Fungicidal and insecticidal activities of various grain extracts against five insect pests and six phytopathogenic fungi

Hoi-Seon Lee* · Gyung-Ja Choi¹ · Kwang-Yun Cho¹ · Sang-Gil Lee² and Young-Joon Ahn³

Institute of Agricultural & Technology and Faculty of Biotechnology, College of Agriculture, Chonbuk National University, Chonju 561-756, Korea

¹Screening Division, Korea Research Institute of Chemical Technology, Taejeon 305-600, Republic of Korea ²Forestry Research Institute, Seoul 130-010, Republic of Korea.

³Division of Applied Biology and Chemistry and the Research Center for New Bio-Materials in Agriculture, College of Agriculture and Life Sciences, Seoul National University, Suwon 441-744, Korea

Abstract: Methanol extracts from 21 grains were tested for fungicidal activities against six phytopathogenic fungi and for insecticidal activities toward five insect pests in a greenhouse. The efficacy varied with both the plant pathogen/insect pest and grain species used. Potent fungicidal activity at 5 mg/pot, were produced from extracts of Elymus sibiricus and Hordeum vulgare var. nudum against Pyricularia grisea and Erysiphe graminis and these of Sesamum indicum (W) and Triticum aestivum against Puccinia recondita and Erysiphe graminis. At 2,500 ppm, potent insecticidal activities were exhibited from the extracts of Fagopyrum esculentum against Myzus persicae and Ischaemum crassipes, and these of Oryza sativa var. glutinosa, Panicum miliaceum, Setaria italica, Sorghum bicolor, and T. aestivum against Tetranychus urticae. All grain extracts revealed weak or no fungicidal and insecticidal effect against Phytophthora infestans, Plutella xylostella and Spodoptera litura. As a naturally occurring fungicide and insecticide, grain-derived materials described could be useful as new fungicidal and insecticidal products against phytopathogenic fungi and insect pests. (Received July 10, 2000; accepted Sep. 15, 2000)

Key words: fungicidal and insecticidal activity, grain, phytopathogenic fungi, insect pest.

Introduction

The economic losses due to pre- and post-harvest fungal diseases and insect pests in crops amount to 5-50%, or even higher in developing countries (Eclert and Ogawa, 1985; Oerke, 1994). Over the several decades, various attempts to control insect pests and fungal diseases have taken for the effective eradication or prevention through the development of synthetic pesticides. However, their continued uses cause many adverse effects such as human intoxication, environmental pollution (Brown, 1978; Hayes and Laws, 1991), resurgences (Ripper, 1956), resistance (Georghiou and Saito, 1983; Georgopoulos, 1987), residue and toxicity to non-target organisms. Both economic consideration and increasing concern on adverse effects of the earlier types of fungicides and insecticides have brought about the need for the development of alternative control methods without or with reduced use of organic pesticides.

Plants may provide an alternative to the pesticides currently used against disease and pests, because they are virtually constituted with a rich source of bioactive chemicals (Swain, 1977; Wink, 1993). Since these are often active against a limited number of pest species, biodegradable to nontoxic products, and potentially suitable for use in integrated management programs, they could lead to the development of new classes of possibly safe disease control agents. Therefore, much efforts have been focused on plant materials for potentially useful products as commercial pesticides or as lead compounds (Balandrin et al., 1985; Benner, 1993; Isman, 1995; Hedin et al., 1997). However, little work has been done on the fungicidal and insecticidal activities of grain extracts in spite of excellent nutritional, pharmacological industrial significance (Namba, 1986; Friggens et al., 1995; Drouillard and Kuhl, 1999).

In the greenhouse studies described herein, we assessed fungicidal and insecticidal activities of 21 grain extracts against five insect pests and six phytopathogenic fungi.

^{*} Corresponding author

Materials and Methods

Plant materials and sample preparation

The grains 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 (100 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 test

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* unable to grow in artificial media, 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 spray method, as previously described (Lee $et\ al.$, 1998). Various grain samples were tested at rates of 5 and 10 mg/pot. Test samples suspended in distilled water with Tween-20 added at the rate of 250 mg/ ℓ were used. Fifty m ℓ 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. Untreated controls received Tween-20 solution. All treatments were replicated three times.

In a test with rice blast (RCB) caused by P. grisea, rice plants at 2nd leaf stage (three plants/pot) were sprayed with each test solution. Treated plants were inoculated with suspension of conidia in distilled water $(1 \times 10^6 \text{ spores/m}\ell)$ 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 for the disease severity. For rice sheath blight (RSB) caused by R. solani, each test solution was sprayed onto rice plants at 3nd leaf stage (three plants/pot). The plants were inoculated by injecting the inoculum at the base of the rice plants. Inoculum was made by culturing mycelial plugs in wheat bran medium at 25°C for 7 days, and macerated in a 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 at 1st leaf stage (one plants/pot) were sprayed with each test solution. The cucumber was inoculated with conidia (1 \times 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 at 2nd leaf stage (two plants/pot). The plants were inoculated with a suspension of 1 x 10⁵ zoosporangia/ml made from 14 days culture of V-8 juice agar medium at 20°C. 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 at 1st leaf stage (four plants/pot) were sprayed with each test solution. The plants were sprayed with a suspension (60 mg /100 m ℓ of 250 ppm Tween 20) of uredospores collected from 2nd 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 collected from the primary leaf of barley and held in a chamber (20°C). The disease severity was rated on 10 DAI.

The control effect of grain extracts on each plant disease was evaluated with control value (CV) calculated by the formula CV (%) = $[(A - B)/A] \times 100$, where A and B represent the disease area on the untreated and treated plants, respectively. The responses were classified as previously described (Lee *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%.

In vivo insecticidal activity test

Laboratory stains of five species of pests were used in this study: brown planthopper (Nilaparvata lugens Stål), diamondback moth (Plutella xylostella L.), green peach aphid (Myzus persicae Sulzer), tobacco cutworm (Spodoptera litura Fab.), and two-spotted spider mite (Tetranychus urticae Koch). They have been reared for several years without exposure to any insecticide in our laboratory. Laboratory rearing procedures were the same as previously described (Kown et al., 1994).

In relation to the search for new bioactive substances against insect pests, we have established a bioassay method suitable for rapid mass-screening of synthetic organic compounds or plant-derived extracts for insecticidal activities with reproducible results,

Table 1. List of plant grains tested

Scientific Name	Chracteristics						
Scientific Name	Family Name	Seed colour	Yield ^{a)} (%)				
Avena sativa	Graminales	White	11.5				
Coix lachryma-jobi var. mayuen	Graminales	White	10.8				
Elymus sibiricus	Graminales	White	15.1				
Fagopyrum esculentum	Polygonaceae	Dark-brown	9.7				
Hordeum vulgare var. hexastichon	Graminales	White	16.8				
Hordeum vulgare var. nudum	Graminales	White	10.8				
Ischaemum crassipes	Graminales	White	12.3				
Oryza sativa var. glutinosa	Graminales	Black	8.9				
Oryza sativa var. Japonica	Graminales	White	9.9				
Panicum miliaceum	Graminales	Yellow	13.2				
Panicum bisulcatum	Graminales	Green	14.6				
Perilla frutescens var. japonica	Labiatae	Dark-brown	11.4				
Schizandra chinensis	Magnoliaceae	Purple	10.6				
Schizandra nigra	Magnoliaceae	Black	15.8				
Secale careale	Graminales	Light-Brown	10.6				
Sesamum indicum(B)	Pedalidaceae	Black	11.3				
Sesamum indicum(W)	Pedalidaceae	White	12.5				
Setaria italica	Graminales	Yellow	7.9				
Sorghum bicolor	Graminales	Dark-Purple	9.4				
Triticum aestivum	Graminales	Light-Brown	10.2				
Zea mays	Graminales	Yellow	12.8				

^{a)}(Dried weight of methanol extract/dried weight of the sample grain) x 100.

using only a minute quantity of compounds. The plant extracts were tested at a concentration of 5,000 ppm for insect pests. If a plant extract exhibited activity, titration studies were performed. Test samples were suspended in distilled water with Triton X-100 added at the rate of 0.1 $m\ell/\ell$.

Spray method was used for the bioassay of N. lugens (BPH). Twenty female adults were transferred onto a test tube (3×20 cm) containing five 'Chucheong' rice seedlings wrapped with cotton and about 20 m ℓ water. Spray was done with a glass spray unit connected to a forced air supply (Pacific Chemical Co., Ltd., Seoul, Korea).

Leaf-dipping method was used aphid, lepidopteran larvae, and mite. Leaves of bean (Glycine max) for M. persicae (GPA), chinese cabbage (Brassica campestris subsp. napus var. pekinensis) for P. xylostella (DBM) and S. litura (TCW), and kidney bean (phaseolus vulgaris var. humilis) for T. urticae (TSSM) from each plant species grown in greenhouse were collected, and disks (3 cm dia.) were punctured from each leaf. Three leaf disks were dipped in the test solution for 30 sec. After evaporation in a hood for 2 hr, 20 2nd larvae of each P. xylostella and S. litura and 20 M. persicae females and 30 T. urticae adults were placed onto the

treated and control leaf disks in Petri dishes.

All treated materials were held in a room at $25\pm1^{\circ}$ C, under $50{\sim}60\%$ RH, and a photoperiod of 16:8 (light/dark). Mortality was counted after 48 hr, and all treatments were done in triplicate. The insecticidal activities were classified as follow: the very strong activity ++++, mortality>90%; strong +++, mortality 81-90%; moderate ++, mortality 61-80%; weak +, mortality 40-60%; and little or no activity -, mortality<40%.

Results

The grains were randomly and anecdotally collected (Table 1). In our study, fungicidal and insecticidal activities of grain extracts in Graminales (15), Labiatae (1), Magnoliaceae (2), Pedalidaceae (2), and Polygonaceae (1) were tested against six plant pathogens and five insect pests.

Fungicidal activity

Fungicidal activities of the test samples against six plant pathogens are shown in Table 2. The responses varied with grain species and pathogen used.

Methanol extracts of Elymus sibiricus, Hordeum vulgare var. hexastichon, Hordeum vulgare var. nudum and Ischaemum crassipes gave 100% control values on P. grisea at a rate of 10 mg/pot. At 5 mg/pot, over CVs 80% were obtained for these grains. Furthermore, at 10 mg/pot, the extracts of Oryza sativa var. glutinosa, Panicum miliaceum, Panicum bisulcatum and Perilla frutescens var. japonica revealed strong activities (CV > 80%). Moderate activities were obtained in the extracts of Setaria italica and Sorghum bicolor. In a test with R. solani (Table 2), potent fungicidal activities were produced from extracts of Avena sativa, Schizandra

chinensis, and Schizandra nigra at the rate of 10 mg/pot, and the extracts also exhibited strong activities at 5 mg/pot. However, other extracts had weak or no activity against *R. solani* when treated at 5 and 10 mg/pot.

In a test with *B. cinerea*, potent fungicidal activities (CV >80%) were produced from extracts of *H. vulgare* var. *nudum*, *P. miliaceum*, *P. bisulcatum*, *Sesamum indicum*(B), *S. italica*, and *Triticum aestivum* when treated at 10 mg/pot whereas the extracts showed moderate activities at 5 mg/pot. Extracts from *Perilla frutescens* var. *japonica* and *S. indicum* (W) exhibited moderate fungicidal activities to gray mold at 5 mg

Table 2. Disease inhibition effects by grain extracts in greenhouse tests.

Grain	RCB ^{a)}		RS	RSB		CGM		TLB		WLR		BPM	
	10 ^{b)}	5	10	5	10	5	10	5	10	5	10	5	
A. sativa	_c)	-	++++	+++	-	-	-	nd ^{d)}	-	-	-	-	
C. lachryma-jobi	+	-	-	nd	++	-	-	nd	+++	+++	-	-	
var. mayuen		•											
E. sibiricus	++++	+++	_	nd	-	-	-	nd	-	-	++++	+++	
F. esculentum	-	-	_	nd	-	-	-	nd	-	-	-	-	
H. vulgare	++++	+++	-	nd	-	-	-	nd	-	-	++++	++	
var. hexasticho													
H. vulgare	++++	+++	-	nd	++++	++	+	nd	++++	+++	++++	+++	
var. nudu													
I. crassipes	++++	+++	-	nd	-	-	-	nd	-	-	++++	++	
O. sativa	+++	++	-	nd	-	-	-	nd	-	-	-	-	
var. glutinosa													
O. sativa	-	-	-	nd	-	-	-	nd	-	-	-	-	
var. Japonica													
P. miliaceum	+++	. ++	-	nd	+++	++	+	nd	++++	+++	+++	++	
P. bisulcatum	+++	++	-	nd	+++	++	-	nd	++	++	++	+	
P. frutescens	+++	++	-	nd	++	+	++	+	++++	+++	++++	++	
var. japonica													
S. chinensis	-	-	++++	++++	-	-	_	nd	-	-	-	-	
S. nigra	-	-	++++	+++	-	-	-	nd	-	-	-	-	
S. careale	-	-	-	nd	-	-	-	nd	++	++	-		
S. indicum(B)	-	-	-	nd	++++	++	++	++	++	++	++++	+++	
S. indicum(W)	-	-	+	nd	++	+	++	+	++++	+++	++++	+++	
S. italica	++	+	-	nd	+++	++	+	nd	+++	+++	+++	++	
S. bicolor	++	+	-	nd	-	-	+	nd	-	-	+++	++	
T. aestivum	-	-	-	nd	+++	++	-	nd	++++	+++	++++	+++	
Z. mays	-	-	-	nd	-	-	-	nd	++++	+++	-	-	

^{a)} RCB, Pyricularia grisea; RSB, Rhizoctonia solani; CGB, Botrytis cinerea; TLB, Phytophthora infestans; WLR, Puccinia recondita; and BPM, Erysiphe graminis.

b) Unit: mg/pot applied.

c) ++++, >90%; +++, 80-90%; ++, 61-80%; +, 40-60%; and -, <40% in control value.

d) Not determined.

/pot whereas weak or no fungicidal activities were observed in the other grains.

Of 21 grain extracts used, at 5 and 10 mg/pot, Coix lachryma-jobi var. mayuen, H. vulgare var. nudum, P. miliaceum, P. frutescens var. japonica, S. indicum (W), S. italica, T. aestivum, and Zea mays showed potent fungicidal activities (CV >80%) against P. infestans (Table 2). At 5 and 10 mg/pot, moderate fungicidal activities were produced from extracts of P. bisulcatum, S. indicum (B), and Secale careale. When treated with 10 mg/pot, extracts of Elymus sibiricus, H. vulgare var. hexastichon, H. vulgare var. nudum, I. crassipes, P. miliaceum, P. frutescens var. japonica, S. indicum (B), S. indicum (W), S. italica, S. bicolor, and T. aestivum

revealed potent fungicidal activities against *E. graminis* (Table 2). At 5 mg/pot, over 80% CVs were obtained from the extracts of *E. sibiricus*, *H. vulgare* var. *nudum*, *S. indicum* (B), *S. indicum* (W), and *T. aestivum*, whereas moderate fungicidal activities were exhibited from the extracts of *H. vulgare* var. *hexastichon*, *P. miliaceum*, *P. frutescens* var. *japonica*, *S. italica*, and *S. bicolor*.

Insecticidal activity

Insecticidal activities of the test samples against five insect pests are shown in Table 3. The responses varied with grain species and insect pests used. Methanol extracts of *H. vulgare* var. *nudum*, *O. sativa*

Table 3. Controlling effect of grain extracts to five insect pests in greenhouse tests

Grain ^{a)}	BP	$H^{b)}$	GPA	A	TS	
	5000°)	2500	5000	2500	5000	2500
A. sativa	_d)	_	-	nd ^{e)}	-	-
C. lachryma-jobi	+	-	-	nd	-	-
var. mayuen						
E. sibiricus	-	-	-	nd	-	-
F. esculentum	-	-	++++	+++	-	-
H. vulgare	-	-	-	nd	-	-
var. hexastichon						
Hordeum vulgare	+++	++	-	nd	-	-
var. nudum						
I. crassipes	-	-	-	nd	+++	+++
O. sativa	. +++	++	-	nd	++++	+++
var. glutinosa						
O. sativa	-	-	-	nd	-	-
var. Japonica						
P. miliaceum	-	-	-	nd	++++	++++
P. bisulcatum	-	-	-	nd	++	+
P. frutescens		-	-	nd	++	++
var. japonica						
S. chinensis	-	-	-	nd	-	-
S. nigra	-	-	-	nd	-	-
S. careale	+++	+	-	nd	++	++
S. indicum(B)	+++	-	-	nd	++	+
S. indicum(W)	+++	-	-	nd	++	+
S. italica	-	-	-	nd	++++	+++
S. bicolor	-	-	-	nd	++++	+++
T. aestivum	+	-	-	nd	+++	+++
Z. mays	-	-	_	nd	++	+

a) All test samples revealed little or no insecticidal activities against Plutella xylostella and Spodoptera litura.

b) BPH, Nilaparvata lugens; GPA, Myzus persicae; and TSSM, Tetranychus urticae.

c) Unit: ppm.

d) ++++, >90%; +++, 80-90%; ++, 61-80%; +, 40-60%; and -, <40%.

e) Not determined.

var. glutinosa, S. careale. S. indicum (B), and S. indicum (W) gave over 80% control values on N. lugens at 5,000 ppm, and, moderate activities were obtained at 2,500 ppm in extracts of H. vulgare var. nudum and O. sativa var. glutinosa. In a test with M. persicae, potent fungicidal activities were produced from extracts of Fagopyrum esculentum when treated at 5,000 and 2,500 ppm, but other test extracts had no insecticidal activities.

When treated with 5,000 and 2,500 ppm, extracts of I. crassipes, O. sativa var. glutinosa, P. miliaceum, S. italica, S. bicolor, and T. aestivum revealed potent insecticidal activities against T. urticae (Table 3). Furthermore, moderate activities were obtained in extracts from Panicum bisulcatum, P. frutescens var. japonica, S. careale. S. indicum (B), S. indicum (W), and Z. mays at 5,000 ppm, whereas these grain extracts had weak activity at 2,500 ppm. The results from P. xylostella and S. litura showed that all test grain extracts had weak or no insecticidal activity when treated at 5,000 and 2,500 ppm.

Discussion

In the greenhouse studies with methanol extracts from 21 grains belonging to the family Graminales, Magnoliaceae, Pedalidaceae, Labiatae, Polygonaceae, many of them showed potent fungicidal and insecticidal activity against the economically important phytopathogenic fungi and insect pests. Fungicidal and insecticidal activity varied with both the grain species and pathogen/insect tested. In a test with phytopathogenic fungi, BPM, CGM, RCB, and WLR were inhibited more effectively by application of methanol extracts of various grains than RSB and TLB, and, when tested with five insect pests, BPH and TSSM were controlled more effectively by the application of methanol extracts of various grains than DBM, GPA and TCW. Jacobson (1989) pointed out that the most promising botanicals as sources of novel plant-based pesticides 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, and 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 alternatives for synthetic pesticides (Hostettman and Potterat, 1997; Hedin, 1982), or lead compounds for new classes of synthetic pesticides such as podoblastin produced from Podophyllum peltatum (Miyakado, 1986; Hostettman and Potterat, 1997). However, little information is available for pesticidal activity of grain plants. Lee et al. (1998) already reported that leguminous seed extracts have fungicidal activity against phytopathogenic fungi.

In this study, 12 grain extracts showed significant fungicidal activity (>80% CV) against P. grisea, P. recondita and E. graminis, although nearly most of all test samples were ineffective to B. cinerea, R. solani and P. infestans. Furthermore, 6 extracts showed significant insecticidal activity (>80% CV) against N. lugens and T. urticae. Especially, the strong activities of E. sibiricus, H. vulgare var. nudum, P. miliaceum, P. frutescens var. japonica, and T. aestivum against P. grisea, P. recondita, and E. graminis, and I. crassipes, O. sativa var. glutinosa, P. miliaceum, S. italica, S. bicolor, and T. aestivum against N. lugens and T. urticae confirm their superiority and usefulness as a potent fungicide and insecticide. These grains may give a new clue for managing these plant pathogens and insect pests in field ecosystem, although their effects on non-target organisms or environment remain unknown. It has been reported that many of 25 leguminous seed extracts used were very effective against P. recondita and E. graminis but exhibited no fungicidal activity against B. cinerea, R. solani, and P. infestans (Lee et al., 1998). These results indicate that biologically active components between grains and leguminous seeds tested may be similar.

In conclusion, the grain-derived materials may be useful for developing new types of fungicides, insecticides, and biorational management agents for controlling plant pathogens and insect pests on crops at same time, although their effects on natural enemies, vegetable qualities, or environment has not been fully clarified.

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다양한 잡곡 추출물의 살균 · 살충활성

이회선 * , 최경자 1 , 조광연 1 , 이상길 2 , 안용준 3 (전북대학교 응용생물공학부, 1 한국화학연구소, 2 임업연구원, 3 서울대학교 응용생물화학부)

요약: 21종 잡곡류의 메탄올 추출물을 대상으로 기주식물상의 온실실험조건에서 6종의 주요 식물병원균의 방제효과 및 5종의 해충에 대한 살충효과를 조사한 효과는 잡곡류의 종류에 따라 커다란 차이를 보였다. 21종의 공시시료를 5 mg/pot 농도로 처리하였을 때 개보리 (E. sibiricus)와 늘보리 (H. vulgare var. nudum)는 벼도열병 및 보리흰가루병, 참깨 (S. indicum (W))와 밀 (T. aestivum)은 밀녹병과 보리흰가루병에 대하여 80% 이상의 방제효과를 나타냈다. 5종의 주요해충에 대한 살충효과는 2,500 ppm의 농도로 처리할 때 메밀 (F. esculentum)은 복숭아혹진딧물, 쇠보리 (I. crassipes), (O. sativa var. glutinosa), 기장 (P. miliaceum), 조 (S. italica), 수수 (S. bicolor) 및 밀 (T. aestivum)은 점박이용에에 대하여 80% 이상의 살충효과를 나타냈다. 그러나, 실험에 사용된 잡곡류는 토마토역병, 배추좀나방 및 담배거세미나방에 대해서는 거의 활성이 없거나 활성이 낮았다. 이상의 결과로부터 벼도열병, 밀녹병 및 보리흰가루병 등에 높은 방제효과를 보인 상기 잡곡 추출물들은 식물병 방제제로서 사용 가능성이 예상되었으며, 또한 복숭아혹진딧물 및 점박이용에에 강한 살충효과를 보인 추출물은 해충방제에 이용할 수 있을 것으로 기대되었다.

*연락저자(Fax: +82-63-270-2550, E-mail: hoiseon@moak.chonbuk.ac.kr)