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Evaluation of weed control potential of meadowfoam (*Limnanthes alba* Benth.) plant

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ABSTRACT

We studied the effects of defatted seed meal of meadowfoam on weed control, but not of other plant parts (leaves, stems, flowers and roots). There were no differences among the extraction methods (water, boiling water, ethanol and fermentation) and plant parts (leaves, stems, above ground parts, seeds and defatted seed meal) in I_{50} values for germination, shoot and root lengths of cucumber and barley. The I_{50} and I_{90} values for shoot and root lengths of *Echinochloa oryzicola* Vasing, *Digitaria ciliaris* Koel, *Artemisia princeps* Pamp., *Cirsium japonicum* var. Maackii Matsum and *Taraxacum platycarpum* Dahlst were lower in leaves, stems, above ground parts, seed meal and defatted seed meal extracts than in flowers and roots. Broad leaf weed species (*A. princeps*, *C. japonicum*, and *T. platycarpum*) were more sensitive to water extracts of tested plant parts than grassy weeds (*E. oryzicola* and *D. ciliaris*). *T. platycarpum* was completely controlled by soil application of stems and defatted seed meal at 600 g/m^2 and was 95% controlled by seed meal at 600 g/m^2. Other weed species (*E. oryzicola*, *D. ciliaris*, *A. princeps* and *C. japonicum*) were 62-91% controlled by stems, seed meal and defatted seed meal at 600 g/m^2. However, total phenol and flavonoid contents may not be related to herbicidal mechanisms in plant parts of meadowfoam. Germination rates, shoot and root lengths of barley and cucumber in *n*-hexane solvent fraction were more inhibited than in chloroform, ethyl acetate, butanol and water fractions. A herbicidal compound, 3-methoxyphenyl acetonitrile, was found present at higher levels in the *n*-hexane fraction than other fractions. Application of 3-methoxyphenyl acetonitrile at 0.1% completely inhibited the germination, shoot and root lengths of lettuce, *E. oryzicola* and *C. japonicum*. Thus, meadowfoam leaves and stems as well as defatted seed meal can be used for weed control on organic farms.

Key words: *Artemisia princeps*, barley, byproducts, *Cirsium japonicum* var. Maackii, cucumber, *Digitaria ciliaris* , *Echinochloa oryzicola*, extraction methods, herbicidal compound, *Limnanthes alba*, meadowfoam, plant parts, *Taraxacum platycarpum*, weed control.

INTRODUCTION

Synthetic pesticides have solved the hunger associated with food (1). But, their overuse had adverse effects on ecosystems as poor water quality soil and contamination of agricultural products etc. (1). Recently the interest in environmentally friendly and organic farming rather than conventional agricultural practices is increasing, particularly everything related to the use of organic materials (8). Eco-friendly materials developed using plant-derived natural materials have the potential to alleviate the problems associated with synthetic pesticides (19). However, these materials are less effective for weed control than synthetic herbicides; thus, we needed to develop effective eco-friendly farming materials (11).

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Meadowfoam (*Limnanthes alba* Hartw. ex Benth.) is an industrial oil seed crop (Limnanthaceae family, order Brassicales), native to southern Oregon and northern California (10). It is well adapted to the relatively mild winters and warm, dry summers of the Pacific Northwest, generally grows in many types of soil, including those that are poorly drained. It is an annual herb that produces an erect or decumbent stem approximately 30 cms long. It yields about 1,875 kg seeds/ha in farmer fields due to disease and pollination problems. Its seeds contain 20-30% oil, rich in 20:1 and 22:1 fatty acids not commonly found in other seed oils (15). The oil is used in cosmetics, lubricants, rubber additives, plastics and biodiesel (3,5,18). Seed meal (by-product of the seed oil extraction process) comprises about 70% biomass of harvested seeds and is commonly used as organic amendments to composts or manures. Defatted seed meal has 25% protein, 22% fiber and 4% glucosinolates (16). High doses (5 to 7%) of defatted seed meal (with high glucosinolate content) act as bioherbicides against various weed species viz., downy brome (*Bromus tectorum* L.) (19) and velvet leaf (*Abutilon theophrasti* Medik.) (21). However, low doses of defatted seed meal stimulates the growth of vegetable crops (21). The herbicidal potency of meadowfoam seed meal strongly correlates with the total amount of nitrile (3-methoxyphenyl acetonitrile), isothiocyanate and thioamide glucosinolate breakdown products present in the defatted seed meal (19). Although the effects of defatted seed meal of meadowfoam on weed control have been investigated (19,20), but not of other plant parts such as leaves, stems, flowers and roots.

This study aimed to (a) investigate plant growth inhibition by various plant parts of meadowfoam obtained using different extraction methods, (b) know the weed control potential after soil application of selected meadowfoam plant parts, (c) determine the effects of different solvent fractions (HPLC) of defatted seed meal on weeds growth and (d) determine the weeds growth inhibition by 3-methoxyphenyl acetonitrile.

MATERIALS AND METHODS

Plant materials

The leaves, stems, flowers, roots, were collected from the Oregon State University farm in Corvallis, USA. At harvest time, the plant parts (leaves, stems, flowers and roots) were separately collected and dried in oven at 40°C for 5 days. These materials were ground in coffee grinder and sieved through 1 mm mesh prior to use. Test crops seeds and defatted seed meal were provided by Oregon State University, Corvallis, USA. In bioassays 3-crops [(*Cucumis sativus* L. var. Neulpureunoi), barley (*Hordeum vulgare* var. Yuhobori) and lettuce (*Lactuca sativa* L. var. Cheonghacheongchima)] and 6-weed spp. [*Echinochloa oryzicola* Vasing, *Digitaria ciliaris* Koel, *Portulaca oleracea* L., *Artemisia princeps* Pamp, *Taraxacum platycarpum* Dahlst and *Cirsium japonicum* var. Maackii Matsum] were used. To break seed dormancy, the seeds were stored at 4°C for 1 month.

Cucumber and barley growth

Dried leaves, stems, flowers, roots, seed meal and defatted seed meal of meadowfoam were extracted as under. Fifty g of each plant part were placed in 500 ml distilled water for 24 h to obtain water extract, or in 500 ml ethanol to obtain ethanol extract. In addition, 50 g of each ground plant species were placed in 500 ml distilled water and boiled at 100°C for 30 min to get boiling water extract or in 500 ml distilled

water kept at room temperature for 14 days to obtain fermentation extract. Each extract was filtered through a Miracloth and then concentrated under reduced pressure, after which the pellet was completely evaporated using a vacuum dryer (Hanbaek Scientific Co. Korea). Then the extracts were dissolved in distilled water to prepare the final concentrations of 0, 1, 3, 5 and 10%. Each concentration was subsequently centrifuged at 6,000 g for 20 min, after which the supernatants were filtered with a 0.45 µm syringe. Next, 5 ml supernatants were placed in a Petri dish (90 mm) and covered with two sheets of filter paper, after which 10 seeds each of cucumber and barley were sown. The petri dishes were kept in the growth chamber [25°C in dark for 3 days followed by 7 days at 14/10 h photoperiod (light intensity: 100 µmol m^{-2} s^{-1}). Thereafter, germination rate, plant height and root length were measured.

Weeds control

(i). Petriplate bioassay : We selected water extract for further study because it showed greater inhibitory effects on cucumber and barley growth. To confirm the inhibitory effects on five weed species (*E. oryzicola*, *D. ciliaris*, *A. princeps*, *T. platycarpum* and *C. japonicum*), we used the same seed bioassay as described in the previous section.

(ii). Pot culture : We selected stems, seed meal and defatted seed meal that showed greater weed control than other plant parts of meadowfoam for soil application experiment. Plastic pots (12×18×10 cm) were filled with upland soil (clay loam). Dried stems, seed meal and defatted seed meal were applied at 150, 300 and 600 g m^{-2} on the soil surface (7.5, 15, and 30 g per pot). These materials were uniformly mixed in the upper 2 to 3 cm soil and irrigated with water. After 1 day, 20 seeds each of *E. oryzicola*, *D. ciliaris*, *A. princeps*, *T. platycarpum*, *C. japonicum* and *P. oleracea* L. were sown per pot. After sowing, the pots were placed in greenhouse [30°C/25°C under a 14/10 h day/night regime and a light intensity of 500 µmol m^{-2} s^{-1}]. The germination rate, plant height and shoot fresh weight were recorded 12 days after sowing.

Chemical analysis

Total phenol and flavonoid contents in meadowfoam plant parts

Total phenol and flavonoid contents were determined in the leaves, stems, flowers, above ground parts and roots at harvest and seed meal and defatted seed meal after harvest.

(i). Phenolics and flavonoids contents: These were analyzed with 0.2 g of ground meadowfoam plant parts, digested in a mortar using 10 ml 80 % methanol and centrifuged at 5,000 × g for 10 min.

(ii). Total phenolics content: One ml extract was mixed with 3 ml distilled water and 1 ml Folin-Dennis' reagent, then shaken for 5 min. The mixed solution was allowed to stand for 1 h at room temperature, afterwards, phenolics were measured colorimetrically using UV spectrophotometer at 640 nm (UV-1601; Shimadzu Co., Kyoto, Japan). A standard, ferulic acid (Sigma Aldrich (St. Louis, MO, USA) was used to prepare the standard curves. A standard calibration curve showed high degrees of linearity ($r^2 > 0.99$) (data not shown).

(iii). Flavonoids concentration: The 0.5 ml of extract was mixed with 1.5 ml ethanol (95%), 10% $AlCl_3$, 1 M potassium acetate and 2.8 ml distilled water. The mixed

solution was then allowed to stand for 40 min at room temperature, afterwards, phenolics were colorimetrically measured using a UV spectrophotometer at 415 nm (UV-1601; Shimadzu Co., Kyoto, Japan). The quercetin (Sigma Aldrich (St. Louis, MO, USA) was used to prepare the standard curves. The standard calibration curves showed high degrees of linearity ($r^2 > 0.99$) (data not shown).

Effects of different solvent fractions water extract of defatted seed meal on growth

Meadowfoam plants yield higher amount of defatted seed than stem or seed meal, hence, we selected defatted seed meal for this study. For water extract, 250 g defatted seed meal was extracted in 5.0 L distilled water for 24 h. These extracts were then filtered through a Miracloth and separated by hexane, chloroform, ethyl acetate and water layers. Each organic layer was then concentrated under reduced pressure, thereafter the pellet was completely evaporated using vacuum dryer (Hanbaek Scientific Co. Korea). Other procedures and seed bioassays were the same as those described in the effect of extracts of different plant parts of meadowfoam on cucumber and barley growth section. HPLC-grade hexane, chloroform and ethyl acetate were purchased from Sigma Aldrich (St. Louis, MO, USA).

3-methoxyphenyl acetonitrile analysis by HPLC

Ten g solvent fraction was dissolved in 3 ml methanol (HPLC grade). The suspension was subsequently filtered through a 0.45 μm syringe filter, thereafter 10 μL filtrate was loaded onto the HPLC system (Agilent 1200 series). Separation was achieved on a 4.6 × 250 mm Luna C-18 5 μm column (Phenomenex, USA). The absorbance of each sample solution was measured with a DAD detector. The mobile phase was 1% formic acid in water (solvent A) and acetonitrile (solvent B). The gradient was 50 min, 0% to 70% (B); 2 min, 70% to 50% (B). The run time was 60 min using a flow rate of 1 mL/min. The 3-methoxyphenyl acetonitrile standard (Sigma Aldrich (St. Louis, MO, USA)) was used to prepare the standard curves. All standard calibration curves showed high degrees of linearity ($r^2 > 0.99$) (data not shown).

Effects of 3-methoxyphenyl acetonitrile on plants growth

To determine the growth inhibitory effects of 3-methoxyphenyl acetonitrile, we moistened the filter paper in petri dishes with 3-methoxyphenyl acetonitrile in concentrations of 0.01, 0.03, 0.05 and 0.1%. Then 10-seeds of each weed species *E. oryzicola* and *C. japonicum* and lettuce (var. Asiajeokoak) were sown. Seed bioassays were same as described in the above section "cucumber and barley growth".

Statistical analysis: The studies were done in completely randomized design with three replications. Data were expressed as the percentages of untreated control to standardize comparisons among extraction methods, plant parts or plant species. A non-linear regression using Polynomial Equation (Cubic) or Polynomial Equation (Quadratic) was used to describe plant inhibition of meadowfoam byproducts in different extraction methods, plant parts and plant species using the Statistical Analysis Systems (17) software.

$$Y = Y_0+aX+bX^2+cX^3$$
$$Y = Y_0+aX+bX^2$$

I_{50} or I_{90} values (Table 1 and 2) between different doses within treatments were calculated from these equations. Significant differences between treatments (Table 3, 4, 5 and Fig. 1) were determined using analysis of variance (ANOVA). Analyses were

performed using Statistical Analysis Systems (17) software. In the case of significant difference, means were separated using Duncan's multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Cucumber and barley growth

The I_{50} for germination rates, shoot and root lengths of cucumber and barley were determined in water, boiling water, ethanol and fermentation extracts of leaves, stems, above ground parts, seed meal and defatted seed meal of meadowfoam (Table 1). In test barley seeds, the extracts from stem and above ground parts decreased the I_{50} values of germination rates, shoot lengths and root lengths more than extracts from defatted seed meal. This was also true in studies, where extracts were made using water, fermentation and ethanol. However, in tests using cucumber seeds, we found that the I_{50} values of germination rates, shoot lengths and root lengths were lower than defatted seed meal tests only, when water extract was used. Other extraction methods were less effective, hence, we selected the water extracts in further studies.

Table 1. Effects of water, boiled water, fermentation and ethanol extracts of various plant parts of meadowfoam on germination, shoot and root lengths of barley and cucumber

Extraction method /Plant part	Barley			Cucumber		
	Germination Rate (I_{50})	Shoot Length (I_{50})	Root Length (I_{50})	Germination Rate (I_{50})	Shoot Length (I_{50})	Root Length (I_{50})
Leaf	8.5^{a}	1.9^{bc}	3.2^{ab}	4.6^{ab}	3.1^{ab}	2.8^{a}
Stem	2.9^{c}	1.5^{c}	2.1^{b}	2.3^{c}	2.0^{b}	1.5^{b}
Above ground part	4.9^{b}	1.7^{c}	3.4^{a}	3.7^{b}	2.3^{b}	2.3^{a}
Seed meal	7.9^{a}	2.6^{a}	2.5^{ab}	4.4^{ab}	2.1^{b}	2.4^{a}
Defatted seed meal	7.2^{a}	2.4^{ab}	2.6^{ab}	5.2^{a}	4.1^{a}	2.5^{a}
Mean	6.3	2.0	2.8	4.1	2.7	2.3
Leaf	5.3^{b}	1.8^{b}	1.9^{cd}	7.5^{b}	3.1^{b}	1.8^{c}
Stem	6.5^{ab}	1.0^{c}	1.7^{d}	9.4^{a}	4.3^{ab}	2.9^{b}
Above ground part	6.7^{ab}	2.6^{a}	3.2^{a}	6.1^{c}	4.0^{ab}	1.7^{c}
Seed meal	10.0^{a}	2.7^{a}	3.1^{ab}	6.3^{c}	3.6^{ab}	6.1^{a}
Defatted seed meal	8.5^{ab}	2.3^{a}	2.5^{bc}	5.6^{c}	6.1^{a}	3.2^{b}
Mean	7.4	2.1	2.5	7.0	4.2	3.1
Leaf	7.8^{b}	2.3^{b}	3.9^{a}	7.7^{b}	3.7^{ab}	2.9^{b}
Stem	7.3^{b}	1.5^{c}	1.9^{d}	6.9^{bc}	3.5^{ab}	2.3^{b}
Above ground part	6.7^{b}	1.6^{c}	2.0^{d}	5.6^{c}	2.6^{b}	2.7^{b}
Seed meal	10.0^{a}	3.4^{a}	2.9^{b}	10.0^{a}	6.1^{a}	5.2^{a}
Defatted seed meal	10.0^{a}	2.7^{b}	2.4^{c}	7.2^{b}	5.9^{a}	3.3^{ab}
Mean	8.4	2.3	2.6	7.5	4.4	3.3
Leaf	9.6^{a}	2.8^{b}	3.2^{a}	10.0^{a}	3.7^{ab}	4.0^{b}
Stem	6.5^{b}	2.4^{bc}	2.6^{ab}	9.3^{a}	4.8^{ab}	4.5^{b}
Above ground part	5.4^{bc}	1.9^{bc}	2.9^{ab}	6.4^{b}	3.2^{b}	5.6^{ab}
Seed meal	4.7^{c}	1.8^{c}	1.9^{b}	5.8^{b}	3.1^{b}	7.8^{a}
Defatted seed meal	9.1^{a}	3.9^{a}	3.2^{a}	7.4^{b}	5.6^{a}	3.6^{b}
Mean	7.1	2.6	2.8	7.8	4.1	5.1

*I_{50}=estimated plant extract concentration that will cause 50% germination rate, shoot and root length of barley and cucumber.

**Means separation within columns by Duncan's multiple range test at 5% level.

This is first study to report that meadowfoam byproducts such as stems and above ground parts have the potential for herbicidal activity, whereas defatted seed meal has been tested extensively (6,7,19,21).

The defatted meadowfoam seed meal applications inhibits > 95% emergence of spiny sowthistle (*Sonchus oleraceus* L.) than control (6). The defatted meadowfoam seed meal inhibited lettuce germination (58%) and growth (72%) compared to control (7). Other defatted seedmeals at 1 % concentration from 15 different glucosinolate-containing plant species such as Indian mustard, money plant and field pennycress also significantly inhibited the wheat and sicklepod (*Cassia obtusifolia*) seedling emergence (22). Our study suggests that like the defatted seed meal, the meadowfoam byproducts also have potential for use as pre-emergence bioherbicide.

Aqueous extracts and Weed control

To confirm herbicidal activities of water extracts of various plant parts of meadowfoam, we calculated the I_{50} and I_{90} values on germination, shoot and root lengths of weeds (*E. oryzicola*, *D. ciliaris*, *A. princeps*, *T. platycarpum* and *C. japonicum*) (Table 2). Regardless of which plant part extracts were used, the I_{50} and I_{90} values for germination rates of above weed species were generally similar. However, the *T. platycarpum* species was an exception. In this species, I_{50} and I_{90} values for germination rates were significantly lower with seed meal and defatted seed meal extracts. Generally, the I_{50} and I_{90} values for shoot and root lengths of above weed species were lower, when leaves, stems, above ground parts, seed meal and defatted seed meal extracts were used, but not when flowers and roots extracts were used. These results indicate that, perhaps, main components such as 3-methoxyphenyl acetonitrile, isothiocyanate and thioamide glucosinolate are present in different concentrations in various plant parts (7,20,21). Regardless of plant part extract used, the I_{50} and I_{90} values for germination rates, shoot and root lengths of broadleaf weeds (*A. princeps*, *T. platycarpum* and *C. japonicum*) were lower than those of grass weeds (*E. oryzicola* and *D. Ciliaris*). In other studies using defatted rice bran, broadleaf weeds [ivyleaf morningglory (*Ipomoea hederacea*) and hemp sesbania (*Sesbania exaltata*)] were also found more sensitive than grass weeds [large crabgrass (*Digitaria sanguinalis*) and barnyardgrass (*E. oryzicola*) (12)].

Specifically, the I_{90} values for root lengths of *A. princeps*, *T. platycarpum* and *C. japonicum* were significantly lower when using leaves, stems, above ground parts, seed meal and defatted seed meal extracts than when using root extracts. In root lengths, we found consistencies between certain plant part extracts and their impact on certain weed species' I_{90} values (as mentioned above). However, shoot length did not gave the same trend. Root length was more sensitive to extracts of all plant parts than shoot length. In another study, Italian ryegrass shoot and root extracts were more inhibitory to root length than shoot length of rice cultivars (9). Thus, meadowfoam leaves, stems and above ground parts used in this study have similar herbicidal potential to defatted seed meal.

Soil incorporated meadowfoam plant parts and Weed control

We added stems, seed meal and defatted seed meal of meadowfoam in the soil to find their herbicidal potential in pot culture at ratios of 150, 300 and 600 g/m^2 (Table 3). Compared to untreated controls, applications of dried stem, seed meal and defatted seed meal at 300 g/m^2, controlled the *T. platycarpum* by 85-90%, *Portulaca oleracea* L. by 77%

(37-56% in tests using stem and seed meal). However, other weed species (*E. oryzicola*, *D. ciliari*, *A. princeps* and *C. japonicum*) were only controlled 34-63% with dried stem, seed meal and defatted seed meal applied at 300 g/m^2. Furthermore, herbicidal activities on these weed species were lower (150 g/m^2 vs. 300 g/m^2). However, *T. platycarpum* was

Table 2. Effects of water extracts of various plant parts of meadowfoam on germination rates and shoot and root lengths of weeds

Weed Species /Plant part	Germination rate		Shoot length		Root length	
	I_{50}	I_{90}	I_{50}	I_{90}	I_{50}	I_{90}
ECORY						
Leaf	9.6^{ab}	>10.0^{a}	9.1^{ab}	>10.0^{a}	8.5^{a}	>10.0^{a}
Stem	>10.0^{a}	>10.0^{a}	7.0^{bcd}	>10.0^{a}	8.3^{a}	>10.0^{a}
Flower	9.0^{abc}	>10.0^{a}	8.3^{abc}	>10.0^{a}	5.4^{b}	9.8^{a}
Above ground part	7.2^{bcd}	>10.0^{a}	5.8^{cd}	9.2^{b}	4.7^{b}	9.7^{a}
Root	9.4^{abc}	>10.0^{a}	9.7^{a}	>10.0^{a}	9.6^{a}	>10.0^{a}
Seed meal	5.8^{d}	9.5^{b}	5.6^{d}	9.6^{a}	2.6^{c}	6.0^{b}
Defatted seed meal	6.9^{cd}	>10.0^{a}	5.9^{cd}	9.3^{b}	2.3^{c}	5.7^{b}
Mean	8.3	9.9	7.3	9.7	5.9	8.7
DIGCI						
Leaf	7.7^{abc}	9.0^{ab}	8.8^{a}	9.9^{ab}	2.4^{cd}	5.8^{cd}
Stem	6.6^{abc}	9.5^{ab}	6.0^{bc}	9.3^{ab}	3.2^{c}	7.4^{b}
Flower	8.4^{ab}	>10.0^{a}	9.5^{a}	>10.0^{a}	8.8^{b}	>10.0^{a}
Above ground part	3.9^{c}	8.2^{b}	4.7^{c}	8.9^{b}	2.6^{cd}	7.0^{bc}
Root	>10.0^{a}	>10.0^{a}	>10.0^{a}	>10.0^{a}	>10.0^{a}	>10.0^{a}
Seed meal	6.8^{abc}	9.4^{ab}	6.7^{b}	9.4^{ab}	3.2^{c}	6.9^{bc}
Defatted seed meal	5.7^{bc}	9.2^{ab}	5.3^{bc}	9.0^{b}	2.0^{d}	5.0^{d}
Mean	7.0	9.3	7.3	9.5	4.6	7.5
ARTPR						
Leaf	4.9^{a}	9.4^{ab}	8.2^{a}	9.7^{a}	1.3^{b}	3.4^{c}
Stem	4.0^{a}	7.7^{bcd}	6.3^{ab}	9.2^{ab}	2.0^{b}	5.1^{bc}
Flower	4.6^{a}	9.3^{ab}	6.0^{abc}	9.4^{ab}	2.6^{b}	6.0^{b}
Above ground part	2.9^{a}	6.7^{cd}	3.2^{cd}	7.1^{c}	1.4^{b}	3.8^{bc}
Root	5.1^{a}	>10.0^{a}	7.2^{a}	>10.0^{a}	5.1^{a}	9.4^{a}
Seed meal	4.6^{a}	8.1^{abc}	3.8^{bcd}	7.4^{bc}	2.7^{b}	5.6^{bc}
Defatted seed meal	2.6^{a}	5.7^{d}	2.6^{d}	5.6^{c}	1.5^{b}	3.6^{c}
Mean	4.1	8.1	5.3	8.3	2.4	5.2
TARPL						
Leaf	4.3^{b}	8.3^{ab}	5.8^{b}	8.9^{ab}	2.0^{b}	4.6^{cd}
Stem	5.3^{b}	8.8^{ab}	5.7^{b}	9.0^{ab}	3.3^{b}	7.3^{ab}
Flower	5.0^{b}	7.7^{ab}	2.7^{cd}	5.8^{cd}	3.3^{b}	6.1^{bc}
Above ground part	3.9^{bc}	6.8^{b}	3.6^{c}	6.6^{bc}	2.0^{b}	4.2^{cd}
Root	8.3^{a}	9.7^{a}	8.8^{a}	9.7^{a}	7.7^{a}	9.1^{a}
Seed meal	1.4^{c}	3.7^{c}	1.4^{d}	3.8^{de}	1.5^{b}	4.1^{cd}
Defatted seed meal	1.4^{c}	3.2^{c}	1.3^{d}	3.1^{e}	1.0^{b}	2.9^{d}
Mean	4.2	6.9	4.2	6.7	3.0	5.4
CIRJA						
Leaf	5.4^{abc}	>10.0^{a}	5.1^{ab}	>10.0^{a}	2.8^{b}	6.3^{bc}
Stem	4.2^{abc}	7.7^{ab}	2.5^{b}	6.5^{bc}	2.7^{b}	5.7^{c}
Flower	7.6^{ab}	9.4^{ab}	7.0^{a}	8.8^{ab}	6.6^{a}	8.7^{ab}
Above ground part	2.0^{c}	3.7^{c}	1.9^{b}	3.6^{d}	1.9^{b}	3.6^{c}
Root	8.1^{a}	>10.0^{a}	8.9^{a}	>10.0^{a}	8.1^{a}	>10.0^{a}
Seed meal	2.6^{bc}	5.9^{bc}	1.6^{b}	4.6^{cd}	1.8^{b}	4.0^{c}
Defatted seed meal	4.5^{abc}	7.7^{ab}	2.1^{b}	4.8^{cd}	1.9^{b}	4.2^{c}
Mean	4.9	7.8	4.2	6.9	3.7	6.1

*ECORY; *Echinochloa oryzicola* Vasing, DIGCI; *Digitaria ciliaris* Koel, ARTPR; *Artemisia princeps* Pamp, TARPL; *Taraxacum platycarpum* Dahlst, CIRJA; *Cirsium japonicum* var. *maackii* Matsum.

$^{**}I_{50}$=Estimated plant extract concentration that will cause 50% germination rate, shoot and rot length of barley and cucumber.

***Means separation within columns by Duncan's multiple range test at 5% level.

completely controlled by stems and defatted seed meal incorporation at 600 g/m^2 and 95% controlled by seed meal. Even stems, seed meal and defatted seed meal incorporation at 600 g/m^2, controlled only 62-91% weeds (*E. oryzicola*, *D. ciliaris*, *A. princeps* and *C. japonicum*). There were no differences in herbicidal activities among stem, seed meal and defatted seed meal.

Table 3. Effects of soil treatment of plant part of meadowfoam on shoot fresh weight of weeds

Plant part	Treatment (g/m^2)	Weed species					
		ECORY*	DIGCI	ARTPR	TARPL	CIRJA	POROL
		(% of control))					
Stem	0	100^{a**}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
	150	66^{c}	68^{bc}	57^{bc}	65^{b}	84^{b}	61^{c}
	300	60^{c}	37^{d}	53^{bc}	10de	55^{cde}	44^{d}
	600	37^{d}	35^{d}	27^{cd}	0^{f}	33^{fg}	22^{e}
Seed meal	150	73^{bc}	61^{c}	57^{bc}	49^{c}	70^{bc}	81^{b}
	300	62^{c}	58^{c}	45^{bcd}	15^{d}	59^{cd}	63^{c}
	600	38^{d}	31^{d}	16^{d}	5^{ef}	19^{g}	15^{ef}
Defatted seed meal	150	82^{b}	81^{ab}	64^{b}	69^{b}	76^{b}	52^{cd}
	300	66^{c}	57^{c}	41^{bcd}	14^{d}	52de	23^{e}
	600	33^{d}	27^{d}	21^{d}	0^{f}	40^{ef}	9^{f}

*ECORY; *Echinochloa oryzicola* Vasing, DIGCI; *Digitaria ciliaris* Koel, ARTPR; *Artemisia princeps* Pamp, TARPL; *Taraxacum platycarpum* Dahlst, CIRJA; *Cirsium japonicum* var. *maackii* Matsum, POROL; *Portulaca oleracea* L.

**Means separation within columns by Duncan's multiple range test at 5% level.

Both studies with water extracts plant parts and soil incorporation of biomass showed herbicidal effects. While the water extracts were used on barley, cucumber and weed species (*A. princeps*, *C. japonicum*, *T. platycarpum, E. oryzicola* and *D. ciliaris*) (Tables 1 and 2). The soil incorporation method was also used on various weed species (*A. princeps*, *C. japonicum*, *T. platycarpum, E. oryzicola*, *D. ciliaris* and *P. Oleracea*) (Table 3), both methods inhibited their respective targets weeds.

In another study, defatted seed meal has the potential as bioherbicide on downy brome and velvetleaf (19,21). Meadowfoam is winter rotation crop in perennial grass seed production systems in the Willamette Valley, Oregon (18). Thus, meadowfoam plant parts (stems and leaves) remaining as crop residues in soil after harvest may help in weed suppression. Furthermore, defatted seed meal has glucosinolate breakdown products that suggest its potential use in agriculture as soil amendment to enhance the plant growth (13), as well as to inhibit the soil pests (nematodes and insects) (4,23) and suppress the weeds (19). Thus, future studies are needed to confirm the inhibitory activities of meadowfoam byproducts such as stems and leaves on soil pests.

Total phenol and flavonoid contents in meadowfoam plant parts

Apart from glucosinolate degradation products, other chemical compounds may be related to the herbicidal potential of plant parts of meadowfoam. Thus, we measured total phenols and flavonoid contents in different plant parts of meadowfoam (Fig. 1). The total phenol contents followed the order : flower (98.0 mg/g DW) > leaf (74.7 mg/g DW) > above ground part (73.1 mg/g DW) > stem (49.3 mg/g DW) > root (15.5 mg/g DW) > defatted seed meal (11.1 mg/g DW) > seed meal (7.8 mg/g DW). Although the phenol

content of defatted seed meal was 8.8 times lower than flower but it was very herbicidal. Flowers were least herbicidal to weeds. Similar to total phenol contents, the total flavonoid contents followed the order : flower (4.68 mg/g DW) > leaf (3.04 mg/g DW) > above ground part (2.55 mg/g DW) > stem (0.83 mg/g DW) > root = seed meal = defatted seed meal (0.2 mg/g DW). The flavonoids content in defatted seed meal was 20-folds lower than in flower. Total phenol and flavonoid contents may not be related to herbicidal potential in plant parts of meadowfoam.

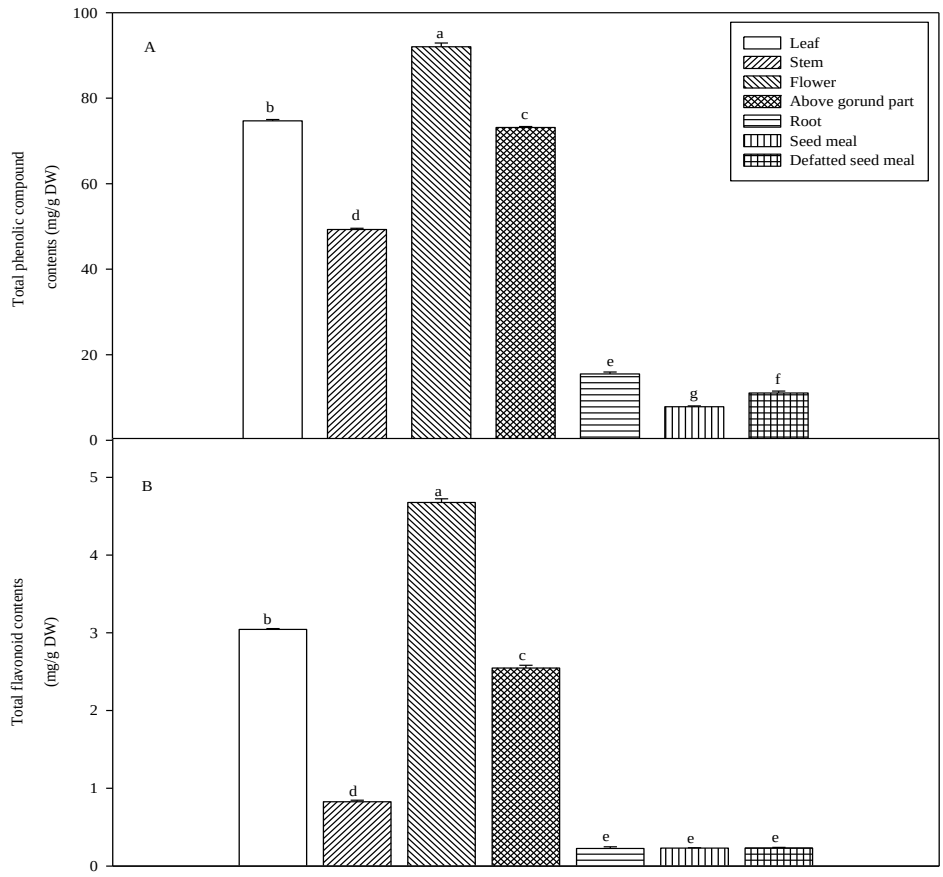

Figure 1. Total phenolic (A) and total flavonoid (B) contents in various plant part of meadowfoam. Means separation within bars by Duncan's multiple range test at 5% level.

Defatted seed meal effects on growth

Hexane, chloroform, ethyl acetate, butanol and water fractions were separated from water extract of defatted seed meal (Table 4). Germination rate, shoot and root lengths of barley and cucumber were more inhibited in response to increasing concentrations of

hexane fraction. The 10 % hexane fraction inhibited the shoot and root lengths by 62 and 65 % in barley and 53 and 43 % in cucumber, respectively. However, the chloroform, ethyl acetate, butanol and water fractions did not influence the shoot and root lengths.

Table 4. Effects of different solvent fractions by water extracts of meadowfoam defatted seed meal on germination rate and shoot and root length of barley and cucumber

Fraction layer		Germination rate	Shoot length	Root length	Germination rate	Shoot length	Root length
		(% of control))					
n-hexane	0	100^{a**}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
	1	90^{abc}	80^{abc}	66^{bc}	97^{a}	82^{ab}	81^{bcd}
	3	87^{abcd}	59^{bcd}	50^{c}	80^{bcd}	78^{ab}	78^{cd}
	5	77^{cd}	58^{cd}	45^{c}	77^{cd}	66^{b}	76^{d}
	10	70^{d}	38^{d}	35^{c}	70^{d}	47^{c}	57^{e}
$CHCl_3$	1	93^{abc}	100^{a}	99^{ab}	97^{a}	97^{a}	94^{ab}
	3	87^{abcd}	98^{a}	98^{ab}	93^{ab}	96^{a}	93^{abc}
	5	83^{abcd}	96^{a}	95^{ab}	93^{ab}	93^{a}	92^{abc}
	10	80^{bcd}	92^{ab}	90^{ab}	90^{abc}	83^{a}	85^{bcd}
EtOAc	1	90^{abc}	99^{a}	100^{a}	100^{a}	99^{a}	93^{ab}
	3	90^{abc}	97^{a}	99^{ab}	97^{a}	95^{a}	90^{abcd}
	5	83^{abcd}	93^{ab}	92^{ab}	93^{ab}	91^{a}	89^{abcd}
	10	80^{bcd}	93^{ab}	89^{ab}	93^{ab}	88^{ab}	86^{bcd}
n-BuOH	1	100^{a}	95^{ab}	99^{ab}	100^{a}	98^{a}	96^{ab}
	3	100^{a}	93^{ab}	96^{ab}	100^{a}	97^{a}	93^{abc}
	5	96^{ab}	92^{ab}	92^{ab}	100^{a}	97^{a}	91^{abc}
	10	96^{ab}	91^{ab}	90^{ab}	93^{ab}	94^{a}	89^{abcd}
Water	1	97^{ab}	95^{ab}	100^{ab}	100^{a}	99^{a}	97^{a}
	3	90^{abc}	94^{ab}	96^{ab}	100^{a}	93^{a}	96^{a}
	5	87^{abcd}	94^{ab}	95^{ab}	100^{a}	92^{a}	93^{abc}
	10	87^{abcd}	93^{ab}	93^{ab}	93^{ab}	90^{a}	87^{abcd}

*Conc.; Concentration.
**Means separation within columns by Duncan's multiple range test at 5% level.

Table 5. Effects of 3-methoxyphenyl acetonitrile (MA) on plant growth

Conc.* (%)	LASAT**			ECORY			CIRJA		
	Germination rate	Shoot length	Root length	Germination rate	Shoot length	Root length	Germination rate	Shoot length	Root length
0	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
0.01	97^{a}	49^{b}	25^{b}	53^{b}	70^{b}	60^{b}	60^{b}	72^{b}	74^{b}
0.03	53^{b}	37^{bc}	20^{b}	27^{c}	25^{c}	22^{c}	40^{c}	41^{c}	31^{c}
0.05	23^{c}	22^{c}	6^{c}	10^{d}	5^{d}	3^{d}	10^{d}	13^{d}	18^{d}
0.10	0^{d}	0^{d}	0^{d}	0^{e}	0^{d}	0^{d}	0^{e}	0^{e}	0^{e}

*Conc., Concentration.**LASAT; *Lactuca sativa* L., ECORY; *Echinochloa oryzicola* Vasing, CIRJA; *Cirsium japonicum* var. *maackii* Matsum.
***Means separation within columns by Duncan's multiple range test at 5% level.

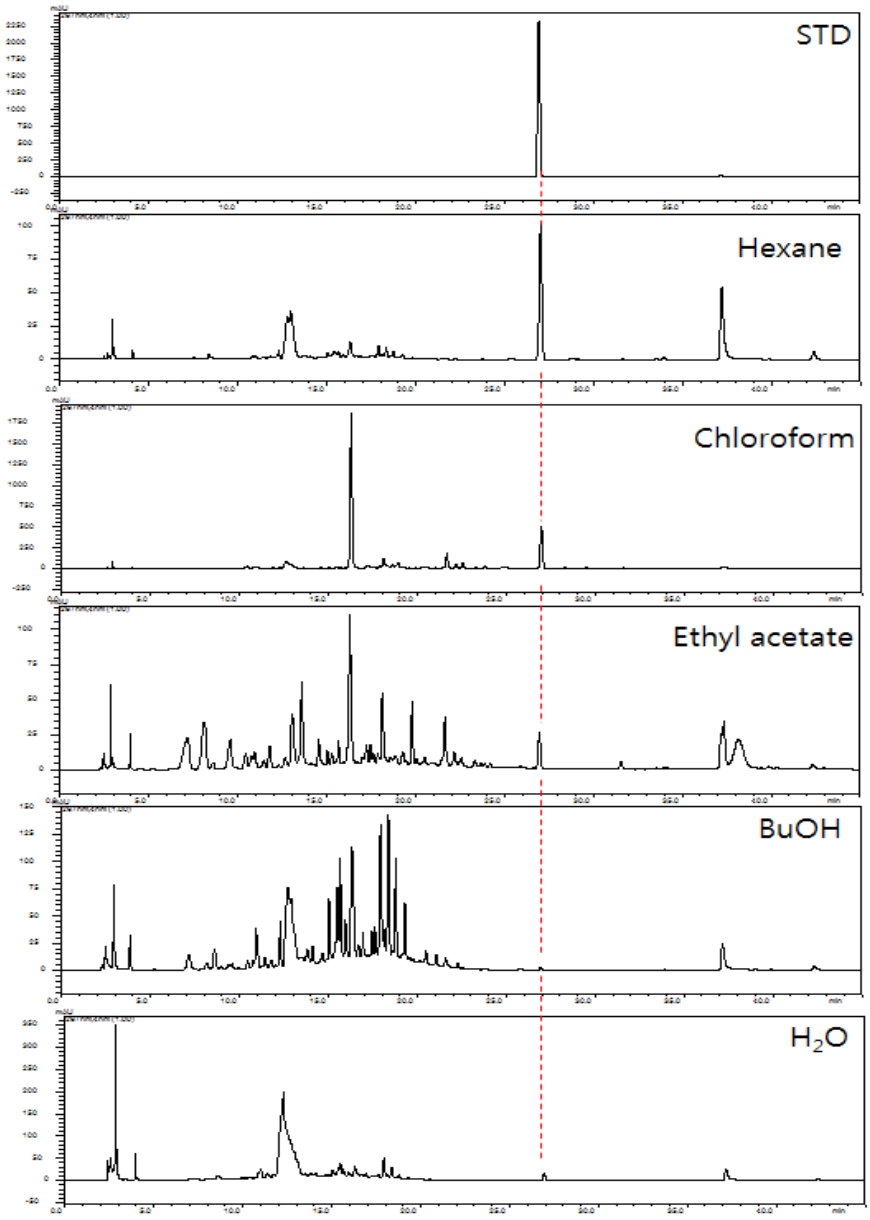

Figure 2. 3-methoxyphenyl acetonitrile in different solvent fractions of defatted seed meal of meadowfoam by HPLC analysis.

3-methoxyphenyl acetonitrile analysis by HPLC

Meadowfoam seeds contain the glucosinolate glucolimnanthin (2), which is not extracted with oil, as it is not soluble in the solvent (hexane) used for oil extraction and thus may be retained in the defatted seed meal. Glucosinolate degradation products include isothiocyanates, 3-methoxyphenyl acetonitrile (nitrile) and thiocyanates, their herbicidal activity differed among the products. We measured only 3-methoxyphenyl acetonitrile among glucosinolate degradation products (Fig. 2). The 3-methoxyphenyl acetonitrile was detected in solvent fractions, *n*-hexane, $CHCl_3$ and EtOAc of water extract in defatted seed meal. However, the 3-methoxyphenyl acetonitrile was present at higher concentrations in the *n*-hexane fraction than other fractions.

3-methoxyphenyl acetonitrile effects on growth

Commercially available 3-methoxyphenyl acetonitrile was used to evaluate the herbicidal activity to lettuce, *E. oryzicola* and *C. japonicum* (Table 5). Inhibition of germination, shoot and root lengths of above plant species increased with increasing 3-methoxyphenyl acetonitrile concentrations. 3-methoxyphenyl acetonitrile at 0.1% showed complete inhibition of germination, shoot and root lengths of above test plant species. Stevens *et al*. (19) reported that meadowfoam 3-methoxyphenyl acetonitrile had greater activity than isothiocyanate at preventing coleoptile emergence of downy brome. Conversely, meadowfoam isothiocyanate inhibited the lettuce germination 14.4 times more effectively than 3-methoxyphenyl acetonitrile (7). In another study, Vaughn *et al*. (21) reported that meadowfoam isothiocyanate had phytotoxic effects on velvetleaf and wheat (*Triticum aestivum* L.) radicle growth. The difference between the studies may have been due to different test plant species. For example, in this study, broadleafed weed species (*A. princeps* and *T. platycarpum*) were more effectively controlled by the above ground parts, seed meal and defatted seed meal of medowfoam than of grass weeds (*E. oryzicola* and *D. Ciliaris*). Furthermore, it is possible that herbicidal effects does not depend on a single chemical compound and that it instead occurs in response to a combination of all glucosinolate degradation products.

CONCLUSIONS

The I_{50} and I_{90} values for shoot and root lengths of *E. oryzicola*, *D. ciliaris*, *A. princeps*, *C. japonicum and T. platycarpum* were lower, with water extracts of leaves, stems, above ground parts, seed meal and defatted seed meal than flowers and roots extracts. Broadleaf weeds were more sensitive to water extracts of all tested plant parts than grass weeds. *T. platycarpum* was completely controlled by soil incorporation of stems and defatted seed meal at 600 g/m^2 and 95% controlled by seed meal at 600 g/m^2. Other weed species (*E. oryzicola*, *D. ciliaris*, *A. princeps* and *C. japonicum*) were 62-91% controlled by stems, seed meal and defatted seed meal at 600 g/m^2. The results of this study increased the current knowledge that stems and leaves of meadowfoam have similar herbicidal activities as defatted seed meal. Thus, the meadowfoam plant parts can be used in organic farms for weed control.

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