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Bioremediation Bentazon using Minari(*Oenanthe stolonifera* DC.) Plant.

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미나리(*Oenanthe stolonifera DC.*)를 이용한 Bentazon의 생물학적 분해

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Abstract : Laboratory experiments were conducted to the potential ability of bioremediation with bentazon such as determining the absorption, translocation, and metabolism of ^{14}C-bentazon in minari after foliar applications.

The absorption and translocation of ^{14}C-bentazon were compared when applied to foliar of minari. In foliar applications, 21% was observed in treated leaves, 66% remained in water extracts of leaf surfaces, and 13% was found in the epicuticular wax layer after 2 d. Translocation of the herbicide from treated leaves to roots was very low(79 to 9 %). Analysis of methanol-soluble extracts of ^{14}C indicated that more than 60% of the foliar-applied herbicide was metabolized in all plant sections after 2 d. However, 77% or more of the bentazon was degraded in roots and shoots 2 d after root absorption. The major metabolite in these experiments was an unknown compound that was less polar than bentazon and 6- and 8-hydroxy bentazon.

Nomenclature : Bentazon, [3-(1-methylethyl,-(1H)-2,1,3,-bentazothia-diazin-4(3H)-one,2,2-dioxide] ; minari, Oenanthe stolonifera DC.

Key words : Uptake, translocation, metabolism.

Introduction

Increased environmental concerns over pesticide use have stimulated interest in developing the bioremediation technique that might be cleaned up the pesticide residues in water and soil. Minari(*Oenanthe stolonifera* DC. or *O. javanica* DC.) is commonly known as water dropwort, dropwort, Japanese parsley, oriental celery, and batjarongi. Minari is a traditional Korean vegetable that has an unique flavor, a special aroma, and medicinal properties. Farmers grow minari in rice paddies for crop rotation and they have the perception that this unique plant has a beneficial effect on paddy soils. Rice is one of the major sources for processed foods in Korea. Studies on the removal of pesticides from rice paddies have been associated with green remediation and we believe that minari has the potential to serve in this capacity, particularly with the metabolism of bentazon.

Bentazon [3-(1-methylethyl,-(1H)-2,1,3-bentazothiadiazin-4(3H)-one,2,2-dioxide] is described as a leacher and toxicological concern by the U.S.A Environmental Protection Agency(EPA) (Goodrich et al., 1991). The selective properties of herbicides often result from from a complex interation of a number of factors. With postemergence materials, the amount of herbicide at the active site is primarily a function of morphological and physiological factors leading to differential uptake, translocation, and metabolism(Mahoney and Penner, 1975 ; Nalewaja and Adamczewski, 1977 ; Penner, 1975). Differential bentazon metabolism has been established as the major basis of selectivity. Bentazon is first hydroxylated and then glycosylated either at the 6- or 8-position of its aromatic ring. In rice, only 6-hydroxy bentazon has been found(Mine et al., 1975) whereas both 6- and 8-hydroxy bentazon have been reported in soybean(Connelly et al., 1988).

The objectives of our studies were to : (1) determine the absorption, translocation, and metabolism of ^{14}C-bentazon in minari after root and foliar applications, and (2) characterize bentazon metabolites.

Materials and Methods

Plant Culture

Minari was cultured in a greenhouse. Root sections from established plants were selected for uniformity and transplanted in perforated 250mℓ styrofoampots containing a sterilized sand. All pots were irrigated with 0.5 strength Hoagland solution as needed until the date for post applications. The experiments were conducted with a randomized complete design with four replications.

Herbicide treatments

The ^{14}C-uniformly-ring-labeled bentazon with a specific activity of 1.6kBq was dissolved in distilled water. A microsyringe was used to apply the ^{14}C bentazon in 200μℓ of solution(100 drops, 2μℓ each) to the adaxial surface of one expended leaf on each plant at the midstem position.

Absorption and translocation of ^{14}C

Minari was harvested 1 and 2 days after treatment (DAT) and sectioned into treated leaves, lower leaves, axillary bud, and roots. Fresh weights of plant parts were recorded. Treated leaves were first washed for 3min in 10mℓ of distilled water to remove bentazon on the leaf surface. The radiolabel in the epicuticular wax was removed by a subsequent washing of the treated leaves for 3 min in 100mℓ of chloroform. Plant parts were homogenized separately in methanol. Cellular debris and precipitated protein were removed by centrifugation(5min at 10, 000×g). Aliquots of the methanol extracts, water washes, and chloroform washes were measured by liquid scintillation spectrometry(Corbin and Swisher, 1986).

Metabolism

The chloroform and water washes from treated leaves and the methanol extracts of treated leaves, lower leaves, axillary bud, and roots from the foliar application and the methanol extracts of shoot, roots, and remain solutions harvested from root application were evaporated under vacuum at 10℃ in a rotary evaporator. A 200μℓ fraction of each ^{14}C-sample was spotted on reversed phase thin layer chromatography(TLC) plates[1)] and were developed in Methanol/Na acetate(40 : 60, v/v). Following development, the plates were radio chromatographically scanned and areas under the radioactive peaks were integrated with a Imaging Scanner. Quantification of the metabolites was based on the distribution of radioactivity in the TLC profiles and verified by scraping of the zones from the plates followed by liquid scintillation spectrometry.

Data analysis

Data were subjected to an analysis of variance and least significant differences(LSD) were calculated to compare treatment means.

Results and Discussions

Absorption and translocation of ^{14}C

Recovery of the foliar applied radiolabeled bentazon was greater than 90% at 1 DAT and 2 DAT. Absorption of ^{14}C-bentazon by minari is illustrated in Table 1. At 1 and 2 DAT 7 and 21% of the bentazon was absorbed. Differences were found in the distri bution of the radiolabel in external(water wash or chloroform wash) and methanol-soluble internal extracts. After 2 d, 66% of the ^{14}C remained on the treated leaf surface and was recovered by water wash and 12% was found in the epicuticular

Table 1. Absorption of ^{14}C-bentazon in Minari 1 and 2 d after foliar application.

Location of ^{14}C	Time	Extracted ^{14}C[a]
	day	%
External		
Water wash	1	86.3
Chloroform wash	1	6.3
Internal[b]	1	7.4
External		
Water wash	2	66.3
Chloroform wash	2	12.5
Internal	2	21.2
L.S.D.(0.05)[c]		4.6

[a]The ^{14}C values are means of the treated leaf external washes and the internal methanol extracts.
[b]Treated leaves, lower leaves, axillary buds, and roots.
[c]Based on Fisher's protected L.S.D.($p>0.05$).

[1)]C_{18} reversed-phase plates; Analteech, Inc., 75 Blue Hen Drive, Newark, DE 19714.

wax layer that was recovered by the chloroform wash. Radioactivity recovered from the internal plant parts increased with exposure time. Our results are in agreement with other studies that showed that uptake of bentazon in soybean leaves increased with time(Wills, 1976). In our experiments with minari, the increase in the percent of ^{14}C absorbed was proportional to the decrease in ^{14}C in the surface water wash of treated leaves. The ^{14}C extracted by the water wash from the leaf surface decreased from 86 to 66% between 1 and 2 d(Table 1). After the foliar application, the treated leaf and axillary bud contained a higher concentration of ^{14}C than any other plant section(Table 2). The distribution of ^{14}C in the axillary bud and roots indicated that the herbicide was translocated both acropetally and basipetally. This finding is in agreement with previous reports with soybean, which showed that foliar applied bentazon was translocated both above and below the treated leaf. The treated leaf was considered to be one of major photosynthetic sources and ^{14}C was translocated with the photosynthesis to the sink areas of the shoot and root meristems. In our experiments with minari a higher percentage of ^{14}C was found in the axillary buds than in the roots after 1 days, but the difference was not observed after 2 d. Only 2% of the observed herbicide was translocated to minari lower leaves. Also, the concentration of ^{14}C (μg/g) in the lower leaves was lower than in all other plant parts(Table 2).

Table 2. Translocation of ^{14}C-bentazon in minari 1 and 2 d after foliar application.

Location of ^{14}C	Time	Extracted ^{14}C content[a]	
	day	— %—	— μg/g—
Treated leaves	1	79	0.17
Lower leaves	1	2	0.02
Axillary buds	1	17	0.15
Roots	1	2	0.02
Treated leaves	2	79	1.36
Lower leaves	2	2	0.03
Axillary buds	2	10	0.18
Roots	2	9	0.15
L.S.D.(0.05)[b]		3	0.17

[a]The ^{14}C values are means of the internal methanol extracts and are percents of the internal fraction listed in Tble 1.
[b]Based on Fiser's protected L.S.D.($p>0.05$).

Metabolism

Betazon was metabolized rapidly by young minari plants with 60 to 95% being degraded in the treated leaves, lower leaves, axillary buds and roots 2 d after foliar application(Table 3). Differences in metabolism in treated leaves were not observed between 1 and 2 d after treatments. Investigators have found that tolerant soybeans metabolized bentazon rapidly, with more than 50% being degraded in the treated leaves and plant apex. Susceptible weed species metabolized the herbicide much more slowly than soybean(Penner, 1975). In our experiments, metabolites were present in higher amounts than bentazon in the treated leaves, lower leaves, axillary buds, and roots after foliar application. The lowest percent of metabolism in plant sections was observed in the lower leaves and differences were not found in the axillary bud and roots(Table 3). Also, metabolism in the internal fraction of the treated leaves was different from metabolism in the cuticle(Table 5). Thus, more herbicide remained in the treated leaves and was not translocated to lower leaves. Parent bentazon was recovered in higher percent in the chloroform wash when herbicide was applied 1 d(74

Table 3. Metabolism of bentazon in minari 2 d after foliar application.

Plant parts	^{14}C product content[a]	
	——%——	
	Bentazon	Metabolites
Treated leaves	5	95
Lower leaves	40	60
Axillary buds	27	73
Roots	27	73
L.S.D.(0.05)[b]	3	2

[a]The ^{14}C values are means of the internal methanol extracts and are percents of the internal fraction listed in Table 1.
[b]Based on Fisher's protected L.S.D.($p>0.05$).

Table 4. Metabolism of bentazon on minari leaf surfaces 1 and 2 d after foliar application.

^{14}C product	Time	^{14}C content[a]	
		Chloroform wash	Water wash
	day	——%——	
Bentazon	1	74	93
Metabolites	1	26	7
Bentazon	2	54	78
Metabolities	2	46	22
L.S.D.(0.05)[b]		3	4

[a]The ^{14}C vaules are means of the internal methanol extracts.
[b]Based on Fisher's producted L.S.D.($p>0.05$).

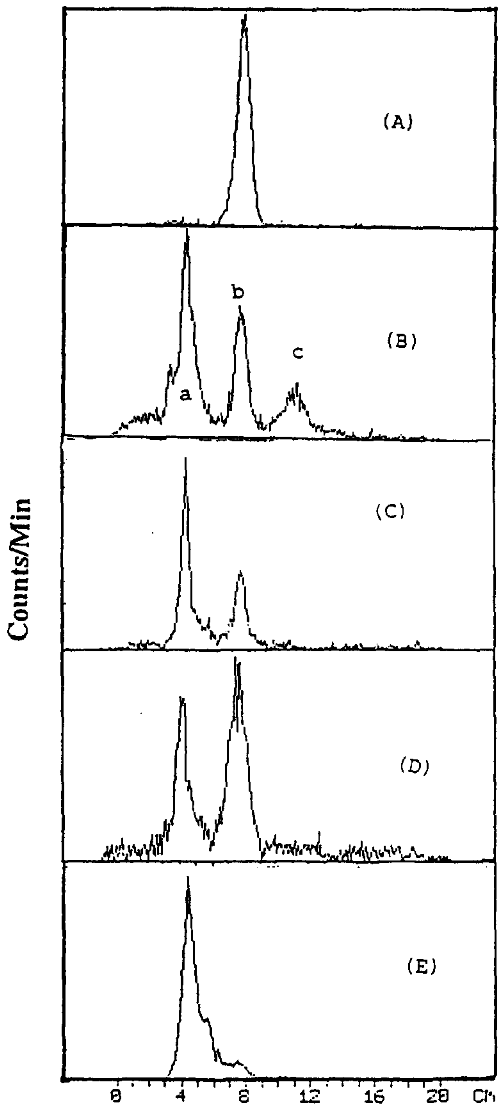

Fig. 1. Radioactivity traces of reversed phase, thin layer chromatogram of ^{14}C-bentazon and its metabolites formed in 1 d after foliage applications. (A) synthetic standard ; (B) roots ; (C) axillary buds ; (D) lower leaves ; (E) treated leaves. Bentazon is associated with b, 6-hydroxy bentazon with c, and unknown metabolite with a.

%) than when applied 2 d(54 %). Metabolites of ^{14}C in the water wash from the treated leaves increased with time(Table 4). Thus, the metabolites of bentazon were exuded back to the leaf surface. An example of metabolites shown in the radiographic traces of the reversed phase chromatogram of plant part extracts from foliar application after 2 d is illustrated in Fig. 1. Chromatography with authentic standards suggested that the peak at Rf 0.45 corresponded to bentazon. A different metabolic pathway was observed when compared to rice and soy bean(Fig. 2). The major metabolite that was formed

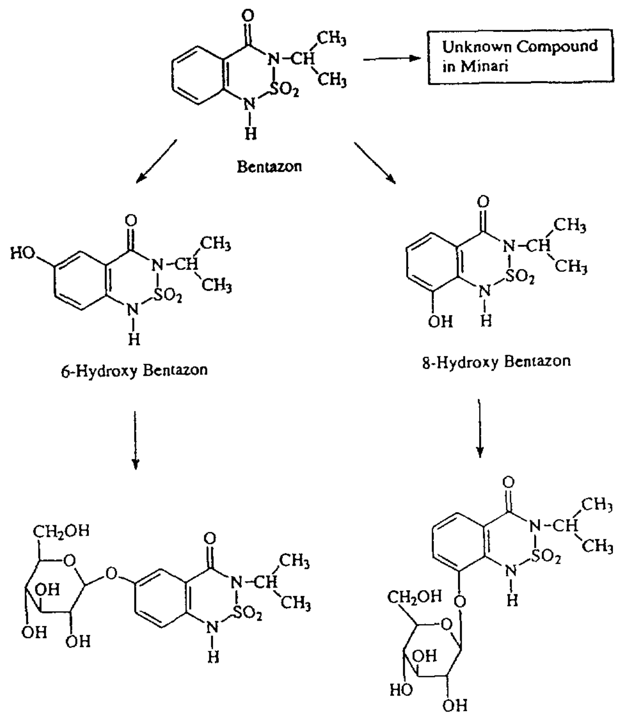

Fig. 2. Suggested pathway of bentazon metabolism in plants.

migrated to Rf 0.08. This major metabolite was an unknown compound that was less polar than the 6- and 8-hydroxy bentazon. This finding is in agreement with previous reports with grain sorghum shoots which showed that small amounts of a less polar metabolite were formed(Morland and Corbin, 1991). However, the identity of this major unknown compound remains to be established.

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요 약

Bentazon의 잔류를 제거하기 위한 미나리의 생물학적 정화능력을 검정코자 ^{14}C-Bentazon의 흡수(uptake)와 이행(translocation), 그리고 분해(metabolism) 실험을 통하여 비교 실험하였다. 잎에 처리한지 2일 후에 있어서 ^{14}C의 21%가 처리된 잎에서 관측되었고, 66%는 잎 표면을 물로 세척한 곳에 잔류하고 있었으며, 나머지 13%는 epicuticu-

lar wax층에서 발견되었다. Bentazon을 처리한 잎으로부터 ^{14}C의 79%가 검출되었으며, 뿌리에는 9%만이 검출되어 잎으로부터 뿌리로의 이행은 매우 적었다. 처리한 2일 후에 ^{14}C의 methanol 추출물 분석에서 잎에 처리된 제초제의 60% 이상이 모든 식물체 부위에서 분해되었다.

본 실험에서 발견된 주요 bentazon의 분해물(Metabolites)은 bentazon이나 6-hydroxy 혹은 8-hydroxy bentazon보다 덜 이온화(Polar)된 알려지지 않은 신 물질(unknown compound)이었다.

References

1. Connelly, J.A., M.D. Johnson, J.W. Gronwald, and D.L. Wyse. 1988. Bentazon metabolism in tolerant and susceptible soybean(*Glycine max*) genotypes. Weed Sci. 36 : 417~501.
2. Corbin, F.T., T.J. Monaco, and L.A. Bjelk. 1991. Environmental toxicology : degradation of herbicides, *In* Carbon Isotope Techniques, D.C. Coleman and B. Fry eds., Academic Press, Inc., New York, p. 101~108.
3. Goodrich, J.A., B.W. Lykins, and R.M. Clark. 1991. Drinking water from agriculturally contaminated ground water. J. Environ. Qual. 20 : 707~717.
4. Mahoney, M.D. and D. Penner. 1975. Bentazon translocation and metabolism in soybean and navy bean. Weed Sci. 23 : 265~271.
5. Moreland, D.E., F.T. Corbin, and J.E. McFarland. 1993. Effacts of safeners on the oxidation of multiple substrates by grain sorghum microsomes. Pestic. Biochem. Physiol. 45 : 43~53.
6. Mine, A., M. Myiakado, and S. Matsunaka. 1975. The mechanism of bentazon selectivity. Pestic. Biochem. Physiol. 5 : 566.
7. Nalewaja, J.D. and K.A. Adamczewski. 1977. Uptake and translocation of bentazon additives. Weed Sci. 25 : 309~315.
8. Penner, D. 1975. Bentazon selectivity between soybean and Canada thistle. Weed Res. 15 : 259~262.
9. Wills, G.D. 1976. Translocation of bentazon in soybeans and common cocklebur. Weed Sci. 24 : 536~540.