ARTEMISIA DUBIA WALL EX BESSER (MUGWORT): A WEED TO CONTROL WEED

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Abstract: The present investigation was carried to screen the phytochemicals presence in *Artemisa dubia* Wall ex Besser and its allelopathic effect. The allelopathic effect of aqueous (decomposed) extract, leachate and solvent extract (hexane, methanol and aqueous) of leaf, stem and root of plant was studied on test seeds of paddy and Barnyard grass focusing on their germination and seedling vigour. Effect of mulch in seedling elongation of test seed was studied in earthen pots. The main groups of chemical constituent were identified by their colour and/or precipitation reaction with specific reagent from different solvent extract. Out of 17 phytochemical tests performed from different solvents (hexane, methanol and H_2O) on this plant, six groups of phytochemicals were detected. Allelopathic effect of all treatments showed significant inhibitory effect on germination of Barnyard grass but had no effect on germination of paddy seeds. All treatments showed significant inhibitory effect on seedling vigour of Barnyard grass but most treatments showed stimulatory effect on seedling vigour of paddy. Leaf mulch significantly retarded the growth of Barnyard grass as compare to paddy. All treatments found to be stimulatory to paddy and inhibitory to Barnyard grass seedling elongation excepting a few inhibitory effect of leaf mulch to seedling growth of Barnyard grass. This finding supported the traditional practice of farmers using leaf mulch of *Artemisia dubia* to control problem of Barnyard grass in their fields.

Key words: Allelopathy; *Artemisia dubia*; Barnyard grass; leaf mulch; phytochemical screening; paddy.

INTRODUCTION

Allelopathy is the effect of plants on one another (including microorganism) due to the release chemicals by the breakdown of their metabolites (Willis, 1994), and the allelopathic effect depnds on the allelochemicals being added to environment (Putnam and Tang, 1986). The allelopathy is a natural phenomenon and chemicals involved in this process get naturally released in amount enough to exert the effect. Allelochemicals vary from species to species (Qasem and Foy, 2001) and varieties of allelochemicals present in plant determine their invasiveness (Putnam, 1985; Zimdahal, 1999).

Although weed account not more than 1% of the total plant species (Qusem and Foy, 2001), nevertheless they cause great problem to humankind by interfering food production, health, economic stability and welfare, Weeds create serious problem to field crop, they reduce the yield, increase the cost of production, degrade the quality of harvested production and reduce the quality of market product. They are not only problematic from an agricultural standpoint but are also a great threat to the plant diversity of near by flora and their subsequent spread reducing the diversity of native plants.

Artemisia dubia Wall ex. Besser is a problematic invasive weed of Nepal found in a wide altitudinal range 1200-3400 masl (Press et al. 2000). Diverse allelochemicals present in aerial portion of mugwort (Lee et al. 1998) influence other ecosystem components which in turn drive interaction that determine the community structure. Since mugwort is not found inside crop field and less concerns regarding its problem from agricultural standpoint. Reversely it is much more concerned to solve the agricultural problem brought by *Echinocloa crus-galli* (Barnyard grass). Hence, it represents an excellent potential source of natural chemicals that may be involved in developing natural herbicides referred to as the future natural pesticides or natural herbicides or bioherbiacide in action (Rice, 1995).

The traditional farmers of Nepal use the leaf mulch of mugwort in their seedbeds of paddy to control the problem of Barnyard grass infestation. This investigation tried to provide the scientific proof to the traditional belief on mugwort as weedicide.

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MATERIALS AND METHODS

Adequate plant (*Artemisia dubia*) materials were collected from the fallow land and marginal land in Kirtipur, Kathmandu. Extraction was done for different parts in different solvent (viz. hexane, methanol and distilled water) of increasing polarity and main groups of chemical constituents were screened following the procedure given by Harborne (1984). For leachate and mulch, plant parts were collected fresh on the same day of experiment. Laboratory based experiments were conducted on seeds of *Oryza sativa* (paddy) and *Echinocloa crus-galli* (Barnyard grass). For studies on seed germination and seedling growth, petridish and earthen pots were used in sterilized condition. Bioassay was carried out in triplicates. These sets were placed at room temperature under continuous light.

Five concentrations (2, 4, 6, 8 and 10%) of aqueous (decomposed extract and leachate and 3 concentrations (0.1, 10 and 1000 ppm) of solvent extract (viz, hexane, methanol and aqueous extracts) and distilled water was used as control. The treatments were carried out for all extracts from all parts (viz, leaf, stem and root). The observations on germination and root and shoot length were made on 7th day of experiment. For pot experiment, 10 g leaf was mulched in each pot having similar type of sterilized soil. Viable seeds of paddy and Barnyard grass were sown in a manner that seeds alone as well as mixed in both mulch and control (soil without mulch) condition, weekly growth of above ground part of seedling was measured up to 4 weeks.

Data were analyzed statistically by using statistical package for social science (SPSS). All means of the same treatment were separated by using homogeneity variance test. Analysis of variance, least significant differences, correlation analysis and paired sample test were taken during the statistical analysis.

RESULTS AND DISCUSSION

Phytochemical Screening

Seventeen phytochemical tests were performed in which six groups of phytochemical gave positive test. These are volatile oils and sterols and triterpenes from hexane extract polyphenols and steroid glycosides from methanol extract and saponin and tannin from aqueous extracts. Result of present study is comparable with essential oils causing allelopathic effect (Friedman and Waller 1985). Coumarin is closely related to phenolic acids (Rice 1984), polysoles (Swain, 1997), tannin, a growth and germination inhibitor to several plants (Harbome, 1984). The bitter taste of sterolic derivatives (Sterols, saponin etc.) protect plant against herbivory (Jha, 2003).

Effect of Extract on the Germination

There was no any significant effect on germination of paddy seed where as germination of Barnyard grass was inhibited by different solvent extracts, aqueous extract and leachates (Table 1). The inhibitory effect was more pronounced in case of leaf extract and inhibition increased with the increasing concentration. This may be due to the reason that only the extract of

Table 1: Effect of aqueous (decomposed) extract from leaf, stem and root on germination of paddy and Barnyard grass.

Parts used	Extract Concentration (%)	Seed Germination (%)			
		Effect of aqueous (decomposed) extract		Effect of leachate	
Leaf	2	100±0.00	90±8.16	100±0.00	70±0
	4	100±0.00	60±8.16	100±0.00	65±0
	6	100±0.00	30±4.08	90±0	80±10
	8	95±4.08	30±4.08	100±0.00	80±10
	10	100±0.00	0	100±0.00	45±15
Stem	2	100±0.00	90±8.14	100±0.00	90±4.08
	4	100±0.00	90±4.08	100±0.00	80±8.16
	6	100±0.00	83.33±9.42	100±0.00	100±0.00
	8	100±0.00	76.66±12.47	80±0	70±4.08
	10	100±0.00	56.66±10.27	100±0.00	90±0
Root	2	100±0.00	80±8.16	100±0.00	90±4.08
	4	100±0.00	90±4.08	100±0.00	70±4.08
	6	100±0.00	90±4.08	100±0.00	60±8.16
	8	100±0.00	80±4.08	100±0.00	90±5
	10	95±4.08	70±8.16	100±0.00	80±4.08
Control	0	100±0.00	100±0.00	100±0.00	100

Note: ± indicates the standard deviation from the mean value.

Table 2: Effect of different solvents extract of leaf, stem and root of *Artemisia dubia* on germination of paddy and Barnyard grass.

Parts used	Extract Concentration (ppm)	Seed Germination (%)					
		Effect of hexane extract		Effect of methanol extract		Extract of aqueous extract	
		Paddy	Barnyard grass	Paddy	Barnyard grass	Paddy	Barnyard grass
Leaf	0.1	100±0.00	80±0.00	100±0.00	90±0	100±0.00	75±4.08
	10	95±2.35	95±4.08	100±0.00	95±2.5	95±4.08	85±4.08
	1000	100±0.00	90±8.16	100±0.00	95±0	100±0.00	75±8.16
Stem	0.1	100±0.00	80±0.00	100±0.00	80±0	95±8.16	80±2.35
	10	100±0.00	90±2.35	100±0.00	90±10	100±0.00	75±0.00
	1000	100±0.00	70±4.08	100±0.00	80±10	95±4.08	75±0.00
Root	0.1	100±0.00	100±0.00	100±0.00	95±2.5	100±0.00	75±0.00
	10	100±0.00	85±4.08	100±0.00	100	100±0.00	80±2.35
	1000	100±0.00	95±4.08	100±0.00	75±5	100±0.00	70±8.16
Control	0	100±0.00	100±0.00	100±0.00	100	100±0.00	100±0.00

Note: ± indicate the standard deviation from the mean value.

higher concentration was able to cause the death of embryo. Similar result was obtained in other studies (Kil and Yun 1992; Malla 2003). The leachate of various parts of plant had no significant effect on germination of paddy seed. Similar results were obtained on different plant extract by Jha and Yadav (1987), Shrestha (2003) and Malla (2003).

The plant extracts of different parts extracted with different solvents were found to exhibit inhibitory effect on germination of Barnyard grass seeds. There was no any significant effect on germination of paddy seeds. Hexane and aqueous extracts showed more inhibitory activity. Among different extracts, aqueous extract caused more inhibitory effect. This may be due to the presence of more water soluble compounds in plants. Leaf and stem extracts had greater inhibitory effect than the root extract. This result is comparable with earlier findings that reported more inhibitory effect of shoot extract than root extract (Qasem, 1995; Jha and Jha 1999; Jha 2003).

Significant inhibition on germination of Barnyard grass seeds by extract of different plant parts supported the farmer's assumption for using *Artemisia* leaf on paddy seed bed to control the Barnyard grass germination and growth.

Effect on Seedling Growth of Test Seeds

The present investigation clearly showed that the extract / decomposition / leachate of leaf, stem and root of plant had little effect on the seedling growth of paddy (except in aqueous [decomposed] and hexane extract) (Tables 3 & 4). These results were a little different than the previously observed by Singh *et al.* (1987), Jha and Dhakal (1990), Jha *et al.* (1996) on seedling growth of paddy on treatment of extract / leachate. The stimulatory effect on the seedling growth of paddy may be due to presence of growth regulatory hormone / chemical present in *Artemisia dubia*.

The aqueous (decomposed) extract of different parts inhibited the seedling growth of paddy which may be because of certain water soluble growth inhibitory substances. Similarly the inhibition of hexane extract on root length of paddy might be due to the direct contact of root with extract. The seedling vigour of Barnyard grass was found to be inhibited by all extracts decomposition / leachate / mulch of all parts viz, leaf, stem and root of plant (Tables 4 & 6). The inhibitory effect seems more significant in aqueous (decomposed) extract and leachate that supported the farmer's traditional practice of leaf mulch on seedbeds.

The strong effect of extract / leachate / on roots in comparison to shoots in the present study may have

Table 3: Correlation of concentration with root and shoot length of seedling of paddy and Barnyard grass on different treatment

Treatment		Paddy		Barnyard grass	
		Root length	Shoot length	Root length	Shoot length
Aqueous (decomposed) extract	Leaf	-0.902**	-0.847**	-0.730**	-0.810**
	Stem	-0.746**	-0.518**	-0.560**	-0.645**
	Root	-0.616**	-0.607**	-0.375*	-0.535*
Leachate	Leaf	0.232	0.274	-0.131	-0.434*
	Stem	0.74	0.128	-0.250	-0.428*
	Root	0.170	0.30	-0.099	-0.07
Aqueous extract	Leaf	0.427	0.590**	-0.162	-0.178
	Stem	0.061	0.410	-0.323	-0.007
	Root	0.323	0.318	-0.449*	-0.177
Hexane extract	Leaf	-0.006	0.176	-0.279	-0.020
	Stem	-0.774	0.423	-0.268	-0.249
	Root	-0.275	-0.067	-0.275	.00
Methanol extract	Leaf	0.103	0.411	-0.246	-0.361
	Stem	-0.071	0.251	-0.628	-0.312
	Root	0.600**	0.630**	-0.209*	-0.021

* Correlation is significant at the 0.05 level (2 tailed)

** Correlation is significant at the 0.01 level (2 tailed)

caused by the fact that roots were in direct contact with extract and consequently its effect on seedling vigour comparable to earlier findings reported by Bhowmik and Doll (1984), Rice (1984) and Qasem (1995).

The result has clearly indicated that the order of effect of various parts of *Artemisia dubia* as leaf > stem > root on germination and seedling growth of paddy and Barnyard grass. Malla (2003) reported the inhibitory activity of leaf extract of *Ageratum conyzoides* on germination and seedling vigour of paddy. Similar results were obtained by Jha and Dhakal (1990), Jha and Yadav (1987), who reported the inhibitory effect of *Eupatorium adenophorum* on the germination and seedling growth of Amaranth and maize. It may be due to the presence of more active substances in leaves than in stem and root to affect the germination and seedling growth.

Among three different organic solvents, hexane extract exhibited the maximum allelopathic effect on germination and seedling vigour of paddy and barnyard grass followed by methanolic and aqueous extract. Same result was reported by Malla (2003) in *Ageratum conyzoides*.

Leaf mulch affected seedling elongation of paddy and barnyard grass (Tables 7 & 8). The result clearly showed stimulatory effect on paddy and inhibitory effect on barnyard grass elongation. However, the effect of mulch varied based on age of seedling and crop condition. The stimulatory effect on paddy seedling was more prominent in early stage (1 week) seedling planted alone, however the effect was more prominent on aged seedling planted mixed with barnyard grass.

The problem brought by barnyard grass due to the similar ecological preferences and similar morphology in young stage (Yabuno, 1966) and paddy mimicry (Barret, 1983) in paddy field can be solved by using leaf mulch, Significant stimulatory effect on paddy and inhibitory effect on barnyard grass supported this possibility.

It appears that different solvent extracts, aqueous (demcomposed) extract and leachate significantly reduced the germination of barnyard grass but there is no any significant effect on paddy germination. However, it seems significant inhibitory effect of aqueous on root / shoot elongation of paddy. This type of effect further supported that the application of plant in seedbeds to check the germination of barnyard grass is more effective than the use of plant as growth stimulator of root / shoot elongation of paddy to suppress the growth of barnyard grass.

CONCLUSION

Artemisia dubia has several allelochemicals causing allelopathic effect on seed germination and seedling elongation of paddy and barnyard grass. It showed the totally different effect on paddy and its associate weed barnyard grass. It showed the positive allelopathic effect on paddy and negative effect on barnyard grass,

Table 4: Effect of aqueous (decomposed) extract and leachate of leaf, stem and root of *Artemisia dubia* on seedling vigour of paddy and Barnyard grass (7 days after germination).

Parts used	Concentration (%)	Effect of aqueous (decomposed) extract				Effect of leachate			
		Paddy		Barnyard grass		Paddy		Barnyard grass	
		Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)
Leaf	2	5.10±1.50d	5.38±0.34d	1.86±0.58b	4.18±1.20a	2.80±0.90a	3.06±0.48a	2.70±1.08a	4.02±1.43ab
	4	3.16±1.34c	3.889±0.26c	2.92±1.12b	4.38±0.72cd	3.74±0.84a	3.82±1.16a	2.34±0.43a	3.60±0.60ab
	6	1.90±0.81bc	3.18±0.91bc	2.00±0.74b	3.42±1.89bc	3.86±0.89a	3.20±0.60a	2.56±1.41a	3.72±0.77ab
	8	1.30±0.34ab	2.96±0.51ab	2.08±0.62b	2.46±0.37b	3.78±0.70a	3.58±1.31a	2.42±0.65a	3.54±0.80ab
	10	0.42±0.16a	2.18±0.56a	0a	0a	3.68±0.21a	3.74±1.28a	2.26±0.97a	3.00±0.93a
Stem	2	4.08±0.62c	1.66±0.58a	3.38±1.71ab	4.62±0.56b	4.28±0.01a	3.38±0.69a	3.46±0.72ab	5.00±1.04b
	4	3.90±0.79c	1.08±0.47a	2.76±1.11a	4.52±1.00b	4.04±0.52ab	3.70±0.62a	3.64±0.39c	3.96±1.05ab
	6	2.42±0.94b	1.94±0.88a	2.22±1.34a	4.36±0.89b	5.04±0.45b	3.64±0.39a	3.06±0.37abc	4.30±0.91ab
	8	1.22±1.17a	1.66±0.65a	2.50±1.17a	4.10±0.66b	4.06±0.60ab	3.30±0.46a	2.78±0.93abc	3.26±1.30ab
	10	2.88±0.53bc	1.80±0.40a	1.94±0.78a	1.98±1.56a	4.20±1.86ab	3.28±0.07a	2.54±0.65a	3.46±0.93a
Root	2	6.14±0.51c	5.16±0.36c	5.60±1.92b	5.66±0.43c	4.56±1.09ab	3.32±0.73a	2.80±0.56a	4.92±0.67a
	4	6.44±1.95c	4.70±0.22c	4.00±0.90b	4.78±0.56c	4.98±0.72b	3.26±0.75a	2.10±0.74a	4.12±0.50a
	6	4.80±0.86bc	5.12±0.47c	4.66±1.90ab	4.96±0.50bc	4.54±0.59ab	3.74±0.55a	2.64±0.50a	4.28±1.02a
	8	1.88±0.48a	2.64±0.41a	2.30±0.95a	2.34±0.42a	4.08±0.62ab	3.36±0.37a	2.60±0.99a	4.26±0.55a
	10	4.48±1.36b	3.82±0.61b	3.961.00a	4.56±0.83b	4.50±0.58ab	3.50±0.54a	2.34±0.57a	4.12±0.27a
Control		6.46±1.21	4.92±0.82	4.56±1.19	5.30±1.16	3.56±0.78	2.88±0.49a	2.60±0.82	4.34±0.50

Note: Mean ± SD followed by the same letter do not differ significantly at p = 0.05 by Homogeneity Variance test.

Table 5: Effect of haxane and methanol extract of leaf, stem and root of *Artemisia dubia* on seedling vigour of paddy and Barnyard grass (7 days after germination).

Parts used	Concentration (ppm)	Effect of haxane extract				Effect of methanol extract			
		Paddy		Barnyard grass		Paddy		Barnyard grass	
		Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)
Leaf	0.1	5.74±1.06a	3.54±0.59a	3.90±0.65a	3.74±0.25a	7.16±1.02a	4.78±0.66a	4.30±0.90a	5.06±0.66a
	10	5.86±1.59a	3.74±1.11a	3.46±0.73a	3.74±0.71a	7.38±1.22a	4.90±0.73a	4.26±1.09a	4.72±1.03a
	1000	5.96±1.15a	3.92±0.34a	3.20±1.17a	3.78±0.31a	7.26±1.75a	5.56±1.32a	3.74±1.10a	4.20±1.29a
Stem	0.1	5.98±0.66b	3.78±1.01a	4.44±0.80a	4.14±0.36a	8.10±2.83a	4.96±0.91a	4.34±0.38b	4.82±0.40a
	10	5.82±0.74b	3.96±0.24a	4.32±1.60a	3.44±0.68a	7.60±2.23a	5.00±0.73a	4.54±1.01b	4.60±1.08a
	1000	4.22±0.25a	4.50±0.61a	3.46±0.86a	3.22±0.41a	7.00±1.18a	5.24±0.76a	3.02±0.98a	4.16±1.52a
Root	0.1	5.94±0.97a	4.04±0.35ab	4.44±1.72a	4.14±0.40a	6.62±1.16a	5.20±0.25ab	4.02±1.22a	5.78±0.43a
	10	5.46±0.48a	4.92±0.27b	4.32±1.79a	3.44±1.46a	7.10±0.96ab	5.52±0.39a	3.64±1.04a	5.06±1.18a
	1000	5.32±1.32a	4.08±0.75ab	3.46±0.96a	3.22±1.31a	8.76±1.34b	6.00±1.09a	3.52±0.71a	5.34±1.15a
Control		6.32±0.92a	3.68±0.60a	4.30±1.60a	3.94±1.08a	6.24±1.68a	4.46±0.67a	4.12±0.44a	5.30±0.96a

Note: Mean ± SD followed by the same letter do not differ significantly at p = 0.05 by Homogeneity Variance test.

representing an excellent strategic source chemicals that may be involved in developing natural herbicides to control the barnyard grass infestation in paddy field, causing a great economic loss to farmers. The study is important and intriguing from weed management view based on hypothesis of "weed for weed control".

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Table 6: Effect of aqueous extract of leaf, stem and root of *Artemisia dubia* on seedling vigour of paddy and Barnyard grass (7 days after germination).

Parts used	Concentration (ppm)	Paddy		Barnyard grass	
		Root (cm)	Shoot (cm)	Root (cm)	Shoot (cm)
Leaf	0.1	4.38±0.54a	3.36±0.31a	4.62±1.34a	4.70±0.65a
	10	5.14±1.09a	3.90±0.47ab	5.98±0.73a	4.38±0.61a
	1000	5.74±0.89a	4.58±0.80b	4.92±0.66a	4.82±0.66a
Stem	0.1	4.32±0.91a	3.24±0.75a	3.32±0.34a	4.44±1.32a
	10	4.70±1.41a	4.14±1.43a	3.00±1.13a	4.18±0.93a
	1000	4.74±1.43a	4.72±1.04a	2.96±0.97a	4.48±1.09a
Root	0.1	5.40±1.51a	3.86±0.35b	4.28±0.90a	4.86±0.65a
	10	5.52±0.29a	4.24±0.19ab	3.80±1.06a	3.96±0.90a
	1000	6.02±0.95a	4.22±0.24ab	3.18±1.44a	4.22±0.78a
Control		4.72±1.32a	3.90±0.29a	5.28±0.80a	4.76±0.61a

Note: Mean ± SD followed by the same letter do not differ significantly at p = 0.05 by Homogeneity Variance test.

Table 7: Effect of mulch of leaves of *Artemisia dubia* on seedling growth of paddy and barnyard grass

Age of seedlings	Length of Paddy (cm)				Length of Barnyard grass (cm)			
	Control		Mulched		Control		Mulched	
	Alone	Mixed	Alone	Mixed	Alone	Mixed	Alone	Mixed
1 Week	4.16±1.59	7.74±1.19	11.48±2.29	10.78±1.16	3.92±2.48	6.50±1.58	4.17±0.52	10.30±2.28
2 Weeks	14.22±1.21	12.42±2.97	18.00±3.08	15.34±3.97	5.72±1.47	17.66±2.33	6.88±1.84	15.99±4.65
3 Weeks	19.16±5.96	15.72±6.34	22.12±2.70	24.02±6.05	13.04±2.86	20.50±2.42	11.58±3.55	17.20±4.36
4 Weeks	19.58±1.40	19.84±2.28	22.20±3.11	25.14±1.27	17.16±4.21	21.50±2.50	13.48±1.23	19.34±1.71

Note: ± indicate the standard deviation from the mean value.

Table 8: Paired sample test for seedling elongation of paddy and barnyard grass due to the effect of leaf mulch on different condition

Paired sample	Pd	T	d.f.	p
PCA-RCM	0.35	0.36	19	0.72
PCA-PMA	-4.16	-4.23	19	<0.001
PCM-PMM	-4.89	-4.82	19	<0.001
BCA-BMA	0.93	1.21	19	0.24
BCM-BMM	0.83	0.86	19	0.002
PCA-BCA	4.32	3.65	19	0.002
PMA-BMA	9.42	13.83	19	<0.001

** Correlation is significant at the 0.01 level (2 tailed)

Note: **pd** = paired differences, **d.f.** = degree of freedom, **p** = probability. *In paired sample,* First letter encodes plants name, **P** = paddy, **B** = Barnyard grass. Second letter encodes treatment, **C** = Control, **M** = Mulched. Third letter encodes condition of cropping, **M** = Mixed, **A** = Alone.

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