한국농약과학회지 제2권 제3호(1998)
The Korean Journal of Pesticide Science
Vol. 2, No. 3, pp.21~27(1998)

Fungicidal activities of leguminous seed extracts toward phytopathogenic fungi

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Abstract: Methanol extracts from 25 leguminous seeds were tested for their fungicidal activities toward six phytopathogenic fungi, using whole plant test in a greenhouse. The efficacy varied with both the plant pathogen and legume species used. At 5 mg/pot, potent fungicidal activities were produced from extracts of *Cassia obtusifolia*, *Glycine max* var. *solitae*, *G. max* var. *yagkong*, *G. max* var. *hooktae*, *Phaseolus multiflorus*, *P. radiatus* var. *aurea*, and *Vigna sinensis* against *Botrytis cinerea*, *Puccinia recondita*, and *Erysiphe graminis*. These seed extracts were highly effective against three *B. cinerea* strains resistant to carbendazim, procymidone, and diethofencarb. All leguminous seed extracts revealed weak or no fungicidal effect against *Rhizoctonia solani*, *Pyricularia grisea*, and *Phytophthora infestans*. As a naturally occurring fungicide, leguminous seed-derived materials described could be useful as new fungicidal products against various plant diseases induced by phytopathogenic fungi.(Received February 26, 1998, accepted December 1, 1998)

Key words : fungicidal activity, leguminous seed, phytopathogenic fungi.

Introduction

The preharvest losses due to fungal diseases in world crop production may be approximately 11.8%, or even higher in developing countries (Oerke *et al.*, 1994; Agrios, 1997). Over the several decades, various attempts to control plant diseases have taken an effort towards effective eradication or prevention through the development of synthetic fungicides. Although effective, their continued or repeated application has disrupted biological control by natural enemies, widespread development of resistance to various types of fungicides (Georghiou and Saito, 1983; Georgopoulos, 1987), toxicity to nontarget organisms, and human health and environmental concerns (Brown, 1978; Hayes and Laws, 1991). Decreasing efficacy and increasing concern over adverse environmental effects of the earlier types of fungicides have highlighted the need for the development of new types of selective control alternatives or of methods of crop protection without or with reduced use of conventional fungicides.

Plants may be an alternative to currently used disease control agents, because they virtually constitute a rich source of bioactive chemicals (Swain, 1977; Wink, 1993). Since these are often active against a limitted number of species including specific target species, are biodegradable to nontoxic products, and potentially suitable for use in integrated management programs, they could lead to the development of new classes of possibly safer disease control agents. Therefore, much efforts have been focused on plant materials for potentially useful products as commercial fungicides or as lead compounds (Balandrin *et al.*, 1985; Benner, 1993; Hedin *et al.*, 1997). However, little work has been done on the fungicidal activity of leguminous seed extracts against phytopathogenic fungi in

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spite of their excellent nutritional, pharmacological and industrial significance (Sharpe, 1984; Namba, 1986; Smith and Huyser, 1987).

In the greenhouse studies described herein, we assessed the fungicidal activities of 25 leguminous seed extracts against six phytopathogenic fungi using whole plant method.

Materials and Methods

Plant materials and sample preparation

The leguminous seeds were randomly and anecdotally collected (Table 1). They were dried in an oven at 60°C for 3 days and finely powdered using a blender. Each sample (50 g) was extracted two times with 500 ml methanol at room temperature and filtered (Toyo filter paper No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated *in vacuo* at 35°C using a rotary vacuum evaporator.

In vivo fungicidal activity

Six phytopathogenic fungi used in this study were *Pyricularia grisea*, *Rhizoctonia solani*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia recondita*, and *Erysiphe graminis*. Except for *P. recondita* and *E. graminis*, the others were routinely maintained on potato dextrose agar (PDA) slants and V-8 agar slants, and kept for stock at 4°C.

The fungicidal activities of test samples against pathogens used were determined by whole plant method, as previously described (Yoo *et al.*, 1998). The leguminous samples were tested at rates of 5 and 10 mg/pot. Test samples suspended in distilled water with Tween-20 (Junsei Chemical, Japan)

Table 1. List of leguminous plants tested

Scientific name	Chracteristics				
	Seed Colour	Flower Colour	Size (cm)	Shape	Yield[a](%)
Amphicarpaea edgeworthii	Purple	Light-purple	0.5	Ellipse	10.7
Arachis hypogaea	Dark-brown	Yellow	1.3	Ellipse	5.3
Canavalia lineata	Brown	Purple	0.9	Rod	12.0
Cassia obtusifolia	Dark-brown	Yellow	0.4	Rod	13.3
Dunbaria villosa	Light-brown	Yellow	0.9	Ellipse	5.6
Glycine max var. *solitae*	Black	White	1.1	Ellipse	10.0
Glycine max var. *yagkong*	Black	White	0.5	Spherical	5.5
Glycine max var. *hooktae*	Black	Purple	0.8	Spherical	6.6
Glycine max var. *bangkong*	Dark-brown	Purple	1.1	Ellipse	5.4
Glycine max var. *geumdu*	Dark-purple	Purple	0.6	Spherical	4.8
Glycine max var. *chungtae*	Light-green	White	0.8	Spherical	11.1
Glycine max var. *wooltalikong*	Purple	Purple	1.1	Ellipse	1.9
Glycine max var. *mejukong*	Yellow	White	0.8	Spherical	7.1
Glycine soja	Brown	Light-purple	2.0	Rod	10.7
Lathyrus japonica	Black	Red	1.5	Ellipse	12.0
Phaseolus multiflorus	Dark-purple	Red	1.2	Rod	5.3
Phaseolus nipponensis	Dark-green	Yellow	2.1	Ellipse	5.7
Phaseolus radiatus var. *geodu*	Black	White	0.5	Spherical	7.8
Phaseolus radiatus var. *aurea*	Green	Yellow	0.5	Rod	5.2
Pisum sativum	Light-green	White-blue	0.7	Spherical	3.6
Rhynchosia volubilis	Brown	Yellow	1.1	Ellipse	5.3
Vicia hirsuta	Black	Light-purple	1.2	Ellipse	11.8
Vicia tetrasperma	Light-purple	Light-purple	1.1	Ellipse	12.3
Vigna angulasis	Red	Yellow	0.6	Spherical	4.8
Vigna sinensis	Light-yellow	Yellow	0.7	Ellipse	6.2

[a](Dried weight of methanol extract/dried weight of sample) × 100.

added at the rate of 250 mg/liter were used. Fifty ml of each test sample solution was sprayed onto two pots on the turntable at the same time. After evaporation in a greenhouse for 1 day, each pathogen was inoculated into treated test plants. Controls received Tween-20 solution. All treatments were replicated three times.

In a test with rice blast (RCB) caused by *P. grisea*, rice plants with 2 leaf stage growth (three plants/pot) were sprayed with each test solution. Treated plants were inoculated with a suspension of conidia in distilled water (1 x 10^6 spores/ml) and kept in a chamber (25°C) for 24 hr under 100% relative humidity (RH). Treated and control plants were then held in a lighted chamber (26±2°C and 85% RH) for 5 days, and rated on the disease severity. For rice sheath blight (RSB) caused by *R. solani*, each test solution was sprayed onto rice plants with 3 leaf stage growth (three plants/pot). The plants were inoculated by injecting inoculum at the base of the rice plants. Inoculum was made by incubating mycelial plugs in wheat bran medium at 25°C for 7 days, and macerated into the mixer. Treated and control plants were held in a lighted chamber (28°C) for 5 days. With cucumber gray mold (CGM) caused by *B. cinerea*, cucumber plants with the first leaf stage growth (one plants/pot) were sprayed with each test solution. The cucumber was inoculated with conidia (1 x 10^6 spores/ml) of *B. cinerea* incubated on PDA medium at 20°C for 15 days by leaf spray and then placed in a chamber (20°C) for 4-5 days. For tomato late blight (TLB) caused by *P. infestans*, each test solution was sprayed onto tomato plants with 2 leaf stage growth (two plants/pot). The plants were inoculated with a suspension of 1 x 10^5 zoosporangia/ml incubated on V-8 juice agar medium at 20°C for 14 days. They were kept in a chamber (18°C) for 4 days and then disease ratings were made. For wheat leaf rust (WLR) caused by *P. recondita*, wheat plants with the first leaf stage growth (four plants/pot) were sprayed with each test solution. The plants were sprayed with a suspension (60 mg/100 ml of 250 ppm Tween 20) of uredospores colonized on the second leaf of wheat and then placed in a moist chamber. One day after inoculation, plants were held in a growth chamber (20°C and 70% RH). The fungicidal activities of the test samples were made on 10 days after inoculation (DAI).

For barley powdery mildew (BPM) caused by *E. graminis*, barley plants with fully expended first leaf (four plants/pot) were sprayed with a suspension of a test material. Treated plants were dusted with conidia of *E. graminis* formed on the primary leaf of barley and held in a chamber (20°C). The disease severity was rated on 10 DAI.

The control effect of test samples on each plant disease was evaluated with control value (CV) calculated by the formula CV (%) = [(A - B)/A] x 100, where A and B represent the disease area on the untreated and treated plants, respectively. The responses were classified as previously described (Yoo *et al.*, 1998): the very strong activity ++++, CV >90%; strong +++, CV 81~90%; moderate ++, CV 61~80%; weak +, CV 40~60%; and little or no activity -, CV<40%.

Control effect on fungicide-resistant strains of *B. cinerea*

For the investigation of their potent fungicidal activity against *B. cinerea* in which recently resistant problem was brought out, effectiveness of eight test samples against fungicide resistant *B. cinerea* was investigated, as mentioned earlier. Four fungicide resistant *B. cinerea* strains were used: 2-18, sensitive to both carbendazim and procymidone but highly resistant to diethofencarb (SSR); P2, sensitive to carbendazim but highly resistant to both procymidone and diethofencarb (SRR); DJ-78, highly resistant to carbendazim and procymidone but sensitive to diethofencarb (RRS); and SDT-17, highly resistant to both carbendazim and diethofencarb but sensitive to procymidone (RSR) (Kim, 1997). Their collection sites and resistance patterns were described in detail elsewhere (Kim, 1997). Fungicidal activities was evaluated at 10 mg/pot.

Results

***In vivo* fungicidal activity**

Fungicidal activities of the test samples against six plant pathogens are shown in Table 2. The responses varied with legume species and pathogen used. Methanol extracts of *Glycine max* var. *yagkong* and *Phaseolus multiflorus* gave 91 and 71% control value on *P. oryzae* at a rate of 10 mg/pot, respectively. At 5 mg/pot, moderate activities were obtained in these plants.

In a test with *B. cinerea* (Table 2), potent fungicidal activities (CV >80%) were produced from extracts of *Cassia obtusifolia*, *G. max* var. *yagkong*, *G. soja*, *Phaseolus radiatus* var. *aurea*, *Pisum sativum*, *P. multiflorus*, and *Vigna sinensis* when treated with 10 mg/pot. Of these legume species, extracts from *C. obtusifolia*, *G. max* var. *yagkong*, and *G. max* var. *hooktae* exhibited strong fungicidal activities at 2,000 ppm whereas moderate activities were observed in the other species.

Of 25 legume seed extracts used, at 10 mg/pot, 14, 3, and 2 test samples showed very strong, strong and moderate fungicidal activities against *P. recondita*, respectively (Table 2). At 5 mg/pot, potent fungicidal activities (CV, >80%) were produced from seed extracts of *C. obtusifolia*, *G. max* var. *solitae*, *G. max* var. *yagkong*, *G. max* var. *hooktae*, *G. max* var. *geumdu*, *G. max* var. *mejukong*, *L. japonica*, *P. multiflorus*, *P. radiatus* var. *geodu*, *Pisum sativum*, and *Vigna angulasis*.

Table 2. Control effect of leguminous seed extracts on some crop diseases, whole plant test

Legume[a]	RCB[b]		CGB		WLR		BPM	
	10[c]	5	10	5	10	5	10	5
A. edgeworthii	-[d]	nd[e]	-	-	++	++	+++	+++
A. hypogaea	-	nd	++	+	+++	++	+++	++
C. lineata	-	nd	+	-	-	-	-	-
C. obtusifolia	+	-	++++	+++	++++	+++	++++	++++
D. villosa	-	nd	-	-	++++	++	+++	++
G. max var. *seolitae*	-	dn	+	-	++++	+++	++++	++++
G. max var. *yagkong*	++++	++	++++	+++	++++	++++	++++	++
G. max var. *hooktae*	-	nd	+++	+++	++++	+++	++++	+++
G. max var. *bangkong*	-	nd	-	-	++++	++	++	+
G. max var. *geumdu*	-	nd	+	+	++++	++++	++++	++
G. max var. *chungtae*	-	nd	-	+	+	-	+	-
G. max var. *wooltalikong*	-	nd	+	-	+	-	+	-
G. max var. *mejukong*	-	nd	+	-	++++	+++	++++	++
G. soja	-	nd	++++	++	+	-	+	-
L. japonica	+	+	-	-	+++	+++	+++	+++
P. multiflorus	++	++	+++	+	++++	++	++++	++
P. nipponensis	-	nd	-	-	+	+	++++	++
P. radiatus var. *geodu*	-	nd	+	+	++++	++++	+++	++
P. radiatus var. *aurea*	+	-	++++	++	++++	++	++++	++
P. sativum	-	nd	++++	++	++++	+++	+++	+++
R. volubilis	-	nd	+	-	++++	++	++++	++
V. hirsuta	-	nd	-	-	+	+	+++	+
V. tetrasperma	-	nd	-	-	++	+	+	-
V. angulasis	-	dn	-	-	+++	+++	++++	+++
V. sinensis	+	nd	+++	+	++++	++	++++	++

[a]All test samples revealed little or no fungicidal activities against *Rhizoctonia solani* and *Phytophthora infestans*.
[b]RCB, *Pyricularia grisea*; CGB, *Botrytis cinerea*; WLR, *Puccinia recondita*; and BPM, *Erysiphe graminis*.
[c]Unit: mg/pot.
[d]++++, >90%; +++, 80~90%; ++, 61~80%; +, 40~60%; and -, <40%.
[e]Not determined.

When treated with 10 mg/pot, 12, 7 and 2 leguminous seed extracts revealed very strong, strong and moderate fungicidal activities against *E. graminis*, respectively (Table 2). At 5 mg/pot, over 80% CVs were obtained in extracts from *C. obtusifolia*, *G. max* var. *solitae*, *G. max* var. *hooktae*, *L. japonica*, *V. angulasis*, *Amphicarpaea edgeworthii*, *L. japonica*, *P. sativum*, and *Vicia hirsuta*. The results from *R. solani* and *P. infestans* showed that all test seed extracts when treated with 5 and 10 mg/pot had weak or no fungicidal activity.

Effectiveness of test samples against fungicide-resistant *B. cinerea*

Because of their excellent fungicidal activity, the control effect of eight test extracts against three *B. cinerea* strains resistant to carbendazim, procymidone, and diethofencarb were determined at a rate of 10 mg/pot (Table 3). Extracts of *C. obtusifolia*, *P. multiflorus*, and *P. sativum* were highly effective against all the strains of *B. cinerea*. Potent fungicidal activity against the SSR, RSS and RSR strains was produced from extracts of *G. max* var. *yagkong*, *G. max* var. *hooktae*, and *G. soja*.

Discussion

In the greenhouse studies with methanol extracts from 25 leguminous seeds belonging to the family Apiaceae, many of them showed potent fungicidal activity against the economically important phytopathogenic fungi. Fungicidal activity varied with both the legume species and pathogen tested. WLR and BPM were controlled more effectively by the application of methanol extracts of various leguminous seeds than RSB, TLB, RCB and CGM. Jacobson (1989) pointed out that the most promising botanicals as sources of novel plant-based pesticides for use at present and in the future are species of the families Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, and Canellaceae. It has been also reported that Annonaceous plant species can be employed as safe, effective, economical, and environmentally friendly pesticides on the home garden, ornamental, greenhouse (Hostettman and Potterat, 1997). Various compounds including phenolics, terpenoids and alkaloids exist in plants (Swain, 1977; Wink, 1993). These compounds jointly or independently contribute to generation of biological activities. About 18,000 secondary plant metabolites have been chemically identified so far (Swain, 1977). Since these plant-derived extracts and phytochemicals act in many ways on various types of disease complex, and may be applied to the plant in the same way as other agricultural chemicals, they are being considered as potential

Table 3. Control effect of leguminous seed extracts on susceptible and fungicide resistant strains of *Botrytis cinerea*, whole plant test

Legume[a]	Strain[b]			
	2-18	P2	DJ-78	SDT-17
C. obtusifolia	++++[c]	++++	++++	++++
G. max var. *yagkong*	++++	-	++++	+++
G. max var. *hooktae*	++++	-	+++	++++
G. soja	++++	++	++++	+++
P. multiflorus	+++	++++	+++	++++
P. radiatus var. *aurea*	-	-	++++	+
P. sativum	++++	++++	++++	++++
V. sinensis	+	++	+++	+++

[a]Exposed at 10 mg/pot.
[b]2-18 (SSR), susceptible to both carbendazim and procymidone but highly resistant to diethofencarb; P2 (SRR), susceptible to carbendazim but highly resistant to both procymidone and diethofencarb; DJ-78 (RRS), highly resistant to carbendazim and procymidone but susceptible to diethofencarb; and SDT-17 (RSR), highly resistant to both carbendazim and diethofencarb but susceptible to procymidone.
[c]For explanation, see Table 1.

alternatives for synthetic fungicides (Hostettman and Potterat, 1997; Hedin, 1982), or lead compounds for new classes of synthetic fungicides such as podoblastin produced by *Podophyllum peltatum* (Miyakado, 1984; Hostettman and Potterat, 1997). However, little information is available for fungicidal activity of leguminous plants. Lee and Ahn (1997) already reported that leguminous seed extracts have growth-promoting activity against some lactic acid bacteria without any adverse effects on them.

In our *in vivo* study, 18 leguminous seed extracts showed significant fungicidal activity (>80% CV) against *P. recondita* and *E. graminis*, although nealry most of all test samples were not effective on *P. oryzae*, *R. solani* and *P. infestans*. Especially, the strong activity of *C. obtusifolia*, *G. max* var. *yagkong*, *G. max* var. *hooktae*, *P. multiflorus*, *P. radiatus* var. *aurea*, *P. sativum*, and *V. sinensis* against *B. cinerea*, *P. recondita*, and *E. graminis* confirms their superiority and usefulness as a potent fungicide. These leguminous seeds might form a new source for managing these plant pathogens in field ecosystem, although their effects on non-target organisms or environment remain unknown. It has been reported that many of 54 oriental medicinal plant extracts used were very effective against *P. oryzae*, *R. solani* and *P. infestans* but exhibited no fungicidal activity against *B. cinerea*, *P. recondita*, and *E. graminis* (Yoo *et al.*, 1998). These results indicate that biologically active components between leguminous seeds and oriental medicinal plants tested might be different.

Current control of plant diseases is primarily based on repeated or continued applications of conventional fungicides. However, their extensive use for the decades has led to widespread development of resistance (Georghiou and Saito, 1983; Georgopoulos, 1987). Therefore, more emphasis has to be given to the need for selective plant disease control agents for use in integrated management. Certain plant-derived materials are found to be highly effective against fungicide-resistant pathogens. For example, natural compounds such as cinnamaldehyde and salicylaldehyde were effective against four strains of *Fusarium sambucinum* resistant to thiabendazole (Vaughn and Spencer, 1994). Based on our results, some leguminous seed extracts were highly effective against the four resistant strains of *B. cinerea*, indicating that they could be useful as a new fungicidal product against field populations of *B. cinerea*.

In conclusion, the leguminous seed-derived materials might be useful products for developing new types of fungicides, or biorational management agents for controlling plant pathogens on crops, although their effects on natural enemies, vegetable qualities, or environment has not been fully investigated.

Acknowledgements

This work was supported by the Ministry of Agriculture and Forestry through the R & D Promotion Center for Agriculture, Forestry and Fishery, and the Korea Science and Engineering Foundation through the Research Center for New Bio-Materials in Agriculture at Seoul National University to YJA.

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콩과식물 종실 추출물의 살균활성

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요약 : 25종 콩과식물 종자의 메탄올 추출물을 대상으로 기주식물상의 온실실험조건에서 6종의 주요 식물병원균에 대해 방제효과를 조사한 그 효과는 콩과식물의 종류에 따라 커다란 차이를 보였다. 25종의 공시종자를 5 mg/pot 농도로 처리하였을 때 결명자 (*C. obtusifolia*), 소리테(*G. max* var. *solitae*), 약콩(*G. max* var. *yagkong*), 흑태(*G. max* var. *hooktae*), (*P. multiflorus*), (*P. radiatus* var. *aurea*)과 (*V . sinensis*) 추출물들은 오이잿빛곰팡이병, 밀붉은녹병 및 보리흰가루병에 대하여 80% 이상의 방제효과를 나타내었을 뿐 아니라, 잿빛곰팡이병의 살균제 저항성 균주에 대해서도 높은 활성을 보였다. 그러나, 공시종자 모두 벼도열병, 벼잎집무늬마름병 및 *Phytophthora infestans*병에 대해서는 거의 활성이 없거나 활성이 낮았다. 이상의 결과로 부터 잿빛곰팡이병, 밀녹병 및 보리흰가루병 등에 높은 방제효과를 보인 상기 종자 추출물들은 식물병 방제제로서 사용 가능성이 예상되었으며, 특히 살균제 저항성 잿빛곰팡이병에 높은 활성을 보여 시설원예재배에서 문제시 되고 있는 잿빛곰팡이병의 방제에도 크게 이용할 수 있을 것으로 기대되었다

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