韓土肥誌(1992)
Vol. 25(2) : 160~167

Allelopathic Inhibition by Extracts and Volatiles from Leaf and Seed of Sicklepod (*Cassia tora* L.)

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決明子(*Cassia tora* L.) 葉과 種子의 抽出物과 揮發成分의 他感性 生育沮害作用

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SUMMARY

The growth of weeds and some other plants has been considered to be inhibited by sicklepod(*Cassia tora* L.) sharing the habitat. The study was conducted, for the first time, to propose that this phenomenon is not only due to competition for physical and nutritional conditions but also due to allelopathy. In addition, autotoxicity of sicklepod was examined.

The results are summarized as follows.

1. Rice sheath length reduced progressively from 18% to 36% with increasing the concentration of treating aqueous extracts of sicklepod seeds, but rice germination was not affected. In contrast, radish hypocotyl length was not reduced by the aqueous extract treatment but radish germination was significantly reduced by 66% at 1 : 10 and 1 : 5 treatment.
2. Total chlorophyll contents in rice seedling decreased from 50% to 65% by treatment of seed aqueous extracts diluted from 1 : 50 to 1 : 5 ratio.
3. Aqueous extracts of sicklepod leaves significantly reduced hypocotyl length and fresh weight in radish and germination in rice, but mung bean was slightly affected by aqueous extracts only in fresh weight.
4. Volatiles from fresh, immature seeds with husk reduced the radish germination and seedling growth and radish root growth appeared to be more sensitive to the exposure to volatiles from fresh immature seeds than both germination and hypocotyl growth.
5. Volatiles from sickepod leaves inhibited germination and growth of radish, rice and mung bean, and seedling growth was more sensitive to volatiles from leaves than germination.
6. Volatiles from sicklepod leaves reduced germination and radicle length of sicklepod itself.
7. Collectively, it is concluded that there are water-soluble and volatile substances responsible for allelopathy in sicklepod.

INTRODUCTION

Allelopathy, in definition, is the biochemical interaction between species involving microbes and plants. Muller[11] has described the interactions between two plants resulting in the growth inhibition of one or both of these plants as interference, which is further subdivided into competition and allelopathy. Competition is defined as the mechanism by which one plant depletes some essential elements for plant growth to the level that is limiting to the growth of second plant sharing that habitat[23]. Allelopathy is interference resulting from the release of phytotoxic substance from one

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plant that causes an inhibition of second plant growth[23]. On the other hand, autotoxicity as defined by Putnam[16] is an intraspecific form of allelopathy. In environment the phenomena of allelopathic inhibition and competition are likely to take place simultaneously in many cases[23].

The important role of allelopathy has been considered to contribute to controlling the distribution and modification of plant species in the ecosystem. Recently allelopathy has received increasing attention as a mean of explaining vegetation patterns in plant communities[11,13,18] and as an important aspect of weed-crop interactions[1,4,5]. Putnam and Duke[17] postulated that wild types of crop plants survive because they are allelopathic, and that the capacity for producing allelopathic chemicals has been lost in the breeding of commercial varieties. Recently, Oleszek[14] showed that allelopathic effects of unknown volatiles from some cruciferae species on lettuce, barnyard grass and wheat growth.

Sicklepod(*Cassia tora* L.) is widely cultivated in tropical Asian Countries. Various parts of the plant are reputed for their medicinal value[26]. The seeds of sicklepod have been used to make Chinese medicine as aperient, antiasthenic and diuretic. In Korea, the hot aqueous extract of the seeds of sicklepod is used for protection of liver and improvement of visual activity. Sicklepod has been considered as one of the most serious weed in much of the soybean[20,21], cotton and peanut-growing areas of the warm, humid southeastern United States[2,3,7]. Therefore, the use of the chemical herbicides[22,24] and a biological control agent such as *Alternaria cassiae*, an indigenous fungal pathogen of sicklepod[23], for management of sicklepod has been recently studied, but it has never been reported that exudates, leachates, decomposition materials, or volatiles from sicklepod contain the biologically active substances responsible for allelopathy in the agronomic ecosystem.

Therefore we hypothesized that the growth of other plants in sicklepod growing field is inhibited not only by competition for physical and nutritional conditions but also allelopathy. Hence, the objective of this study described here was to examine the allelopathic potential which inhibit the germination and seedling growth by aqueous extracts and volatiles from the several parts of sicklepod by performing bioassay experiment and to characterize the self-inhibition (autotoxicity) in sicklepod.

MATERIALS AND METHODS

Plant Materials : Sicklepod(*Cassia tora* L.) seeds were harvested in 1990 at Chunnam Posung, Korea. Seeds were ground in a blender and stored in a vaccum desiccator until extraction. Leaves were collected from sicklepod grown at the experimental farm of Seoul National University during late flowering stage and dried at 50°C. The oven-dried leaves were ground in a wiley mill and stored in a desiccator until used. Leaves and immature seeds used for volatiles bioassay were fresh.

Extraction : Seeds and oven dried, ground leaves were homogenized with MeOH-$CHCl_3$-H_2O(2 : 1 : 0.8)[27] and filtered through Whatman No.2 paper. After reextraction the residue with an equal volume of same solvent and filtration, both of filtrates were combined. By washing the extracts three times with an equal volume of $CHCl_3$, extracts were partitioned into aqueous and $CHCl_3$ layers. Aqueous extracts were evaporated at 65 ± 5 °C to remove methanol and water. The final concentrations of seeds and leaves aqueous extracts were 1g/1ml H_2O and 3GDW(g dry weight)/1ml H_2O, respectively. These extracts were stored at 4°C until assayed.

Extract bioassay : Twenty seeds of radish, rice or mung bean were placed in 9cm-diam. petri dishes filled with vermiculites sterilized at 150°C for 4h and wetted with 50ml of Hoagland's solution and 10ml of diluted extract(1 : 50, 1 : 30, 1 : 10 and 1 : 5(v : v, extract : Hoagland's solution)). After incubation at 28°C in the dark for 48h, the petri dishes were transfered to green house, and watered daily with distilled water and Hoagland's nutrient solution. Germination and seedling growth were examined at 7 days after sowing. All bioassay experiment were replicated three times.

Bioassay with volatiles from sicklepod :
The bioassay with volatiles from immature seeds and fresh leaves were performed with radish seeds in germination chamber. The germination chamber was designed with a modification of Oleszek's idea[14]. Twenty seeds were put on Whatman No.2 paper placed in 15.5cm-diam. glass petri dish and wetted with 14 ml of distilled water. A petri dish containing seeds was placed in 5000ml chamber, and sample was laid on another petri dish which was supported by glass stand at center of the petri dish on the bottom of chamber. Samples were weighed after chopping immature seeds(20g, 100g) and fresh leaves(15g, 30g) into small pieces, then a small amount of sea-sand was added to the sample and the mixture pulverized as fast as possible (0.5~1 min) to avoid the loss of volatiles. Chambers prepared in this way were incubated at 28°C for 48h in darkness and transfered to the condition of all day light(1100 lux). Germination and seedling growth of radish were examined at 7days after sowing, To conduct bioassay with volatiles from oven dried, ground leaves (5g, 10g), ten seeds of radish, rice, mung bean and sicklepod were placed on two layers of 8cm-diam. Whatman No.2 paper in 250ml glass chamber, and moistened with 7ml of distilled water. The other chamber preparation and growing condition were same as described in bioassay with volatiles from fresh leaves and immature seeds.

RESULT AND DISCUSSION

In preliminary experiments, aqueous layer of sicklepod seeds and leaves which extracted by MeOH-$CHCl_3$-H_2O(2 : 1 : 0.8) considerably showed inhibitory effect on germination and growth of radish seed, whereas those from reflux with distilled water or 95% MeOH did not show any effect on germination and growth of radish seed(data not shown). Therefore, all samples were extraced by using MeOH-$CHCl_3$-H_2O(2 : 1 : 0.8).

Rice sheath length was reduced progressively from 18% to 36% with increasing the concentration of treating aqueous extracts of sicklepod seeds, but rice germination was not affected by the aqueous extract treatments(Table 1). In contrast, radish hypocotyl length was not reduced by the aqueous extract treatments but radish germination was significantly reduced by 66% at 1 : 10 and 1 : 5 treatments.

Table 1. The effects of aqueous extracts of sicklepod seeds on germination and seedling growth in rice and radish seed.

Treatment(v : v) (extract : Hoagland sol'n)	Germination	Seedling growth
	% of control	
rice		sheath length
control	100a*	100a
1 : 50	102a	82b
1 : 30	106a	80b
1 : 10	106a	66c
1 : 5	104a	64c
radish		hypocotyl length
control	100a	100a
1 : 50	64ab	92a
1 : 30	61ab	88a
1 : 10	34b	88a
1 : 5	34b	105a

Treatment(v/v) : 1 : 50=0.20g, 1 : 30=0.32g, 1 : 10=0.91g, 1 : 5=1.67g

* Means within column followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test.

Fig. 1 showed that the inhibitory effect of sicklepod seed extracts on the growth of rice seedling. Green color of rice seedling progressively turned pale with increasing concentration of aqueous extract. Seed aqueous extracts decreased total chlorophyll contents from 50% to 65% in rice seedling(Table 2). These results indicated that sicklepod seeds have allelopathic compound which is responsible for the chlorophyll biosynthesis inhibition and the phytotoxin is water souble. Patterson[15] reported that allelopathic chemicals such as *p*-coumaric, t-cinnamic, caffeic, ferulic, gallic and vanillic acids severely reduced net photosynthetic rate and stomatal conductance of single, fully expanded leaves of soybean at concentration of 10^{-3} M and that these same compounds also caused marked reduction in leaf chlorophyll content of 3-week-old soybeans, with net losses of chlorophyll occurring over an 86h period after treatment.

Aqueous extracts of sicklepod leaves significantly decreased hypocotyl length and fresh weight in radish and germination in rice, but did not affect on those of mung bean(Table 3). Radish hypocotyl length and fresh weight were reduced by 36% and 39%, respectively, by the treatment of 5.01 GDW(1 : 5v/v) but radish germination was not affected significantly by any extract treatments. Mung bean was not affected by aqueous extracts in both germination and seedling growth, whereas fresh weight was reduced by 10% of control by the treatment of 0.60GDW(1 : 50 v/v) These indicated that there are allelopathic chemicals in aqueous extracts of sicklepod leaves responsible for inhibitory effect on germination and growth of test plants and that radish is most sensitive to inhibitory effect of the phytotoxins produced from sicklepod leaves. The phenolic acids and related compounds known to be produced by weeds and/or to be released from decaying crop residues have allelopathic effects on plant germination, gro-

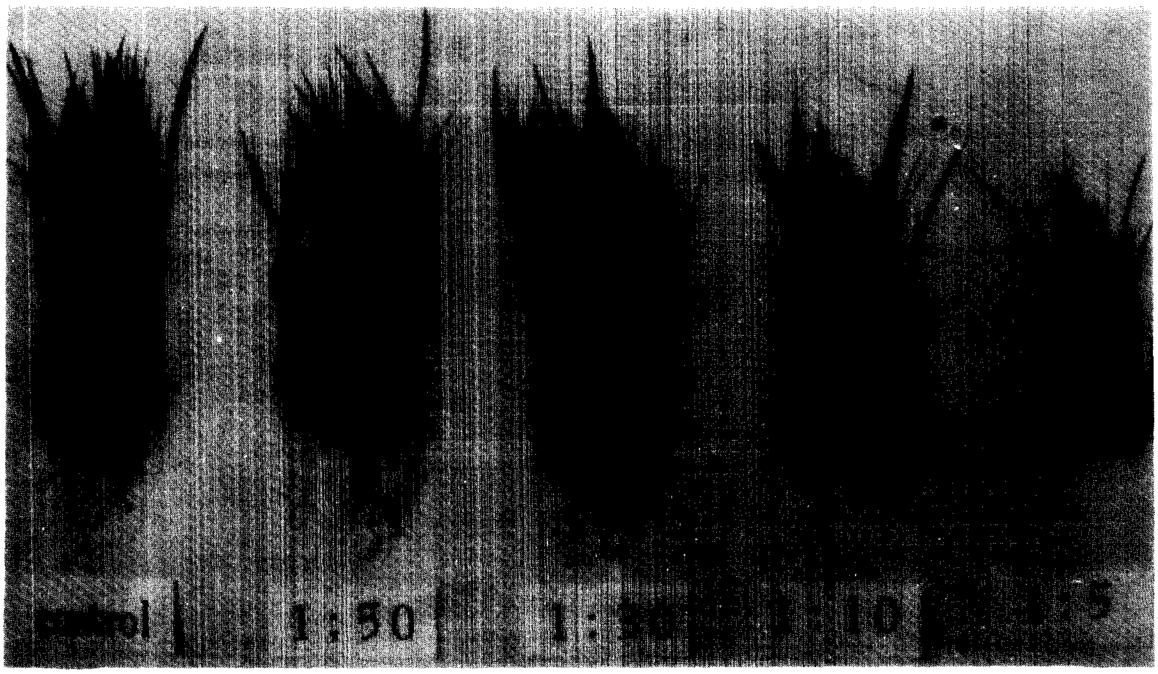

Fig 1. The effect of aqueous extracts of sicklepod seeds on rice seedling
Treatment(v/v) : 1 : 50=0.20g, 1 : 30=0.32g, 1 : 10=0.91g, 1 : 5=1.67g

Table 2. The effects of aqueous extracts of sicklepod seeds on total chlorophyll contents on rice seedings

Treatment (v : v)	Total chlorophyll mg/GFW	% of control
control	1.16	100
1 : 50	0.58	50
1 : 30	0.47	41
1 : 10	0.42	36
1 : 5	0.40	35

Treatment(v/v) ; 1 : 50=0.20g, 1 : 30=0.32g, 1 : 10=0.91g, 1 : 5=1.67g

Table 3. The effects of aqueous extracts of sicklepod leaves on germination and seedling growth.

Treatment (extract : Hoagland sol'n)	Germination	Seedling growth	Fresh weight
	% of control		
radish		hypocotyl length	
control	100a*	100a	100a
1 : 50	100a	87b	84b
1 : 30	98a	67cd	72bc
1 : 10	91a	75c	67c
1 : 5	87a	64d	61c
rice		sheath length	
control	100b	100a	100a
1 : 50	120a	97a	95a
1 : 30	114ab	103a	98a
1 : 10	102b	106a	95a
1 : 5	97b	99a	92a
mung bean		hypocotyl length	
control	100a	100a	100ab
1 : 50	100a	97a	90b
1 : 30	95a	97a	97ab
1 : 10	95a	98a	100ab
1 : 5	97a	100a	112a

Treatment(v/v) ; 1 : 50=0.60GDW, 1 : 30=0.96GDW, 1 : 10=2.73 GDW, 1 : 5=5.01GDW

* Means within column followd by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test.

wth, photosynthesis[15, 19, 25], and representative chemicals in these compounds are salicylic acid, *p*-hydroxybenzoic acid, caffeic acid, umbelliferone, and hydroquinone[19]

Table 4 represented the effect of volatiles from fresh immature seeds with husk and fresh leaves on radish germination and seedling growth. Radish germination, hypocotyl length and root length were reduced by 2%, 60% and 78%, respectively, by the treatment of 20GFW. Radish growth appeared to be more sensitive to the exposure of volatiles from fresh, immature seeds than germination. Radish germination, hypocotyl length and root length were reduced by 20%, 83% and 82%, respectively, by the treatment of volatiles from GFW from fresh leaves. Radish seedling growth was more sensitive to exposure of volatiles from fresh leaves than germination(Fig. 2). These results of radish bioassay referred that volitiles of fresh leaves and immature seeds of sicklepod are considered to have potentially allelopathic effects on the germination and the growth of other plants in vicinity.

Volatiles from dried and ground leaves also inhibited germination and seedling growth of test plants(Table 5). Volatiles from 10g dried and

Table 4. The effects of volatiles from immature seeds and fresh leaves of sicklepod on germination and seedling growth in radish.

Treatment (GFW)	Germination	Hypocotyl length	Root length
	% of control		
immature seeds			
control	100a*	100a	100a
20	98a	40b	22b
100	39b	10c	8b
fresh leaves			
control	100a	100a	100a
15	93a	58b	64b
30	80b	17c	18c

* Means within column followed by the same letter are not significantly different at the 95% confidence level as determined by Duncan's multiple range test.

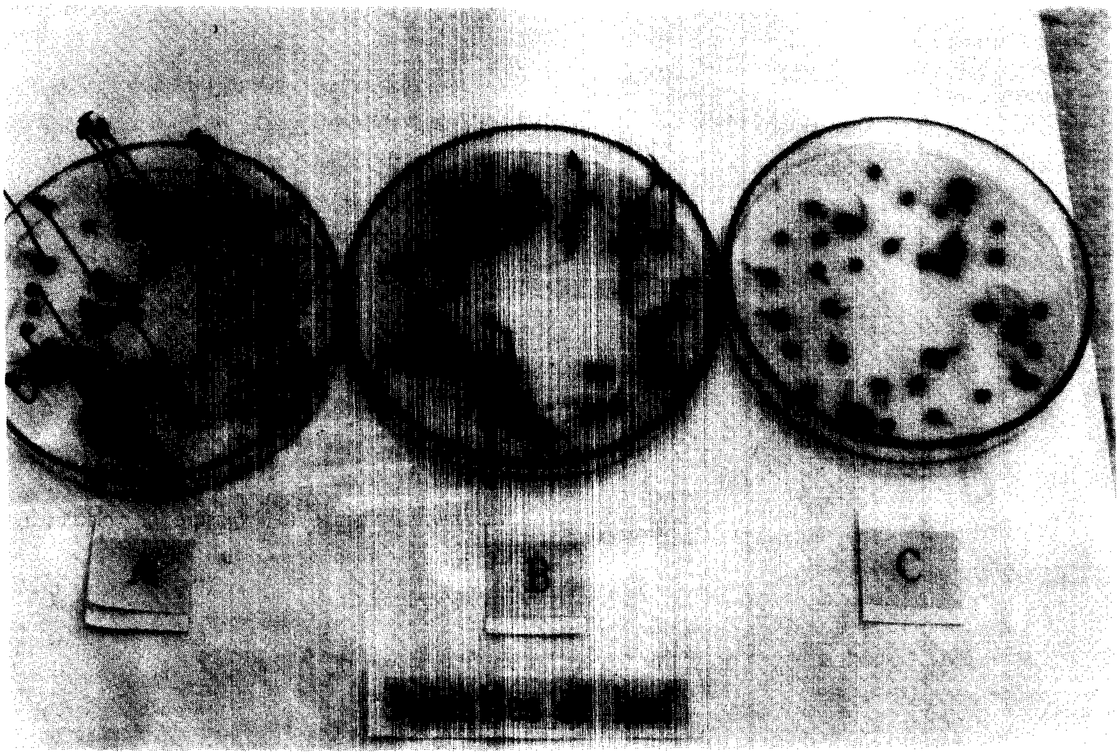

Fig 2. The effect of volatiles of sicklepod leaves on radish germination and seedling growth. Treatment : A. control, B. 15GFW, C. 30GFW

ground leaves decreased radish hypocotyl length and root length by 86% and 82%, respectively, rice sheath and root length by 61% and 79%, respectively, and mung bean hypocotyl and root length by 57% and 41%, respectively. But, germination of all test plants was not affected by volatiles from dried, ground leaves. Mung bean germination was not affected by these volatiles, which was similar to the result of radish bioassay by the treatment of volatiles from fresh leaves. The degree of inhibition of test plants by these volatiles decreased in order of radish, rice and mung bean. Volatiles from sicklepod leaves affected the germination and the growth of sicklepod itself. Germination and radicle length of sicklepod were reduced by 94% and 99%, respectively, by the treatment of volatiles from 5GDW of leaves(Table 5). Halligan[6] reported that phytotoxic terpenoid compounds of plant species are volatile and inhibit the growth of other plant species. Muller[10] suggested that the volatile terpenes may dissolve in the cuticular layer of the epidermis of mesophyll cell and then pass through the plasmodesmata into the cell. Muller and del Moral[12] suggested that volatile terpenes may be adsorbed on colloidal material in soil and come into contact in this way with roots of affected plants. Based on these reports and our studies, it could be hypothesized that volatiles from sicklepod leaves could also contain terpenoid compounds responsible for allelopathic effect on other plants in the ecosystem. The potency of phytotoxic volatiles from immature seeds was difficult to be exactly compared with that of phytotoxic volatiles from leaves, yet it seems that both volatiles from immature seeds and leaves of sicklepod similarily contribute to the allelopathic effect in the nature.

It has been reported that the growth or yield

Table 5. The effects of volatiles from dried leaves on germination and seeding growth.

Tretment (GDW)	Germination	Seedling growth	Root length
		% of control	
radish		hypocotyl length	
0	100a*	100a	100a
5	84a	16b	23b
10	54a	14b	18b
rice		sheath length	
0	100ab	100a	100a
5	108a	35b	20b
10	89b	39b	21b
mung bean		hypocotyl length	
0	100a	100a	100a
5	104a	46b	53b
10	100a	43b	59b
sicklepod		radicle length	
0	100a	100a	
5	6b	1b	
10	6b	1b	

* Means within column followed by the same letter are not significantly different at the 95% confidene level as determined by Duncan's multiple range test.

of some plants, mainly soybean and cotton are inhibited by sicklepod sharing the habitat and traditionally described these phenomena as competition for light, nutrient, water and space[8,9,21]. According to Buchanan *et al*[3], when cotton was grown with densities of sicklepod ranging from 0 to 32 weeds/15m of row, regression of seed cotton yields on weed density revealed a linear decrease in yield with increasing weed densities by competition. However, the study reported here show that water-soluble and volatile phytotoxin from sicklepod have an allelopathic effect on the germination and the early growth of plants, and that, in contrary to the traditional explains on the interaction between sicklpod and other plants in field studies, the inhibitory effect of competition and allelopathy could be certainly caused by the combined interference of sicklepod against other weeds or crops.

The isolation and the identification of water-soluble and volatiles which are biologically active in sicklepod seeds and leaves are in preceeding. In addition, the observation described above indicates that biologically active substance(s) from sicklepod seeds and leaves may have potential for development to natural hericides. Now more research on several mechanisms of allelopathy and autotoxicity by sicklepod in the agronomic ecosystem is needed to understand the role of sicklepod in the ecosystem and to utilize active allelochemicals from sicklepod as useful compounds in agriculture.

摘　　要

본 실험은 決明子(*Cassia tora* L.)의 재배지에서 타작물이나 잡초의 생육이 저해되는 현상이 단순한 물리적 조건의 경쟁뿐 아니라 타감작용(allelopathy)에 기인함을 탐색함과 동시에 결명자의 자가독성을 확인하는 연구로서, 결명자 식물의 종자와 잎의 수용성 추출액 및 휘발성분이 무우(십자화과), 벼(화본과), 녹두(두과)등의 발아와 유묘성장에 미치는 영향을 살펴 본 결과는 다음과 같다.

1. 결명자 종자의 수용성 타감물질에 의한 저해효과에 대해, 벼는 발아보다 유묘성장이 더욱 민감하게 저해되었고, 반면 무우는 발아가 배축의 길이 성장보다 더 민감하게 저해되었다.

2. 결명자 종자의 수용성 추출액 처리에 의해 추출액의 처리농도가 커질수록 벼 유묘의 엽록소 함량은 무처리구의 50%에서 65%까지 감소하였다.

3. 결명자 잎의 수용성 추출액 처리에 의해 무우는 배축길이와 생체량이, 벼는 발아가 저해되었으나, 녹두는 단지 생체량에서 약간의 저해를 보였다.

4. 결명자 미숙종자의 휘발성분은 무우의 발아와 생육에 저해작용이 있으며, 특히 뿌리의 생장이 발아나 배축성장보다 더 민감하게 저해되었다.

5. 결명자 잎의 휘발성 성분은 무우, 벼, 녹두의 발아나 생육에 저해적인 타감작용 효과를 나타냈으며, 특히 발아보다는 유묘의 성장이 더 많이 저해되

었다.

6. 결명자 잎의 휘발성분에 의해 결명자 스스로의 발아와 유근 성장이 저해되는 자가중독 현상이 확인되었다.

7. 결과적으로, 결명자의 종자와 잎에는 일부 타식물에 대하여 타감작용과 아울러 자가중독을 유발하는 수용성 및 휘발성 물질이 존재하는 것으로 인정된다.

LITERATURE CITED

1. Bell, D. T. and Koeppe, D. E. 1972. Noncompetitive effects of giant foxtail on the growth of corn. Agron. J. 64 : 321~325.
2. Buchanan, G. A. and Burns, E. 1971. Weed competition in cotton. I. sicklepod and tall morninglory. Weed Science. 19 : 576~579.
3. Buchanan, G. A., Crowley, R. H., Street, J. E. and Mcguire, J. A. 1980. Competition of sicklepod (*Cassia obtusifolia*) and redroot pigweed (*Amaranthus retro flexus*) with cotton(*Gossypium hirsutum*). Weed Science. 28 : 258~262.
4. Colton, C. E. and Einhellig, F. A. 1980 Allelopathic mechanisms of velvetleaf(*Abutilon theophrasti* Medic., Malvaceae) on soybean. Amer.J.Bot. 67 : 1407~1413.
5. Drost, D. T. and Doll, J. D. 1980. The allelopathic effect of yellow nutsedge (*Cyperus esculentus*) on corn (*Zea mays*) and soybean (*Glycine max*). Weed Science. 28 : 229~233.
6. Halligan, J. P. 1975. Toxic terpenes from *Artemisia californica*. Ecology. 56 : 999~1003.
7. Hauser, E. W., Buchanan, G. A. Ethredge, W. J. 1975. Competition of Florida beggarweed and sicklepod with peanuts.I. Weed-free maintenance and weed competition. Weed Science. 23 : 368~372.
8. Johnson, B. J. 1971. Effect of competition on sunflowers. Weed Science. 19 : 378~380.
9. Monks, D. W. and Oliver, L. R. 1988. Interactions between soybean(*Glycine max*) cultivars and selected weeds. Weed Science. 36 : 770~774.
10. Muller, C. H. 1965. Inhibitory terpenes volatilized from *Salvia* shrubs. Bull. Torrey Bot. Club 92 : 38~45.
11. Muller, C. H. 1966. The role of chemical inhibition (Allelopathy) in vegetational compositions. Bull. Torrey Bot. Club 93 : 332~351.
12. Muller, C. H. and del Moral, R. 1966. Soil toxicity induced by terpenes from *Savia leucophylla*. Bull. Torrey Bot. Club. 93 : 130~137.
13. Muller, C. H., Muller, W. H. and Haines, B. L. 1964. Volatile growth inhibitors produced by aromatic shrubs. Science. 143 : 471~473.
14. Oleszek, W. 1987. Allelopathic effect of volatiles from some cruciferae species on lettue, barnyard grass and wheat growth. Plant and Soil. 102 : 271~273.
15. Patterson, D. T. 1981. Effect of allelopathic chemicals on growth and physiological responses of soybean (*Glycine max*), Weed Science. 29 : 53~59.
16. Putnam, A.R. 1985. Allelopathic research in agriculture : Past highlights and potential. in The chemistry of allelopathy. Thompson, A. C. Washington, DC. pp.1~8.
17. Putnam, A. R. and Duke, W. B. 1974. Biological suppression of weeds : Evidence for allelopathy in accessions of cucumbers. Science. 185 : 370~372.
18. Rice, E. L. 1984. Allelopathy. 2nd ed. Academic. New York. pp. 130~188.
19. Shettel, N. L. and Balke, N. E. 1983. Plant growth response to several allelopathic chemicals. Weed Science. 31 : 293~298.
20. Teem. D. H., Hoveland, C. S. and Buchanan, G. A. 1980. Sicklepod (*Cassia obtusifolia*) and coffee senna (*Cassia occidentalis*) : Geographic distribution, germination, and emergence. Weed Science. 28 : 68~70.
21. Thurlow, D. L. and Buchanan. 1972. Competition of sicklepod with soybeans. Weed Scence. 20 : 379~384.
22. Waldrop, D. D. and Banks, P. A. 1983. Interactions of 2,4-DB, acifluorfen, and toxaphene applied to foliage of sicklepod (*Cassia obtusifolia*). Weed Science. 31 : 351~354.
23. Walker, H. L. and Boyette, C. D. 1985. Biocontrol of sicklepod (*Cassia abtusifolia*) in soybeans(*Glycine max*) with *Alternaria cassiae*. Weed Science. 33 : 212~215.
24. Wilcut, J. W., Wehtje, G. R., Cole, T.A., Vint Hicks, T. and Mcguire, J. A. 1989. Postemergence weed control systems without dinoseb for peanuts (*Arachis hypogaea*). Weed Science. 37 : 385~391.
25. William, R. D. 1982. The effect of naturally occuring phenolic compounds on seed germination. Weed Science. 30 : 206~212.
26. Wong, S. M., Wong, M. M., Seligmann, O. and Wagner, H. 1989. New antihepatotoxic naphto-pyrone glycosides from the seeds of *Cassia tora*. Planta Medica. 55 : 276~280.
27. 植物營養實驗法編集委員會. 1990. 植物營養實驗法. 博友社(東京, 日本). pp.352~359.