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Allelopathy of Rice Husk on Barnyardgrass

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Abstract: Research efforts in seeking allelopathic compounds play an important role in developing natural herbicides. The objective of this research was to investigate the allelopathic effects of rice (*Oryza sativa* L.) husk extracts and to bioassay allelopathic compounds. The husk extracts of seven rice varieties were used to examine allelopathic effects on the growth of barnyardgrass (*Echinochloa crusgalli* (L.) Beauv.). After that, allelopathic substances effective on the growth of barnyardgrass were sought using an open chromatography and analyzed using a GC/MS. Husk extract from Ilpum rice (a national variety in South Korea) showed the prominent allelopathic effect on barnyardgrass. The organic compounds analyzed by the GC/MS were found to be 9-octadecenoic acid; 7-octadecenoic acid; 5, 8, 11-heptadecatriynoic acid and androstan-17-one. The minimum inhibition concentration of the isolated allelochemical compounds was 50 ppm. The results suggest that there is a possibility of developing a rice husk oriented natural herbicide effective on barnyardgrass.

Key words: Allelochemical compound, germination, natural herbicide

INTRODUCTION

Barnyardgrass is one of the most problematic weeds in rice farming. Even though it can be controlled by many herbicides, overusing these herbicides causes environmental problems. This concern makes it necessary to develop alternative weed control methods. One of these would be allelopathy defined as the biochemical interrelationship between plants including microorganisms by Molisch[1]. This phenomenon influences crop growth and production due to inhibition mainly by accumulating biochemical substances in the soil from continuous cultivation and by causing changes of the plant ecosystems[2,3].

The major ways which plants secrete or emit allelochemicals to the environment are as follows: 1) plant residue itself or emission separated by microorganisms; 2) emission of water-soluble substances from plant root into the surrounding environments; 3) secretion of water soluble substances from plants above ground to the soil by rain, mist and/or dew; and 4) emission of gas substances from plant above ground to the atmosphere[4]. Allelopathic substances are involved in secondary reaction in plants and synthesized broadly without any relationship to plant growth and development. The substances found in plants are alkaloids, flavonoids, phenolics, terpenoids, lactones, tannins, cyanogenic glucosides, quinones, polyacetylenes, coumarins, sulfides and plant organic acids[5-7]. Allelopathic substances have been reported in many plants such as rye, oat, wheat, sorghum, alfalfa, tobacco, pine tree and wormwood[8-15].

After the Dilday research team initiated and confirmed the potential allelopathic effect of rice[16-18], this phenomenon has been a subject of continued research for a decade[19,20]. Most allelochemicals reported in rice were phenolic compounds and were found in leaf, straw, stem and rice soil rather than husk[21-24]. In addition, Chung *et al.*[25] reported that water-soluble husk extracts inhibited the germination of barnyardgrass stronger than leaf extracts. The research milestones of rice allelopathy described by Olofsdotter[20] are as follows: 1) there is large variation in allelopathy among rice cultivars; 2) allelopathy plays a role under field conditions; 3) allelopathic rice can suppress both monocot and dicot weed species; 4) progress has been made in identifying rice allelochemicals; 5) quantitative trait loci correlated

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with allelopathy have been identified. As an endeavor to identify allelochemicals, this research was intended to investigate the allelopathic effect of some rice husk extracts on the growth of barnyardgrass and to bioassay the allelopathic compounds from the rice husk.

MATERIALS AND METHODS

Extraction of rice husk: The rice varieties used were Sanghai, Black-Pearl, Ilpum, Seoan, Jangan, Red-rice and Hyangmi, which were produced at Kangwon Agricultural Research and Extension Services in South Korea in 1996. These varieties were chosen from the regional representative varieties. Five hundred grams of each rice husk were filled in 2,000 mL Erlenmeyer flasks. The rice husks in each flask were soaked using 1,000 mL of 70% methanol (MeOH) solution and kept at room temperature (25°C). After 48 h, each extraction from the rice husks dissolved in MeOH was concentrated in a vacuum at 40°C and 70 atm. Six grams of concentrated extract from each rice husk were obtained.

Separation of allelopathic compounds: The Ilpum rice husk was chosen to bioassay allelopathic compounds because it showed the strongest inhibition on the germination and early growth of barnyardgrass. Ilpum rice husk extract was separated using three different solvents such as *n*-Hexane, Ethyl acetate (EtOAc) and n-Butyl alcohol (BuOH) (Fig. 1). An appropriate solvent condition was determined using an aluminum TLC (Kieselgel 60F254, Merk Co.). It was observed that the best solvent condition was n-Hexane 75%: EtOAc 25%. A silica gel column chromatography was performed according to the condition.

The silica gel column chromatography procedure is as follows. The concentrated n-Hexane fraction was absorbed to silica gel (7734, Merk Co.) with the same amount to the fraction. One hundred twenty grams of silica gel were put in the n-Hexane solution and then filled up in an open column (70 cm in length and 5.5 cm in diameter). The silica gel absorbed n-Hexane fraction was then put on top of the open silica gel column. After that, a column chromatography was done with nine different fractions. The conditions of each fraction are presented in Table 1. Each fraction was distributed at 500 mL and concentrated in a vacuum.

GC/MS analysis: The fraction which shows allelopathic effect was analyzed using GC/MS (HP 5890 II and VG Trio-2000MS) to identify the compounds. The instrument conditions were as follows. The column was Rfx-5 (30 m×0.25 mm id×0.25 μm df). The injector temperature was 250°C. Oven temperatures were as follows; initial temp 40°C; initial time 4 min.; rate 120°C min^{-1}; final temp 260°C; final time 10 min. The carrier gas (He) flow rate was 1.0 mL min^{-1}. The ion energy was 70 eV. The source temperature was 250°C. The scan range was 45 to 465.

Table 1: Fraction conditions for silica gel column chromatography and the concentrated amounts of each fraction

Fraction No.	Condition	Concentrated amount (g)
F_1	100% n-Hexane	0.149
F_2	90% n-Hexane to 10% EtOAc	0.709
F_3	80% n-Hexane to 20% EtOAc	0.650
F_4	60% n-Hexane to 40% EtOAc	0.897
F_5	20% n-Hexane to 80% EtOAc	0.133
F_6	100% EtOAc	0.150
F_7	90% EtOAc to 10% MeOH	0.587
F_8	50% EtOAc to 50% MeOH	0.137
F_9	100% MeOH	0.054

Experiment of growth inhibition: The inhibitory activity of rice husk extraction and its column fractions were observed using a 24 well plate assay (Fig. 2). Samples were dissolved in MeOH and prepared at different concentrations. Each MeOH-dissolved sample and MeOH only control solution were put in the wells with four replications. MeOH in the wells was completely dried out and barnyardgrass seeds (10 seeds $well^{-1}$) were placed on the filter paper of the bottom of the wells. The plate was sealed with parafilm and placed in a growth chamber (25°C with a 16 h photoperiod at 400 μM m^{-2} s^{-1} photosynthetically active radiation). After 5 days, germination rates and seedling growths were examined. Statistical analysis of mean separations (Least Significant Difference and Duncan's Multiple Range Test at the α=0.05 level) was performed using SAS software (SAS version 8.1, SAS Institute Inc. Cary, North Carolina).

RESULTS

Growth inhibition of rice husk extracts on barnyardgrass: Ilpum rice husk extract shows strong inhibition of germination of barnyardgrass followed by Jangan and Seoan husk extracts when the extract concentrations increased (Table 2). The strongest inhibition in shoot growth was shown in the Ilpum rice husk extract (Table 3). Radicle growth inhibitions were same as shoot growth inhibitions (data not shown). Because inhibition of germination generally corresponds to shoot and radicle growth inhibitions, only germination data was used in the following process.

Water soluble rice husk extracts were also examined for the growth of barnyardgrass and the strongest germination inhibition was shown in Jangan husk extract (data not shown). However, because of the lower inhibitory effect of the water soluble rice husk extracts

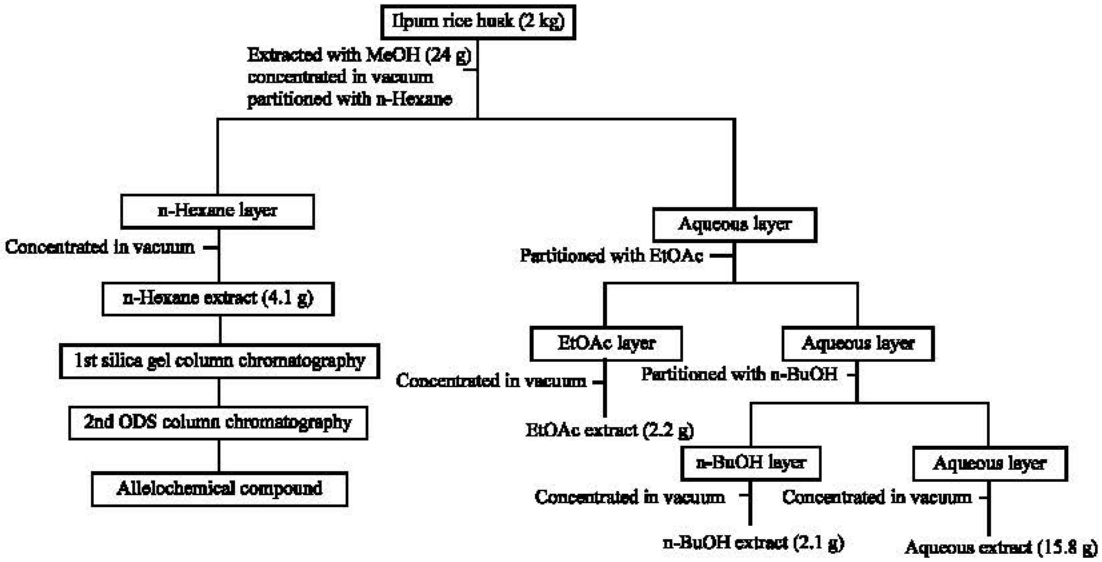

Fig. 1: Diagram of isolating allelopathic compounds partitioned with the different solvents and the chromatographic methods

Table 2: Germinations of barnyardgrass as a function of the different varieties of rice husk MeOH extract at different concentrations

	MeOH extract concentration (μL mL^{-1})			
Varieties	625	1,250	2,500	5,000
	Germination (%)			
Hyangmi	90.0a	60.0cd	70.0b	40.0cd
Ilpum	80.0a	15.0e	0.0d	0.0e
Jangan	85.0a	75.0abc	75.0b	15.0de
Red rice	85.0a	85.0ab	75.0b	60.0bc
Black-pearl	80.0a	90.0a	65.0b	70.0ab
Sanghae	90.0a	55.0d	80.0ab	20.0de
Seoan	90.0a	67.7bcd	65.0b	17.7de
Control	93.3a	93.3a	93.3a	93.3a
LSD (α= 0.05)	14.3	17.3	14.5	25.5

Letter following numbers are DMRT (α= 0.05)

Table 3: Shoot weights of barnyardgrass as a function of the different varieties of rice husk MeOH extract at different concentrations

	MeOH extract concentration (μL mL^{-1})			
Varieties	625	1,250	2,500	5,000
	Shoot weights (mg plant^{-1})			
Hyangmi	1.4c	0.3cd	0.0c	0.0c
Ilpum	0.0d	0.0d	0.0c	0.0c
Jangan	2.3abc	0.7bc	0.0c	0.0c
Red rice	3.1a	2.2a	2.2b	0.8b
Black-pearl	2.2abc	1.0b	0.0c	0.0c
Sanghae	2.2abc	0.0d	0.0c	0.0c
Seoan	1.8bc	0.4cd	0.0c	0.0c
Control	2.8ab	2.8a	2.8a	2.8a
LSD (α= 0.05)	0.9	0.6	0.4	0.4

Letter following numbers are DMRT (α=0.05)

compared to the MeOH ones, those were not considered more in this study.

Bioassay of allelopathic compounds: According to the previous results in this study, the Ilpum rice husk was chosen to bioassay allelopathic substances. The Ilpum rice husk was partitioned with the different organic solvents (Fig. 1) and then the partitioned extracts were used to examine the germination of barnyardgrass. The n-Hexane extract showed the strongest inhibition on the germination of barnyardgrass (Table 4).

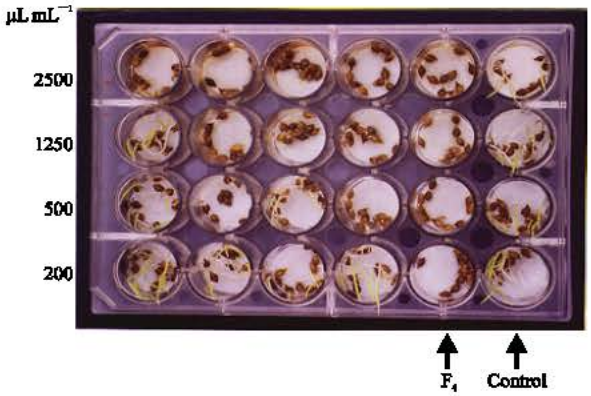

Fig. 2: Twenty four well plate assay showing germination inhibitions on barnyardgrass in the fraction 4 (F_4) at different concentrations

The n-Hexane extract was then partitioned using silica gel column chromatography based on the TLC condition that had been tested to seek an optimum separation condition of the *n*-Hexane extract. After that, the partitioned extracts were used to test the germination inhibition of barnyardgrass. As shown in Table 5 and Fig. 2, the fraction 4 (F_4) showed the strongest inhibition.

A GC/MS analysis was performed for the F_4 to bioassay the allelochemical compounds, identifying four compounds. Each of these was found to be

Table 4: Germinations of barnyardgrass as a function of the partitioned extracts of the Ilpum rice husk using the solvents at different concentrations

	Extract concentration (μL mL^{-1})			
Solvent used	625	1,250	2,500	5,000
	Germination (%)			
Hexane	15.0c	0.0c	0.0d	0.0c
Ethyl acetate	75.0b	65.0b	35.0c	0.0c
BuOH	70.0b	85.0a	90.0a	70.0b
Water 1	80.0ab	85.0a	65.0b	85.0a
Water 2	75.0b	80.0ab	75.0ab	80.0ab
Control	93.3a	93.3a	93.3a	93.3a
LSD (α= 0.05)	16.2	15.6	21.0	12.8

Letter following numbers are DMRT (α= 0.05)

Table 5: Germinations of barnyardgrass as a function of the fractions from silica gel chromatography at different concentrations

	Extract concentration (μL mL^{-1})			
Fraction	200	500	1250	2500
	Germination (%)			
Control	73.3ab	76.7b	76.7b	83.3ab
Hexane extract	80.0ab	30.0d	3.3e	0.0 e
F_1	86.7a	96.7a	73.3bc	93.3a
F_2	70.0b	93.3a	96.7a	90.0a
F_3	86.7a	93.3a	76.7b	70.0b
F_4	0.0c	0.0e	0.0e	0.0e
F_5	76.7ab	60.0c	50.0d	20.0d
F_6	70.0b	83.3ab	93.3a	93.3a
F_7	86.7a	96.7a	60.0cd	50.0c
F_8	70.0b	93.3a	76.7b	80.0ab
F_9	66.7b	90.0ab	66.7bc	3.3e
LSD (α= 0.05)	14.1	13.1	13.7	13.3

Letter following numbers are DMRT (α= 0.05)

Table 6: Compounds found in the Ilpum rice husk analyzed by GC/MS

Retention time (min)	Scientific name of compound	Molecular weight (Dalton)
20.59	9-Octadecenoic acid	239
22.10	7-Octadecenoic acid	371
23.90	5,8,11-Heptadecatriynoic acid	256
30.35	Androstan-17-one	314

Table 7: Minimum Inhibition Concentration (MIC) of the allelochemical compounds to barnyardgrass

Treatment	MIC (μL mL^{-1})
Ilpum rice husk crude extract	2,500
Ilpum rice husk allelochemical compounds	50

9-octadecenoic acid; 7-octadecenoic acid; 5, 8, 11-heptadecatriynoic acid; and androstan-17-one (Table 6). The F_4 was further separated using Octadecylsilyl (ODS) column chromatography and the minimum inhibition concentration of the final active compounds in this study was 50 ppm (Table 7).

DISCUSSION

The n-Hexane solvent is the most non-polar one used to partition the rice husk extracts. Non-polar solvents such as n-Hexane are not soluble with water. The n-Hexane is primarily used to remove the oil and then distilled from the oil for reuse[26]. Considering the fact that the analyzed allelochemical compounds were separated from the n-Hexane layer, the following speculation could be possible. Out of four ways of releasing allelochemicals described by Rice[4], those from rice husk could be released to the environment by plant residue itself or emission separated by microorganisms.

In general, allelochemicals from rice reported so far has been known as phenolic compounds[23]. However, Mattice *et al.*[27] reported that some fatty acids such as linoleic, oleic and searic from rice showed allelopathic effect on the ducksalad growth. This research is similar to Mattice *et al.*[27] that compounds found include fatty acids such as 9-octadecenoic and 7-octadecenoic acids, which showed allelopathic effect on the barnyardgrass growth.

Following the silica gel column chromatography, the ODS column chromatography was carried out to separate and identify the active compound from the fraction 4. However, each single compound could not be separated. It remains further endeavor to isolate and bioassay each compound. We believe that the research efforts to search for allelochemicals should result in developing effective weed control techniques and natural herbicides for the future agriculture.

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