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Article

EXPLORING THE HERBICIDAL POTENTIAL OF *Achyranthes aspera* AGAINST SOME WEEDS

*Identificação do Potencial Herbicida de **Achyranthes aspera** em Relação a Plantas Daninhas Específicas*

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ABSTRACT - The phytotoxic composition of *Achyranthes aspera* was identified through HPLC, and its herbicidal potential was investigated against two narrow leaf weeds viz., *Phalaris minor* Retz. and *Avena fatua* L.; and four broad leaf weeds viz. *Lathyrus aphaca* L., *Vicia sativa* L., *Convolvolus arvensis* L. and *Asphodelus tenuifolius* L. through bioassays. Weed seeds were grown in the aqueous extracts of various plant parts (roots, leaf, stem, fruit and whole plant) of *A. aspera* at 5% (w/v) concentration. The extracts of all plant parts caused significant reductions with differential degree in germination percentage and mean germination time. *Phalaris minor, A. fatua, L. aphaca, V. sativa* and *A. tenuifolius* completely failed to germinate whereas *C. arvensis* showed the lowest GP (20%) in response to 5% fruit extract of *A. aspera*. Inhibition of seed germination of all weeds was higher with the fruit extract than with the root, stem, leaf and whole plant extracts of *A. aspera*. Seed germination of all narrow leaf weeds was completely inhibited at the 5% fruit extract of *A. aspera*. The highest phytotoxic inhibitory effect of *A. aspera* fruit extract was proved to be due to the presence of gallic acid (88.4 mg kg^{-1}), caffeic acid (519.2 mg g^{-1}) and m-coumaric acid (51.4 mg kg^{-1}) as assessed by their HPLC analyses. The study, therefore, showed that *A. aspera* exerts an inhibitory effect on germination of weeds and can be further explored as a pre- or post-emergence herbicide to provide natural alternative to chemical herbicides in the future.

Keywords: allelochemicals, germination bioassay, natural herbicide, weeds.

*RESUMO - A composição fitotóxica de Achyranthes aspera foi identificada através de cromatografia líquida de alta eficiência (CLAE), e o potencial herbicida da espécie foi investigado em relação a duas plantas daninhas de folhas estreitas (**Phalaris minor** Retz. e **Avena fatua** L.) e quatro de folhas largas (**Lathyrus aphaca** L., **Vicia sativa** L., **Convolvolus arvensis** L. e **Asphodelus tenuifolius** L.), através de bioensaios. As sementes das plantas daninhas foram cultivadas nos extratos aquosos de várias partes das plantas (raízes, folhas, caule, frutos e plantas inteiras) de A. aspera na concentração de 5% (p/v). Os extratos de todas as partes da planta causaram reduções significativas, em graus diferentes, na porcentagem de germinação e no tempo médio de germinação. **Phalaris minor, A. fatua, L. aphaca, V. sativa** e **A. tenuifolius** simplesmente não germinaram, enquanto a espécie **C. arvensis** obteve o PG mais baixo (20%) em resposta ao extrato a 5% dos frutos de **A. aspera**. A inibição da germinação de sementes de todas as plantas daninhas foi maior com o extrato dos frutos do que com os extratos de raiz, caule, folha e planta inteira de **A. aspera**. A germinação das sementes de todas as plantas daninhas de folhas estreitas foi completamente inibida no extrato a 5% de **A. aspera**. O maior efeito inibitório fitotóxico do extrato dos frutos de **A. aspera** foi devido à*

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presença de ácido gálico (88,4 mg kg^{-1}), ácido cafeico (519,2 mg kg^{-1}) e ácido m-cumárico (51,4 mg kg^{-1}), conforme as análises de CLAE. O estudo, portanto, mostrou que a espécie ***A. aspera*** *exerce efeito inibitório na germinação de plantas daninhas e pode ser mais bem explorada como um herbicida aplicado em pré ou pós-emergência, a fim de fornecer uma alternativa natural aos herbicidas químicos, no futuro.*

Palavras-chave: aleloquímicos, bioensaio de germinação, herbicida natural, plantas daninhas.

INTRODUCTION

Weeds cause significant losses in crop yield and quality. Weeds are unwanted plants that potentially invade cultivated crops and reduce their production and aesthetic value (Navas, 1991). In addition to competition for inputs and space, weeds cause negative effects on other crops by releasing phytotoxic chemicals commonly known as allelochemicals (Abbas et al., 2014). Ecological interference of weeds refer to a phenomenon by which weed plants release phytotoxic chemicals that inhibit germination and growth of surrounding crops or weed plants (Zimdahl, 2007). The literature has reported a significant inhibitory effect of various weed-released phytotoxins on germination and growth of other weed plants (Abbas et al., 2017b). For instance, *Achyranthes aspera*, *Alternanthera philoxeroides*, *Datura metel* and *Rumex dentatus* cause momentous reductions in germination and growth of invasive weed *Parthenium hysterophorus* (Safdar et al., 2016). Maximum inhibition has been shown by the whole plant extract of *A. aspera*. Abbas et al. (2017b) reviewed the herbicidal potential of different weed species, including *Ageratum conyzoides, Alternanthera, Philoxeroides, Alternanthera sessilis, Echinochloa crus-galli* and *Echinochloa colona*, against other weed species in cultivated land.

Chemical herbicides create spray drift hazards and detrimental effects on crops, ground water, soil and environment, but they cannot effectively control herbicide-resistant weeds (Macias et al., 2001; Abbas et al., 2017a). In addition, herbicidal residues in food commodities, directly or indirectly affect human and animal health (Petroski and Stanley, 2009). Yet no new herbicide with a novel mode of action has been introduced to the market for last 25 years (Duke, 2012). After the first report of herbicide resistance in *P. minor* from Pakistan, weed scientists have become worried about the need to find alternative measures to control this troublesome weed of wheat and other weeds of major crops (Abbas et al., 2016). These problems encourage researchers to search for alternative, eco-friendly methods for sustainable weed management. Plant-derived chemicals offer environmentally safe substitutes to chemical herbicides for weed control because they are biodegradable and comparatively safer for the environment (Duke et al, 2000; Petroski and Stanley, 2009). Plant-released phytotoxins are biodegradable and rarely contain halogenated atoms; they can be used directly as natural herbicides or can provide lead structures for herbicidal discovery (Duke et al, 2000). Studies on half-life of various plant-released phytotoxins have reported that they have enough time before decomposition, and during such time they can produce phototoxic effects against weeds (Cheng and Cheng, 2015). Various allelopathic strategies, e.g., application of allelopathic aqueous extracts, mulches, cover crops, residue incorporation and rotation of an allelopathic crop with a routine crop, can be utilized to control weeds under field conditions (Jabran et al., 2015).

Achyranthes aspera L. (prickly-chaff flower) is a weed of the family Amaranthaceae. It can potentially grow on undisturbed waste land and major weed of cultivated crops including maize, sugarcane and wheat (Shah et al., 2006). *Achyranthes aspera* plants contain various phytotoxic compounds that potentially work as a herbicide to inhibit germination and growth of other weed species (Rameshwar and akito, 2007; Srivastav et al., 2011, Abbas et al., 2017b). Safdar et al. (2016), explored the herbicidal potential of *A. aspera* as a natural herbicide against exotic invasive weed *P. hysterophorus*. To the best of our knowledge, no research has been conducted so far to explore the herbicidal potential of *A. aspera* against *P. minor*, *A. fatua*, *L. aphaca, V. sativa, C. arvensis* and *A. tenuifolius*. Therefore, a study was conducted to test the hypothesis that *A. aspera* plant parts contain various allelochemicals in different concentrations that have a differential potential to inhibit germination and seedling growth of weed species. We also assumed

that the response of broadleaf weeds would be different from that of grassy weeds because of their dissimilar types of germination and seed structure. In addition, HPLC analysis was performed to identify the types and concentrations of phytotoxic compounds in roots, stem, leaf and fruits of *A. aspera.*

MATERIALS AND METHODS

Studies were conducted under laboratory conditions to explore the herbicidal potential of *A. aspera* against six weeds viz., *Phalaris minor* Retz. (littleseed canarygrass), *Avena fatua* L. (wild oat), *Lathyrus aphaca* L. (yellow pea), *Vicia sativa* L. (*common vetch), Convolvolus arvensis* L. (field bindweed) and *Asphodelus tenuifolius* L. (onion weed).

Preparation of aqueous extracts

Achyranthes aspera plants were collected at maturity from the Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan during October, 2013 and were shade-dried at room temperature. The dried plant parts were dipped in distilled water at a ratio of 1: 20 w/v (herbage: water) (on dry weight basis) for a period of 24 hours at room temperature (Hussain and Gadoon, 1981). Aqueous extracts were obtained by filtering the mixture through a 2000 µm sieve and then through a 250 µm sieve; the resulting aqueous extract was considered at a 5% concentration.

Determination of biochemical characteristics of aqueous extracts

Values for pH and electrical conductivity (EC) and pH of each extract were determined with a pH meter (JENWAY 3510 pH Meter) and an EC meter (JENWAY 3510). One ml of water extract of each part was centrifuged in a test tube for 10 minutes at 13,000 rpm. Then, 95% ethanol, distilled water and Folin-Ciocalteu phenol reagent (Sigma Chemical Co., St. Louis, MO) were added at a volume of 1-, 5- and 0.5 mL, respectively. After 5 minutes, 5% Na_2CO_3 at a volume equal to that of the water extract (1 mL) was added and the mixture was kept in the dark for one hour. Absorbance was measured at 725 nm using a UV-spectrophotometer (UV-4000, ORI, Germany) and total phenolic content was expressed as gallic acid equivalents (Randhir and Shetty, 2005) (Table 1). Values for pH and EC of *A. aspera* extract were 8.3-9.6 and 2.72-5.44 dS m^{-1} respectively. For identification and quantification of their suspected phytotoxins, aqueous extracts of different plant parts were chemically analyzed on a Shimadzu HPLC system (Model SCL-10A, Tokyo, Japan). The peaks were detected by a UV detector. Standards of suspected phytotoxins (Aldrich, St Louis, USA) were run similarly for their identification and quantification. Concentration of each isolated compound was determined by the following equation:

$$\text{Concentration (ppm)} = \frac{\text{Peak area in the sample}}{\text{Peak area in the standard}} \times \text{Concentration of the standard} \times \text{Dilution factor}$$

Table 2 shows the phytotoxic composition as mg kg^{-1} of various plant parts of *A. aspera.*

Table 1 - Biochemical characteristics of aqueous extracts of various plant parts of *A. aspera*

Plant part	pH	EC (dSm^{-1})	Total phenolic concentration (expressed as gallic acid equivalent) (mg L^{-1})
Root	8.3	2.72	1398.9
Stem	9.6	3.90	1510.6
Leaf	9.1	5.44	3794.7
Fruit	9.4	3.76	5778.5
Whole plant	9.3	4.65	2875.5

Table 2 - Phytotoxic composition as mg kg^{-1} of various plant parts of *A. aspera* (dry matter)

Sr. No.	Phenolic	Plant part				
		Root	Stem	Leaf	Fruit	Whole plant
1	Gallic acid	-	45.0	239.4	88.4	337.0
2	Caffeic acid	-	319.0	319.4	519.2	148.2
3	4-hydroxy-3-methoxy benzoic acid	-	347.6	1243.8	-	495.4
4	*p*-coumaric acid	157.8	-	155.4	-	-
5	*m*-coumaric acid	-	-	-	51.4	62.6
6	chromotropic acid	-	-	-	-	1276.6
7	vanillic acid	678.8	-	-	-	-
8	syringic acid	-	-	-	-	184.2
9	ferulic acid	517.8	-	-	-	-
10	chlorogenic acid	-	-	-	-	-

Germination studies

In this experiment, 5% (w/v) aqueous extracts of root, stem, leaf, fruit and whole plant of *A. aspera* were used. Ten seeds of each weed species were evenly placed in 9 cm diameter petri plates separately lined with a double layer of sterile filter paper (Whatman No.1) and the 8 mL of each aqueous extract and distilled water (for control) were applied. After planting, the seeds on the petri plates were covered with Parafilm® to prevent evaporation losses. The Petri plates were than arranged under a completely randomized design with four replications. The Petri plates were kept under laboratory conditions for 24 hours with minimum (20 ± 2 °C) and maximum (25 ± 2 °C) temperatures throughout the course of this study. The number of seeds germinated were counted on a daily basis up to a period of 15 days. Mean germination time (MGT) was calculated as per the equation of Ellis and Roberts (1981):

$$MGT = {\Sigma(D_n)}/{\Sigma n}$$

where, n is the number of seeds or emerged seedlings on day D, and D is the total number of days counted from the beginning of germination.

Data analysis

The data of the repeated experiments were pooled for statistical analyses. When the treatment effect was significant at the 5% level, multiple mean comparison treatments were completed and letter groupings were generated, using the least significant difference (LSD) at 5% significance level. The validity of normal distribution and constant variance assumptions on the error terms were checked by examining the residuals (Steel et al., 1997).

RESULTS AND DISCUSSION

Figure 1 shows the effect of aqueous extracts of various plant parts of *A. aspera* on germination percentage (GP) and mean germination time (MGT). The results showed that the extracts of all plant parts significantly inhibited percentage and speed of germination of all test weeds when compared with control (distilled water). Moreover, the response of each weed species was different for aqueous extracts from different plant parts of *A. aspera*. When supplying the whole plant, fruit, leaf and stem extracts of *A. aspera* to *P. minor*, the fruit extract to *A. fatua*, the fruit and stem extracts to *L. aphaca*, the fruit, leaf and stem extracts to *V. sativa*, the whole plant and leaf extracts to *C. arvensis*; the whole plant, fruit and root extracts to *A. tenuifolius,* none of their seeds was able to germinate, resulting in zero GP in these weeds.

Phalaris minor and *A. fatua* (grassy weeds) showed zero germination in response to the 5% fruit extract of *A. aspera* (Figure 1). Furthermore, *P. minor* did not germinate in Petri plates

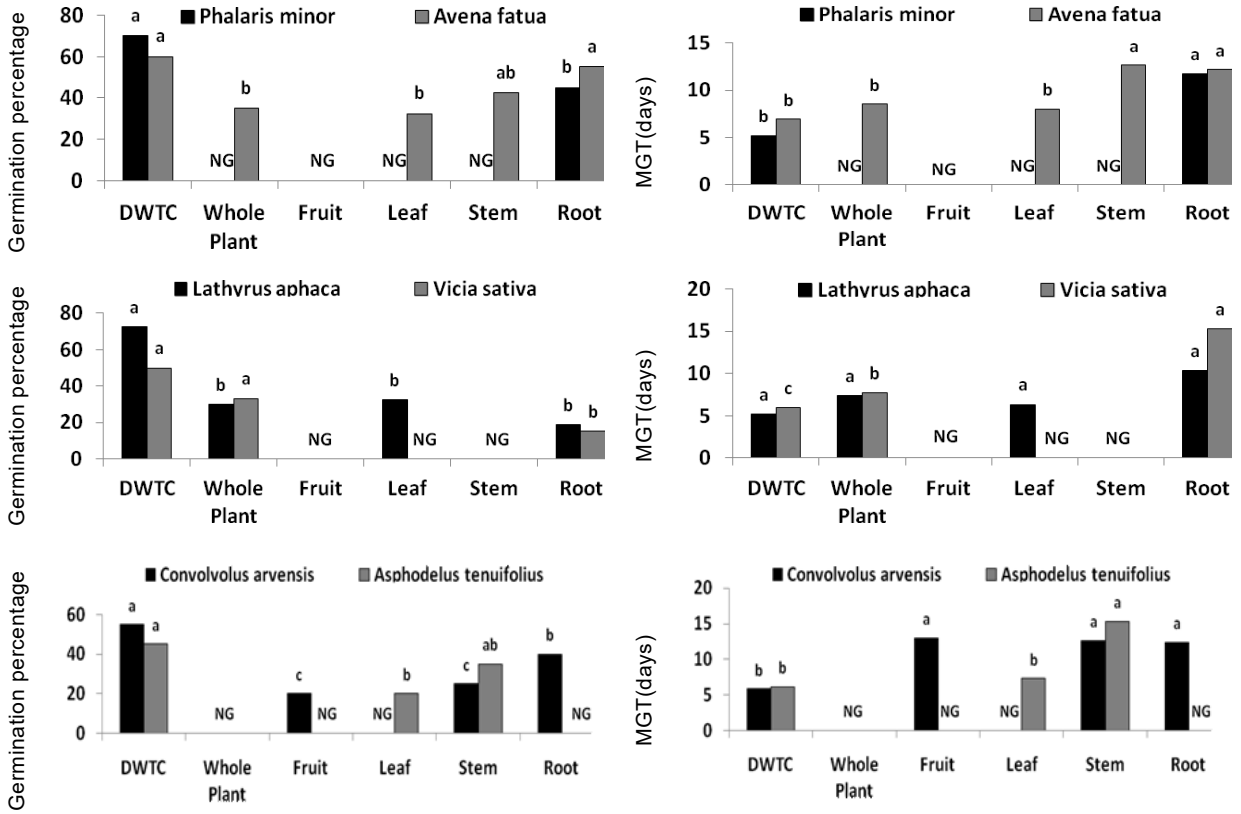


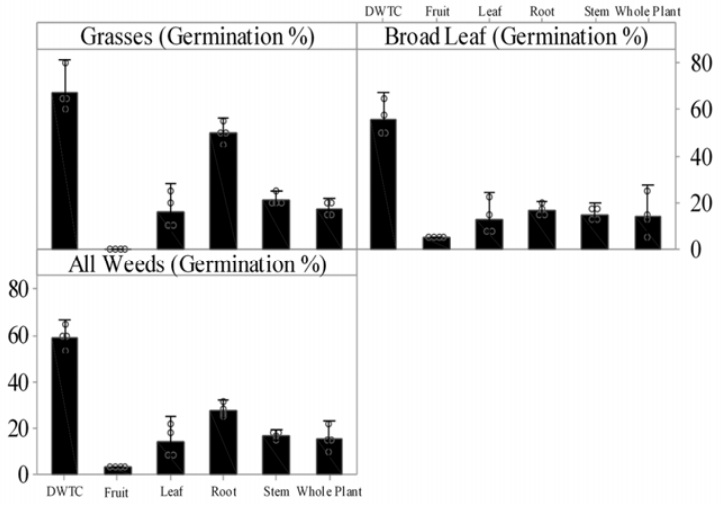
Figure 1 - Germination percentage and MGT of six major weeds as influenced by aqueous extracts (5% w/v) of various plant parts of *Achyranthes aspera* L. DWTC stands for distilled water treated control.

which had been treated with the whole plant, leaf and stem aqueous extracts of *A. aspera*. There was also strong inhibition of germination of broad leaf weeds with the fruit extract of *A. aspera*. Overall seed germination inhibition of all weeds was about 97% with the fruit extract of *A. aspera*, followed by the leaf extract with 86% inhibition. A similar trend was recorded in MGT for all test weed species.

The root extract of *Achyranthes aspera* in *P. minor* and *V. sativa* (45 and 15%), the leaf and whole plant extracts in *A. fatua* (32.5 and 35%), the whole plant and stem extracts in *L. aphaca* (30 and 32.5%), the fruit and stem extracts in *C. arvensis* (20 and 25%) and the leaf extract in *A. tenuifolius* (20%), respectively, caused a significant reduction in GP (Figure 1). A comparison among weed species showed that significantly higher MGT values were found in *P. minor* (11.8 days) and *V. sativa* (15.3 days) when they had been treated with the aqueous extract of *A. aspera* roots; *A. fatua* (12.7 and 12.2 days) and *C. arvensis* (12.6 and 12.4 days) in response to the *A. aspera* stem and root extracts, respectively; and *A. tenuifolius* (15.4 days) in response to the *A. aspera* stem extract (Figures 1, 2 and 3).

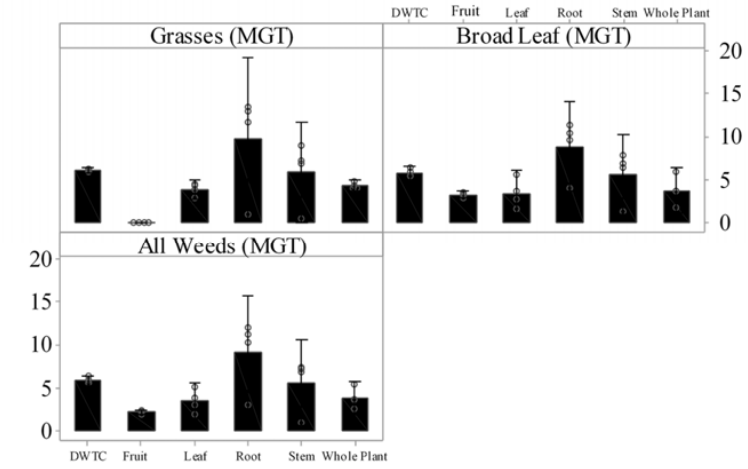
The extraction of *A. aspera* allelochemicals with water is better than using organic solvents (Salam and Kato-Noguchi, 2011). Allelochemicals are secondary metabolites, and they are non-nutritional primary products (Soltys et al., 2013). There are different chemical groups of allelochemicals, e.g., alkaloids, phenolics, saponins, oleonolic acid and dihydroxy ketones (Srivastav et al., 2011: Soltys et al., 2013). The allelopathic influence of various weed species has been explored against field crops and weeds (Abbas et al., 2017b). Inhibition of seed germination of other plants is one of the most common allelopathic strategies employed by *A. aspera* (Khan and Shaukat, 2006; Abbas et al., 2017b).

Germination bioassays are excellent tools to evaluate the influence of any exogenously applied plant-released or synthetic compound (Williams and Hoagland, 2003). Hence, allelopathic experiments were planned to apply aqueous extracts of different plant parts of *A. aspera* at a 5% w/v concentration directly to the germinated seeds of various weeds *in vitro*. There was a differential response of weeds against the aqueous extract obtained from different plant parts of



95% confidence intervals (CI) for the mean. Individual standard deviations were used to calculate the intervals. DWTC stands for distilled water treated control.

Figure 2 **-** Interval plot of grasses (GP), broad leaf (GP) and all weeds (GP).



95% confidence intervals (CI) for the mean. Individual standard deviations were used to calculate the intervals. DWTC stands for distilled water treated control.

Figure 3 **-** Interval plot of grasses (MGT), broad leaf (MGT) and all weeds (MGT).

A. aspera. Phytotoxic inhibition of the *A. aspera* fruit extract was more pronounced when compared with other plant parts. The strong inhibitory potential of aqueous extract obtained from *A. aspera* root was due to presence of vanillic acid (678.8 mg L^{-1}), ferulic acid (517.8 mg L^{-1}) and *p*-coumaric acid (157.8 mg L^{-1}) whereas gallic acid (88.4 mg L^{-1}), caffeic acid (519.2 mg L^{-1}) and *m*-coumaric acid (51.4 mg L^{-1}) were found in the fruit extract (Table 1).

The differential inhibitory potential of different plant parts has been reported in the literature (Abbas et al., 2014). For instance, the leaf extracts of *Xanthium strumarium* and *Amaranthus spinosus* showed more inhibition than their stem and root extracts; and the root extract of *Physalis minima* caused more inhibition than its leaf and stem extracts against germination of *P. hysterophorus* (Sinha and Singh, 2004; Swain et al., 2004), respectively. Chandra et al. (2011), reported inhibition of broad bean (*Vicia faba*) after application of a concentrated aqueous extract of *A. aspera*. Complete failure of seed germination was recorded at > 6% leaf extract of *P. hysterophorus* in *Ageratina adenophora* and at 10% in *Artemisia decbia* (Maharjan et al., 2007). Germination of *A. fatua* and *Bidens pilosa* was reduced by increasing the concentration of *P. hysterophorus* extract (Batish et al., 2002).

The present findings suggest that narrow leaf weed seeds are more sensitive to *A. aspera* extracts as compared to broad leaf weed seeds. In a similar study, it was noted that species varied considerably in their sensitivity to aqueous extracts of *A. aspera* and *P. hysterophorus* for germination (Khan and Shaukat, 2006; Gupta and Narayan, 2010). The leaf biomass of *A. aspera* has been reported to inhibit germination in wheat and pea, but inhibition was increased when the amount of incorporated biomass was increased (Gupta and Narayan, 2010).

Based on the present study, it was concluded that allelochemicals in *A. aspera* possess weed suppressing ability that could be used for development of natural herbicides by obtaining their higher contents through an extraction method. Further studies are needed to clarify the weed control potential and effective dose of *A. aspera* allelochemicals against other weed species and the mechanism behind weed control.

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