ECOPRINT **21:** 23-30, 2014 ISSN 1024-8668
DOI: http://dx.doi.org/10.3126/eco.v21i0.11901
Ecological Society (ECOS), Nepal
www.nepjol.info/index.php/eco; www.ecosnepal.com

ALLELOPATHIC EFFECT OF *ARTEMISIA DUBIA* EXTRACTS ON SEED GERMINATION AND SEEDLING GROWTH OF SOME WEEDS AND WINTER CROPS

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ABSTRACT

Allelopathic effects of aqueous extract of different plant parts (root, stem and leaf) of *Artemisia dubia* on seed germination and seedling growth of two winter crops (*Triticum aestivum* and *Brassica campestries,* and some associated weeds (*Bidens pilosa, Ageratum conyzoides, Galinsoga parviflora* and *Cyperus rotundus*) have been investigated in the present study. Extracts of root, stem and leaves of *Artemisia dubia* showed significant reduction in germination and seedling growth of test crops and weeds. Germination of crop and weed seeds and growth of shoot and root were reduced significantly in test treatments in comparison to the control. The seed germination, shoot length and root length were low at higher concentration. Complete inhibition of seed germination of *Ageratum conyzoides*, *Galinsoga parviflora* and *Cyperus rotundus* at 5 and 10% leaf extract of *A.dubia* was observed. Allelopathic effects were more pronounced with leaf extract than root or stem extract in most cases.The result indicated difference in allelopathic effect on crop seed and weed seed at higher concentrations.

Key words: *Artemisia dubia*, aqueous extract, allelopathy, weed.

INTRODUCTION

Several species of Asteraceae family are known for having alleolpathic compounds which may reduce seed germination and seedling emergence of other plants (Watban and Salama 2012). Genus *Artemisia* is hardy herbaceous shrub which is widely distributed, and consists species between 200 and 400.

The research information is inadequate on allelopathic effect of potential plants in controlling weeds of vegetables. Leaf extract of *A. dubia* caused an inhibitory effect on germination and growth of barnyard grass (Paudel *et al.* 2005). Such information will help to develop organic herbicides which are environmentally safe and cost effective. Identification of suitable plants with herbicidal properties, with their formulation gains special importance in organic farming. Although many plant species are reported to have allelopathic properties but the information on their compatibility with field crops, effective active ingredient, extraction and utilization technology is lacking. Therefore, present work was carried out to investigate the potentiality of *Artemisia* aqueous extract on seed germination and seedling growth of some winter crops and associated weeds.

As some growth inhibitors have been reported from some other species of *Artemisia*, therefore, it is hypothesized that aqueous extract of *Artemisia dubia* possibly will also reduce seed germination of both winter weeds and winter crops due to their allelopathic effects. The adverse effect on seed germination will be more on weed seeds than on crop seeds because the size and reserve food materials in weed seeds are comparatively less.

MATERIALS AND METHODS

Site for seed collection

Experiments were conducted under laboratory conditions at Botany Department, Amrit Campus (Tribhuban University), Kathmandu. The mature weed seeds of *Ageratum conyzoides*, *Bidens pilosa*, *Cyperus rotundus* and *Galinsoga parviflora* were collected from Wheat, Mustard and Radish fields and fallow land from different sites of Kathmandu valley in Kirtipur (Machhegaon, Chhobhar), Bhaktapur (Thimi, Lokanthali, Gatthaghar) and Godavari.

Preparation of extracts of *Artemisia dubia*

Artemisia dubia plants were collected from Kirtipur, Bhaktapur and Kathmandu area for preparing the extracts of stem, root and leaf. Collected plants were air dried and then leaf, stem and roots were separated. To prepare aqueous extract, 2 g of grinded air dried leaf, stem and root were soaked in 20 ml distilled water for 24 h separately. The extracts were filtered using Whatman No.1 filter paper and thus 10% stock solution was prepared. From this stock solution, 5%, 2.5%, and 1.0% concentration was prepared by diluting with distilled water.

Seed germination

Seeds of the dominant weed species (*A. conyzoides*, *B. pilosa*, *C. rotundus* and *G. parviflora*) and the crop seeds of *B. campestris* and *T. aestivum* were soaked in 2-4% Sodium hypochlorite for 2 minutes separately. The seeds were then washed with distilled water thoroughly. The sterilized petridishes were lined with single Whatman No. 1 filter paper and moistened with 5 ml of treatment solution. Uniform size seeds of the crops (*B. campestris*, *T. aestivum)* and weeds *(A. conyzoides*, *B. pilosa*, *C. rotundus* and *G. parviflora*) were selected and five seeds of each tested species were kept in sterilized petridishes containing 0% (control), 1, 2.5, 5 and 10% concentrations of aqueous extracts for 10 days. For control, seeds were grown in filter paper soaked with distilled water. All these experiments were conducted under normal room temperature. Each treatment was replicated four times. The moisture level in the petridish was maintained by adding distilled water as required.

Statistical Analysis

To understand significant difference among the data obtained at each treatment, statistical analysis was done by using IBM SPSS Statistics Version 20. The data were subjected to one way ANOVA followed by Duncan's Multiple range test.

RESULTS

In the present study, seed germination, shoot length and root length was found decreased with the increase in the concentration of aqueous extracts in both crops (*Brassica campestris, Triticum aestivum)* and tested weeds (*Bidens pilosa*, *Ageratum conyzoides, Galinsoga parviflora* and *Cyperus rotundus*) (Tables 1-6). Seed germination of both crops *B. campestris* and *T. aestivum* was found reduced significantly with increase in concentration of root, stem and leaf extract of *A. dubia* (Tables 1 and 2). Insignificant decrease in germination of *Brassica* seeds was observed at 1, 2.5 and 5% root extract but significant reduction was noticed at 5 and 10% concentrations. Similarly, insignificant decrease in *Triticum* seed germination was observed up to 2.5% root and leaf extract treatment. Germination of *Brassica* seeds was found to be higher in root extract than in stem or leaf extracts, but in *Triticum*, reduction in germination of seeds was more with root and stem treatments than with leaf extract. Inhibition of growth i.e. denoted by shoot and root length was highest with leaf extract in *Brassica* but it was more with root extracts in *Triticum* (Table 2).

Table 1. Effect of root, stem and leaf extracts of *Artemisia dubia* on seed germination (%±SD), shoot and root length (cm±SD) of *Brassica campestris* after 10 days.

Concentration (%)	Root extract			Stem extract			Leaf extract		
	Germination	Shoot length	Root length	Germination	Shoot length	Root length	Germination	Shoot length	Root length
0	100 ±0.00c	8.05 ±0.37 d	5.86 ±0.50 e	80 ±16.32 b	6.13 ±0.39 c	4.87 ±0.41c	90 ±11.54 b	3.64 ±1.39c	1.87 ±1.11b
1	80 ±16.32 b	5.71 ±1.39 c	4.77 ±1.17 d	75 ±12.14 b	4.25 ±1.90 b	3.15 ±1.41b	85 ±10.00 b	3.45 ±1.52c	1.61 ±1.14b
2.5	80 ±0.00 b	4.03 ±1.81 b	3.59 ±1.28 c	65 ±19.14 ab	3.13 ±2.38ab	2.38 ±1.83ab	70 ±34.64 ab	2.20 ±1.75 b	0.98 ±0.96 a
5	75 ±10.00 b	2.71 ±1.63 a	2.35 ±1.42 b	50 ±11.54 a	2.27 ±2.38a	1.89 ±1.95a	50 ±25.81 a	1.65 ±1.79ab	0.73 ±0.97a
10	60 ±16.32 a	1.84 ±1.40 a	1.61 ±1.23 a	45 ±10.00 a	1.87 ±2.17a	1.65 ±1.89a	40 ±16.32 a	0.84 ±1.12 a	0.35 ±0.48a

Same letters in the same column after Mean ± SD does not differ significantly according to ANOVA followed by Dunkan's Multiple Range Test at P=0.05.

Table 2. Effect of leaf, stem and root extracts of *Artemisia dubia* on seed germination (%±SD), shoot and root length (cm±SD) of *Triticum aestivum* after 10 days.

Concentration (%)	Root extract			Stem extract			Leaf extract		
	Germination	Shoot length	Root length	Germination	Shoot length	Root length	Germination	Shoot length	Root length
0	85 ±19.14 c	8.89 ±0.45e	6.12 ±0.34 e	100 ±0.00 c	9.00 ±1.18 c	5.27 ±0.27 b	100 ±0.00 b	10.28 ±0.34d	7.58 ±1.05c
1	80 ±0.00 c	7.60 ±0.49d	5.16 ±0.46 d	75 ±10.00 b	6.19 ±3.71 b	4.31 ±2.75 b	100 ±0.00 b	8.58 ±2.98 c	6.56 ±0.84 c
2.5	70 ±11.54 bc	4.54 ±2.36c	3.89 ±2.01 c	70 ±20.00 ab	5.77 ±2.52 b	4.26 ±1.86 b	85 ±10.00 ab	5.18 ±2.45 b	3.92 ±1.76b
5	55 ±10.00ab	3.48 ±2.09 b	2.92 ±1.75 b	55 ±10.00 ab	3.21 ±3.04a	2.25 ±2.20a	75 ±30.00 ab	3.90 ±3.10 ab	3.15 ±2.50 ab
10	40 ±23.09 a	2.31 ±1.76 a	1.96 ±1.53 a	50 ±20.00 a	2.65 ±2.73a	1.66 ±1.71 a	60 ±16.32 a	3.11 ±2.44 a	2.49 ±2.08 a

Same letters in the same column after Mean ± SD does not differ significantly according to ANOVA followed by Dunkan's Multiple Range Test at P=0.05.

Insignificant effect on seed germination was observed up to 2.5% root, stem and leaf extract in *Bidens pilosa* (Table 3) and up to 1% of root, stem and leaf extract in *Ageratum conyzoides* (Table 4). In both weeds, seed germination decreased significantly (P=0.05) with their higher concentrations. The length of shoot and root of seedlings decreased significantly (P=0.05) in all treatments with increase in concentration (Tables 3 and 4). Leaf extract of 5 and 10% concentration was found to be more detrimental for germination of seeds of *Ageratum conyzoides* and no germination was observed.

Table 3. Effect of leaf, stem and root extracts of *Artemisia dubia* on seed germination (%±SD), **shoot and root length (cm±SD) of *Bidens pilosa* after 10 days.**

Concentration	Root extract			Stem extract			Leaf Extract		
(%)	Germination	Shoot length	Root length	Germination	Shoot length	Root length	Germination	Shoot length	Root length
0	85 ±10.00 **c**	4.94 ±1.70**d**	3.85 ±1.38**c**	85 ±10.00 **c**	4.35 ±1.92**c**	3.47 ±1.54**c**	80 ±16.32 c	7.81 ±1.21**c**	6.36 ±1.42**c**
1	70 ±11.54**bc**	4.00 ±1.74 **cd**	3.17 ±1.41**bc**	75 ±10.00 **bc**	3.32 ±1.97**bc**	2.49 ±1.50**b**	70 ±11.54 **bc**	4.61 ±3.15**b**	3.96 ±2.68**b**
2.5	65 ±19.14 **bc**	3.42 ±1.76**bc**	2.77 ±1.45**b**	70 ±11.54 bc	2.75 ±1.87**ab**	2.03 ±1.46**ab**	65 ±13.00 **bc**	4.50 ±3.41**b**	4.05 ±2.80**b**
5	55 ±10.00 **ab**	2.70 ±1.64**ab**	2.32 ±1.41**b**	60 ±16.32**ab**	2.22 ±1.87**ab**	1.64 ±1.39**ab**	50 ±11.54 **ab**	3.24±3.45**a** **b**	2.41 ±2.57**a**
10	40 ±16.32 **a**	1.88 ±1.96**a**	1.34 ±1.40**a**	50 ±11.54 **a**	1.53 ±1.59**a**	1.11 ±1.23**a**	35 ±10.00**a**	0.71 ±2.32**a**	1.55 ±2.30**a**

Same letters in the same column after Mean ± SD does not differ significantly according to ANOVA followed by Dunkan's Multiple Range Test at P=0.05.

Table 4. Effect of leaf, stem and root extracts of *Artemisia dubia* on seed germination (%±SD), **shoot and root length (cm±SD) of *Ageratum conyzoides* after 10 days.**

Concentration	Root extract			Stem extract			Leaf extract		
(%)	Germination	Shoot length	Root length	Germination	Shoot length	Root length	Germination	Shoot length	Root length
0	80 ±16.32 d	2.29 ±1.40b	1.62 ±1.11b	75 ±10.00 c	2.17 ±1.41b	1.44 ±1.03b	70 ±20.00 b	2.00 ±0.52c	1.22 ±0.25b
1	75 ±10.00 cd	2.07 ±1.56b	1.44 ±1.10b	65 ±19.00 bc	1.80 ±1.31b	1.20 ±0.97b	50 ±11.54ab	1.19 ±0.34b	1.02 ±0.05a
2.5	60 ±0.00bc	1.74 ±1.24 b	1.29 ±0.95b	50 ±11.54 ab	1.60 ±1.42b	1.15 ±0.73b	35 ±19.14a	1.30 ±1.11ab	1.11 ±0.01a
5	50 ±11.54 b	1.60 ±0.81a	1.51 ±0.57a	45 ±19.14 ab	1.50 ±0.79a	1.33 ±0.57a	NG	NG	NG
10	30 ±11.54 a	1.35 ±0.39a	1.19 ±0.21a	40 ±0.00a	1.35 ±0.38a	1.32 ±0.38a	NG	NG	NG

Same letters in the same column after Mean ± SD does not differ significantly according to ANOVA followed by Dunkan's Multiple Range Test at P=0.05.NG = No germination

Seed germination of *Galinsoga parviflora* and *Cyperus rotundus* decreased with increase in concentration of root, stem and leaf extracts of *A. dubia* (Tables 5 and 6). In both weeds, seed germination in root extract was more than in extracts of stem and leaves. Lowest percentage of seed germination was observed with leaf extract treatment indicating its more allelopathic effects than root or stem. Significant decrease in shoot length and root length of seedlings were observed with high concentrations. Germination of *Galinsoga* seed was completely inhibited with 10% stem and leaf extract of *A. dubia* (Table 5). Similarly complete inhibition of seed germination with 10% root stem and leaf was observed in *Cyperus rotundus* (Table 6).

Table 5. **Effect of leaf, stem and root extracts of *Artemisia dubia* on seed germination (%**±SD) **shoot and root length (cm±SD) of*Galinsoga parviflora* after 10 days.**

Concentration	Root extract			Stem extract			Leaf extract		
(%)	Germination	Shoot length	Root length	Germination	Shoot length	Root length	Germination	Shoot length	Root length
0	75 ±10.00 c	1.73 ±1.32b	1.39 ±1.03 c	65 ±19.14 d	1.64 ±1.05c	1.28 ±1.00b	60 ±28.28 c	1.40 ±0.93c	1.20 ±0.44b
1	65 ±10.00c	1.49 ±1.39b	1.29 ±0.87 bc	50 ±11.54 cd	1.36 ±0.84 bc	1.17 ±0.82b	45 ±19.14 bc	1.16 ±0.77bc	1.11 ±0.44b
2.5	45 ±10.00 b	1.40 ±1.35 b	1.20 ±0.77 bc	40 ±0.00bc	1.30 ±0.74 bc	1.08 ±0.75b	20 ±16.32ab	1.17 ±0.37ab	1.02 ±0.09a
5	40 ±16.32 ab	1.32 ±0.81a	1.11 ±0.51 ab	30 ±11.54 ab	1.28 ±0.47 ab	1.03 ±0.47 a	15 ±10.00 a	1.20 ±0.52a	1.00 ±0.07a
10	25 ±10.00 a	1.22 ±0.30a	1.10 ±0.19 a	NG	NG	NG	NG	NG	NG

Same letters in the same column after Mean ± SD does not differ significantly according to ANOVA followed by Dunkan's Multiple Range Test at P=0.05;.NG = No germination

Table 6. Effect of leaf, stem and root extracts of *Artemisia dubia* on seed germination (%±SD) **shoot and root length (cm±SD) of *Cyperus rotundus* after 10 days.**

Concentration	Root extract			Stem extract			Leaf extract		
(%)	Germination	Shoot length	Root length	Germination	Shoot length	Root length	Germination	Shoot length	Root length
0	70 ±11.54 c	1.65 ±0.89c	1.48 ±0.66d	65 ±10.00 c	1.42 ±0.98c	1.33 ±0.88c	60 ±16.32 c	1.39 ±0.52c	1.30 ±0.25c
1	50 ±25.81 c	1.54 ±0.80c	1.37 ±0.59 cd	55 ±19.14 c	1.36 ±0.92 c	1.24 ±0.73c	45 ±10.00 c	1.23 ±0.52b	1.17 ±0.21b
2.5	25 ±19.14 b	1.38 ±0.85c	1.29 ±0.44 bc	30 ±11.54 b	1.25 ±0.88 bc	1.20 ±0.48b	25 ±10.00 b	1.18 ±0.34a	1.07 ±0.13ab
5	20 ±0.00ab	1.27 ±0.44b	1.18 ±0.25ab	15 ±10.00 ab	1.22 ±0.48 ab	1.17 ±0.26ab	15 ±10.00 ab	1.12 ±0.08a	1.04 ±0.04a
10	NG	NG	NG	NG	NG	NG	NG	NG	NG

Same letters in the same column after Mean ± SD does not differ significantly according to ANOVA followed by Dunkan's Multiple Range Test at P=0.05;.NG = No germination

Comparing seed germination of *Brassica campestries* in leaf extract (where the allelopathic effect was high) with other tested weed (Tables 1 and 3-6), it was observed that adverse effect on seed germination is low in *B. campestries* than in other tested weeds. Similarly, from comparison of seed germination of *Triticum astivum* (in root and leaf extracts) and other weeds (Table 2-6), it was noted that adverse effect on *Triticum* seed germination was less than in studied weeds.

DISCUSSION

In the present study it was found that seed germination, shoot length and root length decreased in extract of vegetative parts (leaf, stem and root) of *Artemesia* but the effect of leaf extract was more pronounced. Putnam (1988) listed 6 classes of allelochemicals namely alkaloids, bezaoxaziones, cinnamic acid derivatives, Cyanogenic compounds, ethylene and others. More amount of volatile phytotoxic compounds have been recorded from green leafy part in *Artemesia californica (*Halligen 1973) and possibly such phytotoxic chemicals are also present in *A. dubia* and this may be the reason for more allelopathic effect of leaf on seed germination.

The germination of seeds, root and shoot length of crop plant *B. campestris* and *T. aestivum* was inhibited by the aqueous extract of weed *A. dubia*. Among the different treatments, the seed germination was more reduced in 5% and 10% concentration of root, stem and leaf extracts. Similar effects were obtained by Watban and Salama (2012) of *Artemisia monosperma* extract on *Phaseolus vulgaris*. Similarly, decrease in germination, growth and chlorophyll contents in *T. aestivum* was reported by Deef and El-Fattah (2008) when grown on the aqueous extract of *Artemesi aprinces.* Study of the allelopathic effects of some selected weeds (*Phalaris minor L., Chenopodium murale L., Sonchus oleraceus L., Cyanodon dactylon L.* and *Convolvulus arvensis L.*) on seed germination and seedling growth of wheat (*Triticum aestivum L.*) also showed similar inhibitory effects on seed germination; seedling length and seedling dry weight of crop, which increased progressively on increasing the concentration of extracts of weed plants (Gupta and Mittal 2012).

Seed germination and seedling growth of weeds *Bidens pilosa, Ageratum conyzoides Galinsoga parviflora* and *Cyperus rotundus* grown in the aqueous extract of *A. dubia* also revealed the reduction with increase in concentrations. Katoch *et al.* (2012) have also reported that the inhibition of seed germination and seedling growth were dependent on concentration and numerically more inhibition was observed at higher concentration. In present study significant reduction of shoot and root length occurred in all concentrations of leaf extract, which were also observed by many earlier workers while working on other weeds indicated that leaves have more powerful inhibitory allelopathic effect than other vegetative parts (Kanchan 1975, Tefera 2002, Maharjan *et al.* 2007). Passim and Rodrigues (1999) also mentioned that the aqueous extract of *Artemisia velotrum* reduced the seed germination, shoot and root length of some crops (*Zea mays*, *Glycine max*) and weeds like *Bidens pilosa, Galinsoga parviflora, Sida rhombifolia, Amaranthus retroflexus, Ipomoea aristolochiaefolia,* and *Cenchrus echinatus.*

There is total inhibition of seed germination of *Ageratum conyzoides*, *Galinsoga parviflora* and *Cyperus rotundus* with higher concentration of leaf extract. Work by Mittal and Kohli (2010) mentioned that Artemisia oil has bioherbicidal properties as it causes phytotoxicity and interfere with the growth and physiological processes of some weed species. Similarly, in the present sudy, more allelopathic effects were found with leaf extract followed by stem and then root in most cases except in *Triticum aestivum*. Quartey *et al.* (1997) also revealed that leaves of *Artemisia afra* had the most inhibitory effect on seed germination compare to other parts of plant. The aerial parts contain high inhibitory allelochemicals (Gill *et al.* 1996) which may interfere with the processes of plant growth. These allelochemical may be reducing cell division or auxin induced growth of roots (Gholami *et al*. 2011).

Comparison of seed germination of *Brassica campestries* and *Triticum aestivum* with other studied weeds revealed that seed germination in crops was less adversely affected than in weeds. This might possibly be due to large seed size and more reserve food material present in crop seed than in weed seeds.

From the study it can be concluded that the aqueous extract of vegetative parts, particularly leaf contained more growth inhibiting allelochemicals. According to the study the shoot and root length of weeds (*Bidens pilosa*, *Ageratum conyzoides*, *Galinsoga parviflora* and *Cyperus rotundus*) and crops *(Brassica campestris* and *Triticum aestivum)* are significantly reduced at high concentration of *Artemisia dubia* extract. Comparison of seed germination percentage in crops and weed seeds revealed that seed germination is less adversely reduced in crops than in weeds. Artemesinine, a sesquiterpene lactone extracted from *Artemisia annua L.* is a potent plant growth inhibitor (Javaid and Anjum 2006). Similar allelochemicals may be present in *A. dubia* as both of them belong to the same genus. Thus, there is possibility for extraction and using of such allelochemicals from *Artemesia dubia* directly or to develop environment friendly bio-herbicides to control weeds.

ACKNOWLEDGEMENTS

The authors like to thank Campus Chief and Head of Botany Department, Amrit Campus, Tribhuvan University, Kathmandu, Nepal for providing laboratory facilities

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