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Allelopathic effects of decomposed leaf litter from intercropped trees on rape

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Abstract: The allelopathic effect of decomposed litter from trees interplanted with crops is a key problem in the intercrop agroforestry business that could influence the economic benefits and sustainable development of ecoagriculture. In our study, the litter from 12 common intercropped tree species was collected from the Guanzhong Plain (Shaanxi Province, China) and mixed with soil, incubated to allow decomposition for 120 days, and then extracted using water. The water extracts at different concentrations were used for filter paper–dish cultivation of rape (*Brassica napus*) seeds. Indicators of the germination and growth of seedlings were measured to investigate the allelopathic effects of decomposed litter on rape. The results showed that in rape the most sensitive indicators of harmful allelochemicals derived from decomposed litter were the germination speed index and catalase activity of seedlings. Moreover, extracts of decomposed medium (soil) containing litter from *Paulownia fortunei*, *Acer truncatum*, *Zanthoxylum bungeanum*, *Juglans regia*, *Diospyros kaki*, *Prunus persica*, *Prunus armeniaca*, and *Ziziphus jujube* were beneficial to the germination and seedling growth of rape at all concentrations examined, and thus these trees could be safely interplanted with rape. Extracts from *Eucommia ulmoides*, *Populus canadensis*, and *Malus pumila* inhibited the germination and seedling growth of rape, and thus the use of these trees in intercropping should be reduced. Extracts from *Pyrus bretschneideri* showed growth promotion at lower concentrations (10 and 20 mg mL⁻¹) but were growth inhibitive at a high concentration (40 mg mL⁻¹); thus, it could be intercropped with rape but at a low density.

Key words: Allelopathy, decomposition, germination status, leaf litter, rape, water extraction

1. Introduction

Interplanting is an important form of agroforestry. In this system, resources such as light, land, water, and fertilizer are adequately utilized, and thereby improve the productivity of both crop and woods. In addition, interplanting can improve the microclimate (decrease wind velocity and increase temperature and air humidity) and resist adverse environmental changes due to its structural complexity. Hence, it has become a promising agroforestry form for its economic and ecological benefits (Oelbermann et al., 2015). In order to select appropriate trees for intercropping, attention should be paid not only to the competition between the crops and interplanted trees for sunlight, water, and nutrients, but also to the harmful allelopathic effects of trees on crops. Many studies have shown that endogenous allelochemicals released by some trees can have certain disadvantageous effects on other plants in terms of seed germination, seedling growth, and the physiological activity of seedlings. Shen et al. (2009) stated

that *Prunus salicina* showed obvious inhibitory effects on the germination and growth of seedlings of *Zea mays* and other leguminous crops, and that the extent of this effect depended on the concentration of allelochemicals. Moreover, different crops also show differences in their resistance to allelopathy. Wang et al. (2010) investigated the allelopathic effects of *Eucommia ulmoides* on *Glycine max*, *Phaseolus minimus*, and *Capsicum annum*, and found that allelochemicals are present in the leaves of *E. ulmoides*. Furthermore, their inhibitory effects on the stems and leaves of the receptor plants are much stronger than on the roots of these plants. A water extract of *Juglans regia* leaves at low concentration showed an accelerating effect on the plant height, ground diameter, and chlorophyll content of *Atractylodes macrocephala*, but extracts became inhibitory at a high concentration (Li et al., 2011).

Previous research studies regarding allelopathic effects have been carried out by simply extracting the living or dead plant organs of the donor plants using water or an

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organic solvent and then treating the receptor plants. However, under natural conditions, only very small amounts of allelochemicals are leached or volatilized directly and subsequently come into contact with receptor plants (Ni et al., 2011). Furthermore, after they are released into the soil, allelochemicals would be affected by the soil, such as by absorption, migration, or decomposition by microorganisms and enzymes. The studies by Yenish et al. (1995) and Blum et al. (1998) illustrated that phenolic allelochemicals can be decomposed by soil microbes, and thus they do not reach active concentrations. Research by Walker et al. (2003) indicated that ceratiolin, a nonallelopathic active substance secreted by *Ceratiola ericoides*, can be transformed into activated phenylpropionic acid and subsequently converted into a more poisonous chemical, hypnone. Moreover, soil nutrient content might influence the final results of allelopathy. For example, Kong et al. (2002) showed that the transformation of ageratochromene, a type of terpene allelochemical, is controlled by soil nutrient content. This allelochemical initially aggregates into a dimer and then decomposes into small molecules, which can be inactivated by high levels of soil nutrients.

On the basis of the above results, the practical allelopathy of the decomposed leaf litter of 12 commonly planted intercropping tree species on rape, a widely planted oil-bearing crop in the Guanzhong Plain, was investigated in this study. In addition, the results were compared with those of a previous study by Chi (2011), in which rape was treated directly using water extracts of undecomposed leaf litter. This study may provide scientific insight into the allelopathic effects of litter decomposition, and provide a basis for the selection of interplanting trees that can be used in conjunction with rape.

2. Materials and methods

2.1. Leaf litter and soil sample collection

In the late autumn of 2011, leaf litter of the current year from main intercropping mature trees (eucommia bark (*E. ulmoides*), empress tree (*Paulownia fortunei*), Canadian poplar (*Populus canadensis*), maple (*Acer truncatum*), persimmon tree (*Diospyros kaki*), peach tree (*P. persica*), wild pepper (*Zanthoxylum bungeanum*), walnut tree (*J.*

regia), apricot tree (*P. armeniaca*), jujube tree (*Ziziphus jujube*), apple tree (*Malus pumila*), and pear tree (*Pyrus bretschneideri*)) was collected from the ground in the Yangling Region, Shaanxi Province, China. After removing leaves that were decayed or damaged by pests or diseases, the litter was washed rapidly with distilled water, oven dried at 60 °C for 24 h, and then ground to pass through a sieve (Φ 1 mm) to accelerate the subsequent decomposition process.

Soil from a local cropland of rape was gathered using a 5-point sampling method and passed through a sieve (Φ 2 mm) to remove stones, roots, plant debris, and animal debris, and this was used as the decomposition medium. The initial properties of the soil medium are given in Table 1.

2.2. Decomposition incubation and preparation of water extracts

The prepared powder of leaf litter and fresh soil were mixed uniformly at a ratio of 1:8 (the weight of the soil was converted into dry weight according to the measured soil water content). The ratios used in this study were based on the ratio of annual production of leaf litter and the weight of surface soil, which were increased or decreased as appropriate. The soil-leaf litter mixture was placed into plastic pots, and each treatment was run in triplicate. Distilled water was added to the soil mixture to adjust the soil humidity to 60% of the field saturated water capacity, and the pots were then weighed. All pots were then covered with a plastic film containing 2 holes to reduce evaporation. During the incubation period, the pots were weighed every 5 days and distilled water was uniformly added to the pots with a sprayer, according to individual water loss, to maintain a constant soil water content. The soil mixtures were incubated at room temperature (20–25 °C), and at almost consistent humidity for 120 days, until most of the litter was decomposed.

After incubation, the soil medium was mixed with distilled water at a ratio of 9:25 (w/v, which is equal to the ratio of 1 g of litter to 25 mL of water) and extracted by water for 40 h. The soil and water mixtures were then centrifuged and filtered. The concentrations of the extracts were 40 mg (litter weight)/mL. Dilutions of 2 and 4 times the prepared extracts were maintained in a refrigerator at 4 °C (Chen et al., 2014).

Table 1. Initial properties of the soil medium (from rape cropland).

| Soil taxonomy | Texture | pH | Organic matter content % | EC μ S/cm | CEC cmol/kg | Available nutrients content mg/kg | | | Lime content g/kg |
|------------------------|------------|------|--------------------------|---------------|-------------|-----------------------------------|-------|--------|-------------------|
| | | | | | | N | P | K | |
| Eum-Orthric Anthrosols | loamy clay | 7.72 | 2.32 | 27.8 | 22.58 | 332.10 | 10.46 | 118.24 | 74.99 |

2.3. Rape germination and seedling growth experiment

To investigate the effects of the litter extracts on seed germination and seedling growth, the filter paper–dish cultivation method was used as suggested by Zeng (1999). Plump and uniform rape (*Brassica napus*, cultivar: Ganza 1st) seeds without any pest infestation were carefully selected and disinfected with a 5% sodium hypochlorite solution for 10 min, washed, and then soaked in water for 4–5 h. Three layers of filter paper were placed into autoclaved petri dishes containing 6 mL of distilled water or different concentrations of the prepared extracts (10, 20, or 40 mg mL⁻¹) to give 37 types of germination beds (12 litter species × 3 extracted concentrations + 1 control). Then 100 rape seeds were placed uniformly into each petri dish. The seeds treated with distilled water were used as controls. The prepared petri dishes were incubated in an illuminated incubator at 20/26°C (night/day), with a 12 h light/dark photoperiod. An appropriate amount of distilled water or extract (1–5 mL) was added daily to the dishes to maintain a constant humidity. In addition, the number of germinated seeds was noted every 24 h for 5 days. The germination rates were recorded and the germination speed index was calculated using the following equation:

Germination

$$\text{speed index } I = 2(5X_1 + 4X_2 + 3X_3 + 2X_4 + X_5), \quad (1)$$

where X_i is the number of germinated seeds in every 24-h period; that is, X_1 is the number of germinated seeds on the first day, and so on. The criterion used for determining germination was a radicle length of 1–2 mm.

After the germination test, 10 seedlings were randomly selected from each petri dish and shoot height and root length measurements were taken. Seedlings were oven dried at 105 °C to remove excess water, then at 75 °C for 30 min in order to obtain dry weight measurements. The remaining seedlings were used for the determination of physiological properties. Among these, root activity was determined using the tetrazolium red (TTC) method. First, 0.5 g root samples were soaked in 10 mL of 0.4% (w/v) TTC-0.1 M phosphate buffer (pH 7.4 Na₂HPO₄-KH₂PO₄) for 1 h at 37 °C. The soaked samples were then ground with silica sand in 3–5 mL of ethyl acetate and all of them were transferred to 10 mL tubes. The residues were rinsed 2–3 times in ethyl acetate and all solutions were also transferred into tubes. The total extracted solution was brought up to a volume of 10 mL and analyzed with a spectrophotometer (UV-2450 Shimadzu Corporation, Kyoto, Japan) at a wavelength of 485 nm. The root activity was represented as the production of formazan per gram root per hour (Chi, 2011). Chlorophyll (Chl) content was determined using an alcohol extraction method: 0.05 g leaf sample was ground with CaCO₃ and silica sand in 95% ethanol and then the suspension was filtered. The residues and filter paper were washed using ethanol several times. All extraction solutions were transferred into a volumetric flask and the

total solution was brought up to a volume of 25 mL. The final solution was analyzed with the spectrophotometer at 3 different wavelengths (665 nm, 649 nm, and 470 nm). Chlorophyll content was represented as the quantity (mg) of Chl in 1 g of leaf (Chi, 2011). Catalase (CAT) activity was determined using the KMnO₄ titration method. First, 1.0 g leaf sample was ground with CaCO₃ in 0.2 M phosphate buffer (pH 7.8 Na₂HPO₄-KH₂PO₄). The total volume of the suspension was diluted to 25 mL and then centrifuged. The mixture of 2.5 mL of supernatant liquor and 2.5 mL of H₂O₂ was incubated in a 30 °C water bath for 10 min, and then titrated with a 0.1 M K₂MnO₄ solution. CAT activity was represented as disintegrations of H₂O₂ per minute (Chi, 2011). All treatments in this study were replicated 3 times and mean values and standard errors were calculated using SPSS 19.0. Control experiments were also conducted.

2.4. Data analyses

The data were analyzed using SPSS 19.0. The allelopathy index RI (obtained from Eq. (2), Williamson and Richardson, 1988) was used to indicate the allelopathic effects of extracts on the growth and physiological characteristics of seedlings:

$$RI = (T - C) / C, \quad (2)$$

where T is the treatment value and C is the control value. A positive RI value indicated a promotional effect of allelochemicals on rape, whereas a negative value indicated allelopathic inhibition.

SPSS 19.0 was employed to test significant differences between T and C. Furthermore, the least significant difference method was used for post hoc analyses ($P < 0.05$), and the RI values of each index were subjected to comprehensive principal component analysis (PCA). The comprehensive principal component value F was used as an indicator to evaluate the allelopathic effects of the decomposed litter.

3. Results

3.1. Effects of decomposed tree leaf litter on rape seed germination

3.1.1. Rate of germination

Among the tree species tested, extracts of the decomposition medium of *Z. bungeanum* and *Z. jujube* caused a significant decrease (by 2.1%) in the germination rate of rape seeds at a high concentration of 40 mg·mL⁻¹. However, the treatment with *P. canadensis* showed a significant accelerating effect on the germination rate at a low concentration of 10 mg·mL⁻¹, and the treatments with *J. regia* and *M. pumila* accelerated germination at a moderate concentration (20 mg mL⁻¹, 2.1% and 3.1% increases, respectively). Treatments with the litter extracts of the other trees did not significantly influence the germination of rape seeds (Table 2).

Table 2. Effect of the water extracts of leaf litter decomposition medium (soil) on rape germination.

| | Conc ² mg mL ⁻¹ | <i>E.u.</i> ¹ | <i>P.f.</i> | <i>P.c.</i> | <i>A.t.</i> | <i>Z.b.</i> | <i>J.r.</i> | <i>P.b.</i> | <i>M.p.</i> | <i>D.k.</i> | <i>P.p.</i> | <i>P.a.</i> | <i>Z.j.</i> |
|-------------------------|--|---------------------------|--------------------------|---------------------------|--------------------------|---------------------------|--------------------------|---------------------------|---------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
| | 0 | 96.67(1.15) ^{ab} | 96.67(1.15) ^a | 96.67(1.15) ^b | 96.67(1.15) ^a | 96.67(1.15) ^b | 96.67(1.15) ^b | 96.67(1.15) ^{ab} | 96.67(1.15) ^b | 96.67(1.15) ^a | 96.67(1.15) ^a | 96.67(1.15) ^a | 96.67(1.15) ^a |
| Germination rate | 10 | 98.33(0.58) ^a | 97.00(1.00) ^a | 97.00(1.00) ^a | 96.60(1.15) ^a | 96.00(1.00) ^b | 96.00(1.00) ^b | 97.33(1.15) ^{ab} | 98.00(1.00) ^{ab} | 96.00(1.00) ^a | 95.33(0.58) ^a | 95.67(0.58) ^a | 96.33(1.15) ^{ab} |
| % | 20 | 97.00(1.00) ^{ab} | 96.00(1.00) ^a | 97.67(0.58) ^{ab} | 95.00(1.00) ^a | 98.67(0.58) ^a | 98.67(0.58) ^a | 98.67(1.15) ^a | 99.67(0.58) ^a | 97.00(0.58) ^a | 96.33(1.15) ^a | 95.67(1.15) ^a | 95.67(0.58) ^{ab} |
| | 40 | 96.33(0.58) ^b | 97.33(0.58) ^a | 96.00(1.00) ^b | 94.67(0.58) ^a | 97.33(1.55) ^{ab} | 97.33(1.15) ^b | 96.33(1.15) ^b | 97.33(1.15) ^b | 95.67(1.15) ^a | 95.67(0.58) ^a | 96.67(1.15) ^a | 94.67(1.15) ^b |
| | 0 | 1059(8.02) ^a | 1059(8.02) ^a | 1059(8.02) ^b | 1059(8.02) ^a | 1059(8.02) ^a | 1059(8.02) ^a | 1059(8.02) ^a | 1059(8.02) ^a | 1059(8.02) ^b | 1059(8.02) ^a | 1059(8.02) ^a | 1059(8.02) ^b |
| Germination speed index | 10 | 1067(4.36) ^a | 1025(8.60) ^b | 1093(9.02) ^a | 1021(6.26) ^b | 1046(7.21) ^a | 1046(7.21) ^a | 1001(4.16) ^b | 1071(6.21) ^a | 1075(8.70) ^a | 843(7.15) ^b | 992(7.86) ^b | 1073(5.07) ^a |
| I | 20 | 1065(6.00) ^a | 1018(6.27) ^b | 1095(6.53) ^a | 1019(9.29) ^b | 1025(11.63) ^b | 961(9.45) ^b | 987(5.03) ^c | 1023(7.13) ^b | 1088(5.65) ^a | 853(6.61) ^b | 994(9.28) ^b | 991(8.51) ^c |
| | 40 | 1059(1.09) ^a | 976(5.76) ^c | 1064(5.49) ^b | 981(5.20) ^c | 989(7.51) ^c | 945(8.08) ^c | 963(6.43) ^d | 981(4.59) ^c | 1020(10.77) ^c | 847(9.36) ^b | 950(8.04) ^c | 995(8.73) ^c |

¹*E.u.* = *E. ulmoides*, *P.f.* = *P. fortunei*, *P.c.* = *P. canadensis*, *A.t.* = *A. truncatum*, *D.k.* = *D. kaki*, *P.p.* = *P. persica*, *Z.b.* = *Z. bungeanum*, *J.r.* = *J. regia*, *P.a.* = *P. armeniaca*, *Z.j.* = *Z. jujube*, *M.p.* = *M. pumila*, and *P.b.* = *P. bretschneideri*.

²Conc is short for concentration, in mg of dried litter per mL of soil extracts.

³Data are represented as average (standard deviation).

⁴Different letters indicate significant differences between treatments ($P < 0.05$), and the least significant difference method was employed for the comparison tests.

3.1.2. Germination speed index

The speed of rape germination was inhibited by many types of extracts (Table 2). The treatments with *P. fortunei*, *A. truncatum*, *Z. bungeanum*, *P. bretschneideri*, *P. persica*, and *P. armeniaca* led to a decrease in the germination speed of rape at all concentrations, and the germination inhibition strengthened with increasing extract concentration. Inhibition became most apparent at a concentration of 40 mg mL⁻¹, and the decreases in germination speed were 7.8%, 7.3%, 6.6%, 9.1%, 20.0%, and 10.2%, respectively. Moreover, the treatment with *J. regia* resulted in a significant inhibitory effect at moderate and high concentrations, and its influence strengthened with increasing extract concentration. The treatments with *P. canadensis*, *D. kaki*, and *Z. jujube* significantly accelerated the speed of germination at lower concentrations (10 and 20 mg mL⁻¹ for *P. canadensis* and *D. kaki* and 10 mg mL⁻¹ for *Z. jujube*). However, with increasing extract concentration, the acceleration was reduced, until its effect became inhibitory. The treatments with *M. pumila* and *Z. jujube* showed significant inhibitory effects at concentrations of 20 and 40 mg mL⁻¹ and the treatment with *D. kaki* showed a significant inhibitory effect at a concentration of 40 mg mL⁻¹.

3.2. Effects of decomposed tree leaf litter on rape seedling growth

3.2.1. Shoot height

Treatments with the litter of all tree species at different concentrations led to a significant acceleration in shoot height (Table 3), and the effect was dependent on concentration. Among the 12 tree species tested, there were significant differences among the accelerations produced by different concentration of the extracts of *E. ulmoides*, *Z. bungeanum*, *J. regia*, and *P. persica*.

3.2.2. Root length

The promotional effects of the extracts of the litter decomposition medium on rape shoot height were variable compared to the effects on root length (Table 3). The treatment with *E. ulmoides* led to a significant increase in root length ($P < 0.05$, 7.1% increase). The treatments with each of *P. fortunei*, *P. canadensis*, *A. truncatum*, *Z. bungeanum*, *D. kaki*, *P. armeniaca*, and *Z. jujube* accelerated root growth at all concentrations, while *P. fortunei*, *A. truncatum*, *D. kaki*, and *P. armeniaca* produced a significant acceleration at 40 mg mL⁻¹. The treatments with *J. regia* and *P. bretschneideri* produced a significant acceleration at concentrations of 20 and 40 mg mL⁻¹. The treatment with *M. pumila* showed typically significant acceleration ($P < 0.05$) at low concentration, with an increase of 12.9%, but had an inhibitory effect of 7.5% at a high concentration.

3.2.3. Dry weight of shoots

Extracts of the decomposition medium of the 12 types of litter showed variable promotional effects on the dry weight of shoots (Table 3). The treatments with *P. fortunei*, *Z. bungeanum*, *P. bretschneideri*, *D. kaki*, and *P. persica* led to an increase in the dry weight of shoots, the increment becoming more pronounced with an increase in extract concentration, and the dry weights of shoots were significantly higher than that recorded in the control experiment ($P < 0.05$). The treatment with *P. canadensis* accelerated the growth of shoots at a concentration of 10 mg mL⁻¹ (23.6% increase, $P < 0.05$) and this effect was noted only at low extract concentrations. The treatment with *M. pumila* accelerated the growth of shoots at 40 mg mL⁻¹ (23.6% increase, $P < 0.05$), whereas the treatments with *J. regia*, *P. armeniaca*, and *Z. jujube* accelerated the growth of shoots at all concentration levels ($P < 0.05$).

3.2.4. Dry weight of roots

E. ulmoides produced significant ($P < 0.05$) promotional effects on the dry weight of shoots at a concentration of 20 mg mL⁻¹, whereas *P. canadensis* and *M. pumila* produced significant ($P < 0.05$) promotional effects at 10 mg mL⁻¹, and *P. bretschneideri* produced significant ($P < 0.05$) promotional effects at 40 mg mL⁻¹, with the respective increments being 15.2%, 17.2%, 17.2%, and 18.8%. Treatments with *Z. bungeanum* and *D. kaki* produced significant promotional effects at 20 and 40 mg mL⁻¹, whereas treatments with *A. truncatum*, *J. regia*, *P. persica*, *P. armeniaca*, and *Z. jujube* showed significant promotional effects at all concentration levels (Table 3).

3.3. Effects of leaf litter decomposition on rape physiological indicators

3.3.1. Catalase activity

All treatments resulted in inhibitory effects on the CAT activity of seedlings to some extent (Table 4). The extracts of *E. ulmoides*, *P. canadensis*, *Z. bungeanum*, and *J. regia* resulted in significant inhibition of CAT activity only at 40 mg mL⁻¹. *A. truncatum*, *D. kaki*, and *P. armeniaca* resulted in significant inhibition of CAT activity only at 20 and 40 mg mL⁻¹. The treatments with *P. bretschneideri* and *Z. jujube* inhibited CAT activity at all concentrations, and the inhibition was stronger with increasing concentration. The extract of *M. pumila* resulted in acceleration (2.4% increase, $P < 0.05$) at 20 mg mL⁻¹ but inhibition (9.8% decrease, $P < 0.05$) at 40 mg mL⁻¹.

3.3.2. Root activity

There were only a few treatments that influenced root activity and all of these showed promotional effects (Table 4). Among these, the treatment with *E. ulmoides* significantly accelerated root activity only at 10 mg mL⁻¹ (87.3% increase, $P < 0.05$). The extracts of *P. canadensis*, *P. bretschneideri*, and *P. persica* resulted in a significant

Table 3. Effect of the water extracts of leaf litter decomposition medium (soil) on rape seedling growth.

| Conc mg mL ⁻¹ | <i>E.u.</i> | <i>P.f.</i> | <i>P.c.</i> | <i>A.t.</i> | <i>Z.b.</i> | <i>J.r.</i> | <i>P.b.</i> | <i>M.p.</i> | <i>D.k.</i> | <i>P.p.</i> | <i>P.a.</i> | <i>Z.j.</i> |
|-----------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| 0 | 3.74(0.26) ^d | 3.74(0.26) ^c | 3.74(0.26) ^c | 3.74(0.26) ^c | 3.74(0.26) ^d | 3.74(0.26) ^d | 3.74(0.26) ^c | 3.74(0.26) ^c | 3.74(0.26) ^c | 3.74(0.26) ^d | 3.74(0.26) ^b | 3.74(0.26) ^c |
| 10 | 4.44(0.15) ^c | 4.92(0.19) ^b | 4.51(0.09) ^b | 4.99(0.27) ^b | 4.49(0.42) ^c | 4.59(0.08) ^c | 4.97(0.14) ^b | 4.27(0.11) ^b | 5.09(0.13) ^b | 5.22(0.28) ^c | 4.99(0.18) ^a | 5.09(0.38) ^b |
| 20 | 5.52(0.28) ^b | 5.61(0.27) ^a | 5.41(0.16) ^a | 5.80(0.30) ^a | 5.20(0.29) ^b | 5.53(0.31) ^b | 5.25(0.29) ^b | 4.56(0.09) ^b | 5.74(0.18) ^a | 6.43(0.5) ^b | 5.25(0.24) ^b | 5.61(0.14) ^b |
| 40 | 6.23(0.14) ^a | 5.73(0.19) ^a | 5.72(0.35) ^a | 6.06(0.38) ^a | 6.02(0.42) ^a | 6.25(0.41) ^a | 5.96(0.22) ^a | 5.38(0.14) ^a | 5.88(0.34) ^a | 6.99(0.33) ^a | 5.45(0.36) ^a | 6.22(0.32) ^a |
| 0 | 8.85(0.25) ^b | 8.85(0.25) ^d | 8.85(0.25) ^b | 8.85(0.25) ^c | 8.85(0.25) ^b | 8.85(0.25) ^c | 8.85(0.25) ^c | 8.85(0.25) ^c | 8.85(0.25) ^d | 8.85(0.25) ^c | 8.85(0.25) ^d | 8.85(0.25) ^d |
| 10 | 9.02 (0.10) ^{ab} | 9.37(0.23) ^b | 9.64(0.37) ^a | 9.76(0.35) ^b | 9.79(0.36) ^a | 8.88(0.20) ^c | 8.96(0.25) ^c | 9.99(0.21) ^a | 9.44(0.27) ^c | 11.46(0.32) ^a | 9.63(0.28) ^c | 11.72(0.28) ^b |
| 20 | 9.14 (0.22) ^{ab} | 10.30(0.26) ^c | 10.05(0.26) ^a | 10.62(0.52) ^a | 10.27(0.33) ^a | 10.62(0.25) ^a | 10.78(0.20) ^b | 9.12(0.21) ^b | 10.39(0.27) ^b | 11.67(0.29) ^a | 10.54(0.34) ^b | 12.79(0.29) ^a |
| 40 | 9.47(0.31) ^a | 11.15(0.24) ^a | 9.81(0.23) ^a | 11.26(0.36) ^a | 9.70(0.36) ^a | 9.41(0.17) ^b | 12.19(0.27) ^a | 8.18(0.30) ^c | 12.39(0.32) ^a | 10.70(0.27) ^b | 11.59(0.36) ^a | 11.01(0.26) ^c |
| 0 | 2.03(0.15) ^a | 2.03(0.15) ^b | 2.03(0.15) ^b | 2.03(0.15) ^b | 2.03(0.15) ^b | 2.03(0.15) ^c | 2.03(0.15) ^c | 2.03(0.15) ^b | 2.03(0.15) ^b | 2.03(0.15) ^c | 2.03(0.15) ^c | 2.03(0.15) ^b |
| 10 | 2.02(0.10) ^a | 2.28(0.12) ^b | 2.50(0.20) ^a | 2.56(0.29) ^a | 2.14(0.14) ^b | 2.41(0.17) ^b | 2.28(0.15) ^{bc} | 1.92(0.17) ^b | 2.17(0.20) ^b | 2.29(0.19) ^b | 2.67(0.15) ^b | 2.61(0.15) ^a |
| 20 | 2.13(0.16) ^a | 2.58(0.12) ^a | 2.41(0.19) ^b | 2.60(0.28) ^b | 2.63(0.15) ^a | 2.72(0.19) ^b | 2.50(0.09) ^{ab} | 2.03(0.12) ^b | 2.84(0.15) ^a | 2.51(0.13) ^{ab} | 2.93(0.17) ^{ab} | 2.58(0.12) ^a |
| 40 | 2.19(0.19) ^a | 2.79(0.18) ^a | 2.25(0.14) ^b | 2.74(0.29) ^a | 2.85(0.14) ^a | 3.11(0.18) ^a | 2.74(0.17) ^a | 2.43(0.13) ^a | 3.00(0.18) ^a | 2.94(0.20) ^a | 2.99(0.16) ^a | 2.83(0.14) ^a |
| 0 | 2.46(0.15) ^b | 2.46(0.15) ^a | 2.46(0.15) ^b | 2.46(0.15) ^b | 2.46(0.15) ^b | 2.46(0.15) ^b | 2.46(0.15) ^b | 2.46(0.15) ^b | 2.46(0.15) ^b | 2.46(0.15) ^c | 2.46(0.15) ^b | 2.46(0.15) ^b |
| 10 | 2.44(0.25) ^b | 2.59(0.18) ^a | 2.89(0.13) ^a | 2.89(0.17) ^a | 2.69(0.19) ^{ab} | 2.78(0.14) ^a | 2.71(0.15) ^{ab} | 2.89(0.17) ^a | 2.60(0.15) ^{bc} | 2.91(0.16) ^a | 3.06(0.18) ^a | 2.94(0.15) ^a |
| 20 | 2.84(0.17) ^a | 2.65(0.18) ^a | 2.60(0.18) ^b | 3.16(0.15) ^a | 2.93(0.20) ^a | 3.00(0.20) ^a | 2.78(0.21) ^{ab} | 2.54(0.14) ^b | 2.83(0.19) ^b | 3.05(0.18) ^a | 2.99(0.18) ^a | 3.20(0.17) ^a |
| 40 | 2.45(0.16) ^b | 2.73(0.19) ^a | 2.48(0.13) ^b | 2.98(0.23) ^a | 2.84(0.19) ^a | 2.93(0.12) ^a | 2.98(0.15) ^a | 2.27(0.19) ^b | 3.20(0.16) ^a | 3.01(0.13) ^a | 2.94(0.16) ^a | 2.91(0.18) ^a |

E.u. = *E. ulmoides*, *P.f.* = *P. fortunei*, *P.c.* = *P. canadensis*, *A.t.* = *A. truncatum*, *D.k.* = *D. kaki*, *P.p.* = *P. persica*, *Z.b.* = *Z. bungeanum*, *J.r.* = *J. regia*, *P.a.* = *P. armeniaca*, *Z.j.* = *Z. jujube*, *M.p.* = *M. pumila*, and *P.b.* = *P. breitschneideri*. Conc is short for concentration, in mg of dried litter per mL of soil extracts. Data are represented as average (standard deviation). Different letters indicate significant differences between treatments ($P < 0.05$), and the least significant difference method was employed for the comparison tests.

Table 4. Effect of the water extracts of leaf litter decomposition medium (soil) on rape physiological indicators.

| | Conc mg ml ⁻¹ | <i>E.u.</i> | <i>P.f.</i> | <i>P.c.</i> | <i>A.t.</i> | <i>Z.b.</i> | <i>J.r.</i> | <i>P.b.</i> | <i>M.p.</i> | <i>D.k.</i> | <i>P.p.</i> | <i>P.a.</i> | <i>Z.j.</i> |
|--------------------------|-----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| | 0 | 3.03(0.04) ^a | 3.03(0.04) ^{ab} | 3.03(0.04) ^a | 3.03(0.04) ^a | 3.03(0.04) ^a | 3.03(0.04) ^a | 3.03(0.04) ^a | 3.03(0.04) ^a | 3.03(0.04) ^a | 3.03(0.04) ^a | 3.03(0.04) ^a | 3.03(0.04) ^a |
| CAT activity mg/g/min | 10 | 3.02(0.05) ^a | 3.10(0.04) ^a | 3.03(0.03) ^a | 2.98(0.03) ^{ab} | 2.94(0.04) ^{bc} | 3.05(0.03) ^a | 2.92(0.02) ^b | 3.03(0.04) ^b | 2.98(0.03) ^b | 3.04(0.05) ^a | 3.01(0.04) ^{ab} | 2.83(0.03) ^b |
| | 20 | 3.07(0.05) ^a | 2.99(0.06) ^b | 3.04(0.04) ^a | 2.96(0.04) ^b | 3.00(0.02) ^{ab} | 3.00(0.02) ^{ab} | 2.79(0.03) ^c | 3.11(0.03) ^a | 2.88(0.03) ^b | 2.94(0.04) ^a | 2.94(0.05) ^b | 2.75(0.03) ^c |
| | 40 | 2.91(0.03) ^b | 2.98(0.05) ^b | 2.71(0.04) ^b | 2.73(0.03) ^c | 2.91(0.03) ^b | 2.96(0.01) ^b | 2.64(0.04) ^d | 2.74(0.03) ^c | 2.79(0.04) ^c | 3.01(0.03) ^a | 2.55(0.04) ^c | 2.64(0.04) ^d |
| | 0 | 0.14(0.04) ^b | 0.14(0.04) ^a | 0.14(0.04) ^b | 0.14(0.04) ^a | 0.14(0.04) ^a | 0.14(0.04) ^b | 0.14(0.04) ^b | 0.14(0.04) ^a | 0.14(0.04) ^a | 0.14(0.04) ^b | 0.14(0.04) ^a | 0.14(0.04) ^b |
| Root activity mg/g/h | 10 | 0.25(0.03) ^a | 0.13(0.04) ^a | 0.14(0.03) ^b | 0.13(0.02) ^a | 0.17(0.17) ^a | 0.23(0.04) ^{ab} | 0.14(0.04) ^{ab} | 0.14(0.04) ^a | 0.13(0.03) ^a | 0.18(0.03) ^{ab} | 0.15(0.02) ^a | 0.22(0.05) ^{ab} |
| | 20 | 0.15(0.04) ^{ab} | 0.11(0.04) ^a | 0.18(0.04) ^{ab} | 0.12(0.03) ^a | 0.16(0.05) ^a | 0.25(0.04) ^a | 0.18(0.03) ^{ab} | 0.16(0.03) ^a | 0.13(0.04) ^a | 0.21(0.03) ^{ab} | 0.16(0.03) ^a | 0.26(0.02) ^a |
| | 40 | 0.15(0.05) ^{ab} | 0.10(0.03) ^a | 0.24(0.03) ^a | 0.10(0.03) ^a | 0.10(0.03) ^a | 0.24(0.04) ^a | 0.23(0.03) ^a | 0.18(0.03) ^a | 0.15(0.05) ^a | 0.28(0.05) ^a | 0.16(0.03) ^a | 0.25(0.04) ^a |
| | 0 | 1.04(0.04) ^b | 1.04(0.04) ^b | 1.04(0.04) ^b | 1.04(0.04) ^b | 1.04(0.04) ^b | 1.04(0.04) ^b | 1.04(0.04) ^b | 1.04(0.04) ^c | 1.04(0.04) ^b | 1.04(0.04) ^b | 1.04(0.04) ^{ab} | 1.04(0.04) ^b |
| Chl content mg/g | 10 | 1.05(0.03) ^b | 1.10(0.04) ^{ab} | 1.12(0.04) ^{ab} | 1.13(0.06) ^{ab} | 1.10(0.03) ^b | 0.98(0.03) ^{ab} | 1.20(0.05) ^b | 1.03(0.04) ^b | 1.11(0.02) ^a | 1.03(0.02) ^b | 1.11(0.04) ^a | 1.14(0.04) ^a |
| | 20 | 1.12(0.04) ^{ab} | 1.12(0.03) ^{ab} | 1.21(0.04) ^a | 1.18(0.04) ^a | 1.13(0.04) ^b | 0.95(0.04) ^{ab} | 1.20(0.02) ^b | 1.19(0.01) ^a | 1.11(0.02) ^a | 1.05(0.04) ^b | 0.98(0.03) ^b | 1.14(0.01) ^a |
| | 40 | 1.19(0.04) ^a | 1.13(0.03) ^b | 1.18(0.03) ^a | 1.25(0.04) ^a | 1.31(0.04) ^a | 0.89(0.04) ^b | 1.37(0.04) ^a | 1.16(0.05) ^a | 0.97(0.01) ^b | 1.26(0.04) ^a | 0.96(0.06) ^b | 0.93(0.01) ^c |

E.u. = *E. ulmoides*, *P.f.* = *P. fortunei*, *P.c.* = *P. canadensis*, *A.t.* = *A. truncatum*, *D.k.* = *D. kahi*, *P.p.* = *P. persica*, *Z.b.* = *Z. bungeanum*, *J.r.* = *J. regia*, *P.a.* = *P. armeniaca*, *Z.j.* = *Z. jujube*, *M.p.* = *M. pumila*, and *P.b.* = *P. bretschneideri*. Conc is short for concentration, in mg of dried litter per mL of soil extracts. Data are represented as average (standard deviation). Different letters indicate significant differences between treatments ($P < 0.05$), and the least significant difference method was employed for the comparison tests.

acceleration of root activity at 40 mg mL⁻¹, with increments of 78.9%, 70.8%, and 81.3%, respectively. Treatments with *P. persica* and *Z. jujube* resulted in a significant promotion of root activity at concentrations of 20 and 40 mg mL⁻¹.

3.3.3. Chlorophyll content

Extracts of the litter decomposition medium had varying effects on Chl content (Table 4). The treatments with *E. ulmoides*, *P. fortunei*, *Z. bungeanum*, and *P. persica* significantly decreased Chl content in seedlings only at 40 mg mL⁻¹ ($P < 0.05$). The treatment with *P. bretschneideri* increased Chl content in seedlings at all concentrations, and the increment was most pronounced at a high concentration. The treatments with *P. canadensis*, *A. truncatum*, and *M. pumila* showed significant differences from the control experiment at concentrations of 20 and 40 mg mL⁻¹ only. The treatment with *J. regia* resulted in a decreased Chl content (by 14%) only at a concentration of 40 mg mL⁻¹, whereas the treatment with *D. kaki* increased Chl content at concentrations of 10 and 20 mg mL⁻¹ (both with increments of 6.7%, $P < 0.05$). The treatment with *Z. jujube* resulted in an increased Chl content at 10 and 20 mg mL⁻¹, whereas an inhibitory effect on the Chl content of the seedlings ($P < 0.05$) was produced at 40 mg mL⁻¹.

3.4. Comprehensive analyses of the allelopathic effects of decomposed tree litter on rape

SPSS 19.0 was used to perform a PCA analysis on the RI values of all measured indicators of the germination and growth of rape. The comprehensive principal component value equation we used was as follows:

$$F = 0.601F_1 + 0.221F_2 + 0.178F_3 \quad (3)$$

The F values we obtained (Figure) indicate that extracts of the litter decomposition medium of *P. fortunei*, *A. truncatum*, *Z. bungeanum*, *J. regia*, *D. kaki*, *P. persica*, *P. armeniaca*, and *Z. jujube* showed promotional effects on rape at all the concentrations examined. Among these, the acceleration resulting from the treatments with *A. truncatum*, *P. persica*, and *P. armeniaca* become more pronounced with an increasing concentration of extracts. All of these trees can therefore be safely planted alongside rape. The promotional effect of *P. bretschneideri* decreased with increasing extract concentration, and thus this tree should be planted at a lower density when intercropped with rape. The treatments with *E. ulmoides*, *P. canadensis*, and *M. pumila* showed significant inhibitory effects on rape at all concentration levels examined. Among these, the treatment with *M. pumila* resulted in the most pronounced inhibition at 20 mg mL⁻¹, whereas the inhibition caused by *E. ulmoides* and *M. pumila* decreased with increasing extract concentration. Accordingly, these trees should not be used in intercropping with rape.

4. Discussion

Unlike allelochemicals obtained directly by extraction from living organs or tree litter, allelochemicals released from decomposed leaf litter are influenced by soil; thus their concentration, composition, structure, and activity might be extremely different. The important circumstances in which allelopathic effects appear occur

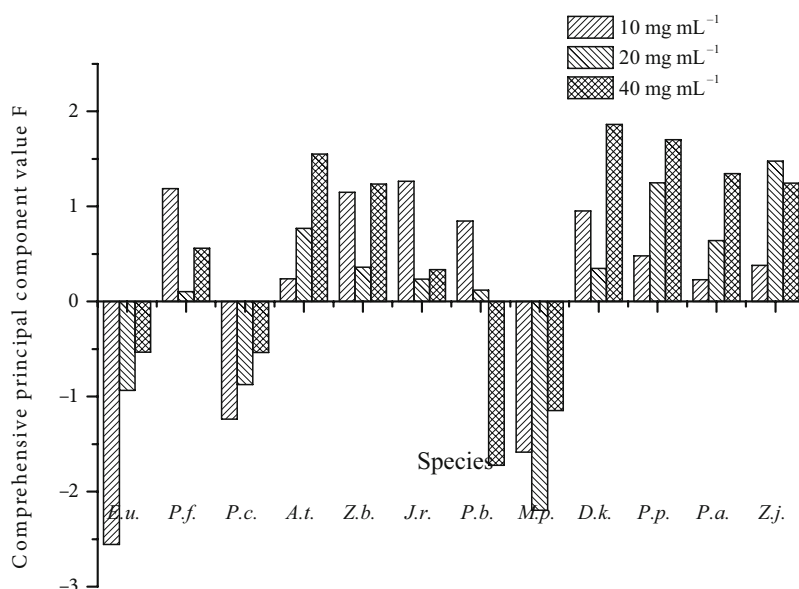


Figure. Comprehensive effects of the leaf litter decomposition of different trees on rape. *E.u.* = *E. ulmoides*, *P.f.* = *P. fortunei*, *P.c.* = *P. canadensis*, *A.t.* = *A. truncatum*, *D.k.* = *D. kaki*, *P.p.* = *P. persica*, *Z.b.* = *Z. bungeanum*, *J.r.* = *J. regia*, *P.a.* = *P. armeniaca*, *Z.j.* = *Z. jujube*, *M.p.* = *M. pumila*, and *P.b.* = *P. bretschneideri*.

when allelochemicals reach the recipient plant in their active structure and at their effective concentration, thus extracts of litter and decomposed litter (or the decomposed medium) often show different allelopathic effects. For example, in this study, extracts of *A. truncatum*, *P. armeniaca*, and *Z. jujube* decomposed medium did not have significant influences on the germination rate of rape seed, whereas those of *Z. bungeanum* and *P. bretschneideri* resulted in a significantly decreased germination rate. However, when the allelochemicals were extracted from undecomposed litter (Chi, 2011), such as extraction from *A. truncatum*, this resulted in a significant increase in the germination rate at concentrations of 0.005~0.1 g/mL. Furthermore, the *P. armeniaca* and *P. persica* litter extracts resulted in a significant decrease in the germination rate, whereas the *Z. bungeanum* and *P. bretschneideri* litter extracts did not influence the germination rate. These results indicate that under the soil biochemical conditions of this study, the allelochemicals that promote or inhibit germination might diffuse, decompose, or accumulate in the soil, and their structure or activities may be altered; consequently their allelopathic ability may become weakened or enhanced. According to Inderjit (2005), soil microorganisms have important modifying effects on allelochemicals, and are able to decompose some of these chemicals into inactive substances. Pollock et al. (2009) stated that when catechins are combined with metal ions, their allelopathic inhibitory effect is strongly accelerated. Different ions show differences in these promotional effects. The investigation of An et al. (2001) also demonstrated that the concentrations of allelochemicals in the residues of *Vulpia* are strongly increased after a short period of decomposition. Similarly, the allelochemicals released from the decomposed litter in this study may have been affected by metal ions in the soil. In addition, common allelochemicals such as phenolic acids and terpene may be transformed by biological and chemical actions in the soil (Blum et al., 1998; Kong et al., 2002).

The above-mentioned two types of extracts showed differences in their allelopathic effects on rape seedling growth. All of the extracts of litter decomposition medium had significant promotional effects on the shoot growth of rape, and this acceleration was positively related to the concentration of the extracts. In contrast, an extract of the undecomposed litter of *J. regia* produced significant inhibition at concentrations of 10~40 mg kg⁻¹, and the inhibitory effect was stronger with an increasing extract concentration (Chi, 2011). Similarly, an extract of the decomposition medium of *E. ulmoides* accelerated the growth of roots, whereas an extract of undecomposed litter of *E. ulmoides* resulted in significant inhibition (Chi, 2011). This might be caused by the decomposition and transformation of allelochemicals (Schmidt, 1988). For

instance, Huang (2013) stated that after decomposition, the main potential allelochemicals in *J. regia* litter change from arachyl alcohol, eicosane, and squalene to sitostenone. Furthermore, the nutrients released from litter weaken the toxic effects of allelopathy, and the humus produced by litter decay also adsorbs allelochemicals (e.g., caffeic acids, ferulic acid, or salicylic acid) and weakens their toxicity (Loffredo et al., 2005; Cayuela et al., 2008). Moreover, humus can accelerate the growth of plants to some extent; therefore, all of these effects can effectively counteract the negative effects of allelopathy. In contrast, allelochemicals may be transformed into more active substances or enriched, as shown by Kong et al. (2002).

The effects of the two types of extracts on physiological properties are also different. In this study, the extract of decomposed medium of *P. fortunei* litter did not show any significant effect on the root activity of rape, whereas an extract of undecomposed litter inhibited root activity at 0.005 g mL⁻¹ (Chi, 2011). A possible mechanism for this phenomenon may be similar to that mentioned above.

This study indicated that the extracts of decomposed medium of some species of tree litter often showed promotional effects on rape at low concentrations and inhibitory effects at high concentrations, which is similar to what has been found in other investigations (Yuan and Hou, 2009; Zhang et al., 2012). These effects of allelopathy depend on the concentration of the allelochemicals (Wang et al., 2009b). One possible explanation for our results may be that the allelochemicals were decomposed by soil microbes (Lankau, 2010), which makes the trophism of the decayed litter more pronounced (Qin et al., 2012). Furthermore, allelochemicals at low concentrations may induce compensatory plant growth, which is similar to what has been shown as a result of low-intensity environmental stresses (Wang et al., 2005; Wang et al., 2009a).

There were differences in the effects of the same species on different indicators of the germination and growth of rape. For example, in all the tree species examined, none of the decomposition medium extracts had significant effects on the germination rate of rape, but they did significantly decrease the germination speed. This indicates that even though biochemical reactions with soil could weaken potential allelopathic effects on germination, they still had inhibitory effects on germination speed. This may be due to allelochemicals that are released from the decayed litter and hinder the physiological and biochemical processes of seed germination (Weir et al., 2004), such as inhibiting water absorption (Turk and Tawaha, 2003), inhibiting the activity of key enzymes (e.g., protease, sucrose dehydrogenase, and succinodehydrogenase) of the germinating process (Einhellig, 1995), and increasing inhibitory substances such as abscisic acid, coumarin, and phenolics (Yang et al., 2005). Furthermore, in addition to

the germination speed index, only the CAT activity of the rape seedlings was hindered significantly among these indicators. This phenomenon indicates that the sensitivity of indicators and organs is different. For rape, germination speed index and CAT activity may be the most sensitive indicators of toxic allelopathy, which is in agreement with the findings of Zeng (1999). The allelopathy of decomposed litter is the result of the comprehensive effects of many allelochemicals. Some chemicals have relatively strong selectivity and specificity, and therefore the sensitivity of indicators of germination and seedling growth is influenced by different allelochemicals (Inderjit, 2006; Yu et al., 2008). Similarly, different types of allelochemicals undergo chemical reactions with each other, which could result in complex additional or antagonistic effects (Kong et al., 1998). All of these factors increase the variability of allelopathy, and we suggest that further studies be undertaken to investigate these factors.

It deserves to be specially noted that temperature and humidity are variable under field conditions, which can cause fluctuations in microorganism populations and activities, and consequently influence the litter decomposition rate and the allelochemicals release and transformation process. Previous studies illustrated that the main kinds of allelochemicals released vary during different decomposition stages (Liu, 2006). On the other hand, microbial activity can change (decrease or increase)

the biotoxicity of allelochemicals due to degradation or resynthesis effects during litter decomposition and allelochemical release (Aziz and Shaukat, 2015). Hence, the fluctuations of microorganism would result in more complex allelopathic processes. To simulate authentic field conditions, further field research should be conducted by sowing rape seeds in litter-covered farmland or soil with buried litters (just mixed with top soil). The fluctuations of soil microorganism and enzymes as well as the dynamics of allelochemicals also need to be studied for better understanding of the allelopathic effects of litter decomposition on rape and their biological mechanisms. Even so, aiming to achieve good comparability in this study, the potential allelopathy of decomposed leaf litter was studied under controlled laboratory conditions, in which the disturbances caused by field variable factors were mostly excluded. Thus, the results could lay a good foundation for further field research and provide a scientific basis for preliminary screening for the suitable interplanting of tree species with rape.

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