

Effects of Rice Straw Steam-explosion Spent Liquor on the Growth of Cabbage Seedlings

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Abstract: The direct use of spent liquor from pulping and papermaking engineering, which is abundant in active organic matter, can avoid resource wastage and environmental pollution. In this study, the effects of soaking solution and filtrate from steam-exploded rice straw on seed germination and the early development of cabbage were investigated. The results have shown that soaking solution and filtrate stimulated early cabbage growth at low concentrations. Optimal germination potential and the rate of soaking solution-treated cabbage seeds increased by 8% and 5%, respectively; meanwhile, that of the filtrate-treated cabbage seeds increased by 11% and 5%, respectively. Promotion of root growth by steam-exploded rice straw spent liquor was expressed in lateral root multiplication. The fresh weight of cabbage seedlings treated with the soaking solution/filtrate increased by more than 50% and the dry weight over 20%. Comparatively, the stimulation of filtrate on seed germination and early development of cabbage is superior to that of the soaking solution. Steam-exploded rice straw spent liquor is rich in bioactive lignin and oligosaccharides, which makes it a promising biostimulant for promoting crop growth.

Keywords: rice straw; steam explosion; spent liquor; cabbage seedlings; stimulation

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1 Introduction

Pretreatment is an important biorefinery step for bioethanol production from lignocellulosic biomass, which aims to disintegrate the structure of plant cell walls and weaken lignin resistance ^[1]. Among pretreatment strategies, steam explosion is one of the most commonly used technologies for the separation of lignocellulose components, especially in gramineous plants ^[2–3]. It has the advantages of low cost, high pulping yield, and low chemical and energy consumptions. However, the spent liquor produced during the steam explosion process generally results in complex processing and may lead to environmental pollution. Spent liquor contains abundant lignin and oligosaccharides. Lignin is a renewable biomacromolecule comprising guaiacyl, syringyl, and *p*-hydroxyphenyl units. The three phenylpropane units are predominantly connected through ether and carbon-carbon bonds ^[4]. As an abundant aromatic resource in nature, lignin has excellent biological activity, biocompatibility, flexibility in structural modification, and amphipathy ^[5]. Oligosaccharides are often used as active components in biomedical engineering. The functional application of steam-explosion spent liquor is an important way to promote the high-value use of the organic matter.

Steam explosion is a procedure in which lignocellulose is treated using high-temperature steam, and hemicellulose is hydrolyzed under acidic conditions to produce soluble oligosaccharides, reducing sugars, and uronic acids. Meanwhile, lignin is partially degraded through the cleavage of aryl ether bonds, generating lignin fractions with low molecular weight and increased phenolic hydroxyl and carboxyl contents ^[6–7]. Lignin fractions combined with nitrogen-containing compounds, ash, and hemicellulose hydrolysate sugars can form humic acid-like substances. Humic acid has been demonstrated to possess high levels of physiological activity in promoting plant growth and development and maintaining soil fertility. With a similar molecular structure, lignin has received extensive attention in recent years as a plant growth regulator because of its desirable physiological activities in accelerating the

meristem of plant tissues, promoting plant photosynthesis, and enhancing stress resistance ^[8–9]. Savy et al ^[10–11] found that water-soluble lignin extracted from asparagus and silvergrass can promote corn seed germination and early seedling growth, and that the phenolic acid-type structure plays a key role. Xylooligosaccharides have been demonstrated to increase plant height, fresh weight, and main root length in tomato seedlings ^[12]. In view of the constituents in steam-explosion spent liquor, its direct use is expected to support high levels of bioactivity in promoting plant growth.

In this study, the effect of rice straw steam-explosion spent liquor on the growth of cabbage seedlings was investigated. The steam-exploded rice straw soaking solution and filtrate were used as active substrates. Seed generation and seedling growth were comparatively analyzed. We anticipate that the direct use of steam-explosion spent liquor will provide a high-value use method for these degraded compounds.

2 Experimental

2.1 Materials

Steam-exploded pulp and spent liquor of rice straw were provided by a paper mill in Hubei province. The steam-explosion conditions were as follows. The temperature was 180–207 °C, pressure was 1.0–1.7 MPa, and the explosion interval was 3.5–9.0 min. Cabbage seeds (*Brassica chinensis*) were purchased from the Nanjing Vegetable Seed Company.

2.2 Preparation of soaking solution and filtrate

The soaking solution was obtained as follows. The washed and air-dried steam-exploded pulp was ground using a Wiley mill, and particles that could go through 20 mesh sieves were collected. The resulting particles (40 g) were fully dispersed in 400 mL deionized water for 48 h at room temperature. The soaking solution was further diluted 5, 10, 20, and 40 times with deionized water, that is, the mass ratios of steam-exploded pulp to deionized water were 1/50, 1/100, 1/200, and 1/400, respectively.

The filtrate was obtained by directly extruding fresh

steam-exploded pulp of rice straw through a gauze bag. After centrifugation, the filtrate was diluted to the same concentration as the soaking solution.

2.3 Composition characterization

Rice straw and steam-exploded pulp were ground using a Wiley mill. Particles of 40–80 mesh were collected and then extracted with ethanol/benzene (1:2, *V/V*) for 48 h to obtain extractive-free samples. The chemical compositions of rice straw, steam-exploded pulp, soaking solution, and filtrate were measured according to the analytical method of the National Renewable Energy Laboratory (NREL) [13]. The hemicellulose content of the steam-exploded pulp was expressed as the pentosan content. The cellulose content was expressed as the holocellulose content after deducting the pentosan content. Holocellulose content was determined using the sodium chlorite method. The acid-soluble lignin content was determined by measuring the absorbance at 205 nm using a UV-vis spectrometer (TU-1810, Persee, Beijing, China).

Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of the filtrate were measured using the TAPPI test methods [14]. The soluble xylooligosaccharide content was analyzed according to the NREL protocol. The ash content was analyzed according to the national standard method [13]. For the filtrate, the COD and BOD contents were based on the filtrate volume (mg/L), and the ash, extractive, acid-soluble lignin, and xylooligosaccharide contents were based on the filtrate solid (%).

2.4 Germination of cabbage seeds

Absorbent cotton with a thickness of 3 mm was placed in each germination box (120 mm × 120 mm × 60 mm). Whatman filter paper was covered in each germination box, and the soaking solution or filtrate was added at concentrations of 1/50, 1/100, 1/200, and 1/400. The Whatman filter paper was fully immersed in a soaking solution or filtrate, and the soaking solution or filtrate was only added once. A germination box with no soaking solution or filtrate was used as the control (ck).

The cabbage seeds were soaked in distilled water

and the plump seeds were chosen by flotation. The seeds were further sterilized for 30 s with 75% ethanol with continuous shaking. The sterilized seeds were fully washed with sterile distilled water for further use.

Thirty seeds were placed in a uniform germination box. The cabbage seeds were then allowed to germinate in an incubator. The temperature was $(22 \pm 2) ^\circ\text{C}$, and the relative humidity was $(75 \pm 5)\%$. There was no light in the first 3 d, after which the cabbage seeds were incubated under light for 16 h at a light intensity of 5500 LX. The germination rate and potential of cabbage seeds were calculated using the following equations:

$$\text{Germination rate} = G_n/T_n \times 100\% \quad (1)$$

$$\text{Germination potential} = LG_n/T_n \times 100\% \quad (2)$$

where G_n is the number of germinated seeds at the end of the experiment, T_n is the total number of test seeds, and LG_n is the maximum number of germinated seeds on a given day. The samples treated with the soaking solution were labeled as 1-1, 1-2, 1-3, 1-4, and ck. The samples treated with filtrate were labeled as 2-1, 2-2, 2-3, 2-4, and ck. All the measurements were performed in triplicate.

2.5 Characterization of cabbage seedlings

After two weeks of incubation, 10 cabbage seedlings were randomly harvested from each germination box. These fresh cabbage seedlings were washed with deionized water, and the surface water was immediately removed using absorbent paper. The fresh and dry weights and length of the seedling leaves and roots were recorded. The dry weights of the samples were measured by obtaining a constant weight at $75 ^\circ\text{C}$. Before this procedure, the seedlings were baked at $105 ^\circ\text{C}$ for 20 min to be de-enzymed.

3 Results and discussion

3.1 Chemical composition analysis

The chemical composition of rice straw before and after steam explosion is shown in Fig. 1. The content of hemicellulose and lignin in rice straw was 24.5% and 13.8% (both acid-soluble and Klason lignin), respectively, with the results similar to that in other typical

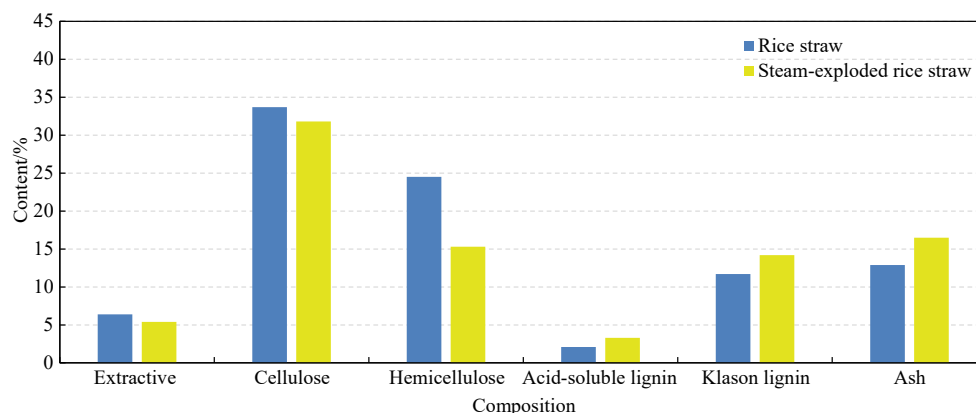


Fig. 1 Comparison of the chemical compositions of rice straw before and after steam explosion

Gramineae plants, such as wheat straw (22.9% and 17.4%)^[15] and switchgrass (26.1% and 17.3%)^[16]. Rice straw has a substantially higher ash content (12.9%) than softwood and hardwood. This was mainly attributed to the high silicon cell content of rice straw^[17].

After the steam explosion, a slight decrease was observed for cellulose in the steam-exploded pulp. The cellulose contents in rice straw and steam-exploded pulp were 33.7% and 31.8%, respectively. This showed that the steam explosion procedure had no pronounced effect on the cellulose structure. Comparatively, rice straw had a lower lignin content (13.8%) than steam-exploded straw (17.5%). This was mainly attributed to the degradation of hemicellulose, resulting in an increase in the relative lignin content. The hemicellulose content decreased from 24.5% to 15.3%. Generally, the acetyl groups of hemicellulose were easily converted to acetic acid under steam and high pressure, resulting in the degradation of hemicellulose to oligosaccharides that were easily dissolved in the spent liquor. Therefore, the oligosaccharide content was expected to be high in the filtrate. The cellulose and hemicellulose contents implied that steam explosion showed high selectivity for the degradation of pentosan. Given the cleavage of aryl ether bonds during steam explosion, the content of acid-soluble lignin with low molecular weight increased compared to that in rice straw. Preferential dissolution of lignin with a low molecular weight and structural condensation of lignin may also occur during this process, leading to a higher content of Klason lignin in the steam-exploded pulp (14.2%) than that in rice straw

(11.7%).

The main composition of the soaking solution and filtrate are shown in Fig. 2. As expected, the degradation of hemicellulose during steam explosion led to a high oligosaccharide content in the steam-exploded spent liquor. Xylan is the main pentose constituent of hemicellulose in rice straw, a typical gramineous plant. Therefore, the pentose in the soaking solution and filtrate was governed by xylooligosaccharides. The xylooligosaccharide content in the filtrate (41.6%) was substantially higher than that in the soaking solution (28.6%). This was mainly because of the milder reaction conditions of water extraction than those from steam explosion.

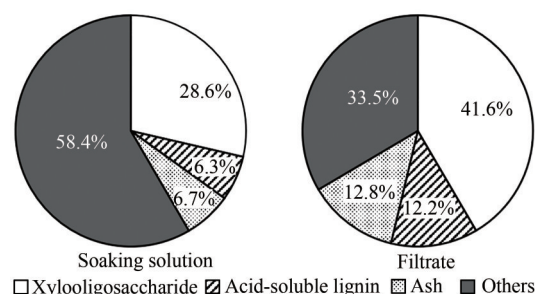


Fig. 2 Main chemical compositions of the soaking solution and steam-exploded rice straw filtrate

Degradation also occurred in lignin, and the acid-soluble lignin content was abundant in the filtrate (12.2%). Under stream and high pressure, the cleavage of β -aryl ether bonds induces an increase in water-soluble groups (phenolic hydroxyl and carboxyl) and a decrease in lignin molecular weight^[18]. Residual lignin with a high molecular weight was mainly

present in the steam-exploded rice straw. Therefore, only minor acid-soluble lignin was observed in the soaking solution.

COD and BOD values are important parameters for evaluating water pollution ^[19]. For steam-exploded rice straw filtrate, the COD and BOD content was 761.1 and 273.8 mg/L, respectively, with the COD/BOD ratio up to 2.78 (Fig. 3). The high COD/BOD ratio has shown that the steam-exploded rice straw filtrate contained large amounts of organic matter and is an important bioresource for subsequent chemical production and agricultural applications.

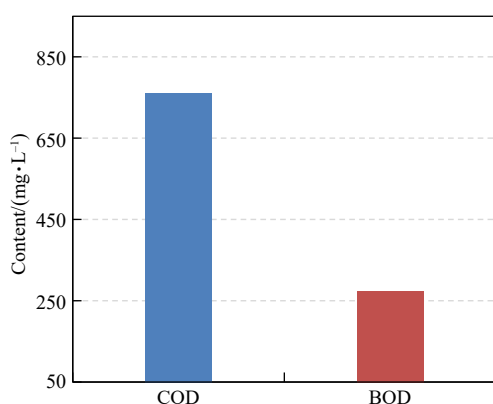


Fig. 3 COD and BOD of steam-exploded rice straw filtrate

3.2 Effect of steam-exploded spent liquor on germination of cabbage seeds

Generally, a high germination potential indicates strong seed vigor. The germination rate reflects the ability of seeds to germinate under suitable conditions ^[20]. Therefore, high germination potential

and high germination rate are desirable for plant growth and development. The effects of steam-exploded rice straw pulp soaking solution and filtrate at different concentrations on cabbage seed germination are shown in Fig. 4. After being soaked in the solution and filtrate, the germination potential of cabbage seeds was higher than that of the control seeds. The germination potential of cabbage seeds treated with the soaking solution was 2%–8% higher than that of the control samples, with an optimal mass ratio of 1/100. In contrast to the soaking solution treatment, cabbage seeds treated by filtrate displayed an optimal germination potential at a low concentration of 1/400.

Similar to the germination potential, the germination rate of cabbage seeds also increased after soaking in the solution and filtrate. This indicated that the spent liquor of steam-exploded rice straw was a promising biostimulant to promote crop growth. The germination rate of cabbage seeds treated with the soaking solution increased at the experimental concentrations (1/400–1/50). Treatment with the filtrate initially increased (0–1/400) and then decreased at higher concentrations (1/400–1/50). Comparatively, the cabbage seeds treated with filtrate showed the highest germination potential and germination rate compared to those treated with the soaking solution. This may be because there were more active substrates in the filtrate than in the soaking solution. Active oligosaccharides and lignin fractions are considered to be the main constituents that promote seed germination.

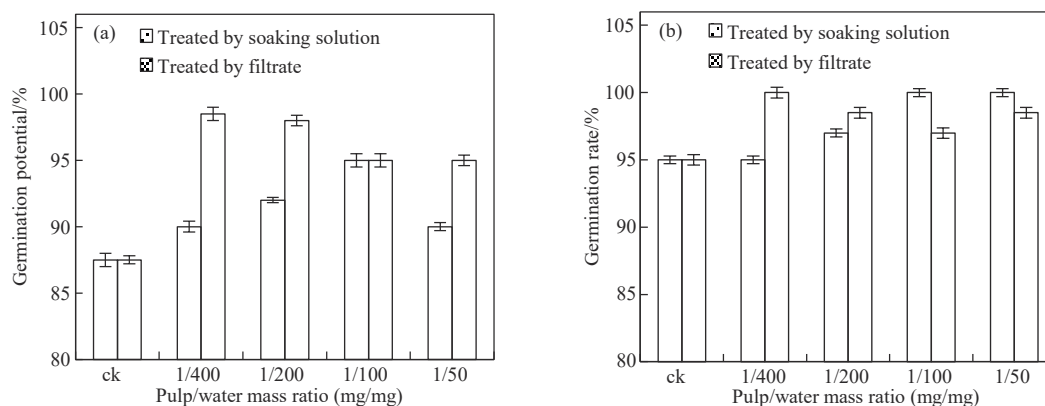


Fig. 4 Effect of steam-exploded rice straw pulp soaking solution and filtrate on cabbage seed germination

3.3 Effect of steam-exploded spent liquor on cabbage seedling length

Effects of steam-exploded rice straw soaking solution and filtrate on cabbage seedling length are shown in Fig. 5. Compared with the control seedlings, the plant length of the cabbage seedlings treated with the soaking solution increased. Plant length rapidly increased at low concentrations and then leveled off at high concentrations. Compared with the control sample, the plant length of

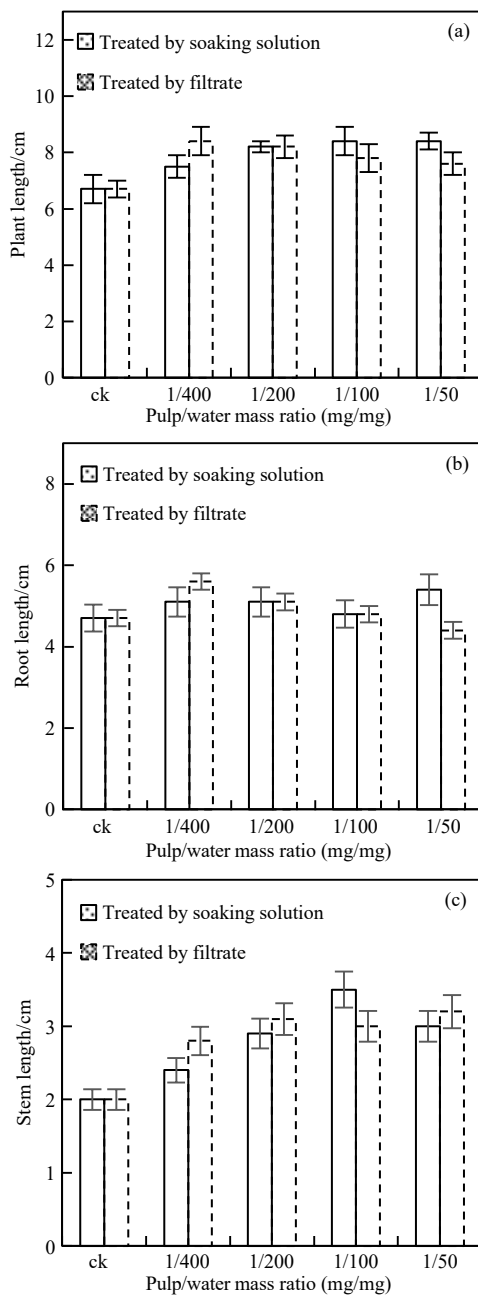


Fig. 5 Effect of steam-exploded rice straw soaking solution and filtrate on cabbage seedling length

cabbage seedlings treated with the soaking solution increased by 25% when the mass ratio was up to 1/100. The root length of cabbage seedlings treated with the soaking solution increased slightly by 4%–15%. The soaking solution mainly promoted increased stem length in the cabbage seedlings. For the filtrate treatment, the plant length of cabbage seedlings increased by 13%–25%. At low concentrations (0–1/400), the root length of the cabbage seedlings treated with the filtrate significantly increased. However, high concentrations had negative effects on the root length. As for other plant growth regulators, plant length was found to be dependent on the concentration of steam-exploded spent liquor.

Fig. 6 shows images of steam-exploded spent liquor-treated cabbage seedlings under different concentrations. After the steam-exploded spent liquor treatment, the plant diameter and length increased significantly. Stimulation of cabbage seedling stems and leaves by steam-exploded spent liquor was greater than that on cabbage seedling roots. The effect of steam-exploded spent liquor on the lateral root was more pronounced than that on the taproot. The promotion of cabbage seedling growth by steam-exploded spent liquor may be attributed to the active

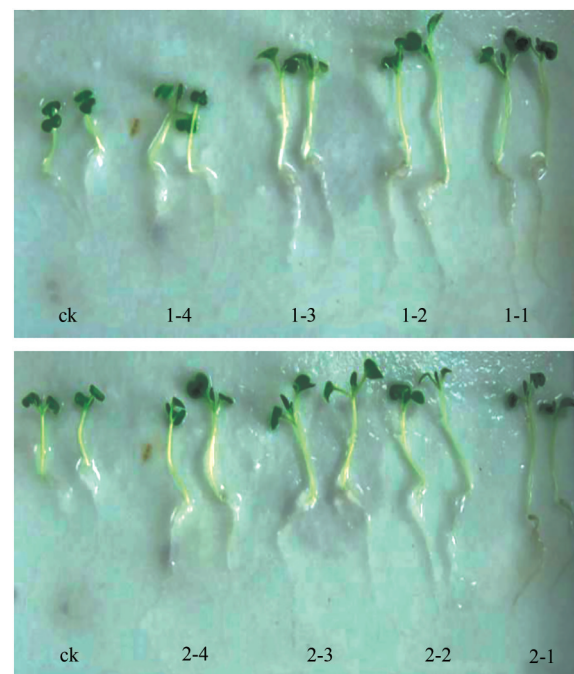


Fig. 6 Images showing the effect of steam-exploded rice straw soaking solution and filtrate on cabbage seedlings

lignin in the spent liquor. Savy et al.^[21] also found that the length of maize seedlings taproot, lateral root, and coleoptile increased after water-soluble lignin treatment by 8%, 40%, and 72%, respectively. The hydrophilicity and active groups (phenolic hydroxyl and carboxyl) of water-soluble lignin are considered to play a critical role, and we concluded that lignin with low molecular weight showed high bioactivity in promoting plant development and growth.

3.4 Effects of steam-exploded spent liquor on fresh and dry weights of cabbage seedlings

Phytomass and dry weight are often used to evaluate plant growth status and quality. The effect of steam-exploded rice straw soaking solution and filtrate on the fresh and dry weights of cabbage seedlings is shown in Fig. 7. Both fresh and dry weights of cabbage seedlings displayed a similar trend. They initially increased at

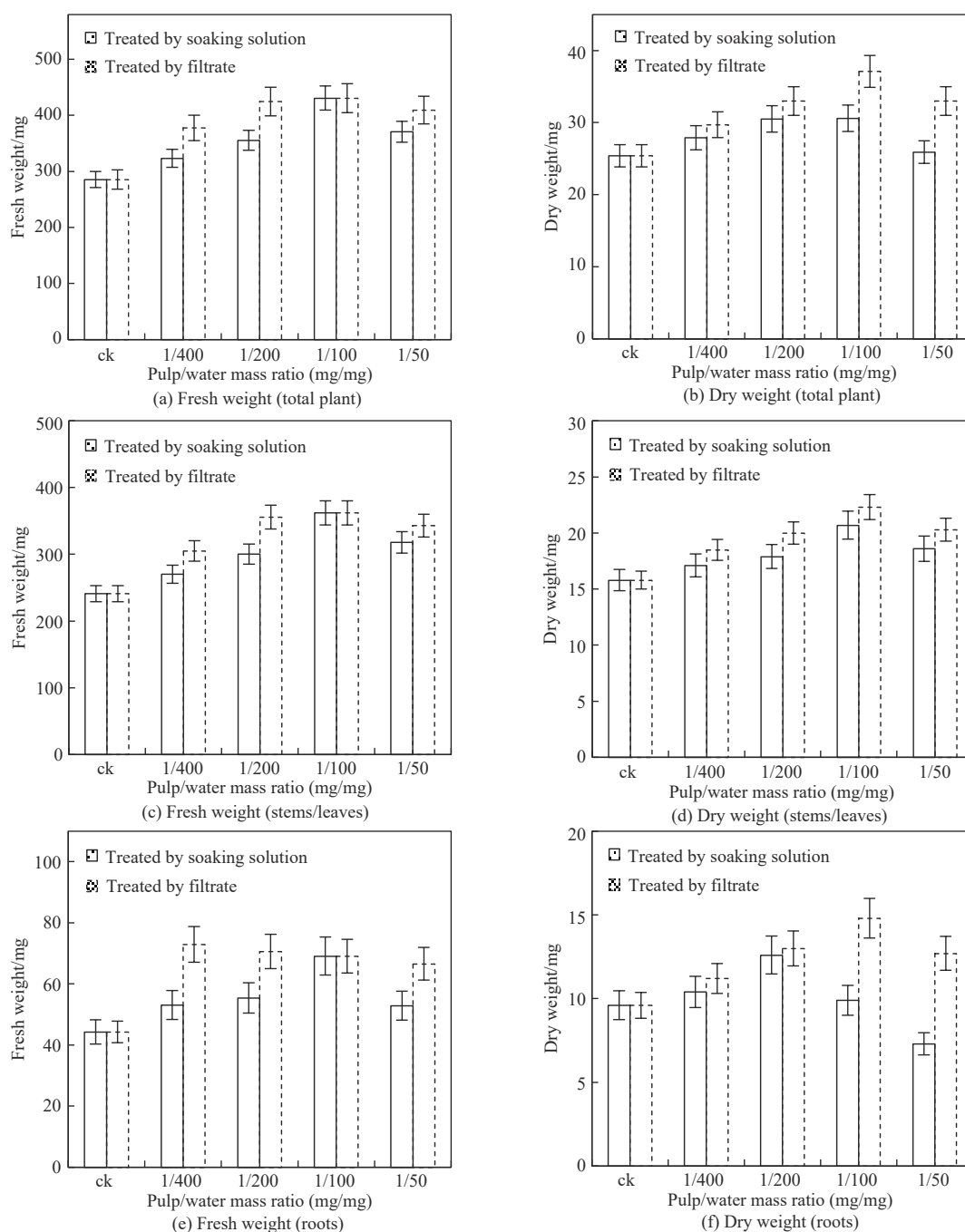


Fig. 7 Effect of steam-exploded rice straw soaking solution and filtrate on fresh and dry weights of cabbage seedlings

low concentrations (0–1/100) and decreased at high concentrations (1/50). The fresh weight of the stems/leaves, dry weight of the roots, and total fresh weight of cabbage seedlings treated with soaking solution and filtrate were higher than those of the control seedlings. The fresh weight of the roots and stems/leaves treated with the soaking solution increased at low concentrations and decreased at high concentrations. When the mass ratio was up to 1/100, the fresh weight of the roots and stems/leaves reached the maximum compared with the control samples, increasing by 56% and 50%, respectively. At other concentrations, the fresh weights of the roots and stems/leaves increased by 19%–25% and 12%–32%, respectively.

Comparatively, under the same concentration, the increase in fresh weight of roots and stems/leaves treated with filtrate was substantially higher than that treated with the soaking solution. The fresh weight of the roots and stems/leaves increased by 50%–65% and 27%–50%, respectively. Similar to the fresh weight of roots and stems/leaves treated with the soaking solution, the optimal concentration of filtrate for promoting cabbage seedling growth was 1/100.

The trend of dry weight was similar to that of fresh weight and increased at low concentrations and decreased at high concentrations. The dry weight of stems/leaves treated with soaking solution was up to the optimum when the mass ratio was 1/100, which was 31% higher than that of the control. The dry weights of roots, stems/leaves treated with filtrate increased by 54% and 41%, respectively. The effect of steam-exploded rice straw spent liquor on phytomass was similar to that of other plant growth regulators. This indicated that the steam-exploded rice straw spent liquor had high levels of physiological activity to regulate plant growth.

During steam explosion, phenolic acid compounds obtained from lignin have shown biological activity similar to that of gibberellins^[22]. The phenolic acid structure of lignin has a molecular skeleton similar to that of indole acids^[23]. Therefore, lignin in steam-exploded spent liquor showed high levels of physiological activity in promoting plant development and growth,

similar to other plant growth regulators and hormones. Within a certain concentration range, steam-exploded spent liquor can increase the germination potential and germination rate of cabbage seeds, increase the phytomass, and promote the growth of hypocotyl and lateral roots.

4 Conclusions

In this study, the effects of soaking solution and filtrate from steam-exploded rice straw on the germination and early development of cabbage were investigated. The soaking solution and filtrate stimulated early cabbage growth at low concentrations. This implies that steam-exploded rice straw spent liquor is a promising biostimulant for promoting crop growth.

4.1 During the steam-explosion treatment, a substantial amount of hemicellulose and a proportion of lignin were degraded, resulting in the accumulation of soluble organic matter with high levels of physiological activity.

4.2 The promotion of cabbage generation and growth by steam-exploded rice straw spent liquor predominantly involves improving the germination rate and germination potential. Generally, steam-exploded rice straw spent liquor with organic matter concentrations of 0.484 – 3.870 g/L significantly promoted the growth of cabbage plant length, roots, and phytomass.

4.3 Comparatively, the stimulation of filtrate on seed germination and early development of cabbage is considerably superior to that of the soaking solution. At the same concentration, the filtrate showed a more pronounced stimulation of cabbage growth and development than the soaking solution.

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