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Research Article

Allellopathic Impacts of Leaf Litters Decomposition from Intercrop Tree Species on Soybean

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Abstract: Foliar litters from 5 commonly planted intercorpped trees were collected and decayed within soil of local farm. These soil samples containing different amount of decomposed litters were then used as culture medium for a germination and seedlings growth testing of soybean. The allelopathic effects of intercropped trees on soybean were assessed. The results indicated that *Eucommia ulmoides*, *Paulownia fortunei* and *Acer truncatum* litters showed promotional effects at relative low concentration (75-150 g litters decomposed in 6.5 kg soil, that is 75-150 g/pot), but inhibitory effects at high concentration (300 g/pot), thus these trees should be planted with soybean with a low intercrop proportion. *Populus canadensis* litters showed promotional effects at low and high concentration (75 or 300 g/pot), but inhibitory effects at moderate concentration (150 g/pot) and this tree should be planted with a moderate intercrop proportion. *Zanthoxylum bungeanum* litters exhibited promoting effects at all concentrations, thus this tree was recommened to be planted with soybean with any tested intercrop proportion.

Keywords: Allelopathy, foliar litter's decomposition, soybean

INTRODUCTION

Agroforestry business pattern has been a promising method to promote the coordinated development of agriculture and forestry, for its properties such as effective natural resources utilization, high crop yield and natural disaster reduction (Rao *et al*., 1997). Tree and crop interplanting is one of the important patterns of agroforestry system, while the selection of species and intercropping proportion were the key factors affecting the results of interplanting. For doing this, not only the competition of sunlight and nutrients between trees and crops, but also the interspecific allelopathy should be considered. Because unsuitable intercropping species or intercropping proportions could lead to obvious inhibitory allelopathic effects on crop germination and growth and thus cause the reduction of crop productivity and economic benefits.

Soybean (*Glycine max*) is one of the most important crops; it is meaningful to investigate the allelopathic effects of intercropping species on it. However, most studies on this subject were carried out by obtaining allelochemicals from the water extracts of intercropping species' organs, while in natural conditions, the main approach of allelochemicals releasing is the decomposition of plant litters (Lin *et al*., 2007). Previous studies demonstrated that the products of decomposed litters caused obvious self-inhibition on grass or tree species and they also showed significant inhibitory allelopathic effects on other species (Li *et al*., 2012). Soil used for long term plantation showed allelopathic effects as well (Hao *et al*., 2011). Thus, it might be a more practical way to investigate the allelopathic effects by carrying out a pot culture experiment of receiver plants using soil medium containing decomposed litters from offering species. Hence, litters from 5 commonly planted intercropping tree species were collected and decomposed in soil. These soils were used as culture medium to plant soybean. The germination, seedlings growth and physiological properties of soybean were measured. Based on these data, the allelopathic effects of intercropping trees were assessed. Our results might provide a scientific basis for selecting appropriate intercropping species and suitable intercropping proportions.

MATERIALS AND METHODS

Litters and soil sampling: In later autumn, fresh foliar litters from commonly used intercropping species, including *Eucommia ulmoides*, *Paulownia fortune*,

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Populus Canadensis, *Acer truncatum* and *Zanthoxylum bungeanum*, were collected in Yangling, China. Litters decayed or infected by diseases and insect pests were removed. Selected litters were rinsed rapidly and air-dried and then smashed and passed through 1 mm sieves for storing. In addition, the top layer (0-10 cm) soil from local farmland was collected (classified as Eum-orthic anthrosols). After picking out plant and animal debris, roots and stones, the homogenized fresh soil was passed through 2 mm sieves and then used as medium for litters decomposition.

Litters decomposition: Prepared fresh soil was divided into subsamples with a weight of 6.5 kg (converted as dry weight here). Smashed foliar litters from every tree species were added into 3 subsamples with weights of 300, 150 and 75 g, respectively. Subsamples containing litters were uniformly mixed and put into plastic pots as culture medium for treatments and the ones which were not added litters were used as culture medium for control testing. Every kind of soil was prepared for 3 parts. Distilled water was uniformly added into soil with a sprayer to adjust the soil moisture to 60% of the saturated field water capacity. Plastic films with four vents (Φ = 1.5 cm) were used to cover the pots to prevent extreme evaporation. The pots were weighed weekly during decomposition processes and distilled water were added into pots according to their mass losses. With these methods, soils were incubated for 120 d at constant moisture and temperature (20-25°C), until the litters were completely decomposed.

Cultivation testing and parameters determination: Plump seeds without pests infections were disinfected by 5% NaClO solution for 10 min, washed for 4 times and soaked in distilled water for 4-5 h. The prepared soil medium subsamples were placed into culture pots. In every pot, a total of 25 soybean seeds were uniformly sowed in the soil, covered with soil of 2 cm. The sown pots were then placed in a phytotron (26°C, 12 h daylight). During the cultivation process, appropriate amount of distilled water were added in pots to maintain the soil moisture constant. The quantity of germinated seeds was noted daily after sowing until no seeds germinated and the data were used for calculating the germination rate and germination speed index *I* Eq. (1):

$$I = 2(5X_1+4X_2+3X_3+2X_4+X_5) \quad (1)$$

where,

X_i = The quantity of germinated seeds in every 24 h, i.e.,

X_1 = The quantity of the first day after sowing

X_2 = The quantity of the second day after sowing and so on

About 15 days after sowing, all seedlings had 2 well grown euphyllas. Ten seedlings were randomly selected in every pot for shoot and root length determination. Seedlings measured were then oven dried at 75°C to constant weight for dry weights of shoot and root determination, after removing water at 105°C for 30 min. The remaining seedlings were used for physiological properties determinations using methods as follows: root activity was measured by triphenyltetrazolium chloride method, Chlorophyll (Chl) content by ethanol-acetone extraction method and Catalase (CAT) activity by $KMnO_4$ titrimetry method. Every indicator was measured for 3 times with an error less than 5%.

Data processing: Allelopathic effect index *RI* Eq. (2) was used as indicator for the allelopathic effects of litters decomposition on the germination, seedling growth and physiological properties:

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

In which,

T = The data obtained in treatment testing

C = That obtained in control testing

A positive *RI* indicates promotional effects and a negative *RI* is the opposite (Williamson and Richardson, 1988).

Data obtained in treatment and control testing was submitted to SPSS 19.0 software for a one-way analysis of variance, in which the least significant difference method was employed for *post hoc* analysis. The *RI* values of every index of treatment testing were analyzed using Integrated Principal Component Analysis (IPCA) and the integrated principal component value *F* was used as the indicator for the overall allelopathic effects of litters decomposition on soybean. A positive *F* revealed promotional effects and a negative one was the opposite.

RESULTS

Allelopathic effects of litters decomposition on germination of soybean: litters decomposition showed variable allelopathic effects on soybean germination among different litters species and concentrations (Table 1).

On germination rate: decomposition of litters from *E.u.* and *P.f.* significantly increased the germination rate only at low concentration (75 g/pot). Decomposition of *P.c.* litters accelerated germination at low and moderate concentration (75 and 150 g/pot) by 9.50-10.77%, while its effects turned out to be not significant at high concentration (300 g/pot). Decomposition of *A.t.* litters increased the germination rate only at low concentration by 10.26%. Decomposition of *Z.b.* litters significantly increased the germination rate at moderate and high concentrations by 10.81-13.51%.

Table 1: Impacts of water extracts from decomposed leaf litters of different tree species on seed germination of soybean

Indicator	Concentration g/pot	*E.u.*	*P.f.*	*P.c.*	*A.t.*	*Z.b.*
Germination ratio %	0	74.00bc	74.00bc	74.00b	74.00b	74.00b
	75	82.45a	82.46a	81.03a	82.11a	78.00ab
	150	77.17ab	77.18ab	81.97a	75.01ab	82.00a
	300	69.77c	69.77c	75.14b	76.03ab	84.00a
Germination speed index *I*	0	390bc	390bc	390b	390b	390c
	75	477a	477a	418ab	435a	447b
	150	404b	404b	409ab	369b	432b
	300	362c	362c	430a	372b	491a

Different letters in the same column indicates significant differences among different concentrations leach liquor from the same tree at 0.05 level; *E.u.*: *Eucommia ulmoides*; *P.f.*: *Paulownia fortune*; *P.c*: *Populus canadensis*; *A.t.*: *Acer truncatum*; *Z.b*: *Zanthoxylum bungeanum*, the same below

Table 2: Impacts of water extracts from decomposed leaf litters of different tree species on seedlings growth indices of soybean

Indicator	Concentration g/pot	*E.u.*	*P.f.*	*P.c.*	*A.t.*	*Z.b.*
Shoot height cm	0	9.38b	9.380c	9.3800b	9.38bc	9.380c
	75	9.88b	10.29b	9.4700b	9.90ab	11.72b
	150	11.77a	12.08a	10.430a	10.46a	11.74b
	300	7.420c	8.700c	10.00ab	8.71c	13.24a
Root length cm	0	5.770b	5.770c	5.7700c	5.77c	5.770b
	75	6.340b	8.370a	6.2200c	7.83b	6.020b
	150	7.590a	8.080a	8.5500a	9.36a	6.39ab
	300	4.910c	6.670b	7.2300b	7.16b	6.940a
Shoot dry weight mg·plant-1	0	25.25c	25.25c	25.250c	25.25b	25.25c
	75	26.58b	26.77b	26.57ab	26.18b	28.69b
	150	28.11a	29.12a	27.550a	27.44a	28.50b
	300	24.50c	24.390c	25.94bc	24.16c	29.90a
Root dry weight mg·plant-1	0	16.80a	16.800b	16.800b	16.80b	16.80b
	75	17.09a	17.23ab	17.16ab	17.03ab	17.25ab
	150	17.78a	17.900a	18.100a	17.830a	17.820a
	300	15.49b	17.05ab	17.32ab	16.75b	18.00a

On germination speed: decomposition of litters from *E.u.*, *P.f.* and *A.t.* significantly increased the germination speed at low concentrations by 22.31%, 22.31 and 11.54%, respectively, while along with the increasing concentration, the promoting effects were reduced until it were not significant. Decomposition of *P.c.* litters increased the germination speed only at high concentration. Decomposition of *Z.b.* litters increased the germination rate significantly at all concentrations, especially at high concentration, the increment of which was 25.90%.

Allelopathic effects of litters decomposition on seedlings growth of soybean: litters decomposition showed variable allelopathic effects on soybean seedlings growth among different litters species and concentrations (Table 2).

On shoot heights: litters decomposition of *E.u.* significantly increased the shoot heights of soybean seedlings by 25.48% at moderate concentration, while it significantly decreased that by 20.90% at high concentration. *P.c.* and *A.t.* litters decomposition significantly increased the shoot heights by 11.19% and 11.51% respectively only at moderated concentration. The promoting effect of *Z.b.* litters decomposition increased with the increasing concentration and it was the most obvious at high concentration, the increment of which was 41.15%.

On root lengths: litters decomposition of *E.u.* significantly promoted the root growth of soybean seedlings by 31.54% at moderate concentration, while it significantly decreased that by 14.90% at high concentration. *P.f.* litters decomposition significantly increased the root lengths at all concentrations, but its promoting effect decreased with the increasing concentration. Decomposition of *P.c.* litters accelerated the growth of roots by 48.18 and 25.30%, respectively at moderate and high concentration. *A.t.* litters decomposition significantly increased the root lengths at all concentrations, especially at moderate concentration (the increment was 62.22%). The significant promoting effect of *Z.b.* litters decomposition only appeared at high concentration (the increment was 20.28%).

On shoot weights: decomposition of *E.u.*, *P.f.* and *P.c.* litters significantly increased the shoot dry weights of soybean seedlings at low and moderate concentrations and the promoting effects were more obvious along with the increasing concentration. *A.t.* litters decomposition significantly decreased the shoot weights only at high concentration, while *Z.b.* litters decomposition significantly increased the shoot weights at all concentrations especially at high concentration (the increment was 18.42%).

On root weights: decomposition of *E.u.* litters significantly decreased the root weights of soybean seedlings by 7.80% at high concentration. Decomposition of *P.f.*, *P.c.* and *A.t.* litters significantly decreased the root weights by 6.55, 7.74 and 6.13%,

Table 3: Impacts of water extracts from decomposed leaf litters of different tree species on physiological indices of soybean

Indicator	Concentration g/pot	*E.u.*	*P.f.*	*P.c.*	*A.t.*	*Z.b.*
CAT activity mg·g-1·min-1	0	3.31a	3.31a	3.31a	3.31a	3.31b
	75	3.12b	3.27a	3.25a	3.24a	3.40ab
	150	3.09b	3.18b	3.15b	3.33a	3.44a
	300	3.09b	3.18b	3.17b	3.24a	3.46a
Root activity mg·g-1·h-1	0	0.13b	0.13c	0.13b	0.13b	0.13b
	75	0.14b	0.15b	0.13b	0.14b	0.14ab
	150	0.16a	0.16a	0.15a	0.15a	0.14a
	300	0.10c	0.12d	0.13b	0.13b	0.15a
Chl content mg·g-1	0	2.12c	2.12b	2.12a	2.12c	2.12b
	75	2.40b	2.19ab	2.13a	2.27b	2.30a
	150	2.62a	2.23a	2.20a	2.36a	2.32a
	300	1.86d	2.02c	2.14a	2.05c	2.35a

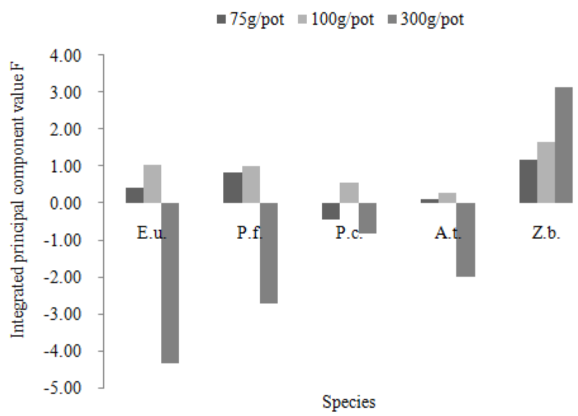

Fig. 1: Integrated principal component analysis of the allelopathic impacts of intercrop leaf litters decomposition on soybean

respectively at moderate concentration. *Z.b.* litters decomposition significantly increased the root weights at moderate and high concentration.

Allelopathic effects of litters decompostion on physiological properties of soybean: CAT activity, root activity and Chl content indicated the ability of active oxygen resistance, nutrient and water untake and photosynthesis. Litters decomposition showed variable allelopathic effects on soybean physiological properties among different litters speices and concentrations (Table 3).

On CAT activity: decomposition of *E.u.* litters significantly decreased the CAT activity of soybean seedlings at all concentration. *P.f.* and *P.c.* litters decomposition significantly decreased the CAT activity at moderate and high concentrations. *Z.b.* litters decomposition significantly increased the CAT activity by 3.93-4.53% at moderate and high concentrations.

On root activity: decomposition of *E.u.* litters significantly increased the root activity of soybean seedlings by 23.08% at moderate concentration, while it significantly decreased the root activity by 23.08% at high concentration. Decomposition of *P.f.* litters accelerated the root activity by 15.38-23.08% at low and moderate concentrations, but it inhibited that by 7.69% at high concentration. *P.c.* and *A.t.* litters decomposition significantly increased the root activity only at moderate concentration. *Z.b.* litters decomposition significantly increased the root activity at moderate and high concentrations.

On Chl content: decomposition of *E.u.* and *A.t.* litters significantly increased the Chl content of soybean seedlings at low and moderate concentrations and the promoting effects increased as the concentrations increased. However, they significantly decreased the Chl content by 12.26 and 3.30% at high concentration respectively. Decomposition of *P.f.* litters increased the Chl content at moderate concentration, but it significantly decreased that by 4.72% at high concentration. *P.c.* litters decomposition did not significantly affected the Chl content at any concentration. *Z.b.* litters decomposition significantly increased the Chl content at all concentrations.

Integrated analysis on the allelopathic effects of litters decomposition on soybean: Decomposition of litters exhibited variable allelopathic effects on the germination, seedlings growth and physiological properties of soybean and even litters from the same species still showed differences. Thus integrated principal component analysis was employed to assess their overall effects on soybean. The integrated principal component value *F* of every treatment was obtained by SPSS 19.0 (Fig. 1). The results demonstrated that *E.u.*, *P.f.* and *A.t.* litters decomposition exhibited integrated promoting allelopathic effects on soybean at low and moderate concentration but inhibitory allelopathic effects at high concentration. *P.c.* litters decomposition exhibited inhibitory allelopathic effects at low and high concentrations, but promoting effects at moderate concentration. *Z.b.* litters decomposition exhibited promoting effects at all concentrations and the effects increased as the concentration increased.

DISCUSSION

Allelochemicals obtained by water extraction or decomposition methods showed differences in their effects. Because after released from litters, these chemicals were transported or retained by soil. In addition, the allelochemicals were usually degraded or re-compounded by microorganisms and enzymes in the litters decomposition processes. Their activities, concentration and chemical constitution were then altered. Consequently, their allelopathic effects might be enhanced or weakened and even totally changed over.

Our results indicated that the germination, seedlings growth and physiological properties of soybean were accelerated at relative low concentrations and inhibited at higher concentration in treatments of *E.u.*, *P.f.* and *A.t.* litters decomposition, which were agreed with the findings of Chi and Liu (2011). Zhao *et al*. (2010) reported that water extracts of *P.f.* litters did not show significant effects on the germination processes on soybean at the concentration of 10-50 mg/mL (equivalent to 75-375 g/pot in this study), which was contrary to our results. These phenomena demonstrated that the allelochemicals were activated by the chemical and biological reactions in soil and thus inhibited the activities of key enzymes in germination processes at relative low concentrations (the highest concentration of this study) (Song *et al*., 2006). Similarly, the activated allelochemicals might influence the abilities of water and nutrient uptake and destroy the organelle and biomembrane system. They also might inhibit the elongation and division of cells and consequently inhibit the germination and seedling growth of soybean (Turk and Tawaha, 2003; Baziramakenga *et al*., 1997; Kaur *et al*., 2005). Han *et al*. (2011) stated that water extracts of *Z.b.* litters exhibited significant inhibitory effects on the germination rate of 3 soybean breeds at the concentration of 20-40 mg/mL (equivalent to 150-300 g/pot in this study), causing sharp decreases in biomass, SOD activity and a significant increase of MDA. However, decomposition of *Z.b.* litters exhibited integrally promoting effects on soybean in present study. These might reveal that the inhibitory allelochemicals, such as abscisic acid, coumarin, hydroxy-3-methoxycinnamic acid and phenolics, were degraded or inactivated by microbes. In addition, humus formed in litter's decomposition processes had adsorptive actions to allelochemicals and the concentrations of these chemicals thus decreased (Loffredo *et al*., 2005). Furthermore, allelochemicals with a low concentration might cause the compensatory growth, which was similar to that caused by cold stress (Duan *et al*., 2012). Simultaneously, the nutrients released from litters might temper the inhibitory effects of allelochemicals and promote the germination and seedlings growth of soybean.

CONCLUSION

In view of allelopathic effects, *E. ulmoides*, *P. fortunei* and *A. truncatum* should be planted with soybean with a low intercrop proportion. *P. canadensis* should be planted with a moderate intercrop proportion. *Z. bungeanum* was recommended to be planted with soybean with any appropriate intercrop proportion.

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