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Full Length Research Paper

Decomposition of blue gum (*Eucalyptus maidenii*) leaf litter may accelerate the maturation and senescence of spinach (*Spinacia oleracea*)

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In order to study the effect of decomposition of eucalyptus leaf litter on crops in agroforestry ecosystems, a pot experiment was conducted with five treatments, that is, 0 (the control), 30, 60, 90, and 120 g of blue gum (*Eucalyptus maidenii*) leaf litter were mixed with 10 kg of soil, namely CK, L30, L60, L90, and L120, respectively. Spinach (*Spinacia oleracea*), the selected receptor, was seeded for each treatment. Several morphological and physiological indicators of spinach were determined 50 days later. The results were shown as follows: (1) Significant stimulation was observed in the bolting length, rate of bloomed plants and rachis length treated with the leaf litter, except L120 in the bolting length. On the contrary, the root shoot ratio (R/S) and leaf area from leaf litter treatments were both considerably lower than those from CK. (2) As the leaf litter increased, the ratio of soluble sugar content to soluble protein content (SS/SP) rose accompanied with the descent of the leaf area ($r=-0.9298$, $n=5$, $p<0.05$). (3) The peroxidase (POD) activity was promoted, while the superoxide dismutase (SOD) and catalase (CAT) activity were inhibited by L60 to L120. The lipid peroxidation determined using the malondialdehyde (MDA) assay was promoted by each leaf litter treatment. Based on the data stated previously, it is suggested that decomposition products of blue gum leaf litter induces the maturation and senescence of spinach via hormones or/and reactive oxygen species (ROS).

Key words: Blue gum, spinach, decomposition, maturation, senescence, allelopathy.

INTRODUCTION

Eucalyptus species, with a series of traits such as fast-growing, adaptable and broad-spectrum, are widely planted, especially in India, Brazil and China (Chen, 2001; Xu and Zhang, 2006; Varghese et al., 2008), which as agroforestry component trees have been used for a long time (Ahmed et al., 2008). In India, paper mills and forest departments, etc., have substantial eucalyptus plantations in farmers' land (Varghese et al., 2008).

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China, the pioneer to practice agroforestry, where eucalyptus is commonly inter-planted with crops after being introduced in (Zhang and Yu 2007 and Lin et al., 2010).

However, the relatively poor understory of eucalyptus plantations has been a concern for a long time (Del Moral and Muller, 1970; Chen, 2001; Liu and Li, 2010). Of all the causes, allelopathy, the active interference between or within species mediated by some particular chemicals (the so-called allelochemicals) (Rice, 1984), is believed to play an important role. Related laboratory bioassays have provided many evidences (Florentine and Fox, 2003; Hill et al., 2006; Gao et al., 2007; Verdeguer et al., 2009), in

which a gradient doses of tissue extraction, volatile oils or even a single component were commonly used. Such bioassays are of great help to recognize the potential and mechanism of allelopathy, yet very different from the field situation. According to Foy (1999), in land ecosystems, any bioassay without involving soil lacks ecological relevance.

Residue decomposition is a major way for a plant to release biologically active compounds, along with volatilization, foliar leaching and root exudation (Inderjit and Nisen, 2003). Del Moral and Muller (1970) reported long before those annual herbs rarely survived to maturity where eucalyptus litter accumulated. Batish et al. (2006) found that the decaying leaf of *Eucalyptus citriodora* contained essential oils that could inhibit the seed germination and root elongation of *Cassia occidentalis* and *Echinochloa crus-galli*. Decomposition of *Eucalyptus camaldulensis* leaves in the soil was proved suppressive to *Phalaris minor* and several forest and agricultural crops, when the dose reached a certain level (Ahmed et al., 2008; Niakan and Saberi, 2009). Recently, our studies suggested that decomposition of *E. grandis* leaf litter resulted in strong allelopathic effects on *Elymus sibiricus* and *Cichorium intybus* based on some morphological and physiological indicators (Chen et al., 2011; Wu et al., 2012).

Leaf senescence is an essential process that occurs as part of plant development, accompanied with the breakdown of chlorophyll, the degradation of proteins and lipids, the catabolism of energy reserves, and induction of senescence-associated genes (Buchanan-Wollaston et al., 2003; Fukao et al., 2012). However, diverse environmental stresses, such as drought stress (Munné-Bosch and Alegre, 2004), salt stress (Chen et al., 2012) may induce the premature senescence in plants. Although enormous efforts have been made on the recognization of the mechanism of senescence (Buchanan-Wollaston et al., 2003), its highly complex molecular regulatory network remains to be dissolved (Lim et al., 2007; Breeze et al., 2011). Allelochemicals were reported to be potential to influence almost any aspect of the receptor from gene to individual level (Wang et al., 2005), but few related studies paid attention to the development of the receptor in the presence of allelopathic materials. This paper, from the standpoint of the development of spinach (*Spinacia oleracea*), a popular horticultural crop all over the world, expounded the effect of decomposition of eucalyptus leaf litter in soil, aiming at providing some theoretical reference for the construction and management of eucalyptus-crop systems.

MATERIALS AND METHODS

Site description

The experiment was implemented in the vinyl house of Teaching and Research Garden of Sichuan Agricultural University, southwest of Sichuan, China (29°58′ 48″ N, 102°59′ 55″ E, 600 m altitude, a. s. l.), where features a subtropical humid monsoon climate with four distinct seasons, insufficient light supply, a small difference in temperature between day and night, and concentrated rains in summer. Several meteorological parameters are shown as follows: The annual average temperature 14.1 to 17.9°C, ≥ 10°C accumulated temperature 5231°C, monthly mean maximum temperature 29.9°C (July), monthly average minimum temperature 3.7 (January), average annual sunshine hours 1039.6 h, frost-free period 298 days, average annual rainfall 1774.3 mm, and average air humidity 79%.

Materials and pretreatment

Leaf litter of blue gum (*Eucalyptus maidenii*) was collected in March 2010 from a 25-year-old blue gum stand in Ya'an, China. Once taken back, the leaf litter was air-dried and cut into pieces with an approximate area of 2 cm^2, mixed fully.

Seeds of spinach "Chuan0165" were provided by Keda Seeds and Seedlings Service of Fucheng District, Mianyang, China. First of all, the seeds were disinfected in 0.5% potassium permanganate ($KMnO_4$) for 30 min, then soaked in tap water for 12 h with an initial temperature of 65°C, finally covered with the wet gauze on the condition of real time room temperature for 12 h.

The container was white plastic pot made from polyvinyl with the upper diameter 29 cm, the bottom diameter 25 cm, and the height 26 cm. The soil was sandy loam collected from local farmland with the field capacity (FC) 246.40 $g \cdot kg^{-1}$, the content of organic matter (OM) 13.527 $g \cdot kg^{-1}$, the content of total nitrogen (TN) 0.132 $g \cdot kg^{-1}$, and the content of hydrolyzable nitrogen 14.478 $mg \cdot kg^{-1}$. After disinfected with carbendazim, the soil was mixed fully and laid flat for 2 days.

Experimental design

According to the design adopted by Chen et al. (2011) and Wu et al. (2012), we roughly investigated the annual leaf litter quantity of the blue gum stand; it is about 6000 $kg \cdot hm^{-2}$, nearly 42 g per pot upper surface area (about 0.07 m^{-2}). Given the fact that different stands (even different quadrats in our statistics) differ greatly in the litter quantity, we finally decided 60 $g \cdot pot^{-1}$ as the base of leaf litter application (denoted by L60), then half the base (30 $g \cdot pot^{-1}$, denoted by L30), one and a half the base (90 $g \cdot pot^{-1}$, denoted by L90) and double the base (120 $g \cdot pot^{-1}$, denoted by L120) were set up. The control group was added no leaf litter in (0 $g \cdot pot^{-1}$, denoted by CK). Considering that the tilling depth 17 cm was recommended by Lu (1998) for spring spinach, the depth of the pretreated soil loaded into the pot was designed 20 cm, about 10 kg in weight (the real time water content was measured 16.67% (w/w)).

As designed above, On April. 7th 2010, 0 (CK), 30, 60, 90 and 120 g of blue gum leaf litter were mixed with pre-weighed 10 kg of soil, respectively, then put into the pot. Six repeats for CK and each leaf litter treatment, so thirty pots in total. After that, spinach seeds were sowed (30 seeds per pot) and kept distribute uniformly on the pot surface. Each pot was covered with 0.7 kg of soil. Afterwards the soil water content was controlled at 80% FC by weighing method; observation and weeded frequently was done. Indicators were determined 50 days after sowing (May 27th, 2010), which was among the appropriate harvest time of spring spinach recommended by Lu (1998).

Items and methods for observation and measurement

Ten plants were randomly chosen from each treatment for the bolting length measurement. For the plant that had bloomed, the

rachis length was measured, and the observations were averaged by treatment. The rate of bloomed plants was calculated as:

The rate of bloomed plants (%) = No. of bloomed plants×100/No. of total plants in the pot (six repeats were done)

Three plants (the root and the shoot were separated) a repeat, water-removed in the oven on the condition of 105°C for 30 min, then dried under 80°C until the weight was constant, and the dry weight of the root and the shoot of a single plant was obtained, three repeats.

The fresh weight (FW) of two leaves (the 7th or 8th euphylla counted from the bottom to the top) a repeat was weighed; Both were punched triple with a puncher and six disks (the area was known, denoted by S_0) were obtained, weighed FW_0, then the area of a single leaf was calculated as:

Leaf area (cm^2) = $3\times FW\times S_0/FW_0$, (three repeats were done)

About 0.1 g of the leaf sample of spinach was weighed with a precise electronic balance, fully grinded with 8 ml 0.05 mol/L pH7.0 phosphate buffer. The homogenate was centrifuged for 10 min on the condition of 5000 rpm and 4°C. The supernatant fluid was the crude protein (enzyme) extract.

Superoxide dismutase (SOD) activity was determined using the method called NBT photochemical reduction (Giannopolitis and Ries, 1977). The reaction system included 0.1 ml enzyme extract, 1.5 ml phosphate buffer, 0.3 ml methionine (MET), 0.3 ml disodium ethylene diamine tetraacetate (EDTA-Na_2), 0.3 ml chlorinated amino tetrazolium (NBT), 0.2 ml distilled water, and 0.3 ml riboflavin. 50% inhibition of the NBT photoreduction was calculated as an activity unit (U). In the detection of catalase (CAT) activity, 1.5 ml phosphate buffer, 1.0 ml distilled water and 0.2 ml enzyme extract were mixed in the cell first, then the reaction was initiated by adding 0.3 ml 0.1 mol/L H_2O_2. The decrement of 0.1 at A_{240nm} under the UV-VIS spectrophotometer was defined as an activity unit (U) (Zhao et al., 2002). Peroxidase (POD) activity was determined by the guaiacol method (Xiong, 2003). 1 ml enzyme extract was added in the cell first, then the reaction was started when 3 ml reaction solution (500 ml 0.01 mol/L pH 6.0 phosphate buffer + 0.28 ml guaiacol + 0.19ml 30% H_2O_2) was mixed in. The increment of 0.01 of A_{470nm} per min was defined as an activity unit (U); The soluble protein (SP) content was determined referring to the Coomassie brilliant blue G250 method (Xiong, 2003). The enzyme extract reacted with Coomassie brilliant blue G250 solution (0.1000 g Coomassie brilliant blue was dissolved in 50 ml 95% ethanol, then 100 ml 85% phosphoric acid was mixed in, afterwards distilled water was added to constant volume 1000 ml). The reaction product was detected; the maximum absorption peak at 595 nm.

The malondialdehyde (MDA) and the soluble sugar (SS) content were determined by spectrophotometry (Xiong, 2003): With 10% trichloroacetic acid (TCA) as the grinding solution, 0.6% thiobarbituric acid (TBA) as the chromogenic agent, then the reaction system (2 ml grinding extract + 2 ml TBA) was colored in 100°C water bath for 15 min. Quickly water-cooled, and detected using the multi-wavelength measurement process under the UV-VIS spectrophotometer. All the physiological indicators were repeated thrice.

Statistical analysis

One-way ANOVA with Fisher's LSD test and Pearson's linear correlation analysis were conducted using SPSS 16.0 statistical analysis software for windows (SPSS Inc., USA). Tabulation and mapping were performed using Microsoft Excel 2003.

RESULTS

Effects of decomposition of blue gum leaf litter on bolting and flowering of spinach

As shown in Table 1 and Figure 5, the bolting length of spinach was significantly elevated by leaf litter treatments (L30 to L90) ($p<0.01$). Especially in L60, the bolting length was about 2.3 times that in CK, while L120 did not promote the bolting length apparently. Each leaf litter treatment stimulated the rate of bloomed plants and the rachis length to a large extent ($p<0.01$), especially in L90, the rate of bloomed plants and the rachis length were approximately 6.5 and 6.2 times those in CK, respectively. Obviously, decomposition of the leaf litter accelerated the bolting and flowering of spinach, despite the fact that the stimulatory effect of L120 was relatively slighter due to its stronger inhibition on the early vegetative growth of spinach.

Effects of decomposition of blue gum leaf litter on leaf area and biomass of spinach

From Table 2, we can see that the leaf litter treatment caused inhibition in the leaf area of spinach, and the significance relied on the amount of leaf litter in the soil. The root dry weight fell with the increase of the leaf litter, significant differences were observed between CK and each leaf litter treatment ($p<0.05$ or $p<0.01$). Of all the four leaf litter treatments, only L120 inhibited the shoot dry weight remarkably ($p<0.05$), while L60 and L90 exerted no apparent effect on it ($p>0.05$), even L30 promoted the shoot dry weight considerably ($p<0.01$). The root shoot ratio (R/S) was reduced by each leaf litter treatment, especially in L30; the R/S ratio was only 40% of that in CK, probably due to its more vigorous reproductive growth in combination with Table 1.

Effects of decomposition of blue gum leaf litter on two soluble substances in spinach leaves

Soluble sugar (SS) content, soluble protein (SP) content and their ratio (SS/SP) are often used for bolting and organogenesis research (Liu et al., 2009; Dai et al., 2010). As shown in Figure 1, the SS content in spinach leaves was significantly higher than that in CK for the presence of the leaf litter (L30 to L120) ($p<0.01$), but the difference between random two leaf litter treatments was small ($p>0.05$). The SP content was observed rose in L30 relative to that in CK ($p<0.05$), but then went down as the leaf litter increased until L120, in which the SP content was significantly suppressed ($p<0.01$). The SS/SP ratio rose with the increase in the leaf litter (Figure 2), which was negatively correlated with the leaf area according to Pearson correlation analysis ($r=-0.9298$, $n=5$, $p<0.05$).

Table 1. Effects of decomposition of blue gum leaf litter on bolting and flowering of spinach.

Treatment	Bolting length (cm)	Rate of bloomed plants (%)	Rachis length (cm)
CK	20.14 ± 1.23^{Cd}	6.16 ± 1.97^{Cc}	2.20 ± 0.72^{Dd}
L_{30}	35.78 ± 2.80^{Bb}	22.73 ± 5.90^{Bb}	8.62 ± 1.14^{Bb}
L_{60}	46.50 ± 7.35^{Aa}	34.26 ± 9.37^{ABab}	12.61 ± 2.07^{Aa}
L_{90}	30.00 ± 5.64^{Bc}	40.08 ± 8.82^{Aa}	13.55 ± 0.91^{Aa}
L_{120}	19.48 ± 1.37^{Cd}	36.32 ± 9.60^{ABa}	6.31 ± 1.12^{Cc}

Different capital and small letters in the same column of the table indicated the difference between the two treatments was significant at $\alpha<0.01$ and $\alpha<0.05$ level, respectively.

Table 2. Effects of decomposition of blue gum leaf litter on leaf area and biomass of spinach.

Treatment	Biomass			Leaf area (cm^2)
	Dry weight of shoot (g)	Dry weight of root (g)	Root shoot ratio (R/S)	
CK	1.1095 ± 0.0488^{bCbc}	0.1855 ± 0.0163^{Aa}	0.168 ± 0.022^{aa}	9.06 ± 0.64^{Aa}
L_{30}	2.0893 ± 0.1344^{aa}	0.1387 ± 0.0309^{aBb}	0.066 ± 0.011^{bb}	6.48 ± 1.10^{Bb}
L_{60}	1.5370 ± 0.4415^{aBb}	0.1260 ± 0.0199^{bCb}	0.086 ± 0.028^{bb}	4.94 ± 0.71^{BCc}
L_{90}	1.0523 ± 0.1840^{bCc}	0.0750 ± 0.0157^{cDc}	0.088 ± 0.020^{bb}	5.18 ± 0.66^{bCbc}
L_{120}	0.4453 ± 0.0235^{cd}	0.0443 ± 0.0085^{Dc}	0.100 ± 0.025^{aBb}	4.15 ± 0.32^{Cc}

Different capital and small letters in the same column of the table indicated the difference between the two treatments was significant at $\alpha<0.01$ and $\alpha<0.05$ level, respectively.

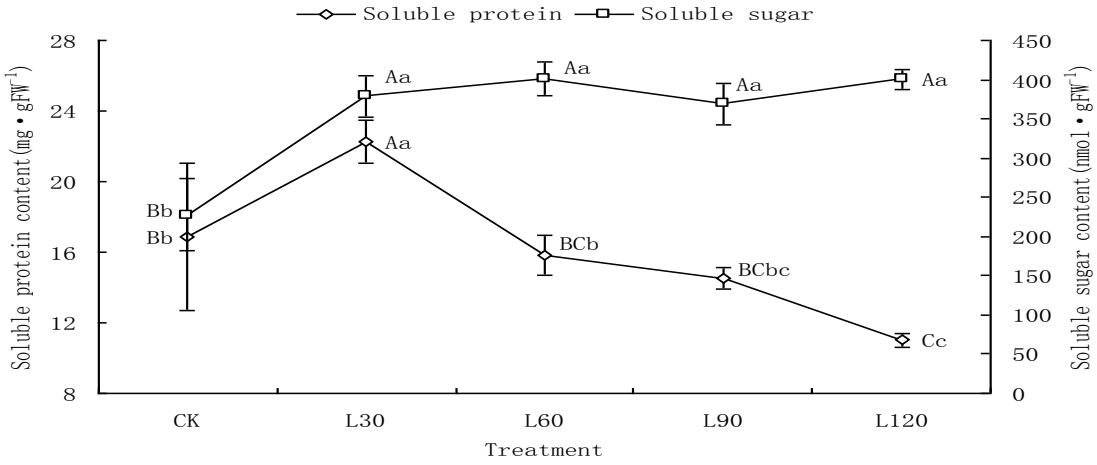

Figure 1. Effects of decomposition of blue gum leaf litter on soluble sugar and soluble protein in spinach leaves. Different capital and small letters on the same line of the Figure indicated the difference between two treatments was significant a t $\alpha<0.01$ and $\alpha<0.05$ level, respectively.

Some relationship might exist between the leaf growth and the changes of SS and SP based on the data stated previously.

Effects of decomposition of blue gum leaf litter on the lipid peroxidation in spinach leaves

Reactive oxygen species (ROS) were widely discussed as the trigger of senescence (Lin et al., 1984; Chang and Kao, 1998; Ruan et al., 2006), the excessive accumulation of which might aggravate the peroxidation of polyunsaturated fatty acids during senescence, resulting in the increase of malondialdehyde (MDA) that has been frequently used as a biomarker for lipid peroxidation. According to Figure 3, the lipid peroxidation determined using the MDA assay was obviously enhanced by leaf litter treatments except L90 ($p<0.05$), the maximum

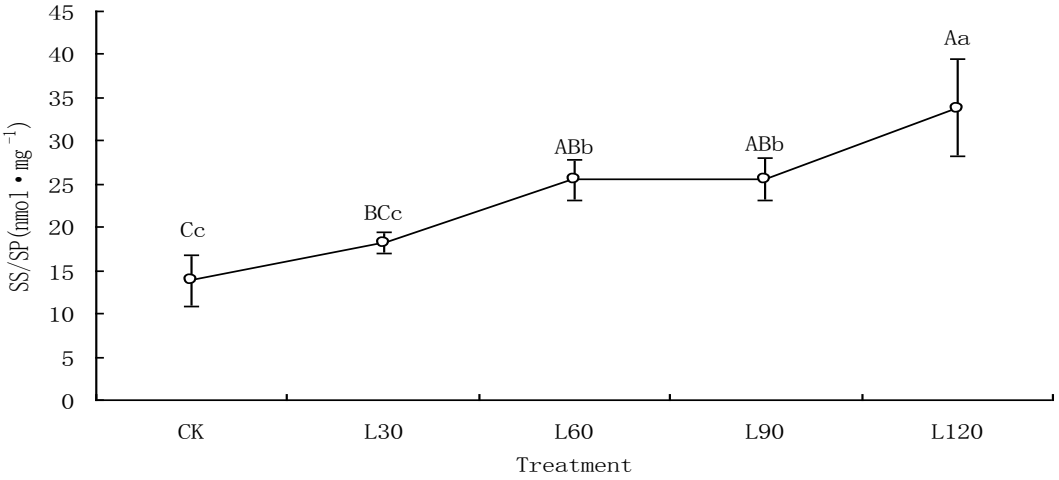

Figure 2. Effects of decomposition of blue gum leaf litter on SS/SP in spinach leaves. Different capital and small letters on the same line of the Figure indicated the difference between two treatments was significant a t $\alpha<0.01$ and $\alpha<0.05$ level, respectively.

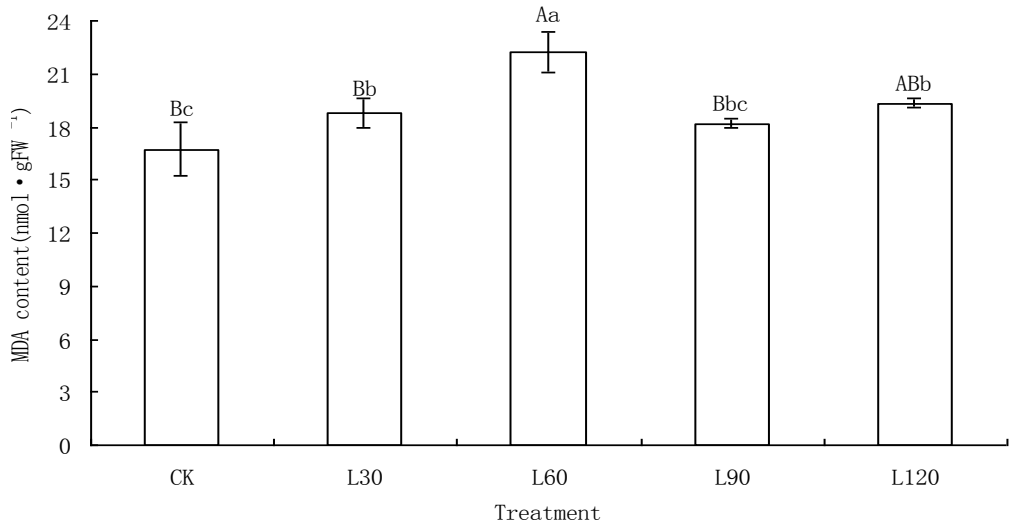

Figure 3. Effects of decomposition of blue gum leaf litter on MDA content in spinach leaves. Different capital and small letters on bars of the figure indicated the difference between random two treatments was at 0.01 and 0.05 level, respectively.

promotion occurred in L60. It was supposed that decomposition of the leaf litter in the soil would aggravate the ROS disorder in spinach leaves.

Effects of decomposition of blue gum leaf litter on three antioxidative enzymes in spinach leaves

Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are important members of ROS enzymatic removing system. It was shown in Figure 4 that the SOD activity was inhibited by four leaf litter treatments, but not significantly ($p>0.05$). The POD activity ascended first and descended then with the increase of the leaf litter, the peak value appeared in L90. The CAT activity was promoted by L30 ($p<0.05$), whereas inhibited by L60 to L120 ($p<0.01$). It seemed that three antioxidative enzymes above did not work well in controlling ROS in combination with Figure 3.

DISCUSSION

Environmental stresses have been commonly reported

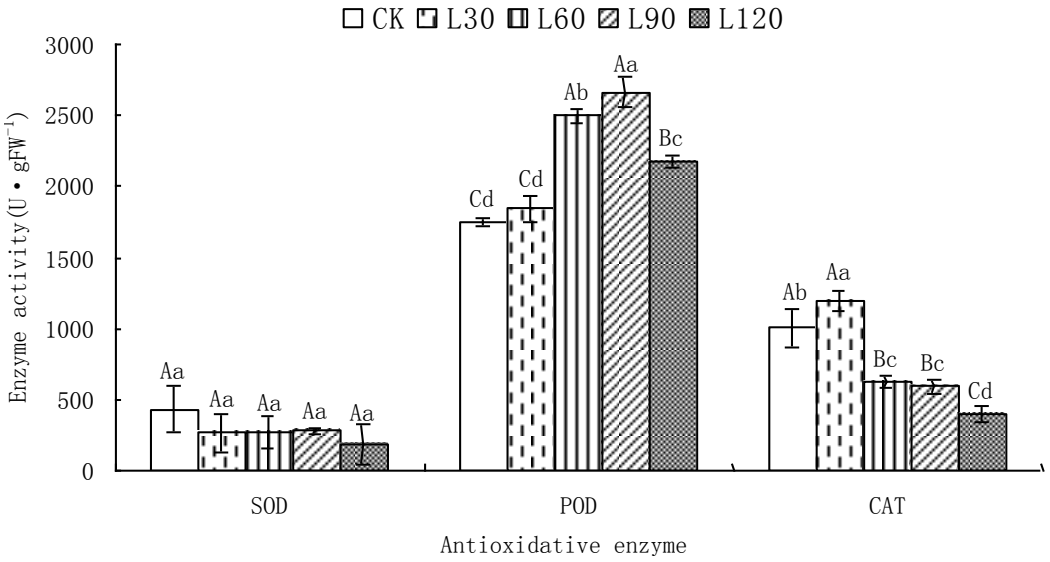

Figure 4. Effects of decomposition of blue gum leaf litter on the activity of antioxidative enzymes in spinach leaves. Different capital and small letters on bars of the figure indicated the difference between random two treatments was at 0.01 and 0.05 level, respectively.

to induce the premature senescence in plants (Irigoyen et al., 1992; Munné-Bosch and Alegre, 2004; Chen et al., 2012). However, the reproductive growth of the receptor has been rarely observed in the investigation of allelopathic effect. That might be something to do with the receptor selected for short-term bioassays. Because some receptors, *Cichorium intybus* for instance, is not easy to be observed flower in a short time as a perennial herb. Even though the distinct characteristics of leaf senescence, that is, chlorophyll breakdown and photosynthetic capacity compromise appeared more early due to the decomposition of *Eucalyptus grandis* leaf litter (Wu et al., 2012), its development was not paid enough attention to. While spinach, as a short-lifecycle herb, matures quickly during warm seasons resembling that in our experiment. So its reproductive growth was observed in our study, and it was visible that it got easier to bolt and bloom in the presence of blue gum leaf litter (L30 to L120) (Table 1 and Figure 5).

From the point of some scholars, leaf senescence benefits the survival of plants suffering stresses via the degradation of macromolecules for the mobilization and recycling of nutrients (Buchanan-Wollaston et al., 2003; Munné-Bosch and Alegre, 2004). Just as the result obtained in present study, the SS/SP ratio in spinach leaves rose with the increase in the leaf litter (Figure 2), and the leaf area was negatively correlated with the SS/SP ratio ($r=-0.9298$, $n=5$, $p<0.05$). This indicated that the protein degradation occurred more strongly in leaves of spinach treated with the leaf litter, which got weaker in vegetative growth. Those were somewhat in accordance with the C/N theory put forth by Klebs (1904) and Kraus and Kraybill (1918), that is, relatively higher carbohydrates to nitrogen compounds ratio (C/N) promotes the plant to turn from vegetative growth to reproductive growth, although it cannot explain the flowering of the short-day plants well according to following researches (Li, 2006).

What caused the alteration of the development of spinach in the presence of the leaf litter? We have carried out a parallel experiment using steamed eucalypt leaf litter, and proved to some extent that it was the chemicals steamed off rather than the change of soil nutrients or/and soil physical structure after application of leaf litter that should be responsible for the results (Wu et al., 2012). By using the gas chromatography - mass spectrometry (GC-MS), more than 100 compounds, including terpenoids, alcohols, esters, phenols, ketones, organic acids, alkaloids and their oxygenated derivatives were detected from blue gum leaf litter (unpublished data), and many of which were regarded as the potential allelochemicals (Rice, 1984). Nevertheless, since they were so various, and some unstable ones were probably transformed by soil microbes during leaf litter decomposition according to Kong et al. (2002), Jilani et al. (2008) and Etzerodt et al. (2008), identification of the contributing chemical(s) remains to be a very hard work.

In spite of the uncertainty of the functional chemicals, it is believed here that they affect the development of spinach via hormones referring to some published papers. Holappa and Blum (1991) showed that the ferulic acid (a commonly accepted allelochemical) stimulated the ABA content in *Cucumis sativa* and *Solanum lycopersicum* just 8 h after treatment. Chi et al. (2011) also pointed out that the juglone affected the root elongation of *Oryza sativa* via ABA, jasmonic acid (JA) and GA. Present data provided some evidence as well, although hormones in spinach leaves were not analyzed.

Figure 5. The appearance of spinach under CK and four leaf litter treatments.

POD was early reported participated in the synthesis of ethylene (ETH) and the oxidation of indole-3-acetic acid (IAA) (Yang, 1969; Beffa et al., 1990), regardless of its function in ROS scavenging. Considering that ETH enhances and IAA delays organ senescence, respectively (Picton et al., 1993; Kim et al., 2011), the stimulation observed in POD activity might indicate the stimulation in ETH level or the inhibition in IAA level in spinach leaves treated with the leaf litter (Figure 4), which was possible to explain the alteration of the development process of spinach.

Other signaling molecules, the ROS, were also reported the regulators that intermediated in the stress-induced senescence in some species (Irigoyen et al., 1992; Munné-Bosch and Alegre, 2004; Chen et al., 2012). Even Navabpour et al. (2003) proved that the expression of some senescence-associated genes was related to the increase of ROS. However, it was also believed that the increase of ROS during senescence was likely to occur after the initiation of protein and lipid degradation (Buchanan-Wollaston et al., 2003). Anyway, it is indeed possible for the allelochemicals to arouse ROS in plants based on many studies (Bais et al., 2003; Singh et al., 2006; Yang et al., 2011; Chi et al., 2011). So we argue here that the promotion in lipid peroxidation (Figure 3) was associated with the disorder of ROS in the presence of the leaf litter, which might subsequently affect the integrity of the membrane according to Antonacci et al. (2011). Despite that CAT has the potential to play a significant role in the cell defense against produced hydrogen peroxide as part of the macromolecules breakdown (Haddad et al., 2004), it did not perform well in the present paper as well as SOD (Figure 4). The increasing production of ROS was able to damage membranes, proteins, nucleic acids, etc. (Wink et al., 1998; Singh et al., 2006; Yang et al., 2011), which resulted in spinach senescence in physiology and appearance.

Conclusion

It is clear that decomposition of blue gum leaf litter caused the degradation of proteins, peroxidation of lipids, etc., in spinach leaves, and resulted in maturation and senescence of spinach in respect of bolting and flowering. The alteration of hormone levels or/and the disorder of ROS caused by decomposition products of blue gum leaf litter might be a primary explanation. For better understanding of the mechanism, it is necessary to carry out a further study on the dynamics of the hormones in spinach, as well as the identification of decomposition products of the leaf litter in the soil. Anyway, spinach is a leafy crop, the edibility of which was greatly influenced by application of blue gum leaf litter. So we suggest seeding spinach after scavenging eucalyptus leaf litter in spinach-eucalyptus systems.

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