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Effects of Plant Extracts on Crop Diseases, Two-Spotted Spider Mites, and Weeds

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ABSTRACT

This research was conducted to evaluate the controlling effects of various plant extracts obtained from 38 agricultural materials of traditional agriculture against rice blast (*Pyricularia oryzae*) in rice plants. We also evaluated the inhibition rates of other crop pathogens, two-spotted spider mites (*Tetranychus urticae*), and weeds by three selected plant extracts. Rice blast was completely suppressed by 3% ethanol extracts of *Rheum palmatum* roots, and suppressed by 97% and 77% in response to treatment with 10% ethanol extracts of onion (*Allium cepa*) bulbs and pine tree (*Pinus densiflora*) leaves, respectively, under *in vitro* conditions. Moreover, a seedling bioassay revealed that rice injuries of two cultivars (Ilmibyeo and Hopyeongbyeo) caused by rice blast were reduced by 40-71, 29-63, and 23-63% in response to treatment with 5% and 10% ethanol extracts of *R. palmatum* roots, *A. cepa* bulbs, and *P. densiflora* needles, respectively, compared with non-treated controls. The selected *R. palmatum* extract also controlled crop diseases caused by *Phytophthora capsici, Bortytis cinerea, Rhizoctonia solani,* and cucumber powdery mildew (*Sphaerotheca fuliginea*). *R. palmatum* extracts at 10% reduced two-spotted spider mites by 46% when compared with each control. A broad leaf weed, *Quamoclit coccinea* Moench and a grass weed, *Echinochloa oryzicola* were inhibited 46-55% by *R. palmatum* roots, *A. cepa* bulbs, and *P. densiflora* needles at 10%. Thus, the selected plant extracts may be used for control of crop diseases and weeds in organically cultivated crop fields.

Keywords: Agricultural organic materials, *Pyricularia oryzae*, Rice blast, Traditional agriculture.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most widely cultivated crop in Asia and an important staple food for approximately 45% of the world's population (Lee, 2001; Li *et al*., 2011). In Korea, rice is grown at an average of 1.08 Mha with a total production of 4.5 million tons and an average yield of 5,000 kg ha^{-1}. However, many diseases, such as rice blast, sheath blight, brown spot, bacterial leaf blight, and leaf streak occur in rice cultivation and cause great reductions in rice yields (Mew and Gonzales, 2002; Sharma and Bambawale, 2008). Among these diseases, rice blast is the most serious, and is prevalent in more than 85 rice producing countries worldwide (Gilbert *et al*., 2004). Rice blast is caused by a filamentous ascomycete, *Pyricularia oryzae*. It infects plants by spores and produces lesions or spots on the leaves, leaf collars, and panicles. The pathogen results in yield losses of 1-50%, depending on the type of cultivated varieties and prevailing environmental conditions (Greer *et al*., 1997). Synthetic chemical fungicides have been routinely applied to control rice blast disease in the field (Gohel *et al*., 2008). However, their continued use leads to the

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development of resistance in strains of fungal pathogens (Avis, 2007).

On the other hand, natural products have been shown to be safe and have less impact on the environment (Dayan *et al*., 2009). Therefore, the demand for, and development of, natural products for disease control has increased, particularly those intended for use in organic agricultural production systems (Dayan *et al*., 2009). Recently, many studies have investigated practical approaches to application of plant extracts to control plant pathogenic fungi such as *P. oryzae* in organically produced rice crops (Choi *et al*., 2006). Furthermore, plant extracts of *Styrax japonica*, *Pittosporum tobira,* and *Carmella japonica* have been shown to have a controlling effect against rice blast (Jang *et al*., 2016).

Rhubarb (*Rheum palmatum*, Polygonaceae) is a medicinal plant that contains considerable amounts of anthraquinones such as chrysophanol, emodin and physcion. The anthraquinone derivatives emodin and chrysophanol have activities against phytopathogens such as cucumber powdery mildew (*Sphaerotheca fuliginea*) and rice sheath blight (*Rhizoctonia solani*) (Choi *et al*., 2004; Izhaki 2002). The needles of pine (*Pinus densiflora* S. and Z.), which belong to the Pinaceae family, produce an essential oil (0.3-1.3%) that contains α-pinene, β-pinene, camphene, phellandrene, limonene, borneol (6.8%), and bornyl acetate (3.8%) (Kim and Shin, 2005). Onion (*Allium cepa* L.) is a bulbous plant widely cultivated in almost every country of the world (Hertog *et al*., 1995) that serves as a major source of dietary flavonoids in many countries. *Allium* plants such as *A. cepa* have high antioxidant, antibacterial and antifungal activities that have mainly been attributed to a variety of sulfur-containing compounds and their precursors (Kim *et al*., 1997; Yin and Cheng, 1998).

In Korea, a total of 1,495 eco-friendly agricultural materials are registered, including 40 plant extracts (RDA, 2016). Agricultural organic materials for controlling diseases and insects in organically produced crops have not been as effective as conventional methods (pesticides). But classical farming systems in South Korea are based on natural resources that are readily accessible in farmland, which may facilitate expansion of the base of current organic farming technologies. To date, traditional farming technology for crop protection has been taught using old agriculture books (Guh *et al*., 2011). However, no studies have reported whether the agricultural organic materials used in traditional farming technology affect crop protection. Thus, this study was conducted to determine the ability of plant extracts obtained from 38 materials used in traditional agriculture using different extraction methods (water, boiling water, and ethanol) to control rice blast (*P. oryzae*) in rice plants. In addition, we evaluated the phytotoxicity of the selected plant extracts for application to rice as well as their inhibition effects on other crop pathogens, namely, the two-spotted spider mites (*Tetranychus urticae*) and weeds.

MATERIALS AND METHODS

Plant Materials

Thirty-eight agricultural materials (see Table 1) used in this study were selected after considering potential effects on rice blast and other crop diseases recorded in an old agriculture book (Guh *et al*., 2011). Some agricultural materials such as rice hull, rice bran, and barley bran were collected from the milling process, and other materials such as pine needle, composts, charcoal power and oil cake were purchased from each Korean Material Cooperation (Suncheon, South Korea). Additionally, bulbs and stems of *A. cepa* and *R. palmatum* root were obtained from Jeollanamdo Agricultural Research and Extension Service and dried in a drying oven at 40°C for 5 days.

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Table 1. Inhibition rate of extracts of various organic materials against *Pyricularia oryzae* under *in vitro* test. Parameter was recorded at 3 days after treatment.

Inhibition rate (%)	5% Extract			10% Extract		
	Water	Ethanol	Boiled water	Water	Ethanol	Boiled water
11-20		Oil cake (Sesame), Charcoal (Pine tree)	Pine tree (Branch)	Barley bran (cv. Geotboli), Barley bran (cv. Huinssalboli), Rice bran (cv. Kanto504), Rice bran (cv, Cheongmoo), Rice straw ash (cv. Hwangeunnodle), Organic compost (Geumkangyoobaek), Pine tree (Branch)	Compos t (Busuck), Oil cake (Sesame), Rice bran (cv, Cheongmoo), Rice hul l(cv. Hwangeunnodle)	Organicfertilizer (Geumkang), Compost (Busuck), Organiccompost (Geumkangyoobaek), Pine tree (Branches), Barley bran (cv. Geotboli), Rice bran(cv. Kanto504)
21-30		Pine tree (branch), Gralic stem (cv. Daeseo)	Onion (cv. Cheonjoojeok), Onion (cv. Maepsihwang)		Charcoal (Pine tree)	
31-40	Pine tree (needle), Onion (cv. Cheonjoojeok)	Pine tree (needle)	Pint tree (needle)		Gralic stem (cv. Daeseo), Pine tree (branch), Barley bran (cv. Geotboli), Barley bran (cv. Huinssalboli)	
41-50	Onion (cv. Maepsihwang), Onion (cv. Deoshingihan)	Onion (cv. Cheonjoojeok)	Onion (cv. Deo shingihan)	Pine tree (needle)		Onion (cv. Maepsihwang), Onion (cv. Cheonjoojeok)
51-60		Onion (cv. Romang)				Onion (cv. Deoshingihan)
61-70		Onion (cv. Deoshingihan)		Onion (cv. Cheonjoojeok)		Pine tree (needle)
71-80	Onion (cv. Romang)		Onion (cv. Romang), Rhubarb root	Onion (cv. Deoshingihan), Onion (cv. Maepsihwang)	Pine tree (needle)	
81-90		Onion (cv. Maepsihwang)		Onion (cv. Romang)	Onion (cv. Deoshingihan), Onion (cv. Cheonjoojeok), Onion (cv. romang)	Onion (cv. Romang)
91-100	Rhubarb root	Rhubarb root		Rhubarb root	Rhubarb root, Onion (cv. Maepsihwang)	Rhubarb root

Effect of Agricultural Materials on Inhibition Rates of Rice Blast

For each type of extraction (water, boiled water, and ethanol), 50 g of ground powder of agricultural materials were used in separate processes to compare the inhibition effect. They were extracted from homogenizer in 1,000 mL distilled water and ethanol for 24 hours for water and ethanol extracts. Fifty g of each grinded agricultural material was placed in 1,000 mL distilled water and boiled at 100°C for 30 minutes for boiling water extract. Thereafter, each extract was filtered through a Miracloth and evaporated under reduced pressure. The remaining liquid was completely evaporated using a vacuum dryer (Hanbaek Scientific Co. South Korea). Afterwards, each extract was resolved in distilled water to ensure that the final concentration was 50% (w/v), which was then diluted with distilled water to attain 5 and 10% concentrations for experiment on inhibition rates of rice blast. To accomplish 5 and 10% final concentrations, the extracts were added to Potato Dextrose Agar (PDA) media in Petri dishes (90 mm). In the control treatment, only distilled water was used . After solidification, a mycelia plug (10 mm diameter) of *P. oryzae* was placed in the center of the Petri dishes and incubated at 26 °C in darkness (Jang *et al*., 2016). Three-day-old cultures of the test fungus grown on PDA medium were used for bioassays. Radial mycelia growth of the test fungus was recorded at 3 days after treatment. The inhibition activity was calculated using colony diameter growth of treated plates compared to the control plates (PDA medium without extract).

Effect of Selected Plant Extract on Inhibition of Rice Blast

Three ethanol plant extracts, namely, *R. palmatum* root, *A. cepa* bulb, and *P. densiflora* needle were selected from the aforementioned experiment to study its inhibition effect on rice blast by extraction methods in various agricultural materials. To confirm concentration responses, inhibition effect on rice blast was determined by the selected plant extracts at 0, 0.5, 1, 3, 5, and 10% concentrations. Other procedures were the same as described above.

Two *P. oryzea* susceptible rice cultivars, i.e. cv. Ilmibyeo and Hopyeongbyeo, were used for rice seedling bioassays (Jang and Kuk, 2017). Ilmibyeo and Hopyeongbyeo plants were sown at a density of 5 seeds per pots (200 mL) filled with commercial potting soil (Pungnong NPKO, Seoul, South Korea). Rice blast was induced by mixing infected and uninfected trays. The plants were grown with day/night mean temperatures of 28/22°C, relative humidity of 70/90%, and a 14/10 hour day/night period. Fifteen-day-old seedlings of Ilmibyeo and Hopyeongbyeo that were 0, 5% or 10% infected with rice blast were sprayed with 0, 5, and 10% of the selected plant extracts. The spray volumes were 5 mL per pot and the control plants were only sprayed with water. Infestation due to the rice blast was evaluated visually (0-100; 0= No damage, 100= Complete death) at 1, 3, 5, and 7 days after treatment while plant height and shoot fresh weight were measured 7 days after treatment.

Inhibition of Crop Pathogens, Two-spotted Spider Mites and Weeds

The inhibition rates of four fungal pathogens, *Bortytis cinerea, Rhizoctonia solani, Phytophthora capsici,* and *Colletotrichum coccodes* (anthracnose), were investigated as described above. The selected *R. palmatum* extracts were sprayed (5 mL per plant) with a hand sprayer at 3 leaf-stage of cucumber (cv. Hodongchungjang) plants in a greenhouse (Jang and Kuk, 2017). Cucumber plants were not inoculated; however, they got natural infections of powdery mildew. These infections covered about 20% of the leaves surface area. The above extracts treated were 0, 3, 5, and 10%, and the extracts were applied

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once or twice with 5-day intervals. Controlling value was evaluated at each 7-day interval after treatment. The calculation of controlling value is as follows:

Controlling value (%)= (1-Symptom area in treated plot/Symptom area in untreated plot)×100

Thirty two-spotted spider mite adult females were inoculated on a kidney bean (*Phaseolus vulgaris* var. *humilis*) host plant. Kidney bean leaf discs (4 cm diameters) were placed bottom-side up, on moistened cotton in a petri dish (9 cm diameter) and then sprayed (5 mL per petri dish) with *R. palmatum* extract at 3, 5, and 10% concentrations with a hand sprayer. The petri dishes were placed in a growth chamber (day/night mean temperatures of 28/22°C, relative humidity of 70/90%, and a 14/10 hour day/night period with 100 μmol nm^2 s^{-1} light intensities). Acaricidal activity was investigated at 1, 3, and 5 days after treatment.

To determine inhibition of seedling growth of *Quamoclit coccinea* Moench and *Echinochloa oryzicola* (barnyard grass), the selected plant extracts were used for Petri dish bioassay. The bioassay used 9-cm-diameter Petri dishes, containing 10 seeds, as experimental units arranged in a completely randomized design with three replications. Seeds were placed in Petri dishes between two sheets of filter paper (Whatman No.1). Five mL of plant extracts at 0.5, 1, 3, and 5% concentrations were applied to each Petri dish, which were then incubated in the dark for 3 days and in the light for 3 days in a growth chamber (conditions mentioned above). Thereafter, the shoot and root lengths of each seedling were measured. Data were expressed as percentage of the non-treated control.

Phytotoxicity of Selected Plant Extracts

Rice seedlings were cultivated by the same method as the previous section. Rice damage (Visual rate: 0-100%, 0= No damage), plant height, and shoot fresh weight were investigated at 1, 3, 5, and 7 days after treatments of ethanol extracts of finally selected *R. palmatum* root, *A. cepa* bulb, and *P. densiflora* needle at 0, 1, 3, 5, and 10% in Ilmibyeo and Hopyeongbyeo at 15-day-old seedlings to confirm rice safety.

Statistical Analysis

Significant differences were determined using Analysis Of Variance (ANOVA). Analyses were performed using Statistical Analysis Systems software (SAS, 2000). In the case of significant difference, means were separated by Duncan's Multiple Range Test at P≤ 0.05.

RESULTS AND DISCUSSION

Effects of Various Agricultural Materials on Rice Blast

The rice blast inhibition rates were investigated following treatment with water, boiling water, and ethanol extracts (all together 84) of 38 agricultural materials at 5 or 10% in *in vitro* bioassays (Table 1). Forty-three of the 84 extracts showed < 10% inhibition of rice blast (data not shown). Rice straw, rice straw ash, rice hull, rice hull ash, rice bran, barley bran, bean cake, charcoal (oak, bamboo, pine tree), pine tree, organic compost, onion stem (cultivars Romang, Cheonjujeog), and manure also showed < 20% inhibition rates, regardless of extraction methods and concentrations. Additionally, ethanol extracts of pine tree, pine tree (ash), and barley bran (cvs. Huinssalboli, Geotboli), as well as water and boiling water extracts of pine needles 5 or 10% showed 21-40% inhibition rates. Water and boiling water extracts of pine needle at 10% showed rice blast inhibition rates of 41-50%. Although the agricultural materials mentioned above appear in old books (Guh *et al*., 2011) regarding potential control of rice blast and other pests, the controlling effect of rice blast from this study was very low. However, rice blast was suppressed by 74% in response to treatment with ethanol extract of pine needles, 100% by ethanol extract of onion (cv. Maebsihwang),

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and 100% by water, boiling water, and ethanol extracts of *P. palmatum.* Choi *et al.* (2006) reported > 90% inhibition of rice blast in response to extracts (1,000 μg mL^{-1}) of *Aleurites fordii* (Hemsl.) Airy Shaw, *Camellia japonica*, *Thuja orientalis* L., *Pittosporum tobira* (Thunb.) Ait., and *Styrax japonica* (snowbell) fruits among 67 plant species. In addition, even though the extraction method differed (boiling water), Jang *et al*. (2016) found 100% inhibition of rice blast in response to 3% extract of *R. palmatum* roots. The quality of a plant extract depended on the plant material, choice of solvents and extraction methods (Abdelgaleil *et al*., 2019; Dhanani *et al*., 2017; Gurjar, 2012; Zlotek *et al*., 2016). This difference may be because of the different physiologically active ingredients among plant species and extraction methods.

Effect of Selected Plant Extracts on Rice Blast in Infected Rice Seedlings

From 38 agricultural materials, we selected plant extracts of three species (*R. palmatum* root, *A. cepa* bulb, and *P. densiflora* needle) that showed the highest inhibitory effects on rice blast for further study (Table 1). Hereafter, these extracts will be referred to as "selected extracts." The order of inhibition of rice blast was *R. palmatum* root> *A. cepa* bulb> *P. densiflora* needle. Rice blast was inhibited by 100% in response to treatment with ethanol extract of *R. palmatum* root at 3% (Figure 1). Jang *et al*. (2016) demonstrated that rice blast was completely suppressed by 3% boiling extracts in *R. palmatum* roots, *C. japonica* stems, *P. tobira* leaves, and *S. japonica* leaves among 20 plant species from 11 families in *in vitro* test. Additionally, rice blast was inhibited by 97 and 77% in response to treatment with 10% ethanol extracts of *A. cepa* bulbs and *P. densiflora* needles, respectively. These results suggest that the selected plant extracts were able to suppress mycelial growth of rice blast in an *in vitro* test.

Thus, to confirm its inhibition ability, two rice cultivars, namely, Ilmibyeo and Hopyeongbyeo, were grown in a greenhouse and infected with the rice blast (see Materials and Methods) (Table 2). Fifteen-

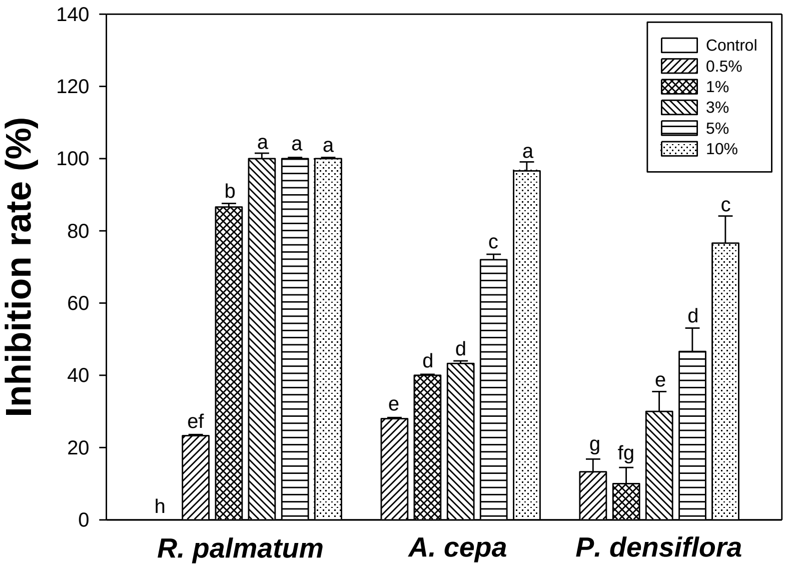

Figure 1. Effects of selected plant ethanol extracts on inhibition rate of *Pyricularia oryzae* under *in vitro* test. Parameter was recorded at 3 days after treatment. Means within bars followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

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Table 2. Effect of selected plant extracts on leaf injury, plant height and shoot fresh weight in *Pyricularia oryzae* infected greenhouse grown 15 days after rice seedlings (cv. Ilmibyeo and Hopyeongbyeo).

Infection rate (%)	Plant extract[a]	Conc. (%)	Ilmibyeo						Hopyeongbyeo					
			Leaf injury (%)				Plant height (cm)	Shoot FW[b] (g pot^{-1})	Leaf injury (%)				Plant height (cm)	Shoot FW (g pot^{-1})
			1 DAT[c]	3 DAT	5 DAT	7 DAT	7 DAT	7 DAT	1 DAT	3 DAT	5 DAT	7 DAT	7 DAT	7 DAT
Control			33.5^{a}	56.5^{a}	75.0^{a}	88.0^{a}	20.5^{c}	2.20^{e}	34.0^{a}	55.0^{a}	82.5^{a}	92.0^{a}	19.5^{d}	2.13^{d}
0	*Rheum palmatum*	5	11.3^{b}	18.5^{c}	30.0^{bc}	38.5^{cd}	29.0^{a}	5.74^{b}	10.5^{bc}	21.0^{c}	37.5^{c}	45.0^{e}	27.0^{b}	5.62^{a}
		10	12.5^{b}	15.5^{c}	23.5^{c}	30.0^{d}	31.0^{a}	6.04^{a}	8.5^{c}	15.0^{d}	27.5^{d}	35.0^{f}	28.0^{ab}	5.86^{a}
	Allium cepa	5	12.5^{b}	28.5^{b}	34.8^{ac}	46.2^{c}	25.6^{ab}	3.89^{d}	11.5^{b}	25.8^{b}	38.7^{c}	62.5^{c}	24.8^{c}	4.26^{b}
		10	10.8^{b}	25.5^{b}	32.0^{bc}	42.5^{c}	27.8^{ab}	4.56^{c}	9.8^{bc}	24.3^{bc}	47.6^{b}	55.4^{d}	30.4^{a}	4.32^{b}
	Pinus deniflora	5	10.8^{b}	26.8^{b}	40.2^{b}	60.5^{b}	26.8^{ab}	3.98^{d}	10.3^{bc}	28.9^{b}	52.9^{b}	70.8^{b}	23.7^{c}	3.89^{c}
		10	8.5^{c}	24.5^{b}	38.2^{b}	57.4^{b}	28.8^{ab}	4.78^{c}	12.5^{b}	23.8^{b}	43.5^{b}	62.7^{c}	27.8^{ab}	4.24^{bc}
Control			5.0^{a}	20.0^{a}	60.0^{a}	87.5^{a}	17.9^{c}	2.16^{d}	5.0^{a}	20.0^{a}	70.0^{a}	87.5^{a}	19.8^{d}	2.16^{e}
5	*Rheum palmatum*	5	0.0^{c}	20.0^{a}	37.5^{bc}	37.5^{c}	22.2^{b}	5.34^{ab}	1.0^{b}	15.0^{a}	37.5^{c}	52.5^{c}	26.0^{bc}	4.98^{c}
		10	1.0^{bc}	10.0^{b}	32.5^{c}	32.5^{c}	25.9^{a}	5.97^{a}	1.0^{b}	15.0^{a}	32.5^{c}	25.0^{e}	29.0^{ab}	8.70^{a}
	Allium cepa	5	0.0^{c}	10.0^{b}	40.0^{bc}	42.5^{b}	25.6^{ab}	3.75^{bcd}	1.5^{ab}	20.0^{a}	45.0^{bc}	62.5^{b}	23.5^{c}	4.08^{cd}
		10	0.0^{c}	10.0^{b}	32.5^{bc}	37.5^{c}	25.5^{ab}	6.51^{a}	1.5^{ab}	20.0^{a}	35.0^{c}	32.5^{d}	29.0a	7.07^{b}
	Pinus densiflora	5	2.5^{ab}	12.5^{b}	40.0^{b}	47.5^{b}	24.0^{ab}	3.95^{cd}	1.5^{ab}	20.0^{a}	55.0^{b}	67.5^{b}	24.1^{c}	3.55d
		10	0.0^{c}	12.5^{b}	32.5^{bc}	32.5^{c}	25.8^{ab}	3.99^{abc}	0.0^{b}	20.0^{a}	40.0^{c}	42.5^{d}	27.8^{ab}	7.57^{b}
Control			15.0^{a}	48.5^{a}	86.5^{a}	92.5^{a}	30.3^{c}	1.50^{f}	15.0^{ab}	58.5^{a}	86.5^{a}	90.0^{a}	21.3^{c}	1.70^{c}
10	*Rheum palmatum*	5	5.0^{b}	42.5^{ab}	53.5^{c}	43.5^{d}	26.9^{c}	3.97^{cd}	6.5^{bc}	36.0^{bc}	55.0^{d}	63.5^{c}	26.8^{ab}	3.07^{bc}
		10	10.0^{ab}	22.5^{d}	22.5^{e}	32.5^{e}	36.4^{a}	8.21^{a}	4.5^{c}	30.0^{c}	42.5^{e}	52.5^{d}	31.4^{a}	5.33^{a}
	Allium cepa	5	7.5^{b}	40.0^{ab}	65.5^{b}	67.5^{b}	26.3^{c}	2.44^{ef}	15.0^{ab}	46.0^{bc}	65.0^{c}	74.0^{b}	22.9^{bc}	2.88^{bc}
		10	5.0^{b}	28.5^{cd}	51.0^{c}	59.0^{c}	34.3^{ab}	4.67^{c}	11.0^{bc}	36.0^{bc}	47.5^{e}	61.5^{d}	26.7^{ab}	4.24^{ab}
	Pinus densiflora	5	12.5^{ab}	44.0^{a}	58.5^{c}	67.5^{b}	26.9^{bc}	2.77^{de}	20.0^{a}	57.5^{a}	77.5^{b}	77.5^{b}	28.7^{ab}	2.27^{bc}
		10	10.0^{ab}	36.0^{bc}	33.5^{d}	35.5^{e}	35.1^{abc}	6.20^{b}	17.0^{ab}	47.5^{b}	55.0^{d}	65.0^{c}	23.0^{c}	3.56^{ab}

[a] When the plant extracts were applied on the rice plants, the infected *Pyricularia oryzae* rates were 0, 5, and 10%; [b] FW: Fresh Weight, [c] DAT: Days After Treatment. [a-d] Means within a column followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

day old seedlings that were 0, 5, and 10% infected with rice blast were chosen for subsequent treatment with 5-10% selected plant extract. After infection, rice blast infections in the control plants (cvs. Imilbyeo and Hopyeongbyeo) increased steadily over time. Their infestations were calculated to be 88-92% at 7 Days After Treatment (DAT). In this context, the control plants treatment refers to spraying only with water. Ilmilbyeo injuries caused by rice blast with different infection levels before treatments were reduced by 55-60, 34-50, and 31-57% in response to treatment with 10% ethanol extracts of, respectively, *R. palmatum* root, *A. cepa* bulb, and *P. densiflora* needle compared with an untreated control at 7 DAT. In addition, treatments with 10% ethanol extracts of *R. palmatum* root, *A. cepa* bulb, and *P. densiflora* needle were, respectively, 20-51, 13-42, and 15-44% better in plant height than the untreated control at 7 DAT. Moreover, treatments with 10% ethanol extracts of *R. palmatum* root, *A. cepa* bulb, and *P. densiflora* needle were, respectively, 171-447, 107-211, and 84-313% better in shoot fresh weight than the untreated control at 7 DAT. The reduction of rice damage caused by rice blast in response to treatment occurred in the order *R. palmatum* root> *A. cepa* bulb= *P. densiflora* needle, regardless of infection levels of rice blast. Hopyeongbyeo injuries caused by different levels of rice blast were reduced by 38-63, 29-55, and 25-45% in response to treatment with 10% ethanol extracts of, respectively *R. palmatum* root, *A. cepa* bulb, and *P. densiflora* needle when compared with the untreated control at 7 DAT. In addition, treatments with 10% ethanol extracts of *R. palmatum* root, *A. cepa* bulb, and *P. densiflora* needle were, respectively, 43-47, 25-55, and 7-42% better in plant height than the untreated control at 7 DAT. Treatments with 10% ethanol extracts of *R. palmatum* root, *Allium cepa* bulb, and *P. densiflora* needle were, respectively, 175-302, 102-227, and 99-250% better in shoot fresh weight than the untreated control at 7 DAT. Similar to Ilmilbyeo, the reduction of rice damage caused by rice blast in response to treatment with extracts occurred in the order *R. palmatum* root> *A. cepa* bulb= *P. densiflora* needle, regardless of infection levels. Overall, the selected plant extracts showed higher control of rice blast in both cultivars, regardless of infection levels. Thus, the effects of the selected plant extracts were also confirmed in greenhouse trials of rice seedlings infected by rice blast.

Inhibition of Crop Pathogens, Two-spotted Spider Mites, and Weeds

In order to confirm the inhibition rates of other crop pathogens, we applied the selected plant extracts (Figure 2). *R. palmatum* extract showed the highest potential of inhibition of rice blast and was also effective in suppressing *B. cinerea*, *R. solani*, and *P. capsici*. Moreover, *P. capsici* was completely suppressed by *R. palmatum* extract at 5%, while *B. cinerea* and *R. solani* were suppressed by 89-94%. Treatment by one application of *R. palmatum* extracts at 3, 5, and 10% led to 36-76% inhibition of cucumber powdery mildew when compared with the control (Table 3). The controlling effect was much higher when applied twice. Specifically, treatment with two applications of *R. palmatum* extracts at 10% reduced cucumber powdery mildew by 90% when compared with the control. The plant height of cucumber plants infected with powdery mildew was similar between control plants and those treated with *R. palmatum* extracts, regardless of application times. However, the shoot fresh weight of cucumber plants subjected to powdery mildew was reduced by 2 times in the control plants relative to those subjected to *R. palmatum* extract treatments. Lee *et al*. (2001) reported that extracts of sunflower seeds, potato and maize leaves showed over 80% controlling effect on barley powdery mildew at 10 mg per pot. Overall, *R. palmatum* extracts showed a greater inhibition of rice blast and

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Table 3. Effect of *Rheum palmatum* ethanol extracts on control of cucumber powdery mildew in cucumber plants in greenhouse.

Treatment	conc. (%)	Control value (%)[a]		Plant height (cm)		Shoot fresh weight (g pot^{-1})	
		1st Application	2nd Application[b]	1st Application	2nd Application	1st Application	2nd Application
Control		0.0[d]	0.0[d]	29.4[a]	39.2[a]	5.42[d]	6.80[c]
Rheum palmatum	3	35.8[c]	57.8[c]	28.6[a]	35.2[a]	8.56[c]	13.94[a]
	5	42.3[b]	71.3[b]	30.0[a]	37.3[a]	9.20[b]	12.97[b]
	10	75.8[a]	90.2[a]	30.4[a]	37.7[a]	10.10[a]	13.31[a]

[a] Parameters were investigated at 7 days after first application or 7 days after second application, [b] Second application was 5 days after first application. [a-d] Means within a column followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test.

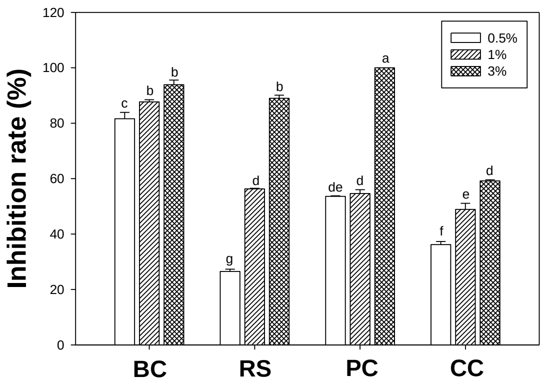

Figure 2. Effect of ethanol extracts of *Rheum palmatum* roots on inhibition rates of four fungal pathogens: (BC) *Bortytis Cinerea*; (RS) *Rhizoctonia Solani*; (PC) *Phytophthora Capsici*, (CC) *Colletotrichum Coccodes*. (Parameter was recorded at 3 days after treatment. Means within bars followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test).

were also effective in suppressing other kinds of fungus diseases tested in this study.

Although *R. palmatum* extracts were not effective in controlling two-spotted spider mites in low concentrations, we observed that higher concentrations led to much higher acaricidal activities. For example, in a trial using a 10% concentration, we observed 46% acaricidal activities against two-spotted spider mites (Figure 3).

The shoot lengths of the broad leaf weed, *Q. coccinea*, were inhibited 46-55% using extracts of *R. palmatum* roots, *A. cepa* bulbs, and *P. densiflora* needles at 10% when compared with the control plants, while root lengths were inhibited by 26-33% (Figure 4). In contrast, the shoot lengths of a grass weed, *E. oryzicola*, were inhibited by 46-54% by *R. palmatum* roots, *A. cepa* bulbs, and *P. densiflora* needles when compared with the control plants, while the root lengths were inhibited by 87-94%. Italian ryegrass shoot and root extracts were also shown to be more effective in root length inhibition than shoot length inhibition of two different rice cultivars (Jang *et al*., 2017).

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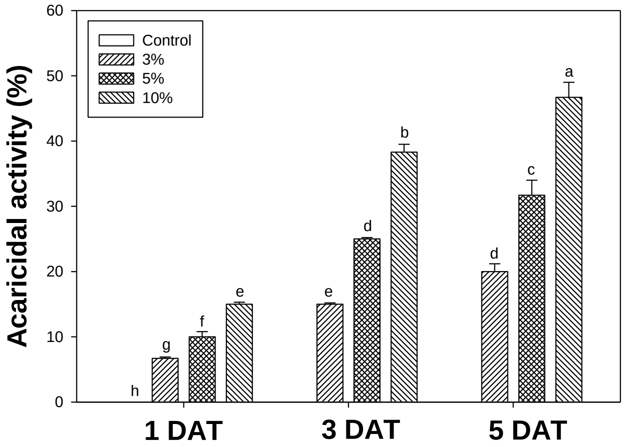

Figure 3. Acaricidal activity of *Rheum palmatm* ethanol extract against *Tetranychus urticae* in a laboratory bioassay. (DAT= Day After Treatment, and means within bars followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test).

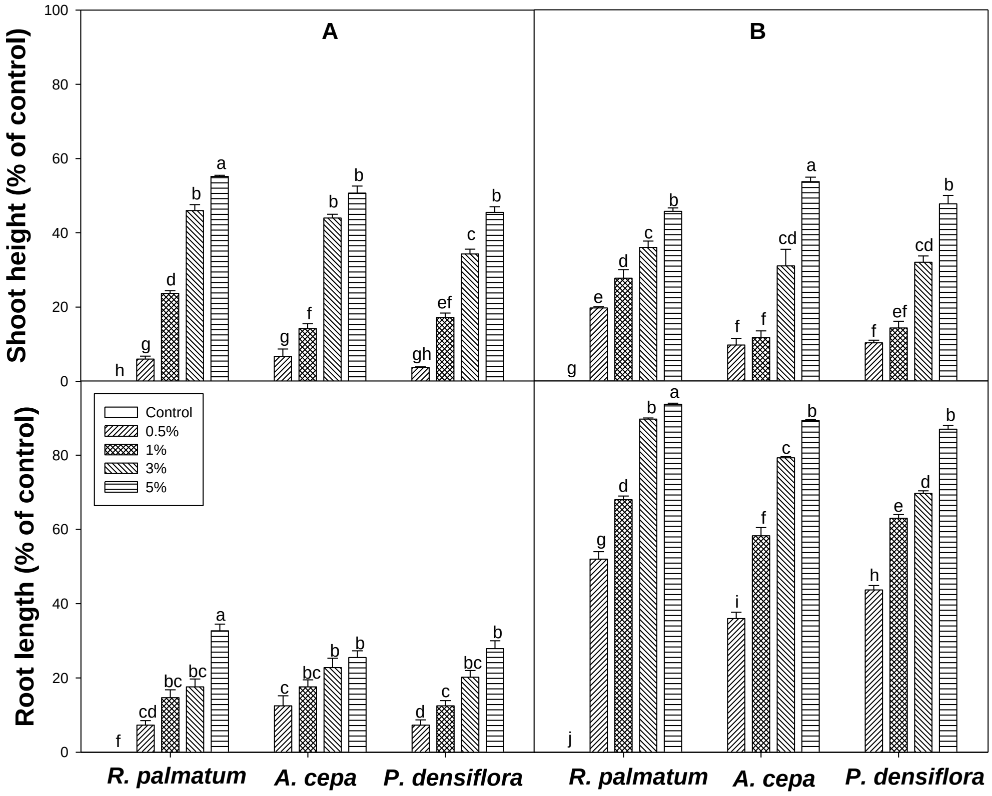

Figure 4. Effect of selected plant extracts on shoot and root lengths of *Quamoclit coccinea* Moench (A) and *Echinochloa oryzicola* (B) in seed bioassay. (Means within bars followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test).

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These results indicate that the controlling effects of plant extracts against weeds differ among weed species and plant parts.

To the best of our knowledge, this is the first report to show the fungicidal, insecticidal, and herbicidal activities of the plant extracts we studied. The main bioactive constituents of *R. palmatum* are anthraquinones, including aloe-emodin, emodin, chrysophanol, and physcion (Wang *et al*., 2008). The anthraquinone derivatives emodin and chrysophanol isolated from *R. palmatum* exert activity against phytopathogens such as cucumber powdery mildew, rice sheath blight, and gray mold (Choi *et al*., 2004; Izhaki, 2002). In addition, emodin extracted from *Polygonum sachalinense* was shown to have inhibitory activities against the growth of *Amaranthus viridis*, and *P. pratense* (Inoue *et al*., 1992). Thus, anthraquinone contents in *R. palmatum* may be related to the inhibition of crop pathogens and weeds. *A. cepa* bulbs have high antioxidant, antibacterial and antifungal activities that have been mainly attributed to a variety of sulfur-containing compounds such as methyl allyl sulfide and diallyl disulfide (Kim *et al*., 1997; Yin and Cheng, 1998). For example, *Collectotrichum* sp. was inhibited by *A. cepa* bulb extracts (2 mg/mL) (Cornago *et al*., 2011). When treated with a 5% concentration of ethanol-extracted *A. cepa* bulb, *P. oryzae* and *C. coccodes* were inhibited at rates of 71% and 78%, respectively (Kim, 2018). Kim (2018) reported that weed species *Q. coccinea*, *E. oryzicola*, and *Digitaria ciliris* were inhibited by five different water-extracted *A. cepa* cultivars. *P. densiflora* needles have antibacterial, antifungal, and herbicidal activities that have been mainly attributed to secondary metabolites including volatile components (Amri *et al*., 2013; Kim and Shin, 2005; Velmurugan *et al*., 2009).

Phytotoxicity of Selected Plant Extracts

Although the selected plant extract treatments resulted in decreased injury caused by rice blast, it is possible that application of the plant extract can inhibit growth or cause injury to plants (Figure 5). Therefore, plant extracts were applied to healthy rice plants and plant height and shoot fresh weight were analyzed at 7 days after treatment. Ilmbiyeo and Hopyungbyeo seedlings treated with plant extracts at 1, 3, 5, and 10% showed no injury at 7 DAT (data not shown). Furthermore, plant height in Ilmibyeo and Hopyeongbyeo was not significantly different in response to the selected plant extracts when compared with the control plant. Shoot fresh weight was not significantly different in response to *A. cepa* bulb and *P. densiflora* needle extracts when compared with the control plant. However, compared to control plants, shoot fresh weight in Ilmibyeo and Hopyeongbyeo was significantly increased by 24-29 and 13-24%, respectively, after treatment with ethanol extracts of *R. palmatum* roots and *A. cepa* bulbs. Jang and Kuk (2019) reported that water extracts of Chinese chive (*A. tuberosum*), soybean (*Glycine max*) leaves, and soybean stems induced 31-45% increase in shoot fresh weight of lettuce compared with the control plants.

Indeed, *R. palmatum* root and *A. cepa* bulbs are widely used as folk medicine for health promoting purposes in Korea, and the *A. cepa* bulb is commonly used as food (Dongeuhak Institute, 1994). Thus, the selected plant extracts can be used in organic rice cultivation because they showed controlling effects on crop diseases, two-spotted spider mites, and weeds without inhibition of rice growth. However, further study of these selected plant extracts is required to clarify the mechanisms underlying the controlling effects on these pests.

CONCLUSIONS

Rice blast was completely suppressed after treatment with 3% ethanol extracts of *R. palmatum* roots, and suppressed by 97 and 77% in response to treatment with 10%

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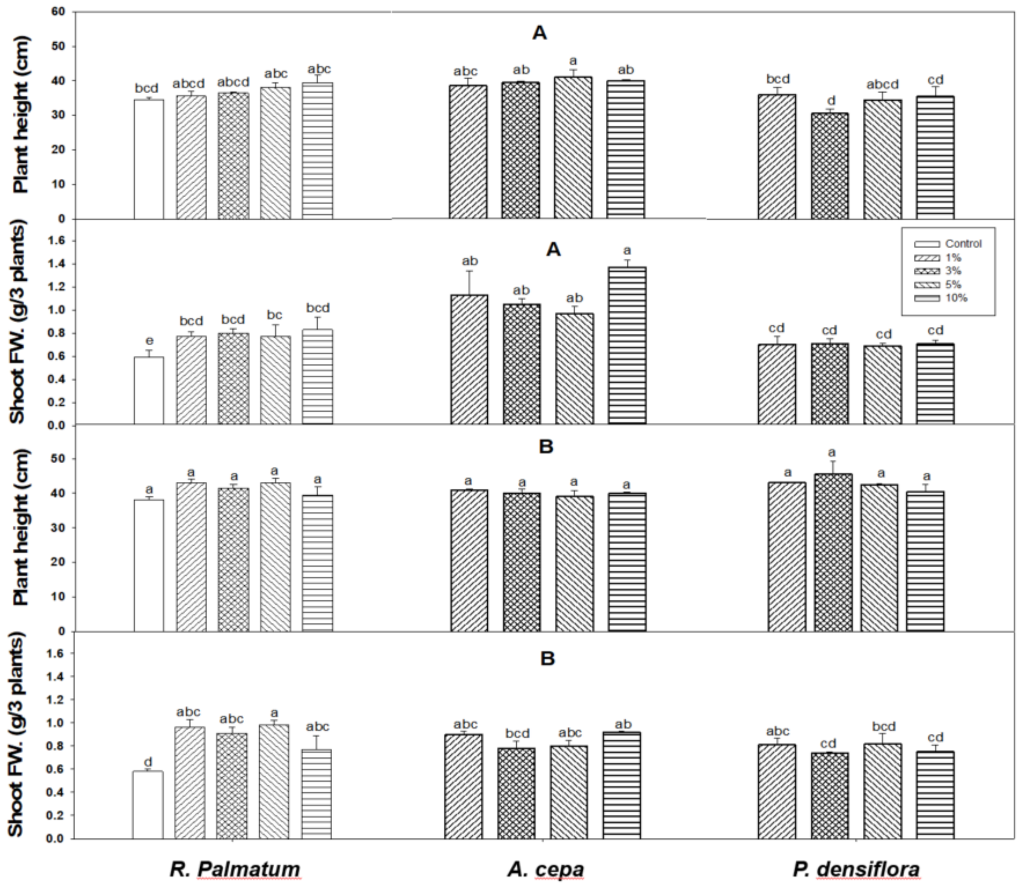

Figure 5. Effect of selected plant extracts on plant height and shoot fresh weight at 15 days after rice seedlings: (A) cv. Ilmibyeo, (B) cv. Hopyeongbyeo. (Parameters were recorded at 7 days after treatment, and means within bars followed by the same letters are not significantly different at 5% level according to Duncan's Multiple Range Test).

ethanol extracts of *A. cepa* bulbs and *P. densiflora* needles, respectively, under *in vitro* conditions. Moreover, a seedling bioassay revealed that rice injuries of two cultivars (Ilmibyeo and Hopyeongbyeo) caused by rice blast were reduced by 40-71, 29-63, and 23-63% in response to treatment with 5 and 10% ethanol extracts of, respectively, *R. palmatum* roots, *A. cepa* bulbs, and *P. densiflora* needles, compared with the untreated controls. The selected *R. palmatum* extract also controlled other crop diseases such as *P. capsici*, *B. cinerea*, *R. solani*, and *S. fuliginea*. *R. palmatum* extracts at 10% reduced two-spotted spider mites by 46% when compared with each control. A broad leaf weed, *Q. coccinea*, and a grass weed, *E. oryzicola*, were inhibited 46-55% by *R. palmatum* roots, *A. cepa* bulbs, and *P. densiflora* needles at 10% when compared with the control plants. Rice plants showed no leaf injuries or growth reduction after treatment with extracts of *R. palmatum* roots, *A. cepa* bulbs, and *P. densiflora* needles. Thus, the selected plant extracts may be used for control of crop diseases and weeds in organically cultivated crop fields.

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اثرات عصاره های گیاهی روی بیماری های گیاهی،کنه عنکبوتی دو نقطه ای، و علف های هرز

س. ج. جنگ، و ی. ا. کوک

چکیده

در این پژوهش اثرات کنترل کننده عصاره های مختلف گیاهی که از ۳۸ محصول کشاورزی به دست آمده بود بر علیه بلاست برنج (*Pyricularia oryzae*) بررسی شد. همچنین، نرخ بازدارندگی (inhibition rate) سه عصاره گیاهی منتخب برای دیگر بیمارگرهای گیاهی، کنه عنکبوتی دو نقطه ای، و علف های هرز ارزیابی شد. در شرایط درون شیشه ای، عصاره به دست آمده با عصاره گیری توسط ۳٪ اتانول از ریشه *Rheum palmatum* اثر بازدارندگی کاملی روی بلاست برنج داشت، در

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حالیکه که بازدارندگی از این مرض در مورد عصاره گیری با اتانول ۱۰٪ از غده پیاز(*Allium cepa*) و برگ سوزنی کاج (*Pinus densiflora*) به ترتیب ۹۷٪ و ۷۷٪ بود. افزون بر این، زیست سنجی گیاهچه آشکار ساخت که صدمات بلاست برنج به دو کولتیوار برنج (Ilmibyeoو Hopyeongbyeo) در واکنش به تیمار کردن بوته ها با عصاره به دست آمده از عصاره گیری با ۵٪ و ۱۰٪ اتانول ریشه *Rheum palmatum*،غده پیاز(*Allium cepa*)، و برگ سوزنی کاج (*Pinus densiflora*) به ترتیب ۷۱-۴۰٪، ۶۳-۲۹٪ و ۶۳-۲۳٪ در مقایسه با شاهد تیمار-نشده کاهش یافت. عصاره انتخابی از *R. palmatum*، بیماری های گیاهی ناشی از*Phytophthora capsici*، *Bortytis cinerea*، *Rhizoctonia solani* و سفیدک پودری خیار *Sphaerotheca* (*fuliginea*)را هم کنترل کرد. عصاره های *R. palmatum*در ۱۰٪ موجب کاهش اثر کنه عنکبوتی دو نقطه ای در حد ۴۶٪ در مقایسه با شاهد شد. نیز، اثر بازدارندگی عصاره ۱۰٪ ی ریشه *Rheum palmatum*،غده پیاز(*Allium cepa*)، و برگ سوزنی کاج (*Pinus densiflora*) روی یک علف هرز برگ پهن به نام *Quamoclit coccinea* Moenchو یک علف هرزعلفی به نام *Echinochloa oryzicola*بین ۵۵-۴۶٪ بود. به این قرار، عصاره های گیاهی منتخب در این پژوهش را می توان برای کنترل بیماری های گیاهی و علف های هرز در مزارعی که کشت و کار به صورت ارگانیک است استفاده کرد.

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