(+)-arabitol and catechol used in the experiment were all analytically pure, while hexane, pyridine and N,O-bis (trimethylsilyl) trifluoroacetamide with trimethylchlorosilanec were chromatographically pure, which all were purchased from Hubei Huawei Scientific Equipment Co.

Effects of autotoxic substances on seed germination and shoot growth

GC–MS results have indicated that autotoxic substances are present in *M. glyptostroboides* litter, but the autotoxic substances are highly selective and specific (Wang et al. 2007; Huang et al. 2021a). Based on the content, species, occurrence frequency of chemical compounds in different *M. glyptostroboides* litter and the reference to the confirmed autotoxic substances, representative autotoxic substances in *M. glyptostroboides* litter were selected to verify their autotoxic effects. The contents of trifluoroacetamide and D-(+)-arabitol were high, both appearing three times. Additionally, benzoic acid and phenol were typical autotoxic substances in many plants (Chaves Lobón et al. 2019; Guo et al. 2019). Therefore, we selected four single substances (catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol) to treat *M. glyptostroboides* seeds to verify their autotoxicity.

Reference to the concentration levels of autotoxic substances in related studies (Chaves Lobón et al. 2019; Huang et al. 2021b), the sensitivity concentration levels of four autotoxic substances in this study were finally determined at limited gradient levels after several pre-tests. Catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol, were set at four concentrations: 5, 1, 0.2, 0.04 mg·mL^{-1}, and distilled water was used as an isolated control (CK). There were three replicates per treatment (concentrations and chemical compounds). Before germination test, *M. glyptostroboides* seeds were sterilized in 0.5% $KMnO_4$ for 30 min, and then rinsed them in distilled water five times. *Metasequoia glyptostroboides* seeds were evenly arranged in petri dishes (Φ9cm, sterilized at 180 ℃ for 2 h before use) with two layers of filter paper. There were three petri dishes for each treatment. Four mL of corresponding solution was added to 50 seeds in each petri dish, placed in SPX-30085H-II biochemical incubator in total darkness (Guo et al. 2018) at 20 ℃ (Referring to the National Meteorological Science Data Center, the average temperature in Lichuan City in April and May is 20 ℃). We replaced the filter paper every three days and added 4 mL corresponding solution to avoid increasing concentrations in each petri dish. The seed germination of *M. glyptostroboides* was recorded every 24 h, and the appearance of radicle was used as the germination marker for continuous observation for 20 days. Meanwhile, 10 seeds were randomly selected from each experimental unit, and their shoot length (i.e., the total length of seed growth after germination) were measured daily with a straightedge for 20 days. On the 20th day of the experiment, the main root lengths of 10 previously randomly selected seeds in each experimental unit were measured. The seed germination rate, germination index, vigor index, allelopathic sensitivity index and synthetical effect of allelopathy index were calculated using the following equations:

(1) Germination rate (Guo et al. 2018): $G = \frac{G_a}{G_t} \times 100\%$

where G_a is the number of seeds that germinate, G_t is the number of tested seeds;

(2) Germination index (Liu et al. 2019): $G_i = \sum \frac{G_p}{G_d}$

In the formula, G_p is the number of germinated seeds per day corresponding to G_d, and G_d is the sequence day of germinated days (20 days in total);

(3) Vigor index (Zhang et al. 2018): $V_i = G \times S_h$.

where S_h is shoot length at the end of the experiment (20 days);

(4) Allelopathic sensitivity index (Williamson and Richarsdson 1988):

$$RI = 1 - \frac{C}{T}(T \geq C)$$

$$RI = \frac{C}{T} - 1(T < C)$$

where RI is the allelopathy sensitivity index, C is the control mean value, and T is the treatment mean value of the different indicators. $RI > 0$ means promotion, $RI < 0$ means inhibition, and the magnitude of the absolute value is consistent with the effect intensity.

(5) Synthetical effect of allelopathy index (Liu et al. 2019):

SE = (*RI* of germination rate + *RI* of germination index + *RI* of vigor index + *RI* of shoot length + *RI* of main root length) / 5. Where *SE* is synthetical effect of allelopathy index, which reflects the intensity of allelopathy.

Statistical analysis

Jaccard similarity coefficient formula was used to compare the similarity of chemical compounds in fresh and natural litter. The formula is as follows:

$$J = \frac{c}{a+b} \times 100\%$$

where J is the Jaccard similarity coefficient, a and b respectively represent the number of unique chemical compounds in fresh and natural litter, c is the number of common chemical compounds. The matching degree of the chemical compounds represented by a, b and c is greater than or equal to 90%.

One-way analysis of variance (ANOVA) and Duncan multiple comparison were carried out to investigate the significant differences in seed germination and shoot growth of *M. glyptostroboides* under different concentrations of the same chemical compound. A logistic model was used to fit shoot length growth data of *M. glyptostroboides* with different concentrations of different chemical compounds by SPSS 18 (Wu et al. 2020). The fitting equation of logistic model is:

$$y = \frac{k}{1 + e^{a-bt}}$$

In the formula: y is the cumulative growth of the shoot length, k is the growth limit value of the fitted shoot length, t is the growth time, a and b are undetermined coefficients.

Results

Chemical compounds of *M. glyptostroboides* litter aqueous extract

The GC–MS analysis maps of ether extract phase, ethyl acetate extract phase and n-butanol extract phase of *M. glyptostroboides* litter aqueous extracts were shown in Fig. 2. The spectral library analysis revealed that 28 chemical compounds were detected in *M. glyptostroboide*s litter aqueous extracts (Table 1). The number of chemical compounds in fresh litter was 5.56% more than that in natural litter. Nineteen major chemical compounds and eighteen major chemical compounds were detected in fresh and natural litter, respectively. The relative contents of trifluoroacetamide and D-(+)-arabitol in fresh and natural litter were the highest, and included 50.59% ~ 95.22% and 79.13% ~ 84.97%, respectively. Meanwhile, the chemical compounds detected in ether extraction phase were the highest, followed by n-butanol extraction phase and ethyl acetate extraction phase. Alcohols, acids and amides were detected in both fresh and natural *M. glyptostroboide*s litter, while sugars and phenols were present only in the fresh litter, and aldehydes and alkanes were unique to the natural litter. N,N-diethylformamide and glycerol appeared most frequently (four times), trifluoroacetamide, D-(+)-arabitol, D-terpineol, succinic acid and phosphoric acid appeared three times, 8 chemical compounds such as 2,3-butanediol appeared twice, while 12 chemical compounds such as 1,2,3-butanetriol appeared only once.

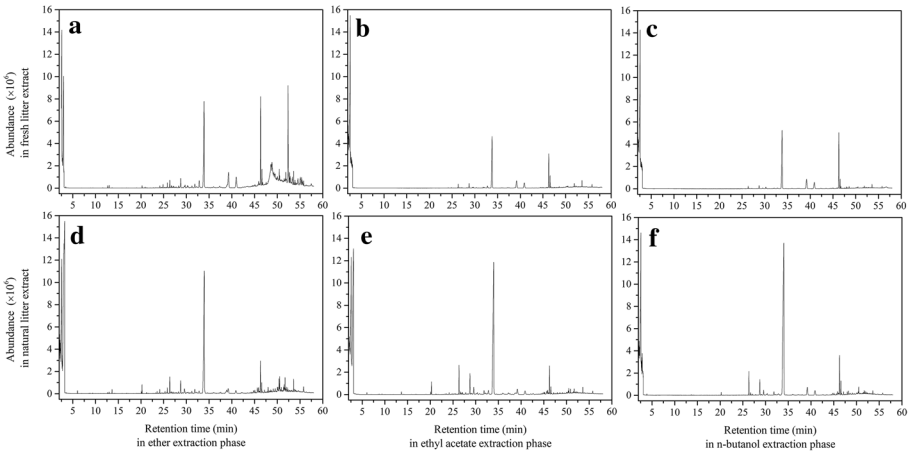

Fig. 2 GC–MS total ion current chromatograms of the organic solvent extraction phases of different *M. glyptostroboides* litter aqueous extracts. These chemical compounds were detected on December 26th, 2020

The Jaccard similarity coefficient of the chemical compounds contained in fresh and natural litter reached 50%, which illustrated a certain difference in the chemical compounds of fresh and natural litter. Trifluoroacetamide, D-(+)-arabitol, D-pinitol, succinic acid, N, N-diethylformamide, lactic acid, glycerol, pentaerythritol and 2,3-butanediol were simultaneously detected in both fresh and natural litter.

Effects of chemical compounds in *M. glyptostroboides* litter on seed germination

Germination rate, germination index and vigor index of *M. glyptostroboides* seeds were significantly affected by different concentrations of catechol, trifluoroacetamide, benzoic acid or D-(+)-arabitol (Table 2, $P < 0.05$). Overall, maximum germination rate, germination index and vigor index were reached in low concentrations, even above CK, and were lower only at the highest concentrations of each chemical compound (Table 3). The germination rate, germination index and vigor index of catechol 0.04 and 0.2 mg·mL^{-1} were not statistically different from CK, while these three seed germination indicators were significantly inhibited at 1 and 5 mg·mL^{-1}. Moreover, all *M. glyptostroboides* seeds failed to germinate at the concentration of 1 and 5 mg·mL^{-1} benzoic acid and 5 mg·mL^{-1} catechol. Compared with CK, trifluoroacetamide and benzoic acid stimulated seed germination and vigor at 0.04 mg·mL^{-1}, but significantly hindered these three seed germination indicators at concentrations higher than 0.02 mg·mL^{-1}. The effect of D- (+)-arabitol on the seed germination was somewhat different from that of the other three chemical compounds. It was worth highlighting that the concentration ranging from 0.04 to 1 mg·mL^{-1} of D-(+)-arabitol showed positive stimulation, although it was not significantly different from CK. The highest germination rate of D-(+)-arabitol was at 1 mg·mL^{-1} (46.00%), while germination rate, germination index and vigor index at 5 mg·mL^{-1} were significantly less than CK by 80.44%, 91.03% and 98.04%, respectively.

Table 1 Major chemical compounds of *M. glyptostroboides* litter aqueous extract

Retention time (min)		Compound name	Compound classification	Relative content (%)					
				Ether extract phase		Ethyl acetate extract phase		*N*-butanol extract phase	
Fresh litter	Natural litter			Fresh litter	Natural litter	Fresh litter	Natural litter	Fresh litter	Natural litter
2.972	3.061	Trifluoroacetamide	Amides	50.59	95.22	–	92.21	–	–
8.937	8.759	N,N-diethylformamide	Amides	0.06	0.16	–	0.08	–	0.04
11.608		Propylene glycol	Alcohols	0.07	–	–	–	–	–
12.682	12.676	2,3-Butanediol	Alcohols	0.40	0.33	–	–	–	–
13.643	13.638	Lactic acid	Acids	0.11	–	–	0.43	–	–
	14.184	Glycolic acid	Acids	–	–	–	0.07	–	0.03
	17.217	Dodecamethylpentasiloxane	Alkanes	–	0.09	–	–	–	–
	19.027	Benzoic acid	Acids	–	0.18	–	–	–	–
	20.077	Phosphoric acid	Acids	–	0.64	–	0.41	–	0.06
20.255	20.238	Glycerol	Alcohols	0.43	2.17	–	1.90	–	0.38
20.653		1,2,3-Butanetriol	Alcohols	0.13	–	–	–	–	–
20.95		Catechol	Phenols	0.21	–	–	–	–	–
21.294	21.205	Succinic acid	Acids	0.22	0.30	–	0.08	–	–
	21.704	Glyceric acid	Acids	–	0.16	–	0.18	–	–
	23.554	2,3-Dihydroxyacrylicacid	Acids	–	0.75	–	–	–	–
	26.167	Meso-Erythritol	alcohols	–	–	–	4.64	–	0.09
27.728		Dodecanoic acid	acids	1.40	–	–	–	1.51	–
28.737	28.737	Pentaerythritol	alcohols	–	–	–	–	2.32	3.16
	30.167	2,3,4,5-Tetrahydroxypentanal	aldehydes	–	–	–	–	–	0.39
31.218		2,6-Di-tert-butyl-4-methylphenol	phenols	0.96	–	–	–	–	–
	31.847	2,3,4,5-Tetrahydroxyhexanal	aldehydes	–	–	–	–	–	1.06
33.788	34.002	D-(+)-Arabitol	alcohols	–	–	84.97	–	79.13	92.04
33.889		Xylitol	alcohols	45.42	–	–	–	–	–

Table 1 (continued)

Retention time (min)		Compound name	Compound classification	Relative content (%)					
				Ether extract phase		Ethyl acetate extract phase		*N*-butanol extract phase	
Fresh litter	Natural litter			Fresh litter	Natural litter	Fresh litter	Natural litter	Fresh litter	Natural litter
40.887	40.875	D-Pinitol	alcohols	–	–	13.92	–	13.32	2.75
42.317		Myristic acid	acids	–	–	–	–	0.39	–
47.172		6-Deoxy-beta-L-Galactopyraniose	sugars	–	–	0.30	–	0.30	–
48.549		Myo-Inositol	alcohols	–	–	0.81	–	1.10	–
50.116		11-Trans-Octadecenoic acid	acids	–	–	–	–	1.93	–

The chemical compounds listed are those retrieved by mass spectrometry database of GC–MS with a matching degree of ≥ 90%. '-'means not detected

Table 2 One-way ANOVA for the effect of different chemical compounds on seed germination and growth

Variance source	Seed germination and growth indicators	Square sum of type III	*df*	Mean square	*F*	*P*
Catechol	Germination rate	3398.933	4	849.733	14.162	0.000
	Germination index	17.658	4	4.414	29.675	0.000
	Vigor index	8.524	4	2.131	44.415	0.000
	Shoot length	6121.904	4	1530.476	476.700	0.000
	Main root length	282.025	4	70.506	93.953	0.000
Trifluoroacetamide	Germination rate	2774.933	4	693.733	45.640	0.000
	Germination index	15.103	4	3.776	39.669	0.000
	Vigor index	9.207	4	2.302	104.465	0.000
	Shoot length	6532.934	4	1633.233	1167.118	0.000
	Main root length	351.836	4	87.959	166.770	0.000
Benzoic acid	Germination rate	5494.400	4	1373.600	59.207	0.000
	Germination index	21.572	4	5.393	64.157	0.000
	Vigor index	8.467	4	2.117	45.385	0.000
	Shoot length	6253.889	4	1563.472	399.261	0.000
	Main root length	280.896	4	70.224	179.979	0.000
D-(+)-arabitol	Germination rate	3166.400	4	791.600	14.769	0.000
	Germination index	12.965	4	3.241	13.729	0.000
	Vigor index	8.295	4	2.074	22.206	0.000
	Shoot length	4558.143	4	1139.536	245.912	0.000
	Main root length	214.535	4	53.634	50.242	0.000

Effects of chemical compounds in *M. glyptostroboides* litter on shoot growth

The logistic model was used to fit the *M. glyptostroboides* shoot length growth because the change of *M. glyptostroboides* shoot length growth followed a 'S'-shaped growth curve (Fig. 3, Table 4). Specifically, it started growing slowly, then growed rapidly, and then tended to slow down. The growth rates of *M. glyptostroboides* shoot lengths were inhibited by different chemical compounds at different concentrations, catechol and benzoic acid also hindered the growth rate of shoot length at low concentrations (0.04 mg·mL^{-1}) (Fig. 3a, Fig. 3c). Low concentrations of trifluoroacetamide and D-(+)-arabitol did not significantly hinder the growth rate of shoot length. Trifluoroacetamide produced negative effects at concentrations higher than 0.04 mg·mL^{-1} (Fig. 3b), and D-(+)-arabitol produced negative effects only at concentrations higher than 0.2 mg·mL^{-1} (Fig. 3d). In the range of 1 ~ 5 mg·mL^{-1}, the growth rate of shoot length and the time to enter the linear growth phase were obviously smaller than CK. Meanwhile, the growth curves of shoot length tended to level off with increasing concentration. The growth process curves of benzoic acid and catechol were lower than those of trifluoroacetamide and D-(+)-arabitol at 1 and 5 mg·mL^{-1} concentrations.

Shoot length and main root length of *M. glyptostroboides* were also significantly affected by different concentrations of catechol, trifluoroacetamide, benzoic acid or D-(+)-arabitol ($P < 0.05$, Table 2). Overall, shoot length and main root length of *M. glyptostroboides* were inhibited at high concentrations, and the inhibition threshold

Table 3 Germination rate, germination index and vigor index of *M. glyptostroboides* seeds grown under different treatments (four chemical compounds and four concentrations)

Chemical compound	Concentration ($mg \cdot mL^{-1}$)	Germination rate (%)	Germination index	Vigor index
Catechol	CK	30.67 ± 2.49ab	2.34 ± 0.21a	1.53 ± 0.10a
	0.04	41.33 ± 6.80a	2.84 ± 0.39a	1.79 ± 0.31a
	0.2	40.67 ± 4.11ab	2.58 ± 0.28a	1.54 ± 0.20a
	1	26.00 ± 11.43b	0.97 ± 0.46b	0.20 ± 0.13b
	5	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00b
Trifluoroacetamide	CK	30.67 ± 2.49b	2.34 ± 0.21ab	1.53 ± 0.10b
	0.04	40.67 ± 4.71a	2.80 ± 0.44a	2.02 ± 0.21a
	0.2	34.00 ± 3.27ab	2.16 ± 0.13b	1.37 ± 0.13b
	1	22.00 ± 3.27c	1.02 ± 0.23c	0.21 ± 0.05c
	5	1.33 ± 0.94d	0.04 ± 0.03d	0.00 ± 0.00c
Benzoic acid	CK	30.67 ± 2.49b	2.34 ± 0.21ab	1.53 ± 0.10a
	0.04	44.67 ± 7.36a	2.84 ± 0.42a	1.78 ± 0.31a
	0.2	38.67 ± 4.11ab	1.98 ± 0.24b	1.11 ± 0.22b
	1	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
	5	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c
D-(+)-arabitol	CK	30.67 ± 2.49b	2.34 ± 0.21a	1.53 ± 0.10a
	0.04	42.67 ± 5.73ab	2.69 ± 0.36a	2.11 ± 0.20a
	0.2	40.67 ± 4.99ab	2.53 ± 0.29a	1.95 ± 0.18a
	1	46.00 ± 10.20a	2.53 ± 0.72a	1.64 ± 0.48a
	5	6.00 ± 3.27c	0.21 ± 0.12b	0.03 ± 0.01b

Values are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences ($P < 0.05$, Duncan's test)

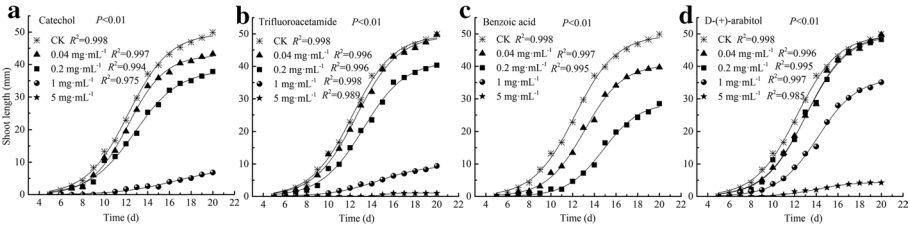

Fig. 3 Curve fitting of *M. glyptostroboides* shoot length treated with different concentrations of same chemical compound. Because seeds failed to germinate at 1 and 5 $mg \cdot mL^{-1}$ of benzoic acid and 5 $mg \cdot mL^{-1}$ of catechol, the relevant data were lacking in the figure

concentration varied among different chemical compounds (Fig. 4). Shoot lengths of catechol and benzoic acid treatments were significantly smaller than CK (Fig. 4a, Fig. 4c), and the shoot lengths decreased with increasing concentration. 0.04 $mg \cdot mL^{-1}$ of trifluoroacetamide was not statistically different from CK, but the shoot length at 0.2, 1 and 5 $mg \cdot mL^{-1}$ was significantly reduced by 19.10%, 81.30% and 98.66% compared with CK, respectively (Fig. 4b). There was no significant difference in shoot length

Table 4 Parameters of height-logistic-equation of *M. glyptostroboides* seedling under different concentrations and chemical compounds

Chemical compound	Concentration ($mg \cdot mL^{-1}$)	Logistic equation parameters					
		Growth limitation (k)	Undetermined coefficient (a)	Undetermined coefficient (b)	Determination coefficient (R^2)	Levene (F)	Significant level (P)
CK	0	49.632	6.386	0.523	0.998	7378.664	0.000
Catechol	0.04	42.918	7.126	0.584	0.997	4158.226	0.000
	0.2	38.390	6.201	0.484	0.994	2243.351	0.000
	1	8.363	6.475	0.392	0.975	387.927	0.000
	5	–	–	–	–	–	–
Trifluoroacetamide	0.04	49.509	6.482	0.515	0.996	3587.936	0.000
	0.2	41.529	6.790	0.514	0.996	3468.168	0.000
	1	9.702	6.655	0.454	0.988	919.075	0.000
	5	1.027	7.744	1.905	0.989	724.354	0.000
Benzoic acid	0.04	40.574	7.463	0.570	0.997	4026.081	0.000
	0.2	28.715	8.793	8.793	0.995	2123.535	0.000
	1	–	–	–	–	–	–
	5	–	–	–	–	–	–
D-(+)-arabitol	0.04	50.020	6.897	0.528	0.996	3271.000	0.000
	0.2	49.782	6.746	0.517	0.995	2829.650	0.000
	1	36.453	7.768	0.549	0.997	3520.510	0.000
	5	4.238	8.023	0.597	0.985	678.492	0.000

Because seeds failed to germinate at 1 and 5 $mg \cdot mL^{-1}$ of benzoic acid and 5 $mg \cdot mL^{-1}$ of catechol, the relevant data were lacking in the table

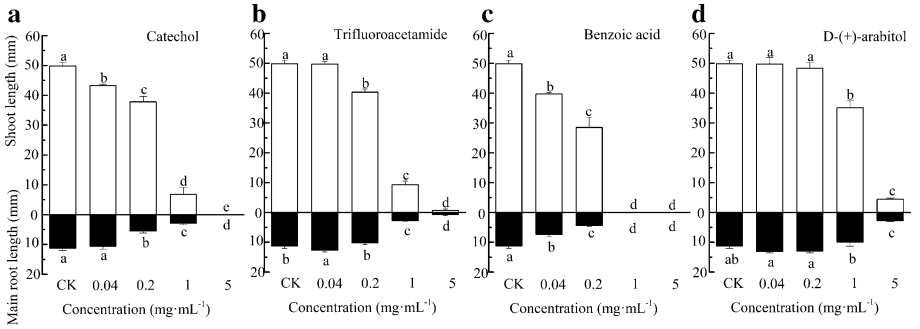

Fig. 4 Effects of the same chemical compound under different concentrations on shoot length and main root length of *M. glyptostroboides*. Different lowercase letters indicate significant differences ($P < 0.05$, Duncan's test). Because seeds failed to germinate at 1 and 5 mg·mL^{-1} of benzoic acid and 5 mg·mL^{-1} of catechol, the relevant data were lacking in the figure

between the 0.04 and 0.2 mg·mL^{-1} of D-(+)-arabitol and CK, but 1 and 5 mg·mL^{-1} treatments decreased by 29.58% and 91.17% compared with CK, respectively (Fig. 4d).

Under catechol treatment, the main root length had no significant difference with CK only at 0.04 mg mL^{-1}, while 0.2, 1 and 5 mg·mL^{-1} treatments significantly decreased by 51.16%, 74.38% and 100% than CK, respectively (Fig. 4a). The main root length of trifluoroacetamide treated with only 0.04 mg·mL^{-1} was 13.13% higher than that of CK, and 1 and 5 mg·mL^{-1} were significantly lower than CK (Fig. 4b). The main root lengths under benzoic acid treatment were all observed as significantly inhibitory effect, and the main root lengths decreased with increasing concentration (Fig. 4c). D-(+)-arabitol significantly inhibited the main root length only at the concentration of 5 mg·mL^{-1}, which was 75% less than CK (Fig. 4d). Moreover, although the main root length was smaller than shoot length, the shoot length was inhibited higher than the main root length with increasing concentrations of the chemical compounds. Shoot growth was severely inhibited by catechol and benzoic acid, and the treatments of trifluoroacetamide and D-(+)-arabitol with 1 and 5 mg·mL^{-1} were also not conducive to the shoot growth.

Allelopathic effect evaluation of chemical compounds in *M. glyptostroboides* litter on seed germination and shoot growth

Important differences in allelopathy sensitivity index (*RI*) at different chemical compound concentrations (Table 5). When the concentration was ≤ 0.2 mg·mL^{-1}, the *RI* of catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol on seed germination rate ranged from 0.10 to 0.31, all of which showed promoting effects. When the concentration was ≥ 1 mg·mL^{-1}, the seed germination rate was inhibited by all treatments (-0.18 ~ -22.00) except 1 mg·mL^{-1} treatment of D-(+)-arabitol. The response of germination index and vigor index to different concentrations of chemical compound showed some differences. Catechol in the range of 0.04 ~ 0.2 mg·mL^{-1} promoted it, while 1 ~ 5 mg·mL^{-1} inhibited it. When the concentration was ≥ 0.2 mg·mL^{-1}, the inhibitory effect of benzoic acid and the negative effect of trifluoroacetamide increased with increasing concentration. The germination index and vigor index were inhibited by D-(+)-arabitol only at 5 mg·mL^{-1}. In addition, shoot length and main root length of *M. glyptostroboides* were all inhibited by catechol and benzoic acid, and the higher the concentration, the stronger the inhibition.

Table 5 Allelopathic sensitivity index (*RI*) and synthetical effect of allelopathy index (*SE*) of different chemical compound concentrations on seed germination and shoot growth of *M. glyptostroboides*

Chemical compound	Concentration ($mg \cdot mL^{-1}$)	*RI* value of germination rate	*RI* value of germination index	*RI* value of vigor index	*RI* value of shoot length	*RI* value of main root length	Synthetical effect of allelopathy index
Catechol	CK	0.00	0.00	0.00	0.00	0.00	0.00
	0.04	0.26	0.18	0.15	−0.15	−0.06	0.08
	0.2	0.25	0.09	0.01	−0.32	−1.05	−0.20
	1	−0.18	-1.41	−6.81	−6.39	−2.91	−3.54
	5	–	–	–	–	–	–
Trifluoroacetamide	0.04	0.25	0.17	0.24	0.00	0.12	0.16
	0.2	0.10	−0.08	−0.11	−0.24	−0.10	−0.09
	1	−0.39	−1.30	−6.33	−4.35	−3.11	−3.06
	5	−22.00	−53.25	−1143.72	−73.77	−15.81	−261.71
Benzoic acid	0.04	0.31	0.18	0.14	−0.26	−0.52	−0.03
	0.2	0.21	−0.18	−0.38	−0.75	−1.57	−0.53
	1	–	–	–	–	–	–
	5	–	–	–	–	–	–
D-(+)-arabitol	0.04	0.28	0.13	0.28	0.00	0.14	0.17
	0.2	0.25	0.08	0.22	−0.03	0.13	0.13
	1	0.33	0.08	0.07	−0.42	−0.13	−0.01
	5	−4.11	−9.94	−59.25	−10.33	−3.00	−17.33

Because seeds failed to germinate at 1 and 5 $mg \cdot mL^{-1}$ of benzoic acid and 5 $mg \cdot mL^{-1}$ of catechol, the relevant data were lacking in the table

Trifluoroacetamide showed an inhibitory effect in the range of 0.2 to 5 mg·mL^{-1}, and the strongest inhibitory effect was observed at high concentration. Shoot growth indicators were inhibited by D-(+)-arabitol in the range of 1 to 5 mg·mL^{-1}.

At 0.04 mg·mL^{-1}, the *SE* values of catechol and trifluoroacetamide were positive, indicating the promotion of seed germination and shoot growth, but when the concentration was higher than 0.04 mg·mL^{-1}, both compounds generated inhibition (−0.09 ~ −261.71) (Table 5). The *SE* values of benzoic acid were all negative, all showing inhibitory effect. D-(+)-arabitol showed promotion at 0.04 and 0.2 mg·mL^{-1}, and inhibition at 1 and 5 mg·mL^{-1} (−0.01, −17.33).

Discussion

Chemical compounds of *M. glyptostroboides* litter aqueous extract

Autotoxic substances in plants are produced by natural means, and in the natural state, there is no solvent other than water to leach out autotoxic substances from plants (Liu et al. 2019). The water extraction method was used to extract allelochemicals from plants in this study, which was more strongly than a simple wash. The natural leaching intensity of litter is difficult to be precise because of its fluctuation and seasonal dependence. The concentration used in our extraction may be higher than under natural conditions, as we aim to identify its chemical compound. Moreover, litter aqueous extracts were extracted by different solvents, but the extracted chemical compounds were all derived from the water-soluble substances in the aqueous extract. Seven types of chemicals (phenols, alcohols, acids, sugars, amides, aldehydes and alkanes) were identified from the *M. glyptostroboides* litter, most of which belonged to reported autotoxic substances of conifer species (Wang et al. 2007; Pan et al. 2009). Because *M. glyptostroboides* is also a conifer species, so water-soluble organic acids (lactic acid, glycolic acid, succinic acid), straightchain alcohols (propylene glycol, 2,3-butanediol, glycerol, 1,2,3-butanetriol, Meso-erythritol, D-(+)-arabitol, xylitol), aliphatic aldehydes (2,3,4,5-tetrahydroxypentanal, 2,3,4,5-tetrahydroxyhexanal), long chain fatty acids (glyceric acid, 2,3-dihydroxyacrylicacid, dodecanoic acid, myristic acid, 11-trans-octadecenoic acid), simple phenols (catechol, 2,6-di-tert-butyl-4-methylphenol), benzoic acid and its derivatives (benzoic acid) may be potential autotoxic substances of *M. glyptostroboides*. More detailed studies on these autotoxic substances are needed. Yu (2008) identified ferulic acid, cinnamic acid and p-hydroxybenzoic acid as the autotoxic substances in *Cryptomeria fortunei* litter, but these autotoxic substances were not included in our findings. This may be due to the uniqueness of the research object, and this also shows that there are differences in the composition of the autotoxic substances in plants of the same family.

The number of chemical compounds in fresh litter of *M. glyptostroboides* was higher than that in natural litter. Fresh litter contains relatively complete and abundant secondary metabolites, because fresh litter is the first to fall off and has not entered the full decomposition stage. Natural litter is a mixture of different decomposing layers, which may eventually lead to the degradation and flushing of certain autotoxic substances from natural ecosystems due to its weathering and leaching over a certain period of time (Araniti et al. 2016; Xu et al. 2021). In addition, the Jaccard similarity coefficient of chemical compounds of fresh and natural litter of *M. glyptostroboides* reached 50%, which was higher than the Jaccard similarity coefficient of chemical compounds in *Lilium davidii* var. *unicolor salisb*

soil (Huang et al. 2021a). This might indicate that *M. glyptostroboides* is richer than other species in recalcitrant chemical compounds with low decomposition rates. The Jaccard similarity coefficient of *M. glyptostroboides* litter is higher because the collection time of fresh and natural litter is relatively close, and the decomposition degree of natural litter is limited to a month. In the future, indoor and field experiments should be carried out based on the dynamic changes of litter composition under natural conditions. The relative content and occurrence frequency of trifluoroacetamide and D-(+)-arabitol were high, which belonged to autotoxic substances (Guo et al. 2019). Although the relative contents of benzoic acid and catechol were low, they were typical autotoxic substances (Wang et al. 2007; Pan et al. 2009). Therefore, these four chemical compounds were selected to test in the subsequent study.

Effects of chemical compounds in *M. glyptostroboides* litter on seed germination and shoot growth

Seed germination is one of the most sensitive and important stages in plant growth cycle (Rajjou et al. 2012), which is of great significance to population reproduction, renewal and dispersal. High concentrations (5 $mg{\cdot}mL^{-1}$) of catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol inhibited the seed germination indicators of *M. glyptostroboides*, while low concentrations (0.04 $mg{\cdot}mL^{-1}$) had no significant or slightly promoting effect. This result is consistent with 'low promotion and high suppression' mass concentration effect of some autotoxic substances on plant growth (Warrag 1995; Wang et al. 2018a, b; Huo et al. 2019; Zhang et al. 2021). This may be because that low concentrations of autotoxic substances activate the plant's antioxidant defense mechanism, inducing plants to take measures such as increasing seed germination rate and growth rate to deal with adverse factors (Graine and Dybzinski 2013). High concentrations of chemical compounds inhibit seed germination and growth of *M. glyptostroboides*, because excessive harmful substances exceed the regulatory threshold of plants, destroy organelles and affect cell division and elongation (Mahdavikia et al. 2017). The medium concentration (1 $mg{\cdot}mL^{-1}$) of D-(+)-arabitol significantly promoted seed germination rate of *M. glyptostroboides,* which was similar to the results of medium concentration of fresh *M. glyptostroboides* litter aqueous extract (Xu et al. 2021). This may be attributed to the fact that different autotoxic substances have different concentration effects, and the same autotoxic substance also shows different action directions at different concentrations (Wang et al. 2018a; Huang et al. 2021b). The higher the concentration of each autotoxic substance, the stronger the inhibition degree of the initial stages of seed germination (Chaves Lobón et al. 2019). The earlier the seed germination, the more conducive to enhance the competitiveness and viability of plants (Shen et al. 2015).

Healthy seedling growth is an important prerequisite for plant species survival and reproduction in natural communities (He et al. 2021), and shoot growth is the initial stage from seed to early seedling establishment. Both catechol and benzoic acid reduced shoot length, main root length and growth curve rate of *M. glyptostroboides*, and their inhibitory effects on seedling growth were generally shown to increase with increasing concentration. This result is consistent with the inhibitory effect of *P. koraiensis* litter extract on the growth of *Lepidium sativum* seedlings (Kimura et al. 2015), and the same as the inhibitory effect of dibutyl phthalate on the growth of *Lactuca sativa* seedlings (Geng et al. 2008). Trifluoroacetamide and D-(+)-arabitol showed a 'promotion at low concentration and inhibition at high concentration' effect on *M. glyptostroboides* shoot growth. This is in line

with the effects of dioctyl terephthalate and 2,2'-methylenebis (6-tert-butyl-4-methyl-phenol) on the growth of *L. davidii* var. *unicolor salisb* seedlings (Huang et al. 2021a). Meanwhile, the 'S'-shaped growth curve describes the growth process of plants under spatial constraints (Wu et al. 2020; Xu et al. 2021). The growth dynamics of shoot length of *M. glyptostroboides* under different concentrations of catechol, benzoic acid, trifluoroacetamide and D-(+)-arabitol also conformed to the 'S'-shaped growth curve ($R^2 \geq 0.975$). The 'S'-shaped growth curve is also suitable for fitting shoot growth (Xu et al. 2021). Moreover, the growth rates of *M. glyptostroboides* seed shoot lengths were significantly inhibited by high concentrations of catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol. This also confirmed that these four chemical compounds were autotoxic substances in *M. glyptostroboides* litter, and their autotoxic effects were concentration-dependent (Liu et al. 2019).

Allelopathic effect evaluation on seed germination and shoot growth

Allelopathic effect evaluation is an important way to measure the intensity of allelopathic effects (Liu et al. 2019). The autotoxic substances in *M. glyptostroboides* litter inhibited the shoot growth, and its inhibition was stronger than the seed germination. This is consistent with the results of a previous study (Xu et al. 2021), because seeds have the protection of seed coat and seed wings, there is a lagging response of seed germination to autotoxic substances (Devaney et al. 2018). The shoots are in direct contact with autotoxic substances, and autotoxic substances in the plant continue to accumulate as the contact time increases, thus reducing the uptake of nutrients and hindering the rapid accumulation of organic matter (Xu et al. 2021). Different autotoxic substances have different degrees and directions of action, because of the differences in the mechanisms of different autotoxic substances and the inconsistent sensitivities of the research objects. This confirms the mass concentration effect, selectivity, specificity and complex diversity of autotoxic substances (Wang et al. 2007; Huang et al. 2021a).

Natural regeneration and litter autotoxicity

Successful natural regeneration of *M.glyptostroboides* seedlings are difficult to be found under existing original and asexual mother forests of *M.glyptostroboides* (Lin et al. 2017). The failure of natural regeneration means that plants have difficulty in achieving genetic recombination, adapting to changing environments, and also reducing their evolutionary potential (Wiegand et al. 2009; D'amato et al. 2009). Successful natural regeneration of plants is closely linked to seed germination, seedling growth and environmental conditions. Among them, plant autotoxicity is a key factor affecting the success or failure of natural regeneration of plants (Alías et al. 2006; Huo et al. 2019). The autotoxic effects of different species, different parts and different decomposing litter are somewhat different and show a concentration effect of "low promotion and high inhibition" or "absolute inhibition effect" (Warrag 1995; Guo et al. 2019; Xu et al. 2021).

The release of autotoxic substances was closely related to the density of the stand in which the autotoxic substances were produced (Sinkkonen 2007). The more original population of *M. glyptostroboides* are now concentrated and relatively dense, the more litter there will be, and the more autotoxic substances will be accumulated, resulting in difficulties in the production and survival of new *M. glyptostroboides* individuals. Furthermore, autotoxicity may also depend on the life history of plants (Jackson and Willemsen 1976;

Zhang et al. 2021). *Metasequoia glyptostroboides* is a long-lived species, reaching into the tens or even hundreds of years. In limited living space, *M. glyptostroboides* may obtain sufficient living space and nutrition by suppressing seedling growth as a survival strategy for adult *M. glyptostroboides*.

Seed germination and seedling establishment are two of the most vulnerable stages in the life history of most plants (Svensson et al. 2013), and litter can have a significant impact on these two stages. In this study, catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol were found to be autotoxic substances in *M. glyptostroboides*. Overall, as the concentrations of all chemical compounds increase, more autotoxic effects occur. In the field experiment, the growth of *M. glyptostroboides* seedlings was significantly inhibited when the litter thickness exceeded 300 $g \cdot m^{-2}$ (0.5 cm). In the indoor seed germination experiment, *M. glyptostroboides* litter aqueous extract inhibited its own seed germination and growth (Xu et al. 2021). These results indicate that a large number of *M. glyptostroboides* litter forms physical barrier and allelopathy inhibitory effects, ultimately limiting the natural regeneration of *M. glyptostroboides*. In the conservation and management of *M. glyptostroboides* populations, all litter in *M. glyptostroboides* forest land should be properly cleared before the peak of the seed rain occurs to help seed contact with the soil. In the process of seedling growth, regularly clean up litter without damaging the seedlings, which can avoid the accumulation of allelochemicals and promote the natural regeneration of *M. glyptostroboides*. But in the actual ecological environment, litter will also undergo chemical-biological reactions with other factors such as soil, and there are spatial and temporal differences in the ecological processes of autotoxicity (Sampietro 2006). Autotoxicity is caused by multiple chemical compounds and their synergy, superposition and antagonism (Blum et al. 1999). Therefore, it is necessary to further study the mixed monomers according to the actual concentration of autotoxic substances in the wild *M. glyptostroboides* litter at different stages, to provide a basis for improving the regeneration capacity of *M. glyptostroboides*. How to effectively promote the natural regeneration of *M. glyptostroboides* also remains to be further explored.

Conclusions

Twenty-eight compounds were detected in the aqueous extracts of *M. glyptostroboides* litter at different decomposition stages, alcohols, acids, phenols, aldehydes and amides were the most important types of autotoxic substances in *M. glyptostroboides* litter. Seed germination and growth of *M. glyptostroboides* were significantly inhibited by the autotoxic substances such as catechol, trifluoroacetamide, benzoic acid and D-(+)-arabitol, but the threshold value of inhibition varied depending on the chemical compound. Overall, the autotoxic effect was concentration dependent, low concentrations were positive and neutral, high and extremely high concentrations were negative for all the chemical compounds. The autotoxic substances in *M. glyptostroboides* litter would significantly affect the seed germination and seedling survival of *M. glyptostroboides*, resulting in rare understory seedlings and seriously limiting their natural regeneration.

Appendix

See Figs. 5 and 6.

Fig. 5 Pictures of *M. glyptostroboides* litter, seeds and seedlings. **a** is the germination experiment of *M. glyptostroboides* seeds. **b** is the growth of *M. glyptostroboide* shoots. **c** is the collection site of *M. glyptostroboides* litter

Fig. 6 Pictures of the original mother tree of *M. glyptostroboides*

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Author contributions LX: Experiment design, operation, data analysis, drafting, revision and editing. LY: Provide research direction, experimental design and revision of paper writing. XA: Design test, modify paper, data analysis. QG: Revise papers, guide experiments. SW: Experimental guidance, data analysis. DZ: Experimental guidance, data analysis. CD: Test operation, data acquisition. XA: Test operation, data acquisition. All authors gave their approval for publication.

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Declarations

Conflicts of interest The authors declare no conflict of interest.

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