Pyrolysis of *Cunninghamia lanceolata* Waste to Produce Wood Vinegar and Its Effect on the Seeds Germination and Root Growth of Wheat

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As the by-product of biomass pyrolysis, wood vinegar (WV) possesses numerous beneficial properties and has been used in many fields. The properties and utilization of WVs are primarily influenced by the type of biomass feedstock and the production techniques. In this paper, WVs were pyrolyzed from fir sawdust waste at 350 to 650 °C to study their growth regulation effect on wheat seed and to investigate the underlying mechanisms. The highest yield of WV was at 450 °C with major components of phenols (37.92%) and acids (24.59%). The concentration of WVs has a major influence on regulation effect, which mainly affected the development of lateral roots. Compared with sterile water (CK), the WV-2 showed the highest seed germination rate and lateral roots growth, which increased nearly 65% and 92%, respectively. The lower concentration of WVs increased the roots vigor (RV) and promoted growth, while the higher concentration increased the content of malondialdehyde (MDA) and inhibited growth. The increased MDA indicated that wheat roots were suffering from oxidative stress. The findings revealed the suitability of WV as growth regulator in sustainable agriculture and also provided an efficient way for biomass waste utilization.

Keywords: Biomass waste; Pyrolysis; Wood vinegar; Seeds germination; Growth regulation

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INTRODUCTION

The full estimated potential of annual global biomass production from forestry and agriculture is 1.08×10^{11} tons, which is nearly 10 times the world's current energy need (Kan *et al*. 2016). The abundant reserves, renewability, and CO_2 neutrality have been the major driving forces for research into the application of biomass. Many countries have promoted renewable natural biomass resources to mitigate global environment issues and fulfill energy needs (Cong *et al*. 2018). Biomass can be converted into chemicals and energy by various thermochemical technologies such as pyrolysis, combustion, high-pressure liquefaction, and gasification. Biomass pyrolysis has a long application history. It is generally defined as thermal decomposition of biomass organic matrix under anaerobic conditions leading to prepare biochar, wood vinegar (pyroligneous acid), and gases (Orgun and Yildiz 2015; Tripathi *et al*. 2016).

Wood vinegar (WV), a recycled natural product, as one of the main products of biomass pyrolysis, has been studied extensively in relation to chemical properties and energy values. WV is a free flowing organic liquid mixture consisting of water and more than 200 chemical compounds, such as acids, phenols, alcohols, aldehydes, ketones, and esters (Creepier *et al.* 2018). The composition of WV depends on the feedstock type (biomass type, particle size), pyrolysis conditions (temperature, heating rate, pressure, residence time), reactor, and variables, such as catalysts. WV has numerous beneficial properties, and its utilization is closely related to its components.

Agriculture is one of the most important application fields of WVs. In organic agriculture, WVs have replaced many toxic chemicals to combat disease and pests, restore the soil environment, and serve as bacteriostatic agents. Mmojieje and Horning (2015) prepared WV from mixed wood biomass and investigated its pesticidal effect on the red spider mite and green peach aphid, finding that WV exhibited more than 90% mortality for both pests. Lashari *et al.* (2013) found that WV has a beneficial effect on leaching soluble salts and decreasing soil pH, resulting in the improvement of crop productivity in saline soils. Jung *et al.* (2007) showed that lower concentrations of WV inhibited *Alternaria mali*, the cause of *Alternaria* blotch of apple, and speculated that furaldehydes and phenols in WV were responsible for its antifungal activity.

Allelochemicals such as polyphenols and phenolic acids extracted from wetland plants have chemical compositions similar to WVs (Temiz *et al.* 2013), and they have been used successfully to regulate seed germination and plant growth (Guo *et al.* 2017). Many studies have demonstrated similar effects of WV on regulating plant growth in sustainable agriculture. Pan *et al.* (2010) found that WV had significant effects on germination and growth of crop seeds, which varied with the concentration of WV, preparation conditions and seed types. Xu *et al.* (2015) found that different concentrations of bamboo vinegar inhibits the seed germination and seedling growth of tobacco, but spraying 100 to 400 times bamboo vinegar solution on leaves promotes growth and improves the quality. Wood vinegar exhibits a regulatory effect on seed germination and plant growth, but the effect is dependent on the WV properties, concentration, and plant species. At present, there are few studies on WV regulation of wheat seed germination and root growth. As the world's largest grain crop with the largest sown area and the widest distribution, wheat plays an important role in agricultural production and food supply. Therefore, it is important to study the regulation of wood vinegar on wheat seed germination and growth. The aims of this study were 1) to characterize the properties of WVs prepared by pyrolysis of fir sawdust, a waste material of wood processing industry; 2) to investigate the effects of WVs on wheat seed germination and root growth; and 3) to illustrate the potential mechanism. These findings will inspire the use of WV-derived plant growth regulators and efficient ways for the utilization of waste biomass resources.

EXPERIMENTAL

Material Preparation

Cunninghamia Ianceolata (Lamb.) Hook. waste (CLW) was collected from Jiangxi, China, and smashed into small pieces (0.5 to 2.0 mm). The WV was prepared by slow pyrolysis; 20 g of CLW was pyrolyzed at 350 to 650 °C for 30 min using a vacuum-tube-furnace (VTL 1200, Nanjing, China) with the heating rate of 10 °C/min under the N_2 flow of 100 mL/min. The WV was obtained at 350, 450, 550, 650 °C from sediment *via* a condenser filled with water-ice-salt mixture, and remarked as WV-350, WV-450, WV-550, and WV-650. Three parallel experiments were performed for each pyrolysis condition, and the yield of WV was the weight ratio of WV to raw material. The WVs were stored at 4 °C in dark.

The density of WV was measured using an optical density meter, and moisture content was measured using a moisture analyzer (ZSD-2J, Anting, China). The chemical composition was characterized using GC-MS (7890A-5975C, Agilent, Palo Alto, USA). The following conditions were used for GC analysis: initial temperature 50 °C for 2 min, ramped at 5 °C/min to 280 °C and held for 20 min, a split ratio of 100:1, and injected sample of 0.2 μL. The MS analysis was obtained under 230 °C at 70 eV. The relative contents of compounds were calculated by corresponding peak areas (Wu *et al.* 2015).

Regulatory Effect Test of WV

The wheat seeds were sterilized using 75% ethyl alcohol solution and washed with sterile water 2 to 3 times before the test. Next, 1 mL of WV, obtained under 450 °C, was diluted in sterile water to the desired concentration of 0.33, 0.50, 0.67, 0.80, 1.00, 1.33, and 2.00 mL/L, and marked as WV-1, WV-2, WV-3, WV-4, WV-5, WV-6, and WV-7; sterile water without WV was denoted as sample CK. The culture experiments were carried out under a light/dark cycle of 12/12 h at 28 ± 1 °C for 7 days in an intelligent incubator (KBWF-720, Binder, Germany). For every culture experiment, 30 wheat seeds were placed on a watch-glass (d=9 cm) with two filter papers, and different concentrations of WV solution were added under sterile conditions with 4 conducted repetitions.

Morphological and Physiological Characterization

The characteristics analyzed in this study were seed germination potential, seed germination rate, relative seed germination rate, seed germination index, main root length, lateral root number, total fresh root mass, root uniformity, root vigor (RV), and malondialdehyde content (MDA), as analyzed following Liu *et al.* (2008).

Statistical Analysis

The culture data were analyzed using analysis of variance (ANOVA), and the results were expressed as the mean values of three repetitions with standard deviation. Different small letters in the tables and figures indicate a significant difference among the various parameters with different concentration WV treatments. The correlation was analyzed using a Pearson test at $P < 0.05$.

RESULTS AND DISCUSSION

Properties of WV

CLW is a tropical biomass consisting of cellulose, lignin, and hemicellulose content of 47.40%, 32.94%, and 10.65%, respectively. Table 1 shows the properties of WVs prepared under different pyrolysis temperatures. With increasing temperature from 350 to 650 °C, the yield of WVs increased from 47.9% to 57.1%, and then it decreased to 51.8%. The results indicated that increasing temperature helped to decompose and precipitate a large amount of volatile gas, which lead to increasing yield. However, under higher temperature (550 or 650 °C), the secondary cracking of volatiles produced small molecular mass molecules to form non-condensable gases, which reduced the yield of volatiles. Furthermore, the moisture content was 54.1 to 57.8%, the pH decreased from 3.47 to 2.49 with increasing temperature, but the density remained stable at 1.010 to 1.025

g/mL, similar to the density of 1.00 to 1.04 g/mL from giant seed (Zhang *et al.* 2018).

Table 1. Physical Properties of WVs from CLW

Sample	Yield (%)	Moisture Content (%)	pH	Density (g/mL)
WV-350	47.9	57.8	3.47	1.025
WV-450	57.1	54.1	3.11	1.013
WV-550	53.9	55.3	2.83	1.010
WV-650	51.8	55.3	2.79	1.008

The main organic components contained in WVs are given in Fig. 1 as acids, phenols, alcohols, kentones, aldehydes, and esters. Phenols and acids showed the higher relative content, followed by ketones, alcohols, aldehydes, and esters. With increased pyrolysis temperature, the content of acids increased and the content of alcohols decreased, while the content of phenols increased first and then decreased. The highest content of alcohols was 29.4% at 350 °C, phenols was 37.9% at 450 °C, and acids was 23.0% at 650 °C. Regardless of the prolysis temperature, the content of ketones, aldehydes, and esters remained steady. Under different prolysis temperature, the degree of decomposition of cellulose, hemicellulose, and lignin were different. Hemicellulose decomposes at 100 to 260 °C, cellulose at 240 to 350 °C, and lignin at 280 to 500 °C (Liu *et al.* 2017). In the slow pyrolysis of biomass, acids are formed from hemicellulose and cellulose, which formed small molecule acids at low temperature and macromolecular acids at high temperature. Phenols were attributed to decomposition and depolymerization of lignin; the maximum rate of degradation was at 370 to 385 °C and continued above 550 °C (Brebu *et al.* 2013).

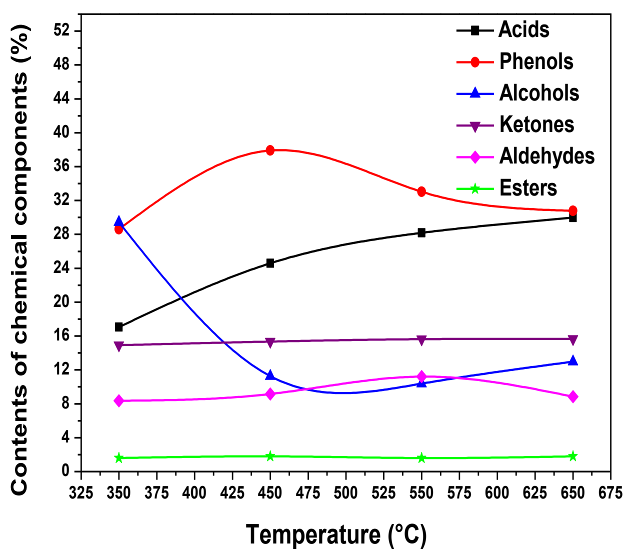


Fig. 1. Contents of organic components contained in WVs at different temperature

A total of 32 chemical components of WVs were identified (Table 2). The main components of acids were acetic acid and hexanoic acid. Acetic acid was the typical product of hemicelluloses pyrolysis under low temperature, while at high temperature long chain acids, such as pentanoic acid were the main component. The major components of phenols were methyl- and methoxy- phenols, which may be due to the pyrolysis of phenylpropane units that are cross-linked by various hydroxyl- and methoxy-structures in lignin. Acetone and cyclopentanedione were the primary compounds of ketones decomposed from hemicelluloses and cellulose. Glycan structures of hemicelluloses disassembled at low temperature produced ketones. For cellulose, the slow release of volatiles in pyrolysis was beneficial to dehydration and secondary reactions of oligosaccharides, which promoted the formation of acetone and cyclopentanedione (Custodis *et al.* 2015; Quan *et al.* 2016).

Table 2. Chemical Components of WV Prepared from CLW

Category	Compounds	Relative contents (%)[a]			
		350 °C	450 °C	550 °C	650 °C
Acids	Acetic acid	7.42	7.38	7.84	7.28
	Propanoic acid	2.30	2.40	2.56	2.67
	2-Methyl propanoic acid	1.42	1.37	1.46	1.43
	Butyric acid	2.37	—	—	—
	Pentanoic acid	—	10.02	16.31	14.09
	3-Methoxy-4-hydroxybenzoic acid	3.55	3.42	—	4.50
Phenols	Phenol	0.99	0.94	1.01	0.59
	2-Methyl phenol	1.09	1.30	1.34	1.31
	2- Methoxy phenol	5.20	6.22	5.82	4.62
	3-Methyl phenol	1.12	1.15	1.19	—
	4-Methyl-2-methoxy phenol	5.64	6.18	3.41	6.23
	1,2-Benzenediol	4.18	6.72	4.77	7.25
	4-Ethyl-2-methoxy phenol	4.23	4.89	4.82	5.24
	Eugenol	—	0.55	—	0.68
	4-Propenyl-2-methoxy phenol	1.76	1.86	1.85	2.22
	4-Propyl-2-methoxy phenol	4.09	7.29	7.89	1.57
	4-Hydroxyalkyl-2-methoxy phenol	—	0.82	0.92	1.06
Alcohols	2-Furfuryl alcohol	1.62	1.59	1.71	2.32
	4-Hydroxy-3-methoxy phenethy alcohol	27.83	9.69	8.67	10.65
Ketones	Acetone	3.30	3.43	3.47	3.53
	2-Furanone	2.45	2.50	2.62	2.51
	1,2-Cyclopentanedione	4.01	4.23	4.31	4.14
	3-Methyl-1,2-cyclopentanedione	3.80	3.80	3.81	3.85
	4-Hydroxy-3-methoxy hypnone	1.36	1.39	1.42	1.62
Aldehydes	Furfural	2.59	2.75	2.75	2.78
	5-Methyl-2-furfural	0.65	0.70	0.66	0.73
	2-Ethyl hexanal	3.53	3.30	6.14	3.49
	5-Hydroxymethyl furfural	—	0.88	—	—
	Vanilline	1.58	1.53	1.65	1.83
Esters	Formic acid, tetrahydrofuryl eater	0.53	0.54	0.55	0.54
	2-Methyl-1-propenoic acid, ethyl eater	1.06	1.26	1.06	1.26

[a]The relative contents of the compounds were determined by the corresponding peak area (Wu *et al.* 2015)

Seed Germination Effect of WVs on Wheat Seed

The germination potential is an important index that indicates the germination speed and viability of seed. Table 3 shows the detail germination characteristics of wheat seed. The seed germination rate of wheat seed increased from WV-1 to WV-2, and then decreased from WV-3 to WV-7. Under the treatment of WV-1 and WV-2, the germination rate reached about 52.5 and 75.8%, and compared with the corresponding CK (45.8%), it increased. The variation trend of germination index was similar to seed germination rate. For germination index, it increased with increasing of WV concentration which reached the highest of 1.79 at concentration of 0.50 mL/L(WV-2), and then decreased. The relative germination rate revealed the effect of WVs on germination rate of wheat seed, in which positive numbers indicated a promoting effect and negative numbers indicated an inhibiting effect. Under the treatment of WV-1 and WV-2, the relative germination rate was positive and reached 14.6 and 65.4%. These results indicated the low concentration of WV promoted the germination of seed and high concentration exhibited inhibitory effect, and the WV-2 had the greatest positive effect on wheat seed germination. Coincidentally, Pan *et al.* (2010) reported that gingko vinegar exhibited higher effect on seed germination and the effect was obvious between different concentration and crops. These results demonstrated that WV could serve as a potential plant growth regulator.

Table 3. Germination Characteristics of Wheat Seed

Number	3rd-Day seed germination potential/%	5th-Day seed germination potential/%	Seed germination rate/%	Relative seed germination rate/%	Seed germination index
CK	16.67 e	40.00 b	45.83 c	-	1.00 c
WV-1	33.33 b	41.67 b	52.50 b	14.55	1.25 b
WV-2	40.00 a	60.83 a	75.83 a	65.45	1.79 a
WV-3	18.33 d	38.33 c	40.00 d	-12.72	0.59 d
WV-4	15.83 e	34.17 d	37.50 e	-18.18	0.44 e
WV-5	10.00 f	32.50 e	34.17 f	-25.44	0.28 f
WV-6	9.17 g	27.50 f	33.33 f	-27.27	0.18 g
WV-7	9.17 g	22.50 g	33.33 f	-27.27	0.17 g

Note: Different small letters represent significant difference among the different concentrations of WVs treatments ($P < 0.05$).

Effects of Root Growth by WVs on Wheat Seed

Main root

The main root length is the major morphological characteristic of root growth. The impact of WVs on main root length is shown in Fig. 2. With increasing concentration, the main root length increased and then decreased. Under the treatment of WV-2, the main root length reached 10.68 cm and was remarkably longer than that of corresponding CK, which increased respectively 1.26 times. Under treatment of WV-7, it decreased to 2.30 cm and only 27% of that treated by CK. Thus, low concentrations of WV promoted the growth of roots, and high concentrations inhibited the growth of roots. This probably resulted from the higher content of acids and phenols leading to increased ABA and impeded the growth of main root.

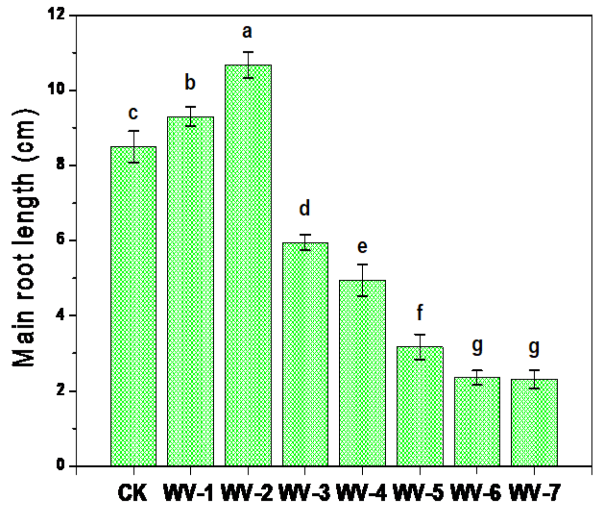
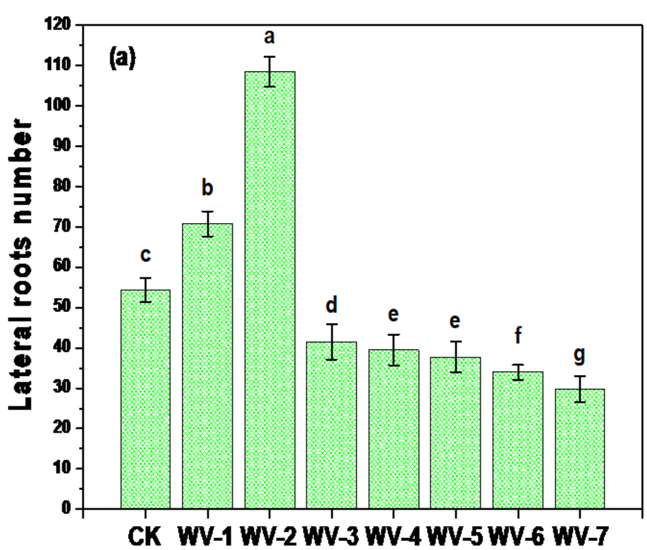


Fig. 2. The main root length of wheat culture treated with CK, WV-1, WV-2, WV-3, WV-4, WV-5, WV-6 and WV-7. Different small letters represent significant difference among the different concentrations of WVs treatments ($P < 0.05$).

Lateral roots

Lateral roots number and roots uniformity were chosen to characterize the lateral roots. Effects of WVs treatment on lateral roots number and roots uniformity of wheat seed are shown in Fig. 3. The lateral roots number for wheat under different WVs was remarkably greater than that of CK with a concentration lower than 0.50 mL/L. At a concentration of WVs higher than 0.50 mL/L, total roots number was fewer than that of CK and the effect was not significant. For the lateral roots number, it increased to 108 treated by WV-2 which was two times of that of CK, while it decreased to 30 treated by WV-7 which was 0.56 times of that of CK. Figure 3(b) shows the variation in root uniformity, which was similar to the change of lateral roots number. The lateral roots number and roots uniformity increased at low concentration of WVs and decreased at high concentration, which indicated the low concentration was conducive to growth of lateral roots.



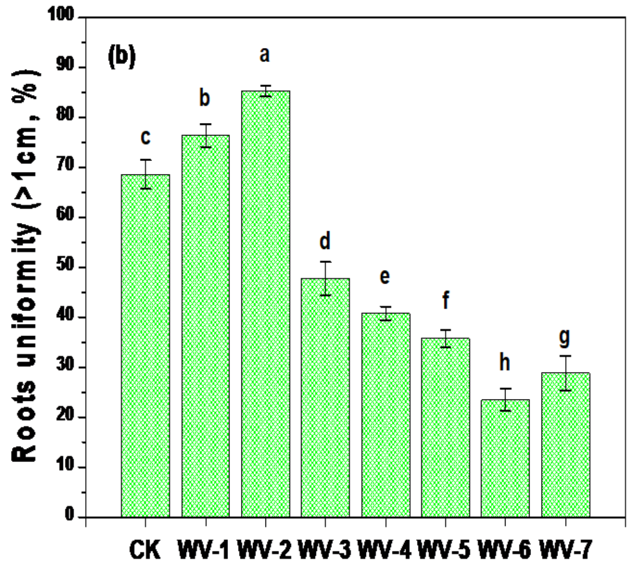


Fig. 3. The lateral roots number (a) and roots uniformity (b) of wheat culture treated with CK, WV-1, WV-2, WV-3, WV-4, WV-5, WV-6 and WV-7. Different small letters represent significant difference among the different concentrations of WVs treatments ($P < 0.05$).

Total fresh mass of roots

Figure 4 shows the influence of different concentrations of WVs on fresh root quality. The total fresh root mass increased by low concentration treatment of WVs, but the trend was not obvious. Treating with a high concentration reduced the total fresh root mass. When the concentration of WVs was lower than 0.50 mL/L, the total fresh root mass showed an increasing trend. Compared with the CK control group, the total fresh root mass increased from 0.497 g to 0.528 g, and reached a maximum at WV-2. When the concentration was more than 0.50 mL/L, the total fresh root mass decreased. The fresh root quality of wheat seeds treated with WV-7 was the lowest (0.170 g), which decreased nearly 66% compared with the CK group.

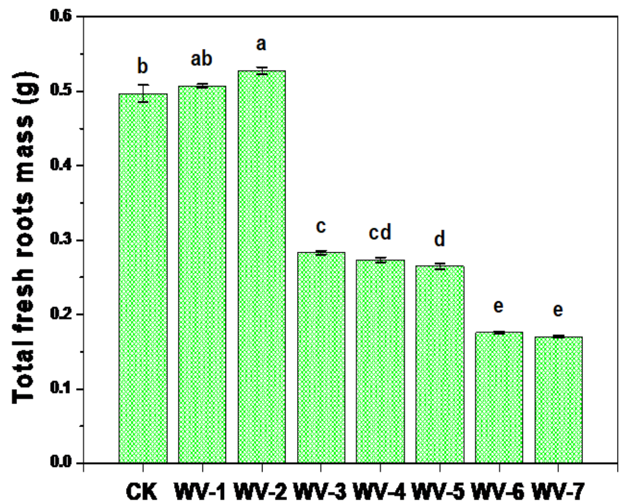


Fig. 4. The total fresh roots mass of wheat culture treated with CK, WV-1, WV-2, WV-3, WV-4, WV-5, WV-6 and WV-7. Different small letters represent significant difference among the different concentrations of WVs treatments ($P < 0.05$).

Effects of WVs on Root Physiological Characteristics

Root vigor (RV)

Root systems anchor the plant and allow uptake of water and nutrients. Root vigor is an index to evaluate the performance of roots, which indicates the absorptive capacity of water and nutrient from soil. There are many enzymes involved in plant growth, and dehydrogenase is an important enzyme involved in photosynthesis and respiratory action. Therefore, dehydrogenase activity was chosen as an indicator of root vigor. Figure 5 shows the impact of RV on roots treated with WVs. The RV content of roots were effected by the concentration of WV, which increased at low concentration and decreased at high concentration. The RV of roots treated with WV-2 reached the highest value of 77.351 μg/g·h, which was 1.82 times higher than CK. The RV of roots treated with WV-7 exhibited lowest amount of 23.19 μg/g·h. ·

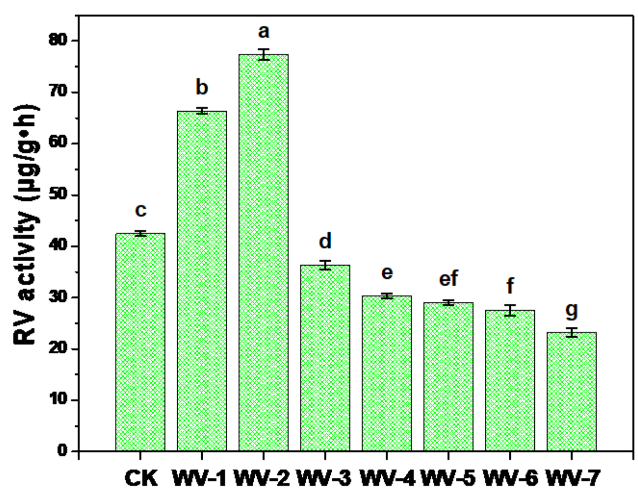


Fig. 5. The RV of wheat culture treated with CK, WV-1, WV-2, WV-3, WV-4, WV-5, WV-6 and WV-7. Different small letters represent significant difference among the different concentrations of WVs treatments ($P < 0.05$).

The root growth indices such as main root length, lateral root number, root uniformity, and total fresh root mass were influenced by treatment with WVs. Therefore, RV and root growth indices were chosen for a correlation analysis study, which is shown in Fig. 6. The R^2 of correlation analysis between RV and lateral roots number nearly to 0.93 indicated that the influence of RV on lateral root number was significant. This was consistent with a previous study on the effects of cotton straw on wheat growth (Zhang *et al.* 2015). As the principal part of the root system, the lateral roots affect the specific surface area, volume, and activity of roots. Increases in the lateral roots could increase the total surface area and volume of the roots system, improving the water and nutrients adsorption of roots and enhancing the roots vigor activity.

Malondialdehyde content (MDA)

MDA is the peroxidation product of cell membrane lipids when the plant grows under stressful conditions and suffers from oxidation stress. Under stress conditions, MDA accumulates continually with cell deterioration and damages cell membranes. Therefore, MDA was chosen as the index of stress level, with a higher MDA content indicating greater cell damage. The MDA content of wheat culture is highly dependent on the WVs concentration. With increasing WVs concentration, MDA content decreased first and then increased. After treatment with WV-2, MDA content decreased to 0.00928 μmol/g, which was reduced nearly 30% compared with CK. When treated with WV-7, it increased to 0.01432 μmol/g.

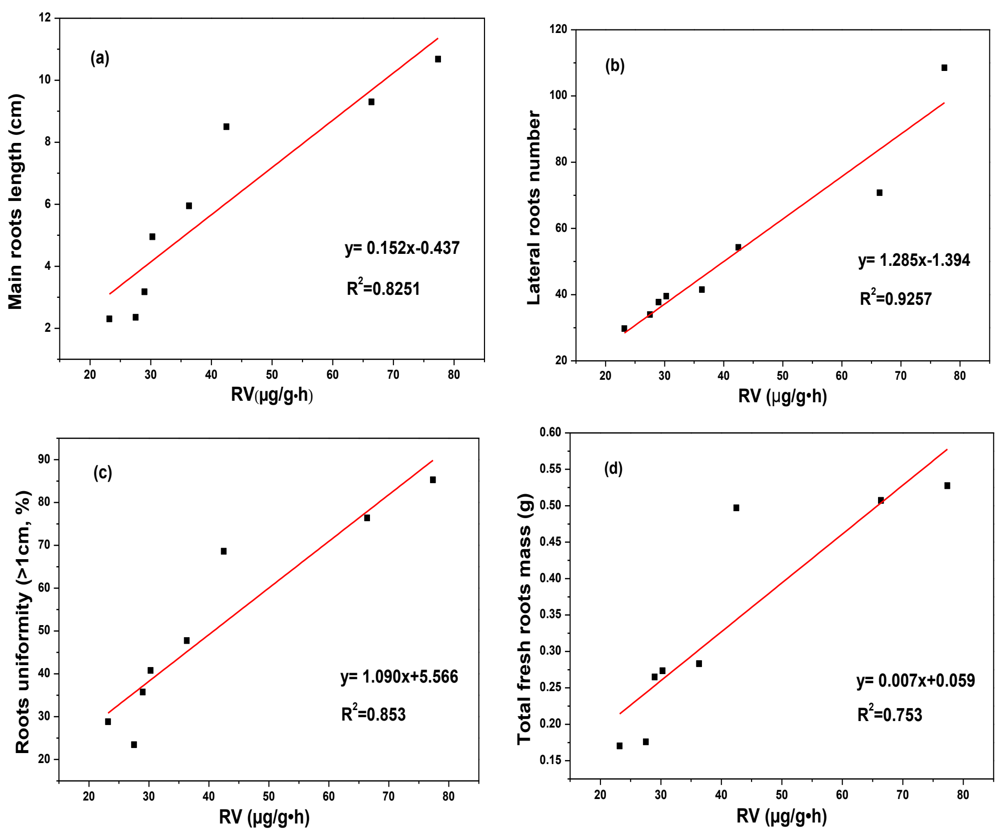


Fig. 6. Correlation analysis between RV activity and main root length (a), lateral roots number (b), roots uniformity (c), and total roots mass (d)

The changing trend of MDA content was in contrast with that of RV activity. This result indicated that the effects of different concentrations on the growth of plant roots were different, and the higher concentration was a stress environment, which caused cell damage and inhibited roots growth.

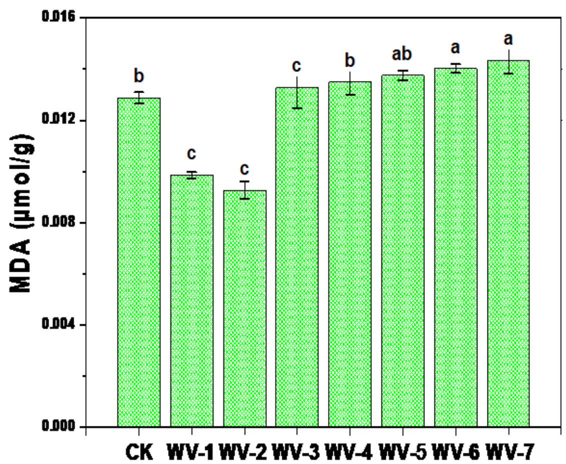
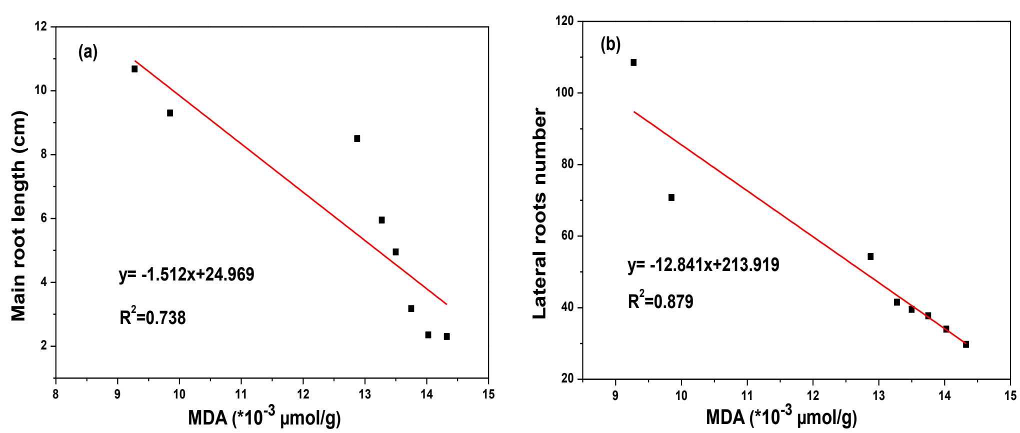


Fig. 7. The MDA of wheat culture treated with CK, WV-1, WV-2, WV-3, WV-4, WV-5, WV-6 and WV-7. Different small letters represent significant difference among the different concentrations of WVs treatments ($P < 0.05$).

Figure 8 shows the correlation analysis on MDA and root growth indices such as main root length, lateral root number, root uniformity, and total root mass. The MDA content was negatively correlated with the four root growth indices, and the order of correlation was: lateral root number, root uniformity, main root length, and total fresh root mass. The effect of MDA content on the number of lateral roots was more significant than the other three indices. The trend of MDA content on root growth indices was similar to that of RV activity. These results demonstrated that a high concentration of WV was the stress condition for wheat root growth and significantly affected the growth of lateral roots.



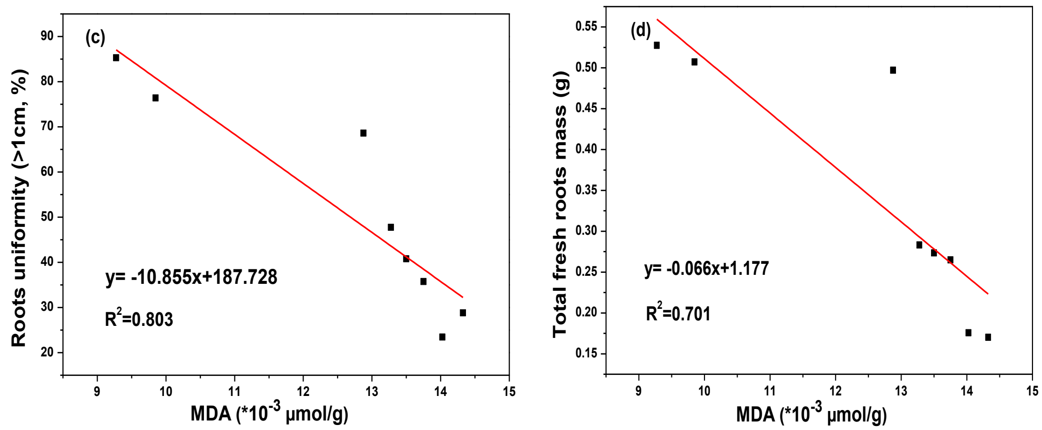


Fig. 8. Correlation analysis between MDA and main root length (a), lateral roots number (b), roots uniformity (c), total fresh roots mass (d)

Discussion

Roots are the first tissues to sense environmental signals and produce corresponding physiological responses, which affect the growth of the aboveground parts. The main root length, lateral root number, total fresh root mass, and root uniformity are the major morphological characteristics that can be used to evaluate the growth qualities of the root. Main root length is beneficial to absorb water and nutrients from deep soil; lateral roots are the principal part of the root system, which influences the total specific surface area and activity of the root system. Wood vinegar appears to stimulate cell growth and acts as the catalyst of microbes and enzyme activation. It has been used in agriculture for seed germination and plant growth.

The results of this study illustrated that the regulation effect of wood vinegar was selective and shown as "low concentration promotion, high concentration inhibition". WV contains abundant organic compounds, such as acids, phenols, and ketones. Treated with WV, the physical interaction between xyloglucan and other compounds in the cell-wall was broken with H^+, which was provided by acids in the WV. In this case, the secretion of H^+ and organic acid of root increased and caused acidification of the cell and breakage of the hydrogen bonds of microfiber between cellulose and hemicelluloses in the cell wall. This resulted in cell differentiation and promoted the growth of roots. Moreover, wood vinegar stimulates plant roots to release more exudates, improve the microenvironment, increase nutrient uptake and dry matter accumulation, and promote roots development (Mungkunkamchao *et al.* 2013). There are abundant phenolic compounds in WV. Monophenol inhibits plant growth, and diphenol or polyphenol promotes plant growth. Phenolic compounds exhibit a two-phase dose-effect relationship of stimulation at lower concentration and inhibition at higher concentration.

Under low concentration, acids, phenol, and other allelochemicals in wood vinegar can increase soluble sugar content, induce some protein expression, and increase root vigor that promote seed germination and root growth. The mechanism involves increasing free radicals at low concentration, which activate protease, regulate synthesis, and induce gene expression, leading to cell proliferation and expressing as growth stimulation effects (Calabrese and Blain 2009; Wang *et al.* 2015; Li *et al.* 2016). WV promoted the growth of plant under low concentration, and its regulation effect was similar to indoleacetic acid

(Guo et al. 2017). Treatment with low concentration of WVs resulted in the increasing of root growth respectively, which may be due to the phenolic acids breaking the hormonal balance.

Under high concentration, the high allelochemicals content and osmotic pressure was an adverse condition. When living in adverse conditions, the key enzymes required for seed germination were inhibited and plant roots adapt to stress by increasing respiration. Long-term high-intensity respiration consumes a large amount of carbohydrates, which may affect respiration due to insufficient respiratory substrates, greatly reducing the respiratory rate, and ultimately inhibiting growth (Renaut *et al.* 2004; Anan and Simm 2015). Treated with low concentration WVs, roots respiration and roots activity of wheat increased, which was an emergency response to an adverse environment. However, treated with high concentration, the respiratory rate decreased, which may be due to the inhibitory effect of WV on root metabolism and ATP production, which could not meet the needs of roots growth, thus leading to the increase of MDA content and the decrease of roots activity (Li *et al.* 2011; Liu *et al.* 2016).

CONCLUSIONS

1. The major chemical components of wood vinegars (WVs) obtained from pyrolysis of *Cunninghamia lanceolata* (Lamb.) Hook. waste were acids, phenols, alcohols, ketones, and esters. After pyrolysis at 450 °C, the WV shown the highest yield and chemical components, especially in acids and phenols.
2. Compared with sterile water (CK), WV at a concentration of 0.05 mL/L strongly promoted seed germination and lateral roots growth, which increased by 1.7- and 2-fold, respectively.
3. The concentration of WVs influenced the regulation effect. Lower concentrations promoted growth, and higher concentrations inhabited growth. The lower concentration of WVs increased the roots vigor and promoted growth, while the higher concentration increased the content of malondialdehyde (MDA) and inhibited growth.
4. WV prepared from *Cunninghamia lanceolata* waste could be a natural and efficient growth regulator in sustainable agriculture.

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REFERENCES CITED

Anan, P. N., and Simm, K. B. (2015). "Effect of *Pistia stratiotes*, cattle manure and wood vinegar (pyroligneous acid) application on growth and yield of organic rainfed rice,"

Paddy & Water Environment 13(4), 337-342. DOI: 10.1007/s10333-014-0453-z
Brebu, M., Tamminen, T., and Spiridon, I. (2013). "Thermal degradation of various lignins by TG-MS/FTIR and Py-GC-MS," *Journal of Analytical & Applied Pyrolysis* 104, 531-539. DOI: 10.1016/j.jaap.2013.05.016
Calabrese, E. J., and Blain, R. (2009). "Hormesis and plant biology," *Environmental Pollution* 157(1), 42-48. DOI: 10.1016/j.envpol.2008.07.028
Cong, H. B., Masek, O., Zhao, L. X., and Yao, Z. L. (2018). "Slow pyrolysis performance and energy balance of corn stover in continuous pyrolysis-based poly-generation system," *Energy & Fuels* 32(3), 3743-3750. DOI: 10.1021/acs.energyfuels.7b03175
Creepier, J., Masle, A. L., Charon, N., Florian, A., Pascal, D., and Sabine, H. (2018). "Ultra-high performance supercritical fluid chromatography hyphenated to atmospheric pressure chemical ionization high resolution mass spectrometry for the characterization of fast pyrolysis bio-oils," *Journal of Chromatography B* 1086, 38-46. DOI: 10.1016/j.jchromb. 2018.04.005
Custodis, V. B. F., Bährle, C., Vogel, F., and Van Bokhoven, J. A. (2015). "Phenols and aromatics from fast pyrolysis of variously prepared lignins from hard- and softwoods," *Journal of Analytical & Applied Pyrolysis* 115, 214-223. DOI: 10.1016/j.jaap.2015.07.018
Guo, W., Xue, Y. W., Yu, S., and Sun, H. Y. (2017). "Effects of phthalic acid and p-hydroxybenzonic acid on seeding growth and antioxidant properties of oat (*Avena nuda*)," *Plant Physical Journal* 53(10), 1885-1892. DOI: CNKI:SUN:ZWSL.0.2017-10-008
Jung, K. H. (2007). "Growth inhibition effect of pyroligneous acid on pathogenic fungus, *Alternaria mali*, the agent of alternaria blotch of apple," *Biotechnology Bioprocess Engineering* 12(3), 318-322. DOI: 10.1007/BF02931111
Kan, T., Strezov, V., and Evans, T. J. (2016). "Lignocellulosic biomass pyrolysis: A review of product properties and effects of pyrolysis parameters," *Renewable & Sustainable Energy Reviews* 57, 126-1140. DOI: 10.1016/j.rser.2015.12.185
Lashari, M. S., Liu, Y., Li, L., Pan, W. N., Fu, J. Y., Pan, G. X., Zheng, J. F., Zhang, J. W., and Xu, X. Y. (2013). "Effects of amendment of biochar-manure compost in conjunction with pyroligneous solution on soil quality and wheat yield of a salt-stress cropland from central china green plain," *Field Crops Research* 144, 113-118. DOI: 10.1016/j.fcr.2012.11.015
Li, W., and Tang, S. H. (2017). "Effects of PHBA (p-hydroxybenzoic acid) on root physiological characteristics and potassium absorption of tobacco," *Guizhou Agriculture Scicence* 45(10), 35-39. DOI: 10.2017-10-009
Li, Z. X., Qin, S. J., Lu, D. G., and Nie, J. Y. (2011). "Research progress in root respiratory metabolism of plant and the environmental influencing factors," *Plant Physical Journal* 47(10), 957-966. DOI: 10.1093/mp/ssq070
Li, H., Zhang, G. C., Xie, H. C., Xu, J. W., Li, C. R., and Sun, J. W. (2016). "The effect of phenol concentration on photosynthetic physiological parameters of *Salix babylonica*," *Bulletin of Botany* 51(1), 31-39. DOI: 10.11983/CBB15028
Liu, R. X., Guo, W. Q., Chen, B. L., and Oosterhuis, D. M. (2008). "Effects of N fertilization on root development and activity of water-stress cotton (*Gossypium hirsutum* L.) plants," *Agricultural Water Management* 95(11), 1261-1270. DOI: 10.1016/j.agwat.2008.05.002
Liu, W. J., Li, W. W., Jiang, H., and Yu, H. Q. (2017). "Fates of chemical elements in biomass during its pyrolysis," *Chemical Reviews* 117(9), 6367-6398. DOI:

10.1021/acs.chemrev. 6b00647
Liu, R. X., Zhang, G. W., Yang, C. Q., Zhang, L., and Ni, W. C. (2016). "Allelopathic effects of wheat straw extract and decomposition liquid on cotton seed germination and seedling growth," *Cotton Science* 28(4), 375-383. DOI: 10.11963/issn.1002-7807.201604009
Mmojieje, J., and Horning, A. (2015). "The potential application of pyroligneous acid in the UK agriculture industry," *Journal Crop Improvement* 29(2), 228-246. DOI: 10.1080/15427528.2014.995328
Mungkunkamchao, T., Kesmala, T., Pimratch, S., Toomsan, B., and Jothityangkoon, D. (2013). "Wood vinegar and fermented bioextracts: Natural products to enhance growth and yield of tomato (*Solanum lycopersicum* L.)," *Scientia Horticulturae* 154(2), 66-72. DOI: 10.1016/j.scienta.2013.02.020
Orgun, S., and Yildiz, D. (2015). "Slow pyrolysis of paulownia wood: Effects of pyrolysis parameters on product yields and bio-oil characterization," *Journal of Analytical & Applied Pyrolysis* 114, 68-78. DOI: 10.1016/j.jaap.2015.05.003
Pan, J. X., Cao, F. L., Wang, G. B., and Zhang, W. X. (2010). "Effects of gingko wood vinegar on seed germination and seedling growth," *Seed* 29(2), 39-44. DOI: 10.3969/j.issn.1001- 4705.2010.02.010
Quan, C., Gao, N. B., and Song, Q. B. (2016). "Pyrolysis of biomass components in a TGA and a fixed-bed reactor: Thermochemical behaviors, kinetics, and product characterization," *Journal of Analytical & Applied Pyrolysis* 121, 84-92. DOI: 10.1016/j.jaap.2016.07.005.
Renaut, J., Iutts, S., Hoffmann, I., and Hausman, J. F. (2004). "Responses of poplar to chilling temperatures: Proteomic and physiological aspects," *Plant Biology* 6(1), 81-90. DOI: 10.1055/s-2004-815733
Temiz, A., Albas, S., and Panov, D. (2013). "Chemical composition and efficiency of bio-oil obtained from giant cane (*Arundo donax* L.) as a wood preservative," *BioResources* 8(2), 2084-2098. DOI: 10.15376/biores.8.2.2084-2098.
Tripathi, M., Sahu, J. N., and Ganesan, P. (2016). "Effect of process parameters on production of biochar from biomass waste through pyrolysis: A review," *Renewable & Sustainable Energy Reviews* 55, 467-481. DOI: 10.1016/j.rser.2015.10.122
Wang, S., Su, Y. H., and Qiao, M. (2015). "Effect of short-term exposure of phenols on root elongation of wheat and barley," *Asian Journal of Ecotoxicology* 10(2), 283-289. DOI: 10.7524/AJE.1673-5897.20140307001
Wu, Q., Zhang, S., Hou, B., Zheng, H., Deng, W., Liu, D., and Tang, W. (2015). "Study on the preparation of wood vinegar from biomass residues by carbonization process," *Bioresource Technology* 179, 98-103. DOI: 10.1016/j.biortech.2014.12.026
Xu, L. J., Yang, S. J., Dong, M., Shi, Y. Y., and Du, X. G. (2015). "Effects of bamboo vinegar on seed germination and seedling growth of different flue-cured tobacco varieties," *Agriculture Science Technology* 16(8), 1660-1665. DOI: 10.3969/j:issn.2095-1191.2014.10.1764
Zhang, G. W., Liu, R. X., Yang, C. Q., and Yang, F. Q. (2015). "Allelopathic effects of cotton straw on wheat growth," *Journal Triticeae Crop* 35(4), 555-562. DOI: 10.7606/j.issn.1009-1041.2015.04.17

Zhang, H., Sun, G. Z., Hou, X. D., Wu, M., Yao, Y., and Li, F. M. (2018). "Pyrolysis of *Arundo donax* L. to produce pyrolytic vinegar and its effect on the growth of dinoflagellate *Karenia brevis*," *Bioresource Technology* 247, 273-281. DOI: 10.1016/j.biortech.2017.09.049

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