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Allelopathic Effect of Some Weed Species Extracts and Residues on Alfalfa

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몇가지 잡초들의 추출물과 잔유물의 알팔파에 대한 타감작용

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ABSTRACT : Dried top and root extracts of seven different weed species, fresh top and root extracts, and various concentrations of extract (0, 5, 10 and 15%, W/V) and residue rate (0.0, 0.25. 0.5, 0.75 and 1.0%) of velvetleaf (*Abutilon theophrasti* Medic.) were used to study their allelopathic effects on alfalfa in the laboratory and greenhouse. Top and root aqueous extracts of common lambsquarter (*Chenopodium album* L.), giant foxtail (*Setaria faberii* Herrm.), redroot pigweed (*Amaranthus retroflexus* L.), velvetleaf, large crabgrass (*Digitaria sanguinalis* L.), canada thistle (*Cirsium arvense* L.) and prostrate knotweed (*Polygonium aviculare* L.) significantly inhibited germination, seedling length, weight, and vigor in alfalfa. Top growth extracts of weeds exhibited greater allelopathic effects than root extracts. Alfalfa test species, WL-320, responded significantly different to the various weed species extracts in terms of allelopathic effect. The regression slopes of various top extracts showed significant variation with respect to germination percentage. Velvetleaf (b=3.69) extracts were the most inhibitory, while large crabgrass (b=2.39) extracts had the least allelopathic effect on alfalfa. When compared the activity of fresh velvetleaf extract to that of dry velvetleaf extract, dry extract was more inhibitory to alfalfa germination and seedling growth. Germination, seedling length and weight of alfalfa were inversely proportional to the concentration of dried velvetleaf extracts.

Seedling emergence and survival percentage was inhibited by velvetleaf residue mixture treatment. Also, more of the toxic effects were observed from the dried top extracts, as compared to extracts from fresh top and root. These results demonstrate the allelopathic activity of different weed species extracts and suggest that weeds may affect crop growth and development due to the inhibitory effects of allelochemicals present in weed tissue.

Key word : Alfalfa, Weed, Allelopathic effect, Aqueous extracts, Germination rate, Seedling growth, Seedling vigor

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Allelopathy, any direct or indirect effect of one plant on another through release or production of chemical compounds by leaching, root exudation and residue decomposition by microorganism that escape into the environment, occurs widely in the plant ecosystem[20]. Although their mechanisms are theoretically distinct, allelopathy and competition are closely related in the field.

Allelopathic chemicals that inhibit crop growth and yield may be released by weeds. Growth inhibition by allelochemicals would be expected to reduce the competitiveness do the inhibited plant for space, water nutrients, and light. It is theorized to be one mechanism by which weeds interfere with crop growth and development.

The allelopathic effects of toxic substances are important among crops and weeds within agricultural ecosystems[19]. Allelopathic effects on crop growth and development have been reported in perennial weeds[3,9,25], and annual weeds[1~4,7,8,10,11,17,23,25,27]. Several of the compounds, chlorogenic acid, isochlorogenic acid, *p*-coumaric acid, gallic acid, tannic acid, and *p*-hydroxybenzoic acid were isolated. Velvetleaf, a commom weed in soybean field, has been suggested to have an inhibitory effect on soybean[8]. A number of extracts from the plant material of velvetleaf produce observable effects on growth and development of soybean in the laboratory and suggest that there is a high probability of allelopathic interference[10]. Root residue of giant foxtail[3,23], crabgrass[17,23], canada thistle[25,27] inhibited the growth of corn. Bell and Koeppe[3] exhibited that height, fresh weight, and dry matter accumulations of corn were inhibited by the exudates of mature giant foxtail roots, leachates of dead roots, and leachates of whole plant residues.

The successful establishment of alfalfa, a major source of high protein forage, depends upon adequate control of competing vegetation, and this is important for no-till seedlings[26]. Aqueous extracts of quackgrass (*Agropyron repens* L.) shoot and root material have been reported to inhibit the growth of alfalfa[16]. They observed that alfalfa is more affected by shoot than root extracts. Also, the length of alfalfa seedlings was more sensitive to quackgrass extracts than was percentage of germination. Shoot extracts were more inhibitory to alfalfa seedling when quackgrass was harvested at the vegetative stage than at later stage.

However, little information is available on the effect of weed extracts 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, a laboratory experiment was conducted to determine the allelopathic effects of weed extracts on alfalfa seed germination and seedling growth. The objectives of this experiment were to evaluate the variation among weed species and relative inhibitory effects of seven different weed species top growth and root extracts, to assess the toxicity of various concentrations of velvetleaf top extracts and to compare whether or not top growth and root extracts of dried and fresh material inhibit germination and seedling growth of alfalfa in the laboratory.

Materials and Methods

1. Collection and preparation of weeds

Seven common weed species were collected at vegetative including roots. All weeds were separated into top growth (leaves + stems) and roots. 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. Half of the samples were air dried for 5 weeks 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 a 5℃ refrigerator until use. Undried tissue was classified as fresh tops and roots, and used immediately for extraction.

2. Top and root extracts

The top (leaves+stems) and root extracts of the seven different weed species were extracted by shaking 5g dried plant materials with distilled water at 24℃ room temperature for 24h in 250ml Erlenmeyer flasks. Extracts were filtered through filter paper (Whatman No. 42) and 0.2μm Nalgene filterware unit (Becton and Dickinson Labware, Lincoln Park, NJ) to prevent fungal contamination during the experiment. Alfalfa seeds, 20g (WL-320), were surface sterilized with 10:1 ratio (water: Clorox) for 5min, then rinsed with distilled water. One hundred sterilized seeds were placed in each heat sterilized 9cm petri-dish with a filter paper (Whatman 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℃ room. Germination was recorded 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 germinating seed each day by the number of days and adding the values[14]. Seedling vigor(SV) was calculated by multiplying to total seed germination and radicle length[21]. After 5d, radicle and hypocotyl length were measured and plants were separated into cotyledons, hypocotyl, and radicle parts to determine dry weight.

The samples were dried in a forced air oven at 70℃ for 6h. Each treatment including control was replicated 4 times using a total of 32 petri dishes in a completely randomized design and the experiment was repeated three times. Seedlings (10) were randomly chosen from each petri dish and measurements used for statistical analysis by Statistical Analysis System (SAS) program[22]. The means were separated on the basis of least significant difference (LSD) at the 0.05 probability level.

3. Study with different extraction level

Another experiment was conducted using water extracts of velvetleaf in petri dishes. Since velvetleaf has the largest effect on alfalfa seed germination and seedling growth as based on top extract results, the inhibition by velvetleaf extract concentration was studied in more detail.

Rates of 0, 5, 10 and 15% were prepared by soaking dried ground top residues with at 24℃ room temperature for 24h in 250ml Erlenmeyer flasks. Extracts were filtered through filter paper (Whatman No. 42) and 0.2μm Nalgene filterware unit. Each treatment (concentration) was replicated four times in a complete randomized design and the experiment was repeated three times. The means were separated by least significant difference (LSD) at the 0.05 probability level.

4. Dried and fresh residue extracts

This experiment was to determine which extracts of dried and fresh velveleaf would more inhibit germination and seedling length of alfalfa seed. This experiment was done in petri dishes using aqueous extracts of fresh tops and roots plus dried tops and roots of velvetleaf at 5% (w/v). The dry extracts (5g) were prepared as described above. The fresh tops and root extracts of the velvetleaf were extracted by homogenizing 5g of plant material in a blender with 5ml distilled water for 15min. Plant material was washed with distilled water and extracts of 5% concentration were made on a fresh weight basis. These extracts were filtered through four layers of cheese cloth, and centrifuged at a low speed (3000rpm) for 4h. The supernatant was filtered through 0.2㎛ Nalgene filterware unit (Becon Dickinson Labware, Lincoln Park, NJ). Each treatment (plant part) was replicated four times in a randomized complete block design and the experiment was repeated twice. The means were separated by least significant difference (LSD) at the 0.05 probability level.

5. Effect on alfalfa survival percentage

The effects of dried velvetleaf residues on alfalfa survival were determined in the following manner. Velvetleaf weed grown in the field was harvested at the vegetative stage. The harvested samples were air-dried at the room timperature (24℃). Dried samples were ground using a Wiley Mill with silica sand (500g) at 0.0, 0.25, 0.5, 0.75 and 1.0% (w/w). Fifty sterilized alfalfa seeds (WL-320) 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 I was added to the saucers as needed to maintain moisture for seed germination. A residue treatment was used as control. Also, the percentage of alfalfa seedling emergence was counted at 10 DAP. A second count was done 20 DAP when cotyledons were extended. Four replications each residue treatment were arranged in a completely randomized design.

Results

1. Top and root extracts

Germination percentage, seedling length and weight, vigor and germination rate as affected by weed top and root extracts are presented in Tables 1 and 2. Top and root extracts of the seven different weed species significantly reduced germination, seedling length, weight and vigor and germination rate of alfalfa compared to the control. Top growth extraction was more inhibitory than root extraction. Among the different weed species studied, velvetleaf had the greatest effect on germination, seedling length and weight, seedling vigor and rate of germination on alfalfa. This trend was follwed 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 top 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 growth extraction are presented in Table 3. The slope of the regression line varied for different weed species. This indicates that alfalfa germination responded differently to each weed species ex-

Table 1. Allelopathic effects of dry top growth extracts from various weeds on alfalfa germination, seedling growth and weight, seedling vigor, and rate of germination

Weed species	GP[1]	RL[1]	HL[1]	CW[1]	RW[1]	HW[1]	SV[1]	RG[1]
	... % ...	..... cm		..... mg				
Canada thistle	74.0	2.3	2.9	1.08	0.33	0.53	170.20	46.38
Crabgrass	78.3	2.5	3.1	1.15	0.33	0.70	195.20	32.63
Giant foxtail	63.8	1.8	2.6	0.85	0.23	0.48	96.5	22.17
Lambsquarter	53.8	1.8	2.6	0.93	0.23	0.50	96.72	28.23
Pigweed	71.5	2.1	2.8	1.03	0.30	0.53	150.15	36.81
Velvetleaf	52.0	1.7	2.4	0.75	0.15	0.43	95.87	22.80
P.knotweed*	69.5	1.9	2.7	0.93	0.23	0.53	134.07	34.98
Control	89.0	3.8	3.4	1.50	0.50	0.80	335.95	61.38
LSD(0.05)	10.15	0.10	0.12	0.07	0.07	0.06	21.79	4.80
CV(%)	10.26	3.19	2.87	4.88	15.64	7.07	9.37	9.07

* Prostrate knotweed.
[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.

Table 2. Allelopathic effects of dry root extracts from various weeds on alfalfa germination, seedling growth and weight, seedling vigor, and rate of germination

Weed Species	GP[1]	RL[1]	HL[1]	CW[1]	RW[1]	HW[1]	SV[1]	RG[1]
	... % ...	..... cm		..... mg				
Canada thistle	77.3	2.5	2.9	1.18	0.35	0.60	195.22	47.23
Crabgrass	80.0	2.6	3.1	1.20	0.35	0.83	206.30	40.42
Giant foxtail	69.3	2.2	2.7	0.95	0.25	0.53	152.35	45.54
Lambsquarter	69.5	2.2	2.8	1.00	0.30	0.53	154.85	36.96
Pigweed	71.8	2.5	2.9	1.13	0.33	0.58	178.90	34.19
Velvetleaf	55.5	1.9	2.7	0.80	0.20	0.40	105.40	26.50
P.knotweed *	71.8	2.3	2.9	1.10	0.33	0.58	167.00	46.88
Control	89.0	3.8	3.4	1.50	0.50	0.80	335.95	61.38
LSD (0.05)	10.79	0.17	0.12	0.09	0.08	0.07	24.82	6.81
CV (%)	10.13	4.54	2.82	5.38	16.01	8.12	9.10	10.99

* Prostrate knotweed.
[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.

Table 3. Total germination of alfalfa as a function of increasing extract concentration (0, 5, 10, 15%) using seven weed species top extracts

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
P.Knotweed*	Y=87.68−3.42x	0.90

* Prostrate knotweed

tract. Velvetleaf (b=3.69) had highest regression value followed by giant foxtail. These species were comparatively the most inhibitory for alfalfa germination.

2. Study with different extraction levels

Significant reduction in total germination, seedling length and weight of alfalfa was observed as the extract concentration increased to 15% (Fig. 1, 2 and 3). Such a reduction response was concentration-dependent.

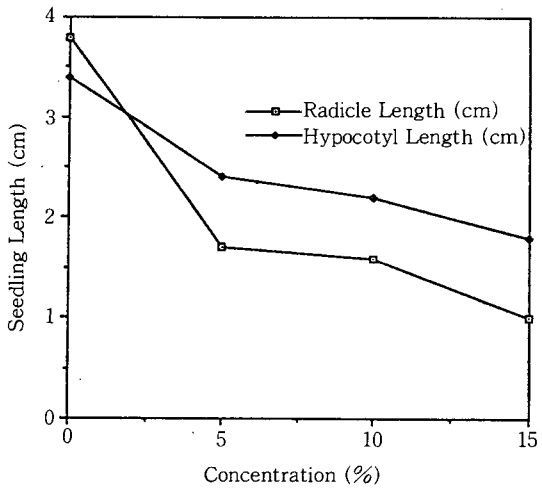

Fig. 1. Effect of dried top velvetleaf extract concentration on the radicle and hypocotyl length of 4 day old alfalfa seedling.

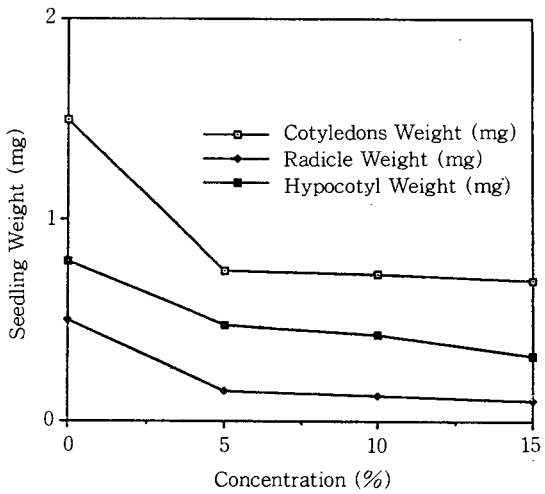

Fig. 2. Effect of dried top velvetleaf extract concentration on the seedling weight of 4 day old alfalfa seedling.

The highest extract concentration of velvetleaf, 15% reduced germination, total seedling length and weight of alfalfa, by 64%, 61% and 53% respectively, when compared to the control.

3. Dried and fresh residue extracts

Extracts of dried and fresh top growth and roots of velvetleaf significantly reduced the germination percentage, seedling length and weight, and germination rate in this study when compared to control (Table 4). Germination percentage and rate of germination of dry (root) and fresh (root) part extracts were 56%, 61% and 27, 33 respectively. Also, extracts of fresh tops and roots had the same effects on percentage germination and rate of germination. Dried top growth and root extracts resulted in a 38%, 42% inhibition of germination percentage and fresh top growth root extracts inhibited percentage germination by 33%, 32% as compared to control.

The rate of germination was significantly

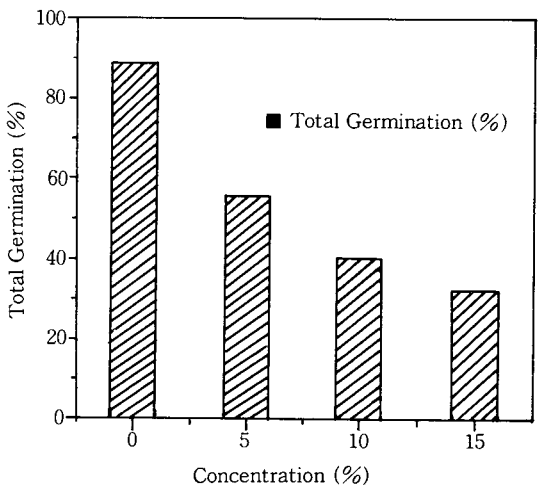

Fig. 3. Effect of dried top velvetleaf extract concentration on alfalfa germination percentage.

different between dried and fresh extracts. Also, dried top growth and root extracts resulted in a similar germination percentage, but dried root extract treatment resulted in a slightly faster germination and emergence than fresh top and root extracts as indicated by the higher germination rate.

Table 4. The effect of velvetleaf fresh and dried top and root extracts on germination, seedling length and weight, and germination rate

Treatment	Germination percentage	Seedling length	Seedling weight	Germination rate
	(%)	(cm)	(mg)	
Dried root	55.5	4.6	1.40	26.5
Dried top	59.0	4.1	1.33	22.8
Fresh root	60.5	5.0	1.83	32.9
Fresh top	59.8	4.4	1.58	29.7
Control	89.0	7.1	2.80	61.38
LSD (0.05)	7.65	0.22	0.08	4.18
CV (%)	7.99	2.96	2.98	8.01

Seedling length and dry weight of alfalfa were inhibited by dried and fresh top growth and root extracts. The dried extracts were more inhibitory to the seedling weight than fresh extracts unlike 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 top growth extract treatment, but was only 30% with fresh tops extracts.

4. Effects on alfalfa survival percentage

The percentage of alfalfa seedling emergence and survival as affected by velvetleaf residues are presented in Table 5. The percentage of seedling emergence and seedling survival after emergence was inhibited by velvetleaf residue silica sand mixture, as the residue rate increased. 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.

Discussion

Allelopathy has been reported to exist between numerous species. Data presented here suggests that allelochemicals are present in the seven different weed species studied using both dried and fresh top growth and root extracts on alfalfa (Tables 1, 2, 4 and Figures 1, 2, and 3). Results indicate that there is variation in allelopathic activity among the weed species studied.

Table 5. Emergence and survival percentage of alfalfa seedling by the different velvetleaf residue treatment

Residue (w/w, %)	Emergence (10DAP)	Survival (20DAP)
0.0	67.0a	85.5a
0.25	61.5b	77.9ab
0.5	49.3cd	72.0b
0.75	51.3c	67.7bc
1.0	44.0d	56.7c

Differential responses are 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.

Also, alfalfa seeds treated with top growth extracts from different weeds had a greater effect than root extracts on germination, seedling length and weight, and seedling vigor of alfalfa species. These results are similar to those of Boner[6], Bieber and Hoveland[5], Muir and Majak[15], and Smith[24]. Borner[6] pointed out that massive exudation of chemicals from plant leaves rather than roots ususlly occur in plants. Bieber and Hoveland[5] reported that extracts from aerial portions of six field crops and four weed species were more inhibitory than those from roots. Muir and Majak[15] reported that the degree of inhibition exhibited by root extracts was less than shoot extracts. Smith[24] reported that leaf extracts

of bitter sneezeweed (*Helenium amarum* L.) were more phytotoxic than stem extracts, and root extracts. Such results suggest that different weed species contain different amounts of water soluble inhibitors.

While root extracts may either contain fewer or less potent chemicals or have lower concentrations of allelochemicals, leaves[7,13] and stems[12] contained more inhibitory compounds. Putnam[18] reported that specialized trichomes on the stems and petioles of velvetleaf plants release toxic chemicals. This study concludes that extracts of top growth result in more inhibitory activity than root extracts.

Results obtained in the concentration study of dried-velvetleaf extracts were similar to that of previous investigations[11]. Velvetleaf extracts inhibited the germination, seedling length and weight of alfalfa as the concentration rate increased. Velvetleaf residue treatment inhibited alfalfa seedling emergence and survival percentage as the residue rate increased (Table 5). The response was probably dependent upon allelochemicals in the extracts of velvetleaf.

In this study, dried top growth aqueous extracts had a greater effect on germination, seedling length and weight of alfalfa than fresh aqueous extracts. The differences between dried and fresh extracts may be either a release of inhibitory substances when the plant tissue and cells were ruptured during drying process, or the drying process may have converted one or more nontoxic compounds into toxic compounds.

Total germination percentage does not provide a complete measure of the effect of toxic substance (Table 4). When germination rate was examined, it showed that germination of seed treated with fresh extracts was faster than seed treated with extracts of dried extracts even though no statistical difference was found in total germination percentage between two treatments. Germination rate under field conditions with various weeds could be a significant factor in the natural environment. Germination, seedling length and weight were more inhibited by dried velvetleaf top growth extract than by other treatments. As previously noted, these results assume that more inhibitory substances are produced from velvetleaf top growth tissue than from roots. The greatest reduction in seed germination, seedling growth and weight occured when alfalfa seeds were treated with dried top extracts, followed in order by extracts of dried root, fresh tops, fresh roots and distilled water.

摘 要

본 실험은 포장에서 주로 발생하는 velvetleaf등을 비롯하여 7종류의 잡초를 마른 상태와 생체상태로 각각 수확하여 지상부와 지하부로 나누어 물질을 추출 이들을 alfalfa 종자 발아와 생육정도 검정에 사용하여 지상부와 지하부의 생육억제정도를 비교하였으며 이 중 억제효과가 제일 큰 velvetleaf의 추출물은 여러 농도로 silica sand와 혼합하여 alfalfa에 대한 타감작용을 검토하였다.

1. 7종류 잡초의 지상부와 지하부의 추출물 처리는 alfalfa의 발아율, 발아세, 유근의 길이, 무게 등을 억제시켰다. 지상부와 지하부의 억제효과를 비교하면 지상부의 추출물이 지하부의 추출물보다 더 큰 타감작용을 보였으며, 이 중에서 velvetleaf 추출물이 가장 억제적이었고, crabgrass 추출물은 가장 낮은 억제 정도를 보였다. 또 alfalfa 발아와 유근의 생육에 대한 velvetleaf의 건조 추출물과 생체 추출물을 비교하면 건조 추출물이 더 억제적 이었다.
2. 농도에 따르는 velvetleaf 추출물 처리에서는

농도가 증가 할수록 alfalfa 발아율, 유근의 길이, 무게 등이 대조구와 비교하여 상대적으로 더 억제되었다.

3. 건조된 velvetleaf 잔기를 silica sand와 혼합처리에서 잔기의 비율이 증가 될수록 alfalfa의 출현율과 생존율이 더 억제되었으며 그 정도는 잔기 비율 1%에서 가장 억제적 이었다.
4. 잡초의 추출물과 잔기의 처리는 alfalfa의 발아와 생육에 억제적으로 작용 하여 타감작용이 인정되었다.

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