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Application of Wood Vinegar for Fungal Disease Controls in Paddy Rice

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Abstract

A survey of an outbreak of fungal diseases of rice variety Phitsanulok2 (PLS91014-16-1-5-1) was conducted in Thailand during June 2014 to January 2015 using a W-random sampling pattern. The study revealed the incidence of several diseases including brown spot (Bipolaris oryzae), narrow brown leaf streak (*Cercospora oryzae*), and dirty panicle (*Alternaria padwickii*, *C. oryzae*, *Curvularia lunata*, *Fusarium semitectum*, and *B. oryzae*). This study evaluated the efficacy of wood vinegar for control of these fungal diseases. A compleel randomized design was used, using the above variety in 3 replications. In the laboratory we found wood vinegar to be effective in inhibiting growth of representative pathogens such as *C. lunata*, *B. oryzae*, *F. semitectum*, and *A. padwickii*, the causal agent of dirty panicle disease. The field results confirmed the efficacy of wood vinegar under greenhouse conditions, with significantly reduced disease incidence of brown spot and dirty panicle, and significantly enhanced germination, seedling vigor, shoot height, root length, and fresh weight, when compared with the untreated control. However, seed treatment and 6 foliar sprays of wood vinegar under field conditions at Ang Thong showed no significant differences from the conventional treatment in suppression of brown spot, narrow brown leaf streak, and dirty panicle. The result demonstrates a promising alternative approach to control of key rice diseases.

Keywords: Agrochemical reduction; Biocontrol; Organic farming; Rice disease

Introduction

In the central part of Thailand, non-photosensitive such as Suphan Buri1 (SPRLR85163-5-1-1-2), Jow Hawm Khlong Luang1 (KLG-83055-1-1-1-2-1-4), Pathumthanil (PTT90 071-93-8-1-1), and Phitsanulok2 (PLS 91014-16-1-5-1) [1] are typically grown. Fungal diseases can cause major losses at all growth stages, and can render the grain unacceptable for consumption as well as for seed. Key fungal

diseases are rice blast (*Pyricularia oryzae*), sheath rot (*Rhizoctonia solani*), dirty panicle (*Alternaria padwickii*, *Cercospora oryzae*, *Curvularia lunata*, *Fusarium semitectum*, and *Bipolaris oryzae*), narrow brown leaf streak (*C.oryzae*) and brown spot (*Bipolaris oryzae*). They tend to infect plants under higher temperatures (25 to 35°C) and high humidity (>80% RH) [1]; however, some pathogens may also remain active after harvest, during storage and transport. Temperatures above 28°C favour rapid infection by all fungal pathogens. Use of contaminated seeds for planting favours infection in the succeeding crop.

Chlorinated hydrocarbons were first used for fungal control, followed by organi-phosphates, then carbamates [4]. However, the high cost of pesticides precluded their use in many cases [4]. Even when farmers could afford to buy pesticides, the health hazards may outweigh the economic benefits. Studies in the Philippines indicated that the effect on farmer health and subsequent lost days of work, and the adverse effects on the environment outweigh the productivity gains from pesticide use [5]. Apart from their cost, toxicity and environmental impacts, chemical insecticides caused secondary pest problems such as resurgence of brown plant hopper, as well as triggering development of resistant populations of pests. In response to these issues, integrated pest management (IPM) has been increasingly adopted, to reduce the dependence on chemicals, maximize use of resistant varieties and conservation of natural enemies, e.g. through use of biologicals and natural control agents [6]. Although there are cases where the judicious use of selective pesticides in rice is necessary, routine, calendar-based applications in a non-IPM context are no longer generally recommended [7].

There are no adequate control measures to manage disease if predisposing factors such as susceptible cultivars and weather conditions favour disease development. However, biological control of plant pathogens is emerging as an important component of plant disease management practices. This alternative control strategy can solve many persistent problems in agriculture including fungicide residues causing environmental pollution and human health hazards, and also inducing pathogen resistance [8-10].

Biological control agents including microorganisms [11] or plant extracts such as wood vinegar are increasingly used. Wood vinegar is a brown condensed acidic liquid which is a by-product of wood charcoal production. Its principal components are acetic acid, methanol, acetone, phenol and tar with a pH of 3.4. It has been widely used in agriculture, in health promotion products and as a wood preservative. In Thailand, wood vinegar is utilized not only in agriculture, but also as a traditional remedy to treat skin infections and dandruff. However, there is no published scientific evidence to support the efficacy of these uses [12]. In agriculture, wood vinegar can also be used to stimulate plant growth. Wood vinegar can improve soil quality, control fungal and insect pests, promote plant growth, and reduce the need for chemical fertilizers. Application of wood vinegar has no side effects and is non-toxic people and animals [13]. Moreover, wood vinegar can retard growth of pathogenic fungi such as *Fusarium, Pythium,* and *Rhizoctonia* [14], and promote growth of plant roots [15-16]. Wood vinegar is a good source for inorganic production in agriculture [17] and has been widely used traditionally in agriculture and daily life in Japan. Of the approximately $4x10^7$ L of wood vinegar produced every year, over half is used in agriculture [18].

Methods

1) Study sites of disease epidemic and fungal isolation

Rice disease incidence was surveyed and sampled at Ayutthaya, Suphanburi, Ang Thong, Singburi, and Chainat during June, 2014 to January 2015 using a W-random sampling with 125 points (Figure 1) per hectare as described by Delp et al. [19]. Fungal samples were taken at all growth stages from seedling to harvest. Samples were placed in sealed plastic bags and stored in an ice box. The causal agents were isolated from the infected plant sample by the tissue transplanting technique. Fungal pathogens were then cultured on potato dextrose agar (PDA) for 7 days old at room temperature (28± 2^0C).

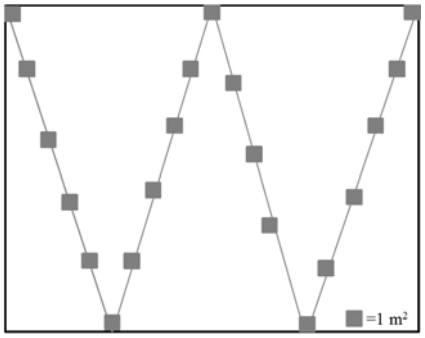

Figure 1 Schematic sampling of diseases incidence (described by Delp et al.) [19], completely random with 125 points/ha

2) Pathogenicity test

Spore suspensions of the representative isolates of *C. lunata*, *B. oryzae, F. semitectum*, *A. padwickii* (the causal agents of dirty panicle) were prepared at 1x10^6 spore/ml concentration as determined by hemocytometer. A pathogenicity test on rice seeds (cv. Pisanulok2, a susceptible variety) was conducted using previously described quantitative methods [5]. Disease incidence on rice seeds was assessed at 7 days after inoculation.

3) Wood vinegar effectiveness test

3.1) Efficacy of wood vinegar under laboratory conditions

Wood vinegar used in this study was prepared from bamboo wood and obtained from the Thailand RURRL Reconstruction Monument (TRRM). The optimum pyrolysis conditions were obtained at a heating rate of 1.4°C/min to a final temperature of 550 C. Wood vinegar was initially infiltrated through Whatman filter paper No. 1, and then sterilized using 0.22 µm filters before assessment for antifungal activity. Antifungal activity of wood vinegar against *B. oryzae*, *C. lunatae*, *A. padwickii*, and *F. semitectum* using poisoned medium technique supplemented with 1, 2, and 5% of wood vinegar were tested and compared with biological control agent (*Bacillus amyloliquefaciens*, KPS46 and *Pseudomonas fluorescens*, SP007s from the Department of Plant Pathology, Kasetsart University, Bangkok) [7], chemical control (carbendazim), and non-treated control [20]. Comparison between the growth of fungi on poisoned medium and PDA at 7 days after inoculation was evaluated as pathogen was taken into consideration.

3.2) The efficacy of wood vinegar under greenhouse conditions

A pot experiment was designed under greenhouse conditions using plastic pots (30 cm, diameter) containing sterilized soil. Ten rice seeds were sown in each pot; five replicate pots were specified for each treatment in a completely randomized experimental design. The experiment comprised the following treatments: T1 (seed treatment plus 6 times interval foliar sprayed with wood vinegar at 15, 30, 45, 60, 75, and 90 days after planting); T2 (seed treatment plus 6 times interval foliar sprayed with fresh cell of *P. fluorecens* SP007s at 15, 30, 45, 60, 75, and 90 days after planting); T3 (seed treatment plus 6 times interval foliar sprayed with fresh cell of *B. amyloliquefaciens* KPS46

at 15, 30, 45, 60, 75, and 90 days after planting); T4 (seed treatment plus 6 times interval foliar sprayed with carbendazim at 15, 30, 45, 60, 75, and 90 days after planting), and T5 (seed treatment plus 6 times interval foliar sprayed with distilled water at 15, 30, 45, 60, 75, and 90 days after planting). All treatment were inoculated with representative dirty panicle pathogens such as *B. oryzae* at 30 days. The pots were kept under greenhouse conditions until the end of the experiment. Disease assessment was conducted weekly.

3.3) Efficacy of wood vinegar under field conditions

Rice seeds (cv. Phitsanulok2) were soaked in sterile water overnight and incubated for 1 day to initiate sprouting at room temperature. Seed treatment of these sprouted seeds was conducted before seeding by broadcasting into the field at a rate of 5 kg/400 m^2 with no transplantation. The field experiment was conducted using a completely randomized design with 4 treatments and 3 replications under farmer's field in Ang Thong province. Plot size was 20x 20 m^2 with a spacing of 4x5 cm^2. Field irrigation and other routine agronomic practices followed conventional standard protocols for all treatments [21]. The 5% of wood vinegar (T1), fresh cell culture of *B. amyloliquefaciens* KPS 46 (T2), and fresh cell culture of *P. fluorescens* SP007s (T3) were each thoroughly mixed in water (100 mL $20L^{-1}$ of water) and 6 time foliar sprayed every 15 days after planting (at 15, 30, 45, 60, 75, and 90 days after planting). The conventional treatment (T4) was applied with a recommended dose of fungicides (propiconazole + difenoconazole applied 4 times during 14-70 days old plant), and insecticides (cypermethrin + dinotefuran and chlorpyrifos at 30, 37, and 45 days). Disease incidence in the entire plots of the naturally infested field was assessed weekly from 14-days after planting, and continued until 2 weeks before harvest at 110-day after planting. Disease rating was performed as disease incidence using W-random sampling as described by Delp et al. [19], Disease reduction was then calculated following Campbell and Madden (1990) [23]. Growth characters (lateral root, plant height and tiller number), yield components (spikelets per panicle), and yield were measured. All data recorded were subjected to analysis of variance (ANOVA) followed by determining the difference among treatment means using Duncan's Multiple Range Test (DMRT) [22].

Results and Discussion

1) Disease epidemic and pathogen isolation

Disease outbreaks of rice in the central region of Thailand were dominated by bacterial leaf blight (*Xanthomonas oryzae* pv. *Oryzae*), rice blast (*Pyricularia oryzae)*, sheath blight (*Rhizoctonia solani*), dirty panicle (*Alternaria padwickii, Cercospora oryzae, Curvularia lunata, Fusarium semitectum*, and *Bipolaris .oryzae*), narrow brown leaf streak (*C. oryzae*) and brown spot (*B. oryzae*). Three main outbreaks of disease were observed: brown spot, narrow brown leaf streak, and dirty panicle, which showed significant incidence 96.3, 82.7 and 74.5%, respectively (Figures 2 and 3). Since dirty panicle disease affects yield quality and quantity, and contaminates the next season's crop [23], we focused on dirty panicle and its control methods in order to support a sustainable rice production system.

2) Pathogenicity test

All of the 30, 41, 28, and 22 isolates of *C. lunata*, *B. oryzae, F. semitectum*, *A. padwickii* caused of dirty panicle symptoms on rice seed cv. Phit-sanulok2. The *C. lunata* isolate W-TU-12, *B. oryzae* isolate W-TU-35*, F. semitectum* isolate W-TU-08, and *A. padwickii* isolate W-TU-15 showed highest significance of disease, with 78.3, 85.2, 67.8, and 64.3%, respectively, at 7 days after inoculation ($p \geq 0.05$) (Figure 4).

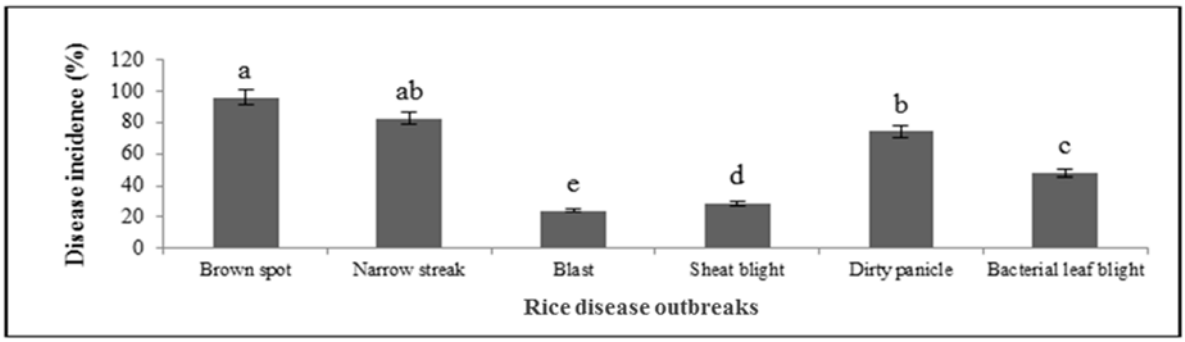

Figure 2 Percentages of rice disease outbreaks in the central production regions of Thailand during June, 2014 to January, 2015. Means followed by the different letters are significantly different ($p \geq 0.05$) by the Duncan's Multiple Range test .Error bars indicate ±SD.

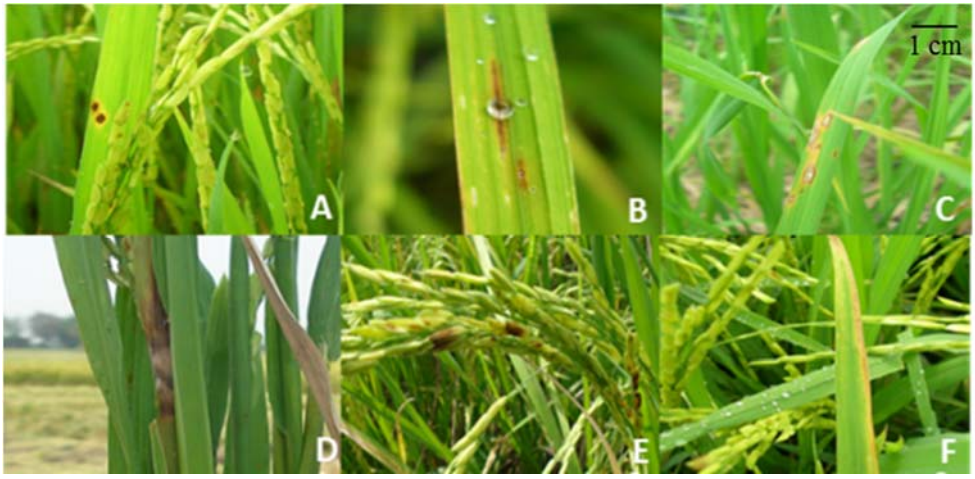

Figure 3 Rice disease outbreaks in the central production regions of Thailand during June 2014 - January 2015 including; brown spot (A), narrow brown leaf streak (B), blast (C), sheath rot (D), dirty panicle (E), and bacterial leaf blight (F). (scale bar=1cm)

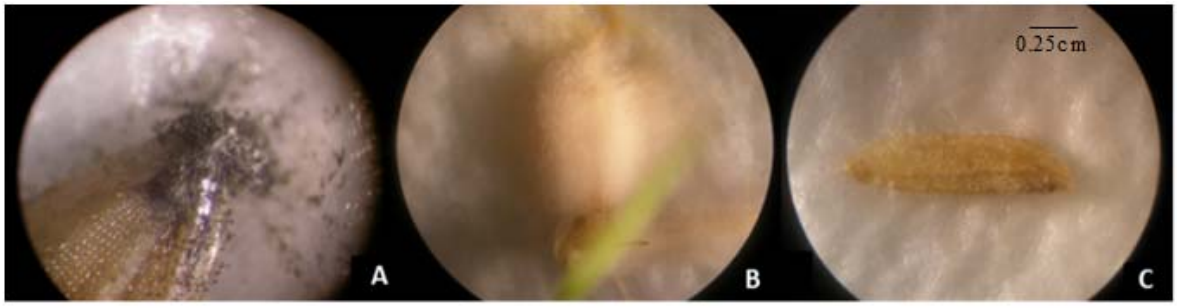

Figure 4 The *Bipolaris oryzae* W-TU-35 (A) and *Fusarium semitectum* W-TU-08 (B) infected on rice seeds compared with non-infected rice seed (C). (scale bar=0.25cm)

3) Wood vinegar effectiveness test

3.1) Efficacy of wood vinegar under laboratory conditions

The colonies of *A. padwickii, C. lunata, F. semitectum*, and *B. oryzae* were inhibited on the poisoned medium (PDA mixed with 5 %wood vinegar) with 70.0, 76.4, 75.3, and 72.8%, respectively when compared with non-treated control (Figure 5). In addition, it has been reported that the degree of concentration determines whether wood vinegar destroys soil microbes or facilitates their growth. At high con-

centrations, wood vinegar has a strong germicidal effect due to its high acidity and the presence of germicidal ingredients. The microbes first killed by wood vinegar are bacilli which have no spores, and some hyphomycetes which are weak or cannot grow under low pH conditions. However, when wood vinegar is diluted it greatly increases the concentration of microbes [24].

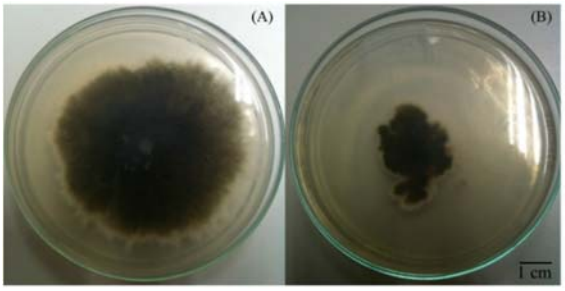

Figure 5 Efficacy of poisoned medium (PDA mixed with 5 %wood vinegar) to inhibited *Bipolaris oryzae* W-TU-35 (B) compared with untreated control (A) under room temperature conditions at 7 days after inoculation (scale bar=1 cm)

3. 2) The efficacy of wood vinegar under greenhouse conditions

Under greenhouse conditions, wood vinegar was shown to be effective in reduction of brown spot and dirty panicle, when wood vinegar was applied as a seed treatment plus foliar sprayed 6 times intervals with 5% wood vinegar every 15 days (T1), compared to antagonistic bacteria (*P. fluorescens* SP007s and *B. Amyloliquefaciens* KPS46) and chemical treatment. Application of wood vinegar resulted in reduction of brown spot and dirty panicle by 70.5 and 72.5%, respectively. Also, wood vinegar enhanced seedling vigor including seed germination, shoot height, root length, and fresh weight (Figures 6 and 7). Especially, T1 increased significantly seed germination and fresh weight when compared with control treatments. However, wood vinegar has the ability to inhibit pathogenic fungi as *B. oryzae* due to its chemical components such as acetic acid, formaldehyde and methanol, as previous reported [25].

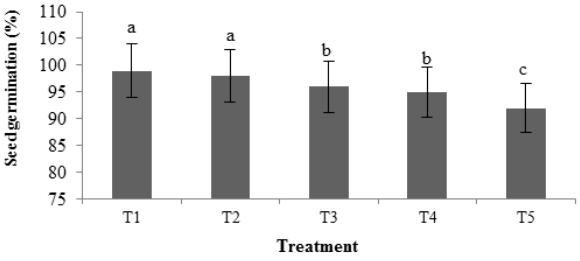

Figure 6 Efficacy of wood vinegar to enhance germination of rice seeds after seed treatment at 7 days. The treatment details listed in Materials and Methods. Means followed by the different letters are significantly different by the Duncan's multiple range test ($p \geq 0.05$). Error bars indicate ±SD. The list of treatment described in Materials and Methods.

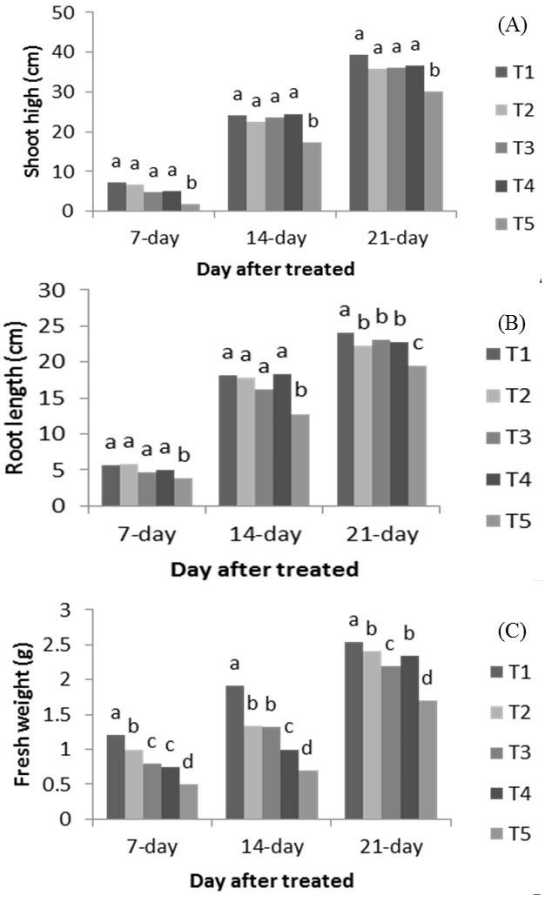

Figure 7 Efficacy of wood vinegar to enhance shoots height (A), root length (B), and fresh weight (C) of rice seedling after seed treatment. Treatment details are listed in Materials and Methods. Means followed by the different letters are significantly different by the Duncan's Multiple Range Test ($p \geq 0.05$).

3.3) Efficacy of wood vinegar under field conditions

Wood vinegar, *B. amyloliquefaciens* KPS46, and *P. fluorescens* SP007s were showed higher records of yield and yield components (spikelets per panicle), when compared with conventional control methods (Table 1). However, the effect of application of wood vinegar was not significantly different from conventional treatments in suppression of epidemic diseases including brown spot, narrow brown leaf streak, and dirty panicle (Table 2). According to previous research of Kishimoto (1991), although wood vinegar seemed to promote seed germination, application of its single principal components such as acetic acid, did not show any promotional effect [24]. Therefore, wood vinegar has potential to contribute to control of fungal pathogens in rice crops, as well as its existing wide use in crop production for plant growth stimulation, seed germination, soil disinfection, and control of weeds, diseases and insect pests [26].

Table 1 Effect of wood vinegar on growth and rice yield under field conditions[1]

Treatment	Growth and yield index				Yield (ton/ha)
	Plant height (cm)	Tiller number (plant/clump)	Lateral root (root/plant)	Spikelets per panicle	
T1	112.8±2.25[a]	10.8±1.10[a]	62.6±2.31[a]	122.5±1.14[a]	6.1±5.21[a]
T2	114.2±2.21[a]	11.4±1.21[a]	65.3±2.25[a]	120.5±1.12[a]	6.1±5.23[a]
T3	111.3±2.33[a]	10.6±1.11[a]	63.4±2.22[a]	100.5±1.16[b]	6.0±5.11[a]
T4	102.3±2.74[b]	9.2±1.20[b]	58.5±214[b]	99.4±1.23[b]	5.8±4.85[b]

[1] Means followed by a same letter in a column are not significantly different according to Duncan's Multiple Range Test ($p \geq 0.05$). Values are means ± SD. Treatment are described under Materials and Methods.

Table 2 Efficacy of wood vinegar application on natural diseases reduction under field conditions[1]

Treatment	Disease reduction%) [1]		
	Brown spot	Narrow brown leaf streak	Dirty panicle
T1	69.8±1.25[a]	72.0±1.56[a]	70.8±1.86[a]
T2	70.8±1.22[a]	72.5±1.23[a]	69.5±1.35[a]
T3	68.2±1.08[a]	68.3±1.77[a]	68.2±1.25[a]
T4	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]

[1] Means followed by a same letter in a column are not significantly different according to Duncan's Multiple Range Test ($p \geq 0.05$). Treatments are described under Materials and Methods.

Conclusion

The results revealed in this study corroborated earlier studies and point to the potential of wood vinegar as a means of promoting growth and health of rice crops. Seed treatment and foliar application with wood vinegar significantly enhanced growth of rice plants and particularly reduced disease incidence. The results suggested that the use of wood vinegar can effectively inhibit fungal diseases in rice high, as compared with agrochemical treatments.

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