ALLELOPATHY OF Taxus chinensis var. mairei ON Camptotheca acuminata SEEDLING GROWTH AND IDENTIFICATION OF THE ACTIVE PRINCIPLES

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ABSTRACT
The root extracts and root exudates from the Taxus chinensis var. mairei had significant promote effects on growth of Camptotheca acuminata seedlings. Four taxane chemicals, cephalomannine, 10-deacetylbacatin III, paclitaxel and 7-Epi-10-deacetyl-paclitaxel, were isolated from T. chinensis var. mairei root extracts and their structures were identified by spectroscopic analysis. The chemical concentrations ranged from 0.01 to 5.90 µg/g. At an approximate concentration determined in T. chinensis var. mairei root exudates, all four chemicals significantly promoted the growth of C. acuminata seedlings. Therefore, four chemicals from T. chinensis var. mairei root exudates may act as allelochemicals accelerating growth of C. acuminata seedling. There were significant relationships between growth of C. acuminata seedlings, soil properties, enzyme activities and microbial community under allelochemicals’ application. The results provide a reference for in-depth understanding of C. acuminata and T. chinensis var. mairei interactions.

1. Introduction
Many plants inhibit or promote with the growth and development of interplants that are grown in their vicinity through the release of allelochemicals (Cheng et al. 2020). Camptotheca acuminata Decne. (Nyssaceae) is listed as the first batch of national secondary key protected wild plants and originally found in the mainland of China (Li et al. 2005; Nacheva et al. 2020; Wu et al. 2021). At present, researchers interest in C. acuminata is still mainly reflected in the study on the development and utilization of camptothecin (CPT) (Jin et al. 2019; Wang et al. 2019). Due to the chemical synthesis of CPT has complex operations steps and high cost, and it is still in the laboratory exploration stage to obtain raw materials through bioengineering methods such as cell suspension, tissue culture and hairy root culture (Xu et al. 2020). At present, C. acuminata is used as a raw material for producing CPT, however the unreasonable exploitation and poor growth status of pure wild-type C. acuminata has significantly reduced the distribution of wild C. acuminata resources (Zhou et al. 2010). It has long been carried out studies on the artificial complex community of C. acuminata, and done a lot of research on ecology for the sustainable utilization of C. acuminata resources (Li et al. 2008). In terms of cultivation methods, it has been proved that the selection of suitable tree species to build a multilayer ecosystem by blending can not only improve the land use index, but also effectively increase the total biomass. Previous studies have shown that the growth of C. acuminata can be improved when it is interplanting with Taxus chinensis var. mairei (Guan 2020). However, the mechanism which T. chinensis var. mairei promotes the growth of C. acuminata remains unclear. Therefore, isolation and identification of responsible allelochemicals are crucial for exploring T. chinensis var. mairei promoting the growth of C. acuminata.

Since plants can self-regulate their distribution and densities in nature via allelopathic interactions, the exploitation of allelopathic responses could provide alternative methods for promoting plant growth (Hierro and Callaway 2021; Zhang et al. 2018; Zhou et al. 2013). Plant allelopathic interactions are mediated by allelochemicals released from plants into the environment, mostly into the soil, and the search for allelochemicals has been pursued extensively (Scavo et al. 2019). A plant may interfere with the growth of neighboring plant through competition inhibition and/or stimulatory effects (Liu et al. 2020). Studies have shown that the low concentration extract of T. chinensis var. mairei seed has a promoting effect on Chinese cabbage (Liu et al. 2020). The results of our research group show that the extract of different parts of T. chinensis var. mairei also has a significant promoting allelopathy effect on C. acuminata seedling growth (Zhang et al. 2018). In order to investigate the promotion

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mechanism of T. chinensis var. mairei on C. acuminata growth, the chemicals with stimulatory activities were evaluated. Accordingly, the objectives of this study were to identify the allelochemicals from T. chinensis var. mairei root extracts and to evaluate their effects on growth of C. camptotheca seedlings combined with the rhizosphere soils chemical properties, enzyme activities and microbial redundancy (RDA).

2. Materials and methods

2.1. Instruments

The UPLC-MS/MS analysis was conducted on an Agilent 1290 Infinity UPLC system coupled to an Agilent 6460 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Agilent Technologies, USA). The NMR spectra were measured with a Bruker AV600 NMR spectrometer (Bruker Instrument Co., Karlsruhe, Germany). An Agilent 1260 HPLC with diode array detector (DAD) (Agilent Technologies, Santa Clara, CA, USA) was used for the quantitation analysis of allelochemicals. A WRS-2A automatic melting point apparatus is used for measuring the melting point of crystalline substance (Hangzhou West Tune Trading Co., Limited).

2.2. Plant materials and chemicals

Preparation of extracts from T. chinensis var. mairei: T. chinensis var. mairei (5-year) and C. acuminata seedlings (1-year) were obtained from the greenhouse, Key Laboratory of Forest Plant Ecology. The T. chinensis var. mairei roots were separated from stems, and left to dry in an airy room for two weeks. Then, they were grinded to obtain a fine powder, 10 g of T. chinensis var. mairei powder were homogenized with 100 mL of distilled water. The homogenate was filtered and the filtrates were used as extracts.

Collection of root exudates of T. chinensis var. mairei by a plant root exudate collection box with double structure (Figure 1) (Zhao 2014). Twenty healthy T. chinensis var. mairei plants were grown in a pot with sand culture. Nutrient solution (KNO₃, 0.61 g/L; Ca(NO₃)₂, 0.95 g/L; (NH₄)₂HPO₄, 0.12 g/L; MgSO₄, 0.50 g/L; pH = 6.0) was irrigated to each pot at start of the pot was collected and filtered after 15 days. The filtrate was concentrated in vacuo, and the concentrated extracts were successively partitioned two times with ethyl acetate and acetone. Each of the extracts was subsequently concentrated, and the concentrate were redissolved in dichloromethane dissolution. The dichloromethane extract was subjected to silica gel column (5 cm × 80 cm) by eluting stepwise with a mixture of 500 mL ethyl acetate / acetone (10:0, 8:2, 6:4, 4:6, 2:8, 0:10, 1:9, 2:8; 3:7, 4:6 and 5:5, v/v). Resulting fractions were screened using a bioassay-guided approach. Finally, different fractions with allelopathic activity were obtained. The obtained components were further purified by prepared liquid chromatography. The chromatographic conditions were as follows: Sino Chorm ODS-BP column (20.0 mm × 250 mm, 5 μm); Mobile phase: methanol–water (50 50, V/V); Detection wavelength: 227 nm; Column temperature: 25°C; Flow rate: 15 mL/min, injection volume: 500 μL. The method of Rimando et al was used for screening, and four active components were obtained (Rimando et al. 2001).

2.3. Isolation and identification of compounds from T. chinensis var. mairei root extracts

A total of 40 g of air-dried T. chinensis var. mairei root were soaked with 70% aqueous MeOH at a temperature of 25°C, extracted for 12 h, and filtered. The filtrate was concentrated in vacuo, and the concentrated extracts were successively partitioned two times with ethyl acetate and acetone. Each of the extracts was subsequently concentrated, and the concentrate were redissolved in dichloromethane dissolution. The dichloromethane extract was subjected to silica gel column (5 cm × 80 cm) by eluting stepwise with a mixture of 500 mL ethyl acetate / acetone (10:0, 8:2, 6:4, 4:6, 2:8, 0:10, 1:9, 2:8; 3:7, 4:6 and 5:5, v/v). Resulting fractions were screened using a bioassay-guided approach. Finally, different fractions with allelopathic activity were obtained. The obtained components were further purified by prepared liquid chromatography. The chromatographic conditions were as follows: Sino Chorm ODS-BP column (20.0 mm × 250 mm, 5 μm); Mobile phase: methanol–water (50 50, V/V); Detection wavelength: 227 nm; Column temperature: 25°C; Flow rate: 15 mL/min, injection volume: 500 μL. The method of Rimando et al was used for screening, and four active components were obtained (Rimando et al. 2001).

3. Results and discussion

The reagents (methanol, chloroform, acetone, dichloromethane and ethyl acetate) used for the extraction and isolation of allelochemicals were purchased from Tianjin Kernel Chemical Reagent Co., Ltd. Paclitaxel, cephalomannine, 10- Decaethylbaccatin III and 7-epi-10-deacetylpaclitaxel reference standards (purity 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Data for cephalomanninine, colorless acicular powder; (C₁₅₅, H₃NO₄)₃, MW: 831.9164, MP: 139–141°C; ¹HNMR (CDCl₃, 300 MHz) δ₁.14 (3H, s, 17-CH₃), 1.28 (3H, s, 16-CH₃), 1.65 (3H, s, 19-CH₃), 1.75 (3H, dd, J = 1.1, 6.9, 4°-CH₃), 1.82(3H, d, J = 1.4, 18-CH₃), 1.80 (3H, d, J = 1.2, 2°-CH₃), 1.84 (1H, ddd, J = 2.2, 10.9, 14.8, 6°-H), 2.24 and 2.36 (each 3H, s, 2xOAc), 2.22 (1H, dd, J = 8.4, 15.4, 14°-H), 2.33 (1H, dd, J = 9.0, 15.4, 14°-H), 2.47 (1H, ddd, J = 6.7, 9.7, 14.8, 6°-H), 3.81 (1H, d, J = 7.3 Hz, 3-H), 4.18 and 4.30 (each 1H, d, J = 8.4 Hz, 20-H₂), 4.33 (1H, dd, J = 6.7, 10.9, 7-H), 4.65 (1H, d, J = 2.8, 2°-H), 4.96 (1H, d, J = 2.2, 9.7, 5-H), 5.64 (1H, dd, J = 2.8, 8.8, 3°-H), 5.65 (1H, d, J = 7.3, 2-H), 6.12, 6.13, 6.15 (1H, brt, J = 8.3, 13-H), 6.25 (1H, s, 10-H), 6.44 (1H, dq, J = 1.2, 6.9, 3°-H), 6.46 (1H, d, J = 8.7), 7.37 (1H, m, Ph-H), 7.40 (4H, m, Ph-H), 7.54 (2H, t, J = 7.9, Ph-H), 7.64 (1H, t, J = 7.5, Ph-H), 8.11 (2H, d, J = 7.9, Ph-H). ¹³CNMR (CDCl₃, 300 MHz): 79.1 (C-1), 74.8 (C-2), 44.6 (C-3), 82.3 (C-4), 85.8 (C-5), 36.6 (C-6), 72.3 (C-7), 57.3 (C-8), 205.1 (C-9), 76.2 (C-10), 133.1 (C-11), 142.1 (C-12), 72.3 (C-13), 37.5 (C-14), 43.1 (C-15), 22.3 (C-16), 26.9 (C-17), 14.9 (C-18), 10.4 (C-19), 76.2 (C-20), 172.2 (C-21), 74.8 (C-22), 57.3 (C-3′), 171.8 (4-O AcC=O), 22.3 (Me), 171.2 (10-O AcC=O), 20.8 (Me), 167.6 (C=O Phl), 129.1 (q-Phl), 129.7 (o-Phl), 128.3 (m-Phl), 133.1 (p-Phl), 131.4 (q-Phl), 127.0 (o-Phl), 128.8 (m-Phl), 129.6 (p-Phl), 167.1 (C-5′), 140.1 (C-6′), 132.4 (C-7′), 13.9 (C-8′), 12.5 (6′Me).

Data for 10-deacetylbaeacatin III (10-DAB III); Colorless acicular crystal (methanol); (C₂₅H₃₆O₁₀), MW: 544.5951, MP: 231–233°C; ¹HNMR (400 MHz, CDCl₃) δ ppm): 8.02 (2H, d, J = 7.2 Hz), 7.6-7.3 (3H, m), 5.4 (1H, d, J = 7.1 Hz), 5.2 (1H, d, J = 4.4 Hz), 5.13 (1H, d, J = 2.2 Hz), 4.95 (1H, d,
The quantitation of allelochemicals in T. chinensis var. mairei root exudates and rhizosphere soils were performed by ultrasonic extraction followed by HPLC. The allelochemicals in rhizosphere soils were extracted with 70% methanol, ultrasonic at 45°C for 30 min, power 100 W and then centrifuged at 6000 r/min for 10 min. The root exudates and soil extracts were evaporated to dryness with rotary evaporator and the concentrate was dried by N2. The concentrate was dissolved in 50% methanol and the content of allelochemicals was analyzed by HPLC. The flow rate was 1 mL/min at a column temperature of 30°C, and the injection volume was 10 μL. For four allelochemicals, the mobile phase was a mixture of acetonitrile and water (45/55, v/v) and detected at 227 nm.

2.5. Effects of allelochemicals on the growth of C. acuminata seedlings

Positive activity of the identified chemicals from T. chinensis var. mairei root on the growth of C. acuminata seedlings was evaluated using the pot-culture test. The identified chemicals were diluted with distilled water to prepare a concentration of similar as concentration root exudates. Thirty C. acuminata seedlings (1-year) were planted in plastic pots (40 × 40 × 23 cm) and then the identified chemicals were used to irrigate each of the treated pots, respectively. The identified chemicals were diluted with distilled water to prepare a concentration of similar as concentration root exudates. The control pots received water only. All pots were placed in an environmental chamber with a temperature of 25°C and 65–90% relative humidity maintained. Pots were watered and randomized once a day. After 15 days, the seedlings were each picked and dried for at least 72 h, and their dry weights were recorded. The test lasted for 60 days.

2.6. Effects of soil carbon, nitrogen, enzyme activities and microorganisms on the growth of C. acuminata seedlings

Soil organic carbon content was determined according to the K2Cr2O7–H2SO4 wet oxidation method of Walkley and Black (Bremner and Jenkinson 2010). Total nitrogen concentration was analyzed by colorimetric method using a continuous flow autoanalyzer (Auto Analyzer III, Bran, Luebbe GmbH, Germany) after the samples were digested with the Kjeldahl method (Winingsih et al. 2019).

Five soil samples were randomly collected from a depth of 5–15 cm in the pots of C. acuminata seedlings irrigated with the above four allelochemicals. The samples were sieved (20 mesh) to remove plant residues and then homogenized, immediately taken back to the laboratory for determination of the soil parameters. The composition of soil microbial population was evaluated by plate colony-counting methods (Kong 2020). All microorganisms were cultured at 28°C.

The six enzymes (urease, sucrase, dehydrogenase, alkaline, protease and phosphatase) were used to assess the potential enzyme activities in soil. The test methods used were those described by Kong (Kong 2007). The controls used distilled water instead of substrates. All six enzyme activities were determined via the colorimetric method and expressed based on each experimental measurement had five replicates.

2.7. Statistical analysis

Data were presented as means standard error (SE) from three independent experiments with three replications for each determination. Analysis of variance (ANOVA) followed by a Tukey test was applied to compare the difference between each plant. All statistical analyses were conducted using SPSS. Redundancy analysis (RDA) were performed for
testing the correlations between soil properties and plant growth.

3. Results and discussion

3.1. Isolation and identification of allelochemicals from T. chinensis var. mairei root extracts

Effect of the root extracts and root exudates of T. chinensis var. mairei on the height (A) and basal diameter (B) of C. acuminata seedlings was tested (Figure 2). The isolation of chemicals from root exudates of T. chinensis var. mairei was conducted by a bioassay-directed fractionation approach. The results showed that the root extract and root exudates from the T. chinensis var. mairei had significant promote effects on growth of C. acuminata seedlings ($p < 0.05$, $p < 0.01$). At 45th day, the plant height and base diameter of C. acuminata seedlings reached the maximum under root extract and root exudates irrigation, with plant height of 77 and 76 mm, basal diameter of 5.9 and 5.8 mm respectively (Figure 2A,B). Their average values were 12.5% and 19.4% higher than those in the control. The above results indicate that there are potential promot- ing chemicals in root extracts that a- ff the growth of T. chinensis var. mairei could release them through the living roots into the surroundings at sufficient concentrations to promote plants grown in its vicinity. To address this, the quantities of cephalomannine, 10-DAB III, paclitaxel and 7-Epi-10-DAT, this did not mean that T. chinensis var. mairei could release them through the living roots into the rhizosphere soils by comparing their retention time with those of the standards of compounds 1–4 (Figure 4). Subsequently, all four chemicals were found in both root exudates and rhizosphere soils. To achieve verification of the allelochemicals released from the T. chinensis var. mairei into soil during its growth, HPLC was used to determine and confirm the existence of the allelochemicals in the rhizo- sphere soil by comparing their retention time with those of the standards of compounds 1–4 (Figure 4). Subsequently, all four chemicals were found in both root exudates and rhizosphere soils, but their concentrations were much greater in the root exudates than that in the rhizosphere soils (Table 1). It maybe that soil microbes degrade allelochemicals (Chen et al. 2010). There is also the possibility of abiotic degra- dation, such as hydrolysis and photodegradation (Tian and Stella 2008). The content of 10-DAB III reached 5.90 μg/g in root exudates and 0.43 μg/g in rhizosphere soil. The content of 7-Epi-10-DAT was the lowest, only 0.15 and 0.01 μg/g was found in root exudates and rhizosphere soil, respectively. The contents of cephalomannine and paclitaxel in root exu- dates were 1.92 and 2.35 μg/g, respectively, and the contents of that in rhizosphere soil were less than 0.01 μg/g. Therefore, there were four chemicals in root exudates and rhizo- sphere soil, which proved that they could enter rhizosphere soil through the root system of T. chinensis var. mairei.

3.2. Quantitation of allelochemicals in root exudate and rhizosphere soil

Substantially different from the phytochemicals in plant tissues, the action of allelochemicals requires their presence in the vicinity of the donor plants (Anh et al. 2021). Although T. chinensis var. mairei plant extracts contained the promoting chemicals, cephalomannine, 10-DAB III, paclitaxel and 7-Epi-10-DAT, this did not mean that T. chinensis var. mairei plant extracts contained the promoting chemicals, cephalomannine, 10-DAB III, paclitaxel and 7-Epi-10-DAT should be released into the soil environment and act as allelochemicals from the plant

3.3. Allelopathy activities of the purified chemicals on C. acuminata seedlings growth

It was verified that chemicals, cephalomannine, 10-DAB III, paclitaxel and 7-Epi-10-DAT should be released into the soil environment and act as allelochemicals from the plant
during its growth and development stage. Plant height and basal diameter can accurately reflect the growth state of a plant (Padjung et al. 2021). The bioassay technique is commonly used to identify whether isolated compounds have allelopathic behavior (Rasul and Ali 2021). Four taxane chemicals, isolated and purified from the root exudate of *T. chinensis* var. *mairei*, were evaluated for allelopathy activities promoting the seedling growth of *C. acuminata* seedlings. We irrigated *C. acuminata* seedlings by simulating the concentration of allelochemicals exudated by roots. Results indicated that all of the compounds increased the relative growth rate of the tested seedlings at different degrees (Figure 5A). From 30 to 45 days, the relative growth rate of *C. acuminata* seedlings height reached the peak under the irrigation of four chemicals, and the highest growth rate was 5.63 ± 0.11% under the irrigation of paclitaxel. As shown in Figure 5B, the change trend of the relative growth rate of *C. acuminata* seedlings basal diameter with time gradient was similar to that of the relative growth rate of plant height, which both increased first and then decreased. Among them, the relative growth rate of the basal diameter of *C. acuminata* seedlings under 7-Epi-10-DAT irrigation was the highest, which was 9.15 ± 0.49%.

Although allelopathic inhibition effect is a common disturbance mechanism, (Xu et al. 2021), allelopathic promotion is also present in some ecosystems. Some studies have found that aqueous extracts from root and bark of *Larix gmelini* at low and moderate concentrations accelerates the growth of the collar diameter and biomass and root/shoot ratio of *Juglans mandshurica* seedlings (Yang 2005). The chemicals released by one plant may promote growth of the other plant at low concentration (Liu et al. 2020). Such as Yuan Hui et al found that some chemicals (2, 4-dihydroxybenzoic acid + ferulic acid, 7-hydroxycoumarin + ferulic acid, ferulic acid + abietic acid rosinic acid) can improve the growth of recipient plants (Yuan et al. 2015). In addition

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**Figure 3.** Chemical structures of taxane. 1 cephalomannine. 2 10-DAB III. 3 paclitaxel. 4 7-Epi-10-DAT.

**Figure 4.** HPLC chromatogram of 10-DAB III, cephalomannine, 7-Epi-10-DAT and paclitaxel in standard mixture (A), the root exudate (B) and rhizosphere soil (C) of *T. chinensis* var. *mairei*. Peak 1, 2, 3 and 4 were 10-DAB III, cephalomannine, 7-Epi-10-DAT and paclitaxel, respectively.
to the above phenolic acid compounds, lactone compounds, maltitol, maltol and (-)-loliolide had stimulating effects on the growth of maize shoots rather than inhibiting effects (Zhou et al. 2013). Monica Scognamiglio et al have found that identification of potential allelochemicals from Arbutus unedo, Medicago minima, Myrtus communis and their synergistic effects of Aegilops geniculata (Scognamiglio and Schneider 2020). The above results show that the different chemicals can accelerate the growth of plant. Similarly, the results of treating C. acuminata seedlings with four taxane chemicals (Cephalomannine, 10-DAB III, paclitaxel and 7-Epi-10-DAT) showed that all increased the relative growth rate of C. acuminata seedlings height at different growth stages and had slightly promote effect on the basal diameter. The results showed that the four taxane chemicals exuded from T. chinensis var. mairei could act as allelochemicals to promote the growth of C. acuminata seedlings.

The change of biomass of C. acuminata seedlings over time is shown in Figure 6A, the biomass of C. acuminata seedlings increased with time under the treatment of four allelochemicals. Among them, the dry weight of C. acuminata seedlings under 10-DAB III irrigation was the largest among all treatments. On the 60th day, the seedling biomass of the 10-DAB III irrigation group reached the peak (12.10 g / plant), followed by that of the paclitaxel irrigation group (9.91 g / plant), and that of the 7-Epi-10-DAT and cephalomannine irrigation groups reached 9.85 g / plant and 9.80 g / plant, respectively.

The total nitrogen content of C. acuminata seedlings was measured by Kjeldahl method (Dai and Wu 1995). The change of total nitrogen content of the whole plant of C. acuminata seedlings with time under the irrigation of four allelochemicals was shown in Figure 6B. The results shown that the total nitrogen content of whole C. acuminata seedlings was also the highest under the irrigation of 10-DAB III. At the 45th day of irrigation, the total nitrogen content of seedlings in the whole plant under 10-DAB III irrigation reached 76.12 mg/plant, which was 1.56 time higher than that in the control group. On the 60th day, the highest nitrogen content was still in the 10-DAB III irrigation group, followed by paclitaxel, 7-Epi-10-DAT group and cephalomannine, which were 1.96, 1.58, 1.50 and 1.16 time of the total nitrogen content in the control group, respectively. At the final stage of irrigation, the whole plant total nitrogen content of C. acuminata seedlings under all irrigation was higher than that of the control, because the soil nitrogen supply of seedlings in each group was the same, so the irrigation of four allelochemicals could promote the absorption of soil nitrogen nutrient of C. acuminata seedlings. This is due to the fact that plants and soils have close interactions such as the exchange of substance and nutrient cycling (Hu et al. 2019).

Allelochemicals are low-molecular-weight compounds released from living or decomposed plant tissues during growth (Iqbal et al. 2019). Many plants could produce various allelochemicals such as phenols, terpenoids, and alkaloids and inhibit the growth of others (Joshi et al. 2019). Taxane compounds, cephalomannine, 10-DAB III, paclitaxel and 7-Epi-10-DAT belong to the terpenoids, mostly released through root exudation into soil to influence the growth of C. acuminata seedlings. Thus, the four allelochemicals mentioned above can significantly increase biomass, this stimulation in growth suggests that the taxane chemicals help C. acuminata seedlings absorb nutrients from the soil.

Nitrogen is the most important mineral element in plant growth and play a key factor (Singh et al. 2019). Nitrogen has a significant effect on chlorophyll, photosynthetic rate, the main enzymes of dark reactions and photorespiration, which directly or indirectly affect photosynthesis and thus further influence plant growth (Sage et al. 1990). It has been reported that allelopathy exists in plants, and the allelopathy increases with the increase of planting time, and allelochemical can change the nitrogen use efficiency (Wei 2013; Chen et al. 2021; Yang et al. 2010). Our results showed that the four allelochemicals, cephalomannine, 10-DAB III, paclitaxel, 7-Epi-10-DAT in T. chinensis var. mairei increased the utilization of nitrogen from C. acuminata seedlings, which was basically consistent with the trend of allelochemicals improving nitrogen content shown by the above researchers. Therefore, application of allelochemicals plays an important role in activating and balancing the nutrients in the plant.

| Table 1 Contents of cephalomannine, 10-DAB III, paclitaxel and 7-Epi-10-DAT in T. chinensis var. mairei root exudates and rhizosphere soils. |
|-------------------------------------------------|
| **Compounds** | Root exudate (μg/g) | Rhizosphere soil (μg/g) |
|----------------|---------------------|------------------------|
| Cephalomannine | 1.92 ± 0.01         | <0.01 ± 0.01           |
| 10-DAB III    | 5.90 ± 0.02         | 0.43 ± 0.01            |
| Paclitaxel    | 2.35 ± 0.01         | <0.01 ± 0.01           |
| 7-Epi-10-DAT  | 0.15 ± 0.01         | 0.02 ± 0.01            |

3.4. Correlation between growth of C. acuminata seedlings and soil properties under allelochemicals irrigation

Soil ecosystem is a complex composition, in which soil enzymes and soil microorganisms play an important role in promoting soil nutrient cycle (Thompson et al. 2021). The physical and chemical properties of soil have a great impact on soil enzyme activity and soil microbial biological activity (Qi et al. 2020). Differing from competition for resources from soil, allelopathy involves the release of allelochemicals from plants into the environment (Wu et al. 2021). Accordingly, the identification of allelochemicals from plants and their environments is key to understand the plant-plant allelopathic interactions (Kong et al. 2019). A series of interactions between allelochemicals and soil abiotic and biotic factors may occur when allelochemicals are released through the soil (Scavo et al. 2019). In particular, soil microbial interactions radically alter the environment and give a much better indication of real effects (Vicua and González 2021). Therefore, it is necessary to explore allelopathy from allelochemicals and soil chemical properties, enzyme activities, microorganisms. At present, there are no reports on the combination of allelochemicals between T. chinensis var. mairei and C. acuminata with soil chemical properties, enzyme activities and microorganisms. We observed significant correlations (all \( p < 0.05 \)) between different growth stage of C. acuminata and soil properties in different allelochemicals irrigation systems (Figure 7). The results of RDA indicated that the cumulative explanatory power of the variables on the axis 1 and axis 2 were 68.08% and 20.04%, respectively. The basal diameter (BD)
of *C. acuminata* seedling under cephalomannine (1), 10-DAB III (2), paclitaxel (3), 7-Epi-10-DAT (4) irrigation were positively correlated with soil urease (SU) content. Based on the length and angle of the arrow, soil dehydrogenase (SD) was positively correlated with 3PTN (plant total nitrogen under 3 irrigation) and 1,2,3 DW (dry weight of plant under 1,2 and 3 irrigation). At the same time, soil organic carbon (SOC), soil total nitrogen (STN), soil fungi (SF), soil bacteria (SB), soil sucrase (SS), soil protease (SPr) and soil phosphatase (SPh) activities significantly promoted the height of *C. acuminata* seedling under 1, 2 and 4 irrigation systems. However, above indicators (1, 2, 4 HIT; 1, 2, 3 DW) were negatively correlated with SF/SB (Figure 7).

Under the simple term effects, the peak explaining power was from 2, 3, 4, 1HIT, and 2, 4, 3, DW ($p < 0.01$), the interpretative quantities were all higher than 42%, and the plant height reached the maximum under 10-DAB III irrigation (Table 2). Meanwhile, the $P$ values in the simple effect table showed that 3,1 DW and 3, 2, 4, 1 PTN were significantly correlated ($p < 0.05$), and the explanatory amount were all higher than 27% (Table 2). Under the conditional term effects, only in 2 HIT and 3 BD, there was a most significant correlation between the interpretative quantities ($p < 0.01$) (Table 3). The comprehensive analysis of all factors showed that soil factors had a most significant positive correlation with the height of *C. acuminata* seedlings under 10-DAB III irrigation ($p < 0.01$) (Table 3). Therefore, compared with the above four allelochemicals and soil factors, 10-DAB III was the most significant allelochemicals affecting the growth of *C. acuminata* seedlings.

While promotion interactions such as allelopathy often are suspected to be a driving force between *C. acuminata* and *T. chinensis* var. *mairei* interactions, few studies have reported the presence and correlation with soil properties of potentially allelochemicals in the rhizospheres of them (Li et al. 2021). This is important to fully explain the interaction of allelochemicals among plant species. Carbon and nitrogen stocks are the basic energy sources for microbes, and they thus play vital roles in regulating microbial biomass, community composition, and diversity. Moreover, the importance of microorganisms in the transport of allelochemicals, and variation in extracellular enzyme activity was also likely driven by the differences in nutrient released by plant roots (Allison and Vitousek 2005; Chen et al. 2007;
Soil phosphatase. SF: Soil fungi; SF/SB: Soil fungi/Soil bacteria; SOC: Soil organic carbon; STN: Basal diameter; DW: Dry weight; PTN: Plant total nitrogen. SB: Soil Bacteria; lomannine, 2:10-DAB III, 3: paclitaxel, and 4: 7-Epi-10-DAT. HIT: Height; BD: Basal diameter; DW: Dry weight; PTN: Plant total nitrogen; SU: Soil urease; SS: Soil sucrose; SP: Soil protease; SPH: Soil phosphatase.

Table 2. Statistical results of simple term effects for the RDA ordinations.

| Name     | Explains (%) | pseudo-F | p   | p (adj) |
|----------|--------------|-----------|-----|---------|
| 2 HIT*   | 64.7         | 18.3      | 0.002 | 0.032  |
| 3 HIT*   | 62.7         | 16.8      | 0.002 | 0.032  |
| 4 HIT*   | 55.9         | 12.7      | 0.002 | 0.032  |
| 1 HIT*   | 51.4         | 10.6      | 0.006 | 0.096  |
| 2 DW*    | 44.5         | 8.0       | 0.006 | 0.096  |
| 4 DW*    | 42.6         | 7.4       | 0.006 | 0.096  |
| 3 DW*    | 42.4         | 7.4       | 0.002 | 0.032  |
| 1 DW*    | 42.0         | 7.3       | 0.012 | 0.192  |
| 3 PTN*   | 33.4         | 5.0       | 0.010 | 0.160  |
| 2 PTN*   | 30.6         | 4.4       | 0.042 | 0.672  |
| 4 PTN*   | 27.1         | 3.7       | 0.036 | 0.576  |
| 1 PTN*   | 27.1         | 3.7       | 0.026 | 0.416  |
| 4 BD*    | 20.4         | 2.6       | 0.084 | 1.000  |
| 3 BD*    | 18.6         | 2.3       | 0.130 | 1.000  |
| 1 BD*    | 17.8         | 2.2       | 0.146 | 1.000  |
| 2 BD*    | 14.1         | 1.6       | 0.178 | 1.000  |

*Means abbreviations are the same as in Figure 7.

Table 3. Statistical results of conditional term effects for the RDA ordinations.

| Name     | Explains (%) | pseudo-F | p   | p (adj) |
|----------|--------------|-----------|-----|---------|
| 2 HIT*   | 64.7         | 18.3      | 0.002 | 0.032  |
| 3 BD*    | 14.9         | 6.6       | 0.002 | 0.032  |
| 3 DW*    | 6.0          | 4.1       | 0.076 | 1.000  |
| 1 HIT*   | 4.6          | 9.4       | 0.06  | 0.960  |
| 2 BD*    | 3.7          | 1.8       | 0.13  | 1.000  |
| 4 HIT*   | 1.9          | 1.4       | 0.334 | 1.000  |
| 1 DW*    | 1.7          | 0.8       | 0.408 | 1.000  |
| 3 PTN*   | 1.1          | 7.0       | 0.11  | 1.000  |
| 2 DW*    | 0.9          | 0.6       | 0.468 | 1.000  |
| 1 BD*    | 0.3          | 4.7       | 0.206 | 1.000  |
| 4 BD*    | <0.1         | <0.1      | 1.0   | 1.000  |

*Means abbreviations are the same as in Figure 7.

4. Conclusion

In this study, T. chinensis var. mairei is proved to be an allelopathic plant. It releases allelochemicals from its root which accelerate C. acuminata seedlings growth. The allelochemicals involved in allelopathic interactions are taxane chemicals, cephalomannine, 10-DAB III, paclitaxel and 7-Epi-10-DAT isolated and identified from T. chinensis var. mairei root. Furthermore, plant height, basal diameter, biomass and total nitrogen content of C. acuminata seedlings were improved using four allelochemicals irrigation. Therefore, this result would provide new insight into the accelerated effects on the growth of C. acuminata seedlings by allelochemicals, especially taxane chemicals play a key role in the mechanism of allelopathic promotion.

Authors’ contributions

Chunjian Zhao is mainly responsible for the ideas of the project and the revision of the paper. Sen Shi is responsible for writing paper and processing data. YinXiang Gao is mainly responsible for experiments and data collection. Jinqing Zhang provided support for the experiment. Jiajing Guan provided support for the experiment. Yujie Fu provided support for the experiment. Chunying Li not only provided support for the translation and revision of the paper. All authors contributed to the drafts and gave final approval for publication.

Disclosure statement

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Notes on contributor

Sen Shi, doctor has been engaged in the study of Plant Ecology and Allelopathy.

Yinxiang Gao, assistant professor has been engaged in the study of natural products from plants.

Chunjian Zhao, assistant professor has been engaged in the study of Plant Chemical Ecology.

Jinqing Zhang, master has been engaged in the study of Allelopathy.

Jiajing Guan, master has been engaged in the study of Botany.
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**Yajie Fu**, professor has been engaged in the study of natural products from plants.

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