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Impact assessment of sulfonylurea herbicides to the diversity of aquatic plants in paddy farming system of Korea

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Abstract : This study was conducted to evaluate the response of the selected aquatic plants to sulfonylurea(SU)-herbicides, which have been used widely in rice-cultivating areas in Korea, and the possibility of using them as bio-indicator species for biodiversity conservation. The aquatic plants identified in Kangwon province where butachlor have been used extensively for many years were much more varied than those in Chonnam where SU-herbicides have been used extensively for many years. The growth responses of *Monochoria korsakowii, Marsilea quadrifolia* and *Salvinia natans* to sulfonylurea herbicides such as bensulfuron-methyl(BSM) and pyrazosulfuron-ethyl(PSE), were much more sensitive than those to butachlor and molinate. The GR_{50} values of BSM and PSE for *M. korsakowii, M. quadrifolia* and *S. natans* were relatively very low when compared with those of butachlor and molinate. The number of internode and dry weight of *M. quadrifolia* which was exposed to herbicides runoff from rice fields were seriously affected. The acetolactate synthase(ALS) activities of *M. quadrifolia* sensitively inhibited by BSM and PSE were, and I_{50}(Inhibition 50%) were 5.6 and 2.1 nM, respectively.(Received March 28, 2005; accepted September 20, 2005)

Key words : ALS, Aquatic plant, herbicide, *Marsilea quadrifolia, Monochoria korsakowii,* runoff, *Salvinia natans,* sulfonylurea.

INTRODUCTION

Rice is a major food in Korea. In 2003, rice production was about 6 M tons from cultivation in about 1.050 M ha. Rice cultivation technologies has been aimed high grain yield because of the shortage of staple food brought about by high input of fertilizers and chemicals for the last decades. The rapid decline of agricultural labor since the 1980's modernized the pattern of rice cultivation, for example, infant-seedling transplantation, direct seeding, no-tillage cultivation and other technologies led to the reduction of production cost in rice cultivation. However, without herbicides, which has saved a large portion of labor, it is impossible to reduce rice production cost. Furthermore, if it had not been for sulfonylurea(SU) herbicides which have high herbicidal activity with a low application rate and good crop selectivity, the new techniques of rice cultivation would be failed due to weed problems(Park *et al*, 1999 ; Itoh *et a*l, 1999).

For weed management, herbicide mixtures containing bensulfuron-methyl(BSM) and pyrazosulfuron-ethyl(PSE) have been extensively used for 15 years as one-shot-herbicides and they are PSE/molinate, BSM/mefenacet, BSM/esprocarb, and PSE/mefenacet in rice paddies(Park, 1999). The high usage rate of SU-herbicides has been a major factor affecting changes of weed population, shifting them from the annuals weeds to perennials(Park *et al.*, 2002).

The biodiversity in the rural ecosystems may have been threatened by the loss of habitats and water pollution as a result of the various agricultural chemicals used in rice fields(Aida *et al.*, 2004). Major aquatic plants surviving mainly in paddy fields, irrigation ditches, and irrigation ponds can be affected seriously by herbicides used in paddy fields(Ueji M *et al.*, 2001). However, susceptibilities of aquatic plants to herbicides have been hardly studied and those, listed as threatened

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species in Japan have been hardly identified to be observed, based on surveys in rice-cultivating areas in Korea(Yamakawa *et al.*, 2003). The present study was conducted to evaluate the response of the selected aquatic plants to SU-herbicides which have been used widely in rice-cultivating areas in Korea and Japan, and the to exam possibility of using them as bio-indicator species for biodiversity conservation.

MATERIALS AND METHODS

Survey system

A survey system was developed and used to ascertain the distribution of aquatic plants by regional groups, and according to herbicides used in paddy farming system of Korea. The survey area was divided into Chonnam and Kangwon provinces where SU-herbicides and butachlor have been used for many years. The survey centers in Chonnam and Kangwon provinces were Naju(35°01'N, 126°51'E) and Pyeongchang(37°31'N, 128°33'E), respectively. Twenty sampling areas in each province, and 20 sites for each sampling area, were surveyed in July, 2000 and 2004, respectively. Sampling sites were paddy fields, irrigation ditches and irrigation ponds.

Subject plants

Three aquatic plants, *M. korsakowii, M. quadrifolia* and *S. natans* were used in this study. *M. korsakowii* is commonly distributed in paddy fields, irrigation ditches and irrigation ponds of Chonnam and Kangwon provinces, while, *M. quadrifolia* and *S. natans* were easily observed in rice fields or irrigation ponds of Kangwon province.

Seeds of *M. korsakowii* were obtained from the reservoir in Pyeongchang(37°31'N, 128°33'E), Kangwon province where herbicides have never been used. Plants of *M. quadrifolia* and *S. natans* were obtained from irrigation pond in Changnyeong(35°28'N, 128°31'E), Kangwon province. Seeds of *M. korsakowii* were stored at 2℃ for two months to break dormancy. Plants of *M. quadrifolia* and *S. natans* were cultured in the glasshouse of the National Institute of Agricultural Science & Technology, Suwon City(37°13'N, 126°55'E), Kyeonggi province.

Exposure of aquatic plants to herbicides

The experiment was conducted with three replications in a growth chamber(E-15, Conviron's, Canada) maintained at 27/22℃(day/night), and 12 hrs photoperiod (day/night). Twenty germinated seeds of *M. korsakowii* having plumule of 2～3 mm, and 15 plants of *M. quadrifolia* having rhizome of 3 cm length with a terminal bud were transplanted to the plastic pots(20 cm×20 cm×15 cm) filled with paddy soil(clay loam) 10 days before herbicide application, respectively. Fifteen plants of *S. natans* with 4~6 leaves were transplanted to plastic pots filled with culture solution 3 days before herbicides application. The water level was maintained at 5 cm above the soil surface.

In Chonnam province practicing infant-rice seedling transplantation and wet-seeded direct seeding rice culture, pyrazosulfuron-ethyl/molinate and bensulfuron-methyl/ molinate have mainly used. However, those of Kangwon province in which machine transplanting of 30-day-old seedling has been used, butachlor have mainly used were used for many years. Considering these situations, BSM, PSE, butachlor, and molinate used in this experiment at the recommended doses of 51, 21, 1,500 and 1,500 g ai ha^{-1} for each herbicide. All the plants were collected 20 days after herbicide application and were oven-dried for 2 days at 60℃ to determine biomass. Data were expressed as percentage of untreated control to standardize herbicides tested. GR_{50} values were calculated from exhibiting 50% reduction of dry weight of aquatic plant treated with different herbicide rates, and all treatments for each measurement were made in triplicates.

Response of the aquatic plant to herbicides runoff from rice fields

This experiment was conducted to investigate impact of herbicide runoff from paddy fields to aquatic plants. Water samples were collected 15 days after herbicides application from irrigation ditches connected to rice fields of 20(sample 1) and 15 ha(sample 2) in which PSE/fentrazamide and azimsulfuron/molinate had been used, respectively. Water collected from irrigation ditches was maintained at 5 cm above the soil of pots which *M. quadrifolia* was transplanted by the same condition as exposure experiments to herbicides. The number of internodes and dry weight of *M. quadrifolia* were determined 20 days after application. The concentrations of herbicides in water were analyzed by the standard method of respective herbicides.

ALS activity of aquatic plants to the SU-herbicides

ALS was extracted from the shoots of 20-day-old seedlings of *M. quadrifolia* which were grown in the growth chamber(E-15, Conviron's, Canada) maintained at 25℃. The shoots were homogenized in 3 volumes of containing 0.1 M K_2HPO_4(pH 7.5), 1 mM sodium pyruvate, 0.5 mM $MgCl_2$, 0.5 mM thiamine-pyrophosphate, 10 μM FAD and 10% v/v glycerol. The enzyme was collected at 25 to 50% saturation by centrifugation and the pellet dissolved in buffer containing 0.1 M K_2HPO_4, pH 7.5, 20 mM pyruvate, and 0.5 mM $MgCl_2$, and desalted on small column of Sephadex G-25 (Pharmacia PD-10) equilibrated with the same buffer. Protein was determined using the method of Lowry *et al.* (1951).

ALS assays were carried out in a final volume of 0.5 ml at 30℃. The final reaction mixture contained 20 mM K_2HPO_4, 20 mM sodium pyruvate, 0.5 mM thiamine-pyrophosphate, 0.5 mM $MgCl_2$, and 10 μM FAD and, its pH was adjusted to 7.0. Then, in the mixtures, 0, 0.0001, 0.001, 0.01, 0.1, 1, 10 μM solutions of BSM and PSE were prepared. ALS activity was determined as described by Westerfield(1945) with some modifications at 525 nm using a spectrophotometer(UV4, Unicam, USA). The I_{50} values for inhibition are defined as the concentration of herbicides which inhibits ALS activity by 50%.

RESULTS AND DISCUSSIONS

Distribution of aquatic plants

The biodiversity including aquatic plants in paddy farming system has been threatened due to the loss of habitats and water pollution caused by the runoff of agrochemicals and the pavement of irrigation ditch, and others and mostly by because of herbicides treatment. However, aquatic plants can be affected differently depending on the herbicides used. Tables 1 and 2 show distribution of main aquatic plants in paddy farming system of Chonnam and Gangwon provinces where SU-herbicides and butachlor have been used extensively for many years. The aquatic plants observed in

Table 1. Distribution of main aquatic plants in paddy farming system of Kangwon province, Korea

Family	Species[a)]	Distribution		
		Paddy	Irrigation	
			Ditch	Pond
Azollaceae	**Azolla imbricata* (Roxb.)Nakai	○	○	○
Commelinaceae	*Aneilema keisak* Hassk.	○	○	
Hydrocharitaceae	**Blyxa aubertii* L.C.Rich	○	○	
Hydrocharitaceae	*Blyxa japonica*	○	○	
Hydrocharitaceae	**Blyxa echinospema* Hook.f.	○	○	
Lemnaceae	*Spirodela polyrhiza* (Linn) Schleiden	○	○	○
Lythraceae	**Rotala leptopetala* (Blume) Koehne *var. Littorea*(Mig.) Koehne	○		
Marsileaceae	*Marsilea quadrifolia L.	○	○	○
Menyanthaceae	*Nymphodes coreana* (Lveill)Hara	○		○
Nymphaeaceae	*Brasenia schreberi* J.F. Gmelin			○
Nymphaeaceae	*Euryale ferox* Sailsbury			○
Potamogetonaceae	*Potamogenton oxyphyllus* Mig.		○	○
Pontederiaceae	*Monochoria korsakowii Regel et Maack	○	○	○
Ranunculaceae	**Ranunculus kadzusesis* Makino			○
Salviniaceae	**Salvinia natans* (L.)All.		○	○
Saxifragaceae	**Penthorum chinense* Pursh		○	○
Trapaceae	*Trapa japonica* Flerov		○	○

[a)]Asterisks indicate aquatic species listed as threatened species by the Environment Agency of Japan.

Table 2. Distribution of main aquatic plants in paddy farming system of Chonnam province, Korea

Family	Species[a]	Distribution		
		Paddy	Irrigation	
			Ditch	Pond
Azollaceae	**Azolla imbricata* (Roxb.)Nakai	O	O	O
Commelinaceae	*Aneilema keisak* Hassk.	O	O	
Lemnaceae	*Spirodela polyrhiza* (Linn) Schleiden	O	O	O
Lythraceae	**Rotala leptopetala* (Blume) Koehne var. *Littorea*(Mig.) Koehne		O	
Menyanthaceae	*Nymphodes coreana* (Lveill)Hara			O
Nymphaeaceae	*Brasenia schreberi* J.F. Gmelin			O
Nymphaeaceae	*Euryale ferox* Sailsbury			O
Potamogetonaceae	*Potamogenton oxyphyllus* Mig.			O
Pontederiaceae	**Monochoria korsakowii* Regel et Maack	O	O	
Saxifragaceae	*Penthorum chinense* Pursh	O		
Trapaceae	*Trapa japonica* Flerov		O	O

[a]Asterisks indicate aquatic species listed as threatened species by the Environment Agency of Japan.

Kangwon were much more varied compared to Chonnam province, and they were also observed in paddy, irrigation ditch and pond. Nine species of aquatic plants listed as threatened species by the Environment Agency of Japan were confirmed in Kangwon province and six species of these nine aquatic plants occurred in rice fields. However, 10 species which include only three threatened species as listed by the Environment Agency of Japan were confirmed in Chonnam province.

Responses of aquatic plant to herbicides

The responses of *M. korsakowii* treated with the aforementioned four herbicides are shown in Fig. 1. Dry weights of *M. korsakowii* to the BSM and PSE was reduced by about 60~70% even at the concentration of 1/00 of recommended dose and almost completely

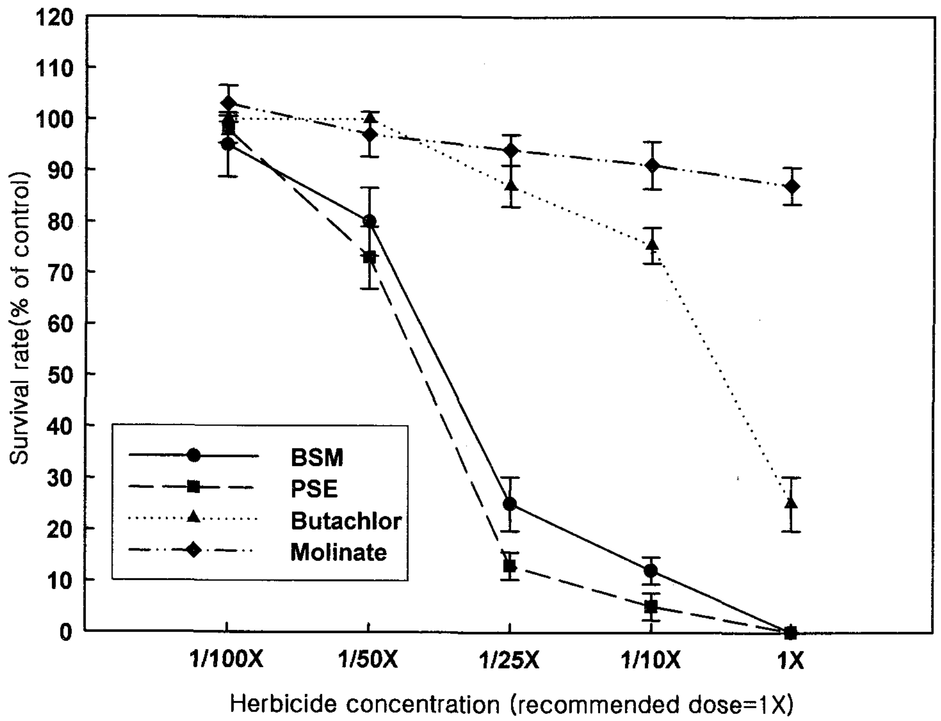

Fig. 1. Dry weight expressed as a percent of control *M. korsakowii* to bensulfuron-ethyl(BSM), pyrazosulfuron-ethyl(PSE), butachlor and molinate 10 days after transplanting. The recommended doses of BSM, PSE, butachlor and molinate are51, 21, 1,500 and 1,500 g ai ha^{-1}, respectively. The vertical bars represent standard errors of the mean.

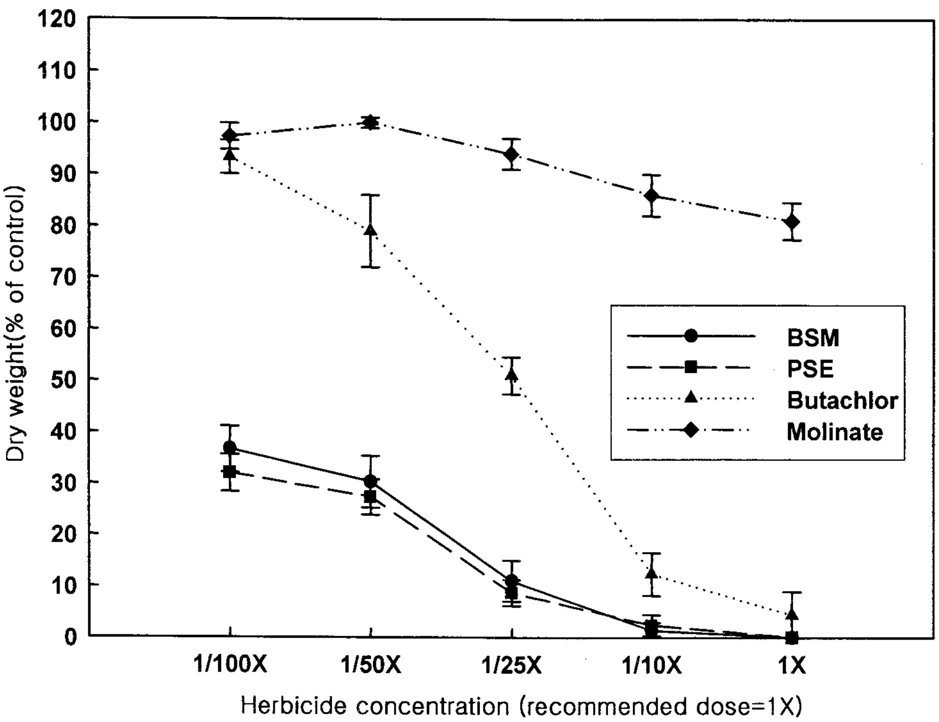

Fig. 2. Survival rate is expressed as a percent of control *Monochoria korsakowii* to bensulfuron-methyl(BSM), pyrazosulfuron-ethyl(PSE), butachlor and molinate 10 days after transplanting. The recommended doses of BSM, PSE, butachlor and molinate are 51, 21, 1,500 and 1,500 g ai ha^{-1}, respectively. The vertical bars represent standard errors of the mean.

reduced at 1/10 times of the recommended rate. The response of *M. korsakowii* to butachlor was less sensitive compared to the BSM and PSE. Dry weight at 1/10 times of the recommended rates of butachlor was reduced by about 85 %. However, among the four herbicides tested, molinate did not affect *M. korsakowii,* even at the recommended rate. The survival rates of *M. korsakowii* against BSM, PSE, butachlor and molinate were somewhat different from to the changes of dry weight.

The survival rates of *M. korsakowiito* against BSM, PSE started to decrease steeply at 1/50 times of the recommended rate of each herbicide, and completely controlled at the recommended rate. However, the survival rate to butachlor and molinate were much less sensitive compared with those of BSM and PSE, especially not almost affected by molinate even at the recommended rate (Fig. 2).

Fig. 3 shows the dry weight expressed as a percent of control of *M. quadrifolia* to BSM, PSE, butachlor and molinate 10 days after transplanting. Dry weighs of *M. quadrifolia* to BSM and PSE, SU-herbicides, began to decrease sharply from 1/500 times of the recommended doses, and completely controlled at 1/10 times. In contrast, dry weighs of *M. quadrifolia* were hardly affected by butachlor and molinate enough to show 70% and 90% of untreated plant dry weights even at the recommended dose, respectively.

The response based on dry weight of *M. quadrifolia* to four herbicides tested shows very similar trend compared with that of dry weight: The internodes of *M. quadrifolia* from over 1/25 times herbicide dose of BSM and PSE were almost completely inhibited, but those of butachlor and molinate ranged from approximately 75% to 90% of the control (Fig. 4).

Fig. 5 shows the number of internodes expressed as a percent of control of *S. natans*, floating aquatic plants, to BSM, PSE, butachlor and molinate 10 days after transplanting. Dry weighs of S. natans to BSM and PSE, SU-herbicides, began to decrease sharply from 0.01 ppb, and controlled completely at 10ppb. Responses to butachlor were relatively much less sensitive than those

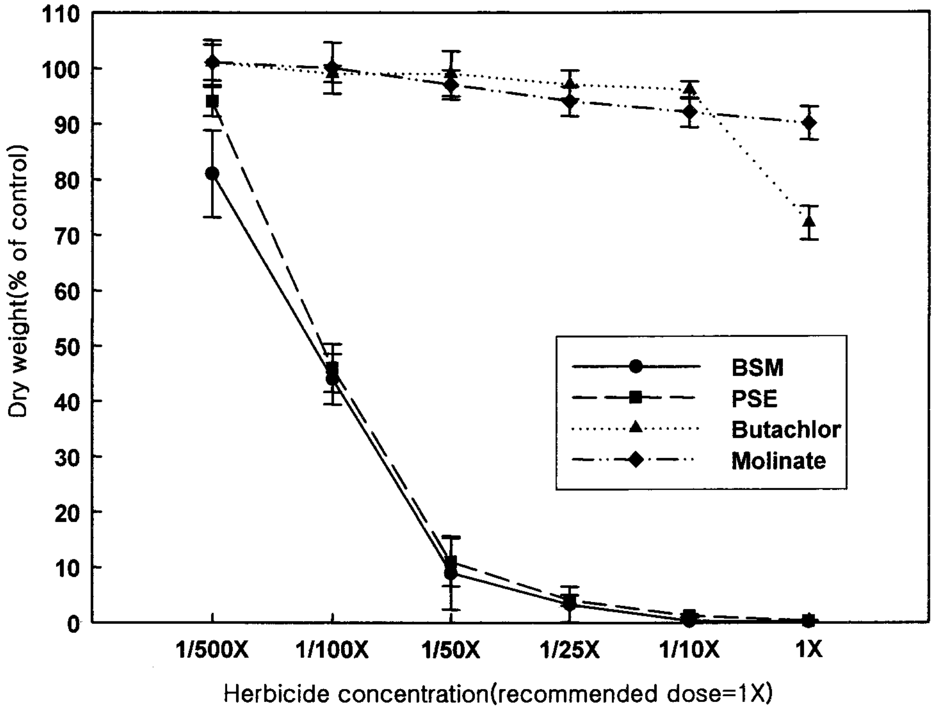

Fig. 3. Dry weight expressed as a percent of control of *Marsilea quadrifolia* to bensulfuron-methyl(BSM), pyrazosulfuron-ethyl(PSE), butachlor and molinate 10 days after transplanting. The recommended doses of BSM, PSE, butachlor and molinate are 51, 21, 1,500 and 1,500 g ai ha^{-1}, respectively. The vertical bars represent standard errors of the mean.

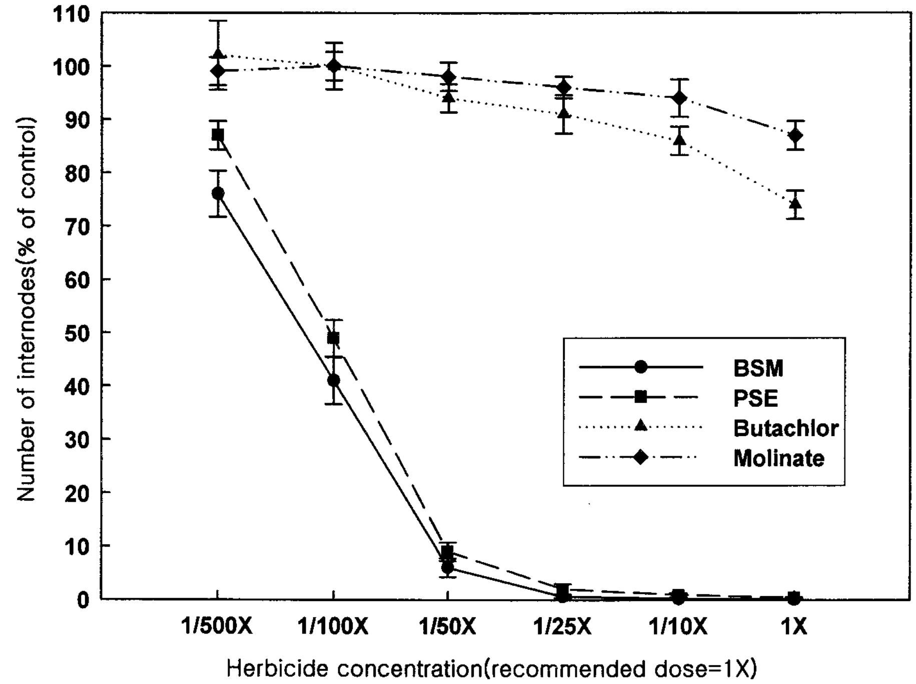

Fig. 4. Number of internodes expressed as a percent of control of *Marsilea quadrifolia* to bensulfuron-methyl(BSM), pyrazosulfuron-ethyl(PSE), butachlor and molinate 10 days after transplanting. The recommended doses of BSM, PSE, butachlor and molinate are 51, 21, 1,500 and 1,500 g ai ha^{-1}, respectively. The vertical bars represent standard errors of the mean.

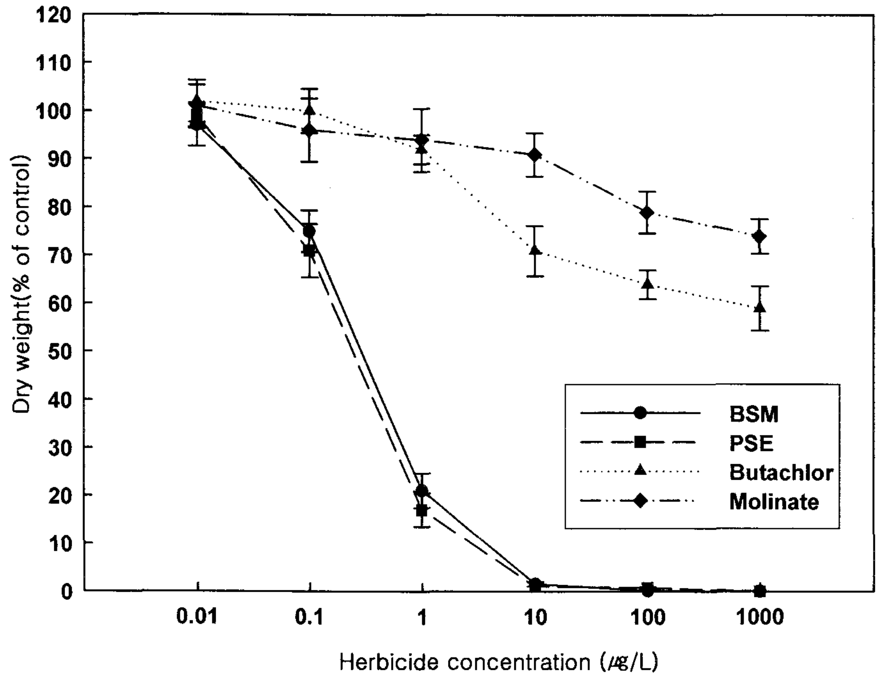

Fig. 5. Dry weight expressed as a percent of control of *Salvinia natans* to bensulfuron-methyl(BSM), pyrazosulfuron-ethyl(PSE), butachlor and molinate 10 days after transplanting. The recommended doses of BSM, PSE, butachlor and molinate are 51, 21, 1,500 and 1,500 g ai ha^{-1}, respectively. The vertical bars represent standard errors of the mean.

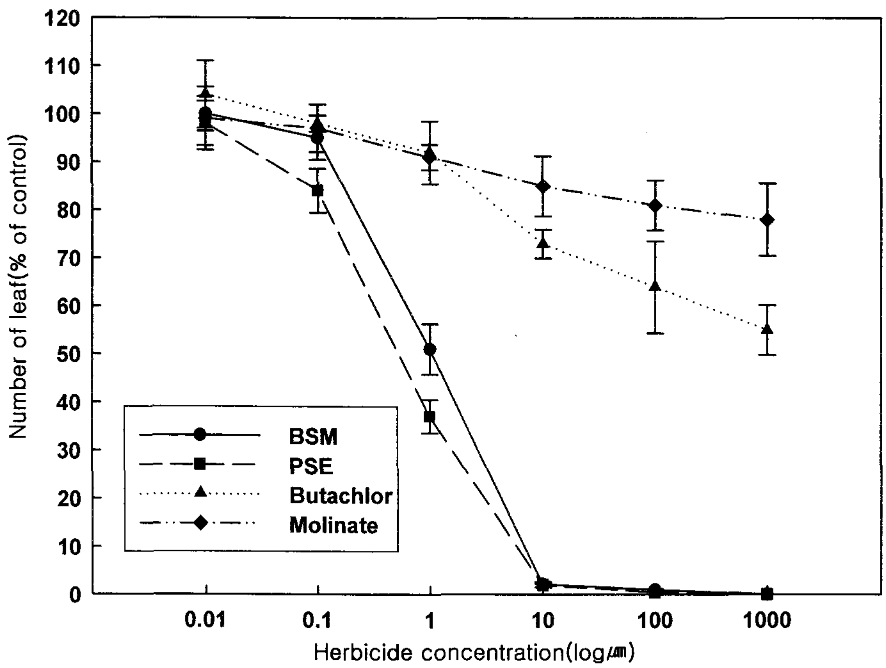

Fig. 6. The number of *S. natans* leaves expressed as a percent of control in pot treated with bensulfuron-methyl(BSM), pyrazosulfuron-ethyl(PSE), butachlor and molinate at 10 days after transplanting. The recommended doses of BSM, PSE, butachlor and molinate are 51, 21, 1,500 and 1,500 g ai ha^{-1}, respectively. The vertical bars represent standard errors of the mean.

Table 3. GR_{50} of bensulfuron-methyl(BSM), pyrazosulfuron-ethyl(PSE), butachlor and molinate for *Monochoria korsakowii, Marsliea quadrifoli* and *Salvinia natans*

Species	GR_{50}a			
	BSM[1)]	PSE[2)]	Butachlor	Molinate
Monochoria orsakowii	<0.51	<0.21	67.4	>1,500
Marsilea quadrifolia	0.6	0.2	>1,500	>1,500
Salvinia natans	0.5	0.3	>1,000	>1,000

[a)]The units for GR_{50} are g ai h^{-1} for *Monochoria korsakowii* and *Marsilea quadrifolia*, and ppb for *Salvinia natans.*

of SU-herbicides tested: Dry weights ranged from 60% to 70% of the untreated plant dry weights at 10~1,000 ppb. However, dry weights of *S. natans* were hardly affected by molinate enough to show about 80% of the untreated plant dry weights even at 1,000ppb.

The number of leaves of *S. natans* per pot treated with four herbicides has no significant difference compared to changes of dry weights. The number of leaves of plants treated with BSM and PSE, was completely inhibited in 10ppb, but those treated with butachlor and molinate have leaves of approximately 60 and 80% of the untreated plant leaves (Fig. 6).

Regression equations derived from the dry weights of *M. korsakowii, M. quadrifolia* and *S. natans* and BSM, PSE, butachlor and molinate were used to calculate GR_{50} values(Table 3). The GR_{50} values of BSM and PSE, for M. korsakowii were relatively very low <0.51 and <0.21 compared to 67.4 and >1,500 of butachlor and molinate, respectively. Also the GR_{50} values of BSM, PSE, butachlor and molinate for *M. quadrifolia* and *S. natans* were similar to those of *M. korsakowii.*

In runoff tests, the number of internode and dry weight of *M. quadrifolia* in samples 1 and 2 were seriously affected. The number of internodes of *M. quadrifolia* in samples 1 and 2 were 2.1 and 1.7%, and dry weights in samples 1 and 2 were 1.4 and 0.9% of untreated plants, respectively(Table 4).

Table 4. The responses of *Marsilea quadrifolia* in runoff water at 15 days after herbicides application

Sample	Number of internode	Dry weight
	---------- % of control	--------
Sample 1[a)]	2.1	1.4
Sample 2	1.7	0.9

[a)]Sample 1 and 2 were collected from irrigation ditches connected to treated with pyrazosulfuron(PSE)/ fentrazamide and azimsulfuron/molinate.

ALS activity of aquatic plants to the SU-herbicides

The results confirmed that BSM and PSE, are a

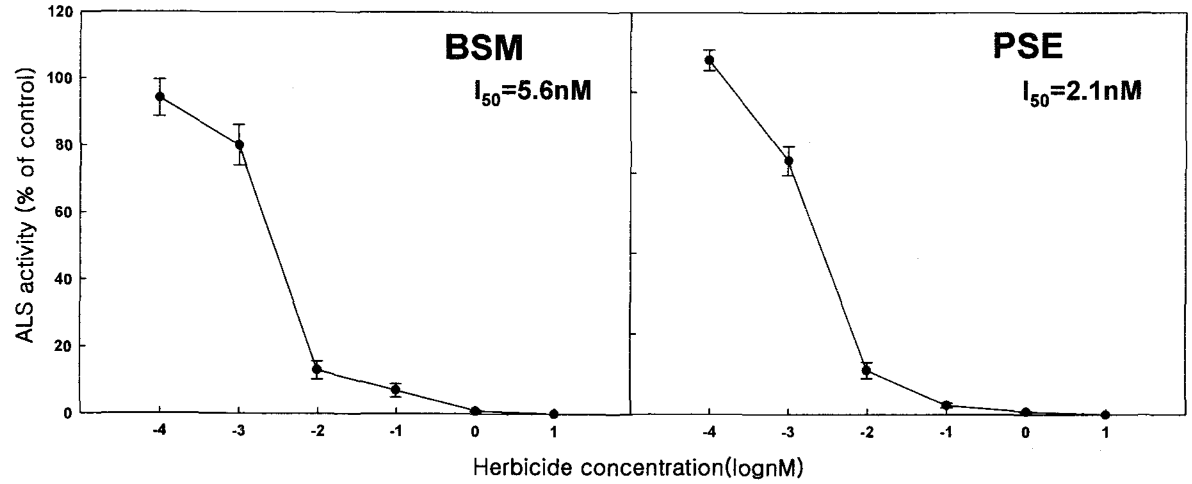

Fig. 8. ALS activity of *M. quadrifolia* by bensulfyron-methyl(BSM) and pyrazosulfuro-ethyl(PSE) at a range from 0.0001 to 10 μM. The vertical bars represent standard errors of the mean. The I_{50}values were the BSM and PSE concentrations that reduced ALS activity by 50%.

potent inhibitor of ALS of *M. quadrifolia*. The ALS activities of *M. quadrifolia* was reduced proportionally to SU-herbicides, and concentrations of BSM and PSE required to inhibit enzyme activity by 50%(I_{50}) value were 5.6 and 2.1 nM, respectively.

Literature cited

Aida, M., K. Itoh, H. Ikeda, N. Harada, Y. Ishi and K. Usui (2004) Susceptibilities of some aquatic ferns to paddy herbicide bensulfuon-methyl. Weed Biology and Management 4, 127~135.

Itoh, K., G. X. Wang, H. Shibaike and K. Matsuo (1999) Habitat management and inhertance of sulfonylurea resistance in *Lindernia micratha*, an annual paddy weed in Japan. Proc. 17th APWSS Conf. 537-543, Bangkok. Lowry, O. H., N. J. R. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. J. Biol Chem. 193:265~275.

Park, J. E., I. Y. Lee, T. S. Park, and B. C. Moon (2002) Occurrence Characteristics of weed flora in rice field in Korea. Kor.J. Weed Sci. 22(3):267~272.

Park, T. S., C. S. Kim, J. P. Park, Y. K. Oh, and K. U. Kim (1999) Resistant biotype of *Monochoria korsakowii* against sulfonylurea herbicides in the reclaimed paddy fields in Korea. Proc. 17th APWSS Conf. 251~254, Bangkok

Ueji, M. and K. Inao (2001) Rice paddy field herbicides and their effects on the environment and ecosystems. Weed Biol. Manag. 1:71~79.

Wang, G. X., H. Kohara and K. Itoh (1997) Sulfonylurea resistance in a biotype of *Monochoria korsakowii*, an annual paddy weed in Japan. Brighton Crop Protection Conference-Weeds 1:311~318.

Westerfield, W. (1945) A colorimetric determination of blood acetoin. J. Biol Chem. 161:495~502.

Yamakawa S., M. Wang and H. Yamaguchi (2003) A field survey of phenology and resproductive features in *Blyxa echinosperma* in the paddy fields of Kii-Ooshima Island. J. Weeds Sci. Technol. 48:109~116.

한국 논 생태계에서 수생식물 다양성에 대한 Sulfonylurea계 제초제의 영향평가

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요약 : 본 연구는 국내 논에 광범위하게 사용되고 있는 설포닐우레아계 제초제에 대한 몇몇 수생식물의 반응과, 생물다양성 보존위하여 제초제 특히 sulfonylurea계 제초제가 농업환경에 미치는 영향평가를 위한 지표식물의 가능성을 구명하고자 실시하였다. sulfonylurea계 제초제와 부타크르를 각각 다년간 사용하고 있는 전라남도와 강원도의 논 생태계에서 수생식물의 분포를 조사한 결과 강원도에서 훨씬 다양한 수생식물이 확인되었다. 수생식물인 물옥잠, 네가래, 그리고 생이가래는 비설포닐우레아계 제초제인 butachlor와 molinate 보다 bensulfuron-methyl과 pyrazosulfuron-ethy에 훨씬 민감하게 반응하였다. 물옥잠, 네가래, 그리고 생이가래의 건물중을 50%억제하는 GR_{50}값은 bensulfuron-methyl과 pyrazosulfuron-ethyl이 butachlor와 molinate 보다 훨씬 낮았다. 논으로부터 유출된 제초제에 대하여 네가래의 생육은 심하게 억제되었다. 그리고 네가래에 대한 bensulfuron-methyl과 pyrazosulfuron-ethy의 I_{50} 수치는 각각 5.6 및 2.1nM을 보였다.

Key words : 설포닐우레아, 수생식물, 유출, 제초제, ALS, *Marsilea quadrifolia*, *Monochoria korsakowii*, *Salvinia natans*.

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