Korean J. Plant Res. 31(6):695-703(2018)
https://doi.org/10.7732/kjpr.2018.31.6.695

Print ISSN 1226-3591
Online ISSN 2287-8203

Original Research Article

Anemarrhena asphodeloides Extract Inhibits the Mycelial Growth of *Magnaporthe oryzae* and Controls the Rice Blast Disease

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Abstract - Previously, we have reported a plant extract isolated from *Lysimachia foenum gracum* Herba as a new environment friendly biopesticide that has the mycelial growth inhibition effect on *Magnaporthe oryzae,* the pathogenic fungus of the rice blast disease. For the finding of additional biopesticide candidate, we tested the mycelial growth inhibitory effects about 700 species of plant extracts on PDA media. Among them, the extract of *Anemarrhena asphodeloides* showed prominent inhibitory effect of which IC_{50} was 139.7 ㎍/㎖. Mycelial radii of *M. oryzae* were measured on PDA medium containing the four organic solvent fractions isolated from total extract from *A. asphodeloides*. Ethyl acetate fraction showed the impressive inhibitory effect of IC_{50}, 54.12 ㎍/㎖. In the subsequent rice field test for the total extract of *A. asphodeloides*, we obtained encouraging 62.0% control rate of rice blast disease without any phytotoxicity. It is almost equivalent to that of chemical pesticides implying the applicability of the extract as a new biopesticide. In further study, the analysis of active ingredients of the extract would be necessary for the development of a new biopesticide and for the verification of cellular mechanism by which the mycelial growth of *M. oryzae* inhibited.

Key words − Biopesticide, Control rate, Mycelial inhibition, Phytopathogenic fungi, Plant extract

Introduction

Rice blast disease occurs by filamentous pathogenic fungus of ascomycete, *Magnaporthe oryzae*. Though, rice is the most important crop plant, but the decrease of the rice crop production causes serious problems of food supply and starvations in worldwide regions. The disease is divided into seedling blast, leaf blast, and neck blast that cause the biggest damages to rice itself and crop production (Dean *et al*., 2005; Ribot *et al*., 2008). It occurs in almost all areas of the world including Korea where the rice is domestically cultivated as a crop. Many kinds of chemical pesticides are being used for the control of the disease, but their overuses cause the drastic environmental problems by their persistent toxicity. Consequently, the overuses of chemical pesticide cause expenditures for the disease control, and importantly, the drastic decrease of rice crop production, and environmental pollutions (Couch *et al*., 2005). In present, screening of pesticide resistant varieties of the *M. oryzae* and the improvement of fertilization method are being adopted for the control of rice blast disease, but chemical control by pesticides are being preferred domestically in Korea because of their high control efficiencies for their respective environment (Skamnioti and Gurr, 2009). But it has been gradually known that the efficiency of chemical controls was greatly threatened, and its usages are being restricted by the increase of the harms of environmental toxicity, and the advent of resistant fungal races against various pesticides were caused by pathogenically mutated new races (Chen *et al*., 2005; Koh *et al*., 1986). Those results have been caused by the fact that

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microorganisms including *M. oryzae,* generally have basic capabilities to resist to various natural toxicants, (Dixon *et al*., 1994; Dixon and Lamb, 1990; Cho *et al.*, 2018, Jang *et al.*, 2018) against chemical pesticides are also observed in various pathogenic fungi of rice plant to survive the toxic ecological environments. As for the molecular mechanisms of the pesticide resistances, a mutation or overproduction of target protein, and detoxification of treated pesticides are reported so far (Gulshan and Moye-Rowley, 2007). Recently, ABC (ATP Binding Cassette) transporter family proteins of plant pathogenic fungi are reported to be involved in multi-drug resistance (MDR) mechanism by which chemically unrelated pesticides or compounds secreted from plant cell out of their cells are effluxed for the defense of themselves (Davidson *et al.*, 2008; Di Pietro *et al.*, 1999; Li and Wang, 2006; Zolnerciks *et al.*, 2011). In the case of *M. oryzae*, about forty kinds of ABC transporters genes are known to be existed in its genome which was firstly revealed complete nucleotide sequences of genome in plant pathogens and are estimated to exist 11,109 genes (Dean *et al*., 2001; Jones and George, 2004), so the *M. oryzae* is being recognized as the model of molecular researches of plant pathogens. In our previous reports, ABC transporter, *ABC2*, of *M. oryzae* are involved in pesticide resistance by the pesticide efflux mechanism (Lee *et al*., 2005), and also we recently proved that the plant extract of *Lysimachai foenum gracum* Herba inhibit the mycelial growth of *M. oryzae,* and it caused decrease of ABC transporter genes expression of *M. oryzae.* Furthermore, when the extract is combinationally treated to mycelia of *M. oryzae* with blasticidin S, a pesticide for rice blast disease, IC_{50} of blasticidin S was dramatically decreased and the decrease of ABC transporter gene expression was also simultaneously accompanied (Lee, 2016). From those results, we found that the plant extract can increase the sensitivity of *M. oryzae* for blasticidin S through the inhibition of ABC transporter expressions of *M. oryzae*. There are also early supporting reports that antifungal activity of *L. foenum gracum* Herba inhibits the mycelial growth of *M. oryzae* and *Botrytis cinerea* (Iida *et al*., 1999; Park *et al*., 2003). Based on our previous reports and our preliminary experimental result for the extracts of *L. foenum gracum* Herba, we concluded that unknown active ingredients of *L. foenum gracum* Herba could contributed to the inhibitory mycelial growth of *M. oryzae.* Likewise, it is noticeable to the enormous variety of plant extracts that might have abilities of overcoming pesticide resistance of plant pathogens like *M. oryzae*.

In this study, we focused on the enormous diversity of plant extract from various species, so additionally screened 700 kinds of plant extracts on PDA (Potato Dextrose Agar) medium with high dose (500 ㎍/㎖) of plant extracts from various species. To determine the mycelial growth inhibitory effects of each plant extract on *M. oryzae*, we measured their mycelial radii after 5 day incubations after their treatments. Among them, the extract of *A. asphodeloides* showed the highest inhibitory effect. Fractionation experiment was also performed for the extract to determine organic solvent fraction containing putative active ingredients concerned with mycelial growth inhibitory effect. Finally, we certified the control rate for the rice blast disease in rice crop field test to verify the field adaptability of *A. asphodeloides* extract that could be a new candidate of biopesticide for the control of rice blast disease. In this study, we are sure that our experimental data can provide the possibility of plant extract of the diverse species as being a new material for the development of a new biopesticides.

Materials and Methods

Strains and culture conditions

M. oryzae strain *KJ201* was distributed by KNRRC (Korea National Research Resource Center, Seoul national university) and was cultured on potato dextrose agar (PDA, Potato dextrose broth (Acumedia) 24 g, agar 1.5 g, per liter) and incubated at 28℃ incubator (Wooshin. 9109). All the pesticides and plant extracts were treated 3 hrs earlier in PDA.

Plant extracts and chemicals (toxicants)

About 700 kinds of plant extracts were supplied from Plant Extract Bank in Korea by dry weight of 20 ㎎ (Table 1). The total extract and solvent fractions was prepared from the leaves of *A. asphodeloides*.

Mass screening of plant extracts

1) Test of mycelial growth inhibition

The mycelial growth inhibition for *M. oryzae* was tested on PDA media in 24 well plates (SPL Life Science) containing each of plant extracts. To determine the mycelial growth inhibitory effect of 700 kinds of plant extracts, Mycelia of *M. oryzae* were roundly cut by Cork borer (KOREA Ace, 5 ㎜) and were placed on center of PDA media amended by 500 ㎍/㎖ of plant extracts and incubated for five days at 28℃ incubator (DAEHAN LABTECH. LSI-3016R).

2) IC_{50} determination of total extract

Plant extracts showing below 0.3 ㎝ of mycelial radii on PDA media were selected for further determination of IC_{50} (50% inhibition concentration). IC_{50} was calculated by methods of Valgas *et al.* (2007) and Magaldia *et al.* (2004) and the value of mycelial radii measured on PDA media amended by *A. asphodeloides* extracts or other pesticides were used for the IC_{50} determination by five day incubations at 28℃ incubator. All tests were performed in triplicate.

Fractionation of plant extract

1) Isolation of total extract of *A. asphodeloides*

1,000 g of completely dried leaves of *A. asphodeloides* was grinded by grinder (HMF-340, Hanil, Korea) and was mixed with absolute methanol (HPLC grade, Burdick & Jackson) with changing the solvent three times through the incubation at 50℃ for 24 hrs to extract the plant constituents completely. Methanol extract was filtrated by Whatmman, No. 2 filter and was vaporized by Rotary evaporator N-1000 (Eyela, Japan) at 45℃ for a week.

2) Preparation of fraction

Methanol concentrates of total extract of *A. asphodeloides* were suspended by 200 ㎖ of sterilized water, and was orderly fractionated by adding equal volume of hexane, chloroform, ethyl acetate and n-butanol and each of them was concentrated by vaporization.

3) Measurement of mycelial growth inhibitory effect of each fractions

Mycelia were placed on PDA containing 10, 20, 50, 100 and 200 ㎍/㎖ of each fractions in six well plates and were incubated for five days. The mycelial growth inhibitory effects were determined by IC_{50} calculated by measured values of mycelial radii. The experiments were performed in triplicate.

Field test of control rate of rice blast disease

Control rate of *A. asphodeloides* extract for rice blast disease was determined by Seounmyeon (Ansung, Gyeonggi-Do, Korea) using rice strain of *Chucheng* strain from the 20th, August to the 19th, September, 2012. Nursery bed test was performed by *A. asphodeloides* extract, diluted by 500 times and foliage was treated in flood season (2012/8/27, 2012/9/3) at intervals of 7 days. Phytotoxicity test were performed on the 27th, August, 2012 by 250 folds and 500 folds of standard amount. The nursery plants were transplanted by machine on the 23th, 26th, May. Plant spacing was 30 x 14 ㎝ and ear emergence was on the 20th, August. Plant extracts were treated by general use of practices but, other pesticide's sprays were banned. The area of test plot of control rate was 30 ㎡, nursery bed test plot, 10 ㎡ and the area of total test plot was 270 ㎡, respectively. Each test was performed in triplicate by randomized block design (RBD). Nursery bed test was investigated on the degree of diseased panicle after 30 days to heading from 30 rice of each plot. Phytotoxic test was performed by disease assessment on after 3, 5, and 7 days (the 30th, August, the 1th, 3th, September) from treatment day of extract of *A. asphodeloides*.

Statistical analysis

ANOVA tests using Graphpad Prism were performed for statistical analysis regarding the measured mycelial radii in comparison to each experimental control (treatment with plant extract, pesticide). All experiments were performed in triplicate. $P < 0.05$ is considered statistically significant. In field study of rice plant, Duncan's multiple range tests (DMRT) using ARM 8 software of Gylling Data Management, INC. were performed for statistical analysis regarding the infected panicle of rice plant in comparison to each experimental control (treatment with plant extract, no treatment). All experiments were performed in triplicate. $P<0.05$ is considered statistically significant.

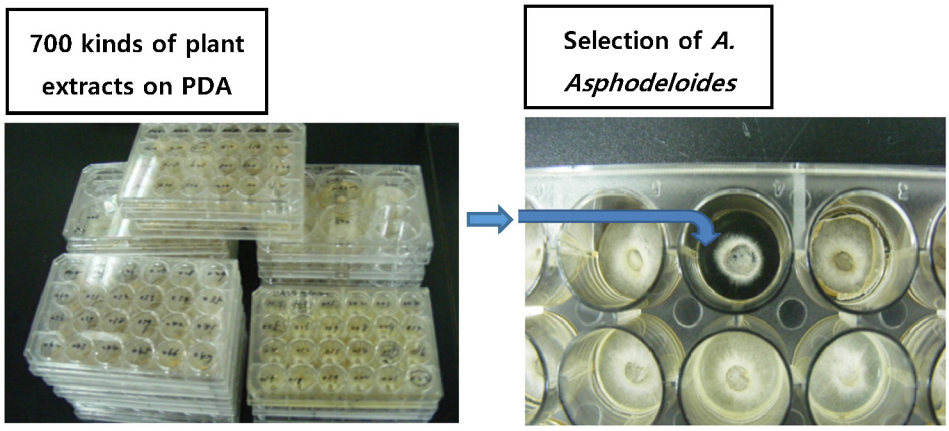


Fig. 1. Massive screening for the mycelial growth inhibitory effects of 700 kinds of plant extracts on *M. oryzae.* Plant extracts provided by plant extract bank (Korea) were tested on PDA solid media in 24 well plates by empirical concentration of 500 ㎍/㎖ of each plant extracts.

Results and Discussion

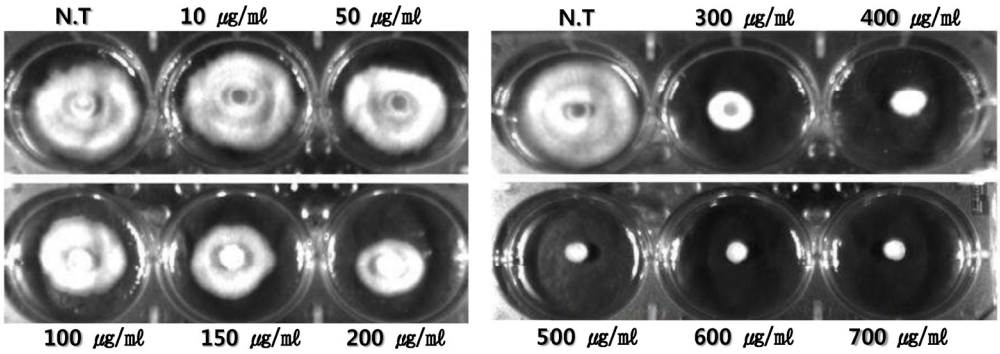
Mycelial growth inhibitory effect of extract of *A. asphodeloides*

We have investigated possibilities that the extracts of numerous plant species have antifungal activities to mycelial growth of *M. oryzae*. As we previously reported the inhibitory effect of *Lysimachia foenum gracum* Herba on the mycelial growth *M. oryzae* (Lee, 2016), likewise in this study, additional 700 kinds of plant extracts (data not shown) were massively tested for investigation of mycelial growth inhibitory effects about *M. oryzae.* Among them, *A. asphodeloides* extract was one of the plant species having the mycelial growth inhibitory effect (Fig. 1). Since the extract showed prominent mycelial growth inhibitory effect on *M. oryzae* (Fig. 2A), we treated the extract to *M. oryzae* on PDA media by following concentrations of none treatment, 10, 50, 100, 150, 200, 300, 400, 500, 600 and 700 ㎍/㎖ in dose dependent manner, and determined the IC_{50}, 139.7 ㎍/㎖ based on the measured values of mycelial radii of *M. oryzae* (Fig. 2B). Comparing the IC_{50} with the chemical pesticide, tricyclazole, being used for the control of rice blast disease, over the concentration of 300 ㎍/㎖ of tricyclazole are needed to reduce the 24% spore growth of *M. oryzae* (Froyd *et al*., 1976). Therefore, the IC_{50}, 139.7 ㎍/㎖ of the total extract is very encouraging value for proceeding further studies because it is needed higher concentration of tricyclazole to inhibit mycelial growth, in this study, than to reduce spore growth in above previous report. From this data, it is possible to suggest that the extract of *A. asphodeloides* might have a possibility of being a new candidate material of substituting the chemical pesticides used for the control of rice blast disease. So we consider that we found another candidate extract of biopesticide after we previously reported for the case of the extract of *L. foenum gracum* Herba that had shown the high control rate for rice blast disease (Lee, 2016).

Fractions of *A. asphodeloides* extract have antifungal activities

From above results, it could be possible to suppose that some specific active ingredients from total extract of leaves of *A. asphodeloides* might have antifungal activities against *M. oryzae*. So, we prepared fractions of four organic solvents for the total extract, hexan, n-butanol, ethyl acetate, chloroform (Fig. 3) and treated the fractions to mycelia of *M. oryzae*, respectively, by the concentration of 10, 20, 50, 100, and 200 ㎍/㎖ in dose dependent manner. Among those fractions, ethyl acetate fraction showed most efficient growth inhibitory effects by IC_{50}, 54.12 ㎍/㎖, lower than IC_{50}, 139.7 ㎍/㎖ of total extract *A. asphodeloides*. (Fig. 4A, 4B). Remarkably, the IC_{50} value of ethyl acetate is near the level of the IC_{50}, 54.25 ㎍/㎖, of isoprothiolane, a conventional pesticide being used for the control of rice blast disease (data not shown). So, it would be needed to compare the IC_{50} of

(A)



(B)

Extract conc. (㎍/㎖)	N.T	10	50	100	150	200	300	400	500	600	700	IC_{50}
Radius (㎝)	1.5	1.3	1.1	1	0.9	0.7	0.6	0.4	0.3	0.3	0.3	139.7 ㎍/㎖

Fig. 2. Mycelial growth inhibitory effect of extract of *A. asphodeloides* on *M. oryzae* in dose dependent treatment. (A) None treatment. Extract was treated by the concentrations of 10, 50, 100, 150, 200, 300, 400, 500, 600, and 700 ㎍/㎖, respectively. Mycelia of *M. oryzae* cut by cork borer were incubated at 28℃ on the PDA solid media for 5 days. (B) The diameters or radii were determined by mean value of individual measured values. Those experiments were performed in triplicate (p<0.05, Anova test).

ethyl acetate with other conventional pesticides for rice blast disease, presumably supposing that they are comparable enough one another. These results suggest that active ingredients are highly concentrated in ethyl acetate fraction than in total extract, and became possible to explain why the total extract has higher IC_{50} than that of ethyl acetate. Furthermore, compared to IC_{50}, 139.7 ㎍/㎖ (Fig. 2B), of total extract of *A. asphodeloides*, the fractions of hexan and chloroform also showed efficiently low enough IC_{50} of 91.08 and 90.49 ㎍/㎖, respectively, though not to the extent of ethyl acetate fraction. So it is furtherly needed to investigate the possibility of existence of active ingredients in those fractions in the future study. From these results, we could presumably consider that some active ingredients involved in the mycelial inhibitory effects were mainly existed in ethyl acetate fraction, but are also distributed in other fractions.

Control effect for rice blast disease of the extract of *A. asphodeloides*

Since we have studied the mycelial growth inhibitory effect of total extract of *A. asphodeloides* on *M. oryzae*, subsequently, we performed the examination for the control rate of total extract *A. asphodeloides* on rice blast disease in the field of rice cultivation. The completion method was used and the evaluation of standard was performed by comparison to none test port of over 15% of rate of diseased panicle. The rice cultivar. of *Chucheong* strain was used for its high sensitivity for rice blast disease and two volumes of nitrogenous manure was used more than conventional culture to enhance the occurrence of rice blast disease. Compared to an untreated control plot, we identified 62.0% control rate against rice blast disease (Fig. 5, Table 1). This control rate was confirmed to be statistically significant and the damages to sclerophyll of rice plant was in the standard range and the drainage amount were not found (Fig. 5, Table 2). From these result that total extract *A. asphodeloides* have very high control rate for the rice blast disease equivalent to that of chemical pesticide for the disease. It could have shown more obvious control rate (>70%) if the total extract were spread before the disease occurrence. It is very meaningful that the extract showed high antifungal activity against *M. oryzae* in both of the laboratory and crop fields conditions, and we anticipate that ethyl acetate fraction would show higher control rate than that of the total extract of *A. asphodeloides.*

In conclusion, we found that the total extract of *A. asphodeloides* has the mycelial growth inhibitory effect on *M. oryzae*. So the four different fractions of organic solvents were isolated from the total extract, and identified that ethyl acetate fraction has the highest growth inhibition effect, almost equivalent to that of chemical pesticides. The control rate of the total extract was remarkably, 62.0%, equivalent to that of chemical pesticides, in the rice field for rice blast

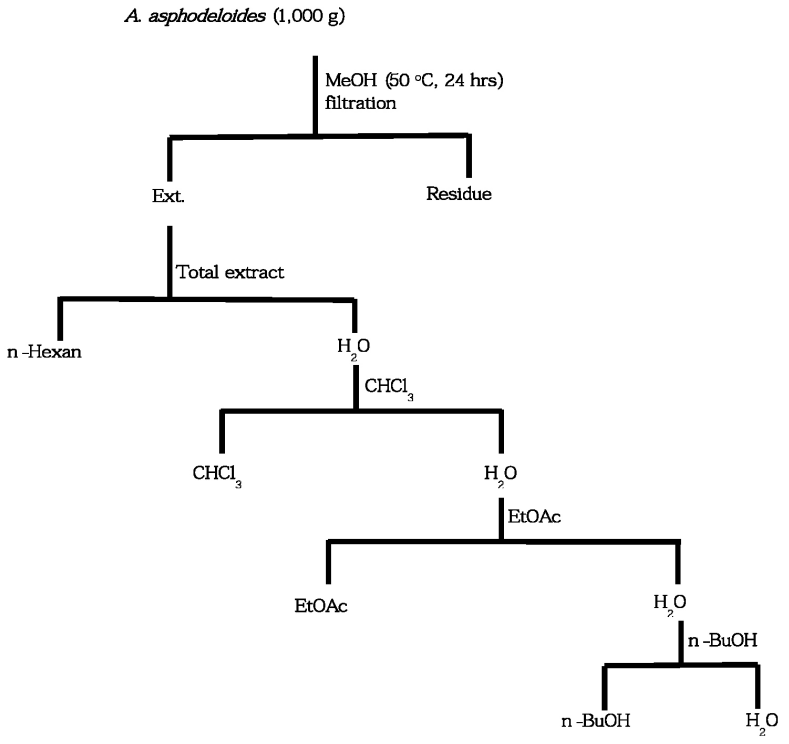
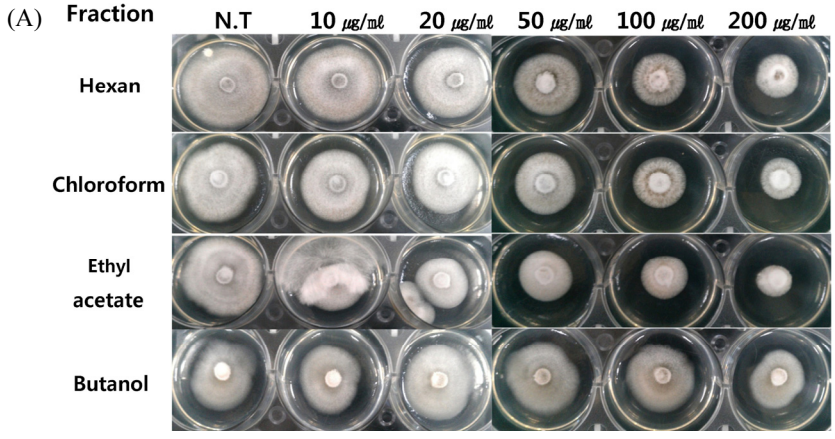


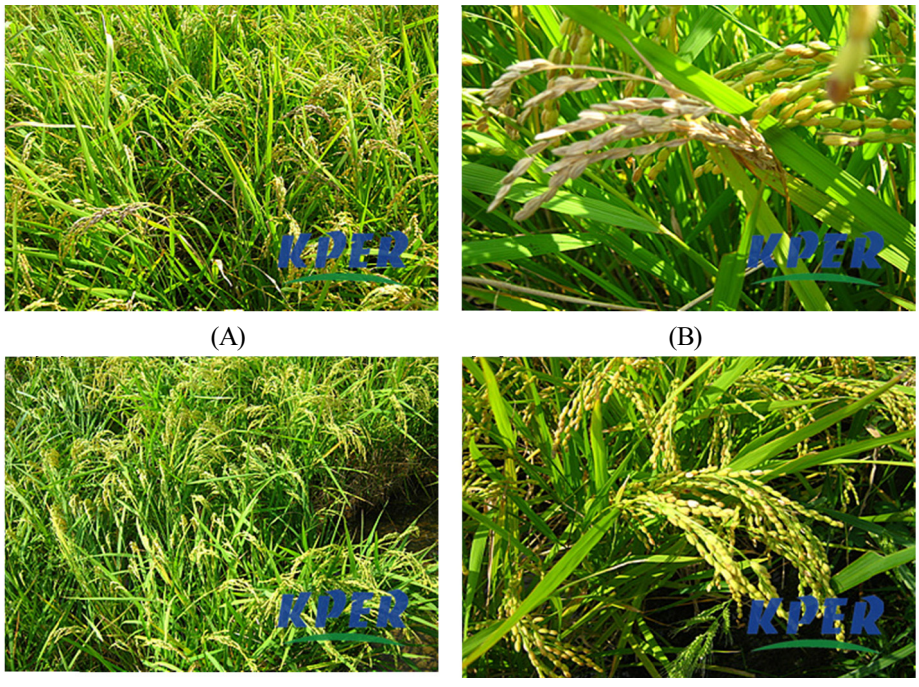
Fig. 3. Fractionation of methanol total extract of *A. Asphodeloides* extract. Each fraction was prepared by orderly treatment of n-Hexan, chloroform, ethyl acetate and n-butanol and were vaporized for the treatment of mycelial growth inhibitory test.



(B) * Radii of mycelium treated by each fraction

Fraction Radius (㎝) / Conc. (㎍)	N.T	10	20	50	100	200	IC_{50} (㎍/㎖)
Hexan	1	1.0	0.8	0.6	0.5	0.4	91.08
Chloroform	1.5	1.8	1.3	1.1	0.9	0.8	90.49
Ethyl acetate	1.5	1.2	1.0	0.9	0.8	0.6	54.12
Butanol	1.8	1.5	1.5	1.3	1.4	1.3	310.7

Fig. 4. Mycelial growth inhibitory effect of fraction. (A) Hexane, Chloroform, Ethyl acetate, and n-butanol fraction were tested by the concentration of 200 ㎍/㎖ of each fraction. (B) Mycelial radii of *M. oryzae* were measured on the PDA solid media for 5 days after the treatment of ethyl acetate fraction. Those experiments were performed in triplicate ($p<0.05$, Anova test).



(A) (B)

(C) (D)

Fig. 5. View of test port of rice blast disease field test of rice plant, *Chucheng*. (A) whole view of testport of none treatment, (B) Symptoms of rice blast disease in none treatment test port by *M. oryzae*, (C) Whole view of treatment testport, (D) Test port of *A. asphodeloides* extract treatment. Those experiments were performed in triplicate.

Table 1. Effect of *A. asphodeloides* extract on control rate of rice in field test

Materials	Rate of diseased panicle (%)				Significant Difference (DMRT)	Control value (%)
	Replicated plot I	Replicated plot II	Replicated plot III	Average		
A. asphodeloides	8.8	8.7	9.6	9.0	a	62.0
None Treatment	23.1	22.2	25.8	23.7	b	-
C. V.(%) ---------- 6.0.						

Table 2. Effect of *A. asphodeloides* extract on phytotoxicity of rice in field test

Materials	Testing varieties (Strain)	Phytotoxicity (0~5)		Note
		Standard amount	Silution	
A. asphodeloides	Rice (*Chucheng*)	0	0	No Phytotoxicity

disease. Finally, it is very meaningful that the extract of *A. asphodeloides* has high field applicability maintaining high antifungal activity against *M. oryzae* both of tests, of laboratory and field. Further research would be needed to find active ingredients having antifungal activity leading to the development of new biopesticide and also to verify the cellular mechanisms including the MDR efflux of ABC transporters (Lee *et al.,* 2005; Lee, 2016) concerned with the mode actions between pesticides and the active ingredients of plant extracts.

Acknowledgement

Mass screening of plant extract were performed by Pil Son, Choi, Professor of Department of Oriental Pharmaceutical Development, The Nambu University, Gwangju 62271,

Korea, and were assisted by Yu Mi, Yeo, Researcher of Department of Agricultural Biotechnology, National Institute of Agricultural Science, Rural Development Administration, Jeonju 54874, Korea. All the study was guided by Kwang Yeol, Yang, Professor of Department of Plant Biotechnology, Chonnam National University, Gwangju 61186, Korea. The preparations of total extract and organic solvent fractions of *A. asphodeloides* were performed by Jae Hyeok, Lee, Professor of Department of Oriental Pharmaceutical Development, The Nambu University, Gwangju 62271, Korea.

References

Chen, S., L. Wang, Z. Que and R. Pan. 2005. Genetic and physical mapping of Pi37(t), a new gene conferring resistance to rice blast in the famous cultivar St. No. 1. Theor. Appl. Genet. 111(8):1563-1570.

Couch, B.C., I. Fudal, M.H. Lebrun, D. Tharreau, B. Valent, P. van Kim, J.L. Notteghem and L.M. Kohn. 2005. Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. Genetics 170(2):613-630.

Cho, J.S., L.W. Chi, B.K. Jang, H.S. Jeong and C.H. Lee. 2018. Anti-microbial activities of ten Lauraceae species against *Propionibacterium acnes*. Korean J. Plant Res. 31(5):423-432.

Davidson, A.L., E. Dassa, C. Orelle and J. Chen. 2008. Structure, function, and evolution of bacterial ATP-binding cassette systems. Microbiol. Mol. Biol. Rev. 72(2):317-364.

Dean, M., A. Rzhetsky and R. Allikmets. 2001. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res. 11(7):1156-1166.

Dean, R.A., N.J. Talbot, D.J. Ebbole, M.L. Farman, T.K. Mitchell, M.J. Orbach, M. Thon, R. Kulkarni, J.R. Xu, H. Pan, N.D. Read, Y.H. Lee, I. Carbone, D. Brown, Y.Y. Oh, N. Donofrio, J.S. Jeong, D.M. Soanes, S. Djonovic, E. Kolomiets, C. Rehmeyer, W.Li,M. Harding, S. Kim, M.H. Lebrun, H. Bohnert, S. Coughlan, J. Butler, S. Calvo, L.J. Ma, R. Nicol, S. Purcell, C. Nusbaum, J.E. Galagan and B.W. Birren. 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. Nature 434(7036):980-986.

Di Pietro, A., G. Dayan, G. Conseil, E. Steinfels, T. Krell, D. Trompier, H. Baubichon-Cortay and J. Jault. 1999. P-glycoprotein-mediated resistance to chemotherapy in cancer cells: using recombinant cytosolic domains to establish structure-function relationships. Braz. J. Med. Biol. Res. 32(8):925-939.

Dixon, R.A., M.J. Harrison and C.J. Lamb. 1994. Early events in the activation of plant defense responses. Annu. Rev. Phytopathol. 32(1):479-501.

Dixon, R.A. and C.J. Lamb. 1990. Molecular communication in interactions between plants and microbial pathogens. Annu. Rev. Phytopathol. 41(1):339-367.

Froyd, J.D., C.J. Paget, L.R. Guse, B.A. Dreikorn and J.L. Pafford. 1976. Tricyclazole: a new systemic fungicide for control of *Pyricularia oryzae* on rice. Phytopathology 66(1):135-131.

Gulshan, K. and W.S. Moye-Rowley. 2007. Multidrug resistance in fungi. Eukaryot. Cell 6(11):1933-1942.

Iida, Y., K.B. Oh, M. Saito, H. Matsuoka, H. Kurata, M. Natsume and H. Abe. 1999. Detection of antifungal activity in *Anemarrhena asphodeloides* by sensitive BCT method and isolation of its active compound. J. Agric. Food Chem. 47(2):584-587.

Jones, P.M. and A.M. George. 2004. The ABC transporter structure and mechanism: perspectives on recent research. Cell Mol. Life Sci. 61(6):682-699.

Jang B.K., L.W. Chi, J.S. Cho and C.H. Lee. 2018. Antimicrobial activity screening of sixty-four evergreen woody species according to extraction conditions against *Trichophyton mentagrophytes.* Korean J. Plant Res. 31(4):330-341.

Koh, Y.J., B.K. Hwang and H.S. Chung. 1986. Screening of rice cultivars for adult-plant resistance to *Pyricularia oryzae*. Korean J. Plant Pathol. 2(2):69-81.

Lee, Y.J. 2016. *Lysimachia foenum-graecum* Herba extract, a novel biopesticide, inhibits ABC transporter genes and mycelial growth of *Magnaporthe oryzae*. Korean J. Plant Pathol. 32(1):8-15.

_____, K. Yamamoto, H. Hamamoto, R. Nakaune and T. Hibi. 2005. A novel ABC transporter gene *ABC2* involved in multidrug susceptibility but not pathogenicity in rice blast fungus, *Magnaporthe grisea*. Pestic. Biochem. Phys. 81(1):13-23.

Li, G. and Y.Q. Wang. 2006. Progress in the research on the *ABCA* gene family of vertebrates. Yi Chuan. 28(8):1015-1022.

Magaldi, S., S. Mata-Essayag, C. Hartung de Capriles, C. Perez, M.T. Colella, C. Olaizola and Y. Ontiveros. 2004. Well diffusion for antifungal susceptibility testing. Int. J. Infect. Dis. 8(1):39-45.

Park, H.J., J.Y. Lee, S.S. Moon and B.K. Hwang. 2003. Isolation and anti-oomycete activity of nyasol from *Anemarrhena asphodeloides rhizomes*. Phytochemistry 64(5):997-1001.

Ribot, C., J. Hirsch, S. Balzergue, D. Tharreau, J.L. Notteghem, M.H. Lebrun and J.B. Morel. 2008. Susceptibility of rice to the blast fungus, *Magnaporthe grisea*. Plant Physiol. 165(1):114-124.

Skamnioti, P. and S.J. Gurr. 2009. Against the grain: safe-guarding rice from rice blast disease. Trends Biotechnol. 27(3):141-150.

Valgas, C., S.M.d. Souza, E.F. Smânia and A. Smânia Jr. 2007. Screening methods to determine antibacterial activity of natural products. Braz. J. Microbiol. 38(2):369-380.

Zolnerciks, J.K., E.J. Andress, M. Nicolaou and K.J. Linton. 2011. Structure of ABC transporters. Essays Biochem. 50(1):43-61.

(Received 26 November 2018 ; Revised 18 December 2018 ; Accepted 19 December 2018)