Effect of Smoke-water on Seed Germination and Resistance to *Rhizoctonia solani* Inciting Papaya Damping-off

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Key words: Smoke-water, 2(5H)-Furanone, Phenolic compounds, Imidazole, Damping-off disease

Abstract

Smoke-water derived from burning dry rice straw and then bubbling the smoke through water was used for this study. The objectives of the present study are (1) to evaluate the efficacy of smoke-water on the germination rate of papaya seedling grown in peat moss infested with *Rhizoctonia solani*, (2) to inquire the antifungal efficiency of *Rhizoctonia solani* caused damping-off disease further and (3) to characterize and identify the compounds of smoke-water. Results showed that 0.1% and 0.2% of smoke-water increase the germination of papaya seed. Germination of papaya seedling grown in peat moss infected with *Rhizoctonia solani* found that the all smoke-water treatments (1%-6%) were stimulated the seed germination, whereas the control treatment failed to germination of seeds. Moreover, 4%-6% of smoke-water significantly reduced *Rhizoctonia solani* damping-off and improved survival of papaya seedlings. Characteristic of smoke-water, the smoke-water solution is acidic and contains substantial amounts of plant nutrients such as high level of NH_4^+, an important source of nitrogen for papaya seedling growth. It also has a high content of phenolic compounds which are especially known for antifungal activity that may be inhibit growth of *Rhizoctonia solani* caused damping-off disease. The compounds of smoke-water were

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investigated by gas chromatography-mass spectrometry (GC-MS), and 30 compounds were identified: alcohols, lactones, aldehydes, acid, ketones, alkaloid and hydroxybenzenes. Among the identified compounds, 2(5H)-Furanone, may be the one stimulating the germination of papaya seeds. Furthermore, GC-MS of smoke-water revealed the presence of 9 phenolic compounds that may be inhibiting the growth of several fungi. Another compound present in smoke water is imidazole, specifically 1H-Imidazole,1-methyl-4-nitroso-5-phenyl- which has the ability to inhibit and kill fungal pathogens.

Introduction

Fire is well established as a major evolutionary driving force in seed biology and mediated via both physical and chemical cues involved in the germination process (Van Staden *et al*., 2000). The stimulatory effect of fire may results from a number of different effects, including the physical one of dry heat on seed coat structure, the physiological effect of dry heat on seed embryos, and the dormancy-breaking effects of volatile compounds such as ethylene (Keeley and Fotheringham, 2000).

Plant-derived smoke plays an important role in breaking the dormancy and stimulating the seed germination of many plant species around the world (De Lange and Boucher, 1990) including Australia, South Africa, North America and Europe (Chiwocha *et al*., 2009). The key germination stimulant from the smoke of burned plant-derived material (van Staden *et al*., 2004) and cellulose (Flematti *et al*., 2004), was first isolated and identified by Flematti *et al*. (2004) as the butenolide, 3-methyl-2H-furo[2,3-c]pyran-2-one. This naturally occurring germination stimulant displays activity in a variety of species at concentrations as low as one-part-per-billion (Dixon *et al*., 2009). Recently it has been referred to as 'karrikinolide' (Commander *et al*., 2008). The action of smoke in promoting the germination of seed of many species is mainly attributed to the presence of this compound (Soos *et al*., 2009). Smoke-responsive species occur from phylogenetically diverse higher plant groups and in a variety of ecosystems including, mediterranean-type vegetation, desert, alpine and wetlands (Chiwocha *et al*., 2009). Experimentally, the germination response to smoke is most easily studied using smoke-water, generated by bubbling of smoke through water (Lloyd *et al*., 2000).

Smoke from a wide variety of biotic sources, including wood, branches, leaves, straw, mixtures of dry and fresh plant material (Brown and van Staden, 1997) and charred wood but not the ash of burnt wood, contain potent cues that stimulate seed germination (Keeley *et al*.,1986). In addition, smoke components may have properties that protection against microbial attack (Roche, 1997).

Rhizoctonia solani damping-off cause serious diseases on many hosts by affecting the roots, stems, tubers, corms, and other plant parts that develop in or on the ground. *Rhizoctonia solani* is found in most agricultural soils and survives between crops on plant residues and as microsclerotia (Franklin, 2001). Development of this disease is available for high temperature, high humidity, soil moisture, poor aeration, high levels of fertilizer of nitrogen application and closely sowed seed (Agrious, 2005). Control of damping-off diseases is difficult, that must be anticipated and provented by using seed and transplant treatments before the seed or plants are planted in the field (Franklin, 2001).

However, this study used dry rice straw (*Oryza sativa*) for plant material in preparing a smoke-water stock solution. The objectives of the present study are (1) to evaluate the efficacy of smoke-water on the germination rate of papaya seedling grown in peat moss infested with *Rhizoctonia solani*, (2) to inquire the antifungal efficiency of *Rhizoctonia solani* caused damping-off disease further and (3) to characterize and identify the compounds of smoke-water.

Materials and Methods

Smoke-water preparation

A smoke-water solution was prepared by igniting dry rice straw (*Oryza sativa*) material (5 kg) in a 20 L metal drum. Using compressed air, the smoke was continuously bubbled through a 500 ml graduated cylinder of distilled water for 45 min. Solutions of this smoke extract (500 ml) were filtered through Whatman No.1 filter paper and was used as the stock solution. A similar method of preparing the smoke-water with different plant material has been described in Boucher and Meets (2004). The apparatus for producing smoke-saturated water has been illustrated by De Lange and Boucher (1990) and Van Staden *et al*. (2004).

Characteristics of smoke-water

pH value was measured by a pH meter. Electrical conductivity (EC) was measured by EC Meter. The concentration of K, Ca, Mg, Fe, Mn, Zn and Cu were measured by Atomic absorption spectrophotometer, Hitachi Z-2300. The concentration of N was determined by Kjeldahl method while P, B, total phenolic compound, total soluble sugar and total free amino acid were determined by the spectrophotometer. Glucose, fructose, tartaric acid, NO^{3-}, SO_4^{2-}, NH^{4+}, F^- and Cl^- were measured by high-performance liquid chromatography (HPLC). Ethylene was measured by Gas-chromatography, Shimadzu Model GC-8A.

Analysis of smoke-water compound by gas chromatography mass spectroscopy (GC-MS)

Smoke-water was exhaustively extracted with dichloromethane. The combined organic extracts were washed with aqueous NaOH, followed by washings with H_2O to a neutral pH. The extract was subjected to vacuum liquid chromatography and eluted with a hexan:ethyl acetate gradient. The smoke-water compound was isolated and identified as per procedures described by Van Staden *et al*. (2004).

Effect of smoke-water irrigation on the percentage germination

The seeds were soaked in distilled water for 24 h and rinsed for 1 min in three changes of sterile water then sown in pots. The control pots were irrigated with water three times weekly. The treatment pots were irrigated twice weekly with smoke-water concentrations (0.1%, 0.2%, 1%, 2%, 3%, 4%, 5%, 7% and 10% v/v) and once with water. Each treatment consisted of five replicates of 30 seeds. The pots (dimensions: 10 cm × 8.5 cm × 7 cm) were contained peat moss, Tref (pH: 5.5, electrical conductivity: 2.5 mS cm^{-1}, organic matter content: 0.85%, Total N: 160 µg g^{-1}, P: 78.66 µg g^{-1}, K: 166 µg g^{-1} and Mg: 80 µg g^{-1}) 200 g of peat moss/pot. Pots were placed in the growth chamber at 8 h light, 30℃, 16 h dark 25℃, and 75 % humidity. Irradiance was provided at 40 µmol $m^{-2}s^{-1}$ using daylight fluorescent lamps. Germination counts were made daily for 15 days. The seedling vigor index was calculated as SVI = [shoot length (mm) + root length (mm)] × percentage germination (Dhindwal *et al*., 1991).

Efficacy of smoke-water against *Rhizoctonia solani* damping-off disease

The seeds of papaya were first soaked in distilled water (the control) or different concentration of smoke water (1%, 2%, 3%, 4%, 5% and 6% v/v) for 24 h; decontaminated with 0.5% NaOCl for 2 min and then rinsed for 1 min in three changes of sterile water. The peat moss, Tref was autoclaved at 121℃ for 1 h and contained in plastic pots. The treated seeds were placed on top of the peat moss in each pot; 1 g of peat moss inoculated with *Rhizoctonia solani* was added and then covered with a 1 cm layer of peat moss. The control pots were water-irrigated three times weekly but the smoke-water pots were irrigated with water once weekly and irrigated with smoke-water concentrations twice weekly. Each treatment consisted of five replicates of 30 seeds. Pots were arranged randomly in plant growth chambers. Germination was recorded daily until 15 day after sowing and experiment was continued for 21 days. Average germination time (AGT) was calculated by using the equation: AGT = $\sum(ti.ni)/ \sum n$, where ti = time of seed germination (days), ni = number of seeds germination in the time t_i (not the accumulated number but the number correspondent to the seeds germinated on that particular day), and $\sum n$ = maximum germination of a seed lot. At the end of the experiment, plant disease incidence was expressed as the percentage of the number of plants showing typical symptoms caused by *Rhizoctonia solani* and percentage of survival was calculated by the number of survival seedling × 100 then divided by the maximum germination of the seed lot.

Statistical analysis

The data of the experiment were performed of statistical analysis by using SAS 9.2 (Institute Inc, 2002) and subjected to one-way analysis of variance (ANOVA) in a completely randomized design (CRD) statistical model. Mean values among treatments were compared by Duncan's multiple range tests at the 1% ($p \leq 0.01$) level of significance.

Results

Characteristics of smoke-water

The analyses made of smoke-water were summarized in Table 1. Its physical and chemical characteristics were pH: 4.6, EC 2.17 ms/cm, sucrose: 42 ppm, fructose: 25 ppm, glucose: 71 ppm, tartaric acid: 720 ppm, NO_3^-: 2.96 ppm, SO_4^{2-}: 309.85 ppm, NH_4^+: 6135 ppm, Total soluble sugar: 1.98 %, C_2H_4 1.7 nmole C_2H_4/ml SW, P: 110 ppm, K: 200 ppm, Ca: 2.4 ppm, Mg: 0.53 ppm, Mn: 0.03 ppm, Zn: 0.52 ppm, B: 6.51 ppm, F^- : 85.25 ppm, Cl^-: 12.17 ppm, Total phenolic compound: 1,471 ppm and Total free amino acid: 0.392 µmole/ml.

Identification of smoke-water

The smoke-water extract was tested to determine the chemical compounds by GC-MS analysis. The results revealed that 30 compounds were present in smoke-water solution (Table 2). There were different types of compounds, including alcohols (2-Propanone, 1-hydroxy, 2-Hydroxyethyl formate, 1-Hydroxy-2-butanone, 2-Furanmethanol), lactones (2-Cyclopenten-1-one, 2-Cyclopenten-1-one,2-methyl-, 3-Methyl-2-cyclopenten-1-one, 2,3-Dimethyl-2-cyclopenten-1-one, 2(3H)-Furanone, dihydro-, 3-ethylcyclopent-2-en-1-one-, 2-Furanone,2,5-dihydro-3,5-dimethyl, 4-Methyl-

2(5H)-furanone, 2(5H)-Furanone, 2-Cyclopenten-1-one,2-hydroxy-3-methyl-, 2-Cyclopenten-1-one, 3-ethyl-2-hydroxy), aldehyde (2-Furancarboxaldehyde), acid (2-Propanone, 1-(acetyloxy)-), ketones (Ethanone, 1-(2-furanyl)-, 2-Butanone, 3,3-dimethyl-, 2-Butanone, 1-(acetyloxy)), alkaloid (1H-Imidazole, 1-methyl-4-nitroso-5-phenyl-) and hydroxybenzenes (Phenol, 2-methoxy-, 2-Methoxy-4-methylphenol, Phenol, Phenol, 4-ethyl-2-methoxy-, Phenol, 4-methyl-, Phenol, 3-methyl-, Phenol, 4-ethyl-, 2-Methoxy-4-vinylphenol, Phenol,2,6-dimethoxy-).

Table 1. Characteristics of smoke-water

Parameter	Value		Parameter	Value	
pH	4.6		P	110	ppm
EC	2.17	mS/cm	K	200	ppm
Sucrose	42.00	ppm	Ca	2.4	ppm
Fructose	25.00	ppm	Mg	0.53	ppm
Glucose	71.00	ppm	Mn	0.03	ppm
Tartaric acid	720	ppm	Zn	0.52	ppm
NO_3^-	2.96	ppm	B	6.51	ppm
SO_4^{2-}	309.85	ppm	F^-	85.25	ppm
NH_4^+	6,135	ppm	Cl^-	12.17	ppm
Total Soluble Sugar	1.98	%	Total phenolic compound	1,471	ppm
Ethylene	1.7 nmole C_2H_4/ml SW		Total free amino acid	0.392 µmole/ml	

The data showed retention time, peak area, peak identifications, quality assurance, molecular formula, molecular weight and chemical structure. Among the identified compounds, 2(5H)-Furanone (the compound with its characteristic retention time at 21.00 min, peak area 138671, quality assurance 72%, molecular formula $C_4H_4O_2$ and molecular weight 84.07 g mol^{-1}) was what may be stimulating the germination of papaya seeds.

Furthermore, GC-MS analysis of the smoke-water revealed the presence 9 compounds, Phenol,2-methoxy-, 2-Methoxy-4-methylphenol, Phenol, Phenol, 4-ethyl-2-methoxy-, Phenol, 4-

methyl-, Phenol, 3-methyl-, Phenol, 4-ethyl-, 2-Methoxy-4-vinylphenol, and Phenol,2,6-dimethoxy-, The presence of these phenolic compounds may be inhibiting the growth of several fungi. In addition, 1H-Imidazole,1-methyl-4-nitroso-5-phenyl- with its characteristic retention time at 15.22 min, peak area 88496, quality assurance 64%, molecular formula $C_{10}H_9N_{3O}$ and molecular weight 187.19 g mol^{-1} is the compound that is present in many fungicides, antifungals and antibacterials.

Effect of smoke-water irrigation on the percentage germination of papaya seeds

Results indicated that papaya seeds irrigated with 0.1% and 0.2%, v/v of smoke-water resulted significantly increase germination (74.7% and 74.0%, respectively) but was not significantly different from the control (66.7%) (Table 3). However, high concentrations of smoke-water irrigation (1% v/v and up) were significantly reduced the percentage germination ($\leq$ 60%). The AGT was best at low concentrations of smoke-water irrigation (0.1% and 0.2% v/v), with an AGT of 8 days. Whereas at high concentration of smoke-water irrigation (3% v/v and up), the AGT were not reduced, with the AGT 10-11 days. The seedlings at low concentrations of smoke-water irrigation (0.1% and 0.2% v/v) also showed a significantly increase the SVI (7015 and 6886, respectively) in comparison with the control (5377).

Effect of smoke-water on percentage germination of papaya seedlings grown in peat moss infected with *Rhizoctonia solani*

Results showed that all smoke-water treatments were significantly higher the percentage germination of papaya seedlings on 15 days after sowing (62.67-76.00%) than the control (27.33%). However, the average germination time of seedlings was not difference among the treatment (11 days) (Table 4).

Effect of smoke-water on percentage *Rhizoctonia solani* damping-off and survival of papaya seedlings, was summarized in Table 5. Results showed that the percentage of *Rhizoctonia solani* damping-off disease of papaya seedling was significantly reduced by concentration of smoke-water 4%, 5% and 6% v/v (percentage damping-off 75.33%, 71.33% and 61.33%, respectively) in comparison with control (100%). In addition, the percentage survival of papaya seedlings at 21days after sowing was significantly greatest in 6% smoke-water treatment (53.59%) compare with the control (0%, seedlings died).

Table 2. Identification of smoke-water

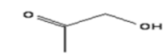
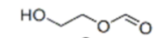
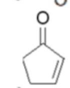
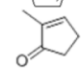
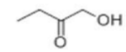
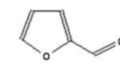
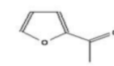
No.	Retention time (min)	Peak area	Peak Identifications	Quality Assurance (%)	Molecular formula	Molecular weight	Chemical structure
1	9.36	471352	2-Propanone, 1-hydroxy	72	$C_3H_6O_2$	74.0785	
2	9.81	57455	2-Hydroxyethyl formate	72	$C_3H_6O_3$	90.0779	
3	10.98	270596	2-Cyclopenten-1-one	86	C_5H_6O	82.1000	
4	11.38	81091	2-Cyclopenten-1-one,2-methyl-	94	C_6H_8O	96.1300	
5	11.49	136325	1-Hydroxy-2-butanone	64	$C_4H_8O_2$	88.1051	
6	13.93	271228	2-Furancarboxaldehyde	93	$C_5H_4O_2$	96.0841	
7	14.02	262396	2-Propanone, 1-(acetyloxy)-	72	$C_5H_8O_3$	116.1152	
8	15.05	111193	Ethanone, 1-(2-furanyl)-	81	$C_6H_6O_2$	110.1106	

Table 2. (Continued)

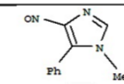
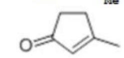
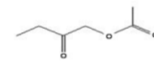
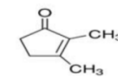
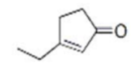
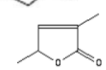
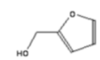
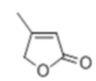
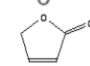
9	15.22	88496	1H-Imidazole,1-methyl-4-nitroso-5-phenyl-	64	$C_{10}H_9N_3O$	187.1980	
10	15.44	204924	3-Methyl-2-cyclopenten-1-one	90	C_6H_8O	96.1300	
11	15.71	59570	2-Butanone, 3,3-dimethyl-	49	$C_6H_{12}O$	100.1589	
12	15.83	64465	2-Butanone, 1-(acetyloxy)	47	$C_6H_{10}O_3$	130.1418	
13	16.01	125455	2,3-Dimethyl-2-cyclopenten-1-one	87	$C_7H_{10}O$	110.1560	
14	18.09	155932	2(3H)-Furanone, dihydro-	86	$C_4H_6O_2$	86.0900	
15	18.21	108117	3-ethylcyclopent-2-en-1-one-	80	$C_7H_{10}O$	110.1537	
16	18.65	65758	2-Furanone,2,5-dihydro-3,5-dimethyl	62	$C_6H_8O_2$	112.1265	
17	19.01	394461	2-Furanmethanol	96	$C_5H_6O_2$	98.0999	
18	20.21	83367	4-Methyl-2(5H)-furanone	80	$C_5H_6O_2$	98.1000	
19	21.01	138671	2(5H)-Furanone	72	$C_4H_4O_2$	84.0700	

Table 2. (Continued)

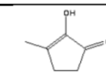
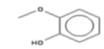
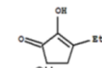
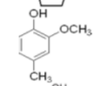
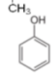
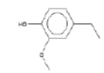
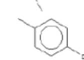
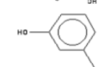
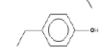
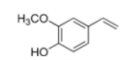
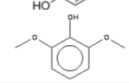
20	22.77	472586	2-Cyclopenten-1-one,2-hydroxy-3-methyl-	95	$C_6H_8O_2$	112.1265	
21	23.39	608430	Phenol, 2-methoxy-	97	$C_7H_8O_2$	124.1372	
22	24.19	205682	2-Cyclopenten-1-one,3-ethyl-2-hydroxy	96	$C_7H_{10}O_2$	126.1531	
23	25.38	168129	2-Methoxy-4-methylphenol	94	$C_8H_{10}O_2$	138.1662	
24	26.38	1195701	Phenol	93	C_6H_6O	94.1100	
25	26.88	117515	Phenol, 4-ethyl-2-methoxy-	87	$C_9H_{12}O_2$	152.1904	
26	27.88	435442	Phenol, 4-methyl-	97	C_7H_8O	108.1378	
27	28.03	309949	Phenol, 3-methyl-	95	C_7H_8O	108.1378	
28	29.63	256928	Phenol, 4-ethyl-	91	$C_8H_{10}O$	122.1644	
29	29.98	86248	2-Methoxy-4-vinylphenol	80	$C_9H_{10}O_2$	150.1745	
30	31.25	577767	Phenol,2,6-dimethoxy-	96	$C_8H_{10}O_3$	154.1632	

Table 3. Effect of smoke-water irrigation on the percentage germination of papaya seeds.

Smoke-water	% Germination	Average germination time (AGT)	Seedling Vigor index (SVI)
Control	66.7 ± 1.8 ab	9 ± 0.3	5377 ± 239 b
0.1%	74.7 ± 4.5 a	8 ± 0.3	7015 ± 471 a
0.2%	74.0 ± 4.6 a	8 ± 0.2	6886 ± 356 a
1%	60.0 ± 3.9 bc	9 ± 0.5	4732 ± 451 b
2%	61.3 ± 0.8 bc	9 ± 0.1	4769 ± 394 b
3%	59.3 ± 3.7 bc	10 ± 0.1	4741 ± 312 b
4%	57.3 ± 1.9 bc	10 ± 0.3	4337 ± 169 b
5%	51.3 ± 3.9 c	11 ± 0.2	3761 ± 489 b
7%	52.7 ± 2.5 c	11 ± 0.7	3931 ± 195 b
10%	50.0 ± 2.1 c	11 ± 0.4	3898 ± 380 b

Values represent mean ± standard error. Values in each column with the same letter are not significantly different at 1% level of significance.

Table 4. Effect of smoke-water on percentage germination of papaya seedling at 15 days after sowing grown in peat moss infected with *Rhizoctonia solani*.

Smoke-water	Germination (%)	Average germination time (days)
Control	27.33 ± 6.53 b	11 ± 0.16
1%	62.67 ± 5.91 a	11 ± 0.32
2%	70.00 ± 5.68 a	11 ± 0.19
3%	75.33 ± 7.27 a	11 ± 0.15
4%	66.67 ± 3.16 a	11 ± 0.28
5%	76.00 ± 1.94 a	11 ± 0.08
6%	72.67 ± 2.87 a	11 ± 0.10

Values represent mean ± standard error. Values in each column with the same letter are not significantly different at 1% level of significance.

Table 5. Effect of smoke-water on percentage *Rhizoctonia solani* damping-off and survival of papaya seedling at 21 days after sowing.

Smoke-water	Percentage of damping-off of disease papaya seedling (%)	Survival (%)
Control	100 ± 0.00 a	0.00 ± 0.00 b
1%	98.67 ± 0.82 a	1.79 ± 1.11 b
2%	92.67 ± 2.21 a	9.63 ± 2.70 b
3%	93.33 ± 1.83 a	8.04 ± 2.15 b
4%	75.33 ± 2.71 b	37.86 ± 5.40 a
5%	71.33 ± 1.70 bc	37.80 ± 2.38 a
6%	61.33 ± 4.79 c	53.69 ± 7.11 a

Values represent mean ± standard error. Values in each column with the same letter are not significantly different at 1% level of significance.

Discussion

Characteristics of smoke-water

Smoke-water solution is acidic and contains substantial amounts of plant nutrients such as high level of NH_4^+ which important nitrogen sources for plant growth, increasing seed, fruit productions and improving the quality of leaf and vegetable crops. It has been noted that preferential uptake of NH_4^+ is documented for wetland plant species particularly, *Oryza sativa*, the biotic source of smoke-water in this study. In addition, smoke water contains a high content of antifungal phenolic compounds which may exhibit growth inhibition of microbial.

Identification of smoke-water

The compounds of smoke-water derived from burnt dry rice straw (*Oryza sativa*) were analyzed through GC-MS and 30 compounds were identified. It was observed that some compounds have a chemical structure similar to the simplest butenolide (the main germination active compound in plant-derived smoke) and is colloquially called "butenolide" in the context of natural product synthesis (Van Staden *et al.*, 2004). One example is 2(5H)-Furanone, a heterocyclic organic compound. Classified as a lactone, this colourless liquid is a common natural product synthesized by biochemical pathways. (Jan, 1993)

Butenolides are a class of lactones with a four-carbon heterocyclic ring structure. They are sometimes considered oxidized derivatives of furan. The simplest butenolide is 2-furanone, which is a common component of larger natural products and is sometimes referred to as simply "butenolide". Butenolide derivatives known as karrikins are produced by some plants upon exposure to high temperatures due to brush fires. In particular, 3-methyl-2H-furo[2,3-c]pyran-2-one was found to trigger seed germination in plants whose reproduction is fire-dependent. (Joule and Mills, 2000).

Smoke-water can promote germination or inhibit, depending on the concentration and species-specific manner. Prolonged smoke treatment or high concentrations of smoke-water have been shown to inhibit germination in other species (Dreweres *et al*., 1995) and may be leached to promotive levels through irrigation (De Lang and Boucher, 1993) Result indicated that the seeds of papaya showed a positive response a low concentration of smoke-water to stimulated germination. Thus, smoke-water may be acting on the seed coat in a way similar to scarification, whereby the passage of water and oxygen into the dormant embryo is made easier (Egerton-Warburton, 1998).

Germination of papaya seedling grown in peat moss infected with *Rhizoctonia solani*.

Results that the all smoke-water treatments can be stimulating the seed germination, whereas the control treatment fail to germination of seeds. Indication that the fungi that cause this disease can attack the seed or the seedlings before it emerges above the soil surface, causing a seed rot or pre-emergent damping-off. When this happens, the result is poor stand that may be mistakenly ascribed to poor seed quality or seed maggots rather than to the presence of a disease (Franklin, 2001). Moreover, smoke-water has reduced *Rhizoctonia solani* damping-off and improved survival of papaya seedlings. The significant reduction of the papaya seedlings with showing symptoms *Rhizoctonia solani* damping-off disease by using smoke-water to treated seeds and irrigated could be due to the property of the phenolic and imidazol compounds.

The antifungal activity of smoke-water extract may be due to the presence of phenolic compounds, 9 of which were identified through gas chromatography mass spectroscopy (GC-MS). The phenolic compounds can be either simple phenols and flavonoids, or polyphenols which result from polymerization of the simple phenols, which are toxic to microorganisms (Paraskeva and Diamadopoulos, 2006) and may be inhibiting the growth of several fungi. These compounds are building blocks for cell wall structures and serve as defense against pathogens (Hahlbrock and Scheel, 1989) which damage the cell by altering the cell wall structure. Compounds from smoke water are able to react with proteins by producing a very strong protein-cross-linking and protein-denaturing effect (Ciafardini and Zullo, 2003).

The inhibition of growth of several fungal revealed that the probable presence of antifungal compounds in the smoke-water extract, namely 1H-Imidazole,1-methyl-4-nitroso-5-phenyl-. Imidazole is an organic compound. This aromatic heterocyclic is a diazole and is classified as an alkaloid. Imidazole refers to the parent compound, where imidazoles are a class of heterocycles with similar ring structure, but varying substituents. This ring system is present in important biological building blocks, such as histidine, and the related hormone histamine. Imidazole can serve as a base and as a weak acid. Many drugs contain an imidazole ring, such as antifungal drugs, nitroimidazole and fungicides (Grimmett, 1997).

In summary, smoke-water is obtained by burning dry rice straw (*Oryza sativa*) and then bubbling the smoke through water. Three types of compounds are produced, those are 2(5H)-Furanone that may be stimulating the germination of papaya seeds, phenolic compounds that may be inhibiting the growth of *Rhizoctonia solani*, caused damping-off disease and 1H-Imidazole,1-methyl-4-nitroso-5-phenyl- that is able to inhibit and kill several fungal pathogens.

The present study indicated that smoke-water was found to be effective in improving seed germination and controlling the papaya damping-off disease.

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煙燻水對番木瓜種子萌芽及 *Rhizoctonia solani* 幼苗猝倒病抗性之影響

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關鍵字：煙燻水、2-(5H)-呋喃酮、酚類化合物、咪唑、猝倒病

摘要：煙燻水母液製備是利用乾燥稻草燃燒產生煙霧，通入水中收集之溶液，以供本試驗用。本研究的目的是(1)評估煙燻水對種植於接種 *Rhizoctonia solani* 泥炭土之番木瓜幼苗之發芽率(2)調查煙燻水對 *Rhizoctonia solani* 引起番木瓜幼苗猝倒病之抗真菌能力(3)分析煙燻水的特性及成分。結果顯示，0.1%、0.2%之煙燻水會增加番木瓜種子的發芽率。而煙燻水 1%-6%會刺激接種 *Rhizoctonia solani* 泥炭土盆中之番木瓜幼苗之種子發芽。此外，4%-6%的煙燻水會顯著降低 *Rhizoctonia solani* 引起猝倒病，提升番木瓜幼苗存活率。分析煙燻水的特性，水溶液呈酸性，並富含大量植物所需營養元素，例如植物重要氮源 NH_4^+，並含抗真菌物質，如酚類化合物，會降低 *Rhizoctonia solani* 所引起之猝倒病之罹病率。煙燻水的成分以氣相層析質譜儀檢測，共測得 30 種化合物，主要是醇、內酯、乙醛、酸、酮、生物鹼和酚類等。其中 2(5H)-呋喃酮，可刺激番木瓜種子萌芽。進一步發現，煙燻水中含 9 種酚類化合物，可抑制數種真菌生長。此外，化合物中含咪唑殺真菌劑 1H-Imidazole, 1-methyl-4-nitroso-5-phenyl-，可抑制並殺死真菌病原。

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