Impacts of adding different components of wood vinegar on rape (Brassica napus L.) seed germination

Xue Shan, Xia Liu, Qian Zhang*
Institute of Coastal Environmental Pollution Control, Key Laboratory of Marine Environmental Science and Ecology, Ministry of Education, College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China

E-mail address: start2633264901@163.com

Abstract. In recent years, wood vinegar has been widely used in the agricultural production. It can be used as the soil amendment, antibacterial agent and organic fertilizer. This study investigated the effect of wood vinegar on rape (Brassica napus L.) seed germination. The results in this study showed that 1% (v/v) wood vinegar had the greatest inhibition effect on the seed germination of rape (Brassica napus L.). The wood vinegar (WV) and the distilled wood vinegar at 98 - 130 °C (D2) significantly inhibited seed germination by 100%, compared to the control treatment. However, the distilled wood vinegar (D1) had significantly increased the shoot length and root length by 58.4% and 31.7%, respectively. These positive effects could be attributed to the improved soil fertility, increased nutrient supply, and further stimulated plant growth. Overall, the D1 could be a promising soil amendment to promote plants growth and enhance crop yields.

1. Introduction
Wood vinegar is the organic product from plants with distillation by the process of condensate, recovery and separation from natural gas mixture [1]. Acids, alcohols, phenols, esters, carbonyl and furan by nearly 200 different compounds had been identified in the wood vinegar [2]. The different chemical compositions of wood vinegar complex usually has different characteristics and roles. Tar material in wood vinegar is not conducive to plant growth [3]. Therefore, it is needed to remove harmful and unstable compounds in crude wood vinegar by rectification to improve the safety and stability of wood vinegar. After a simple rectification process, wood vinegar could be used in agriculture as plant growth promoter, soil conditioner, antibacterial agents and organic fertilizers [4]. Studies have shown that wood vinegar is beneficial to plant germination. Wang et al. studied the effects of wood vinegar on the germination and root growth of carrot, rape and cabbage and the results showed that wood vinegar could significantly promote the germination and root growth of carrot and cabbage seeds, while the effects on rape seed germination was not obvious, and wood vinegar had no significant effect on seedling growth [5]. And wood vinegar enhanced the crop yields and the nutrient uptake by plants followed by its application into agricultural soils [6]. However, studies on the effects of wood vinegar on seed germination are scare and the corresponding mechanisms are still unclear. Thereby, the major objective of this study are to investigate the effects of the wood vinegar on the growth of rape (Brassica napus L.) seed germination and the associated mechanisms, and provide theoretical evidences for the further utilization of wood vinegar as a potential plant growth promoter.
2. Materials and methods

2.1 The preparation of samples
The surface soils (0 - 20cm) used in this study were collected from a farmland in Qingdao, Shandong province. The soil samples were thoroughly mixed, and air dried for 7 days, then passed through a 2-mm sieve. Wood vinegar was the liquid by-product collected from the cooling systems after pyrolyzing poplar (*Populus trichocarpa*) at 450 °C for 6h in a vacuum tube furnace (O-KTF1200, Spring Thunder, Jiangsu). The wood vinegar was stood for 6 months in the darkness and then the upper component was filtered through 0.22 μm membrane for further analyses. Three layers including a thin layer of oil on the top, a thick layer in the middle, and a thin layer of ropy wood tar in the bottom. The middle layer was siphoned off by pipettes. It was refined and purified by the distillation method [7]. The different-times distilled wood vinegar was prepared by atmospheric distillation at 0 - 98 °C, 98 - 130 °C and 130 - 220 °C, respectively.

2.2 Germination tests of rape
The seeds of rape (*Brassica napus* L.) were sterilized for 10 min with 10% hydrogen peroxide, and then soaked in deionized water for 2 h [8]. Fifteen prepared seeds were placed on the filter paper (8.5 cm in diameter) in the clean petri dishes (diameter 9cm, depth 1.5cm) and moistened with either 5 mL deionized water (control groups) or 5 ml diluted wood vinegar solution at 100 times (experimental groups); the whole experiments were performed in four replicates. All dishes were covered and incubated in the light at 25 °C ± 0.5 °C for 7 days [9], and the germination potential, germination rate, root length, and shoot length were recorded daily. The treatment of CK, WV, D1, D2 and D3 indicated the rape seeds treated with deionized water, the non-distilled wood vinegar and the different distilled components of wood vinegar at 0 - 98 °C, 98 - 130 °C and 130 - 220 °C, respectively. Germination rate and germination potential were calculated as the formula below.

\[
\text{Germination rate}=\frac{N_1}{N}\times100% \quad (1)
\]

\[
\text{Germination potential}=\frac{N_2}{N}\times100% \quad (2)
\]

\(N_1\) is the number of germination on the seventh day; \(N_2\) is the number of germination on the seventh day; \(N\) is the total number of seed.

2.3 Statistical analysis
All data in the figures were presented as mean values (n = 4). Error bars in the results represented the standard deviations. Significant differences among the treatments were analyzed using one-way analysis of variance (ANOVA) with Duncan’s multiple range test \((P = 0.05)\) using Statistical Product and Service Solutions Software 20.0 (SPSS 20.0).

3. Result and discussion

3.1 Effect of wood vinegar on rape seed germination
The germination rates of rape were shown in Figure 1, the WV and D2 additions significantly decreased both germination potential and germination rate by 100% compared to the CK treatment \((P < 0.05)\), suggesting that the added WV had the greatest inhibition effects on the rape germination compared with CK treatment. Mu et al. also found that the high concentrated wood vinegar inhibited seed germination [10]. The inhibition effect was mainly attributed to two reasons. Firstly, the pH value of wood vinegar was 2.5 to 3.8 and the high acidic conditions were not conducive to the seed germination of plants. Secondly, the main component of wood vinegar was acetic acid and phenols, which were harmful to the rape seed germination [11]. Moreover, the other treatments (D1 and D3) showed no significant effects on the seed germination of rape, indicating that the rectification of wood vinegar could remove the harmful components to the seed germination.
3.2 Effects of wood vinegar on the rape growth

The effects of wood vinegar on the rape growth were shown in Figure 2, the WV and D2 additions significantly inhibited the seedling growth by 100%, compared to the CK treatment. It was mainly attributed to the wood vinegar, acetic acid and other organic acid in WV and D2 were toxic to the rape seed germination. However, the distilled wood vinegar (D1) obviously increased the shoot and root length by 58.4% and 31.7%, respectively, relative to the CK treatment. It could be explained by the organic components of wood vinegar provide nutrients (e.g., N and P) for the growth of rape seedlings. However, the distilled components of wood vinegar (D3) had no effect on the rape growth compared with the CK treatment, indicating no nutrient component in D3 to promote seedling growth.

Figure 2. Effects of adding wood vinegar on the shoot length (A) and root length (B) of rape. CK, WV, D1, D2 and D3 indicated that the rape seeds treated with deionized water, the non-distilled wood vinegar and the different distilled components of wood vinegar at 0 - 98 °C, 98 - 130 °C and 130 - 220 °C, respectively. The different letters among different treatments indicate the significant differences, which were analysed by Duncan’s test ($P = 0.05$) using SPSS 20.0. Error bars represented standard errors of the mean ($n = 4$).
4. Conclusion
The results revealed that the distilled wood vinegar (D1) could increase seed germination and promote the seedling growth of rape, while the original wood vinegar (WV) and the distilled wood vinegar (D2) had inhibition effect on seed germination. Distillation of wood vinegar could remove harmful components of wood vinegar, and high concentration of original wood vinegar were not conducive to the seed germination. However, the mechanisms of wood vinegar on plant germination and seedling growth still deserve further research. The study could provide a theoretical evidence for the further utilization of wood vinegar as a potential plant growth.

Acknowledgements
This work was financially supported by Natural Science Foundation of China (41573089).

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