



Allelopathic interference of *Ageratum conyzoides* L. Against Rice Varieties

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Abstract

Ageratum conyzoides L. is an invasive weed that has severely infested cultivated lands and interferes with the growth of crops. In this study aqueous extracts of 50 and 100% concentration were prepared from fresh and air dried plant parts (Leaves and roots) of *A. conyzoides* and their allelopathic effect was observed on seed germination and seedling growth of two rice varieties namely Sava and Geru. In Sava variety, a significant reduction in seed germination, seedling length and dry weight was recorded under dry leaf extract while under fresh leaf extract seed germination and seedling length was increased at 50% concentration as compared to control. In Geru variety both fresh and dry leaf extracts resulted in decreased seed germination and seedling growth with increasing concentration. Similar patterns were recorded for fresh and dry root extracts. The inhibitory effect of leaf extracts was more pronounced than root extract and overall dry aqueous extracts of leaf and root were more inhibitory than fresh aqueous extracts. Among varieties, Sava was least affected as compared to the Geru. This study indicated that *A. conyzoides* exert allelopathic effect towards rice crop by releasing water soluble phytochemicals.



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Introduction


Ageratum conyzoides L. commonly known as billy goat weed is an alien weed species native of Central America and Mexico. As a member of Asteraceae family, the plant is herbaceous in habit, found throughout tropic and subtropic regions around the world including India.¹ It is widespread

across different agroecosystems and natural ecosystems²⁻⁴ owing to its wide ecological amplitude and adaptability.⁵ Production of extensive numbers of seeds and its rapid spread to distant places helps in its encroachment to wider areas. *A. conyzoides* forms dense stands which out-compete the native species in terms of space and resource utilizations, affecting

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the biomass of native species. The loss of biomass or productivity results in disruption of the local ecosystem in terms of structure and functioning.⁶⁻¹⁰

Allelopathy is an interference mechanism in which plant release secondary metabolites into the environment that could have either inhibitory or stimulatory effect on the growth of nearby plants.^{6,11,12} Secondary metabolites such as alkaloids, flavonoids, phenolics, chromenes and essential oils have been identified from *A.conyzoides*¹³, some of which are considered as putative allelochemicals.¹⁴⁻¹⁶ Allelopathy is regarded as one of the reasons for imparting invasiveness by *A. conyzoides*.^{4,17,18} Water soluble phenolics as putative allelochemicals have been reported in number of studies in significant amount, deleteriously affected the early growth of rice, wheat^{14,18}, chickpea¹⁹ and pea.²⁰ The leaves were used by Chinese farmers to increase the soil fertility for paddy fields.¹⁶ Weed debris is reported to enrich the soil nutrients especially nitrogen.^{2,3} Enhanced phytotoxic effects are observed when conditions are extremely unfavourable for its growth.¹⁵

A. conyzoides is one of the weeds (locally known as "Bhuvanijhad") that infest rice based (*Oryza sativa* L.) agroecosystems in Kumaun Himalayas. Rice is the main food crop of India^{21,22} adversely affected by this weed. In terraced rice cultivation especially in Himalayan belt, seeds are directly sown which develop together with weeds and could compete for space and nutrient resources. In addition, photochemicals released by weeds cause severe reduction in crop yield.

Therefore, the objectives of present study were to assess the phytotoxic effect of fresh and air (shade) dried aqueous extracts of leaves and roots of *A. conyzoides* against two rice varieties (Sava and Geru) and to assess the allelopathic tolerance potential of selected rice varieties.

Materials and Methods

Preparation of Extract

For preparation of fresh aqueous extract of leaf and root, green leaves and roots of *A. conyzoides* were collected from field and grounded to fine paste. 20 g paste of each plant part (leaf and root) was soaked in 200 ml distilled water in 1:10 w/v at

room temperature for 48 hours. Soaked materials were occasionally shaken and the contents were filtered through Whatman filter paper No.1. The prepared stock solution was considered as 100% concentration and 50% concentration was prepared by diluting with distilled water. A treatment of distilled water was set as the control. For preparation of dry aqueous extract, the collected leaves and roots were air (shade) dried for 10 days and then grinded to fine powder by mortar and pestle. 100% and 50% concentration of each plant part (leaf and root) was prepared from method described for fresh aqueous extracts. The extracts prepared hereafter were named fresh leaf extract (FLE), fresh root extract (FRE), dry leaf extract (DLE) and dry root extract (DRE).

Seed Bioassay

An experiment was conducted in factorial arrangement form using a completely randomized design with three replicates. In this experiment, two varieties of rice *viz.* Sava and local landrace Geru were collected from local farmers and kept under two levels of extract concentrations treatment (50% and 100% concentration of extract) and distilled water as control. This experiment was carried out in the Department of Botany, DSB Campus, Kumaun University, Nainital (Uttarakhand), India.

Ten healthy, uniform sized seeds of both selected varieties were sterilized, washed with distilled water and were put in Petri dishes (with 9 cm diameter) with moistened filter paper in three replications. The Petri dishes were covered to prevent the loss of moisture by evaporation. Germination test were conducted under condition of 12h light/dark cycle with 14°C minimum and 24°C maximum temperature. Numbers of germinated seeds were recorded for upto 17 days. After 17 days the seedling were harvested. Germination percentage was determined by counting the number of germinated seeds every day divided by total number of tested seeds. Seed vigor index was calculated by:

$$\text{SVI} = \text{Germination Percentage} \times \text{Mean of Seedling Length}$$

Inhibition percentage was calculated by:

$$\text{Inhibition \%} = \left(\frac{\text{Control} - \text{Sample Extract}}{\text{Control}} \right) \times 100$$

Shoot and root length, fresh and dry (oven drying at 60°C) weight were recorded.

Statistical Analysis

The parameters measured were analyzed by Analysis of Variance (ANOVA) and if there any differences in the means were observed it was further analyzed by Duncan's multiple range tests at level of 95%. Statistical analysis was performed using SPSS version 16.

Results

The statistical analysis showed that aqueous extracts obtained from both leaf and root of *A. conyzoides* significantly affected germination and seedlings length of rice varieties in laboratory bioassay. For leaf extract differences between varieties were significant only for shoot length while for root extracts, differences between varieties were significant for all observed parameters at $p \leq 0.05$ (Table 1).

Table 1: Analysis of Variance (ANOVA) for Traits Investigated for Two Varieties of Rice in Response to Leaf and Root Extract of *A. conyzoides*.

Parameters		Mean squares					
		Df	SL	RL	GP	FW	DW
Leaf	Extract	1	23.62**	25.99**	16044.44**	0.00ns	0.00 ^{ns}
	Varieties	1	12.09*	5.51ns	900ns	0.01**	0.01**
	Treatment levels	2	1.71 ^{ns}	5.32ns	2905.55 ^{ns}	0.02 ^{ns}	0.00 ^{ns}
Root	Extract	1	10.67**	14.56**	1225.00*	0.01**	0.01**
	Varieties	1	9.96**	4.22*	1225.00*	0.001**	0.00 ^{ns}
	Treatment levels	2	1.27 ^{ns}	4.65ns	72.22 ^{ns}	0.00 ^{ns}	0.00 ^{ns}

** : Significant at $p \leq 0.01$, * : Significant at $p \leq 0.05$ and ns: Not significant. SL: Shoot length, RL: Root length, GP: Germination percentage

Effect of leaves on Seed Germination

FLE induced slight promotion (-5%) at 50% concentration in Sava variety, whereas 50% germination was inhibited at 100% concentration of Geru variety. In FLE, the seed vigour index of Sava variety slightly increased (-8.2%) at both treatment levels except Geru variety (Table 1). Compared to control, DLE induced reduction in the germination percent of both rice varieties. The DLE had shown significant difference ($p \leq 0.05$) in germination percent of Sava variety. In both varieties germination as well as seed vigour were completely controlled in DLE of 100% concentration (Table 1).

Effect of Leaves on Seedling Growth

50% FLE concentration slightly promoted shoot length (-8.7%) and both shoot (-13.3%) and root length (-3.4%) at 100% concentration in Sava variety (Fig 1a). In Geru variety, both shoot and root length were inhibited with increasing concentrations

(Table 2). Compared to control, DLE reduced both shoot and root length in both rice varieties (Fig 2a). The DLE had shown significant difference ($p \leq 0.05$) in both shoot and root length of Sava variety. Complete inhibition (100%) in seed growth was observed in both varieties at 100% DLE concentration (Table 2).

Effect of Roots on Seed Germination

FRE reduced germination percent and seed vigour index at both concentrations in Sava variety whereas in Geru variety, 100% concentration showed slight promotion in germination percent (-16.7%) and seed vigour index (-4.9%) as compared to control (Table 3). In Sava variety, DRE showed slight inhibition in germination percent at both treatments. However, in Geru variety germination was slightly promoted at 50% and 100% concentration. Reduction in seed vigour index was observed for both varieties at both DRE concentrations (Table 3).

Table 2: Effect of *A. conyzoides* Leaves on Seed Germination, Growth and Seed Vigour Index of Two Rice Varieties

Variety	Treatment	FLE				DLE			
		GP	SL	RL	SVI	GP	SL	RL	SVI
Sava	Control	66.67±0.67	2.74±0.45	2.73±0.59	3.65	53.33±0.88	1.42±0.13	1.62±0.18	1.62
	50%	70.00±0 (-5.0)	2.98±0.18 (-8.7)	2.65±0.50 (2.7)	3.95 (-8.2)	36.67±0.88 (31.3)	0.31±0.10 (77.8)	0.1±0.1 (93.8)	0.15 (90.7)
	100%	66.67±0.67 (0.0)	3.11±0.28 (-13.3)	2.82±0.45 (-3.4)	3.95 (-8.2)	0 (100)	0 (100)	0 (100)	0 (100)
Geru	Control	73.33±1.45	1.37±0.21	2.08±0.47	2.53	43.33±2.33	0.408±0.26	0.75±0.66	0.5
	50%	66.67±1.20 (9.1)	0.95±0.23 (30.2)	1.57±0.39 (24.9)	1.68 (33.6)	3.33±0.33 (92.3)	0.1±0.1 (75.5)	0 (100)	0 (100)
	100%	36.67±0.33 (50.0)	0.79±1.73 (42.0)	0.82±0.27 (60.7)	0.59 (76.7)	0 (100.0)	0 (100)	0 (100)	0 (100)

FLE= Fresh leaf extract, DLE= Dry leaf extract, GP= Germination percentage, SL= Shoot length, RL= Root length, SVI= Seed vigor index. The values in the parenthesis are inhibition percent.

Table 3: Effect of *A. conyzoides* Root on Seed Germination, Growth and Seed Vigour Index of Two Rice Varieties

Variety	Treatment	FRE				DRE			
		GP	SL	RL	SVI	GP	SL	RL	SVI
Sava	Control	76.67±0.33	2.75±0.08	2.72±0.10	4.20	63.33±0.88	1.87±0.26	2.58±0.20	2.62
	50%	63.33±0.88 (17.4)	3.28±0.25 (-19.1)	3.56±0.12 (-30.6)	4.33 (-3.1)	63.33±0.88 (0.0)	1.24±0.08 (34.3)	1.08±0.07 (52.0)	1.47 (43.9)
	100%	60±0.57 (21.7)	2.22±0.45 (19.4)	2.49±0.58 (8.8)	2.82 (32.8)	60±1.53 (5.3)	0.94±0.09 (50.1)	0.88±0.17 (60.9)	1.09 (58.4)
Geru	Control	60±0.57	1.50±0.24	2.53±0.37	2.42	40±0.58	0.84±0.23	1.74±0.23	1.03
	50%	56.67±0.88 (5.56)	1.22±0.33 (19.0)	1.28±0.18 (49.5)	1.42 (41.32)	43.33±0.33 (-8.3)	0.45±0.14 (46.4)	0.85±0.35 (50.8)	0.50 (51.5)
	100%	70±0 (-16.7)	1.45±0.31 (3.5)	2.17±0.40 (14.2)	2.54 (-4.9)	46.67±0.33 (-16.7)	0.53±0.10 (36.5)	0.30±0.01 (82.3)	0.40 (61.2)

FRE=Fresh root extract, DRE= Dry root extract GP= Germination percentage, SL= Shoot length (cm), RL= Root length (cm), SVI= Seed vigor index. The values in the parenthesis are inhibition percent.

Effect of Roots on Seedling Growth

Both shoot (-19.1%) and root length (-30.6%) of Sava variety were promoted at 50% FRE and significant reduction in root length was observed in Geru variety

as compared to control. DRE significantly reduced both shoot and root length in Sava variety (Fig. 2b) and root length in Geru variety (Table 3).

Table 4: Effect of *A. conyzoides* leaves and roots on fresh and dry weight of two rice varieties

Variety	Treatment	FLE		DLE		FRE		DRE	
		FW (g)	DW(g)	FW(g)	DW(g)	FW(g)	DW(g)	FW(g)	DW(g)
Sava	Control	0.053	0.018	0.046	0.021	0.053	0.018	0.047	0.018±0
		±0.001	±0.001	±0.034	±0.00	±0.002	±0.0007	±0.00	
	50%	0.054	0.017	0.038	0.024	0.059	0.019	0.039	0.019±0
		±0.002	±0.0004	±0.002	±0.00	±0.003	±0.0005	±0.00	
	100%	(-1.9)	(5.6)	(17.4)	(56.8)	(-11.3)	(-5.6)	(17.0)	(-5.6)
		0.055	0.019	0	0	0.049	0.019	0.047	0.025±0
	±0.002	±0.0005			±0.005	±0.0003	±0.00		
	(-3.8)	(-5.6)	(100)	(100)	(7.5)	(-5.6)	(0.0)	(-39)	
Geru	Control	0.066	0.029	0.037	0.022	0.069	0.028	0.056	0.036±0
		±0.005	±0.0083	±0.019	±0.01	±0.003	±0.0008	±0.01	
	50%	0.059	0.028	0.016	0.010	0.058	0.027	0.055	0.033±0
		±0.002	±0.0005	±0.016	±0	±0.002	±0.0002	±0.00	
	100%	(10.6)	(3.5)	(-14.3)	(54.5)	(15.9)	(3.6)	(1.8)	(8.3)
		0.056	0.029	0	0	0.064	0.031	0.052	0.037±0
	±0.001	±0.0002			v	±0.001	±0.01		
	(15.2)	(0.0)	(100)	(100)	(7.2)	(-10.7)	(7.1)	(-2.8)	

FW=Fresh weight (g), DW=Dry weight (g), FLE= Fresh leaf extract, DLE= Dry leaf extract, FRE=Fresh root extract, DRE= Dry root extract. The values in the parenthesis are inhibition percent.

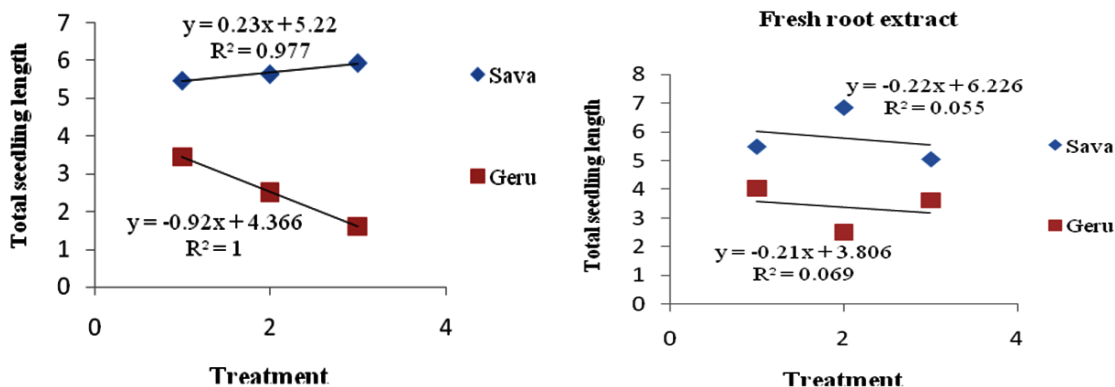


Fig. 1(a,b): Regression analysis of the allelopathic effect of fresh extract of *Ageratum conyzoides* L. on total seedling length (cm) of rice varieties

Effect of Extracts on Fresh and Dry Weight

The fresh and dry weight of Sava and Geru variety in FLE showed no significant difference with control. DLE significantly reduced fresh and dry weight at both concentrations (Table 4). FRE induced no significant change in the biomass of Sava variety,

whereas in Geru variety significant increase in dry weight at 100% concentration was observed. In DRE, both varieties showed no significant changes in fresh weight at both concentrations however, dry weight of Sava variety was promoted (-39%) at 100% concentration (Table 4).

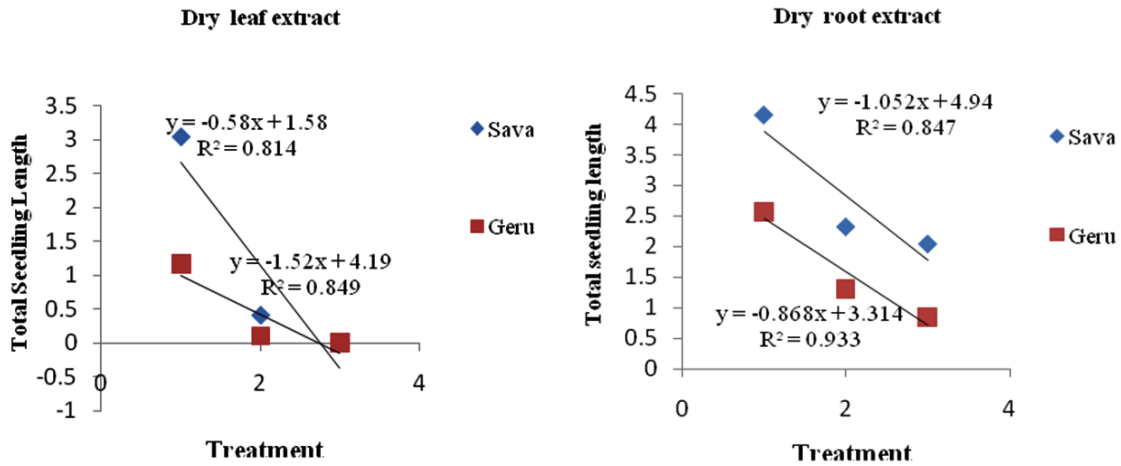


Fig. 2(a,b): Regression analysis of the allelopathic effect of dry aqueous extracts of *Ageratum conyzoides* L. on total seedling length (cm) of rice varieties

Discussion

Invasive plants are reported to outcompete plants growing in its vicinity by releasing inhibitory phytochemicals into the environment.²³⁻²⁵ In number of studies, water soluble phenolics having potential phytotoxic activity, are widely implicated in allelopathic studies.^{2,16,26,27} Batish *et al.*,^{2,3} reported the presence of phenolics such as gallic, coaumatic, protocatechuic, catechin and p-hydroxybenzoic acid from the leaf debris and the presence of ferulic acid as main phenolic compound from root exudates and root residues. In our study, the inhibition observed could be due to presence of phenolics.

In the present study, dry leaf extract (DLE) negatively affected germination parameters of both rice varieties resembles with the previous studies.^{2,16} The reduction was observed concentration dependent. Phenolic compounds are responsible for affecting the cell membrane permeability of the recipient plant which affects its nutrient uptake capacity, physiology, alter enzymatic activity and cell division pattern ultimately leading to reduce growth and development.²⁸

Fresh leaf and root extract (FLE and FRE) and dry root extract (DRE) exerted some positive effects at lower or both concentration levels in some germination parameters of rice varieties. The stimulatory effect observed could be due to growth

promoting substance in tissues.²⁹ The leaf extracts showed much inhibition than the root extracts, this could be due to high amount of phenolics reported in leaf than any other parts¹⁶, even in the leaf debris and debris amended soil.² Dry extracts were more phytotoxic than fresh extract and showed similarity with earlier findings of^{28,30} Overall the Sava variety was more tolerant to the allelopathic effect than local land race Geru variety.

Conclusion

The effects of *A. conyzoides* fresh aqueous extracts were positive in certain cases which might have practical use to increase agricultural yield and can be potential green manure. The dry aqueous extracts reduced germination parameters with increasing concentration. Based on the present study, the differences observed in laboratory are insufficient to establish allelopathic effect of extracts and treatments on both rice varieties needs further evaluation in the field conditions and should be further tested in a natural environment.

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Conflict of Interest

There is no known conflict of interest related to this study.

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