Efficacy of Natural Plant Products on the Control of Aggregate Sheath Spot of Rice

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ABSTRACT

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Aqueous extracts from ginger, pepper, basil, and garlic plants and essential oils from neem, garlic, lemongrass, and cinnamon were evaluated for their antagonistic effects against *Rhizoctonia oryzae-sativae*, the cause of aggregate sheath spot of rice. The compounds in 5% concentrations in water or agar were tested on several *R. oryzae-sativae* isolates. Cinnamon oil, the most efficacious plant product in vitro, was further tested in the greenhouse for the control of the disease on two rice cultivars inoculated with *R. oryzae-sativae*. One milliliter of each of four cinnamon oil concentrations (12.5, 37.5, 62.5, or 87.5%) diluted in vegetable oil was applied to the surface of the water in constantly flooded pots. Cinnamon oil failed to reduce the disease caused by one of the isolates at any concentration. Cinnamon oil suppressed the disease caused by the other isolate on one of the cultivars at a concentration of 37.5%, and on both cultivars at a concentration of 62.5 and 87.5%. However, cinnamon oil at 87.5% was phytotoxic. Cinnamon oil has potential to control aggregate sheath spot but relatively high concentrations were required for disease suppression.

Rhizoctonia oryzae-sativae, the cause of aggregate sheath spot disease on rice (*Oryza sativa* L.), occurs in California in the United States and in countries including Japan, China, Vietnam, India, Thailand, Iran, Venezuela, Uruguay, and Australia (4,9,19,20,33). Aggregate sheath spot became an important disease in California after susceptible semidwarf, high-yielding cultivars were introduced and widely grown (10). Disease symptoms are characterized by oval lesions with green or gray centers surrounded by a distinct brown margin. Several lesions can occur together, resulting in an aggregation of lesions. The fungus also attacks panicles, which results in discolored grain (9,10,23). Overwintering sclerotia or mycelia in soil or crop residue serve as reservoirs of inocula (9,10,21,23,25). A subsequent rice crop is infected by floating sclerotia in the rice paddy (9,10,23). Aggregate sheath spot caused yield losses of 20% in Australia (18) and 4 to 9% in Uruguay (20). However, significant yield losses have not been documented in California (11).

Organic rice growers have relatively few options for the management of aggregate sheath spot. Currently, all rice cultivars grown in California are susceptible to aggregate sheath spot at some level (23). Although removing and destroying crop residues is a somewhat effective strategy for managing the disease, applications of the fungicide azoxystrobin are recommended in conventionally grown crops when the disease is severe (i.e., when lesions spread to the flag leaf; 40). Alternative disease control options that may benefit organic rice growers include application of natural plant products, which have been used in other organic crop systems (5). Plant extracts and plant oils with antifungal activity against a variety of fungi have been reported. For example, Wilson et al. (42) found that garlic extracts, pepper extracts, and essential oils of palmarosa, red thyme, and cinnamon leaf had high antifungal activity against *Botrytis cinerea*. Garlic oil inhibited growth and sclerotium formation of *R. solani* (36). Lemongrass oil has antimicrobial activity against *R. solani*, *Sclerotinia rolfsii*, and *Sclerotinia sclerotiorum* (13). Advantages of natural plant products include the ease with which they biodegrade and their frequently systemic nature (15). Oil products also float on water, which would allow direct contact with the floating sclerotia of *R. oryzae-sativae*. This would increase the opportunity of oil products to penetrate and react directly with floating sclerotia before infection.

The objectives of this research were to determine the ability of various plant extracts and essential oils to inhibit vegeta-

tive growth, sclerotia production, and sclerotia germination of *R. oryzae-sativae* and the efficacy of cinnamon oil to control *R. oryzae-sativae* under greenhouse conditions.

MATERIALS AND METHODS

Plant product preparation. Extracts from ginger, pepper, basil, and garlic plants and plant essential oils, including neem, garlic, lemongrass, and cinnamon (leaf) oils, were used in this study. For plant extracts, 150 g of ginger rhizomes, pepper fruit, basil leaves, or garlic cloves from a retail grocery store were washed with distilled water and macerated in 150 ml of sterile water. The suspensions were then filtered through two layers of Miracloth (Calbiochem, Gibbstown, NJ) followed by serial passage through 1.6- μ m GF/A filter paper (Whatman, Florham Park, NJ), 0.8- and 0.45- μ m filter membranes, and a 0.22- μ m filter membrane under vacuum. Plant essential oils obtained from the manufacturer (Cosmic Living Inc., Naperville, IL) were sterilized by passage through a 0.22- μ m filter.

Antifungal activity assay. About 20 ml of each plant extract diluted to 5% in potato dextrose agar (PDA) was poured into sterile 9-mm-diameter petri plates. PDA with sterile water served as the control. Agar plates with plant essential oils were prepared according to the modified method of Banerjee et al. (1). Tween-20 (1 ml) was mixed with 49 ml of water and then sterilized by passage through a 0.22- μ m filter. In all, 50 ml of each plant essential oil was emulsified with this sterile Tween-20 solution and vigorously mixed with 900 ml of PDA. About 20 ml of the mixture was then poured into a sterile petri plate. PDA with 0.1% Tween-20 served as the control. Eleven isolates of *R. oryzae-sativae* from California rice fields were grown for 5 days on PDA and a 4-mm-diameter mycelial disc of each isolate was transferred to each of four replicate agar plates containing the plant products. Radial growth of *R. oryzae-sativae* was recorded after 3 days at room temperature (approximately 25°C). The production of sclerotia was rated after 7 days based on the following scale: 0 = no sclerotia production and 1 = sclerotia covering less than 25%, 2 = 25 to 50%, 3 = 50 to 75%, and 4 = more than 75% of the petri plate surface. There were four replicate plates per isolate per plant product in each of two independent trials.

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Inhibition of sclerotia germination was determined by a modified method of Banerjee et al. (1). Ten sclerotia, harvested from each of 11 *R. oryzae-sativae* isolates grown on PDA for 14 days, were transferred to PDA after the sclerotia were soaked for 10 min in each 5% plant extract in sterile water or each 5% plant essential oil in a sterile aqueous solution of 0.1% Tween-20. After incubation at room temperature for 3 days, the number of germinated sclerotia was counted. Sclerotia soaked in sterile water or 0.1% Tween-20 served as controls. The experiment was repeated once with four replicate plates in each trial.

LD₅₀ and exposure time. The LD₅₀ of lemongrass oil and cinnamon oil were determined. Ten sclerotia, harvested from each of seven 14-day-old *R. oryzae-sativae* isolates, were soaked for 10 min in 5, 0.5, 0.05, and 0.005% concentrations of each oil. Treated sclerotia were transferred to PDA and the number of germinated sclerotia was counted after 3 days. There were four replicates per isolate in each of two independent trials. The exposure time of cinnamon oil to kill three *R. oryzae-sativae* isolates was determined by exposing sclerotia to 0.5% cinnamon oil for 10, 20, 40, and 80 min.

Greenhouse assay. The ability of cinnamon oil to control aggregate sheath spot was evaluated in a greenhouse maintained at 21 to 32°C. There were two greenhouse assays: the objective of the first experiment was to estimate effective cinnamon oil concentrations and the second experiment was conducted based on the results of the first experiment. Treatments were replicated four times in a completely randomized design and both experiments were repeated once.

In the first experiment, treatments were no oil, 1 ml of vegetable oil but no cinnamon oil (control), and 1 ml of 12.5% (0.125 ml of cinnamon oil/pot) or 87.5% (0.875 ml of cinnamon oil/pot) cinnamon oil diluted with vegetable oil (a generic soybean-based oil from a local market). Two rice cultivars (M-205 and M-206), two *R. oryzae-sativae* isolates (3B and 13B), and a noninoculated control were included in all experiments, for a total of 24 treatment combinations. Three-week-old rice seedlings were transplanted into 25 cm-diameter pots containing a sterilized potting mix of 50% sphagnum peat moss, 50% washed sand, and dolomite lime at 2.43 kg/m³, oyster shell lime at 0.872 kg/m³, superphosphate at 0.872 kg/m³, calcium nitrate at 0.344 kg/m³, potassium nitrate at 135 ml/m³, and potassium sulfate at 90 ml/m³. Five seedlings were transplanted per pot. Potting mix was added into the pots 8 cm from the bottom and the water level was maintained 6 cm from the top of the pot and 8 cm above the potting mix surface.

Four weeks after transplanting, 0.25 g of sclerotia of *R. oryzae-sativae*, which

floated on the water surface, were added to each pot. Inoculum preparation was modified from the procedure described by Gunnell and Webster (12). Briefly, unhulled rice and rice hulls were mixed in a ratio of 10:1 (vol/vol) and 350 ml of the rice mixture was then added to a 1-liter bottle. The center of the lid was removed and replaced with two layers of filter paper (Whatman mo. 1). After 35 ml of distilled water was added to each bottle, the bottles were autoclaved twice at 121°C for 90 min with a 24-h interval. Sterile water (100 ml) was added in each bottle and mixed with the rice mixture. Five 1.5-cm-diameter agar plugs of a 7-day-old *R. oryzae-sativae* culture were then added to each bottle. The cultures were incubated at room temperature for 1 month and periodically shaken. After the colonized rice grains were air dried, sclerotia were sieved from the grain and stored at 6°C for 1 day before inoculation. Treatments were applied on the water surface with a pipette 1 day after sclerotia were added. Beginning at 2 weeks after inoculation, the length of the longest lesion on each rice plant was recorded weekly for 3 weeks. At the end of the experiment (8 weeks after inoculation), rice plants were air dried and the dry weight of the plants aboveground in each pot was determined.

Based on the results of the first experiment, the second experiment was conducted only with isolate 3B with four oil treatments. Treatments included no oil, 1 ml of vegetable oil but no cinnamon oil (control), and 1 ml of 37.5% (0.375 ml of cinnamon oil/pot) or 62.5% (0.625 ml of cinnamon oil/pot) cinnamon oil. Cinnamon oil at 87.5% was not used because it was phytotoxic in the first experiment. The length of the longest lesion on each rice plant and the plant dry weights were recorded as above.

Data analyses. All statistical analyses were performed by JMP (version 7.0; SAS Institute Inc., Cary, NC). Because the results from both trials were consistent and there were no interactions between trials and treatments, data from both trials were combined and analyzed together. The differences in vegetative growth and sclerotia germination between the treatments with plant products and the controls were tested by paired *t* test because the same source of inocula was used. The effect of the plant products on sclerotia production was analyzed by ordinal logistic model. Bonferroni correction was employed for multiple pairwise comparisons to limit type I error. Treatments with no response on vegetative growth, sclerotia production, or sclerotia germination were excluded from statistical analyses.

The LD₅₀ of cinnamon oil on killing sclerotia was estimated by regression fitting of the percent kill of sclerotia from each *R. oryzae-sativae* isolate and the natural logarithm of the concentrations of each plant essential oil. The difference of

the LD₅₀s between lemongrass oil and cinnamon oil for the seven *R. oryzae-sativae* isolates was assessed by Student's *t* test. The exposure time was estimated by regression fitting of the percent kill of sclerotia from each of three *R. oryzae-sativae* isolates and the natural logarithm of different exposure times to 0.5% cinnamon oil.

Disease severity was analyzed by repeated measures in multivariate analysis of variance. The canonical scores of the main effects were generated and subjected to the Tukey-Kramer highly significant difference (HSD) test for mean comparisons. Each pot was treated as a replicate and each plant was a subsample. Because there were no interactions between trials and cinnamon oil treatments, data from both trials were combined and trial was treated as a block term. Plant dry weights were analyzed by analysis of variance and compared among treatments by the Tukey-Kramer HSD method.

RESULTS

Garlic extract and all plant essential oils except neem oil completely inhibited vegetative growth and, therefore, sclerotia production of *R. oryzae-sativae* (Table 1). The effect of neem oil was highly significant and resulted in a reduction in mycelial growth and a reduction in sclerotia production compared with the control. In contrast, three plant extracts, ginger, pepper, and basil, significantly increased vegetative growth of *R. oryzae-sativae*. Pepper and basil extracts increased sclerotia production but the ginger extract had no effect. None of the plant extracts suppressed sclerotia germination. In contrast, there was no germination of sclerotia treated with lemongrass oil or cinnamon oil. Neem oil and garlic oil significantly decreased sclerotia germination.

Because lemongrass oil and cinnamon oil completely inhibited vegetative growth, sclerotia production, and sclerotia germination of *R. oryzae-sativae*, the LD₅₀ of each was determined. The LD₅₀ of each *R. oryzae-sativae* isolate was estimated from regression fitting of the percent kill of sclerotia and the natural logarithm of the plant essential oil concentrations (Fig. 1). The average LD₅₀ of lemongrass oil (1.49%) was significantly higher than the average LD₅₀ of cinnamon oil (0.34%) (Table 2).

There was a significant quadratic polynomial relationship between sclerotia mortality and exposure time to 0.5% cinnamon oil for isolates 11A and 12B but not isolate 14A (Table 3; Fig. 2). However, because the regression curves of all three isolates were not significantly different (*P* = 0.0723), a general regression fitting model from combined data for *R. oryzae-sativae* was determined. In general, there was also a significant quadratic polynomial relationship between the percent kill of scler-

rotia and exposure time to 0.5% cinnamon oil (Table 3; Fig. 3). Based on the general model, a population of sclerotia would be completely killed after 32 min of exposure to 0.5% cinnamon oil.

In the first greenhouse experiment, there were significant interactions between cinnamon oil concentration and cultivars ($P = 0.0022$) and isolates ($P = 0.0155$); there-

fore, the results were interpreted separately. Vegetable oil without cinnamon oil had no effect on the disease of either cultivar (Table 4). No concentration of cinnamon oil inhibited disease caused by isolate 13B relative to the vegetable oil control. Relative to the vegetable oil control, disease severity of plants inoculated with isolate 3B was reduced by 87.5% cinna-

mon oil but not at the concentration of 12.5%. Disease severity caused by either isolate was higher in cv. M-205 than M-206 ($P < 0.0001$). In general, neither the controls (vegetable oil) nor 12.5% cinnamon oil reduced rice dry weights (Table 4). However, 87.5% cinnamon oil significantly reduced plant weight relative to the controls, and chlorotic stripes on the leaf sheaths of some tillers developed.

In the second greenhouse experiment, there was also a significant interaction between cinnamon oil concentration and cultivars ($P = 0.0456$); therefore, the results were interpreted separately in each cultivar. Vegetable oil without cinnamon oil had no effect on lesion expansion caused by isolate 3B (Table 5). Cinnamon oil at 62.5% reduced disease severity of isolate 3B in both cultivars compared with the control. The 37.5% cinnamon oil, however, suppressed lesion expansion only in M-206. Lesion expansion by isolate 3B was greater in cv. M-205 than M-206 ($P < 0.0001$). For both cultivars, rice dry weights were not affected by either concentration of cinnamon oil (Table 5).

DISCUSSION

In general, plant essential oils were more active against *R. oryzae-sativae* than plant extracts. All plant essential oils and the garlic extract had antifungal activity with different degrees of intensity. Lemon-grass oil and cinnamon oil were the most efficacious plant products because they completely inhibited vegetative growth, sclerotia production, and sclerotia germination of *R. oryzae-sativae*. The greater activity of cinnamon oil ($LD_{50} = 0.34\%$) against *R. oryzae-sativae* relative to lemon-grass oil ($LD_{50} = 1.49\%$) was similar to a report on the relative activity of cinna-

Table 1. Antifungal activity of 5% (vol/vol) plant products on mean vegetative growth, sclerotia production, and sclerotia germination on 11 *Rhizoctonia oryzae-sativae* isolates^u

Plant products	Colony diameter (cm) ^{v,w}	Sclerotia production rating ^x	Germinated sclerotia per 10 sclerotia ^{w,y}
Extracts			
Control (water)	5.29	2.1	9.6
Ginger	5.46***	2.1	9.4
Pepper	5.63***	3.3***	9.3
Basil	5.96***	2.9***	9.5
Garlic ^z	0.00	...	9.4
Essential oils			
Control (0.1% Tween-20)	4.65	1.5	9.9
Neem oil	1.87***	0.1***	6.9***
Garlic oil ^z	0.00	...	6.1***
Lemongrass oil ^z	0.00	...	0.0
Cinnamon oil ^z	0.00	...	0.0

^u Significant difference between plant products and the controls at $P \leq 0.05$, 0.01, and 0.001 is indicated by *, **, and ***, respectively; means from combined data of two trials with four replicates in each trial.

^v Radial growth of *R. oryzae-sativae* on potato dextrose agar (PDA) after 3 days.

^w Differences between the mean of plant products and the controls were analyzed by paired *t* test.

^x Production of sclerotia was estimated at 7 days based on the following scale: 0 = no sclerotia production and 1 = sclerotia produced on less than 25%, 2 = 25 to 50%, 3 = 50 to 75%, and 4 = more than 75% of the petri plate surface. Differences between the mean of plant products and the controls were analyzed by ordinal logistic model; ... indicates that sclerotia production was not evaluated because there was no vegetative growth for those treatments.

^y Sclerotia were soaked in each 5% plant extract or oil for 10 min and incubated on PDA for 3 days.

^z Treatments with no response on vegetative growth, sclerotia production, or sclerotia germination were excluded from statistical analyses.

Fig. 1. Regression fitting of the percent kill of sclerotia and the natural logarithm (ln) of cinnamon oil concentrations to determine LD_{50} of cinnamon oil against *Rhizoctonia oryzae-sativae* isolate 11A.

Table 2. LD_{50} of lemongrass and cinnamon oils on killing sclerotia of seven *Rhizoctonia oryzae-sativae* isolates^y

Isolate	LD_{50} (%)	
	Lemongrass oil	Cinnamon oil
3B	1.62	0.14
11A	1.63	0.76
12B	1.55	0.69
13B	1.61	0.16
14A	1.63	0.31
16A	1.18	0.16
17B	1.23	0.17
Mean ^z	1.49	0.34

^y Sclerotia were soaked for 10 min in 5, 0.5, 0.05, and 0.005% concentrations of lemongrass oil or cinnamon oil. The LD_{50} of each *R. oryzae-sativae* isolate was derived from regression fitting of percent kill of sclerotia from each *R. oryzae-sativae* and natural logarithm of plant essential oil concentrations with combined data from two trials.

^z Mean LD_{50} of seven *R. oryzae-sativae* isolates for each plant essential oil. There was a significant difference of LD_{50} s between lemongrass oil and cinnamon oil ($P < 0.0001$) assessed by Student's *t* test.

mon and lemongrass oils against *B. cinerea* (42) and *Fusarium proliferatum* (39). The major chemical constituent in cinnamon (leaf) oil is eugenol (34); in lemongrass oil, the major constituents are limonene (42) and citral (30). At a concentration of 1%, eugenol, limonene, and citral were antagonistic to *Cercospora arachidicola*, *Phaeoisariopsis personata*, and *Puccinia arachidis* (16). These components may potentially contribute to the antifungal activity against *R. oryzae-sativae*.

Both garlic extract and garlic oil completely inhibited growth and sclerotia production of *R. oryzae-sativae* but only garlic oil significantly reduced sclerotia germination. Garlic oil also inhibited growth and sclerotium formation by *R. solani* (36). The other materials (neem oil, ginger, pepper, and basil) either had little effect on *R. oryzae-sativae* or increased its growth. In contrast, a 10% dilution of garlic and pepper extracts exhibited greatest inhibition of vegetative growth and spore germination of *B. cinerea* among 345 plant extracts (42).

The sensitivity of fungi and even isolates of the same species to plant extracts and essential oils may vary. For example, neem oil partially suppressed sclerotia germination of California isolates of *R. oryzae-sativae* in this study but completely inhibited sclerotia germination of *R. oryzae-sativae* from India (1). In the current study, cinnamon oil reduced aggregate sheath spot caused by one isolate of *R. oryzae-sativae* but not another. Sensitivity to cinnamon oil may be genetically influenced because isolate 3B and 13B are in distinct somatic compatibility groups (P. Chaijuckam and R. M. Davis, unpublished data).

In this study, cinnamon oil was applied within 24 h after sclerotia of *R. oryzae-sativae* were added to the pots to mimic a natural pathosystem. In a field, sclerotia would be present before an application of any kind of fungicide could be made. Vegetable oil was used to dilute cinnamon oil in this study because it was documented as a good floating and spreading agent for fungicides active against *R. solani* in rice (27). In general, vegetable oil had no effect on disease severity and rice growth. Cinnamon oil at a concentration of 12.5% did not decrease aggregate sheath

spot and 32.5% cinnamon oil was only effective on one rice cultivar, M-206, which was less susceptible to *R. oryzae-sativae* than M-205. Thus, a lower concentration of cinnamon oil may offer some disease control in a cultivar partially resistant to *R. oryzae-sativae*. At a concentration of 87.5%, cinnamon oil decreased disease development but was phytotoxic because rice plant weight was reduced and chlorotic stripes on the leaf sheaths of some tillers developed. The optimal concentration of cinnamon oil to reduce lesion expansion caused by *R. oryzae-sativae* in this study was 62.5% because it was effective in both cultivars and had no effect on rice dry weight. A relatively high rate of cinnamon oil was apparently required because sclerotia are thick-walled structures able to resist unfavorable environments (40).

Although cinnamon oil completely inhibited vegetative growth, sclerotia production, and sclerotia germination of *R. oryzae-sativae* in vitro, the disease was not

completely controlled in vivo. Many factors may have influenced these results. For the experiment in vitro, the temperature was a constant 25°C and the surface area of an agar plate was small. Therefore, the cinnamon oil was stable and came into direct contact with the sclerotia. In the greenhouse experiment, temperatures fluctuated between 21 and 32°C and sunlight may have degraded the oil.

In other studies, cinnamon oil suppressed *Phytophthora* blight and reduced population densities of *Phytophthora nicotianae* (3). Cinnamon oil also inhibited spore germination of *Phaeoisariopsis personata* and mycelial growth of *Aspergillus niger* and reduced late leaf spot and crown rot of peanut (16). Furthermore, there are several other reports of the antagonistic properties of cinnamon oil to plant-pathogenic fungi (34,42), bacteria (8), and nematodes (17). In addition, cinnamon oil inhibited mycotoxin production of *A. flavus* (31), repelled insects from bean seed (28), and had insecticidal activity against

Fig. 2. Regression fitting of the percent kill of sclerotia and the natural logarithm (ln) of exposure times at 0.5% cinnamon oil on three *Rhizoctonia oryzae-sativae* isolates.

Table 3. Regression models of the exposure time of sclerotia of three *Rhizoctonia oryzae-sativae* isolates to 0.5% cinnamon oil^w

Isolate	Regression model ^x	R ²	SE ^y	P
11A	%kill = 16.04 + 24.89 ln time – 24.72 (ln time – 3.34) ²	0.89	7.58	0.0224
12B	%kill = 31.09 + 20.86 ln time – 22.48 (ln time – 3.34) ²	0.90	6.02	0.0135
14A	%kill = 64.76 + 8.84 ln time – 4.55 (ln time – 3.34) ²	0.66	4.78	0.3844
Combined ^z	%kill = 37.30 + 18.20 ln time – 17.25 (ln time – 3.34) ²	0.73	4.55	0.0011

^w Sclerotia were soaked in 0.5% cinnamon oil for 10, 20, 40, and 80 min.

^x Regression curve of each *R. oryzae-sativae* isolate was derived from regression fitting of percent kill of *R. oryzae-sativae* sclerotia and natural logarithm of different exposure times at 0.5% cinnamon oil with combined data from two trials.

^y Standard error.

^z Regression curves of all three isolates were not significantly different ($P = 0.0723$); therefore, data were combined.

Fig. 3. Regression fitting of the percent kill of sclerotia and the natural logarithm (ln) of exposure times at 0.5% cinnamon oil on the combined data from three *Rhizoctonia oryzae-sativae* isolates.

Table 4. Effect of cinnamon oil treatments on disease severity and plant dry weight of two rice cultivars and two *Rhizoctonia oryzae-sativae* isolates (first greenhouse experiment)^v

Cultivar, isolate	Treatment ^x	Mean of lesion length (cm) ^w			Scores ^y	Dry weight (g) ^z
		2 weeks	3 weeks	4 weeks		
M-205						
3B	No oil	3.45	6.39	7.71	0.64 a	34.25 ab
	Control	3.55	5.59	7.14	0.60 ab	36.29 a
	12.5%	2.11	4.25	5.66	0.44 bc	33.98 ab
	87.5%	1.33	2.78	4.91	0.33 c	28.70 b
13B	No oil	3.45	5.47	7.19	0.59 a	34.18 ab
	Control	2.39	4.55	5.95	0.47 ab	36.40 a
	12.5%	1.71	2.99	4.61	0.34 b	33.53 ab
	87.5%	1.88	3.71	4.54	0.37 b	31.33 b
Noninoculated	No oil	...	...	...	...	44.75 a
	Control	...	...	...	...	36.14 bc
	12.5%	...	...	...	...	37.13 ab
	87.5%	...	...	...	...	30.39 c
M-206						
3B	No oil	0.97	2.54	5.32	0.32 ab	35.08 ab
	Control	2.23	4.40	5.53	0.44 a	39.93 a
	12.5%	1.56	2.98	4.10	0.32 ab	38.86 a
	87.5%	1.31	2.35	3.78	0.27 b	30.19 b
13B	No oil	1.74	3.77	4.52	0.37 a	37.30 a
	Control	1.09	2.14	4.15	0.27 ab	38.91 a
	12.5%	1.51	2.71	4.09	0.31 ab	36.56 a
	87.5%	1.10	1.84	3.01	0.22 b	28.78 b
Noninoculated	No oil	...	...	...	...	40.93 a
	Control	...	...	...	...	41.43 a
	12.5%	...	...	...	...	40.14 a
	87.5%	...	...	...	...	32.81 b

^v Within each cultivar and each *R. oryzae-sativae* isolate, means in the same column followed by the same letter are not significantly different at $\alpha = 0.05$ according to the Tukey-Kramer highly significant difference test.

^w Length of the longest lesion of each rice plant was recorded weekly for 3 weeks (2, 3, and 4 weeks after inoculation).

^x Four cinnamon oil treatments were no oil, 1 ml of vegetable oil but no cinnamon oil (control), 1 ml of 12.5% cinnamon oil, and 1 ml of 87.5% cinnamon oil.

^y Canonical scores were calculated from lesion length data by repeated measures in multivariate analysis of variance procedure (32).

^z Aboveground.

the rice weevil (22). Cinnamon oil is likely a broad-spectrum compound against many pests. In addition to fungi, rice can be damaged by bacteria, nematodes, viruses, and insects (41). Thus, cinnamon oil could benefit rice growers by managing several pests.

Natural plant products may reduce disease development by direct toxicity to pathogens (15,42), induction of systemic resistance (15), stimulating beneficial organisms (2), or inactivation of toxins produced by pathogens (35). Bowers and Locke (3) proposed that synthetic cinnamon oil (30% cinnamaldehyde) may not reduce pathogen populations directly but, instead, may enhance the activity of beneficial organisms. In contrast, cinnamon oil directly affected *R. oryzae-sativae* by inhibiting vegetative growth and sclerotia germination in this study. It was probably because the major chemical constituent in cinnamon leaf oil used in this study was eugenol (34), whereas the active ingredient of cinnamon oil in the other study was different. However, whether cinnamon leaf oil could also induce systemic resistance in rice or inactivate a toxin produced by *R. oryzae-sativae* was still unknown.

The impacts of essential oils on humans and the environment have been reviewed (14). The toxicity of eugenol based on 96 h-LC₅₀ is about 1,500 times less toxic than pyrethrum, a botanical insecticide, and 15,000 times less toxic than azinphos-methyl, an organophosphate insecticide (14,37). Essential oil constituents are degradable in water and soil (24) and the residues of essential oil-based pesticides on crops should not be harmful to humans (14). Some plant essential oils may help prevent cancer in humans (26), reduce cholesterol (6,7), have anti-inflammatory properties (38), and act against human foodborne pathogens, including *Escherichia coli* O157 and *Listeria monocytogenes* (29).

In this research, cinnamon oil showed potential for the control of aggregate sheath spot on rice and may be an alternative to synthetic fungicides for organic rice growers. However, a relatively high rate of cinnamon oil was necessary to control aggregate sheath spot. At a concentration of 62.5% cinnamon oil, approximately 0.625 ml/pot or 1.3 $\mu\text{l}/\text{cm}^2$ (13 ml/m²) was required to reduce lesion expansion by *R. oryzae-sativae*. This amount is about 150 times higher than the recommended rate of azoxystrobin (40). Temperature, humidity, rainfall, and surface areas of rice fields may influence the degradation rate and evaporation rate of cinnamon oil in the field. These factors, which vary by geographic region, may affect the efficacy of cinnamon oil, the rate and frequency of its application, and cost. Moreover, cinnamon oil would have to be used with caution because high concentrations were phytotoxic. Future research should include study

Table 5. Effect of cinnamon oil treatments on disease severity caused by *Rhizoctonia oryzae-sativae* isolate 3B and plant dry weight of two rice cultivars (second greenhouse experiment)^v

Cultivar, isolate	Treatment ^x	Mean of lesion length (cm) ^w			Scores ^y	Dry weight (g) ^z
		2 weeks	3 weeks	4 weeks		
M-205						
3B	No oil	2.64	3.53	4.51	0.54 a	21.94 a
	Control	2.53	3.21	3.65	0.47 a	22.81 a
	37.5%	1.73	2.14	2.85	0.34 ab	21.88 a
	62.5%	0.80	0.83	0.93	0.13 b	21.38 a
Noninoculated	No oil	...	...	...	...	24.94 a
	Control	...	...	...	...	22.75 a
	37.5%	...	...	...	...	22.44 a
	62.5%	...	...	...	...	22.50 a
M-206						
3B	No oil	1.78	2.49	2.78	0.35 a	23.44 a
	Control	1.73	1.98	2.14	0.29 a	23.88 a
	37.5%	0.21	0.36	0.39	0.05 b	24.75 a
	62.5%	0.68	0.73	0.90	0.12 b	22.94 a
Noninoculated	No oil	...	...	...	...	24.94 a
	Control	...	...	...	...	24.88 a
	37.5%	...	...	...	...	24.69 a
	62.5%	...	...	...	...	23.69 a

^v Within each cultivar and each *R. oryzae-sativae* isolate, means in the same column followed by the same letter are not significantly different at $\alpha = 0.05$ according to Tukey-Kramer HSD test.

^w Length of the longest lesion of each rice plant was recorded weekly for 3 weeks (2, 3, and 4 weeks after inoculation).

^x Four cinnamon oil treatments were no oil, 1 ml of vegetable oil but no cinnamon oil (control), 1 ml of 37.5% cinnamon oil, and 1 ml of 62.5% cinnamon oil.

^y Canonical scores were calculated from lesion length data by repeated measures in multivariate analysis of variance procedure (32).

^z Aboveground.

in the field and the potential of cinnamon oil to control a wide range of rice diseases.

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