

Jun Mu · Tohru Uehara · Takeshi Furuno

Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants

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Abstract Two kinds of bamboo vinegar from madake bamboo (*Phyllostachys bambusoides*) and moso bamboo (*Phyllostachys pubescens*) were prepared to analyze their components by gas chromatography (GC). The original vinegar, distilled vinegar, ether-extracted vinegar, and three fractions including acidic, neutral, and phenolic fractions separated from ether-extracted vinegar were diluted with distilled water 10^2 to 10^7 times. These diluted vinegar solutions were used to investigate the effect of bamboo vinegar on the germination and radicle growth of seed plants. High concentrations of all kinds of treated bamboo vinegars (e.g., 10^2 of original vinegar and 10^3 of ether-extracted vinegar) showed strong inhibition against germination of the seeds. However, an appropriate dilution of bamboo vinegar showed an obvious promotional effect on germination and radicle growth for the four kinds of tested seeds (lettuce, watercress, honewort, chrysanthemum).

Key words Bamboo vinegar · Germination · Radicle growth · Seed plants · Promotion effect

Introduction

Bamboo vinegar, a by-product of bamboo carbon, is a brown-red transparent liquid collected during pyrolysis of bamboo carbon. Bamboo vinegar is composed of nearly 90% water plus other chemical components. Of these, there are 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.

J. Mu · T. Uehara (✉) · T. Furuno
Faculty of Science and Engineering, Shimane University, Matsue
690-8504, Japan
Tel. +81-852-32-6565; Fax +81-852-32-6123
e-mail: uehara@riko.shimane-u.ac.jp

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Bamboo vinegar was found to have a regulatory effect on the germination and radicle growth of seed plants,¹ with many studies having researched this area.^{2–6} However, there has been no systematic research on the chemical analysis and application of bamboo vinegar. Japan and other South-east Asian areas are rich in bamboo resources that have not been sufficiently utilized. In Japan, moso and madake bamboo comprise about 80% of the entire bamboo population. The excellent qualities of bamboo carbon encourage the use of bamboo itself as well as bamboo vinegar. Moreover, when considering the protection of wood resources and environmental balance, bamboo and its products show good prospects for application.

This study examined bamboo vinegar for its characteristics and applications. Further research on the effective components of bamboo is ongoing.

Experimental

Madake bamboo (*Phyllostachys bambusoides* Sieb. et Zucc.) vinegar and moso bamboo (*Phyllostachys pubescens* Mazel) vinegar (Shyowa Kikaku Co.) were the materials evaluated.

Treatment of material

Original bamboo vinegar was distilled under nitrogen, and distillation fractions up to 200°C were collected. Original vinegar was also extracted with ether to obtain ether-extracted vinegar, which was then further separated into acidic, neutral, and phenolic fractions in the usual way.

Physical and chemical characteristics

The specific gravity, refractive index, pH, and acidity of the original vinegar, the ether-extracted vinegar, and the distilled vinegar were measured. The acidity is expressed as the weight percent of acetic acid in the bamboo

vinegar, which was converted by titration with 0.1 N sodium hydroxide.

Gas chromatography analysis

The conditions for gas chromatography (GC) analysis were as follows: column was Shimadzu Hicap CBP20-M25 (0.25 mm i.d. × 25 m; PEG-20M); temperature 60–200°C increased at 5°C/min; splitter ratio 60:1; carrier gas was helium; detector was a flame ionization detector (FID).

Components were identified by the retention time of their peaks compared with those in the literature.⁷ Authentic compounds were also used to compare and identify the components. The components were quantified based on the area percent.

Bioassay

The original vinegar (OV), distilled vinegar (DV), ether-extracted vinegar (EEV), acidic fraction of the vinegar

(AFV), neutral fraction of the vinegar (NFV), and phenolic fraction of the vinegar (PFV) were the tested solutions, all of which were diluted 10² to 10⁷ times with distilled water. Distilled water was used as the control. Tested solvent (10 ml) was poured into a petri dish (diameter 9 cm, depth 1.5 cm) that contained two pieces of filter paper (Advantec no. 2). Twenty seeds were scattered on the filter paper and were allowed to stand in a dark room controlled at 20°C and 60% relative humidity (RH). The bioassay period was 4 days from the first day of germination. The radicle growth

Table 1. Fractionation of bamboo vinegars

Component	Madake bamboo vinegar (%)	Moso bamboo vinegar (%)
Ether-extracted vinegar	12.18	12.83
Acid fraction	3.09	4.41
Neutral fraction	0.23	0.18
Phenolic fraction	1.64	1.49

Values are the percents of the gathered weight/weight of the original vinegar

Table 2. Physical and chemical characteristics of madake and moso bamboo vinegar

Sample	Specific gravity (d ₂₀ ²⁰)	Refractive index (n _D ²⁰)	pH	Acidity (%)
Original vinegar				
Madake bamboo	1.0246	1.3491	2.8	5.88
Moso bamboo	1.0257	1.3492	2.8	6.95
Distilled vinegar				
Madake bamboo	1.0130	1.3409	2.4	5.35
Moso bamboo	1.0095	1.3410	2.4	6.37
Ether-extracted vinegar				
Madake bamboo	1.0031	1.4197	2.0	35.05
Moso bamboo	0.9666	1.4110	2.0	38.23

d₂₀²⁰, value of vinegar weight at 20°C to the weight of distilled water at 20°C measured with pycnometer; n_D²⁰, refractive index measured at 20°C by Abbe refractometer using D-line

Table 3. Components in fractionated madake and moso bamboo vinegar liquor^a

Component	Acidic fraction (%)		Neutral fraction (%)		Phenolic fraction (%)	
	Madake	Moso	Madake	Moso	Madake	Moso
2-Cyclopentenone	–	3.11	–	2.20	–	–
Acetic acid	38.35	43.3	–	–	–	–
Furfural	–	–	3.28	6.18	–	–
2-Acetylfuran	–	–	0.68	4.31	–	5.71
Propionic acid	13.17	5.97	–	–	–	–
3-Methyl-2-cyclopentenone	–	–	1.56	7.21	–	–
<i>n</i> -Butanoic acid	2.20	0.54	–	–	–	–
5-Methylfurfural	–	–	2.73	4.07	–	–
Acetophenone	1.09	1.54	9.49	23.97	–	2.24
2,4-Dimethyl-2-butenolide	3.95	4.24	–	–	–	–
Guaiacol	–	–	–	–	6.70	8.00
4-Methylguaiacol	–	–	–	–	15.98	1.83
Phenol+ <i>o</i> -cresol	0.86	0.87	7.39	1.82	19.89	32.14
4-Ethylguaiacol	–	–	–	–	0.76	1.15
<i>p</i> -Cresol	–	–	5.96	3.00	2.98	4.67
<i>m</i> -Cresol	–	–	–	–	1.67	2.37
2,3-Xylenol	–	–	9.26	4.61	1.79	3.59
Syringol	1.90	3.15	–	–	8.65	7.01
Total ^b	61.52	62.72	40.35	57.37	54.82	68.71

^a Figures are the percents based on all peaks as 100% in each fraction

^b Sum of percents of components identified in each fraction

was measured and compared with the controls after 4 days. The bioassay test was repeated three times for each sample. The tested seeds were lettuce (*Lactuca sativa* L.), watercress (*Rorippa nasturtium-aquaticum* Hayek), honewort (*Cryptotaenia japonica* Hassk.), and chrysanthemum (*Chrysanthemum coronarium* L.).

Results

Characteristics of bamboo vinegar

Table 1 shows the percents of ether-extracted bamboo vinegars and the three fractions of the ether-extracted vinegar. There were 12.18% and 12.83% of ether-extracted vinegars for madake and moso bamboo vinegars, respectively. Of the bamboo vinegars, the acidic fraction was the main fraction

followed by the phenolic fraction. Compounds in the neutral fraction were present in trace amounts. Table 2 shows the physical and chemical qualities of madake and moso bamboo vinegars. Based on our measurements, the pH of distilled vinegar was lower than that of the original vinegar owing to the loss of water during distillation. The ether-extracted vinegar showed strong acidity because most of compounds in it were in the acidic fraction. The refractive index of the treated vinegar increased after ether extraction.

GC analysis

Table 3 shows the main compounds identified from GC analysis in the acidic, neutral, and phenolic fractions of madake and moso bamboo vinegars. In the acidic fraction, acetic acid was the main component (present in large

Table 4. Germination rates for watercress with madake and moso bamboo vinegars

Vinegar and dilution	Madake bamboo vinegar		Moso bamboo vinegar	
	1st Day	4th Day	1st Day	4th Day
OV				
10 ²	0	0	0	0
10 ³	96 (5.7)	100 (3.5)	109 (4.5)	100 (5.8)
10 ⁴	119 (5.5)*	110 (4.3)	113 (4.1)*	105 (2.4)
10 ⁵	103 (7.8)	101 (6.9)	90 (1.8)*	83 (3.0)*
10 ⁶	113 (2.7)*	102 (2.0)	109 (2.0)*	100 (3.2)
10 ⁷	96 (2.2)	94 (6.6)	95 (5.2)	95 (8.9)
DV				
10 ²	0	0	0	0
10 ³	95 (5.5)	102 (5.6)	83 (2.3)*	86 (4.2)
10 ⁴	105 (2.3)	105 (8.7)	91 (2.3)*	91 (4.9)
10 ⁵	119 (2.7)**	106 (8.9)	109 (2.9)*	104 (4.9)
10 ⁶	97 (4.3)	92 (3.8)	98 (5.5)	95 (2.9)
10 ⁷	107 (6.7)	103 (3.8)	87 (3.8)*	98 (1.7)
EEV				
10 ³	0	0	0	0
10 ⁴	81 (6.7)	102 (4.9)	49 (4.6)**	93 (6.4)
10 ⁵	100 (4.0)	108 (9.0)	89 (1.6)	86 (3.4)
10 ⁶	99 (1.9)	92 (4.9)	101 (6.5)	99 (1.9)
10 ⁷	106 (5.7)	108 (7.4)	85 (6.0)	95 (2.9)
AFV				
10 ³	0	0	0	0
10 ⁴	91 (5.5)	102 (2.0)	107 (2.3)*	101 (8.7)
10 ⁵	116 (9.8)	104 (3.7)	92 (3.8)	80 (4.2)*
10 ⁶	124 (9.1)*	110 (2.1)*	104 (2.9)	98 (6.4)
10 ⁷	101 (10.3)	102 (5.6)	87 (2.8)	93 (6.4)
NFV				
10 ³	0	0	0	0
10 ⁴	29 (4.4)*	95 (14.3)	16 (3.2)**	82 (6.9)
10 ⁵	105 (4.7)	100 (6.3)	79 (2.9)*	95 (2.9)
10 ⁶	104 (2.2)	102 (2.0)	107 (0.3)**	102 (2.0)
10 ⁷	108 (6.1)	104 (2.0)	93 (9.9)	99 (4.7)
PFV				
10 ³	0	0	0	0
10 ⁴	37 (10.1)*	89 (3.2)	33 (13.3)*	78 (4.2)*
10 ⁵	111 (9.0)	105 (8.7)	92 (4.2)	97 (3.3)
10 ⁶	121 (8.4)	108 (1.7)*	92 (2.7)	88 (4.0)
10 ⁷	106 (4.4)	110 (5.5)	73 (4.7)*	89 (2.6)

Results are percentages based on the control; figures in parentheses are the standard error. OV, original vinegar; DV, distilled vinegar; EEV, ether-extracted vinegar; AFV, acidic fraction of ether-extracted vinegar; NFV, neutral fraction of ether-extracted vinegar; PFV, phenolic fraction of ether-extracted vinegar.

* $P < 0.05$; ** $P < 0.01$

quantities) followed by propionic acid, 2,4-dimethyl-2-butenolide, and *n*-butanoic acid. In the neutral fraction furfural, 2-acetylfuran, 3-methyl-2-cyclopentenone, 5-methylfurfural, and acetophenone were identified as neutral compounds. In the phenolic fraction guaiacol, 4-methylguaiacol, phenol + *o*-cresol, 4-ethylguaiacol, *p*-cresol, *m*-cresol, and syringol were identified as the main components.

Bioassay test

Germination of watercress

The germination rates of watercress in madake and moso bamboo vinegars are shown in Table 4. For watercress seeds, the OV and DV showed a strong inhibitory effect on germination at a dilution of 10^2 , as did the EEV and its three fractions at a dilution of 10^3 . According to the measurements of the components of madake bamboo vinegar

(Table 1) there was 12.2% ether-extracted vinegar; therefore, when the content of the ether-extracted organic components of the test solution comprised more than 0.012% of the vinegar, there was a strong inhibitory effect on seed germination. Obviously, a high concentration of vinegar inhibited germination. The same germination effect appeared with two of the original vinegars: A dilution of 10^4 had the highest germination ratio, whereas dilutions of 10^3 and 10^7 had only a slight inhibitory effect. A dilutional change produced a different effect on the germination rate of watercress.

A 10^5 dilution of distilled vinegar produced a higher germination rate than other dilutions. That distilled vinegar accelerated the germination at a higher dilution compared to the OV might be due to the fact that it has a lower pH than the OV (Table 2).

The ether-extracted vinegar and its three fractions showed a promotional effect on germination at a dilution of 10^6 . Among the solutions tested, the acidic fraction had the

Table 5. Germination rates of lettuce with madake and moso bamboo vinegars

Vinegar and dilution	Madake bamboo vinegar		Moso bamboo vinegar	
	1st Day	4th Day	1st Day	4th Day
OV				
10^2	0	0	0	0
10^3	90	98 (1.7)	97 (3.3)	100 (2.9)
10^4	98 (1.7)	100	100 (2.9)	102 (1.7)
10^5	92 (1.7)	97 (1.7)	102 (1.7)	102 (1.7)
10^6	98 (1.7)	100	100 (2.9)	100 (2.9)
10^7	95 (2.9)	95 (1.7)	98 (1.7)	102 (1.7)
DV				
10^2	0	0	0	0
10^3	93 (1.7)	98 (1.7)	89 (4.7)	102
10^4	97 (1.7)	98 (1.7)	97 (1.7)	102 (1.7)
10^5	100	100	98 (1.7)	100 (2.9)
10^6	98 (1.7)	100	102 (1.7)	102 (1.7)
10^7	97 (1.7)	97 (1.7)	90 (5.2)	100 (5.0)
EEV				
10^3	0	0	0	0
10^4	80 (8.7)*	97 (3.3)	49 (7.0)*	83 (14.9)
10^5	93 (1.7)	97 (3.0)	100	102 (1.7)
10^6	95 (2.9)	95 (2.9)	98 (1.7)	98 (1.7)
10^7	100	100	90 (3.2)	98 (1.7)
AFV				
10^3	0	0	0	0
10^4	13 (1.7)**	60 (2.9)**	98 (3.3)	102 (1.7)
10^5	97 (1.7)	98 (1.7)	95	95
10^6	97 (1.7)	100	102 (1.7)	102 (1.7)
10^7	98 (1.7)	100	98 (1.7)	100 (2.9)
NFV				
10^3	0	0	0	0
10^4	5 (2.9)**	5 (4.4)**	0	9 (6.3)**
10^5	88 (6.7)	98 (1.7)	97 (3.3)	100 (2.9)
10^6	100	100	96 (3.7)	100
10^7	98 (1.7)	100	100 (2.9)	100 (2.9)
PFV				
10^3	0	0	0	0
10^4	32 (8.3)**	70 (6.7)*	90 (7.6)	98 (4.4)
10^5	97 (3.3)	98 (1.7)	97 (1.7)	98 (1.7)
10^6	100	100	100	102 (1.7)
10^7	95 (2.9)	100	100 (2.9)	102 (1.7)

Results are percentages based on the control; figures in parentheses show the standard error
* $P < 0.05$; ** $P < 0.01$

highest germination rate (124%) on the first day, indicating that the acidic fraction had more promotional effect on the initial stage of germination of watercress seeds.

Germination of lettuce

The germination rates for lettuce in the presence of madake and moso bamboo vinegars are shown in Table 5. Lettuce seeds had a nearly 100% germination rate by themselves, so the effects of vinegar on germination were not obvious. For lettuce seeds the madake bamboo vinegar caused more inhibition than moso bamboo vinegar. Furthermore, all of the NFV at a dilution of 10^4 showed a strong inhibitory effect on germination with both madake and moso bamboo vinegars.

Germination of honewort

Table 6 shows the germination rate of honewort in the presence of madake and moso bamboo vinegars. Honewort

seeds in madake bamboo vinegar showed a high germination rate on the first day with an appropriate dilution: especially with 10^4 dilution of OV and DV, 10^7 dilution of EEV, 10^5 dilution of the AFV, and 10^6 dilution of NFV. The AFV exhibited a higher germination rate on both the first and fourth days. Based on the fact that madake bamboo vinegar increased germination at an early stage, the components in madake bamboo vinegar may be said to have an effect on the initial germination of honewort seeds. In contrast, moso bamboo vinegar produced a stronger inhibitory effect on the first germination day, although it did not cause a decrease in germination by the fourth day.

Germination of chrysanthemum

Table 7 shows the germination rate of chrysanthemum in the presence of madake and moso bamboo vinegars. The effect of vinegars on the germination of chrysanthemum seeds appeared to be somewhat different from that for the other three seeds. Chrysanthemum seeds had slight germi-

Table 6. Germination rates of honewort with madake and moso bamboo vinegars

Vinegar and dilution	Madake bamboo vinegar		Moso bamboo vinegar	
	1st Day	4th Day	1st Day	4th Day
OV				
10^2	0	0	0	0
10^3	126 (2.9)*	104 (2.8)**	98 (4.1)	85 (4.1)
10^4	138 (7.3)	97 (2.1)	80 (2.6)**	104 (0.9)
10^5	94 (3.0)	104 (5.4)*	93 (3.5)	82 (2.1)*
10^6	92 (3.5)	102 (4.2)	97 (4.0)	100 (2.7)
10^7	182 (9.6)	105 (7.9)	55 (5.8)**	102 (2.8)
DV				
10^2	0	0	0	0
10^3	106 (4.6)	103 (1.3)	75 (2.9)**	98 (1.5)
10^4	166 (3.8)*	111 (3.8)**	56 (3.2)**	94 (2.0)
10^5	96 (2.9)	109 (5.9)*	96 (3.2)	92 (3.2)
10^6	102 (6.2)	95 (6.0)	71 (2.9)**	106 (1.8)
10^7	71 (2.3)*	100	108 (3.9)	103 (2.3)
EEV				
10^3	0	0	0	0
10^4	79 (2.3)	94 (3.1)	38 (3.6)**	86 (1.0)
10^5	124 (2.1)*	99 (7.8)	76 (2.6)**	98 (2.0)
10^6	116 (8.1)	100 (9.4)	65 (4.8)**	92 (2.3)
10^7	159 (2.3)*	96 (7.4)	86 (2.0)*	98 (2.9)
AFV				
10^3	0	0	0	0
10^4	50 (4.4)*	91 (8.5)	63 (4.0)**	97 (2.3)
10^5	174 (3.5)*	110 (5.8)	44 (3.0)**	95 (2.6)
10^6	129 (5.9)	104 (4.3)	65 (2.9)**	104 (1.5)
10^7	162 (10.1)**	96 (5.5)	93 (5.8)	96 (4.1)
NFV				
10^3	0	0	0	0
10^4	42 (2.3)*	92 (4.9)	58 (2.3)**	92 (3.5)
10^5	94 (4.1)	100 (6.3)	32 (7.8)**	98 (2.3)
10^6	157 (3.3)*	102 (2.0)	38 (4.0)**	100 (1.5)
10^7	121 (8.3)*	102 (5.5)	54 (2.2)**	90 (2.6)
PFV				
10^3	0	0	0	0
10^4	87 (3.3)*	75 (7.5)*	33 (3.7)**	95 (2.0)
10^5	113 (6.7)	106 (0.3)**	100 (5.1)	106 (3.2)
10^6	85 (4.7)	105 (1.2)**	80 (5.0)*	92 (6.9)
10^7	111 (5.7)	96 (9.0)	70 (5.5)*	107 (4.5)

Results are percentages based on the control; figures in parentheses show the standard error
* $P < 0.05$; ** $P < 0.01$

Table 7. Germination rates of chrysanthemum with madake and moso bamboo vinegars

Dilution	Madake bamboo vinegar		Moso bamboo vinegar	
	1st Day	4th Day	1st Day	4th Day
OV				
10 ²	0	0	0	0
10 ³	97 (5.7)	85 (2.2)	110 (5.8)	101 (2.3)
10 ⁴	81 (0.9)**	79 (6.2)*	106 (4.3)	121 (3.8)**
10 ⁵	77 (2.2)**	75 (4.4)*	99 (3.1)	113 (3.1)*
10 ⁶	86 (2.9)*	79 (4.0)*	94 (0.7)	99 (4.7)
10 ⁷	91 (5.6)	79 (2.7)*	92 (6.2)	109 (2.1)*
DV				
10 ²	0	0	0	0
10 ³	86 (2.9)*	72 (4.4)**	67 (3.3)**	95 (1.7)
10 ⁴	100 (2.8)	76 (5.2)*	71 (6.1)*	108 (4.4)
10 ⁵	62 (4.4)**	62 (7.3)*	52 (2.3)**	85 (4.0)
10 ⁶	72 (5.2)*	81 (4.3)	76 (3.1)**	104 (2.4)
10 ⁷	74 (5.0)*	85 (3.3)	113 (3.3)*	87 (3.2)
EEV				
10 ³	0	0	0	12 (3.2)**
10 ⁴	95 (2.3)	90 (2.2)	70 (5.8)*	88 (3.7)
10 ⁵	112 (2.7)*	98 (5.3)	88 (2.3)*	107 (3.8)
10 ⁶	87 (7.3)	74 (5.6)*	102 (4.2)	123 (1.2)**
10 ⁷	71 (7.0)*	68 (7.3)*	72 (3.9)*	113 (2.3)*
AFV				
10 ³	0	0	13 (3.3)**	69 (9.5)*
10 ⁴	77 (6.4)*	72 (5.2)*	48 (3.7)**	68 (3.5)*
10 ⁵	71 (2.0)**	66 (5.1)*	74 (5.7)	121 (6.4)*
10 ⁶	89 (1.9)	81 (4.1)*	104 (6.3)	134 (2.1)**
10 ⁷	81 (3.2)*	77 (5.4)*	63 (3.0)**	109 (5.5)
NFV				
10 ³	0	0	27 (7.3)*	72 (3.9)*
10 ⁴	21 (2.7)**	40 (8.5)**	50 (5.8)*	56 (5.7)**
10 ⁵	49 (9.2)*	62 (2.6)**	71 (4.7)*	97 (7.3)
10 ⁶	87 (2.3)	76 (6.2)*	110 (2.9)*	104 (6.1)
10 ⁷	82 (0.3)	75 (3.2)*	83 (3.0)**	107 (3.1)
PFV				
10 ³	22 (7.4)**	29 (7.2)**	0	46 (5.6)**
10 ⁴	56 (5.2)**	85 (2.7)	63 (3.5)*	77 (2.6)**
10 ⁵	89 (3.1)	83 (1.2)	78 (2.0)*	110 (9.0)
10 ⁶	73 (4.8)*	73 (6.4)*	72 (2.9)**	115 (5.0)
10 ⁷	64 (5.6)**	73 (4.5)*	116 (3.2)*	131 (6.3)*

Results are percentages based on the control; figures in parentheses show the standard error
* $P < 0.05$; ** $P < 0.01$

nation with a 10³ dilution of EEV, AFV, NFV, and PFV in some cases, whereas the other three seeds had no germination at all with the same dilutions. It was with the acidic fraction of moso bamboo vinegar at a 10⁶ dilution that the germination rate of chrysanthemum seeds was highest (134%).

Radicle growth with madake bamboo and moso bamboo vinegar

Figure 1 shows the radicle growth rate of four kinds of seeds in the presence of madake bamboo vinegar. Figure 2 shows the effect of moso bamboo vinegar. Madake bamboo vinegar caused obvious comprehensive promotion in the radicle growth of the seeds tested, except watercress seeds, of which exhibited the highest radicle growth rate based on that of the control (>100%). The effect on radicle growth of the four seeds tested with moso bamboo vinegar seem to be nearly the same as with madake bamboo vinegar, but the promotional effect was not obvious as with madake bamboo vinegar.

Although germination was inhibited, especially with high concentrations of ether-extracted vinegar and its three fractions, radicle growth was not. NFV of madake bamboo vinegar markedly promoted radicle growth at a dilution of 10⁴ for chrysanthemum seeds, in which radicle length was more than twice that of the control. AFV and PFV at 10⁴ dilution also showed good promotion of radicle growth for lettuce and chrysanthemum seeds.

Although a promotional effect on radicle growth was recorded, there was no clear relation between the compounds and the effect because of the complexity of the compounds in each fraction. We inferred that the compounds in AFV, NFV, and PFV might have qualities similar to those of hormonal substances such as auxin and gibberellin, or the compounds might be precursors that induce hormone-like substances in the seeds to affect radicle growth.^{8,9}

The four tested seeds showed different reactions, reflected in the bioassay of radicle growth. The promotional effects on chrysanthemum and lettuce were greater than those on watercress or honewort. This difference may be

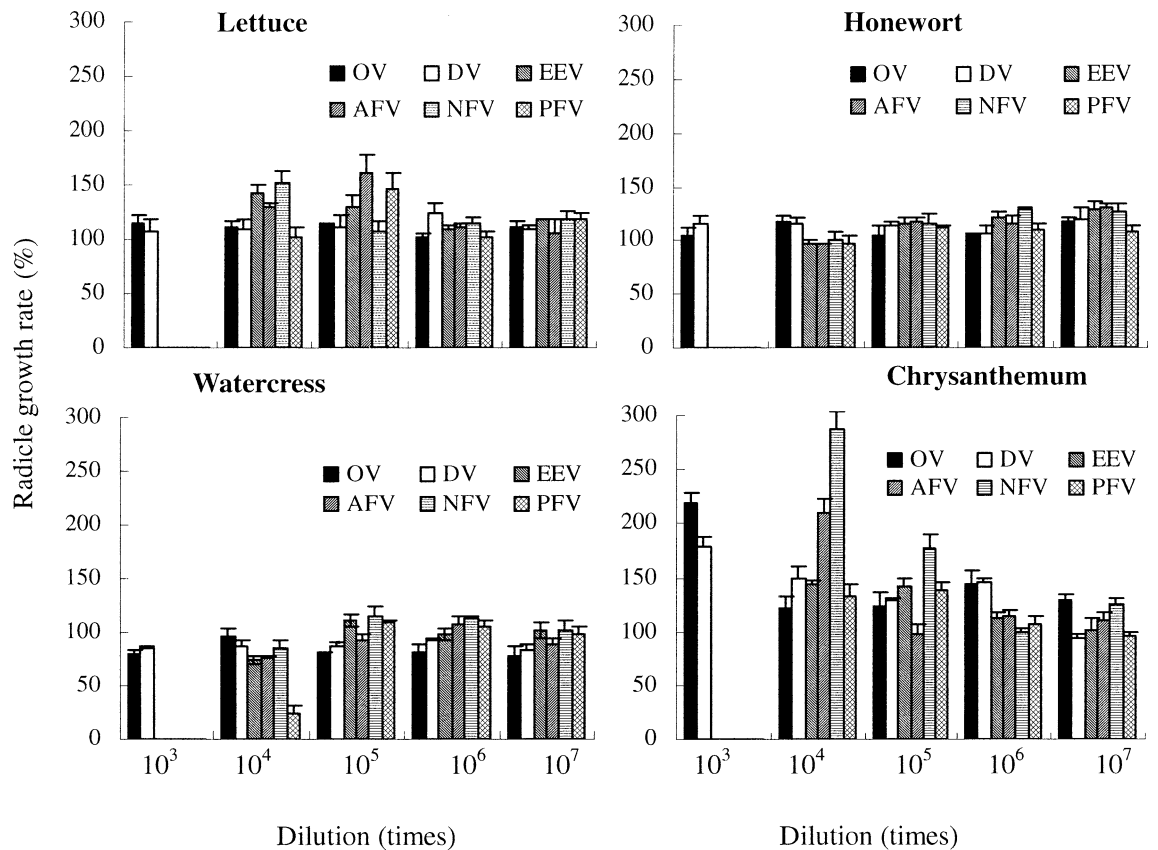


Fig. 1. Radicle growth of four kinds of seeds with madake bamboo vinegar. *OV*, original vinegar; *DV*, distilled vinegar; *EEV*, ether-extracted vinegar; *AFV*, acidic fraction of ether-extracted vinegar; *NfV*,

neutral fraction of ether-extracted vinegar; *PFV*, phenolic fraction of ether-extracted vinegar. T-bars show the standard error

due to the character of the seeds, causing different degrees of sensitivity to the bamboo vinegar.

Discussion

Many biologically active substances are found in the extractives of trees, such as terpenoids, phenolics, alkaloids, and fats.¹⁰ Some substances have a regulatory effect on plant growth. Bamboo vinegar, unlike the extractives from trees, contains numerous low-molecular-weight compounds, many in only trace amounts, along with about 90% water, but they appeared to have a regulatory effect on the seeds' growth.

According to the bioassays of four seeds, the dilution of bamboo vinegar played an important role in regulating germination and radicle growth of these seeds. The promotional effect of bamboo vinegar appeared at a low concentration. This is in accord with hormonal substances, which have an effect at only trace concentrations. High concentrations of bamboo vinegar inhibited germination and radicle growth.

As for the acidic, neutral, and phenolic fractions, it is difficult to determine the action of single components because of the difficulty of separating the many components that are present in only trace quantities. We had thought

that regulation was due to the comprehensive effect of compounds of all the fractions in bamboo vinegar. However, according to previous research, although wood vinegar seemed to promote seed germination, single components, such as acetic acid, did not show any promotional effect.¹¹

In bamboo vinegar, *AFV* appeared to promote seed germination at a higher rate, which could be explained by its stimulating a break in the seeds' dormancy and softening the seed skin. *PFV* showed more inhibition on germination as a neutral fraction than did the *AFV*. Chemicals with aromatic structures were thought to inhibit plant growth more than promote it.¹² However, *NfV* and *AFV* obviously promoted radicle growth, which may be due to their inducing hormone-like substances to affect the growth of the seeds. Original bamboo vinegar had the largest promotional effect due to the organic components at various concentrations.

We compared the effects of wood vinegar versus bamboo vinegar on the regulation of seed growth. Bamboo vinegar did not postpone the time of germination, whereas wood vinegar postponed germination by 1–2 days for the same kinds of seeds. Furthermore, bamboo vinegar showed an obvious promotional effect on radicle growth versus that of wood vinegar for the same seeds. For example, chrysanthemum seeds had radicle growth rates of 219% and 174% with *OV* of madake and moso bamboos, respectively, whereas the same seeds had a radicle growth rate of 106%

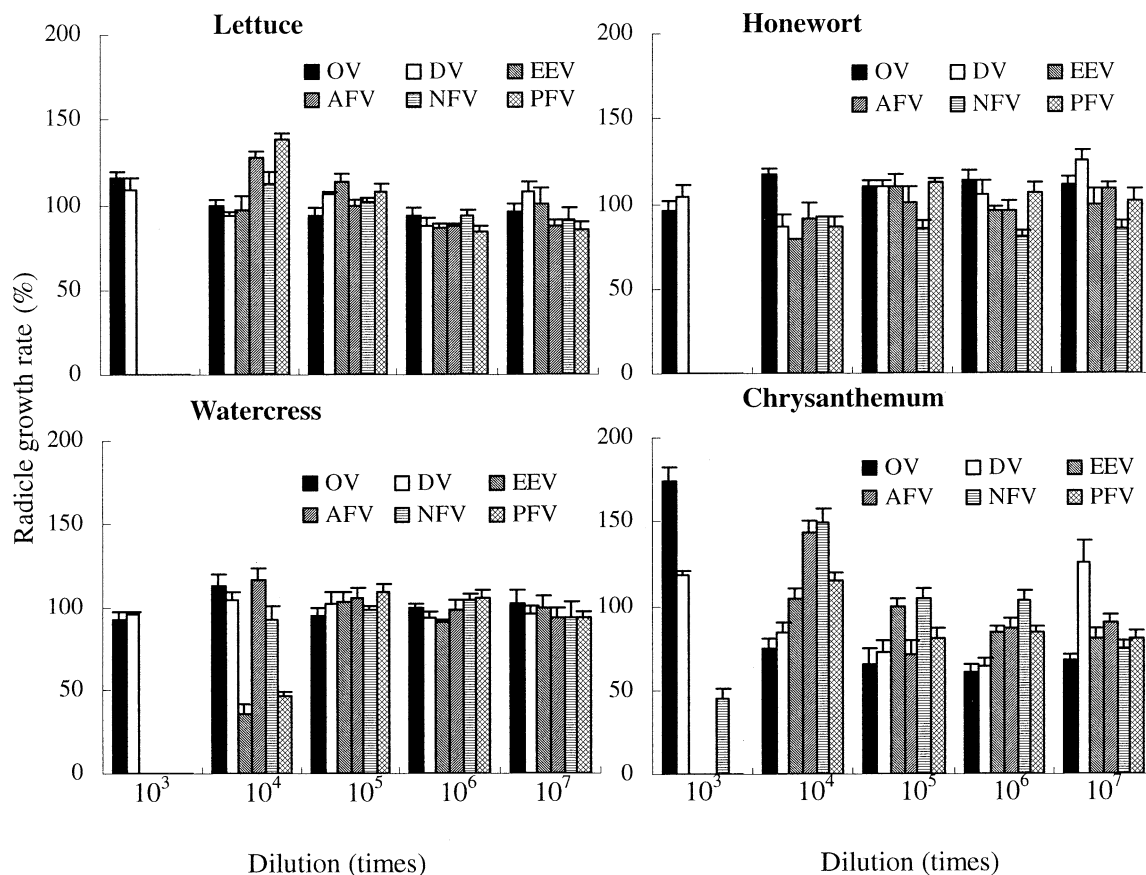


Fig. 2. Radicle growth of four kinds of seeds with moso bamboo vinegar

with wood OV.² The different effects of wood vinegar and bamboo vinegar on seeds might be due to their physical and chemical characteristics. Bamboo vinegar contains more acidic components with a higher acidity value than wood vinegar. In addition, bamboo vinegar has fewer high-boiling-point materials (e.g., tars) than wood vinegar, and they are toxic to most plants' growth.¹ Bamboo vinegar and wood vinegar had nearly the same main pyrolytic components; the quantity difference was due to the difference in three main chemicals: cellulose, hemicellulose, and lignin.¹³ Bamboo had more hemicellulose with pentosanas as the main component, which would produce more compounds contained in AFV and NFV during pyrolysis. On the other hand, enough material resources of bamboo and its similar chemical quality supported the use of bamboo vinegar with its more stable quality than wood vinegar. Bamboo vinegar also exhibits some quality differences depending on the method used to produce carbon, the collection temperature, and the refinement method, all of which should be taken into account when bamboo vinegar is being used.

Conclusions

Bamboo vinegar has an obvious acceleration effect on germination and radicle growth, provided it is present in an

appropriate dilution in accordance with the bioassays of four types of seeds. These experiments indicated that the dilution of the vinegar was an important factor for germination and radicle growth. A too-high concentration of vinegar inhibits germination and radicle growth of the seeds.

In bamboo vinegar, AFV showed less inhibition of germination than NFV and PFV. NFV and AFV showed more obvious promotion of radical growth than PFV at the same dilution.

It can therefore be concluded that the promotional effect of bamboo vinegar stimulates the hormone-like substances to regulate germination and radicle growth of the seeds with a synergistic effect on components in the bamboo vinegar.

Bamboo vinegar exists in nature, and it has demonstrated its usefulness by allowing the use of less or no fertilizer in the agricultural sector. It is destined to play an important role in people's lives in the near future.

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