Effect of Fractionated Palm Fruit Shell Bio-Oil on Seed Germination

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Abstract

Palm fruit shell was pyrolyzed in a closed simple reactor. The physical and chemical properties of the pyrolytic oil (bio-oil) were characterized. The effect of original and fractionated bio-oil was analyzed as a germination promoting agent. Raw palm shell bio-oil character differed from that of wood and other biomass. The pyrolysis temperature caused differences in yield and acetic acid content, but there were no significant differences in specific gravity, pH, or the major chemical components of shell bio-oil. The effect of shell bio-oil on germination and radicle growth of seeds depended on pyrolysis temperature, fraction, and dilution rate. Original shell bio-oil had the best effect on germination and radicle growth. Dilutions of 10^2 and 10^3 inhibited germination and radicle growth for three kinds of seeds.

The US Department of Agriculture (USDA) reported that global palm oil and palm oil derivative consumption reached a record 37.3 million metric tons in 2006 and 2007, representing an increase of 13.2 million metric tons since 2001 (USDA 2006). Palm oil production creates waste, such as fronds, fibers, mesocarp, kernel cakes, palm shells, and liquid waste, in huge quantities annually, which requires proper handling to avoid polluting the environment (Saono and Sastrapradja 1983, Sheshadri 1983). The governments of palm oil–producing countries are responsible for establishing standards for enforcement of waste management practices through their regulations, while the private sector is responsible for using technologies that minimize waste creation.

Recently, there is an increasing awareness that biomass and organic wastes are valuable feedstocks to substitute for dwindling petrochemical resources through pyrolytic processes that produce bio-oil, bio-char, and charcoal (Mohan et al. 2006, Vasile and Brebu 2006, Abdullah and Gerhauser 2008, Phan et al. 2008, Carrier et al. 2010). These processes could address environmental problems and ensure the efficient use of natural resources by recycling abandoned biomass.

Pyrolysis is described as the thermal degradation of materials in either the complete absence or the inadequate presence of oxygen (Bridgwater et al. 1999). In general, pyrolysis of organic materials produces gaseous and liquid products and leaves a solid residue rich in carbon. One useful product of pyrolytic processes is bio-oil (a liquid product), which has interested scientists from ancient to modern times (Nakai et al. 2007).

Bio-oil is composed of a very complex mixture of oxygenated hydrocarbons with an appreciable proportion of water, and it is similar to biomass in elemental composition. Typically, bio-oil is reactive, and its properties change rapidly during condensation and storage (Piskorz et al. 1988). Scientists use bio-oil in many applications. (Keely and Pizzorno 1986, Girard 1992, Jager et al. 1996).

Following reports of seed germination stimulated by bio-oil, scientists have attempted to characterize and isolate the chemicals in plant-derived bio-oil responsible for its phenomenal promotion of germination. Keely and Pizzorno (1986) noted that aqueous bio-oil extract prepared from a range of plants or from extracts prepared by heating agar and cellulose (200°C) contained compounds that stimulated the germination of lettuce seeds. Jager et al. (1996) demonstrated that a wide range of plant materials produced bio-oil that stimulated the germination of lettuce seeds. Further analysis by Flemmatti et al. (2004) revealed that butenolide, contained in bio-oil, was the major active component that stimulated seed germination.

Because palm fruit shell is abundant, we initiated this study with the objective of ascertaining whether it could

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yield an agent stimulating seed germination. The study included characterization of the raw material, palm shell bio-oil properties, and the effect of the bio-oil on germination and radicle growth.

Materials and Methods

Material preparation

Clean (free from impurity) oil palm fruit shell with equilibrium moisture content was obtained from North Sumatra Province, Indonesia. The shells were crushed and sieved to obtain a homogeneous 0.2- to 0.5-cm particle size. Proximate analysis of palm fruit shell was carried out in triplicate following the ASTM Standard D1762-84 (ASTM International 2007b). Soxhlet extraction was conducted to determine holocellulose and alpha-cellulose content following D1104-56 and D1103-60, respectively (American Society for Testing and Materials [ASTM] 1977, 1978), while ASTM D1106-56 (ASTM International 2007a) was followed to determine lignin content (Sjostrom 1981, Fengel and Wegener 1984).

Pyrolysis of oil palm shell

The pyrolysis of shell was carried out using an apparatus consisting of a TK-HV vacuum thermal decomposition unit (Taika Industry Co., Ltd., Matsue, Japan) under two different temperatures, 250°C and 300°C, at a heating rate of 1°C/min for 4 hours. Exhausted gas was condensed by an ice-water column to obtain the pyrolytic liquid.

Physical characteristics of crude shell bio-oil

Specific gravity.—Specific gravity of bio-oil was measured using 10-mL pycnometer glass (ASTM International 2002).

pH.—In this test, bio-oil pH was determined using a Horiba B212 Twin Compact pH meter (Horiba Ltd., Japan) from a single drop of bio-oil on the apparatus.

Acetic acid content.—Acetic acid concentration (percent) in bio-oil was determined by titration. Sodium hydroxide (0.1 N) was reacted with diluted bio-oil in a 10-fold volume of distilled water. Phenolphthalein was used as an indicator to determine the endpoint of titration (Mu et al. 2003).

Fractionation of the crude shell bio-oil

Liquid–liquid extraction was conducted to separate compounds based on their relative solubility in two immiscible liquids. In this work, the raw palm fruit shell bio-oil was fractionated into three fractions: acidic (strong acid), phenolic (weak acid), and neutral by ether extraction following the method in Figure 1 (Mu et al. 2003). Fractionation was performed to determine which fraction gave effects in bioassays.

Gas chromatography

The chemical components of the fractionated palm fruit shell bio-oil were identified by a Shimadzu GC-14A gas chromatograph (GC; Shimadzu Corp., Japan). Operating conditions used were a Shimadzu Hi-cap CBP20-M25 column (0.25-mm inside diameter by 25-mm PEG-20M), helium as the carrier gas, and a flame ionization detector with a temperature range of 60°C to 200°C, a rate of increase of 5°C/min, and a splitter ratio of 60:1. Components were identified by comparing the retention times of peaks with authentic compounds and those in the literature.

Bioassays

Promoting or inhibitory effects that occurred on application of shell bio-oil were evaluated for 20 sample seeds scattered on filter paper (Whatman no. 1) wetted with the shell bio-oil. The bio-oil was diluted 10^2 to 10^7 times with distilled water and used as testing solvent. Deionized water was used as a control. Seeds of species *Cryptotaenia canadensis* (honewort), *Chrysanthemum* sp. (chrysanthemum), and *Nasturtium officinale* (watercress) were incubated in a darkroom at 20°C and 50 percent relative humidity and monitored for 7 days.

The germination and radicle growth rates were assessed as the ratio of germinating seeds showing radicle growth among the tested seeds relative to those among the control seeds. Data obtained were subjected to analysis of variance (ANOVA) and Tukey's multiple range test at a significance level of $P = 0.05$.

Results and Discussion

Characterization of raw material

Proximate analysis revealed that palm fruit shell contained 73.06 percent volatile matter, 11.29 percent moisture, 2.37 percent ash, and 13.3 percent fixed carbon. Tests for holocellulose, lignin, alpha-cellulose, and extractives resulted in 78.16, 6.98, 5.41, and 1.37 percent, respectively. This analysis showed that the volatile matter of palm fruit shell was not as high as that of wood in general and was similar to many kinds of shells, namely, macadamia shells, hazelnut shells, coconut shell powder, and cotton shells (Parikh et al. 2005). The holocellulose content of palm shell is higher than hardwood, but the lignin content is lower (Fengel and Wegener 1984). Based on its composition, palm fruit shell meets the main criterion for bio-oil production raw material because it tends to give a high yield of liquid product (Parikh et al. 2005, Mohan et al. 2006).

Yield and physical characteristics of bio-oil

The yield and physical properties of bio-oil obtained from palm fruit shells under two pyrolysis temperatures (250°C and 300°C) are given in Table 1. In this study, palm fruit shell bio-oil properties strongly depended on the pyrolysis temperature. Yield of bio-oil depends on many factors, including composition of raw material and pyrolysis temperature. The degraded material mixed with moisture released during pyrolysis creates a microemulsion that determines the yield (Hon and Shiraishi 2001, Demirbas 2004, Zhang et al. 2006).

Bio-oil is formed by depolymerizing and fragmenting cellulose, hemicelluloses, and lignin under high temperature and with limited oxygen (Mohan et al. 2006). Holocellulose (cellulose and hemicellulose) is partially degraded at 250°C. Pyrolysis rearranges guaiacols, catechols, syringols, vanillins, furancarboxaldehydes, isoeugenol, pyrones, acetic acid, formic acid, and other carboxylic acids (Piskorz et al. 1988, Zhang et al. 2006).

Hemicellulose, which decomposes at 200°C to 260°C, yields significant acetic acid, which plays an important role in determining the yield and directly influences the pH and acetic acid content of bio-oil (Mohan et al. 2006). Measurements of bio-oil produced at a pyrolysis temperature of 250°C and 300°C, respectively, resulted in a 12.4 and 3.9 percent acetic acid content, while pH values ranged from 2.76 to 2.78. There was a correlation between acetic acid

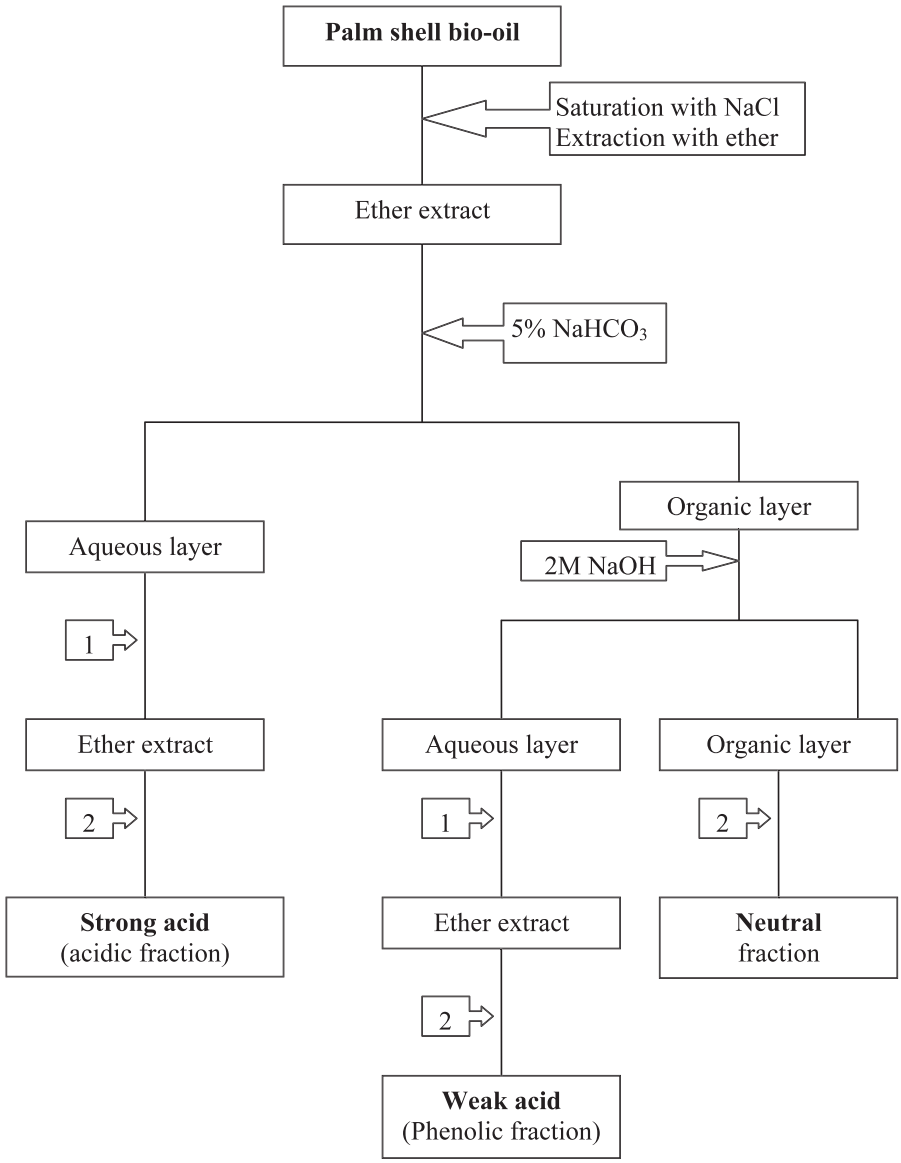

Figure 1.—Diagram of palm fruit shell bio-oil fractionation. (1) Neutralization with 2 N HCl to pH 2 to 3 and extraction with ether. (2) Washing with distilled water and drying with Na_2SO_4, filtration, and distilling out of ether.

content and pH value of palm fruit shell bio-oil. According to Zhang et al. (2006), the amounts of carboxylic, acetic, and formic acids led to low pH values of 2 to 3. The specific gravity of palm fruit shell bio-oil ranged from 1.00 to 1.02, which means it was slightly heavier than water and significantly heavier than fuel oil and the bulk density of the original biomass.

Shell bio-oil fractionation and GC analysis

Fractionation by ether extraction resulted in phenolics fraction being the most widely obtained compared with acidic and neutral fractions of bio-oil produced at temperatures 250°C and 300°C. Table 2 summarizes the relative content of phenolic, acidic, and neutral fractions obtained by ether extraction.

Chemical analysis by GC indicated that only a portion of the fractionated bio-oil could be detected via GC. Complete chemical characterization of fractionated bio-oil was impossible because it contains higher-molecular-weight species, including degradation products of pentoses, hexoses, and lignin (Mohan et al. 2006). In this work, GC analysis

Table 1.—Physical characters of palm fruit shell bio-oil.

Pyrolysis temp (°C)	Yield (%)	Specific gravity	pH	Acetic acid content (%)
250	16.91	1.0041	2.78	12.40
300	29.45	1.0189	2.76	13.90

Table 2.—Relative content of phenolic, acidic, and neutral fractions of ether-extracted palm fruit shell bio-oil.

Pyrolysis temp (°C)	Fraction (%)			Relative to bio-oil (%)
	Phenolic	Acidic	Neutral	
250	41.13	3.42	4.96	49.51
300	41.03	8.69	7.2	56.92

of fractionated bio-oil focused on 16 peaks in chromatograms of the three fractions (Table 3). The results showed eight major peaks in phenolic fraction chromatograms: guaiacol, 4-methylguaiacol, phenol + *o*-cresol, 4-ethylguaiacol, *p*-cresol, *m*-cresol, 2,3-xylenol, and syringol. Phenol + *o*-cresol was the major component found in the phenolic fraction. The acidic fraction had eight peaks: acetic acid, propionic acid, 3-methyl-2-cyclopentenone, *n*-butanoic acid, acetophenone, 2,4-dimethyl-2-butenolide, phenol + *o*-cresol, and syringol. Acetic acid and phenol + *o*-cresol were the major components of the acidic fraction. The neutral fraction contained six peaks: acetic acid, 2-acetylfuran, 3-methyl-2-cyclopentenone, phenol + *o*-cresol, 2,3-xylenol, and syringol, with the most abundant component being syringol. The three fractions had similar phenol + *o*-cresol and syringol content. Fractionated shell bio-oil components produced at a higher temperature (300°C) were generally similar to those produced at a lower temperature (250°C). However, higher amounts of several components, e.g., propionic acid, 3-methyl-2-cyclopentenone, 2,4-dimethyl-2-butenolide, phenol + *o*-cresol, and syringol, were found at higher a pyrolysis temperature. Some scientists reported that increasing the temperature during pyrolysis increases the concentration of phenolic compounds in the filtrates (Guo and Lua 2001, Mohan et al. 2006).

Bioassay: effect of shell bio-oil on plant germination and radicle growth

Seed germination is a complicated event including many reactions with different phases affected by both internal and external factors. Generally, five major types of hormones, auxin, cytokinin, gibberellin, abscisic acid, and ethylene, endogenously regulate plant growth and development including seed germination (Heeyoung 2008). Numerous external factors, such as mechanical, thermal, and chemical treatments, are used to stimulate seed germination and have been studied by scientists. Chemical treatments such as gibberellic acid, sodium hypochlorite, and potassium nitrate are widely used for breaking seed dormancy and promoting seed germination (Cetinbas and Koyuncu 2006, Vashistha et al. 2009, Bhan and Sharma 2011).

The effects of organic compounds contained in palm fruit shell bio-oil (chemical treatment) on seed germination and radicle growth are shown in Tables 4 through 6.

Honewort.—Most fractions of palm shell bio-oil had an inhibitory effect on honewort germination. It can be seen in Table 4 that the higher the concentration (lower dilution rate) of neutral, acidic, and phenolic fractions, the stronger the inhibition. Germination of honewort after treatment with fractionated bio-oil ranged from 0 to 110 percent of control seeds. We found that low concentrations had a promoting effect on germination; specifically, these were the neutral fraction (10^7-fold dilution at 250°C pyrolysis temperature), acidic fraction (10^4- to 10^6-fold dilutions at both pyrolysis temperatures), phenolic fraction (10^5- to 10^6-fold dilutions at both pyrolysis temperatures), and original shell bio-oil (10^4- and 10^6-fold dilutions at both pyrolysis temperatures). Statistically, two factors in this bioassay test, the fraction and the dilution rate, had significant effects on germination ratio: the original shell bio-oil had the lowest inhibitory effect on honewort germination (Table 5). The germination ratio of honewort in our study was lower than those of Mu et al. (2003), which were obtained for the same type of seed but of a different origin.

The length of the radicle emerging from the seeds that were treated by bio-oil ranged from 0 to 112 percent of

Table 3.—Components of fractionated palm fruit shell bio-oil produced at pyrolysis temperatures of 250°C and 300°C.

Component	Phenolic fraction (%)		Acidic fraction (%)		Neutral fraction (%)	
	250°C	300°C	250°C	300°C	250°C	300°C
Acetic acid	—	—	20.44	15.6	18.41	—
2-Acetylfuran	—	—	—	—	1.73	6.26
Propionic acid	—	—	0.94	1.06	—	—
3-Methyl 2-cyclopentenone	—	—	0.69	1.66	0.93	4.61
n-Butanoic acid	—	—	0.45	0.59	—	—
5 Methyl furfural	—	—			—	—
Acetophenone	—	—	0.34	0.41	—	—
2,4-Dimethyl-2-butenolide	—	—	0.84	1.72	—	—
Guaiacol	0.35	1.12	—	—	—	—
4-Methylguaiacol	0.23	0.28	—	—	—	—
Phenol + *o*-cresol	15.05	37.59	19.13	17.97	3.9	—
4-Ethylguaiacol	0.38	0.25	—	—	—	—
p-Cresol	3.10	0.40	—	—	—	—
m-Cresol	0.63	—	—	—	—	—
2,3-Xylenol	0.89	0.38	—	—	0.36	1.01
Syringol	21.47	6.03	6.14	16.18	7.39	19.72
Total	42.10	46.05	48.97	55.19	32.72	31.57

Table 4.—Germination ratio of three kinds of seeds treated with fractionated palm fruit shell bio-oil for up to 7 days.

Dilution rate (fold)	Germination ratio (%)					
	Honewort		Chrysanthemum		Watercress	
	250°C	300°C	250°C	300°C	250°C	300°C
Neutral						
10^2	13	4	2	9	0	0
10^3	0	0	29	0	0	0
10^4	86	77	70	79	80	96
10^5	92	99	90	79	56	94
10^6	97	90	92	92	44	88
10^7	101	86	90	84	46	94
Acidic fraction						
10^2	11	13	0	9	0	0
10^3	0	0	0	0	0	0
10^4	73	105	84	86	100	100
10^5	97	103	101	90	86	104
10^6	103	95	81	79	100	102
10^7	73	88	99	99	92	98
Phenolic fraction						
10^2	9	6	0	0	0	0
10^3	0	0	0	0	0	0
10^4	62	60	92	103	106	86
10^5	103	105	105	101	104	100
10^6	105	101	95	97	104	96
10^7	95	110	95	90	104	104
Original bio-oil						
10^2	0	0	7	11	0	0
10^3	82	73	90	94	96	98
10^4	86	108	81	90	102	100
10^5	88	98	81	97	96	90
10^6	103	110	92	99	102	102
10^7	99	103	86	101	98	108

Table 5.—Summary of significant differences among the means of germination ratio.[a]

Factors	Levels	Honewort	Chrysanthemum	Watercress
Fractions	Phenolic	9.778 A	9.806 A	11.167 A
	Acidic	9.883 A	9.194 A	10.861 A
	Neutral	9.368 A	8.917 A	8.306 B
	Original bio-oil	12.333 B	11.694 B	13.778 C
Dilution rate (fold)	10^2	1.063 A	0.625 A	0 A
	10^3	3.125 B	4.042 B	4.042 B
	10^4	12.750 C	13.000 C	16.042 C
	10^5	15.208 D	13.750 C	15.208 C
	10^6	15.583 D	13.833 C	15.375 C
	10^7	14.240 D	14.167 C	15.500 C
Pyrolysis temperature	250°C	10.236 A	9.778 A	10.528 A
	300°C	10.420 A	10.028 A	11.528 B

[a] The mean difference is significant at the 0.05 level. Within a column, the same letters indicate no significant difference according to Tukey's honestly significant difference test at the 0.05 α level.

control seeds (Table 6). Roots longer than those of control seeds were only found in seeds treated with the original bio-oil. Statistical analysis of the radicle length ratio indicated a significant effect of pyrolysis temperature, dilution rate, and type of fraction (Table 7). As a result, bio-oil prepared at a pyrolysis temperature of 300°C had radicle growth promoted slightly more than material prepared at 250°C, and the original shell bio-oil gave a better result than the other fractions, with the lower concentration being best.

Table 6.—Radicle length ratio for three kinds of seeds treated with fractionated palm fruit shell bio-oil for up to 7 days.

Dilution rate (fold)	Radicle length ratio (%)					
	Honewort		Chrysanthemum		Watercress	
	250°C	300°C	250°C	300°C	250°C	300°C
Neutral						
10^2	0	0	0	0	0	0
10^3	0	0	19	0	0	0
10^4	62	72	86	97	63	99
10^5	71	80	50	82	76	115
10^6	78	89	74	77	58	85
10^7	79	76	60	79	64	102
Acidic fraction						
10^2	0	0	0	0	0	0
10^3	0	0	0	0	0	0
10^4	60	71	90	85	91	82
10^5	64	94	69	53	102	93
10^6	77	76	69	73	111	109
10^7	78	81	69	75	88	95
Phenolic fraction						
10^2	0	0	0	0	0	0
10^3	0	0	0	0	0	0
10^4	38	45	56	50	85	62
10^5	90	96	83	76	111	107
10^6	76	96	74	115	95	100
10^7	71	78	91	77	99	97
Original bio-oil						
10^2	0	0	0	0	0	0
10^3	76	66	114	88	55	67
10^4	98	104	78	79	111	124
10^5	83	87	86	87	109	112
10^6	102	92	90	55	97	124
10^7	103	112	57	43	109	98

Chrysanthemum.—The germination response of chrysanthemum seeds to bio-oil was similar to that of honewort. Germination varied from 0 to 105 percent of control seeds. Statistically, the factors with significant effects were the same. The tendency was for a higher dilution to give a higher percentage of germination. Unfortunately, germination rate was generally lower than control samples; most of the fractions were inhibitory to chrysanthemum germination. The best combination of factors was achieved by treating seeds with the phenolic fraction at a 10^4-fold dilution (see Table 4). This dilution yielded 105 percent of the control seed germination, a 5 percent promotion of germination. Statistically, only the pyrolysis temperature had no significant effect on radicle growth of chrysanthemum (see Table 7). The combination of the original shell bio-oil with a moderate to high dilution rate yielded the best results.

Watercress.—All treatment factors in this study had a significant effect on the germination of watercress. The response of seed germination to bio-oil varied from 0 to 104 percent of control seeds. Promotion and inhibition of watercress germination were fairly balanced. Pyrolysis at 300°C yielded bio-oil with a greater effect on germination than at 250°C; the original shell bio-oil was better than the phenolic, acidic, or neutral fractions, and a 10^4-fold dilution had the same effect as dilutions of 10^7, 10^6, and 10^5 but differed from results at dilutions of 10^3 and 10^2 (see Table 4).

The same tendency occurred for radicle growth; bio-oil prepared at a pyrolysis temperature of 300°C had a greater effect than that prepared at 250°C (see Table 6). The other factors had no significant effect on radicle growth (see Table 7). There were obvious inhibitory effects on radicle growth, especially for the higher concentrations of fractions, namely, the fractions diluted 10^2- and 10^3-fold, for all tests.

Most of the bioassay data was below 100 percent, suggesting that palm bio-oil acts more as an inhibitory agent. However, at very low concentrations, bio-oil might show a stimulatory effect on germination and radicle growth. Butenolide, which was found in the acidic fraction, might not affect seed germination but may still have a role

Table 7.—Summary of significant differences among the means of radicle length ratio.[a]

Factors	Levels	Honewort	Chrysanthemum	Watercress
Fractions	Phenolic	49.175 A	51.847 A	63.028 AB
	Acidic	49.897 A	48.319 A	64.306 A
	Neutral	50.664 A	51.861 A	55.131 B
	Original bio-oil	77.036 B	65.822 B	83.736 C
Dilution rate (fold)	10^2	0 A	0 A	0 A
	10^3	17.783 B	27.646 B	15.229 B
	10^4	68.804 C	77.446 C	89.688 C
	10^5	83.242 D	71.842 C	102.933 D
	10^6	85.642 D	80.200 C	97.388 CD
	10^7	84.688 D	69.642 C	94.063 CD
Pyrolysis temperature	250°C	54.389 A	55.189 A	63.501 A
	300°C	58.997 A	53.736 A	94.063 A

[a] The mean difference is significant at the 0.05 level. Within a column, the same letters indicate no significant difference according to Tukey's honestly significant difference test at the 0.05 α level.

in germination promotion; however, interactions with other chemicals may have weakened its effect. Light et al. (2009) said that the effectiveness of butenolide is a chance coming together of chemistry and biological pathways. Downes et al. (2010) reported that not all species are responsive to butenolide.

Our results also suggested that other chemicals in palm fruit shell bio-oil may inhibit seed germination and radicle growth. El-Barghathi and Asoyri (2007) noted that some natural phenolic compounds are inhibitors of germination and radicle growth but others show stimulatory properties. Keely and Pizzorno (1986) found that extracts from cellulose heated at 175°C did not promote germination of *Erriophyllum confertiflorum*.

The low pH and high content of acetic acid in palm fruit shell bio-oil might be a clue to its inhibitory tendency. Seeds may not germinate as a result of high acidity. Vera et al. (2010) reported that a pH value that is too low can delay germination, decrease the germination rate, and inhibit radicle growth.

Conclusions

The material properties and composition of oil palm fruit shell are different from wood and other biomass. Volatile matter and ash content were lower, fixed carbon was higher, and the content of holocellulose was higher than that of hardwoods in general (Mohan et al. 2006). Based on its chemical composition, palm fruit shell meets the main criterion for raw material useful for bio-oil production (Parikh et al. 2005, Mohan et al. 2006). Differences in pyrolysis temperature caused obvious differences in bio-oil yield and acetic acid content, but there were no significant differences in specific gravity, pH, or major chemical components of shell bio-oil.

Palm fruit shell bio-oil (original and fractionated) had inhibitory properties, rather than stimulatory properties, on germination and radicle growth of tested seeds. However, at very low concentrations, palm fruit shell bio-oil might be useful to stimulate seed germination and radicle growth. Specifically, the dilution rate and kind of fraction affected honewort and watercress germination and radicle growth. The original (unfractionated) bio-oil had promoted germination and radicle growth. Dilutions of 10^2- and 10^3-fold inhibited germination and radicle growth of all three kinds of seeds tested.

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