SUPPLEMENTARY MATERIAL

Chemical constituents of *Cinnamomum septentrionale* leaf litter and its allelopathic activity on the growth of maize (*Zea mays*)

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Abstract: A pot experiment was conducted to study the effect of decomposing *Cinnamomum septentrionale* leaf litter on the growth of maize. In this study, the morphological traits of maize were significantly inhibited when the leaf litter amount reached or beyond 40 g per pot; Furthermore, during the early growth stage or with a large amount of litter addition, the pigment contents were inhibited by *C.septentrionale* leaf litter. Gas Chromatography Mass Spectrometry was used to determine the volatiles of leaf litter, and thirty-four compounds were identified, several of which were reported to be phytotoxic. In conclusion, the leaf litter of *C.septentrionale* showed a strong allelopathic effect on the growth of maize. Thus, it is better to avoid the growing of maize under or near the *C. septentrionale* plantation unless the leaf litter could be eliminated timely or other effective leaf litter processing methods could be implemented.

Key words: *Cinnamomum septentrionale*, Maize, allelopathy, growth, GC/MS

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Experimental details

Planting materials

During the peak fall time of *C. septentrionale* leaf, fresh fallen leaves were collected from a 30-year old *C. septentrionale* plantation in the Teaching and Research Base of Sichuan Agricultural University. The voucher specimen number is Y140321 and the voucher specimen is deposited in Herbarium of Sichuan Agricultural University. The leaf litter were cut into small pieces of approximately 1 cm² for easily mixing with soil after natural withering. The variety of maize seeds is Chuandan 19 purchasing from Zhenghong Seeds Co. Ltd. This maize seed variety was bred by researchers at Sichuan Agricultural University.

Planting soil and container

The soil texture is typical sandy loam with pH 7.85, which was collected from the local farmland. The organic matter content is 24.38 g kg⁻¹. The soil fertility status is as follows: total nitrogen 0.66 g kg⁻¹; total phosphorus 0.67 g kg⁻¹; total potassium 5.57 g kg⁻¹; rapidly available phosphorus 14.73 mg kg⁻¹ and rapidly available potassium 15.15 mg kg⁻¹. Polyethylene pots with a 35 cm caliber and a 30 cm height were used.

Experimental design

The pot experiment was performed in a greenhouse which was located at Yaan City in Sichuan province, China. The annual amount of *C. septentrionale* leaf litter was approximately 7.2 t ha⁻¹ (Huang et al. 2012). This amount was equivalent to the 80g pot⁻¹ dosage based on the area of pot used in this experiment. But because of the disturbances, such as stand density, wind power, and human activities, the litter amount in specific location in the field was different in the plantation and stands. Therefore, four levels of leaf litter addition (CK: 0 g pot⁻¹, A1: 40 g pot⁻¹, A2: 80 g pot⁻¹ and A3: 160 g pot⁻¹) were set for the experiment. The *C. septentrionale* leaf litter fragments were mixed with soil (weight: 12 kg pot⁻¹) and added into pots. Subsequently, 10 seeds of maize were sowed per pots with 6 replicates in each treatment. The seedlings were thinned regularly, and two seedlings were left eventually for the test of corn yield. The receptors were watered regularly with the HH2 soil moisture analyzer (ML2x, GBR) to monitor the soil moisture and maintain the soil volumetric moisture content persistently at 18% (Equivalent to 80% of field water holding capacity).

Indices Measuring

In the preliminary test, we found that the influence of decomposition leaf litter on the growth of

receptor plants began to appear at about 20 days after germination, The plant size, leaf color, and the physiological indexes of leaf began to show symptoms of inhibition which displayed the process from weak to strong and then to weak, and this phenomenon lasted until about 80 days. Therefore, we design 20 days as a measurement interval in the test for exploring the effect of leaf litter decomposition on receptor plants.

On 12th April 2014, the seeds of maize were sowed in pots, and the seeds germinated on 16th-18th April 2014 (T_0). The plant height, basal diameter, biomass and chlorophyll content of fresh function leaves were measured at 20, 40 and 60 days (denoted as T1, T2 and T3 respectively) after germination. The measurement were performed as described below.

Pigment extraction was conducted according to Arnon (1949). The extracting solution comprised of ethanol, acetone, and distilled water (5:4:1, by volume). Absorbance was recorded at 470 nm, 646 nm, and 663 nm simultaneously on an UV-3200PC spectrophotometer (Mapada Inc., China). The contents of total chlorophyll (chl a+b), chlorophyll a (chl a), chlorophyll b (chl b), and carotenoid (car) were calculated by the method of Porra et al. (1989).

The shredded leaf litter was powdered, weighed (20 g), ultrasonically extracted for 20 min with 40 mL solvent (*n*-hexane, ethyl acetate and ethanol), and filtered. The residue was re-extracted with an additional 40 mL solvent and filtered. The obtained filtrates were mixed and concentrated by rotary evaporation. The extracts were made up to a final volume of 5 mL and stored at 4 °C for further analysis. A Hewlett Packard 6890/5973 GC-MS (Hewlett-Packard Co., USA) equipped with a quadrupole mass spectrometer and an HP-5MS column (30 m×0.25 mm×0.25 μm) was used. The injection volume was 1 μl with a split ratio of 1:5. Helium (He) was used as the carrier gas at a flow rate of 0.8 mL min⁻¹. The injector and detector temperature were set at 280°C. The temperature program was as follows: 50°C for 18 min, increased to 146 °C at the rate of 8 °C min⁻¹, increased to 200 °C at the rate of 3 °C min⁻¹, increased to 250 °C at the rate of 5 °C min⁻¹, and increased to 280 °C at a rate of 3 °C min⁻¹, with a final hold time of 20 min. The ionization energy was 70 eV and the mass scan range was 20-550 amu. The identification of compounds was based on an MS library search (NIST and WILEY) and on comparisons with existing literature (Adams 2007).

Statistical analysis

Using the rate of inhibition (*RI*) (Williamson and Richardson 1998), the synthesis allelopathic effect of *C.septentrionale* leave litter on maize seedlings was measured by the function as follows.

$$RI = 1 - C/T \ (T \geq C) \text{ or } RI = T/C - 1 \ (T < C)$$

In the model, T is the processing value and C is the contrast value. The range of *RI* is from -1 to +1. *RI* > 0 means facilitation, and *RI* < 0 means inhibition. The positive value indicates stimulation by treatment, whereas the negative value indicates inhibition by treatment, comparing with the control.

An analysis of variation (ANOVA) of the results was performed using one-way ANOVA and LSD test for multiple comparisons. All data were checked for normality using One-Sample Kolmogorov-Smirnov test and the homogeneity test of variances, and log-transformed to correct deviations from these assumption when necessary. The statistical analysis was performed using SPSS version 20.0 software (SPSS Inc, USA). The results were considered statistically significant at *P* < 0.05.

References

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Table S1. Effect of decomposing leaf litter of *C. septentrionale* on the height, basal diameter and biomass of maize.

Days after sowing/d	Treatment	Height/cm	Basal diameter/mm	biomass/g
20 (T1)	CK	44.74 ± 2.19 ^a	4.63 ± 0.44 ^a	5.287 ± 1.192 ^a
	A1	44.70 ± 2.36 ^a (-0.001)	4.33 ± 0.28 ^{ab} (-0.065)	4.005 ± 0.651 ^b (-0.242)
	A2	41.79 ± 1.25 ^b (-0.066)	3.98 ± 0.19 ^b (-0.141)	4.011 ± 0.603 ^b (-0.241)
	A3	27.79 ± 3.18 ^c (-0.379)	3.14 ± 0.31 ^c (-0.321)	1.447 ± 0.880 ^c (-0.694)
40 (T2)	CK	90.65 ± 3.24 ^a	11.40 ± 1.01 ^a	30.27 ± 11.43 ^a
	A1	86.96 ± 2.12 ^a (-0.041)	9.83 ± 0.52 ^b (-0.138)	21.38 ± 6.16 ^{ab} (-0.294)
	A2	72.09 ± 5.59 ^b (-0.205)	8.03 ± 0.40 ^c (-0.296)	14.02 ± 0.18 ^b (-0.537)
	A3	48.06 ± 9.80 ^c (-0.470)	5.61 ± 1.07 ^d (-0.508)	12.08 ± 5.27 ^b (-0.601)
60 (T3)	CK	111.26 ± 2.70 ^a	10.84 ± 0.50 ^a	83.00 ± 12.74 ^a
	A1	107.88 ± 8.03 ^a (-0.030)	8.62 ± 1.21 ^b (-0.205)	51.66 ± 14.92 ^b (-0.378)
	A2	96.33 ± 4.34 ^b (-0.134)	8.01 ± 1.23 ^{bc} (-0.261)	38.19 ± 5.51 ^{bc} (-0.54)
	A3	85.74 ± 10.61 ^c (-0.229)	6.77 ± 1.09 ^c (-0.375)	16.77 ± 6.60 ^c (-0.798)

Note: Each value is the mean ± SE. Different lowercase letters in the same column and same time indicate significant difference at 0.05 probability level (one-way ANOVA, $N = 3$, $P < 0.05$, $a > b > c$).

The data in the parentheses show the response index (RI) to specific leaf litter treatment.

Table S2. Effect of decomposing leaf litter of *C. septentrionale* on pigment content of maize.

Decomposing duration/d	Treatment	Chl (mg/g FM)	Car (mg/g FM)	Chla/Chlb	Chl/Car
20 (T1)	CK	0.704±0.031 ^b	0.122±0.006 ^b	5.048±0.041 ^a	5.779±0.080 ^b
	A1	0.786±0.037 ^a (0.107)	0.133±0.006 ^a (0.083)	5.018±0.061 ^a (-0.006)	5.933±0.102 ^{ab} (0.026)
	A2	0.654±0.028 ^b (-0.070)	0.108±0.005 ^c (-0.115)	4.753±0.353 ^a (-0.058)	6.076±0.022 ^a (0.049)
	A3	0.379±0.016 ^c (-0.461)	0.064±0.002 ^d (-0.475)	4.752±0.029 ^a (-0.059)	5.900±0.139 ^{ab} (0.021)
40 (T2)	CK	3.138±0.290 ^a	0.663±0.059 ^a	2.220±0.016 ^c	4.733±0.019 ^a
	A1	1.910±0.0238 ^b (-0.391)	0.408±0.052 ^b (-0.385)	2.280±0.020 ^c (0.026)	4.681±0.137 ^{ab} (-0.011)
	A2	1.606±0.114 ^b (-0.488)	0.349±0.027 ^b (0.474)	2.534±0.045 ^b (0.124)	4.605±0.028 ^{bc} (-0.027)
	A3	1.803±0.04 ^b (-0.426)	0.395±0.016 ^b (-0.404)	2.611±0.042 ^a (0.150)	4.571±0.079 ^c (-0.034)
60 (T3)	CK	2.437±0.132 ^{ab}	0.426±0.024 ^b	4.595±0.040 ^a	5.724±0.032 ^a
	A1	2.578±0.042 ^a (0.054)	0.467±0.017 ^a (0.088)	4.547±0.157 ^a (-0.011)	5.522±0.124 ^b (-0.035)
	A2	2.282±0.043 ^b (-0.064)	0.412±0.010 ^b (-0.033)	4.705±0.055 ^a (0.023)	5.547±0.083 ^b (-0.031)
	A3	2.341±0.105 ^b (-0.040)	0.413±0.014 ^b (-0.031)	4.731±0.118 ^a (0.029)	5.669±0.064 ^{ab} (-0.010)

Note: Each value is the mean ± SE. Different lowercase letters in the same column and same time indicate significant difference at 0.05 probability level (one-way ANOVA, $N = 3$, $P < 0.05$, $a > b > c$). The data in the parentheses show the response index (RI) to specific leaf litter treatment.

Table S3. Compounds identified in *C.septentrionale* leaf litter as revealed by GC-MS analysis.

No.	Compound	Molecular formula	Relative content (%)
1	Camphor	C ₁₀ H ₁₆ O	30.53
2	Geranial	C ₁₀ H ₁₆ O	11.10
3	1,8-Cineole	C ₁₀ H ₁₈ O	10.85
4	Cis-Citral	C ₁₀ H ₁₆ O	8.33
5	α -Pinene	C ₁₀ H ₁₆	4.25
6	Citronellol	C ₁₀ H ₂₀ O	3.90
7	α -Terpinenol	C ₁₀ H ₁₈ O	3.73
8	Sabinene	C ₁₀ H ₁₆	3.09
9	Camphene	C ₁₀ H ₁₆	2.84
10	Terpinen-4-ol	C ₁₀ H ₁₈ O	2.29
11	β -Pinene	C ₁₀ H ₁₆	2.20
12	Caryophyllene oxide	C ₁₅ H ₂₄ O	2.20
13	Spathulenol	C ₁₅ H ₂₄ O	1.97
14	Myrcene	C ₁₀ H ₁₆	1.50
15	Borneol	C ₁₀ H ₁₈ O	1.49
16	<i>trans</i> -Nerolidol	C ₁₅ H ₂₆ O	1.30
17	1-Caryophyllene	C ₁₅ H ₂₄	0.92
18	α -Caryophyllene	C ₁₅ H ₂₄	0.69
19	L-Borneol acetate	C ₁₂ H ₂₀ O ₂	0.68
20	3-Thujene	C ₁₀ H ₁₆	0.49
21	γ -Terpinene	C ₁₀ H ₁₆	0.34
22	Geraniol	C ₁₀ H ₁₈ O	0.33
23	p-Menth-1-en-3-one	C ₁₀ H ₁₆ O	0.24
24	6-Methyl-5-hepten-2-one	C ₈ H ₁₄ O	0.22
25	Bicyclogermacrene	C ₁₅ H ₂₄	0.21
26	Terpinolene	C ₁₀ H ₁₆	0.19
27	p-Mentha-1,5-diene	C ₁₀ H ₁₆	0.16
28	<i>trans</i> -Rose oxide	C ₁₀ H ₁₈ O	0.15
29	Perillene	C ₁₀ H ₁₄ O	0.12
30	α -Pinene epoxide	C ₁₀ H ₁₆ O	0.10
31	<i>trans</i> -Sabinene hydrate	C ₁₀ H ₁₈ O	0.08
32	α -Cubebene	C ₁₅ H ₂₄	0.08
33	δ -Cadinene	C ₁₅ H ₂₄	0.08
34	Tricyclene	C ₁₀ H ₁₆	0.07

Figure S1. Site for experiment.

10 days of decomposition

20 days of decomposition

40 days of decomposition

60 days of decomposition

Figure S2. The appearance of maize as affected by decomposing *C. Septentrionale* leaf litter after different exposure time.

Note: The points of red arrows indicated the higher amount of leaf litter (in each figure the treatment is A3, A2, A1, CK from left to right)