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Full Length Research Paper

Effect of some plant extracts on *Rhizoctonia* spp. and *Sclerotium hydrophilum*

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Soilborne phytopathogens affect rice production by inhabiting inoculum permanently in the soil. Pesticides of plant origin are preferred in order to reduce the risks involved in chemical control measure. The present study was conducted to find out bioresource to control some rice pathogens. Sixteen naturally available phytoextracts were tested *in vitro* for their potential to control phytopathogens of rice, such as *Rhizoctonia solani*, *Rhizoctonia oryzae*, *Rhizoctonia oryzae-sativae* and *Sclerotium hydrophilum*. All of the tested fungal growths were suppressed 100% by using clove extract. Neem leaf, rosemary and pelargonium extracts were found to give the second best suppression against the tested fungi. Neem leaf extract inhibited the growth of *R. solani* by 87.5%, *R. oryzae* by 92.5% and *R. oryzae-sativae* by 80%. However, the same extract inhibited *S. hydrophilum* by only 49.1%. Rosemary extract gave an inhibition of 67.7% for *R. solani*, 88.0% for *R. oryzae*, 86.0% for *R. oryzae-sativae* and 73.89% for *S. hydrophilum*. The inhibitory effect of pelargonium on the tested fungi showed 48.1% for *R. solani*, 90.8% for *R. oryzae*, 84.4% for *R. oryzae-sativae* and 83.3% for *S. hydrophilum*. Among the tested phytoextracts, cloves, neem leaf, rosemary and pelargonium are potential phytoextracts to control the tested soilborne phytopathogens.

Key words: Rice, *Rhizoctonia solani*, *Rhizoctonia oryzae*, *Rhizoctonia oryzae-sativae*, *Sclerotium hydrophilum*, sheath diseases, phytoextract.

INTRODUCTION

Sheath diseases of rice caused by *Rhizoctonia solani*, *Rhizoctonia oryzae*, *Rhizoctonia oryzae-sativae* and *Sclerotium hydrophilum* are important phytopathogens distributed worldwide and cause yield losses in rice growing countries (Matsumoto, 2003). Rice is grown as an important cereal crop all over the world, but mostly in Southeast Asian countries. Among different diseases that attack rice crops, sheath diseases have become one of the dominant diseases causing significant reduction in rice yield. Many methods of plant disease control are presently being used to control sheath diseases of rice such as physical, chemical, cultural methods etc., however, chemicals can effectively control several plant diseases. There is an increasing awareness about the risks involved in chemical pesticides, and therefore much attention is being focused on alternative methods of plant pathogen control. On the other hand, the world market continues to be extremely competitive and the growers compete to supply high quality, organic products. Pesticides of plant origin are preferred in countries where farmers practice organic farming. Several higher plants and their constituents have been used successfully in plant disease control, and also have been proven to be harmless and non-phytotoxic unlike chemical fungicides (Singh et al., 1993).

There is evidence from earlier works that several plant species possess antifungal and antibacterial properties (Hasan et al., 2005; Ogbo and Oyibo, 2008; Dubey et al., 2009). The use of plant extracts as a soil treatment measure have produced good results against various soil borne fungi like *Pythium aphanidermatum* and *R. solani* (Khan et al., 1974), *Fusarium oxysporium* (Kannaiyan and Prasad, 1981) and *Colletotrichum atramentarium* (Singh, 1986). Some plant extracts act as contact fungicides; some disrupt cell membrane integrity at different stages of fungal development, while the others inactivate important enzymes and interfere with metabolic

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Table 1. Effect of distilled water and 70% ethanol on efficiency of growth inhibition on *R. solani, R. oryzae*, *R. oryzae-sativae* and *S. hydrophilum*

Treatments	Growth inhibition (%)							
	R. solani		*R. oryzae*		*R. oryzae-sativae*		*S. hydrophilum*	
	DW	ET	DW	ET	DW	ET	DW	ET
Euclyptus	6.3±1.0	35.8±1.5	1.1±0.6	23.5±1.2	0.0±0.0	7.7±4.7	1.6±1.6	34.2±1.1
Neem leaf	6.4±1.0	9.1±0.6	4.3±1.1	15.6±6.5	4.7±3.3	14.8±7.7	0.0±0.0	22.1±5.7
Neem fruit	12.7±4.1	31.0±4.3	0.0±0.0	6.1±1.9	0.00±0.0	0.0±0.0	0.0±0.0	31.3±3.7
Clove	0.0±0.0	100.0±0.0	0.0±0.0	100.0±0.0	20.0±8.2	100.0±0.0	0.0±0.0	84.0±6.7
Worm wood	9.0±3.5	51.6±3.0	5.5±0.1	30.2±1.1	0.0±0.0	37.9±4.8	8.1±2.9	25.9±3.9
Rosemery	0.0±0.0	91.9±0.5	0.0±0.0	72.7±6.6	10.0±2.5	43.7±3.5	8.0±4.9	67.3±2.9
Sage	0.0±0.0	24.5±3.9	1.1±1.1	1.6±0.9	0.0±0.0	3.1±1.9	4.0±2.1	10.8±3.4

Values are the mean of five times testing ± standard error; DW = Distilled water and ET = 70% ethanol.

processes. Adityachaudhury (1991) mentioned that the use of plant extracts and phyto-products is gaining attention due to their biodegradability, low toxicity and minimum residual toxicity in the ecosystem (Ogobo and Oyibo, 2008). Accordingly, natural products are considered to control fungal diseases in plants as an interesting alternative to synthetic fungicides due to their comparatively smaller negative impact on the environment.

As sheath disease pathogens of rice are soilborne pathogens, the adverse effect of chemicals on a range of soil inhabiting beneficial microbes is becoming a threat. In addition, soil borne diseases can contaminate the soil by establishing its inocula permanently in the soil. Therefore, it is important to find out the control measures that are environmentally safe to reduce the incidence of these pathogens. The aim of the present study was to investigate the effect of different plant extracts on the growth of *R. solani*, *R. oryzae*, *R. oryzae-sativae* and *S. hydrophilum in vitro.* Moreover, it was aimed to compare the effect of extracting media, ethanol and water on the inhibiting efficiency of plant extracts.

MATERIALS AND METHODS

Preparation of plant materials

Plant materials used in this experiment are listed in Table 1. The extracts were prepared from different plant parts such as leaves, fruits and flowers. Plant samples were washed thoroughly with tap water followed by drying at 45°C for 2 days in a drying oven. Dried plant parts were ground by using a force mill. Powdery plant materials were mixed with 70% ethyl alcohol or water at 1:1 (w/v) and were placed under 45°C for 72 h. Next, the extract was separated from the plant materials by centrifuging at 15000 rpm for 10 min. The supernatant was transferred to the new tubes and filtered by using a disposable syringe filter unit equipped with 0.45 µm cellulose acetate. Freshly prepared phytoextracts were used to study their inhibitory effects on phytopathogens.

Preparation of fungal isolates

Isolates of our previous study preserved at the Institute of Tropical Agriculture, Kyushu University were used in this experiment (Aye et al., 2008). The isolates, *R. solani*, *R. oryzae*, *R. oryzae-sativae* and *S. hydrophilum*, preserved on PDA slants, were sub-cultured on a water agar (WA) media. All of the cultured isolates were incubated at 28°C. Three days after sub-culturing, the branching hyphal growth could be observed on the WA plates and were used as test isolates.

Assessment of extracting media on inhibition efficiency of plant extracts on fungal growth of *R. solani, R. oryzae, R. oryzae-sativae* and *S. hydrophilum*

One hundred millimeters of each of the plant extracts was spread on one half of the PDA media in a Petri dish, and the other half was kept untreated as the control. The Petri dish containing treated media was allowed to dry for 5 min. A four millimeter mycelial disc was transferred to the center of the Petri dish. The radial growth (cm) of the tested fungus was measured in all treated and untreated halves of the cultured Petri dishes. The radial growth of the tested fungus in the treated plates was measured in centimeters in all treatments when the pathogen growth touched the edge of the controlled Petri dishes. Growth inhibition (%) was compared among treatments. Calculated by the

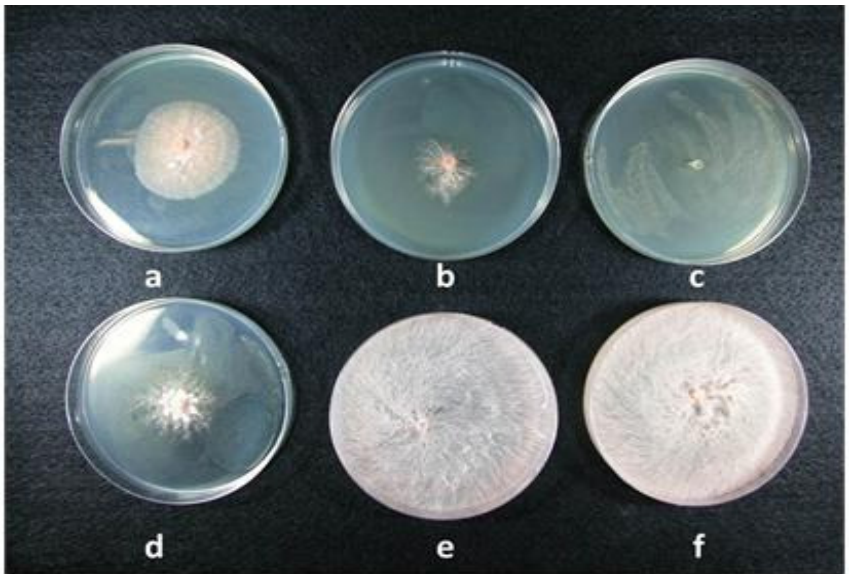

Figure 1. Effect of ethanol extract of rosemary on the growth of *R. oryzae*.

formula:

100× (C-T)/C,

where C = growth in the untreated portion and T = Growth in the treated portion (Dubey et al., 2009; Satish et al., 2007).

Responses of *R. solani*, *R. oryzae*, *R. oryzae-sativae* and *Sclerotium hydrophilum* to some plant extracts *in vitro*

The effect of plant extracts on the growth of a range of phytopathogens was studied based on radial growth of fungi. Each of the plant extracts of 200 µl was spread on a PDA media using spreaders. For the controlled plates, a PDA media was not treated with any phytoextract. Each plate was incubated with an agar block (No. 2 cob borer) of each pathogen growing on a WA plate. The isolated pathogen on the WA plate was transferred to a PDA plate treated with one of the plant extracts. Another set of PDA plates were prepared to comparatively observe the treated and the untreated plates. All the inoculated Petri dishes were incubated at 28°C. The radial growth of the test fungus in the treated plates was measured in centimeters in all treatments when the pathogen growth touched the edge of the controlled Petri dishes. Growth inhibition (%) was compared among treatments. Statistical analysis was carried out by XLSTAT-Pro 7.5.

RESULTS

Assessment of extracting media on inhibition efficiency of plant extracts on fungal growth of *R. solani, R. oryzae*, *R. oryzae-sativae* and *S. hydrophilum*

The higher inhibitory percentages on the growth of *R. solani*, *R. oryzae*, *R. oryzae-sativae* and *S. hydrophilum* were examined on ethanolic extracts of test plant extracts compared to those of water extracts. According to Table 1, the highest inhibitory growth of *R. solani*, *R. oryzae*, *R. oryzae-sativae*, and *S. hydrophilum* was caused by the ethanolic extract of clove as 100.0, 100.0, 100.0 and 84.0%, respectively. In contrast, there was less inhibition on the growth of *R. solani*, *R. oryzae*, *R. oryzae-sativae*, and *S. hydrophilum* caused by the water extract of clove as 0.0, 0.0, 20.0 and 0.0%, respectively. The ethanolic extract of rosemery inhibited 91.8% of the growth of *R. solani* and 72.6% of *R. oryzae* (Figure 1). In contrast, that of the water extract had no effect on the growth of *R. solani* or *R. oryzae*. Inhibitory growth of 43.7% was recorded for the growth of *R. oryzae-sativae* and 67.3% of *S. hydrophilum* by the ethanolic extract of rosemary. On the other hand, the water extract of rosemary retarded the growth of *R. oryzae-sativae* and *S. hydrophilum* by 10.0 and 8.0%, respectively.

In our experiment, the plant extract with the least inhibitory growth was the sage extract. The ethanolic extract of the sage showed a higher inhibitory growth value of *R. solani* 24.5%, *R. oryzae* 1.6%, *R. oryzae-sativae* 3.1% and *S. hydrophilum* 10.8% compared to the lower inhibitory values of the water extracts. The other plant extracts with weaker inhibitory growths, such as eucalyptus, neem leaf, neem fruit and worm wood, also expressed the same trend of a stronger effect with the ethanolic extracts compared to those of the water extracts. The growth inhibition percentages of the ethanolic extract of eucalyptus were 35.7 for *R. solani*, 23.5 for *R. oryzae*, 7.7 for *R. oryzae-sativae* and 34.2 for *S. hydrophilum*. However, a lower inhibitory percent of each fungus was recorded in those of the water extracts of eucalyptus (Table 1). According to results of this

Table 2. Effect of plant extracts on inhibitory growth of *R. solani, R. oryzae, R. oryzae-sativae* and *S. hydrophilum.*

Scientific names	Common names	*R. solani*	*R. oryzae*	*R. oryzae-sativae*	*S. hydrophilum*
Eucalyptus	Euclyptus	32.9^{cdef}	65.2^{abc}	65.0^{abc}	55.0^{cd}
Pelargonium cucullatum	Pelargonium	48.1^{bcde}	90.8^{ab}	84.4^{ab}	83.3^{ab}
Mentha suaveolens	Apple mint	35.0^{cdef}	25.0^{cde}	52.9^{bc}	18.8^{ef}
Lavandula spica L.	Lavender	28.1^{ef}	29.3^{cde}	36.8^{c}	35.0^{de}
Azadirachta indica	Neem fruit	31.8^{def}	33.4^{cde}	39.8^{c}	47.8^{d}
Alpinia zerumbet	Shell ginger	53.6^{bcde}	49.3^{c}	71.3^{abc}	50.0^{d}
Artemisia capillaris Thunb	Worm wood	63.7^{bcd}	81.6^{ab}	82.7^{ab}	73.6^{bc}
Nandina domestica	heavenly bamboo	28.1^{ef}	29.0^{cde}	33.3^{cd}	19.4^{e}
Rosmarinus officinalis	Rosemary	67.7^{abc}	88.0^{ab}	86.0^{ab}	73.9^{bc}
Aloe vera	Aloe	16.7^{ef}	28.1^{cde}	31.5^{cd}	18.1^{ef}
Syzygium aromaticum	Clove	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}
Cymbopogon citratus	Lemongrass	14.3^{ef}	0.0^{e}	25.0^{cd}	0.0^{f}
Salvia officinalis	Sage	14.3^{ef}	0.0^{e}	25.0^{cd}	11.1^{ef}
Azadirachta indica	Neem leaf	87.5^{ab}	92.5^{ab}	80.0^{abc}	49.2^{d}
Centella asiatica	Pennywort	14.3^{ef}	14.3^{de}	0.0^{d}	10.0^{ef}
Azadirachta indica	Neem cake	38.1^{bcdef}	28.6^{cde}	0.0^{d}	16.7^{ef}
	Ethanol	2.1^{f}	37.1^{cde}	51.8^{bc}	0.0^{f}
	Control	0.0^{f}	0.0^{e}	0.0^{d}	0.0^{f}

Numbers within a column followed by the same letter are not significantly different at (p = 0.05).

experiment, it was found that 70% ethanol is a better extraction media compared with water for studying the inhibitory effects of different plant extracts on *R. solani, R. oryzae, R. oryzae-saticvae* and *S. hydrophilum*.

Responses of *R. solani*, *R. oryzae*, *R. oryzae-sativae* and *Sclerotium hydrophilum* to some plant extracts *in vitro*

All plant extracts used in this study expressed a better inhibitory effect over the untreated control and reduced the growth of *R. solani* (Table 2). The growth of *R. solani* touched to the wall of the Petri dish 3 days after the start of incubation, and data were recorded. The tested plants had the ability to control *R. solani* which showed 100.0% inhibition of *R. solani* by clove extract, whereas the lowest inhibitions were recorded for the extracts of pennywort (14.3%), Sage (14.3%), lemongrass (14.3%), and Aloe (16.7%). Among the plant extracts showing higher inhibitory effects, the use of clove, neem leaf and rosemary did not significantly differ from each other, the values of which were 100.0, 87.5 and 67.7%, respectively. Next to the rosemary extract, phytoextracts of worm wood, shell ginger and pelargonium gave 63.7, 53.6 and 48.1% inhibitory growth, respectively. In addition, moderate efficiencies were recorded in heavenly bamboo (28.1%), lavenda (28.1%), neem fruit (31.8%) and eucalyptus (32.9%). Full growths of *R. oryzae* in untreated plates were found 3 days after the start of incubation, and data was recorded. The growth of *R. oryzae* was reduced by all of the tested plant extracts except lemongrass and sage. The highest inhibitory effect was recorded in clove extract (100.0%) followed by neem leaf (92.5 %), pelargonium (91.0%), worm wood (81.6%) and eucalyptus (65.2%) (Figure 2). Slight reduction in fungal growth of *R. oryzae* was observed in the rest of the plant extracts. Ethanol had an inhibitory effect of 37.1% on the growth of *R. oryzae*. The inhibitory growth of *R. oryzae* caused by shell ginger, neem fruit, lavenda, heavenly bamboo, aloe, neem cake, mint and pennywort were found as 49.3, 33.4, 29.3, 29.0, 28.6 and 25.0%, respectively.

The growth of *R. oryzae-sativae* in the control plates touched the wall of the Petri dish at 4 days after incubation, and the data was recorded on that day. Among the tested plant extracts, some of the extracts expressed higher inhibitory effects, such as clove 100.0%, rosemary 86.0%, pelargonium 84.4%, neem leaf 80.0%, shell ginger 71.3% and eucalyptus 65.0%. The growth of *R. oryzae-sativae* was significantly retarded by the aforementioned plant extracts. However, lower inhibition percents were noted in mint 52.9%, neem fruit 39.8%, lavenda 36.8%, heavenly bamboo 33.3%, aloe 31.5%, lemongrass 25.0% and sage 25.0%. In addition, there were some plant extracts those did not inhibit the growth of *R. oryzae-sativae*. The inhibitory effects of pennywort and neem cake on *R. oryzae-sativae* were found as 0.0%. Our data revealed that 70% ethanol also influences the growth of *R. osyzae-sativae* by 51.8% (Table 2).

Growth on the control plates of *S. hydrophilum* touched

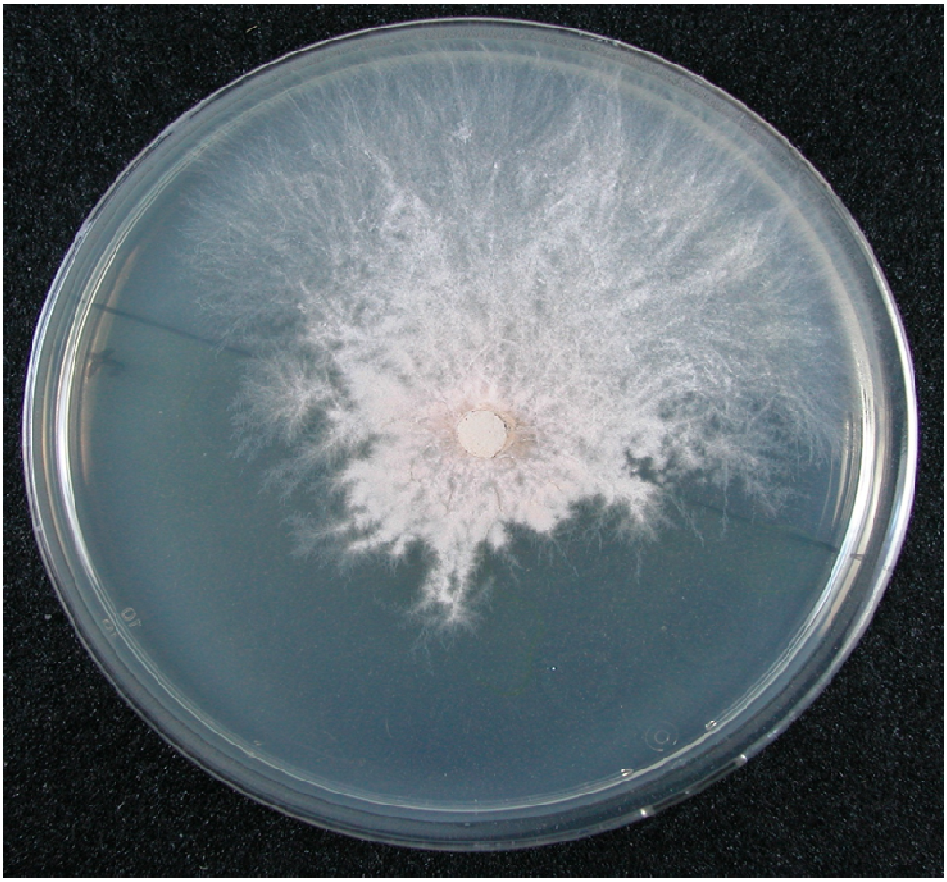

Figure 2. Effect of plant extracts (a) wormwood (b) pelargonium (c) clove (d) rosemery (e)lemongrass (f) Control on the growth of *R. oryzae.*

the wall of the Petri dishes at 4 days after incubation. The phytoextracts of clove and pelargonium on *S. hydrophilum* were found as 100 and 83.3, respectively. Next to pelargonium, phytoextracts of rosemary and wormwood effectively inhibited *S. hydrophilum* with inhibition percents of 73.9 and 73.6, respectively. The other plant extracts with weaker effects on *S. hydrophilum* were eucalyptus 55.0%, neem fruit 47.8%, shell ginger 50.0% and lavenda 35.0%. The rest of the plant extracts had almost no inhibitory effects, such as heavenly bamboo 19.4%, neem cake 16.7%, aloe 18.1% mint 18.8%, sage 11.1% and lemongrass 0.0%.

According to the present data, the higher inhibitory growth of *R. solani* over the control plates was found in all of the plant extracts. However, some plant extracts completely cannot inhibit the growth of *R. oryzae, R. oryzae-sativae* and *S. hydrophilum*. Overall data indicates that the highest inhibitory growth is found in cloves followed by rosemary, pelargonium and neem leaves. These plant extracts have potential as a source of sheath disease control phytochemicals. Extracts of clove, neem leaf, rosemary and pelargonium are potential phytoextracts to control the tested soilborne phytopathogens.

DISCUSSION

Sheath diseases of rice are caused by *R. solani, R. oryzae, R. oryzae-sativae* and *S. hydrophilum*. Nowadays, alternative materials to the commonly used fungicides are being researched to control sheath diseases of rice in a more environmentally friendly way. Several researchers have reported on the fungicidal and bactericidal effects of plant extracts on specific plant pathogens (Dubey et al., 2009; Joseph et al., 2008; Hasan et al., 2005). One of the natural plant product, eucalyptus has been found to possess a wide spectrum of biological activity against fungi, bacteria, insects, mites, and weeds and provide simple, inexpensive, and environment friendly (non-polluting and lesser or no toxicological concerns) alternative pest control (Batish et al., 2008). Sehajpal et al. (2009) studied the effect of plant extracts against *R. solani* causing sheath blight of rice. However, this was the first experiment on the study of various plant extracts on sheath diseases of rice.

In our experiment, some different findings and some similar findings from the previous published authors were found. Hasan et al. (2005) reported that water extract has the ability to control seed born fungi. However, water extracts had almost no effect to inhibit fungal growth of sheath diseases of rice in our experiment. It may be due to different extraction methods and the use of different microbes between the present experiment and the former reported paper. The same author pointed out that *Azadirachta indica* (Neem) has the ability to control seed borne fungi of wheath var. kanchan. Neem leaf extract inhibited the growth of *Fusarium solani* f. sp. Melongenae (Joseph et al., 2008). Similarly, our data expressed satisfactorily inhibitory growth of sheath disease fungi by using neem leaf extract. The ideal agrochemical effect of *Euclyptus citriodora* was published by Ramezani et al. (2002), the authors cited that essential oil of *E. citriodora* possesses strong antifungal activity on several plant pathogens. According to Joseph et al. (2008) *Euclyptus globulys* leaf extract and *Artemisia annua* extract on radial growth of *F. solani* f. sp. *melongenae* indicated that these were effective in reducing the growth of fungi.

Similarly, the present finding pointed out that eucalyptus can reduce fungal growth of *R. solani* 32.9%, *R. oryzae* 65.2%, *R. oryzae-sativae* 65.0% and *S. hydrophilum* 55.0%. In the same way, significant inhibition on radial growth of sheath diseases fungus by extract of wormwood was also recorded in our research. Shutong et al. (2007) reported that Salviae officinalis did not have significant control effects on potato late blight compared with the control treatment. Similarly, our findings indicate that sage cannot significantly inhibit the growth of causal agents of sheath diseases of rice over the control treatments. Our data revealed that clove extract completely inhibited the growth of rice sheath pathogens. The results obtained are in agreement with those of Al-Askar and Rashad (2010) who tested clove with cinnamon, anise and black seed against R. solani. They found that the highest antifungal activity was recorded for clove extract, which causes complete inhibition at a concentration of 1%.

In our research, we found out two new sources of phytoextracts for the use of rice sheath disease control. Studies mentioned that several species of Pelargonium can be used for medicinal purposes of human and animal diseases (El-Wahab et al., 2009; Bereznoy et al., 2003; Jeyabalan, 2003). To our knowledge, there have been no reports on Pelargonium spp. for their effects on plant disease control measures. Our experiment indicated pelargonium to be one of the best plant extracts to control *R. oryzae, R. oryzae-sativae* and *S. hydrophilum*. These extracts exhibited significant inhibitory effects on the growth of *R. solani*. Several reports have been mentioned about the use of rosemary in health care and as an antimicrobial compound (Rozman and Jersek, 2009; Posadas et al., 2009). Our data indicated the potential of rosemary as one of the best sources of phytochemicals to control *R. solani*, *R. oryzae* and *R. oryzae-sativae.* It placed second best among the tested plant extracts in the inhibition of the growth of *S. hydrophilum*.

Indrayan et al. (2009) mentioned that *Alpinia zerumbet* has inhibition ability to certain gram positive and gram negative human pathogens. In the results of our experiment, shell ginger had an inhibitory effect of 53.6% to *R. solani*, 49.3% to *R. oryzae*, 71.3% to *R. oryzae-sativae* and 50.0% to *S. hydrophilum*. Our findings suggest that apple mint, lavender, heavenly bamboo, aloe, lemongrass and pennyword have some inhibitory growth effects on *R. solani.* In all the previous data, extracts from pelargonium, rosemary, and cloves seemed to have potential for sheath diseases of rice control as they are biologically based and environmental safe alternatives. Chemical pesticides have bad effects on human health, plants, fish, other animals, etc., which are harmful to our environment. It is evident from reports that plant extracts are effective antimicrobial substances against soil born fungi and do not produce any residual effects. Our findings can be further exploited for formulating integrated disease management schedules of sheath diseases of rice. Further experiments *in vitro* should be performed to know the efficacy of these plant extracts in the field condition. Disease control using plant extracts are generating a good deal of interest, yet very few materials are commercially available. Further research on naturally available plant disease control measures remains to be done.

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