

NOTE

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Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants II: composition of moso bamboo vinegar at different collection temperature and its effects

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Abstract Moso bamboo vinegar was treated with extractive and separation methods. The acidic, neutral, and phenolic fractions separated from ether-extracted vinegar were analyzed by gas chromatography and gas chromatography-mass spectrometry to identify the major components in moso bamboo vinegar. The compositions of eight moso vinegar fractions collected over different temperature ranges from 100°C to 480°C were also analyzed and their effects on regulation of germination and growth were studied by bioassay with seeds of watercress and chrysanthemum. The results showed that moso bamboo vinegar fractions with collection temperatures up to 250°C promoted radicle and hypocotyl growth and this effect became larger with increasing collection temperature for chrysanthemum. Moso bamboo vinegar collected from 250°C to 400°C had a strong inhibition on germination and radicle growth for both seed types when tested at 10³ dilution.

Key words Moso bamboo vinegar · Collection temperature · Bioassay · Seed plants

Introduction

Bamboo vinegar is a condensed liquor that is collected during the pyrolysis of bamboo. Bamboo vinegar is composed of more than 200 chemical components, with acetic acid being the main one. The other organic components in bamboo vinegar also play an important role in the practical application even when present in trace quantities.

Although there are many reports on the composition and application characters of wood vinegar,^{1–5} bamboo vinegar has not been studied to the extent of wood vinegar. Bamboo is called a wood material for the 21st century because of its similar chemical and physical qualities to those of wood. It is important to study the characters of bamboo and its by-products in order to make good use of them. Bamboo vinegar, as one of the by-products of bamboo charcoal, shows more application prospects than wood vinegar because of abundant bamboo resources as well as consistent quality. Bamboo vinegar is receiving more and more attention in agriculture and in daily life because of its favorable environmental effects and ecological sustainability. For example, it can be used as a deodorizer, in food processing, and in soil disaffection as a natural material.^{6,7} In one study, bamboo vinegar has been found to have regulation effects on the germination and radicle growth of seed plants.⁸ However, the composition of bamboo vinegar at different collection temperatures in the production process has not yet been studied. This aspect is thought to be important for product quality and its further application. The present study investigated the characters of moso bamboo vinegar with different collection temperatures and their regulation effects on seed plants.

Materials and methods

Moso bamboo (*Phyllostachys pubescens*) vinegars with collection temperatures from 100° to 480°C were used. Production conditions were as follows: 4 to 5-year-old moso bamboo culms of diameter 70–150mm were cut into 1200-mm sections and were further partitioned into four or six sections. These were carbonized in a ST1215 furnace (Showa Kikaku, Ø1200 × 1500mm) with a LPG burner. The carbonization temperature was raised to 550°–600°C at ca. 100°C/h for 5–6h, after which the heating was stopped and the furnace allowed to cool. The gas in the furnace was composed of CO₂, H₂O, and N₂ due to the burning of LPG and the existence of air at the beginning of pyrolysis.

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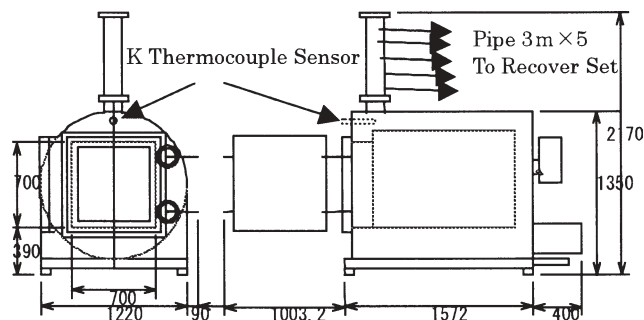


Fig. 1. Outline schematic drawing of the furnace (ST1215, Showakikaku) for bamboo vinegar. Dimensions in millimeters

Bamboo vinegars were condensed by a water-cooled condenser and collected according to the temperature measured by a thermocouple which was positioned at the entrance of a smoke funnel inside the upper portion of the furnace as shown in Fig. 1. The vinegars were classed into eight groups from 100°C to 480°C with a range of 50°C based on the collection temperature.

Treatment of material

Crude bamboo vinegar was extracted with ether to obtain ether-extracted vinegar, which was then further separated into acidic, neutral, and phenolic fractions using literature methods.^{9,10}

Physical and chemical characteristics

The specific gravity, refractive index, pH, and acidity of each vinegar with different collection temperatures were measured. The acidity is expressed as the weight percent of acetic acid in the bamboo vinegar, which was determined by titration with 0.1N sodium hydroxide solution. The ultraviolet-visible (UV-VIS) absorption spectrum of each vinegar at a concentration of 0.1% was recorded by a Jasco V-560 UV/VIS spectrophotometer from 600nm to 200nm with a scanning speed of 400nm/min.

Gas chromatography and gas chromatography-mass spectrometry (GC-MS) analysis

The conditions for gas chromatography (GC) analysis were as follows: column Shimadzu Hicap CBP20-M25 (0.25 mm i.d. × 25 m PEG-20M); temperature 60°–200°C increased at 5°C/min, holding for 22 min at 200°C; splitter ratio 60:1; carrier gas helium; flame ionization detection (FID). Components were identified by comparing the retention times of their peaks with authentic compounds and those in the literature.⁹ Quantification of the components was based on peak areas. The injected dose of ether-extracted bamboo vinegar was 1 μl at 5% concentration in diethyl ether. GC-MS was performed on a Jeol BU20 GC mate system. A GC equipped with a DB-WAX capillary column was connected

Table 1. Quantity of ether-extracted vinegar

Vinegar fraction ^a	Ether-extracted vinegar (%) ^b
<100°C	8.99
100°–150°C	15.89
150°–200°C	12.78
200°–250°C	17.56
250°–300°C	29.14
300°–350°C	29.93
350°–400°C	28.50
400°–480°C	16.41
<480°C	12.83

^a Temperatures are collection temperatures of vinegars measured by a thermocouple at the entrance of a smoke funnel inside the upper portion of the furnace

^b Values show the percentage based on the weight of crude vinegar of moso bamboo

directly to the mass spectrometer. The analytical conditions for GC were the same as stated above. The operating conditions of the mass spectrometer were as follows: ionization voltage 70 eV, and temperature of ionization source 250°C. Identification of the peaks was based on the published MS spectra data.¹¹

Bioassay

Moso bamboo vinegars with different collection temperatures were used as test solutions. All the tested solutions were diluted 10² to 10⁵ times with distilled water. Distilled water was used as the control. Test solution (10 ml) was poured into a petri dish (diameter 9 cm, depth 1.5 cm), which contained two pieces of filter paper (Advantec No. 2). Twenty seeds were scattered on the filter paper and the dishes were allowed to stand in a dark room at 20°C and 60% relative humidity (RH). The bioassay period was 4 days from the first day of germination. The radicle and hypocotyl growth were measured and compared with the controls after 4 days. The bioassay test was conducted three times for each sample. The tested seeds were watercress (*Rorippa nasturtium-aquaticum* Hayek) and chrysanthemum (*Chrysanthemum coronarium* L.).

Results and discussions

Characteristics of moso bamboo vinegar

Moso bamboo vinegar was collected according to the temperature measured by a thermocouple at the entrance of a smoke funnel inside the upper portion of a furnace during pyrolysis and the collected vinegars were classed into eight groups with a range of 50°C from 100°C to 480°C. The groups were collected at <100°C, 100°–150°C, 150°–200°C, 200°–250°C, 250°–300°C, 300°–350°C, 350°–400°C, and 400°–480°C.

Table 1 shows the quantity of ether-extracted vinegar based on the crude vinegar. Ether-extracted vinegars com-

prised 12.83% of moso vinegar collected up to 480°C. However, the content of ether-extracted vinegar varied according to the collection temperature. There are higher contents of ether-extracted vinegars at 250°–400°C than at other temperatures, which means that more pyrolytic components were produced in this temperature range. Table 2 shows the physical and chemical characters of moso bamboo vinegars with different collection temperatures. The specific gravity, refractive index, pH value, and acidity showed some relations with the collection temperature. With increasing collection temperature, the specific gravity and acidity of the vinegar increased from 100° to 350°C and then decreased from 350° to 480°C with a little exception. The vinegar collected at 250°–350°C had higher acidity and lower pH than other collection fractions. The vinegar had the lowest pH (1.9) and highest acidity (8.75%) at 250°–300°C. Each vinegar showed a maximum absorption peak (λ_{\max}) at 268–273 nm due to phenolic substances. The absorbance of vinegars at λ_{\max} increased with the collection temperature up to 350°C and was highest for vinegar collected at 300°–350°C. The absorbance of vinegars showed the same variation with collection temperature as other physi-

cal and chemical qualities, and appeared to have a sudden change at 250°–300°C. The characteristics of vinegars demonstrated that the collection temperature was an important factor for the quality of bamboo vinegar.

Composition of moso bamboo vinegar with different collection temperatures

The ether-extracted vinegar and acidic, neutral, and phenolic fractions were analyzed by GC and GC-MS. Eighteen kinds compounds were identified as main components. Of these compounds, acetic acid, propionic acid, *n*-butanoic acid, furfural, 2-cyclopentenone, 5-methylfurfural, phenol + *o*-cresol, *p*-cresol, *m*-cresol, 2,5-xyleneol, guaiacol, 4-methylguaiacol, 4-ethylguaiacol, and syringol were identified by comparing retention times and mass spectra with those of the authentic compounds. These compounds were found as common components in bamboo vinegar and wood vinegar.^{8,9,12} γ -Butyrolactone (M^+ 86 (50%), m/z 42 (100%)) and 2-hydroxy-3-methyl-2-cyclopentenone (M^+ 112 (100%), m/z 97, 84, 69, 55) were identified based on mass spectra and had the same retention times as standard samples. These two compounds have been previously identified in bamboo vinegar.¹² 1-(4-Hydroxy-3-methoxyphenyl)-2-propanone (M^+ 180 (25%), m/z 137 (100%), 122, 94, 77, 65, 51, 43) was first identified in moso bamboo vinegar. This compound was present in each vinegar fraction in high content and might be the decomposed product from lignin or a secondary product of the pyrolysis.

The vinegars with different collection temperatures had very similar compositions according to the chromatographs. This result was in agreement with the reports on carbonization of wood and bamboo that the composition of vinegar showed little change when the carbonization temperature was over 400°C.^{13,14} However, the relative concentrations of the main components varied with the collection temperatures. Table 3 shows the relative contents of main compo-

Table 2. Physical and chemical characteristics of moso bamboo vinegar at different collection temperatures

Vinegar fraction	Specific gravity d_{20}^{20}	Refractive index n_D^{20}	pH	Acidity (%)	Absorbance ^a
<100°C	1.0188	1.3432	2.8	3.61	0.31
100°–150°C	1.0191	1.3433	2.1	4.46	0.43
150°–200°C	1.0176	1.3439	2.0	5.01	0.46
200°–250°C	1.0197	1.3452	2.0	6.07	0.46
250°–300°C	1.0302	1.3522	1.9	8.75	0.88
300°–350°C	1.0381	1.3571	2.0	7.57	0.89
350°–400°C	1.0315	1.3534	2.1	5.93	0.73
400°–480°C	1.0249	1.3479	2.9	3.82	0.51

^a Values show absorbance measured at λ_{\max} for each vinegar fraction at 0.1% concentration

Table 3. Percentage composition of vinegar fractions collected at different temperatures (%)

Component	<100°C	100°–150°C	150°–200°C	200°–250°C	250°–300°C	300°–350°C	350°–400°C	400°–480°C
Acetic acid	39.36	49.94	48.96	57.78	62.64	53.12	55.80	47.92
Propionic acid	14.11	12.69	6.30	6.81	6.21	5.69	6.37	6.87
<i>n</i> -Butanoic acid	1.46	1.47	1.66	1.64	1.51	1.99	2.03	2.21
Phenol + <i>o</i> -cresol	5.18	4.88	6.09	5.20	4.02	4.31	4.64	6.97
<i>p</i> -Cresol	1.14	0.93	1.21	0.88	0.67	0.70	0.71	0.94
<i>m</i> -Cresol	0.80	0.72	0.78	0.70	0.48	0.40	0.39	0.57
2,5-Xyleneol	1.75	1.62	2.15	1.94	1.73	2.21	2.31	1.84
Guaiacol	2.11	2.35	2.64	2.25	1.90	2.16	1.86	1.50
4-Methylguaiacol	1.18	0.83	1.11	0.61	0.47	0.57	0.45	0.46
4-Ethylguaiacol	0.33	0.26	0.72	0.27	0.22	0.24	0.19	0.17
Syringol	1.85	1.72	2.05	2.04	1.61	1.99	1.43	2.26
2-Cyclopentenone	1.31	2.42	2.24	2.52	3.12	3.64	3.02	1.96
Furfural	0.58	0.80	0.96	1.09	1.49	2.27	0.87	0.44
5-Methylfurfural	0.33	0.61	0.84	0.52	0.38	0.43	0.30	0.35
γ -Butyrolactone	0.88	0.88	0.86	0.89	0.68	0.94	0.98	1.20
2-Hydroxy-3-methyl-2-cyclopentenone	2.58	2.15	2.27	1.99	1.70	2.18	2.29	2.79
1-(4-Hydroxy-3-methoxyphenyl)-2-propanone	5.16	2.82	3.39	1.47	1.04	1.71	2.56	5.37
Total	80.49	87.43	84.61	88.87	90.10	84.84	86.47	84.09

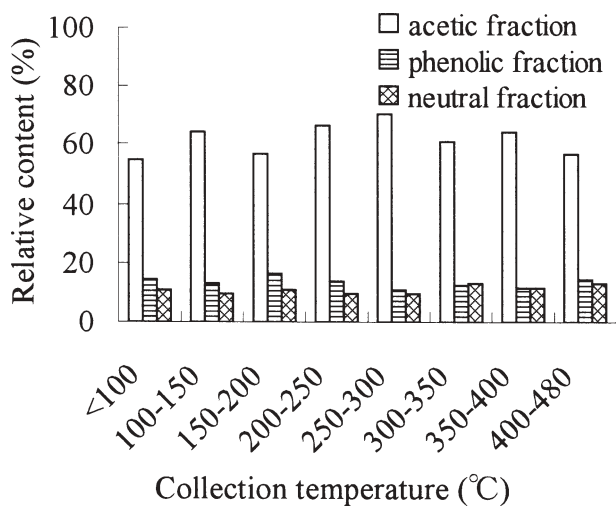


Fig. 2. Relative contents of acetic, neutral, and phenolic fractions of ether-extracted moso bamboo vinegar collected at different temperatures. Temperatures were measured by a thermocouple at the entrance of a smoke funnel inside the upper portion of the furnace

nents identified in the vinegars. Acetic acid was the main component in the vinegar and its relative concentration increased with the collection temperature and reached a maximum of 62.64% at 250°–300°C. This means that acetic acid accumulated during the pyrolysis. In wood pyrolysis, acetic acid was found to reach its maximum relative content at 650°C.¹⁵ On the contrary, propionic acid occurs in higher content at low collection temperature and decreases with increasing temperature. The contents of other compounds appeared to have a little variation with collection temperature. The total contents of identified compounds in each vinegar were 80%–90% of all peaks. Figure 2 shows the relative contents of acetic and neutral and phenolic fractions of ether-extracted vinegars with different collection temperature. Compounds belonging to the acetic fraction accounted for over 50% of the ether-soluble fraction at any collection temperature. The phenolic fraction content was higher than the neutral fraction content below 250°C and was about the same as the neutral fraction over 250°C. The changing content of compounds in each vinegar was thought to cause the effect on the seed bioassay.

Effect of moso bamboo vinegar with different collection temperatures on germination and radicle and hypocotyl growth of seeds

Moso bamboo vinegar promoted germination and radicle growth at an appropriate dilution according to our previous former study.⁸ In this study, the regulation effects of crude vinegar with collection temperatures from 100°C to 480°C on germination, radicle growth, and hypocotyl growth of seed plants were investigated. The tested seeds were watercress and chrysanthemum. The vinegar fractions were diluted 10^2 to 10^5 times.

The effect of crude vinegar with different collection temperatures on germination is shown in Table 4. At 10^2 dilu-

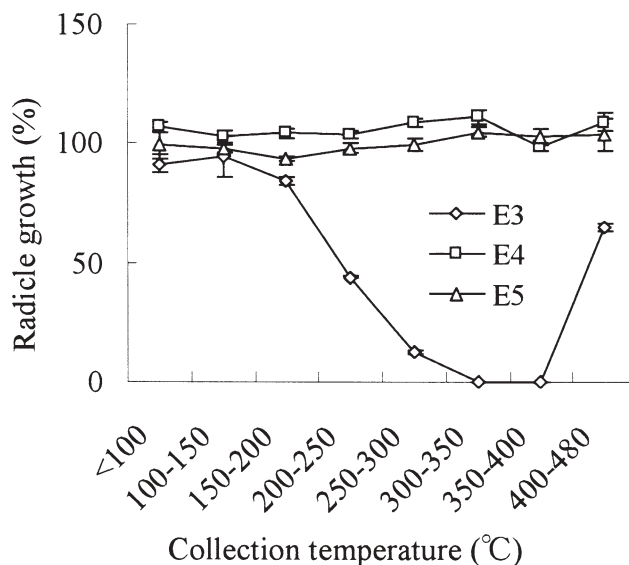


Fig. 3. Effect of moso bamboo vinegar with different collection temperatures on radicle growth of watercress. Bars show the standard error. E3, 10^3 times dilution; E4, 10^4 times dilution; E5, 10^5 times dilution

tion, two seeds of watercress and chrysanthemum showed germination with the vinegars collected up to 150°C. No germination was observed for vinegar fractions collected above 150°C. Collection temperature exerted an obvious influence on germination according to the bioassay results. When the dilution was 10^3 times, there was strong inhibition of germination for watercress with the vinegar fractions collected at 250°–400°C for the first day, although germination rates were similar to that of the control on the fourth day. For chrysanthemum, germination was markedly promoted for the first day and was similar to that of the control on the fourth day. Of the two seed types, germination of chrysanthemum was promoted more strongly than that of watercress.

Radicle and hypocotyl growth were measured 4 days after germination and compared with the control for the two seed types. Results are shown in Figs. 3–6.

Figures 3 and 4 show the effects of vinegars on radicle and hypocotyl growth of watercress, respectively. For watercress, there were strong inhibition effects on radicle growth and hypocotyl growth at 10^3 dilution, especially from fractions collected at 250°–400°C. No growth for watercress was observed for fractions collected at 300°–350°C and 350°–400°C. When the dilution was increased to 10^4 and 10^5 times, the inhibition effect was decreased. Hypocotyl growth was more inhibited than radicle growth for watercress. There was no obvious promotion effect, which coincided with previous results for moso bamboo vinegar.⁸ However, strong inhibition from fractions collected at 250°–400°C reflected the change of the vinegars during the pyrolysis. The characteristics of vinegars such as the content of ether-extracted vinegar, pH, and acidity showed that there were more compounds produced at this collection temperature. The last fraction of 400°–480°C showed less inhibition

Table 4. Effect of moso bamboo vinegar with different collection temperatures on germination

Vinegar fraction	Dilution	Watercress ^a		Chrysanthemum ^a	
		1st day	4th day	1st day	4th day
<100°C	10 ²	0	23 ± 1.8**	0	62 ± 3.5*
	10 ³	95 ± 7.4	95 ± 8.0	144 ± 5.6*	101 ± 8.7
	10 ⁴	114 ± 13.8	98 ± 7.0	103 ± 6.9	95 ± 6.7
	10 ⁵	94 ± 10.4	97 ± 4.6	158 ± 12.7*	107 ± 7.7
100°–150°C	10 ²	0	7 ± 1.8**	0	45 ± 8.2**
	10 ³	73 ± 6.8*	97 ± 3.5	122 ± 12.2	102 ± 8.6
	10 ⁴	96 ± 11.5	98 ± 1.8	147 ± 13.8	104 ± 5.3
	10 ⁵	108 ± 8.5	100 ± 3.0	150 ± 9.6*	107 ± 6.9
150°–200°C	10 ²	0	0	0	0
	10 ³	85 ± 8.3	94 ± 3.2	150 ± 9.5*	118 ± 5.6
	10 ⁴	76 ± 12.6	96 ± 3.7	139 ± 5.6*	102 ± 6.1
	10 ⁵	51 ± 7.9*	106 ± 3.2	89 ± 11.1	101 ± 5.5
200°–250°C	10 ²	0	0	0	0
	10 ³	84 ± 9.6	86 ± 1.7	150 ± 5.4*	106 ± 9.8
	10 ⁴	122 ± 13.7	98 ± 4.7	122 ± 6.4	96 ± 6.1
	10 ⁵	54 ± 12.0*	95 ± 3.2	183 ± 16.6*	115 ± 6.9
250°–300°C	10 ²	0	0	0	0
	10 ³	46 ± 11.1*	87 ± 5.0	89 ± 9.4	101 ± 8.2
	10 ⁴	71 ± 12.5	96 ± 1.9	250 ± 18.8*	117 ± 13.7
	10 ⁵	91 ± 13.4	102 ± 3.6	117 ± 16.6	106 ± 4.3
300°–350°C	10 ²	0	0	0	0
	10 ³	21 ± 4.9*	71 ± 3.6	94 ± 4.2	104 ± 4.4
	10 ⁴	41 ± 15.1*	86 ± 3.3	194 ± 6.4*	121 ± 6.4**
	10 ⁵	76 ± 7.1	89 ± 3.3	161 ± 5.4*	106 ± 2.7
350°–400°C	10 ²	0	0	0	0
	10 ³	41 ± 6.3*	96 ± 1.8	83 ± 4.7	105 ± 9.0
	10 ⁴	49 ± 11.4*	86 ± 1.7	178 ± 11.1*	114 ± 10.1
	10 ⁵	46 ± 11.1*	102 ± 4.8	78 ± 11.1	104 ± 5.3
400°–480°C	10 ²	0	0	0	0
	10 ³	70 ± 1.6	98 ± 1.8	172 ± 4.7*	116 ± 4.8
	10 ⁴	136 ± 10.9*	96 ± 1.8	133 ± 13.3	100 ± 6.1
	10 ⁵	86 ± 5.8	98 ± 1.9	112 ± 5.5	112 ± 7.3

* $P < 0.05$

** $P < 0.01$

^a Values are percentages relative to the control. Each determination was made with three replicates of 20 seeds

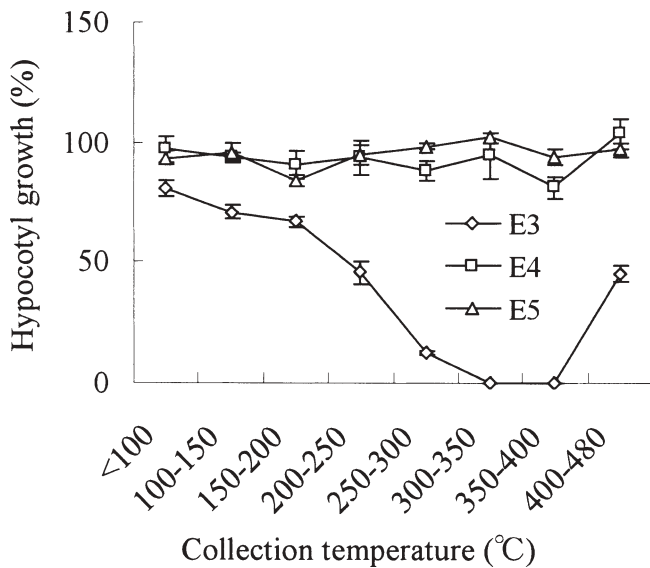


Fig. 4. Effect of moso bamboo vinegar with different collection temperatures on hypocotyl growth of watercress

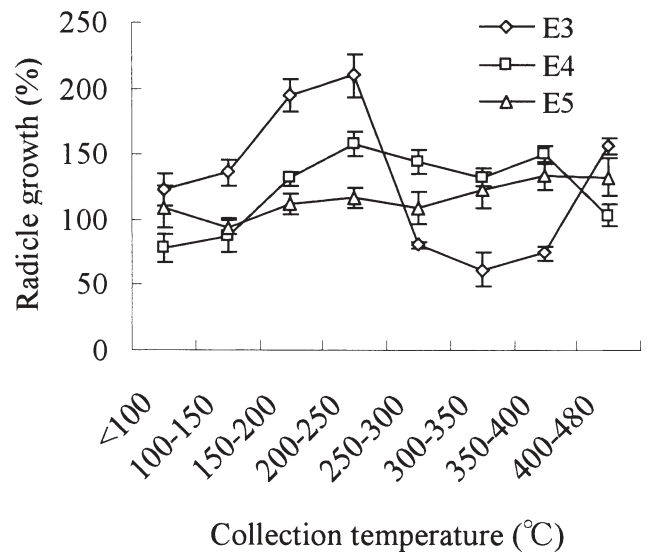


Fig. 5. Effect of moso bamboo vinegar with different collection temperatures on radicle growth of chrysanthemum

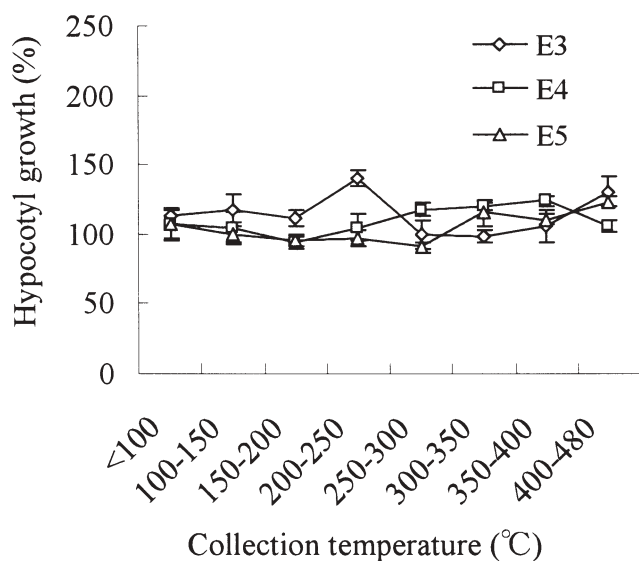


Fig. 6. Effect of moso bamboo vinegar with different collection temperatures on hypocotyl growth of chrysanthemum

than the fraction collected at 250°–400°C, which suggests the pyrolysis was near completion and the concentration of compounds were low in this period.

Figures 5 and 6 show the effects of vinegars on radicle and hypocotyl growth of chrysanthemum, respectively. For chrysanthemum, radicle growth was markedly promoted at 10^3 dilution, although it was inhibited with the fraction collected at 250°–400°C. The promotion effect increased with the collection temperature from 100° to 250°C for radicle growth. The vinegar fractions collected at 250°–300°C, 300°–350°C, and 350°–400°C showed inhibition of radicle growth, although a promotion effect appeared for 10^4 dilution for these temperature ranges. A promotion effect for hypocotyl growth of chrysanthemum was not as obvious as it was for radicle growth. Moreover, hypocotyl growth of chrysanthemum was not obviously inhibited with vinegars collected at 250°–300°C and 300°–350°C. It was with the fraction collected at 200°–250°C that chrysanthemum showed the highest radicle growth rate (210.6%) and hypocotyl growth rate (139.8%) based on the control.

From the results for the two seed types with moso bamboo vinegars collected at different temperatures, the radicle and hypocotyl growth of both seeds were inhibited with the fractions collected at 250°–400°C at 10^3 dilution. This is in accordance with the physical and chemical characters of vinegars at this temperature. Watercress was more strongly inhibited than chrysanthemum. Promoting effects appeared for watercress at 10^4 dilution and for chrysanthemum at 10^3 dilution for all kinds of vinegar. Regarding the temperature ranges, the vinegars collected at under 250°C show good promotion effects on radicle and hypocotyl growth for chrysanthemum.

According to changes of the main compounds in the vinegars, the content of acetic acid increased with the collection temperature. Excessive concentration of acetic acid might be the cause of inhibition of the 250°–400°C fraction.

Greater dilution would decrease this inhibition effect. Although at low content compared with acetic fractions, the neutral substance would cause inhibition of growth because its content increased over 250°C. The promotion effect of bamboo vinegar on germination and radicle growth was thought to be a synergetic effect of many components. The acetic fraction, especially acetic acid, acts as a good solvent for other components and has its own tendency to soften seed bark and induce the production of hormone substances. Low molecular weight components may also make water more accessible so that it can be easily absorbed by seeds. Water is thought to be an important substance for germination and initial growth of seeds. On the other hand, organic components in bamboo vinegar may cause loosening of the cell wall of seed plants and promote radicle and hypocotyl growth of seeds. Despite with these hypotheses, the cause of the promotion effects of bamboo vinegar is still unclear and requires further investigation.

Conclusions

The physical and chemical characteristics of moso bamboo vinegars from different collection temperatures reflected the changing composition of the vinegar during pyrolysis. Although the vinegars from different collection temperatures showed almost the same composition, the relative contents of the main compounds in the vinegar appeared to vary with collection temperature, especially for acetic acid and the acetic fraction. The results of bioassays on two seed types with vinegars from eight collection temperature ranges showed the tendency of vinegars collected at 250°–400°C to inhibit radicle and hypocotyl growth of seeds. Chrysanthemum germination and initial growth of radicle and hypocotyl were markedly promoted with vinegar collected at 200°–250°C. Watercress was promoted less than chrysanthemum. In consideration of the bioassay results of the two seeds, vinegar collected under 250°C had good regulation effect at 10^3 dilution for chrysanthemum and 10^4 dilution for watercress. Collection temperature could be an important factor for controlling the quality of bamboo vinegars in its production and to aid its further application.

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