

1 vinegar and without foliar application and rice soaked in water with foliar application
2 of wood vinegar, respectively. An effect of seed quality was observed in seed vigor
3 from soaking and combination of both soaking and foliar application. It can enhance
4 the seed germinability even after stress test (AA test). The effect on growth, yield and
5 yield traits and seed quality highlights that the wood vinegar is an efficient soaking
6 agent, and growth enhancer in rice. It can reduce the use of chemical fertilizers to
7 enhance the seed growth in future.

8
9 **Keywords:** pyroligneous acid, derived smoke, seed germination, osmoconditioning,
10 priming

11 12 **1. INTRODUCTION**

13 Wood vinegar is a byproduct from charcoal production. It is a liquid
14 generated from the gas and combustion of fresh wood burning in airless condition.
15 Wood vinegar has been introduced in agriculture more than a hundred years in Japan.
16 It has started to be used for organic agriculture nationwide in Thailand. Many research
17 works are explored by the experts on the use of wood vinegar in the organic
18 agriculture. The main focus was to use the wood vinegar as an organic fertilizer and
19 pesticide. It has been used to improve soil fertility, to eliminate pests and to control
20 plant growth, but slightly toxic to fish and very toxic to plants if too much is applied.
21 It enhances the growth of roots, stems, tubers, leaves, flowers, and fruit (FFTC, 2005).

22 Wood vinegar consists mainly of 80-90% water and 10-20% organic
23 compounds including more than 200 chemical components. The main components are
24 organic acids, phenolic, alkone, alcohol and ester compounds with acetic acid being a
25 main component (Mu et al., 2003). Toward reduction of chemical insecticide overuse
26 and provision of information on pest control potentially in an environmentally-
27 friendly way, wood vinegar could be beneficial for the control of insect pests (Kim et
28 al., 2008). It has also been used such as odor remover, animal feed additives and
29 agricultural use such as soil- or foliar fertilizer. In crop production, it has been used
30 towards soil quality improvement, pest elimination, plant growth stimulation (FFTC,
31 2005), promotion of rice growth through the development of branched roots (Tsuzuki

1 et al., 1989), promotion of vegetative growth and slight improvement of some yield
2 components of rice crop (Ruamtakhu, 2007) and acceleration of sweet corn growth
3 (Pangnakorn, 2008). Grain yield of rice with average yield of 5.13 t ha⁻¹ and its
4 components increased when rice foliarly applied with wood vinegar at 300 times
5 dilution combined with chicken manure (Tipparak et al., 2007).

6 Using wood vinegar at 500-800 times dilution as foliar fertilizer increases
7 yield of cucumber, lettuce and cole between 18.8-20.2% compared to control (Jun et
8 al., 2006). Mixing charcoal and wood vinegar in planting materials improves growth,
9 branching and survival rate of zinnia (Kadota and Niimi, 2004) and increases yield of
10 Hiratake mushroom (*Pleurotus ostreatus*) by 21-42% when mixed wood vinegar at
11 the concentration between 0.1—6% in sawdust medium (Yoshimura et al., 1995). In
12 Japan, distilled wood vinegar is approved as feed additive. The effectiveness of
13 activated charcoal containing wood vinegar liquid against intestinal infection with
14 *Samonella entrica* has been reported by Watarai and Tana (2005). Wood vinegar
15 contains phenolic compounds which is toxic to microbial activity if used in high
16 concentration. Zagori (1981) reported that smoke was effective in reducing the
17 incidence of alfalfa anthracnose, barley powdery mildew, and cotton damping off as
18 well as in reducing the number of propagules of *Pythium ultimum* in field soil.
19 However, if wood vinegar is used at the appropriate concentration, it can be used as
20 soil fertilizer. It has been reported that charcoal and wood vinegar stimulate the soil
21 microbial community (Steiner et al., 2007).

22 In fire prone habitats in the regions with Mediterranean-type vegetation such as
23 Western Australia, California and South Africa, smoke has been reported in its
24 effectiveness in enhancing seed germination in many species. Consequently, there
25 have been attempts for decades in elucidating the chemical component that is a
26 germination clue in such environment. In recent scientific breakthrough, Gavin
27 Flematti and colleagues successfully identified the compound in plant-derived smoke
28 that induce germination in plant species and the compound was shown to be
29 butenolide 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (Flematti et al., 2004). Various
30 forms of smoke have been used in promoting seed germination of horticultural- and
31 agricultural crops. Soaking maize seed in smoke water at 500 times dilution for 1 h
32 improves plant height and survival rate of maize seedlings (Staden et al., 2006). Mu

1 et al. (2004) reported that wood vinegar made from moso bamboo promotes radicle
2 and hypocotyls growth of watercress and chrysanthemum. The promoting effect was
3 evident when moso bamboo vinegar was collected at temperature of 100-250°C,
4 higher collecting temperature at 250-400°C, wood vinegar showed inhibitory effect on
5 germination and radicle growth when applied at higher concentration.

6 The foliar application of 300 times diluted wood vinegar with 1875 kg ha⁻¹ or
7 3750 kg ha⁻¹ of chicken manure increased tiller number and seed yield and the
8 application of diluted wood vinegar 300 times and chicken manure at the rate of 1875
9 kg ha⁻¹ gave the highest yield of 5131.25 kg ha⁻¹ (Tipparak et al., 2007). Hok et al.
10 (2009) reported that a sole used of wood vinegar slightly enhanced seed yield but
11 significantly increased seed yield of Khao Dawk Mali 105 (KDML 105, aromatic
12 rice) when applied together with farmyard manure. In the fire prone habitats in the
13 regions with Mediterranean- type vegetation such as Western Australian, California
14 and South Africa, smoke has been reported in its effectiveness in enhancing seed
15 germination in many species.

16 Thailand is a world's biggest rice exporting country (FAO, 2004).
17 Transplanting rice has been practiced by most of farmers in Thailand. However, direct
18 seeding rice production has been drastically increased during the past decades due to a
19 high labor cost and competitiveness. The use of wood vinegar as soaking agent may
20 enhance the root growth and early seedling development, and foliar fertilizer may lead
21 to the increased of seed yield in direct seeding rice production. Therefore the
22 objectives of this study were to find out the effect of wood vinegar as soaking agent
23 on seedling development and on subsequent growth and seed yield of rice and to find
24 out the effects of wood vinegar used as a foliar fertilizer on growth, yield and seed
25 quality of rice.

26

27 **2. MATERIALS AND METHODS**

28 **2.1 Experimental site**

29 The experiment site was conducted in farmer's field in Nong Ben under
30 Amphur Maung of Khon Kaen province, Northeast of Thailand during the dry season
31 under irrigated condition from February to June 2009.

32 **2.2 Experimental design**

1 Two factors were determined, soaking methods and foliar fertilizer. Soaking
2 methods were seed soaked in water for 48 hrs before sowing and seed soaked in wood
3 vinegar 300 times dilution for 48 hrs before sowing. Foliar fertilizer methods were
4 with or without 300 times dilution of wood vinegar as foliar fertilizer. The
5 combinations of four treatments were arranged in plots following the Randomized
6 Complete Block design (RCBD) with three replications. The plot sizes were 3m x 5m.
7 The plots were separated by a 1m strip. Plots layout was shown in Figure 1. The four
8 treatments of the experiment were consisting of:

9 T₁: Seed soaked in water for 48 hrs before sowing and sprayed wood vinegar
10 at 300 times dilutions as a foliar fertilizer at two weeks interval at the rate
11 of 1270 L ha⁻¹

12 T₂: Seeds soaked in water for 48 hrs before sowing and without using of wood
13 vinegar as foliar fertilizer.

14 T₃: Seed soaked in 300 times dilution of wood vinegar for 48 hrs and sprayed
15 wood vinegar at 300 times dilutions as a foliar fertilizer at two weeks
16 interval at the rate of 1270 L ha⁻¹

17 T₄: Seed soaked in 300 times dilution of wood vinegar for 48 hrs and without
18 using the wood vinegar as foliar fertilizer.

19 **2.3 Crop management**

20 **2.3.1 Land preparation**

21 The land was ploughed and harrowed two times. Then, the chicken manure was
22 used as a basal application at the rate of 1875 kg ha⁻¹

23 **2.3.2 Rice cultivar**

24 Pathum Thani 1, popular aromatic rice which is less photo sensitive and grown
25 mostly in irrigated conditions, especially in the central part of Thailand was used for
26 the experiment.

27 **2.3.3 Rice sowing**

28 Rice seeds were soaked as per the given treatments and sown at the seeding
29 rate of at the rate of 125 kg ha⁻¹

30 **2.3.4 Wood vinegar application**

31 Wood vinegar was used as a soaking agent in T₁ and T₃. Wood vinegar was
32 used as a foliar fertilizer in T₂ and T₃ with 300 times dilutions. The first foliar spray

1 was carried out two weeks after sowing and was continued after every two weeks
2 thereafter. The last foliar spray was made two weeks before the harvest. The
3 application rate was 1270 L ha⁻¹.

4 **2.3.5 Other cultural practices**

5 Weed was controlled manually. Water level was maintained at 10 cm. level
6 throughout the growing season until two weeks before harvesting. No insecticide was
7 applied

8 **2.4 Data collection**

9 **2.4.1 Growth**

10 The data for plant height, leaf area, root length, total dry weight of stem and
11 leaves, root area and root dry weight were monitored at 30 and 60 days of planting
12 (DAP) and at harvesting in ten plants from each replicates randomly.

13 **2.4.2 Yield and yield components**

14 The data on yield per plot, numbers of panicle/plant, numbers of seed per
15 panicle, weight of 1000 seeds, percent of filled and unfilled seeds, and seed weight
16 per plant were collected randomly from different plots and harvested area was 2 m x 3
17 m.

18 **2.4.3 Seed Quality:**

19 Seeds from all treatments was cleaned, dried and then stored in the plastic bag
20 under favorable conditions. Quality was tested including seed moisture content,
21 standard germination test and laboratory seed vigor test was carried out using the
22 following procedures:

23 ***Seed moisture content (SMC)***

24 Two replicates of 10 g of each seed lot were used for the determination of seed
25 moisture content (SMC). Samples were ground and oven-dried at 130⁰C–133⁰C for 2
26 hours (ISTA, 2004), and was cooled down in desiccators for 30 – 45 minutes before
27 weighting. Then, seed moisture content was calculated using the following formula:

$$29 \text{ SMC (\%)} = \frac{\text{Seed weight before drying} - \text{seed weight after drying}}{\text{Seed weight before drying}} \times 100$$

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32 ***Standard germination test (GT)***

1 Four replicates of 100 seeds were taken at random from a pure seed fraction of
2 each seed lot. Seeds were then germinated using rolled paper (RP) method as
3 described by ISTA (2004). Seeds were incubated at 25 °C with 12 hours of light and
4 12 hours without light. Seedling evaluation was done according to ISTA (2004) at 5
5 and 14 days after incubation.

6 *Laboratory vigor test*

7 *Accelerated ageing test (AA test)*

8 To artificially age the seed, four replicates of 100 seeds of each seed lot was
9 placed on wire mesh trays and placed in an artificial aging chamber for 84 hours. The
10 temperature was controlled at 41 °C with nearly maximum relative humidity. At the
11 end of the ageing period, the samples were removed and the seeds were set out for
12 germination test. At the end of the germination test, evaluation was made according to
13 ISTA (2004).

15 **2.5 Data analysis**

16 All the agronomic traits were analyzed statistically in Factorial in Randomized
17 Block design (RCBD) by the analysis of variance (ANOVA) techniques. The seed test
18 was carried out using Factorial in Complete Randomized Design (CRD). Means
19 comparison was performed using Least Significance Difference (LSD) Test.
20 Statistical analysis was done using Statistix 8.

22 **3. RESULTS**

23 **3.1 Growth and growth traits**

24 The seeds soaked in wood vinegar for 48 hrs before sowing had significant
25 effect on leaf area/plant (79.89 cm²) as compared to seeds soaked in water for 48 hrs
26 (57.52 cm²) (farmers' practice). The effect of wood vinegar used as a foliar fertilizer
27 had an equal significant effect on leaf area/plant. It is noted that the seeds which were
28 soaked in wood vinegar and rice seedlings/plants which were given foliar spray of
29 wood vinegar at 300 times dilution at the rate of 1271 L ha⁻¹ after every 14 days have
30 attended the mean leaf area/plant of 100.66 cm². It clearly indicated that there was an
31 effect of wood vinegar used as a soaking agent as well as a foliar fertilizer on leaf area

1 as compared to other growth parameters at 30 DAP (Table 1). There was no
2 significant effect on root development by wood vinegar as a soaking agent and foliar
3 fertilizer as compared to the water but had a highly significant when there was an
4 interaction of foliar and spray of wood vinegar on root length, root area and root
5 biomass (dry weight) (Table 2).

6 The effect of wood vinegar as a soaking agent and foliar fertilizer was highly
7 significant on leaf area/plant with the mean leaf area of 112.51 and on biomass. A
8 significant effect of wood vinegar as a foliar fertilizer on plant height and biomass
9 was observed (Table 3). A significant effect of soaking with wood vinegar was
10 observed in root length, root area and root biomass. There was a significant effect of
11 interaction of soaking and foliar of wood vinegar in root length, root area and root
12 biomass but no effect of foliar on root length and root area but highly significant on
13 root biomass (Table 4). A significant effect of soaking, foliar application and
14 combination of both soaking and foliar application on plant height and total dry
15 weight were observed. The maximum effect was observed both on plant height and
16 total dry weight with the interaction of soaking and foliar application respectively
17 (Table 5). Effect of wood vinegar as soaking agent was more pronounced at harvest as
18 rice soaked with wood vinegar had highly significant root length, root area and root
19 dry weight, compared to seed soaked with water only. Additional effect of foliar
20 application of wood vinegar on root development was evident (Table 6).

21

22 **3.2 Yield and yield traits**

23 A highly significant effect from soaking was observed on the numbers of
24 panicles/plant and significant effect on seed weight/plant. Foliar application had a
25 significant effect on numbers of seeds/plant. The combination of soaking and foliar
26 application of wood vinegar had a significant effect on numbers of panicles/plant,
27 numbers of seed/panicle and seed weight/plants. However, effect of wood vinegar
28 used as soaking agent and foliar fertilizer did not significantly increase seed yield of
29 Pathum Thani 1 rice. Slightly increased in seed yield was found in seed soaked with
30 wood vinegar and applied with wood vinegar as foliar fertilizer, with the average
31 yield of 3787 kg ha⁻¹ (Table 7).

32

1 **3.3 Seed quality**

2 The seeds soaked with wood vinegar had the highest seed vigor as shown by
3 germination percentage of 94.33% even after the AA test. The highest germination
4 percent of 95.17% was observed in seeds which received the combination of both
5 soaking and foliar application of wood vinegar even after the AA test. No significant
6 effect was observed on seed moisture content and standard germination test from all
7 the treatments.

8

9 **4. DISCUSSION**

10 Wood vinegar used as soaking agent showed a significant effect on root
11 development of rice variety Pathum Thani 1 and effect on root development was even
12 more pronounced as plant growth advanced. Effect of wood vinegar on root
13 development has been reported Jothityangkoon et al. (2007) who found that there was
14 a clear evident of the promotional effect of wood vinegar on germination and seedling
15 development. Wood vinegar has also been reported in its effectiveness in promoting
16 germination and radicle growth in many horticultural and agricultural crops (Mu et
17 al., 2003; 2004) and plant-derived smoke promotes germination of many Australian
18 and African native flowers and herbs in the fire prone habitats (Brown et al., 1993;
19 Brown and Staden, 1998; Sparg et al., 2005; Staden et al., 2006). The compound in
20 plant-derived smoke that induces germination has been successfully identified
21 (Flematti et al., 2004), but the regulatory mechanism is not understood. The identified
22 chemical compound may be associated with cell division and cell elongation as it
23 promotes germination and seedling development. The effect the compounds in wood
24 vinegar or smoke water may not be limited to the regulation of seed germination as
25 wood vinegar enhanced the stem length and root length of rice seedling. This finding
26 was similar to previous work done by Staden et al. (2006).

27 Significant effect of soaking seed with wood vinegar on vegetative growth or
28 above ground part was evident. Better root development when soaked with wood
29 vinegar might have contributed to a better vegetative development. Wood vinegar
30 used as foliar fertilizer significantly promoted vegetative growth. Wood vinegar
31 increased number of leaves per plant (data not shown) and therefore, consequent leaf
32 area and biomass accumulation. The mechanism underlying how wood vinegar

1 enhanced vegetative growth was not clearly understood. Hormonal effect may have
2 its role as rice applied with wood vinegar had more leaves and more panicles.

3 There was significant effect wood vinegar on the some yield component traits but
4 not significant in yield. This was similar to the findings made by Hok et al. (2009).
5 The wood vinegar had enhanced the vegetative growth and the full development of
6 seeds could not be completed as we found more percentage of unfilled seeds in plants
7 treated with wood vinegar. However, the advantage of using wood vinegar in rice
8 was found in seed as seed can be stored longer as there was highest seed germination
9 even after the accelerated aging test (AA test). The finding was similar to the work
10 done by Jothityangkoon et al. (2007).

11

12 **5. CONCLUSIONs**

13 Wood vinegar can enhance the vegetative growth, and root development which
14 is highly significant as the plant growth advances. This effect can help to reduce or
15 stop using the inorganic fertilizers in rice production. There is a potential that it can
16 increase the yield as wood vinegar has enhance the growth of panicles/plant which
17 will help in producing more seeds. The harvesting period should be observed so that
18 harvesting is done at the right time. The seeds can be stored for longer duration as
19 compared the seeds produced without using wood vinegar. More detailed studies are
20 required in different locations and varieties to show the use of wood vinegar in the
21 field of organic agriculture.

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Table 2 Effect of wood vinegar used as a soaking agent and foliar fertilizer on root development of Pathum Thani 1 direct seeding rice at 30 days after planting (DAP).

Treatments	At 30 DAP		
	Total root length (cm/plant)	Total root area (cm ² /plant)	Root dry weight (g/plant)
Soaking method			
Soaking in water for 48 hrs. (A1)	505	68.04	0.272 A
Soaking in wood vinegar for 48 hrs. (A2)	529	69.62	0.237 B
Wood vinegar application			
Foliar spray after every 14 days. (B1)	586 A	78.63 A	0.246
No foliar spray. (B2)	448 B	59.02 B	0.263
Soaking x foliar			
A 1 x B 1	538 AB	74.30 C	0.177 C
A 1 x B 2	472 B	61.78 BC	0.315 B
A 2 x B 1	635 A	82.97 A	0.367 A
A 2 x B 2	424 B	56.27 C	0.158 C
C.V (a) (%) (Soaking)	46.93	47.14	38.44
C.V (b) (%) (foliar)	44.96	44.88	38.72
C. V (a x b) (%)	44.71	44.94	15.70
Soaking	ns	ns	*
Foliar	**	**	ns
Soaking x Foliar	**	**	**

5 ns= non significant; *, **= significantly different at p≤ 0.05 and 0.01, respectively
6 Means in the same column with different letters are significantly different at p≤0.05

1 **Table 3** Effect of wood vinegar used as a soaking agent and foliar fertilizer on growth
 2 traits above the ground of Pathum Thani 1 direct seeding rice at 60 days after
 3 planting (DAP).

Treatments	At 60 DAP		
	Plant height (cm)	Leaf area/ plant (cm ²)	Total dry weight (g/plant)
Soaking method			
Soaking in water for 48 hrs. (A1)	44.25	93.12 B	3.369
Soaking in wood vinegar for 48 hrs. (A2)	44.19	104.41 A	3.573
Wood vinegar application			
Foliar spray after every 14 days. (B1)	44.88 A	105.16 A	3.717 A
No foliar spray. (B2)	43.56 B	92.37 B	3.225 B
Soaking x foliar			
A 1 x B 1	45.17 A	97.82 B	3.183 BC
A 1 x B 2	43.33 B	88.43 C	3.555 B
A 2 x B 1	44.60 AB	112.51 A	4.251 A
A 2 x B 2	43.78 AB	96.31 B	2.894 C
C.V (a) (%) (Soaking)	6.67	9.18	33.02
C.V (b) (%) (foliar)	6.50	8.64	32.35
C. V (a x b) (%)	6.53	6.20	29.88
Soaking	ns	**	ns
Foliar	*	**	*
Soaking x Foliar	ns	**	**

4 ns= non significant, *, **= significantly different at $p \leq 0.01$

5 Means in the same column with different letters are significantly different at $p \leq 0.05$

6

1 **Table 4** Effect of wood vinegar used as a soaking agent and foliar fertilizer on root
 2 development of Pathum Thani 1 direct seeding rice at 60 days after planting
 3 (DAP).

Treatments	At 60 DAP		
	Root length (cm)	Root area (cm ²)	Root dry weight (g/plant)
Soaking method			
Soaking in water for 48 hrs. (A1)	723.74 B	99.28 B	1.20 B
Soaking in wood vinegar for 48 hrs. (A2)	931.45 A	127.88 A	1.74 A
Wood vinegar application			
Foliar spray after every 14 days. (B1)	860.61	116.83	1.83 A
No foliar spray. (B2)	794.58	110.33	1.12 B
Soaking x foliar			
A 1 x B 1	732.58 B	99.24 B	1.51 B
A 1 x B 2	714.89 B	99.33 B	0.89 D
A 2 x B 1	988.63 A	134.42 A	2.14 A
A 2 x B 2	874.27 A	121.33 A	1.34 C
C.V (a) (%) (Soaking)	33.26	36.56	31.30
C.V (b) (%) (foliar)	35.39	38.63	26.96
C. V (a x b) (%)	33.16	36.65	19.28
Soaking	**	**	**
Foliar	ns	ns	**
Soaking x Foliar	**	**	**

4 ns= non significant, **= significantly different at p≤0.01

5 Means in the same column with different letters are significantly different at p≤0.05

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1 **Table 5** Effect of wood vinegar used as a soaking agent and foliar fertilizer on growth
 2 traits of Pathum Thani 1 direct seeding rice at harvesting.

Treatments	At harvesting	
	Plant height (cm)	Total dry weight (g/plant)
Soaking method		
Soaking in water for 48 hrs. (A1)	59.43 B	3.94 B
Soaking in wood vinegar for 48 hrs. (A2)	64.46 A	4.83 A
Wood vinegar application		
Foliar spray after every 14 days. (B1)	65.41 A	4.70 A
No foliar spray. (B2)	58.47 B	4.07 B
Soaking x foliar		
A 1 x B 1	62.57 B	3.76 B
A 1 x B 2	56.29 C	4.13 B
A 2 x B 1	68.26 A	5.64 A
A 2 x B 2	60.65 B	4.02 B
C.V (a) (%) (Soaking)	8.76	21.72
C.V (b) (%) (foliar)	7.83	22.89
C. V (a x b) (%)	6.69	17.03
Soaking	**	**
Foliar	**	**
Soaking x Foliar	**	**

3 ns= non significant, **= significantly different at $p \leq 0.01$

4 Means in the same column with different letters are significantly different at $p \leq 0.05$

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1 **Table 6** Effect of wood vinegar used as a soaking agent and foliar fertilizer on root
 2 development of Pathum Thani 1 direct seeding rice at harvesting.

Treatments	At harvesting		
	Root length (cm)	Root area (cm ²)	Root dry weight (g/plant)
Soaking method			
Soaking in water for 48 hrs. (A1)	1133 B	145 B	1.347 B
Soaking in wood vinegar for 48 hrs. (A2)	1350 A	169 A	1.902 A
Wood vinegar application			
Foliar spray after every 14 days. (B1)	1293	160	1.989 A
No foliar spray. (B2)	1191	154	1.260 B
Soaking x foliar			
A 1 x B 1	1175 BC	147 BC	1.666 B
A 1 x B 2	1091 C	143 C	1.028 D
A 2 x B 1	1420 A	174 A	2.311 A
A 2 x B 2	1290 AB	165 AB	1.493 C
C.V (a) (%) (Soaking)	28.81	26.84	28.14
C.V (b) (%) (foliar)	29.86	27.89	23.96
C. V (a x b) (%)	28.75	26.98	16.39
Soaking	**	**	**
Foliar	ns	ns	**
Soaking x Foliar	**	*	**

3 ns= non significant, **= significantly different at $p \leq 0.01$

4 Means in the same column with different letters are significantly different at $p \leq 0.05$

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Table 7 Effect of wood vinegar used as a soaking agent and foliar fertilizer on yield and some yield parameters of Pathum Thani 1 direct seeding rice.

Treatments	Yield and some yield components						
	Panicle (no./plant)	Seeds (no./panicle)	Seed weight/ plant (g)	1000 seed weight (g)	% of filled grain	% of filled seed	Seed yield (kg ha ⁻¹)
Soaking method							
Soaking in water for 48 hrs. (A1)	2.1 B	45.1	2.52 B	25.93	88.8	11.2	2,943
Soaking in wood vinegar for 48 hrs. (A2)	2.7 A	49.3	3.34 A	27.18	89.6	10.4	3358
Wood vinegar application							
Foliar spray after every 14 days. (B1)	2.5	49.8 A	3.15	26.78	87.5	12.5	3084
No foliar spray. (B2)	2.4	44.6 B	2.70	26.33	90.9	9.0	3217
Soaking x foliar							
A 1 x B 1	2.1 C	47.0 AB	2.57 B	26.33	85.5	14.5	2381
A 1 x B 2	2.1 BC	43.2 B	2.47 B	25.52	92.2	7.8	3505
A 2 x B 1	2.8 A	52.6 A	3.74 A	27.23	89.5	10.5	3787
A 2 x B 2	2.5 AB	46.0 B	2.93 B	27.13	89.7	10.3	2929
C.V (a) (%) (Soaking)	9.50	8.94	15.37	3.90	4.09	33.89	24.24
C.V (b) (%) (Foliar)	17.03	8.01	20.95	4.74	3.37	27.82	25.56
C. V (a x b) (%)	9.38	6.47	11.15	4.23	2.87	23.72	16.71
Soaking	**	ns	*	ns	ns	ns	ns
Foliar	ns	*	ns	ns	ns	ns	ns
Soaking x Foliar	*	*	*	ns	ns	ns	ns

ns= non significant, **= significantly different at $p \leq 0.01$

Means in the same column with different letters are significantly different at $p \leq 0.05$.

Table 8 Consequent effect of wood vinegar used as a soaking agent and foliar fertilizer on SMC, germination and seed vigor by AA test of Pathum Thani 1 rice seed.

Treatments	Seed quality		
	SMC (%)	Germination (%)	Seed vigor by AA test (%)
Soaking method			
Soaking in water for 48 hrs. (A1)	11.97 A	92.08	92.58 B
Soaking in wood vinegar for 48 hrs. (A2)	11.57 B	92.58	94.33 A
Wood vinegar application			
Foliar spray after every 14 days. (B1)	11.70	92.92	93.42
No foliar spray. (B2)	11.84	91.75	93.50
Soaking x foliar			
A 1 x B 1	12.03	91.67	91.67 C
A 1 x B 2	11.91	92.50	93.50 B
A 2 x B 1	11.37	94.17	95.17 A
A 2 x B 2	11.77	91.00	93.50 B
C.V (a) (%) (Soaking)	2.44	1.79	1.25
C.V (b) (%) (foliar)	2.99	1.68	1.61
C. V (a x b) (%)	2.24	1.29	0.79
Soaking	*	ns	*
Foliar	ns	ns	ns
Soaking x Foliar	ns	ns	**

ns= non significant; *, **= significantly different at $p \leq 0.05$ and 0.01 , respectively
Means in the same column with different letters are significantly different at $p \leq 0.05$

References

- Brown, N.A.C., G. Kotze and P.A. Botha. 1993. The promotion of seed germination of Cape Erica species by plant-derived smoke. *Seed Science and Technology* 21:573-580.
- Brown, N.A.C. and J. Van Staden. 1998. Plant-derived smoke: an effective seed pre-soaking treatment for wildflower species and with potential for horticultural and vegetable crops. *Seed Science and Technology* 26:669-673.
- FAO (Food and Agriculture Organization). 2004. International Year of Rice 2004. Accessed February 8, 2009 at <http://www.fao.org/rice2004/en/rice2.htm>
- FFTC (Food & Fertilizer Technology Center). 2005. Wood Vinegar. Retrieved February 2, 2009, from <http://www.ffc.agnet.org/library/pt/2005025/>
- Flematti, G. R., E. L.Ghisalbert, K W. Dixon, and R. D. Trengove, 2004: A compound from smoke that promotes seed germination. *Science* 305, 977.
- Haefele, S.M., K. Naklang, D. Harnpichitvitay, S. Jearakongman, E. Skulkhu, P. Romyen, S. Phasopa, S. Tabtim, D. Suriya-arunroj, S. Khunthasuvon, D. Kraisorakul, P. Youngsuk, S.T. Amarante and L.J. Wade. 2006. Factors affecting rice yield and fertilizer response in rainfed lowlands of northeast Thailand. *Field Crops Research* 98:39-51.
- Hok, Lyda, Darunee Jothityangkoon, and Anan Polthanee. 2009. Yield and nutrient accumulation of KDML105 rice as influenced by farmyard manure and wood vinegar. In *Agricultural Annual Seminar 2009*, Faculty of Agriculture, Khon Kaen University, Thailand. 26-27 January 2009. Faculty of Agriculture, Khon Kaen University, Thailand. pp. 368-372.
- International Seed Testing Association (ISTA). 2004. *International Rules for Seed Testing*. International Seed Testing Association. Bassersdorf, Switzerland.
- International Seed Testing Association (ISTA). 1995. *Handbook of Vigour Test Methods*. International Seed Testing Association. Zurich, Switzerland.
- Jothityangkoon, D., C. Ruamtakhu, S. Tipparak, S. Wanapat and A. Polthanee. 2007. Wood vinegar enhances seed germination and seedling development of rice. *In Proceedings of the 2nd International Conference on Rice for the Future*, 5-9 November 2007, Bangkok, Thailand.

- Jun, M., Y. Zhi-ming, W. Wen-qiang and W. Qing-li. 2006. Preliminary study of application effect of bamboo vinegar on vegetable growth. *Forestry Studies in China* 8(3):43-47.
- Kim, D.H., H.E. Seo, S.C. Lee and K.Y. Lee. 2008. Effects of wood vinegar mixed with insecticides on the mortalities of *Nilaparvata lugens* and *Laodelphax*
- Mu, Jun, Tohru Uehara and Takeshi Furuno. 2003. Effect of bamboo vinegar on regulation of germination and radicle growth of seed plants. *Journal of Wood Science* 49:262-270
- Mu, Jun, Tohru Uehara and Takeshi Furuno. 2004. 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. *Journal of Wood Science* 50:470-476.
- Pangnakorn, U. 2008. Utilization of wood vinegar by-product from Iwate kiln for organic agricultural system. *In Technology and innovation for Sustainable Development Conference, 28-29th January 2008, Khon Kaen University,*
- Ruamtakhu, C. 2007. Effect of Wood Vinegar on Yield and Seed Quality of KDML 105 rice. M.Sc. Thesis. Khon Kaen University, Thailand.
- Sparg, S.G., M.G. Kulkarni, M.E. Light and J. Van Staden. 2005. Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresearch Technology* 96:1323-1330.
- Steiner, C., K.C. Das, M. Garcia, B. Förster and W. Zech. 2007. Charcoal and smoke extract stimulate the soil microbial community in highly weathered xanthic Ferralsol. *Pedobiologia* 51:359-366.
- Staden, J. van, S. G. Sparg, M. G. Kulkarni and M. E. Light 2006. Post germination effects of the smoke-derived compound *3-methyl-2H-furo [2,3-c]pyran-2-one*, and its potential as a preconditioning agent. *Field Crops Research* 98:98-105.
- Tipparak, S., D. Jothityangkoon and A. Polthanee. 2007. Effect of wood vinegar and farmyard manure on growth and yield of KDML 105 rice. *Khon Kaen Agriculture Journal* 35(Suppl.):6-19.
- Watarai, S. and Tana. 2005. Eliminating the carriage of *Salmonella enterica* Serovar Enteritidis in domestic fowls by feeding activated charcoal from bark containing wood vinegar liquid (Nekka-Rich). *Poultry Science* 84:515-521.

- Yoshimura, Hisashi, Hisako Washio, Sadao Yoshida, Takao Seino, Mitsuho Otaka, Kazunori Matsubara and Matsutoshi Matsubara. 1995. Promoting effect of wood vinegar compounds on fruit-body formation of *Pleurotus ostreatus*. *Mycoscience* 36:173-177.
- Zagori, D. 1981. *Microbial Toxicity of Smoke*. Ph.D. Thesis. University of California, Berkeley, USA.