

Assessment of Allelopathic Potential of Some Weed Species on Alfalfa (*Medicago sativa* L.) Germination and Early Seedling Growth

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알팔파 발아와 초기생육에 대한 잡초종의 Allelopathic 잠재성 평가
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ABSTRACT

Greenhouse and laboratory studies were conducted to investigate allelopathic potential of some weed species on alfalfa (*Medicago sativa* L.) germination and seedling growth. In the comparison between top(leaves+stems) and root extracts, top extract exhibited greater allelopathic effects on alfalfa germination than that of root. The various weed species extract differently responded to alfalfa test species, WL-320, in terms of allelopathic effect. Top and root aqueous extracts of lambsquarter (*Chenopodium album* L.), giant foxtail (*Setaria faberii* Herrm.), redroot pigweed (*Amaranthus retroflexus* L.), velvetleaf (*Abutilon theophrasti* Medic.), crabgrass (*Digitaria sanguinalis* L.), canada thistle (*Cirsium arvense* L.) and prostrate knotweed (*Polygonium aviculare* L.) significantly inhibited germination, seedling length, weight, vigor, and rate of germination of alfalfa. The regression slopes of various top extracts showed that velvetleaf (b=3.69) extracts were the most inhibitory, while large crabgrass (b=2.39) extracts had the least allelopathic effect on alfalfa germination. Germination, seedling length and weight of alfalfa were inversely proportional to the concentration of dried velvetleaf extracts. Also, more of the toxic effects were observed from the dried extracts compared to the fresh extracts. Residue of velvetleaf inhibited significantly alfalfa emergence and survival percentage compared to the control. The emergence and survival percentage of alfalfa were 44%, 57% at 1.0% residue treatment, respectively. When weed residues were mixed with silica sand with incubation time, velvetleaf residue most inhibited alfalfa growth. The degree of inhibition increased as incubation time increased. An incubation for 72h caused the

Key Words : Allelopathy, Weed Residue, Germination Percentage, Seedling Growth, Seedling Weight

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greatest inhibition of alfalfa growth. These results demonstrate the different allelopathic activity of weed species extracts on alfalfa and suggest that weed may affect alfalfa growth and development through the inhibitory effects of allelochemicals present in weed tissue.

INTRODUCTION

Allelopathic effects on crop growth and development have been reported in perennial weeds^{5,10,27} and annual weeds^{2,3,5,8,9,11,25,28}. Velvetleaf, a common weed in soybean field, has been suggested to have an allelopathic interference on soybean^{9,11}. Root residue of giant foxtail^{5,25}, crabgrass^{19,25}, canada thistle^{27,28}, inhibited the germination and growth of maize.

The successful establishment of alfalfa, a major source of high protein forage, depend upon adequate control of competing vegetation, and this is important for no-tillage seedlings. Aqueous extracts of alfalfa shoots and root material have been reported to inhibit the growth of alfalfa¹⁸. They observed that alfalfa is more affected by shoot than root extracts. However, little information is available on the effect of weed in establishing alfalfa. When alfalfa stands deteriorate through winter kill and disease, weeds become quickly established and directly competitive for growth resources. There have been no studies reporting the effects of weed species extracts on alfalfa. Therefore, an experiment was conducted to determine the allelopathic effects of weed extracts on alfalfa seed germination and seedling growth.

The objectives of this experiment were (I) to evaluate the variation among weeds species and relative inhibitory effects of seven different weed species top and root extracts ; (II) to assess the toxicity of various concentration of velvetleaf top extracts and the rate of velvetleaf residue ; (III) to compare between extracts of dried and fresh on alfalfa germination and seedling growth ; (IV)

to evaluate the effect of residue incubation time on alfalfa growth.

MATERIALS AND METHODS

Seven common weed species, lambsquarter (*Chenopodium album* L.), giant foxtail(*Setaria faberii* Herrm.), redroot pigweed(*Amaranthus retroflexus* L.), velvetleaf(*Abutilon theophrasti* Medic.), crabgrass(*Digitaria sanguinalis* L.), canada thistle(*Cirsium arvense* L.), and prostrate knotweed (*Polygonium aviculare* L.) were collected at vegetative stage including roots. All weeds were separated into top(leaves+stems) and root parts. Excess soil was shaken from the roots and residual soil and other adherents were removed from the roots with a brush. The roots were not washed with water to avoid losing any water soluble toxic substances that might be present. The tops and roots were divided into dry and fresh residue. A Half of the sample were air dried for 5weeks under room temperature. The dried roots and tops were ground in a Wiley mill to pass through a 40 mesh screen. Ground samples were stored in plastic bottles in 5°C refrigerator until use. Undried tissue as fresh sample was used immediately for extraction.

Laboratory Study

1) Toxicity Comparison of Dried Top and Root Extract

The top(leaves+stems) and root extracts of the seven different weed species were extracted by shaking 5g dried plant materials with distilled water(100ml) at 24°C room temperature for 24h in 250ml Erlenmeyer flasks. Extracts were filtered through filter paper(Whatman No.42) and 0.2µm

Nalgene filterware unit(Becton & Dickinson Labware, Lincon Park, NJ) to prevent fungal contamination during the experiment. Alfalfa(WL-320) seeds, 20g(WL-320), were surface sterilized with 10 : 1 ratio(water : Clorox) for 5 min, then rinsed several times with distilled water. One hundred sterilized seeds were placed in each heat sterilized 9cm petri-dish with a filter paper (What-man No.4). Extract(10ml) was added to a petri-dish. Distilled water was used as a control. The petri-dishes were placed in a 25°C room. Germination was measured daily for 4d at 24h intervals. To avoid confusion, a seed was considered to be germinated only when the radicle totally protruded from the seed coat. Rate of germination was calculated by dividing the number of germination seed each day by the number of days and adding the values¹⁶⁾. Seeding vigor(SV) was calculated by multiplying total seed germination and radicle length²³⁾. After 5d, 10 seedlings were randomly chosen from each petri-dish for measurements. Radicle and hypocotyl length were measured and plants were separated into cotyledons, hypocotyl, and radicle parts to determine dry weight. The samples were dried im a forced air oven at 70°C for 3h. Also, variation with respect to germination percentage, aqueous extract of progressively increasing concentration were prepared using 5.0, 10.0, 15.0, and 20.0g of seven dried weed tissues per 100ml distilled water.

2) Concentration Study with Velvetleaf Extraction

This experiment was conducted using water extracts of velvetleaf in petri dishes. Since velvetleaf has the greast inhibitory effect on alfalfa seed germination and seedling growth based on top extract results, the inhibition by velvetleaf extract concentration was studied in more detail. Rates of 0, 5.0, 10.0, 15.0, 20.0% (w/v) were prepared by soaking dried ground top

residues with 100ml distilled water at 24°C room temperature for 24h in 250ml Erlenmeyer flasks. Extracts were filtered through filter paper (Whatman No.42) and 0.2µm Nalgene filterware unit.

3) Comparison of Dried and Fresh Residue Extract

This experiment was to compare extract of dried and fresh velvetleaf would inhibit germination and seedling length of alfalfa seed. This experiment was done in petri dishes using aqueous extract of fresh(tops+roots) and dried (tops+roots) of velvetleaf at 5%(w/v). The dried extract(5g) was prepared as described above. The fresh extract of the velvetleaf was extracted by homogenizing 5g of plant material in a blender with 5ml distilled water for 15min. These extracts were filtered through four layers of cheese cloth, and centrifuged at a low speed (3000rpm) for 4h. The supernatant was filtered through 0.2µm Nalgene filterware unit(Becton Dickinson Labware, Lincoln Park, NJ).

Greenhouse Study

1) Effects on Alfalfa Survival and Emergence Percentage

Rates of 0, 0.25, 0.5, 0.75, and 1.0%(w/w) of velvetleaf residue were mixed thoroughly with silica sand(500g). One hundred sterilized alfalfa (WL-320) seeds were planted uniformly 1cm deep in each pot. Each pot was placed on a brown plastic saucer in the green house and covered with moistened filter paper to reduce water loss. Hoagland solution I¹⁴⁾ was added to the saucers as needed to maintain moisture for seed germination. A no residue treatment was used as the untreated control. The percentage of alfalfa seedling emergence was counted at 5DAP (days after planting). A second count was done 10 DAP when cotyledons were extended. Survival rate was calculated by dividing second count by

first count.

2) Incubation Treatment of Weed Species

Dried and powdered alfalfa residue(5g) from seven weeds species was mixed with silica sand (500g) and each pot was placed on a brown plastic saucer. Hoagland solution I¹⁴⁾ was supplied to the mixture for decaying. These mixtures were placed in the green house 25C for 2, 4, and 6 day, respectively. Fifty sterilized alfalfa(WL-320) seeds were planted uniformly 1cm deep in each pot and covered with moistened filter paper to maintain moisture for seed germination. A no residue treatment was used as the untreated control.

Statistical Analysis

Dried top and root aqueous extract comparison experiments of seven different weed species and concentration study with velvetleaf extract and comparison experiment between fresh and dried residue of velvetleaf repeated three and four times with four replication, respectively in the laboratory. Residue rate decaying study was repeated twice with eight replications in the greenhouse. These experiments were arranged in a completely randomized design. Averages for

each replication of the these experiments were polled and subjected to statistical analysis. Analysis of variance for all data was carried out in the general linear model procedure of the all data was carried out in the general linear model procedure of the statistical analysis system (SAS) program²⁴⁾. The pooled mean values for the treatment were separated using least significant difference(LSD) at the 0.05 probability level.

RESULTS

Laboratory Study

1) Toxicity Comparison Dried Top and Root Extract

Germination percentage, seedling length and weight, vigor, and germination rate as affected by weed tops and root extracts were presented in Tables 1 and 2.

Top and root extract of the seven different weed species significantly reduced germination, seedling length, weight, and vigor, and germination rate of alfalfa compared to the control. Top degree of allelopathic activity was different among the weed species in this study. Among

Table 1. Allelopathic effects of various weeds aerial part extract on alfalfa germination, seedling growth and weight, seedling vigor, and rate of germination inhibition.

Weed Species	GP ¹	RL ¹	HL ¹	CW ¹	RW ¹	HW ¹	SV ¹	RG ¹
	—%—	cm		mg			—%—	
Canada Thistle	17.0b	40.0c	15.1b	28.0b	34.0b	34.1c	50.0c	25.1b
Crabgrass	13.2b	35.2b	9.0b	24.0b	34.0b	13.2b	42.6b	47.3d
Giant Foxtail	29.0c	53.4f	24.1e	44.0e	54.0d	40.0e	72.8e	36.0c
Lambsquarter	40.5d	53.0f	24.3d	38.0d	54.0d	38.5d	72.4e	55.1e
Pigweed	20.1b	45.0d	18.4c	32.0c	40.0c	34.7c	56.0c	41.7d
Velvetleaf	42.9d	56.0g	30.1f	50.0f	70.0e	47.3f	72.3e	63.2f
Prostrate Knot	22.0b	50.0e	21d	38.0d	54.0d	34.8c	61.4d	44.0d
Control	0a	0a	0a	0a	0a	0a	0a	0a

¹ GP, Germination Percentage ; RL, Radicle Length ; HL, Hypocotyl Length ; CW, Cotyledons Weight ; RW, Radicle Weight ; HW, Hypocotyl Weight ; SV, Seedling Vigor ; RG, Rate of Germination.

* Values within a column followed by the same letter are not significantly different at the 0.05 level as determined by least significant difference(LSD).

Table 2. Allelopathic effects of various weeds root extract on alfalfa germination, seedling growth and weight, seedling vigor, and rate of germination inhibition.

Weed Species	GP ¹	RL ¹	HL ¹	CW ¹	RW ¹	HW ¹	SV ¹	RG ¹
	—%—	cm		mg			—%—	
Canada Thistle	14.2b	35.1b	15.4c	22.1b	30.2b	25.1c	42.1b	24.0b
Crabgrass	11.0b	32.0b	9.0b	20.3c	30.0b	3.2b	39.7b	35.1c
Giant Foxtail	23.0c	43.5c	21.3e	37.5d	50.1e	34.2d	55.1c	26.1b
Lambsquarter	22.1c	43.0c	18.0d	34.6d	40.3d	34.1d	54.3c	40.3d
Pigweed	20.0c	35.6b	15.2c	25.0c	34.1c	28.6c	51.0c	45.6d
Velvetleaf	38.5d	50.5d	21.0e	47.8e	60.7c	50.8e	69.8d	57.1e
Prostrate Knot	20.3c	40.7c	15.0c	27.5c	34.2c	28.4c	51.2c	24.1b
Control	0a	0a	0a	0a	0a	0a	0a	0a

¹ GP, Germination Percentage ; RL, Radicle Length ; HL, Hypocotyl Length ; CW, Cotyledons Weight ; RW, Radicle Weight ; HW, Hypocotyl Weight ; SV, Seedling Vigor ; RG, Rate of Germination.

* Values within a column followed by the same letter are not significantly different at the 0.05 level as determined by least significant difference(LSD).

Table 3. Total germination of alfalfa as a function of increasing extract concentration using dried seven weed species extracts¹⁾.

Weed Species	Regression Equation Y=a+bX	R ²
Canada Thistle	Y=88.68-2.59X	0.82**
Crabgrass	Y=89.93-2.39X	0.88**
Giant Foxtail	Y=81.35-3.50X	0.82*
Lambsquarter	Y=83.28-3.40X	0.76**
Pigweed	Y=89.50-3.43X	0.95***
Velvetleaf	Y=81.08-3.69X	0.87***
Prostrate Knotweed	Y=87.68-3.42X	0.90*

*p<0.05 ; **p<0.01 ; ***p<0.001

1) Concentration : 0, 5, 10, 15%(w/v)

the different weed species studied, velvetleaf had the greatest inhibitory effect on germination, seedling length and weight, seedling vigor, and rate of germination on alfalfa. This trend was followed by giant foxtail.

Germination percentage, radicle and hypocotyl length, and cotyledons, radicle and hypocotyl weight, vigor, and rate of germination of alfalfa were significantly inhibited by tops and root extracts of both velvetleaf and giant foxtail. The extraction of large crabgrass was found to have the least allelopathic effect on alfalfa.

Regression equations for germination of all weed species top extraction were presented in Table 3. The slope of the regression line was varied for different weed species. This indicated that alfalfa germination responded differently to each weed species extract. Velvetleaf(b=3.69) had the highest regression value followed by giant foxtail. These species were comparatively the most inhibitory for alfalfa germination. Crabgrass (b=2.39) and canada thistle(b=2.59) were less inhibitory for alfalfa germination.

2) Concentration Study with Velvetleaf Extraction

Significant reduction in total germination, seedling length and weight of alfalfa was observed as the extract concentration increased up to 20%(Table 4). Such a reduction response was concentration dependent. The highest extract concentration(20%, w/v) of velvet leaf reduced germination, total seedling length and weight of alfalfa, by 60%, 61% and 53% respectively, when compared to the control. In seedling growth, root growth was more sensitive than shoot growth. These result was similar to those obtained top and root extract.

Table 4. The effects of concentration of velvetleaf on alfalfa germination and seedling growth inhibition.

Concentration Weed (%, w/v)	GP ¹	RL ¹	SL ¹	CW ¹	RW ¹	SW ¹
	— % —	cm		mg		
Control	0	0	0	0	0	0
5.0	13.8	30.2	11.8	36.2	38.7	18.4
10.0	34.3	44.3	26.5	52.6	48.6	38.3
15.0	51.1	53.7	39.0	55.9	62.7	57.1
20.0	59.6	71.1	48.5	60.6	69.8	66.3
LDS(0.05)	3.87	0.20	0.16	0.15	0.13	0.07
CV(%)	4.22	6.07	4.27	9.54	2.43	8.37

¹ GP, Germination Percentage ; RL, Radicle Length ; SL, Shoot Length ; CW, Cotyledons Weight ; RW, Radicle Weight ; HW, Hypocotyl Weight

Table 5. The comparison of fresh and dried extracts of velvetleaf on germination and seedling length and weight, and germination rate.

Treatment ¹	Germination Percentage(%)	Seedling Length(cm)	Seedling Weight(mg)	Germination Rate(%)
Dried Extract	53.8	4.4	1.40	24.7
Fresh Extract	68.2	5.4	1.96	32.3
Control	89.0	7.1	2.80	61.34
LSD(0.05)	3.83	0.12	0.04	2.10
CV(%)	4.00	1.54	1.50	4.00

3) Comparison of Dried and Fresh Residue Extract

Dried and fresh extract of velvetleaf significantly reduced the germination percentage, seedling length and weight, and germination rate in this study when compared to control(Table 5). Germination percentage and rate of germination of dry(top+root) and fresh(top+root) part extract were 54%, 68%, and 25%, 32%, respectively.

Seedling length and dry weight of alfalfa were inhibited by dried and fresh extracts(Table 5). The dried extracts were more inhibitory to the seedling weight than fresh extract like the germination percentage and rate. The dry weight inhibition was greater than that of seedling length. The dry weight reduction was 53% of the control under the dried extract treatment, but

was only 30% with fresh extracts.

Greenhouse Study

1) Effect on alfalfa emergence and survival percentage

The percentage of alfalfa seedling emergence and survival as affected by velvetleaf residues were presented in Table 6. Seedling emergence was significantly reduced by weed residue silica sand mixture. In addition, the percentage of seedling survival after emergence was significantly inhibited as compared to the control. The apparent explanation is that toxic substances are released directly from the velvetleaf residue or their indirect release through the interaction of some microbes and residues.

Table 6. Emergence and survival percentage of alfalfa seedling by the different velvet-leaf residue treatment.

Residue (w/v,%)	First Count (10DAP)	Second Count (20DAP)	Survival Rate(%)
0.0	67.0	57.3	85.5
0.25	61.5	47.8	77.9
0.5	51.3	35.5	72.0
0.75	49.3	34.5	67.7
1.0	44.0	25.0	56.7
LSD(0.05)	5.43	6.24	5.68
CV(%)	2.36	3.53	2.19

2) Incubation Treatment of Weed Species

There were significant changes in the height of alfalfa treated with incubated weed residue (Table 7). Alfalfa height was inhibited in response to an increase in incubation time of the residue. The greatest inhibition(78%) in alfalfa height occurred with velvetleaf when this residue was subjected to a 72h incubation time.

DISCUSSION

Data presented suggest that some weed species had allelopathic potential on alfalfa germination and seedling growth(Table 1 and 2). Results indicate that there was variation in allelopathic activity among the weed species studied. The results also showed that the aqueous extracts of top and root weed species were inhibitory to seed germination and seedling growth of alfalfa. Also, alfalfa seeds treated with top extracts from different weeds had a greater effect than root extracts on germination, seedling length and weight, and seedling vigor of alfalfa species. Borner⁷⁾ pointed out that massive exudation of chemicals from plant roots does not usually occur in plant. Bieber and Hoveland⁶⁾ reported that extracts from aerial portions of six field crops and four weed species were more inhibitory

Table 7. Early height of alfalfa growth in pots with different decaying time of different weed species

Weed	Incubation Time(h)		
	24	48	72
Plant height(cm)			
Canada Thistle	1.87	13.7	9.4
Crabgrass	19.9	13.9	11.8
Giant Foxtail	22.3	15.0	9.2
Lambsquarter	18.4	12.6	9.5
Pigweed	17.1	14.6	7.4
Velvetleaf	15.3	13.3	5.6
Prestrate Knotweed	17.6	16.9	8.8
Control	25.9	25.9	25.9
LSD(0.05)	1.38	1.58	1.25
CV(%)	7.11	10.00	11.42

than those from roots. Muir and Majak¹⁷⁾ reported that the degree of inhibition exhibited by root extracts was less than shoot extracts. Smith²⁶⁾ reported that leaf extracts of bitter sneezeweed(*Helenium amarum* L.) were more phytotoxic than stem extracts, and root extracts. Such results were similar to those of this study. Such results suggest that different weed species contained different amounts of water soluble inhibitors. While root extracts may either contain fewer or less potential chemicals or have lower concentrations of allelochemicals, leaves^{8,15)} and stems¹³⁾ contained more inhibitory compounds. Putnam²¹⁾ reported that specialized trichomes on the stems and petioles of velvetleaf plants release toxic chemicals.

This study concludes that extract of top contained more allelochemicals than that of roots. Thus, top extracts result in more inhibitory activity than root extracts. Results obtained in the concentration study of dried velvetleaf extract were similar to those of previous investigations¹²⁾.

Differential responses were supported by linear

regression equations(Table 3). The velvetleaf species had the greatest slope($b=3.69$) and appeared to be the most inhibitory to alfalfa, whereas large crabgrass had the lowest slope value($b=2.39$), and thus the least inhibitory among the weed species studied.

Velvetleaf extracts inhibited the germination, seedling length and weight of alfalfa as the concentration rate increased(Table 4). Also, alfalfa seedling emergence and survival rate were inhibited by velvetleaf residues(Table 6). The response was probably dependent upon allelochemicals in the extract of velvetleaf.

In the dried and fresh extract comparison, dried aqueous extracts had a greater effect on germination, seedling length and weight of alfalfa than fresh aqueous extracts(Table 5). The differences between dried and fresh extracts may be either a release of inhibitory substance when the plant tissue and cells were ruptured during drying process may have converted one or more nontoxic compounds into toxic compounds.

Increasing the incubation time of alfalfa dry inhibited the germination percentage of six weed species(Table 7). This was in agreement with results from Patrick and Koch²⁰, Al-Naib and Rice¹. Patrick and Koch²⁰ reported that phytotoxic substances may be released during residue decomposition in the soil. Al-Naib and Rice¹ observed that the seed germination and seedling growth of all eight test species were significantly inhibited by the decomposition of sycamore(*Platanus occidentalis* L.) leaves. The decrease of weed seed germination with incubation time may be due to a release of more water soluble toxic substances from decomposing plant tissue or to the formation of toxic chemicals by microorganisms during residue decomposition⁴. This effect may be due to a greater release of phytotoxic substance with progress of decomposition of weed residue.

However, this conclusion may not be definite since several factors, such as microbial activity, play a role in allelochemical production²². Also, it is possible that the incubation process in the soil as shown by Patrick and Koch²⁰ could explain the differences in results. The current study may demonstrate phytotoxicity of some weed species on alfalfa yield and quality in the field.

摘 要

본 실험은 알팔파 발아와 초기생육에 대한 canada thistle 등 7종잡초의 allelopathic 잠재성을 평가하기 위해서 실시하였다.

1. 7종류 잡초의 지상부와 지하부 추출물의 알팔파 발아와 생육에 대한 억제효과를 비교하면 두 추출물이 공히 알팔파 발아와 생육에 억제적으로 작용하였으나, 지상부의 추출물이 지하부의 추출물보다 더 큰 억제작용을 보였으며, 이 중에서 velvetleaf 추출물이 가장 억제적이었고($b=3.69$), crabgrass 추출물은 가장 낮은 억제정도를 보였다($b=2.39$).
2. 농도에 따른 velvetleaf 추출물 처리에서는 농도가 증가할수록 alfalfa 발아율, 유근의 길이, 무게 등이 대조구와 비교하여 상대적으로 더 억제되었으며, 알팔파 발아와 유근의 생육에 대한 velvetleaf의 건조 추출물과 생체 추출물을 비교하면 건조 추출물이 더 억제적으로 작용 하였다.
3. 건조된 velvetleaf를 silica sand와 혼합처리시 혼합비율이 증가될수록 알팔파의 초기 출현율과 생존율이 더 억제되었으며 혼합비율 1%에서는 출현율과 생존율이 44.0%, 56.7%로 가장 낮았다.
4. 잡초 잔기의 처리시간에 따른 알팔파 초기 생육에 대한 억제작용은 처리시간의 증가와 더불어 알팔파 생장이 억제되었고, 그 억제 정도는 velvetleaf 72시간 처리시 가장 높았다.

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