Allelopathic Activity and Determination of Allelochemicals from Sunflower(*Helianthus annuus* L.) Root Exudates.

I. Allelopathic and Autotoxic Effects of Sunflower Root Exudates

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해바라기(*Helianthus annuus* L.) 根分泌物質의 他感作用 및 他感物質의 同定

1. 해바라기 根分泌物質의 他感作用 및 自家抑制作用

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ABSTRACT

There was no significant difference in the final germination percentages(Experiment 1-3) as affected by sunflower root exudates between the control and the treated for the test species. In general(Experiment 1), however, germination onset was delayed the treated bottles. Germination rate was, also, reduced for both radish(*Raphanus sativus* L.) and rice(*Oryza sativa* L.). Therefore, the germination index was low in the treated bottles but germination gradually increased with time in the greated bottles in all test species so that the final germination percentages were similar between treatments. The root exudates of sunflower had significant inhibitory effects(Experiment 1-3) on the lengths of the shoots and roots of all the test species. Fresh weight was also significantly reduced in all test species.

Sunflower seedlings(Experiment 3) in the treated(with the XAD-4 resin column) were larger and healthier than those in the control(without XAD-4 resin column) because of the removal of allelochemicals. The fresh weight of sunflower seedlings was markedly inhibited by sunflower root exudates. These mean that sunflower probably is an autotoxic crop.

INTRODUCTION

H. annuus is one of the important crops in the world. It is reported to be an allelopathic species (Wilson and Rice, 1968 ; Hall et al., 1982) and has considerable variations for this trait(Leather. 1983 b).

Many studies have been done which demonstrate the allelopathic nature of the effects of weeds on crop growth and development(Tukey, 1969 ; Putnam and Duke, 1978 ; Rice, 1979, 1984). In such studies considerable attention has been given to the role of allelopathic interactions between different crop plants or the inhibitory effects of phytotoxins produced by weeds on crops.

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Allelochemicals present in all plant tissues may be released in different ways including root exudation, leaching, decomposition and volatilization. Root exudation may play a major role on the allelopathic effect in agroecosystems. Much of the evidence for root-mediated allelopathy has come from studies where solutions passing by the root systems of one plant are recycled into media containing the indicator species.

A study on root exudation by Tang and Young (1982) successfully utilized an adsorptive column (XAD-4) to selectively trap organic and hydrophobic root exudates while allowing nutrient ions and other hydrophilic compounds to pass through. amberlite XAD-4 is more effective ($725m^2/g$) in adsorbing nonpolar solutes from polar solvents compared to the most commonly used solvents (e. g., methanol and acetone) which are water miscible (Putnam and Tang, 1986). This continuous root exudate trapping system (Tang and Young, 1982) makes possible the trapping of fixed compounds in the root system while being free of physical disturbance.

The objectives of this study were to dertermine the inhibitory effects of sunflower root exudates on early growth of *Echinochloa colona* (L.) Link, radish, and upland rice and to evaluate autotoxicity from the root exudates of sunflower.

MATERIALS AND METHODS

Experiment 1. Inhibitory Effect of Germinating Sunflower Seeds

This experiment was conducted *in vitro*. Bottles (266ml) containing 1.5% (w/v) agar, with cylindrical rings (25% 10mm) cut from stainless steel tubing fixed at the center of each dish, were autoclaved. Seeds of sunflower, *E. colona*, radish, and upland rice (cv. UPLRi-5) were sterilized for 30 minutes in 2% chlorox, then rinsed with distilled water. One sunflower seed together with six seeds of either *E. colona*, radish, of rice were planted inside the inner cylindrical ring. Outside the inner ring, sixteen seeds of *E. colona*, radish, and rice were planted as controls (Fig. 1).

The bottles were kept under continuous light in an incubator at 28℃ and 80% relative humidity. Each treatment was replicated 10 times in a completely randominized design.

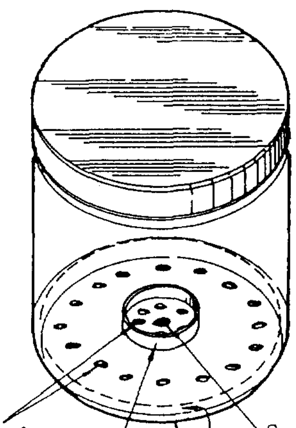

Fig. 1. Experimental setup to examine the effect of germinating sunflower on test species growth.

After 10 days. percentage germination, lengths of shoots and roots, fresh weights of shoots and roots, and total fresh weight of each test species were measured. Data were analyzed using least significant difference test and Duncan's multiple range test.

Experiment 2. Seedling Growth of Upland Rice. *Echinochloa colona* (L.) Link, and radish as Affected by Inhibitory Exudates from Germinating Sunflower

A continuous trapping apparatus (Fig. 2) was installed at the International Rice Research Institute (IRRI) phytotron under the following conditions : temperature (day/night), 28/21℃ ; relative humidity, 70% ; light, natural. A continuous root exudate collecting system developed by Tang and Young (1982) was modified and used to grow donor plants.

This experiment consisted of three parts : One had sunflower as the donor and upland rice as the receiver, another had sunflower as the donor and *E. colona* as the receiver, and the third had sunflower as the donor and radish as the receiver.

The seedbeds were filled with a 20mm layer of

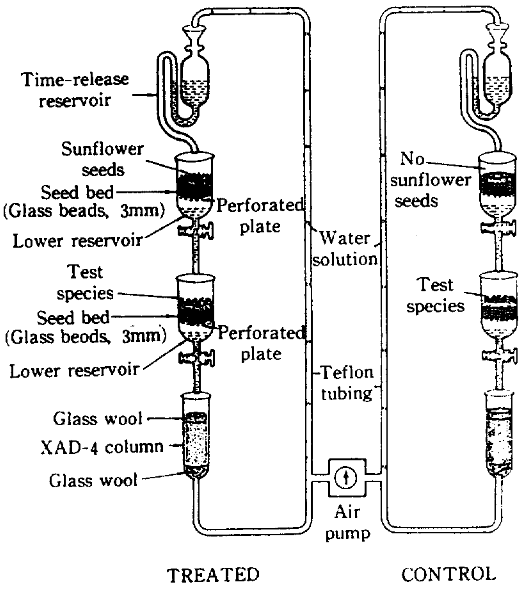

Fig. 2. The continuous hydrophobic exudate trapping apparatus.

glass beads (outer diameter, 3mm). The columns were filled with XAD-4 resin (Fig. 2). The XAD-4 resin which was contaminated with aromatic impurities was cleaned by Soxhlet extraction using acetone (HPLC grade), acetonitrile (HPLC grade), and methanol (HPLC grade), each for 24h and then the column (22×183mm) was packed with 12g of resin as an aqueous slurry. The residual methanol was removed by washing with distilled water and the column was dried at 70℃.

Seeds (sunflower, upland rice, radish, and *E. colone*) of uniform size and appearance were selected. These were sown on the surface of the appropriate seedbeds (Fig. 2).

The columns filled with resin were then connected to a circulating attachment with Teflon tubing connected to an air pump (Nippon Jisei Sangyo Co., Japna) and a Soxhlet type time-release reservoir. Distilled water was circulated at a rate of about 15ml/min by an air pump and seeds were irrigated by water released periodically from the time-release reservoir.

The water flooded the seedbed (donor, sunflower) briefly and then drained into the lower seedbed (receivers ; upland rice, radish, and *E. colona*) and finally drained into the lower XAD-4 resin column. Hydrophobic exudates leached from the germinating sunflower seeds through a continuous trapping apparatus were adsorbed by the XAD-4 column while water was transported back to the Soxhlet time-release reservoir using the air pump.

Water was replenished twice daily with distilled water to compensate for aspiration and evaporation losses. The total volume of circulating water was maintained at about 150ml. The flow rate of water was adjusted to 4ml/min.

The seeedbed was protected from light by aluminum foil, which eliminated possible photoconversions of exudates.

This experiment was replicated three times. Data (percentage germination, lengths of shoots and roots, fresh weights of shoots and roots, and total fresh weights) were collected 10 days after seeding.

Experiment 3. Autotoxicity from the Root Exudates of sunflower

A continuous root exudate collecting system developed by Tang and Young (1982) was modified (Fig. 3). The experiment was conducted at the IRRI phytotron under the following conditions : temperature (day/night), 28/21℃ ; relative humidity, 70% ; light, natural. Sunflower seeds of uniform size and appearance were used.

The seedbeds were filled with a 20mm layer of glass beads (outer diameter, 3mm). Column preparation was the same as in Experiment 2. A control

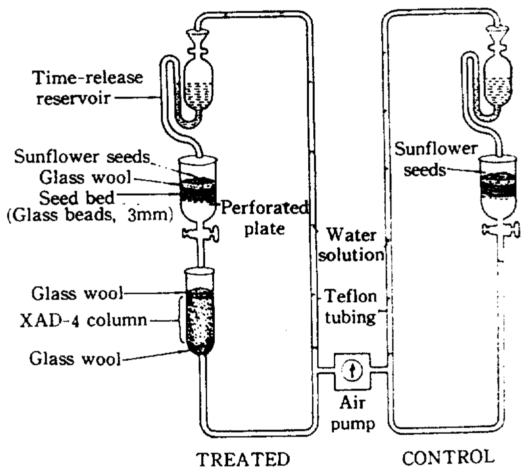

Fig. 3. Continuous exudate trapping apparatus used to determine autoxicity of sunflower.

treatment was set up without an XAD-4 resin column. The column and circulating attachment were then connected to the bottom of the lower reservoir using Teflon tubing.

The solution was circulated at a rate of about 15 ml/min by an air pump and the seeds were irrigated by water released periodically from a Soxhlet type time-release reservoir. This experment was replicated three times. Data (percentage germination, lengths of shoots and roots, fresh weights of shoots and roots, and total fresh weights) were collected at 15 days after seeding.

RESULTS AND DISCUSSION

Experment 1. Inhibitory Eeffect of Germinating Sunflower Seeds

Germination

When sunflower seed was planted in the center of bottles containing agar, a light brown exudate was observed. However, there was no significant difference in the final germination percentages between the control and the treated for the test species (Fig. 4). The highest concentrations reduced the rate of germination, but had no effect on total percent germination after 10 days.

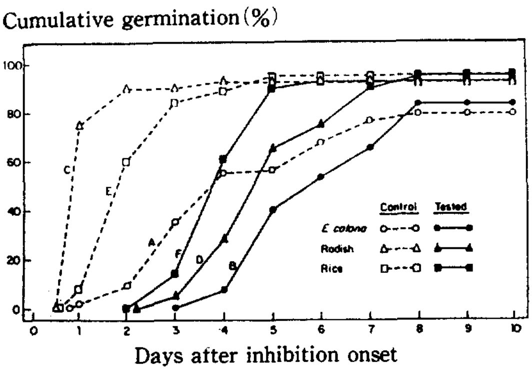

Fig. 4. Cumulative germination of test species as affected by sunflower root exudate.

In general, germination onset was delayed in the treated bottles (Fig. 4 and Table 1). Germination rate was, also, reduced for both radish and rice. Therfore, the germination index was low in the treated bottles but germination gradually increased with time in the treated bottles in all test species so that the final germination percentages were similar between treatments. This implies that the concentration of inhibitor in the exudates was low enabling the test species to recover with time.

The total percentage of seed that germinate does not necessarily give an accurate measure of the effects of an inhibitor. When germination rate, and germination index were examined, it was observed that germination of seed treated with distilled water was markedly different from seed treated with exudates of sunflower roots (Table 1).

In addition, Schon and Einhellig (1982) reported that leaf leachates, decaying litter, root extract and field soil of cultivated sunflower significantly reduced the germination and seedling growth of yellow soybean.

Seedling growth and fresh mass accumulation

The root exudates of sunflower had significant inhibitory effects on the lengths of the shoots and roots of all the test species (Table 2). Based on shoot and root lengths (Table 2), there was a greater inhibitory effect on *E. colona* and radish than rice. Fresh weight was also significantly reduced in all test species (Table 3). Among the three species, radish was the most affected, followed by *E. colona*, and rice.

Leather (1983a) reproted that seedling growth of *Abutilon theophrastic* Medic., *Datura stramonium* L., *Ipomoea purpurea* (L.) Roth., and *Brassica kaber* (DC.) L.C. Wheeler was inhibited by leachates of Hybrid 201 sunflower leaf and stem tissue (Leather, 1983b) and root exudates inhibited

Table 1. Germination index of different treatments as affected by sunflower root exudates.

PARAMETERS	CURVES					
	A	B	C	D	E	F
Maximum germination, %	79	83	93	95	95	92
Germination onset, days	0.8	3.0	0.5	2.2	0.6	2.0
Germination rate, % day^{-1}	10.9	16.6	26.6	16.4	21.6	23.0
Germination	1076	459	4948	708	3420	1058

Table 2. Effect of root exudates of sunflower seedlings on seedling growth of test species[a].

TEST SPECIES	SHOOT LENGTH (mm)			ROOT LENGTH (mm)		
	Control	Test	Difference	Control	Test	Difference
Echinochloa colona	29.3	19.6	9.7**	20.1	12.7	7.4**
Radish	88.3	66.6	21.7**	55.8	34.0	21.8**
Rice	45.0	36.7	8.3**	49.2	39.3	9.9**

[a] Average of 30 seedlings.
** Significantly different from the control at the 1% level.

Table 3. Effect of root exudates of sunflower seedlings on fresh weight of test species[a].

TEST SPECIES	FRESH WEIGHT (mg/plant)								
	Shoot			Root			Total		
	Control	Test	Difference	Control	Test	Difference	Control	Test	Difference
Echinochloa colona	3.0	2.2	0.8*	1.8	1.1	0.7*	4.8	3.3	1.5*
Radish	170.7	102.0	68.7**	30.7	13.69	16.8*	201.4	115.9	85.5**
Rice	13.8	11.3	2.5*	12.4	10.8	1.6*	26.2	22.1	4.1*

[b] Average of 30 seedlings.
* Significantly different from control at the 5% level.
** Significantly different from the control at the 1% level.

Abutilon theophrastic Medic. and *Datura stramonium* L. growth. Field studies (Leather, 1987) were conducted to determine if season-long weed control could be achieved by combining the use of a herbicide with the natural allelochemicals produced by cultivated sunflower. The weed biomass was reduced equally in plots planted with sunflower, whether or not herbicide was applied. Also, Curtis and Cottam (1950) reported that sunflower influences succeeding generations through decomposition and release of allelochemicals from underground plant parts.

Experiment 2. Inhibition of Seedling Growth of Tesh Species by Root Exudates of Sunflower

Germination and seedling growth

The root exudates of sunflower had no effect on germination of all the test species. However, sunflower significantly inhibited seedling growth of *E. colona* and radish. Rice was not affected (Table 4). The tesh species varied in response to the root exudates of sunflower, with radish being most sensitive (Fig. 5). E.Colona showed clear differences in shoot growth (% of control : 90) and root growth (% of control : 86) between treatments (Fig. 5).

Stevens and Tang (1985) reported that *Bidens pilosa* L. root exudates significantly inhibited growth of lettuce (*Lactuca sativa* L.), beans (*Phaseolus vulgaris* L.), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* (L.) Moench).

Fresh weight accumulation

Fresh weights closely agreed with the results for the lengths of shoots and roots (Table 5). Of the test

Table 4. Effect of sunflower root exudates on seed germination and seedling growth of test species[a].

TEST SPECIES	SEED GERMINATION (%)			MEAN LENGTH (mm)					
				Shoot			Root		
	Control	Test	Difference	Control	Test	Difference	Control	Test	Difference
Echinochloa colona	67.0	69.3	+2.3ns	45.2	40.6	4.6*	65.9	56.6	9.3*
Radish	93.3	95.7	+2.4ns	83.2	68.7	14.5*	109.3	79.2	30.1**
Rice	97.0	93.1	3.9ns	116.2	119.3	+3.1ns	81.2	82.3	1.6ns

[a] Average of 30 seedlings.
* Significantly different from the control at the 5% level.
** Significantly different from the control at the 1% level.
ns = Not significant.

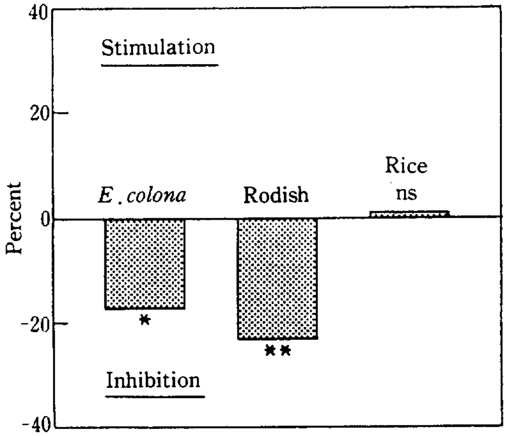

Fig. 5. Percentage inhibition of total fresh weights of test species by sunflower root exudates. (*Significantly different from the control at the 5% level, **Significantly different from the control at the 1% level, ns=Not significant)

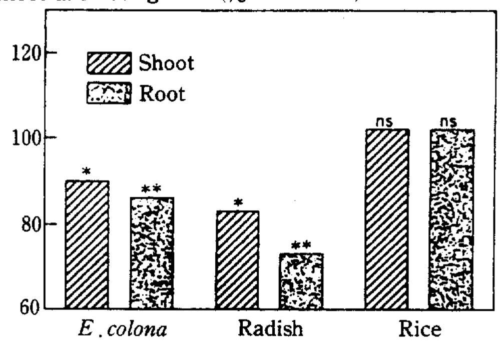

Fig. 6. Effect of sunflower root exudates on seedling growth of test species. (*Significantly different from the control at the 5% level, **Significantly different from the control at the 1% level, ns=Not significant)

species, radish was most affected. Sunflower exudates reduced fresh weight of radish by 23% which was significant at the 1% level. Fresh weight of *E. colona* was reduced by 17% which was significant at the 5% level (Fig. 6) The fresh weight of rice was not affected by the sunflower exudates.

Lovett and Jokinen (1984) reported that live *Agropyron repens* (L.) Beauv. affected the length of barley roots and total dry weight of barley from the commencement of growth and the effects were evident in the morphological and physiological characteristics at harvest. Interference (competition+allelopathy) effects of fescue (*Festuca arundinacea* Shreb.) on the growth of sweetgum (*Liquidambar styraciflua* L.) were tested in the greenhouse (Walters and Gilmore, 1976). Fescue seeded into pots containing sweetgum seedlings resulted in sweetgum dry weight reduction from 29 to 95%. Elimination of the competitive effect through the use of a stairstep apparatus implied an allelopathic mechanism in the interference of sweetgum growth by fescue. Leachates from the rhizosphere of live fescue resulted in reduction of up to 60% in dry matter production of sweetgum seedlings.

Water soluble phytotoxins may be leached from roots or aboveground plant parts (Bell and Koeppe, 1972) or actively exuded from living roots on environmental stimulus.

Bell and Koeppe (1972) found that when maize was seeded into pots with 6 week-old *Setaria faberii* Herrm, maize height, fresh weight, and dry weight were reduced by as much as 90% when compared to comparable plants grown in monoculture. Mature *S. faberii* inhibited the growth of maize approximately 35% through an allelopathic mechanism.

Table 5. Effect of sunflower exudates on fresh weight of test species[a].

TEST SPECIES	FRESH WEIGHT (mg/plant)								
	Shoot			Root			Total		
	Control	Test	Difference	Control	Test	Difference	Control	Test	Difference
Echinochloa colona	16.3	13.8	2.5*	12.2	10.2	2.0*	29	24	5.1*
Radish	236.0	180.7	55.3**	24.8	20.3	4.5**	261	201	60.0**
Rice	437.8	450.0	+12.2[ns]	285.0	288.0	+3.0[ns]	723	738	15.0[ns]

[b] Average of 30 seedlings.
* Significantly different from control at the 5% level.
** Significantly different from the control at the 1% level.
[ns]=Not significant.

Experiment 3. Autotoxicity from the Root Exudates of Sunflower

Sunflower seedlings in the treated (with the XAD-4 resin column) were larger and healthier than those in the control (without XAD-4 resin column) because of the removal of allelochemicals (Table 6). Without the column, however, seedlings in the control were stunted and the roots appeared brownish.

There is some evidence indicating that plant exudates can inhibit or stimulate the growth of other plants and microorganisms (Rice, 1984 and 1979; Hale et al., 1978), but little is known of autotoxicity by root exudates. Autotoxicity could be significant in pure stands of long-term crops and in some continuous cropping systems, particularly cash crops. For example, Tang and Young (1982) reported that asparagus residues can inhibit the shoot and root growth of their own species. Therefore, autointoxication from root exudates of asparagus can be a problem in continous cropping of asparagus.

Germination and seedling growth

There was no difference in percentage germination between treatments which may be due to the low concentration of inhibitors (Table 6). However, mean lengths of shoots and roots differed between treatments. Compared to the treated, the mean lengths of the shoots and roots in the control were reduced by about 25%.

Fresh weight

The fresh weight of sunflower seedlings was markeldy inhibited by sunflower root exudates (Table 7). The inhibition of shoot fresh weight was greater than that of root fresh weight. Compared to the treated, total fresh weight of the control was reduced by 42%. These data suggest that sunflower is an autoinhibited species whose root exudates inhibit its own growth.

Table 7. Inhibtion of fresh weight of sunflower seedligns by sunflower root exudates.[a]

TREATMENT	FRESH WEIGHT (mg/plant)		
	Shoot	Root	Total
Control (Without XAD-4 resin)	318 b (48)	159 b (26)	477 b (42)
Treated (With XAD-4 resin)	608 a	216 a	824 a

[a] Average of 10 seedlings. In a column, means followed by the same letter are not significantly different by DMRT at the 5% level. Percent inhibition indicated in parenthesis.

Based on demonstrated weed inhibition by sunflower in field plots, Leather (1987) conducted an experiment to determine if season-long weed control could be achieved by exploiting the use of herbicides for weed control early in the season and the allelopathic effects of cultivated sunflowers for late-season weed control. Weed control diminished in the second year in all plots. This was attributed to reduced emergence of sunflower in second-year plots, as a result of autotoxicity from sunflower in second-year plots, as a result of autotoxicity from sunflower crop residues remaining after the first-year harvest.

A replant problem in continuous cropping or rotation systems suggests a chemical effect of substances from previous crops or their residuces in the soil. Among the possible causes, self-allelopathy or autointoxication has often been suggested (Dommergues, 1978). Wang and his associates (1967) reported the decline of sugarcane yield in Taiwan, and found that five growth inhibitors were present in the soil. They indicated that these inhibitors were plant-originated and came from the debris of sugarcane decomposing in the soil. Similarly, the productivity

Table 6. Inhibition of growth of sunflower seedlings by sunflower root exudates[a].

TREATMENT	SEED GEMINATION (%)	SHOOT LENGTH ((mm)	ROOT LENGTH (mm)
Control (Without XAD-4 resin)	81.7 a	52.5 b	52.7 b
Treated (With XAD-4 resin)	83.6 a	69.7 a	72.9 a

[a] Average of 10 seedlings. In a column, means followed by the same letter are not significantly different by DMRT at the 5% level. Percent inhibition indicated in parenthesis.

of rice in the second crop is generally lower than that in the first crop.

摘　要

해바라기의 他感作用(Allelopathy) 및 天然除草劑로서의 開發可能性에 관한 試驗을 1988-1991年에 걸쳐 國際米作硏究所(IRRI) : 農學, 生化學部 및 國立필리핀 大學校 化學科(天然物 硏究室)에서 遂行하여 얻은 結果中 해바라기의 生育中 뿌리에서 分泌되는 他感作用 및 自家抑制作用에 關한 硏究結果는 다음과 같다.

1. 해바라기의 根分泌物質은 對照區에 비해 전체적인 發芽率에는 有意的인 差異가 인정되지 않았지만 發芽始등에는 다소 큰 影響을 미쳤음.
2. 生物檢定材料의 地上部, 地下部 生長 및 전체 生體重에는 유의적인 減少가 있었음.
3. XAD-4 Resin을 부착한 試驗 3에서 해바라기는 對照區에 비해 전반적인 生長量이 많은 것으로 보아 自家抑制作用이 인정되었으며 이는 Resin에 의한 他感物質(Allelochemicals)의 坂着效果로 推定된다.

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