ALLELOPATHIC EFFECTS OF LITTER LEACHATE ON SOYBEAN (*GLYCINE MAX*) SEED GERMINATION AND SEEDLING GROWTH UNDER DIFFERENT LIGHT INTENSITIES

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(Received 22nd Aug 2024; accepted 18th Mar 2025)

Abstract. Soybean (*Glycine max*) is a protein-rich crop that also fixes nitrogen and improves soil quality. In China, soybean is cultivated on a large area, however, productivity is not enough to meet local needs. The litter produced from soybean is incinerated which is a major cause of air pollution. Therefore, this study investigates the effects of shading and litter leachate (from *Salix* and *Zea mays*) on germination and growth traits of soybean. The study was comprised of different concentration of leachate of willow and corn stalk litter; control (water), C_1: 10 g/L, C_2: 20 g/L and C_3: 40 g/L and no shading (Li0: shading 0%), mild shading (Li1: shading 84.19%), moderate shading (Li2: 88.75%), and severe shading (Li3: 98.62%). The results indicated that increasing concentration of leachate produced from willow and corn stalk significantly inhibited the seed germination, germination and vitality index, root and shoot growth, and root and shoot water contents. Furthering increasing shading intensity also significantly decreased seed germination, germination and vitality index, root and shoot growth, and root and shoot water contents, chlorophyll synthesis and plant height. In conclusion, the leachate from the willow and corn stalk litter and increasing shading intensity significantly reduced the growth and germination of soybean.
Keywords: *allelopathy, germination, leachate, wild corn, soybean*

Introduction

Soybean (*Glycine max*) is an important oil and grain crops of China and it is used to make different daily life products such as; soybean oil, and soy sauce (Liu et al., 2020; Xu et al., 2020; Płonka et al., 2022).

Soybean is a short-day crop and it enters the light stage after one week of emergence (Płonka et al., 2022). Soybean requires limited short sunshine in the range of 9~18 h and shorter light period can better promote the development of reproductive organs. Opposite to longer growth period inhibit the development of reproductive organs and leading to better plant growth (Wang et al., 2022). The increase in shading intensity and reduction in light intensity reduces leaf growth, ambient temperature, photosynthetic traits and

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2305_94879501

carbon fixation leading to poor assimilates production and subsequent plant growth (Sales et al., 2023).

Plants also produce a significant amount of litter which affect the functioning of ecosystem (Giweta, 2020). Litter layer is one of the important functions of forest hydrological layer as it can reduce the impact rainfall on soil surface, and prevent soil erosion, runoff, slow down soil moisture evaporation (Giweta, 2020). At the same time, the litter also affects the growth and development of plants owing to fact it stores most of the products of plant photosynthesis and rich in nutrients (Wang et al., 2021). The litter has the effect of water conservation and reducing water evaporation, which can improve the growth environment of plants and promote plant growth (Wang et al., 2021). After the decomposition of dead litter, the nutrients return to the soil through the decomposition which increasing the content of soil organic matter, soil nutrient availability and subsequent plant growth and development (Xu et al., 2020; Wang et al., 2021).

Litter imposes variable impacts of plant growth, for instance Krishna and Mohan (2017) found the variable impacts of extracts made from litter of walnut and wheat. Likewise, Song et al. (2019) lower concentration of extract made from litter of small bean showed no obvious impacts on the growth, physiological response and nitrogen availability in soybean. Many other authors found that litter improves the water holding capacity, nutrient availability and growth of plants (Smith et al., 2021; Zheng et al., 2024). Litter also affects the seed germination through the joint action of physical and chemical factors (Eckstein et al., 2005). Some researchers documented that litter has toxic impacts on growth of plants (Bonanomi et al., 2016), while other found a significantly positive impacts of litter on growth and nutrient availability (Wang et al., 2021; Popa et al., 2022).

In recent years, with the increasing amount of litter, the application research of litter is deepening, and it is very important to study whether litter promotes the growth and development of plants. At the same time, as one of the important grains in China, the demand for soybean is increasing, so how to improve the soybean yield has become the key issue of research. There is knowledge gap concerning the impacts of light intensity and leachates from litter on the germination and seedling growth of soybean. Thus, this study was conducted with determine the comprehensive effects of litter and light intensity on seed germination and seedling growth and development process of soybean.

Materials and methods

Experimental materials

The soybean variety Jiyu 507 was used as test material and it has an average 100 seed weight of 20.5 g. The seeds of this variety are oval, with yellow seed skin surface shiny, umbilical yellow; and average plant height of 100 cm (Wang et al., 2017).

Experimental site

Indoor experiment

The study was conducted at the Geography Building 305 (north latitude 45°36’ N east longitude 122°48’ E) of Baicheng Normal University, Baicheng China. The seeds of soybean were soaked in water for 24 h and then 15 seeds were placed in each petri dish and divided into 7 groups with four replications. The solution of leachates was prepared from litters of *Salix* and *Zea mays.* The solution have following concentrations; control 10 g/L, 20 g/L and 40 g/L respectively was prepared. For preparing different

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concentrations of leachates, a 40 g/L of mother liquor was prepared. For which 200 g litters was taken and placed in 5000 ml for 24 h. Thereafter, this liquor was prepared and concentration of 10 and 20 g/L were prepared from the 40 g/L solution.

Different solutions were added into petri dishes and then placed in incubator (12 h light, 12 h dark, temperature 20°C, 25% humidity). The leachate solution was applied to petri dishes daily and after 11 days, seedling growth in petri dishes were removed to measure the root and shoot length and their fresh and dry biomass.

Outdoor experiment

Thirty seeds of soybean were spread into pots and divided into 28 groups having three replications. Thereafter, seeds were covered with 2 cm soil and water was applied. The shading treatment was divided into: no shading Li0 (shading 0%), mild shading Li1 (shading 84.19%), moderate shading Li2 (88.75%), and severe shading Li3 (98.62%). On day 21, the photosynthetic index was measured. For this a 0.1 g of leaves were homogenized by using 5 ml ethanol and absorbance was read to determine chlorophyll-a and chlorophyll-b concentration. Ten seedlings from each pot was taken and above and underground parts were removed and their lengths were measured. Later they were weighed to determine fresh weight and dried to determine dry weight.

Determination of indicators

Seed germination rate was determined as:

$$\text{Germination rate} = [\text{number of normal germination grains (within the end of germination)} / \text{number of supplied seeds}] \times 100 \tag{Eq.1}$$

The germination index was determined as:

$$\text{Germination index} = Gt/Dt \tag{Eq.2}$$

where: Gt is the total number of germination grains, and Dt is the corresponding days of germination.

The vitality index was calculated as:

$$\text{Vitality index} = \Sigma\ (Gt/Dt)\ Sx \tag{Eq.3}$$

Here Sx is the average length of the seedlings.

The seed germination potential (%) was calculated with following formula:

$$\text{Seed germination potential (\%)} = [\text{number of normal germination grains at the initial stage of germination (within the specified date)} / \text{number of tested seeds}] \times 100 \tag{Eq.4}$$

Moreover, root crown ratio was measured with following formula:

$$\text{Root crown ratio} = \text{root fresh weight (dry weight)} / \text{ground fresh weight (dry weight)} \tag{Eq.5}$$

Additionally, chlorophyll A content (mg/g) was measured as:

$$\text{Chlorophyll A content (mg/g)} = (12.7D_{665} - 2.69D_{649}) \times V / 1000\ W \tag{Eq.6}$$

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On the other hand, chlorophyll B content (mg/g) was measured as:

$$\text{Chlorophyll B content (mg/g)} = (22.88D_{649}\text{-}4.76D_{665})\ V / 1000\ W \qquad \text{(Eq.7)}$$

V is the extract volume, W is the leaf fresh weight.

Data analysis

The data was statistically analyzed by SPSS26.0 software (SPSS, Chicago, IL, USA). Before data analysis, the homogeneity of data variance was tested. The effects of shading intensity and leachates on growth and germination was determined by two-factor ANOVA, and the differences among different treatments were compared by Turkey's multiple comparison.

Results

Effect of different concentration of leachates on the germination process of soybean seeds

The germination rate of the control group was higher than that of all the experimental groups, and the concentration of the extract was C1 (10 g/L), C2 (20 g/L) and C3 (40 g/L) had lower germination rate than the control. The germination rate of soybean seeds cultivated with willow and corn stalk litter was lower than that of soybean grown with clear water, indicating that the extract inhibited the germination of soybean seeds

The germination potential of soybean seeds exposed to 10 g/L, 20 g/L and 30 g/L was lower as compared with the control group (*Fig. 1B*). The corn straw solution (10 g/L) significantly decreased the germination potential as compared to willow litter. The germination potential of soybean seeds under the influence of willow litter leaching solution increased weakly with the increase of concentration, indicating that the negative effect of the increasing concentration on seed germination was gradually reduced.

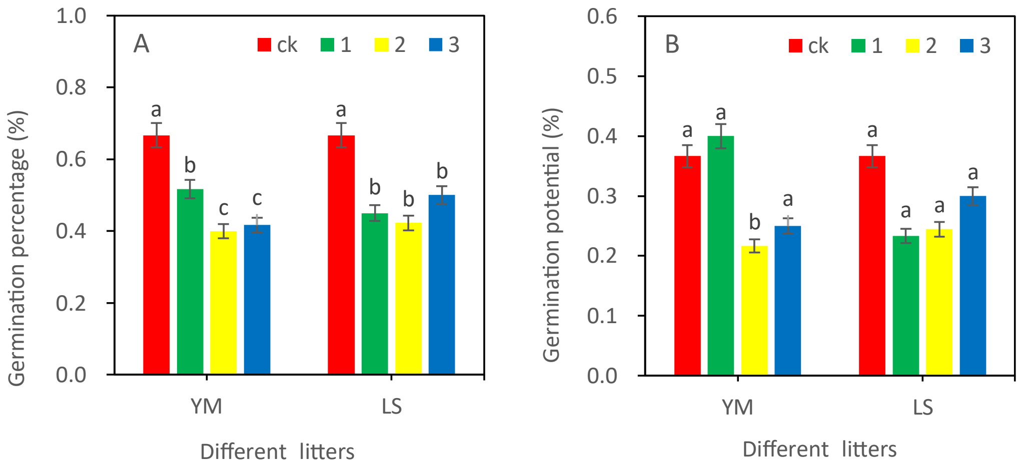

***Figure 1.** Effect of different litter concentration and light intensities on soybean germination rate and potential. Concentrations of CK, C1, C2 and C3 were 0 g/L, 10 g/L, 20 g/L and 40 g/L, respectively, and different lower-case letters indicate significant differences in soybean seed germination rate between different concentrations of the same litter extract (P < 0.05)*

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Effect of different litter solutions on the germination index and vitality index of soybean seeds

Different leachates concentration C1 (10 g/L), C2 (20 g/L) and C3 (40 g/L) reduced the germination index of soybean seeds as compared to control group. The inhibited impacts of leachates on germination index soybean seeds, increased with increasing concentration of willow and corn litter (*Fig. 2A*). However, when the concentration of the two litter was C3 (40 g/L), the germination index of soybean seed was slightly higher than C2 (20 g/L), and the concentration of willow litter was C3 (40 g/L), the germination index of soybean seed was higher than that of C1 (10 g/L) and C2 (20 g/L).

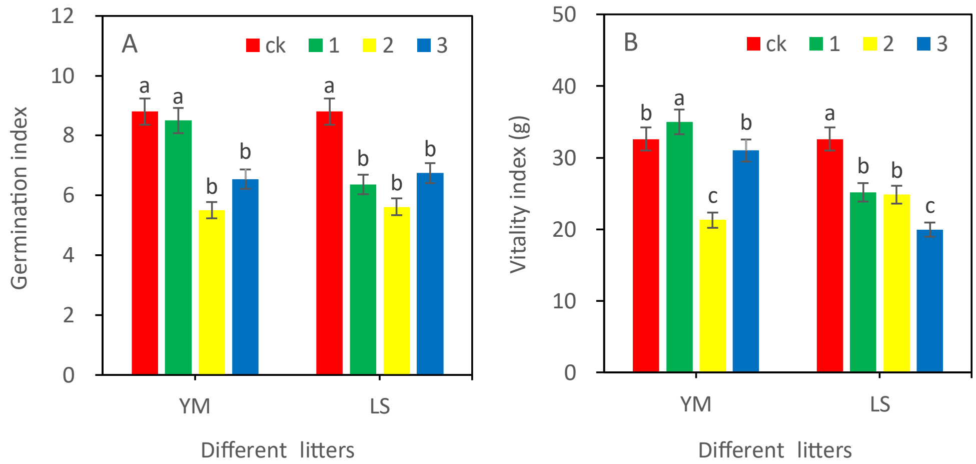

Figure 2. *Effect of different litter samples on soybean germination index and vitality index. Concentrations of CK, C1, C2 and C3 were 0 g/L, 10 g/L, 20 g/L and 40 g/L, respectively, and different lower-case letters indicate significant differences in soybean seed germination rate between different concentrations of the same litter extract (P < 0.05)*

The viability index of soybean seeds exposed to willow litter leaching solutions (10 g/L, 20 g/L and 30 g/L) was lower than that of the control group (*Fig. 2B*). The seed viability index of the extraction solution of corn straw at C1 (10 g/L) was higher than that of the control group, then the viability index decreased significantly with increasing concentration. The viability index of soybean seed increased with increasing concentration, indicating that the inhibitory effect of increasing concentration on seed viability gradually decreased.

Effects of different litter solutions on aboveground and underground length of soybean seed germination

The above-ground portion of soybean showed an increasing trend with the increase of the concentration of corn litter leaching solution. Under the treatment of willow litter, the above-ground length of soybean increased first and then decreased with the increase of the concentration of willow litter leaching solution (*Fig. 3*). Under the influence of different litter leaching solutions, the above-ground length of soybean had obvious changes. The underground length increased first and then decreased with the increase of the concentration of corn straw leaching solution. Under the treatment of willow litter, there was a trend of first increasing and then decreasing with the increase of the concentration of willow litter leaching solution.

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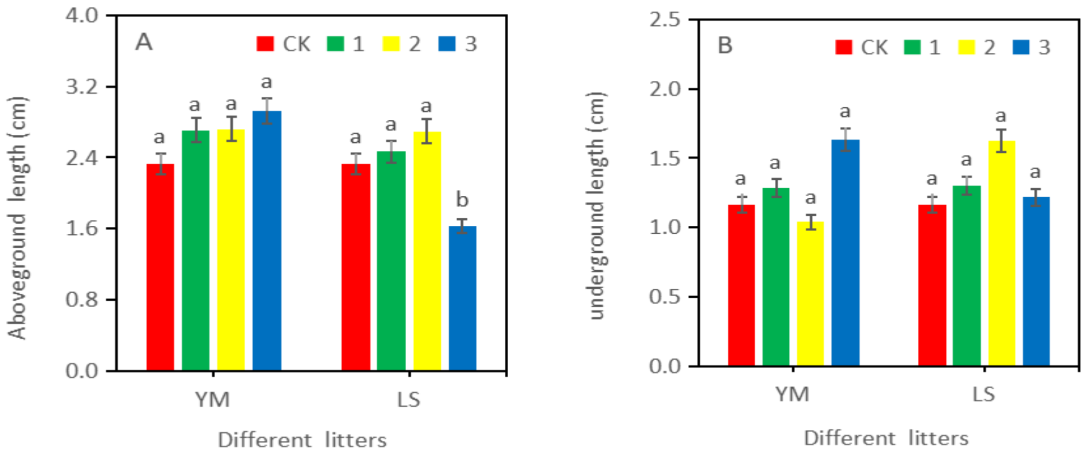

Figure 3. *Effects of different litter solutions on aboveground and underground length of soybean seed germination. Concentrations of CK, C1, C2 and C3 were 0 g/L, 10 g/L, 20 g/L and 40 g/L, respectively, and different lower-case letters indicate significant differences in soybean seed germination rate between (P < 0.05)*

Effects of different litter solutions on aboveground and underground water content of soybean seed germination

With the increase of the concentration of the two litter leaching solutions, the underground water content showed a weak trend of first increasing and then decreasing, and the trend was slightly obvious in the willow litter treatment group compared with the corn litter treatment group (*Fig. 4*).

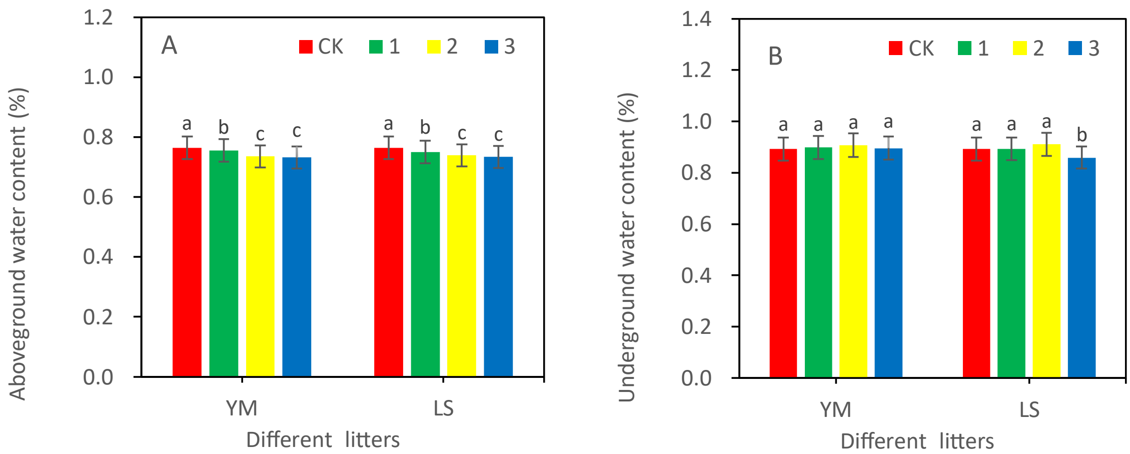

Figure 4. *Effects of different litter solutions on aboveground and underground water content of soybean seed germination. Concentrations of CK, C1, C2 and C3 were 0 g/L, 10 g/L, 20 g/L and 40 g/L, respectively, and different lower-case letters indicate significant differences in soybean seed germination rate between (P < 0.05)*

Effects of different litter solutions on fresh weight root shoot ratio of soybean seed germination

The effects of different litter on the germination root-shoot ratio of soybean were significantly different. Under corn litter treatment, the root shoot ratio of soybean

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seedlings decreased with increasing concentration, but under low concentration corn litter treatment, the root shoot ratio was greater than that in control group. Under the treatment of willow litter, the root shoot ratio showed a slight increase at first and then a decrease with the increase of the concentration (*Fig. 5*).

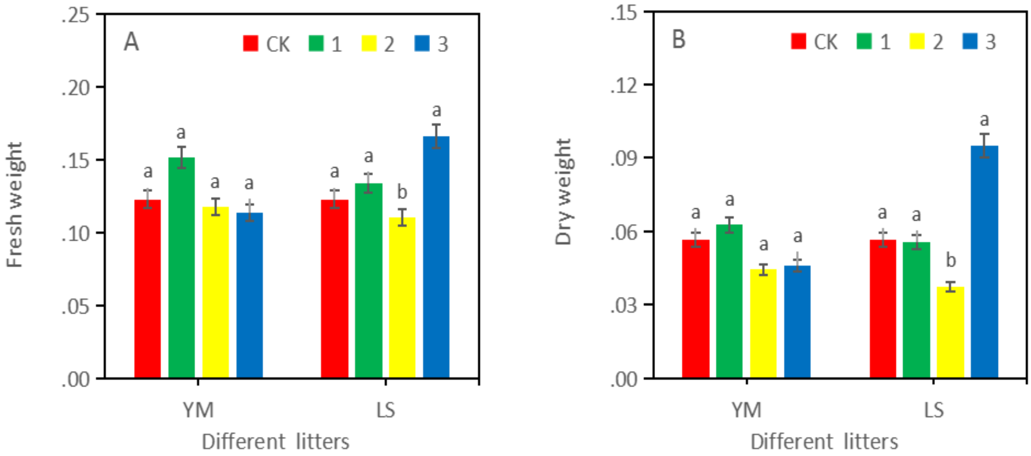

Figure 5. *Effects of different litter solutions on fresh weight and root shoot ratio of soybean seed germination. Concentrations of CK, C1, C2 and C3 were 0 g/L, 10 g/L, 20 g/L and 40 g/L, respectively, and different lower-case letters indicate significant differences in soybean seed germination rate between ($P < 0.05$)*

Effects of different litter extract concentration under different light intensity on soybean seedlings

However, with the decrease of light intensity, the above-ground length of soybean showed a downward trend (*Fig. 6*). Under the severe shading condition, the above-ground length of soybean was significantly different from the other two groups, indicating that the decrease of light intensity had an inhibitory effect on the above-ground length of soybean.

In the same light intensity background, the moderate shading and control group showed the increase in the root length of soybean with the increase of corn extract concentration. Heavy shading is the trend of first decrease before growth. Under the same light intensity background, except for the above-ground length of the heavily shaded soybean, the other three groups decreased with the increase of the concentration of willow litter leaching solution.

In corn straw leachate except 30 g/L, the underground length of soybean is lower than the control group, (*Fig. 7A*). Under the condition of the same concentration, the underground length of the control group decreased with the light intensity, and the difference between the other three groups was significant. There was no significant difference between the three groups: when the concentration of the willow leaching solution was 10 g/L, 20 g/L and 30 g/L.

Under the same light intensity background, the underground length of soybean increased first with the increase of the extract concentration of corn straw, indicating that the concentration of the extraction solution of corn straw at C1 (10 g/L) promoted the underground length of soybean, while the promoting effect decreased with the increase of concentration.

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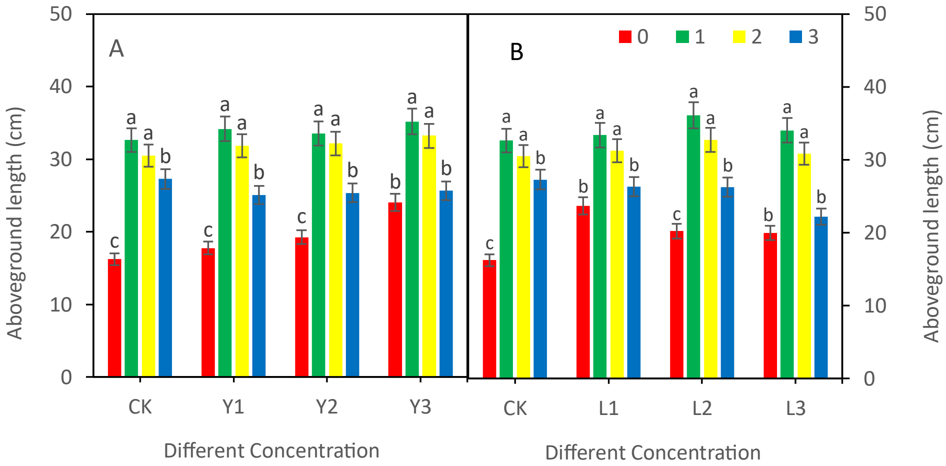

Figure 6. *Effect of different light and litter on the aboveground length of soybean seedlings. L, Y represent willow litter and corn litter respectively; CK, Y1, L1, L2, Y2, Y3, L3 concentrations are 0 g/L, 10 g/L, 20 g/L, 40 g/L; 0, 1, 2, 3, light shading, moderate light shading, heavy light shading in the same concentration under different light intensity conditions (P < 0.05)*

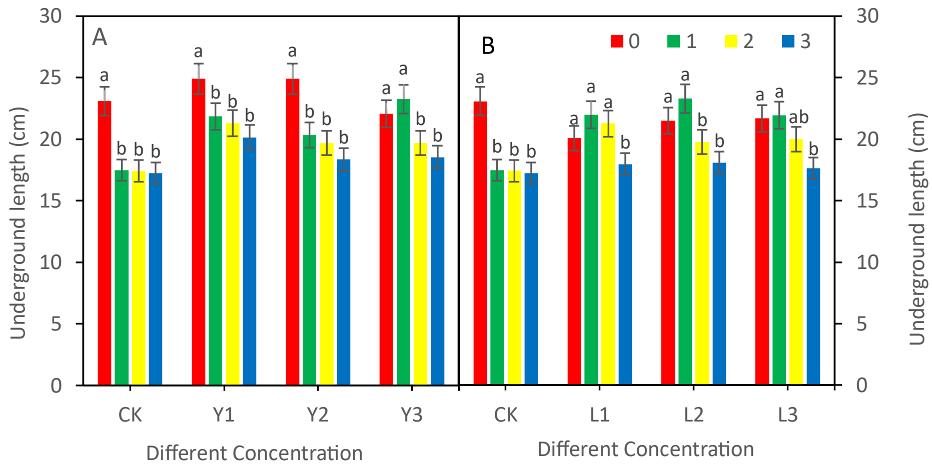

Figure 7. *Effect of different light and litter on underground length of soybean seedlings. L, Y represent willow litter and corn litter respectively; CK, Y1, L1, L2, Y2, Y3, L3 concentrations are 0 g/L, 10 g/L, 20 g/L, 40 g/L; 0, 1, 2, 3, light shading, moderate light shading, heavy light shading in the same concentration under different light intensity conditions (P < 0.05)*

The inductive effect of different litter extract under different light intensity on the crown ratio of fresh heavy root of soybean

When concentration of the extraction solution of the control group and the corn straw was 30 g/L, the ratio of fresh root to light decreased first (*Fig. 8*). When the concentration was 10 g/L, 20 g/L and 30 g/L, the difference between the group and the other three groups; the control group were significantly different from the other two groups, indicating that the weakening of light intensity mainly inhibited the ratio of soybean crop.

Under the same light intensity background, the medium shading of the control group is significantly different from other different concentrations. The control group was

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higher than other groups, the overall downward trend, indicating that the increase with the concentration of the medium shading, the soybean fresh heavy root crown ratio decreased, the crown ratio of soybean.

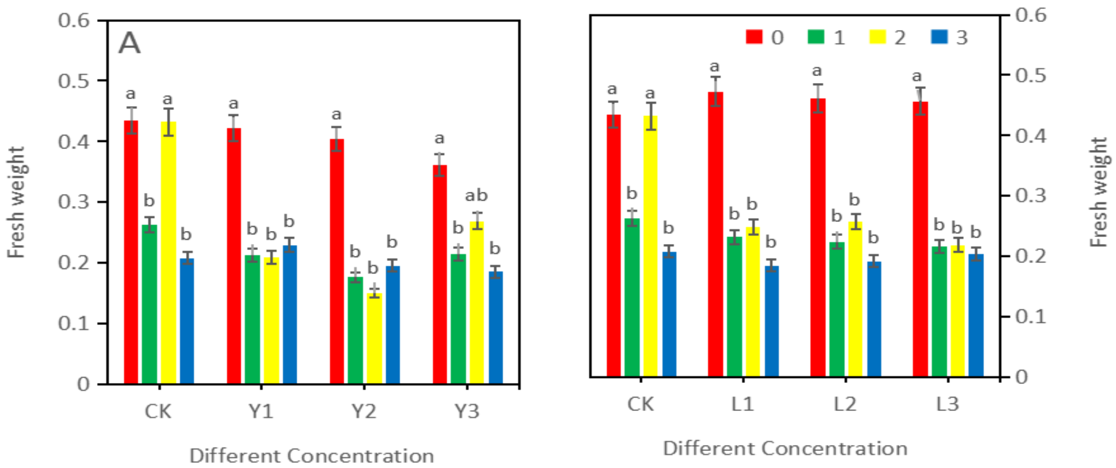

***Figure 8.** Effect of different light and litter on fresh heavy root crown ratio of soybean seedlings. L, Y represent willow litter and corn litter respectively; CK, Y1, L1, L2, Y2, Y3, L3. concentrations are 0 g/L, 10 g/L, 20 g/L, 40 g/L; 0, 1, 2, 3, light shading, moderate light shading, heavy light shading in the same concentration under different light intensity conditions ($P < 0.05$)*

The inductive effect of different light and litter on the ratio of dry weight root and crown of soybean

The dry weight root crown ratio of soybean with shading, or weakened light intensity, is lower than that of the control group, and the difference is significant, indicating that the weakened light intensity suppresses the dry weight root crown ratio of soybean (*Fig. 9*).

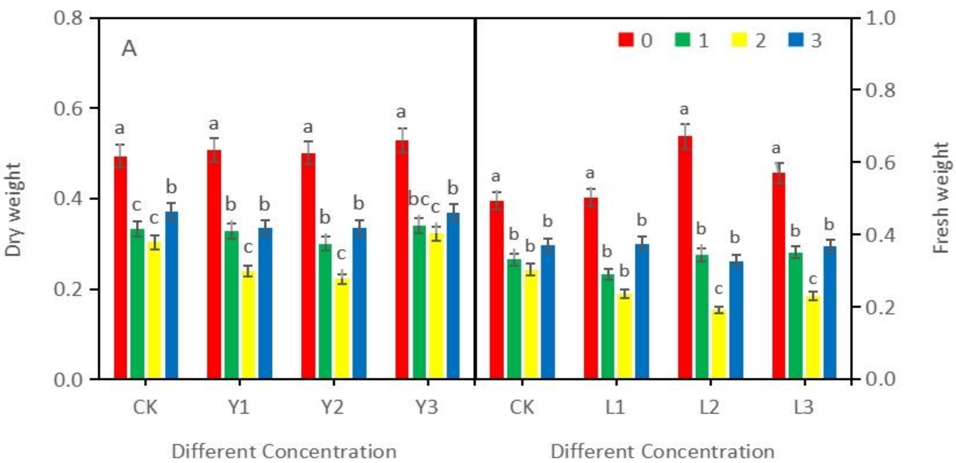

***Figure 9.** Effect of different light and litter on dry heavy root crown ratio of soybean seedlings. L, Y represent willow litter and corn litter respectively; CK, Y1, L1, L2, Y2, Y3, L3 concentrations are 0 g/L, 10 g/L, 20 g/L, 40 g/L; 0, 1, 2, 3, light shading, moderate light shading, heavy light shading in the same concentration under different light intensity conditions ($P < 0.05$)*

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Under the same light intensity background, the two extrusions had little effect on the crown ratio of the dry weight root of soybean, and the change of the soybean dry weight root crown ratio increased and decreased with the increase of the leach concentration.

The inductive effect of different lecontent on soybean water content

During severe light shading, the decrease of light intensity decreased soybean water content. Under the same light intensity background, the two leachates had little effect on soybean water content, and the change of soybean dry root crown ratio increased and decreased with the increase of leach concentration, and there was no significant difference, indicating that the two leachates had no obvious effect on the change of soybean water content (*Fig. 10*).

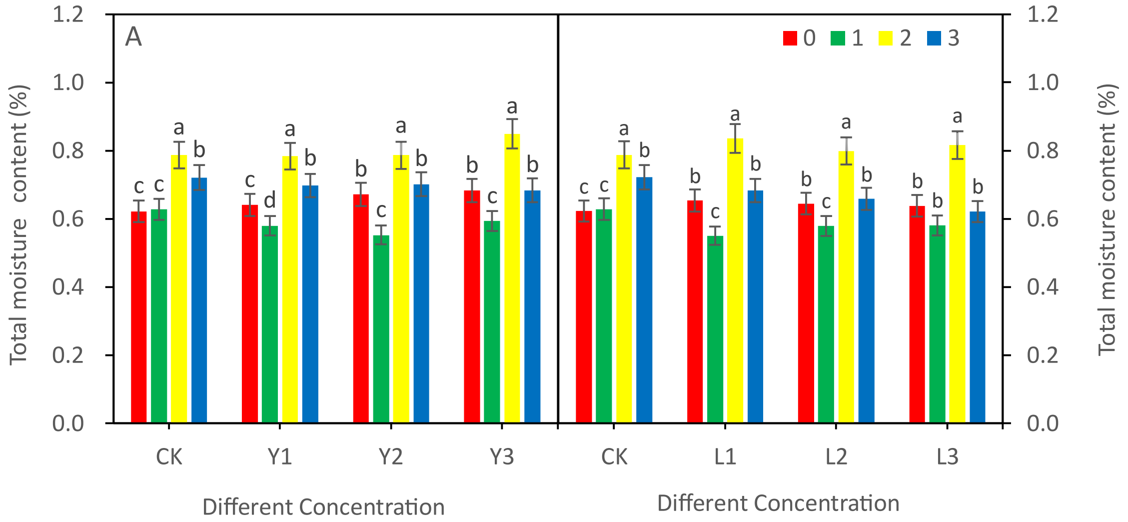

Figure 10. *Effect of different light and litter on water content of soybean seedlings. L, Y represent willow litter and corn litter respectively; CK, Y1, L1, L2, Y2, Y3, L3 concentrations are 0 g/L, 10 g/L, 20 g/L, 40 g/L; 0, 1, 2, 3, light shading, moderate light shading, heavy light shading in the same concentration under different light intensity conditions (P < 0.05)*

Effect of light and litter on chlorophyll a in soybean under different light intensity

When the concentration of the extraction solution was 10 g/L and 20 g/L, the concentration of the moderate light intensity proved beneficial to enhance of chlorophyll A content, but the enhancement of chlorophyll A content with the decrease of light intensity was weakened (*Fig. 11*).

Under the same light intensity background, the content of chlorophyll A increased with the increase of the extract concentration of corn straw.

The effect of leextract on chlorophyll B in soybean under different light intensity

Under the same light intensity background, with increasing concentration, there was no significant difference in the content of chlorophyll B in no and severe shading group, the highest when the concentration was 30 g/L, and the highest amount of chlorophyll B in the concentration was 10 g/L, indicating that the improvement of chlorophyll B content was enhanced (*Fig. 12*).

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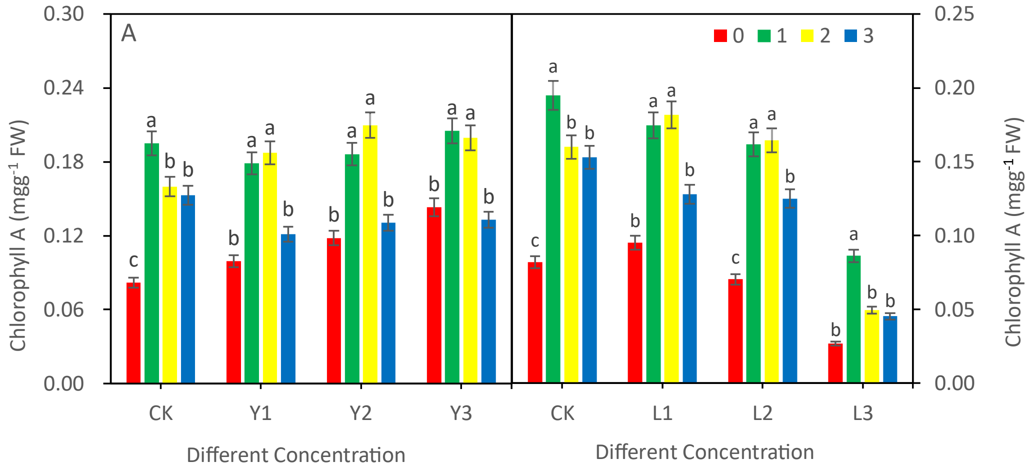

***Figure 11.** Effect of different light and litter on chlorophyll A in soybean seedlings. L, Y represent willow litter and corn litter respectively; CK, Y1, L1, L2, Y2, Y3, L3 concentrations are 0 g/L, 10 g/L, 20 g/L, 40 g/L; 0, 1, 2, 3, light shading, moderate light shading, heavy light shading in the same concentration under different light intensity conditions (P < 0.05)*

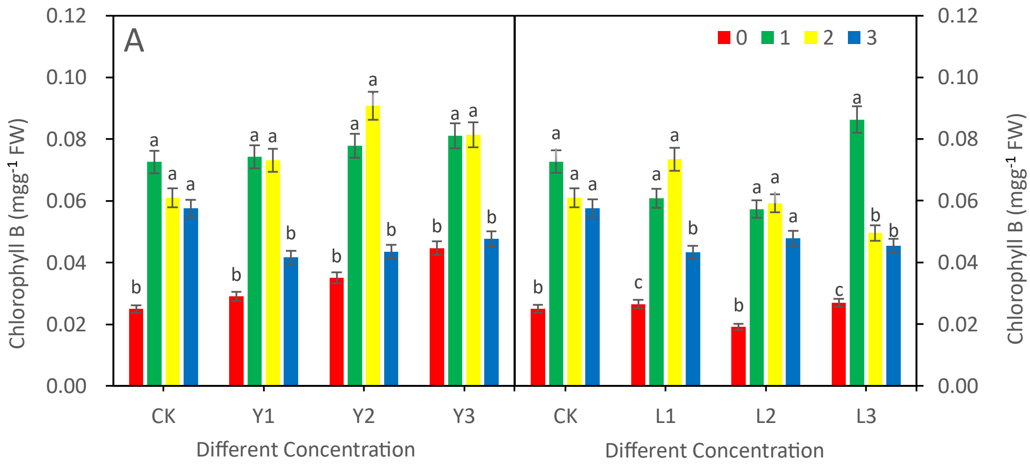

***Figure 12.** Effect of different light and litter on chlorophyll B in soybean seedlings. L, Y represent willow litter and corn litter respectively; CK, Y1, L1, L2, Y2, Y3, L3 concentrations are 0 g/L, 10 g/L, 20 g/L, 40 g/L; 0, 1, 2, 3, light shading, moderate light shading, heavy light shading in the same concentration under different light intensity conditions (P < 0.05)*

Discussion

Effect of different litter leachate extracts on soybean seed germination

In this study, two kinds of litter species (willow and maize) from common Plants in rural areas were selected to determine their impacts of soybean seed germination and seedling growth.

Germination potential and germination rate are one of the main indicators reflecting the good quality of seeds (Jiang et al., 2023). Low concentration of corn straw litter leach significantly increased the germination potential of soybean seeds. As the concentration of willow litter leach increased to the intermediate concentration, the germination potential was significantly reduced. Like, the germination rate, the germination trend

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rebounded to a certain extent when the concentration of the two litter leaching solutions reached the maximum. This phenomenon was significant in the corn straw litter leaching group, but not significant in the willow litter leaching group. The low concentration of corn straw leaching solution did not significantly inhibit the germination index, and the significant inhibitory effect was achieved at the medium concentration. Both the low and medium concentration of the willow litter immersion solution significantly reduced the germination index, and the high concentration of the leaching solution made the germination index increased slightly.

The corn straw leaching solution significantly improved the vitality index at the low concentration, because the plant itself has a certain stress resistance, and significantly reduced the vitality index in the medium concentration. Further, at the high concentration, the vitality index did not decrease significantly, relative to the medium concentration, but returned to the level of the control group.

Effects of different litter leachate extracts under different light intensity on soybean seedlings

Compared with the control group, under the condition of shading, the plant length was significantly increased, but with the increase of the number of shading, the increase is gradually decline (Schulz et al., 2019). This speculated that shade reduced soybean seedling leaf temperature, and make more pores available to obtain carbon dioxide in the air, increased photosynthesis resulting in growth promotion (Wang et al., 2021). After eliminating the low light conditions, the length of the ground was higher than that of the control group after the treatment of both litter extract. It is speculated that the components in the extract of corn and willow litter have a certain Promotion effect on the growth of soybean seedlings within a certain concentration range (Wang et al., 2021).

In corn stalk leaching group plant root length under the condition of each shading is significantly reduced. It is speculated that light shading inhibits the growth of the underground part of soybean seedlings. After eliminating the low light conditions, the underground length is the concentration of the control group. It is speculated that the components in the immersion solution of corn and willow litter have a certain effect on promoting the underground growth of soybean seedlings within a certain concentration range (Jiang et al., 2023).

The significant decrease in the ratio of fresh heavy roots with the number of shading layers compared to the control group indicates that the shading conditions inhibited plant root growth to varying degrees. The overall trend showed the maximum inhibition under severe shading. On the other hand, although the crown ratio of dry heavy root is similar to that of fresh heavy root crown ratio, there is one difference in that the inhibitory effect reaches the maximum under different concentrations of immersion solution. Compared with the control group, it can be seen that different degrees of shading are conducive to the absorption and utilization of water in soybean seedlings, because the shading conditions reduce the temperature of seedling leaves, inhibit their transpiration and then reduce the loss of water. With the increase of the concentration of the leachates solution, the fresh root crown ratio of soybean decreased, while the dry weight did not change significantly, and the leachates solution had a significant inhibitory effect on the fresh root crown ratio of soybean.

Under the same concentration of litter extract, the chlorophyll content of different light groups was significantly different. When the light intensity was reduced, it was conducive to the improvement of chlorophyll content. Under the same light intensity, the

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DOI: http://dx.doi.org/10.15666/aeer/2305_94879501

concentration of litter extract had different effects on chlorophyll. The overall effect of willow litter extract was inhibitory on chlorophyll a and promoted on chlorophyll b.

Conclusion

To sum-up lower concentration of corn stalk and willow litter favored the germination and growth of soybean, while higher concentrations showed inhibitory impacts on germination of soybean. Further, root length also increased with increasing of shading layers due to increase in photosynthesis, chlorophyll synthesis and transform and use more light energy. Looking forward, more molecular and in-depth studies are needed to explore the impacts of different leachates on the soybean germination, seedling growth and final productivity.

Fundings. National characteristic oil industry technology system for improving sunflower varieties (No.CARS-14-1-11). The research is supported by National Natural Science Foundation of China (No.31770520); Key Project of Science and Technology Research in the 13th Five-Year Plan of Education Department of Jilin Province (No.41 of Jijiao Kehe, 2016); Talents of Jilin Province Supported Project (2020040); Baicheng Science and Technology Development Plan Project (201920). Key R&D Project of Jilin Province Science and Technology Development Plan. Utilization of Stress-Resistant Indigenous Forage Grass Resources and Breeding of New Varieties (No. 20240303065NC).

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