ISSN: 2347-4688, Vol. 8, No.(2) 2020, pg. 108-117



Current Agriculture Research Journal

www.agriculturejournal.org

Germination and Early Seedling Growth of Two Rice Varieties as Affected by Invasive Ageratina adenophora

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Abstract

Ageratina adenophora is a perennial herb of family Asteraceae expanding as a serious threat to ecological integrity and biodiversity. This study aims to assess the allelopathic potential of fresh and dry leaf and root aqueous extracts of this invasive species on germination, early seedling growth, seedling biomass and seed vigor of two rice (*Oryza sativa* L.) varieties namely 6444 and 2245. The result revealed that with increasing concentration of extract, inhibition effect also increases. Leaf extract was more inhibitory than root extract and dry plant extracts had higher allelopathic impact in comparison to fresh plant extracts. Among the two investigated rice varieties 6444 was more resistant to the fresh and dry leaf extract while 2245 was more resistant to fresh and dry root extract. This study will be helpful in assessing the effect caused by A. adenophora to rice crop and could be used in devising weed control strategy.



Article History

Received: 27 April 2020 Accepted: 27 July 2020

Keywords:

Ageratina adenophora, Allelopathy, Germination, Inhibition, Invasive, Resistant, Rice varieties.

Introduction

The word allelopathy is derived from Greek word 'allelo' and 'pathy' which means mutual harm/ suffering.^{1,2} Allelopathy is an intricate phenomenon in which plant releases its secondary metabolites into the surrounding which alter the germination and life processes of plants present in its vicinity.³ Secondary metabolites are the phytochemicals produced by the plant for its defense (either biotic or abiotic) and communication purposes such as to attract pollinators and are not used in their growth and development. Some of the invasive species releases these phytochemicals into the surrounding which alters the growth of surrounding community. This helps the former to establish itself via suppressing the other species.⁴

Eupatorium adenophorum Spreng. Syn. *Ageratina adenophora* (Spreng.) King and Robinson, also known as crofton weed (Asteraceae) is an invasive

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perennial herbaceous plant native to Maxico and Central AmericA.^{5–8} The production of large number of small seeds with high germination rate enabled this species to spread as a serious weed almost all over the world and established in various types of ecosystems. The photochemical screening disclosed the existence of various types of phytotoxins in *A. adenophora* which shows suppressive effect against other plants.^{7–8}

This plant is invading almost all types of ecosystems either it is a forest ecosystem, grassland ecosystem or the agroecosystem very rapidly, which can alter soil fertility, grain yield and productivity. With the introduction of invasive species, the native biodiversity is being disturbed, which can ultimately lead to biodiversity loss.9-12 Many studies have shown the allelopathic effect of A. adeonophora on the germination and growth processes of different plant species including rice.13-15 However the impact of A. adeonophora on the seed germination and early seedling establishment of rice (Oryza sativa L.) varieties in Central Himalaya is not well understood. Therefore, this study was accomplished to ascertain the allelopathic effects of water extracts of A. adeonophora on two rice varities. Rice crop was selected because it is a staple food for more than half of the world's population¹⁶ and grown on161 million hectares with an average annual production of 678,7 million tones.¹⁷⁻¹⁸ The objectives of this study were:

- To compare the allelopathic potential of aquous extracts of leaf (fresh and dry) and root (fresh and dry) on seed germination and early seedling growth of selected rice varieties.
- To compare the resistant potential of selected rice varieties against leaf (fresh and dry) and root (fresh and dry) extracts of *A. adeonophorA.*

Materials and Methods Collection of Rice Seed Varieties

The seeds of two rice varieties viz. 6444 and 2245 (hereafter referred to as V I and V II, respectively) were collected from G.B. Pant University of Agriculture & Technology. Pantnagar, Uttarakhand, and tested for seed viability. The healthy seeds of each variety were selected, washed and sterilized. Experiments were conducted in the Ecology laboratory, Department of Botany, DSB Campus, Kumaun University, Nainital, Uttarakhand, Indi*A*.

Preparation of aqueous leaf extract

Leaves were separated from healthy plants of *A. adenophora* and ground to fine paste. For dry extract preparation, mature and healthy green leaves were collected, air-dried at room temperature and ground to fine powder. Following Das *et al.*, ¹⁵ 20g paste/powder was soaked in 200 ml distilled water separately at room temperature (20°C±5°C) for two days to obtain fresh and dry leaf extracts. The leaf extracts were filtered through Whatman filter paper No. 1 to remove impurities. These extracts were used as stock solution of 100% concentration and diluted to 50% concentration for conducting the experiment at two different concentrations. A treatment of distilled water was set as the control.

Preparation of Aqueous Root Extract

To prepare fresh root extract, roots of *A. adenophora* were collected from the field, washed to remove soil particles and ground to a fine paste. 20 g paste was soaked in 200 ml distilled water at room temperature for two days to obtain the root extract. The extract was filtered through Whatman filter paper No. 1. To prepare the dry root extract, washed roots were cut into pieces and air dried at room temperature (20°C±5°C). The dried roots were ground in a grinder and after sieving a fine powder was prepared, stored in air tight plastic bags. Further aqueous extracts of roots were prepared similar to the method followed in fresh extract preparation. From the 100% stock solution, 50% solutions were then made. A treatment of distilled water was set as the control.

Experimental Design

Petri-dishes were washed with tap-water, dried and sterilized with acetone. These Petri-dishes were then kept in oven at 100°C for 1 hour for further sterilization. Ten seeds were spread on sterile filter paper in each Petri dish (9 cm diameter/lined with two sterile filter paper). The Petri-dishes were arranged in a complete randomized block design with 10 seeds per Petri dish and three replicates per treatment. 5 ml aqueous extract of each concentration (50% and 100%) was added to each Petri-dish separately. Similarly, the controls were treated with distilled water. Germination tests were conducted in 12 h light/dark cycles for 15 days with 15°C minimum and 25°C maximum temperature. The germination data were recorded on daily basis. A seed was considered germinated when the length of emergence reached 2 mm long. The root and shoot length were measured on the 15th day. Shoot and root dry weights were recorded after oven drying at 60°C for 48h. Germination percentage and germination rates were calculated following Raun *et al.*,¹⁹, relative water content following Sumithra *et al.*,²⁰, seed vigor index according to Abdul *et al.*,²¹and Response index following Williamson *et al.*,²².

Statistical analysis

The average data recorded was analyzed with ANOVA (Analysis of Variance) and Duncan's test was used to compare the difference between means. Statistical analysis was performed by using SPSS version 16.

Results and Discussion Germination and Seedling Vigor

The aqueous extracts of A. adenophora exerted an inhibitory allelopathic effect on seed germination and seedling growth parameters as compared to the control (Table 1 and 2). It was observed that with increasing the concentration of both fresh and dry leaf extracts, the inhibitory effect also increased. As compared to fresh extracts the dry aqueous extracts were more inhibitory (Table 1) and the seed of both the varieties could not germinate at 100% concentration extract of dry leaf. Seed germination, germination rate and seed vigour was increased by treatment with root aqueous extract at 50% concentration, while it decreased at 100% concentration as compared to control. Fresh aqueous extract of root at 50% concentration also increased seedling growth while dry aqueous extract caused decrease in all the observed parameters with increasing concentration (Table 2).

Hypocotyl and Radicle Length

Hypocotyl and redicle length of both the rice varieties were significantly affected by fresh and dry leaf and root aqueous extracts (Table 1 and 2). Maximum length of hypocotyl and radicle was reported at control treatment and decreased significantly (p<0.05) with increasing concentration of aqueous extracts of leaf and root. Leaf extract was found to be more inhibitory than the root extract

and inhibition caused by dry extract was more as compared to fresh extract.

Seedling Biomass

Fresh aqueous extract of leaves reduced the fresh weight (FW) of seedling in both varieties with increasing extract concentration while the effect on dry weight (DW) was not recorded. Dry leaf extract significantly affected the fresh and dry weights in both varieties. Seedling biomass showed upto 80% inhibition in fresh leaf extract and up to 100% inhibition in dry leaf extract at 100% concentration (Fig. 1).Fresh root extract showed variable results in both varieties while dry root extract decreased the fresh as well as dry weight of the seedling with increasing concentration (Fig. 2). Control treatment showed maximum fresh and dry weight.

When the data was analyzed for fresh weight percentage reduction (FWPR) and dry weight percentage reduction (DWPR) in seedlings due to allelopathic effect of *A. adenophora*, maximum reduction percentage was observed in 100% dry leaf extract in variety 6444 following fresh leaf treatment and dry weight percentage reduction did not show any significant result (Fig.1). Dry root extract showed increasing pattern of FWPR and DWPR in both the varieties where 100% treatment showed maximum inhibition. Fresh root extract increased the fresh weight as well as dry weight of variety 6444 while in variety 2245 the inhibition was higher due to the fresh root extract. In the fresh root extract, the dry weight of both the varieties showed increasing pattern (Fig.2).

Other Parameters

This study shows that response of rice varieties along the concentration gradient i.e., response breadth (RB) in fresh leaf and root extract was high (\approx 0.97-0.99) while dry leaf and root extract had low values (\approx 0.59-0.63). The dry leaf extract had the lowest values of RB in comparison to the dry root extract. Relative water content (RWC) decreased with increasing concentration of fresh leaf as well as root extract while in dry leaf extract RWC was highest in 50% concentration followed by control and 100% solution showed lowest RWC (0%) in both the varieties. Treatment with dry root extract showed increasing pattern of RWC in both varieties with increasing concentration.

Table 1: Effect of aqueous extract of <i>A. adenophora</i> leaf at different concentrations on seed germination, seed vigor and seedling growth parameters in two rice varieties
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/ariety	Variety Extract			Fresh	sh						Dry				
		GP (%)	GR	HL (cm)	RL (cm)	FW (g)	(B)	SVI	GP (%)	GR	HL (cm)	RL (cm)	FW (g)	(g)	SVI
6444	Control 93 b *		1.45 b	3.5 b	3.6 b	0.054 b	0.017 a	6.62	83 C	1.2 c	1.5 b	2.7 b	0.245 b	0.113 a	4.01
	50%	70.ab	±0.07 0.66 a	±0.02 1.0 a	±0.00 0.5 a	±0.0005 0.036 a	±0.0004 0.018 a	1.03	40.07 40 b	±0.10 0.4 b	±0.35 0.4 ab	±0.01 0.2 a	±0.004 0.158 a	±0.000 0.049 a	0.23
		±0.58		±0.35	±0.32	±0.0016	±0.0007		±1.15	±0.10	±0.12	±0.10	±0.004	±0.021	
	100%	67 a	0.61 a	0.7 a	0.6 a	0.030 a	0.018 a	0.87	0 a	0 a	0 a	0 a	0 a	0 a	0
		±0.88	±0.08	±0.14	±0.05	±0.0002	±0.0007								
2245	Control	83 a	0.98 b	1.9 a	1.9 b	0.045 c	0.019 c	3.15	63 c	0.8 c	1.9 c	2.2 b	0.225 b	0.0907 a 2.58	2.58
		±0.33	±0.07	±0.30	±0.22	±0.0009	±0.0007		±0.33	±0.03	±0.07	±0.21	±0.006	±0.0013	
	50%	83 a	0.80 ab	1.6 a	0.7 a	0.040 b	0.019 b	1.93	40 b	0.4 b	0.4 b	0.2 a	0.272 ab	0.0513 a 0.24	0.24
		±0.33	±0.08	±0.44	±0.11	±0.0034	±0.0002		±1.00	±0.13	±0.14	±0.19	±0.04	±0.0178	
	100%	70 a	0.62 a	0.7 a	0.3 a	0.036 a	0.019 a	0.69	0 a	0 a	0 a	0 a	0 a	0 a	0
		±0.58	±0.09	±0.40	±0.19	±0.0002	±0.0008								

vigor index, (values are given as Mean±standard error), * Mean ±SD in the same column followed by the same letter does not differ significantly according to Duncan's Multiple Range Test at p≤0.05 followed after ANOVA.

extract of A. adenophora root on seed germination, seed vigor	ling growth parameters in two rice varieties
le 2: Effect of aqueous extract of A. ade	1
Table 2:	

/ariety	Variety Extract			Fresh	sh						Dry				
		GP (%)	GR	HL (cm)	RL (cm)	FW (g)	DW (g)	SVI	GP (%)	GR	HL (cm)	RL (cm)	FW (g)	(6)	SVI
6444	Control 67 b*	67 b *	0.91 b	2.3 b	2.2 b	0.045 b	0.017 a	2.95	q 27	77 b 1.1 b 1.9 a	1.9 a	2.7 b	0.230 b	0.0930 a 3.54	3.54
		±1.09	±0.23	±0.62	±0.66	±0.0063	±0.0007		±0.66						
	±0.06	±0.21	±0.19	±0.0062	2	±0.0025									
	50%	70 ab	1.03 a	2.8 a	1.8 ab	0.052 b	0.017 a	3.20	47 a	0.6 b	0.6 b 1.6 a	2.2 ab	0.199 ab	0.199 ab 0.0747ab 1.75	1.75
		±0.94	±0.14	±0.15	±0.23	±0.0019	±0.0007		±0.67	±0.14	±0.48	±0.86	±0.0162	±0.0084	
	100%	70 a	0.81 a	1.3 a	0.5 a	0.045 a	0.018 a	1.25	67 b	0.9 ab	1.2 a	0.8 a	0.185 a	0.0580a 1.33	1.33
		±0.94	±0.10	±0.11	±0.10	±0.0030	±0.0002		±0.33	±0.07	±0.24	±0.19	±0.0075	±0.0096	
2245	Control	80 a	1.05 b	2.1 a	2.4 b	0.171 a	0.018 a	3.63	70 a	0.9 a	2.1 b	2.5 b	0.226 a	0.0913 a 3.20	3.20
		±0.47	±0.11	±0.16	±0.31	±0.1227	±0.0002		±0.58	±0.06	±0.16	±0.11	±0.0041	±0.0020	
		83 a		2.7 a	2.1 a	0.048 a	0.018 a	4.0	90 a	1.1 a	1.6 ab	2.0 ab	0.220 a	0.0807 a 3.30	3.30
	±0.27	±0.15		±0.16	±0.0033	33	±0.0005		±0.58	±0.06	±0.23	±0.12	±0.0087	±0.0066	
		70 a	0.76 a	1.4 a	0.8 a	0.043 a	0.020 b	1.52	57 a	0.7 a	1.1 a	1.3 a	0.195 a	0.0683	1.36
		±0.82	±0.11	±0.38	±0.53	±0.0030	±0.000		±1.45	±0.18	±0.30	±0.40	±0.0193	±0.0107	

GP= Germination percentage, GR=Germination rate, HL=Hypocotyl length, RL=Radicle length, FW=Fresh weight, DW=Dry weight, SVI=Seed vigor index, (values are given as Mean±standard error), *Mean ±SD in the same column followed by the same letter does not differ significantly

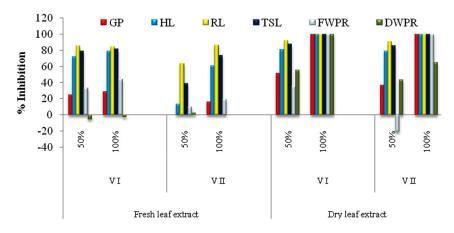


Fig.1: Inhibitory effects of leaf extract on GP (germination percentage), HL (Hypocotyl length), RL (Radicle length), and TSL (Total seedling length) on selected rice varieties

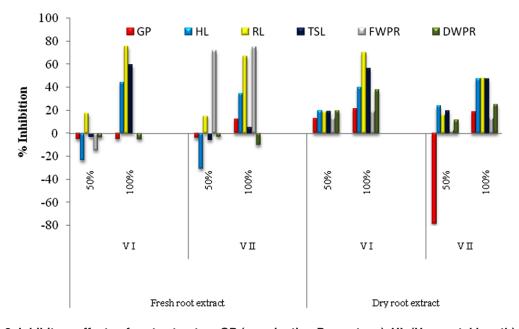


Fig.2: Inhibitory effects of root extract on GP (germination Percentage), HL (Hypocotyl length), RL (Radicle length), and TSL (Total seedling length) on selected rice varieties

This study demonstrated that under allelopathic effect, significant variations were observed among different varieties of the same species. Seed germination is an essential process in plant development that results in high crop production. The present study showed the suppressive effect of *A. adenophora* on the germination, fresh weight, dry weight, relative water content, hypocotyl length, radicle length, total seedling length and seed vigor index of rice var. 6444 and 2245. The inhibition

effect was significantly increased with increasing concentration of the solution, indicating that the effect of plant allelopathy depends on the concentration of the extract solution. Many authors^{23–28} also reported increased inhibitory effect with increasing concentration of extract. This study also showed that the effect of fresh and dry leaf extract was more allelopathic in comparison to the fresh and dry root extract. Similar results were also reported by Namvar *et al.*,²⁹. In this study, although inhibition

effects were recorded in the rice varieties mentioned above, surprisingly seed germination and seedling growth was found to be enhanced by the fresh root extract. Achakzai *et al.*,³⁰ reported that weed imposed inhibitory as well as stimulatory allelopathic effect on crop plants and more phytochemicals are present in leaves as compared to roots of same plant species.

When data of fresh and dry extracts were compared, aqueous extract of dry leaves had more potent inhibitory effect on germination percentage and seedling growth in both varieties of rice. ANOVA (Table 3) of leaf extract showed that treatments (extract concentrations) had significant effect on HL, RL, GP, and GR; extract type (Fresh and dry) showed significant impact on HL, GP, GR, FW and DW. Muhammad and Majeed³¹ also reported that air dry sunflower (*Halianthus annuus* L.) aqueous extracts caused higher inhibition on growth performance of wheat (*Triticum aestivum* L.) as compared to the fresh extract. Similarly, the brown dry leaf extract of *Psidium guajava* had more inhibition on *Cassia occidentalis* L. than the green fresh extract.³² According to Achakzai *et al.*,³⁰ dry plant material gives free bonding of solvent with the photochemicals and increase surface area for release of photochemicals as compared to fresh plant material.

Table 3: Analysis of Variance	(ANOVA) for A. ad	lenophora leaf and root	extract allelopathy	investigated for two rice varieties.

					Mean Se	quare						
		Leaf Ex	tract					Root Ex	tract			
	GP	GR	HL	RL	FW	DW	GP	GR	HL	RL	FW	DW
Extract type	14400.0*	1.428*	7.067*	1.377 ^{ns}	0.018*	0.012*	277.78 ^{ns}	0.029 ^{ns}	2.210 ^{ns}	0.632 ^{ns}	0.182*	0.032*
Variety	44.444 ^{ns}	0.087 ^{ns}	0.087 ^{ns}	1.152 ^{ns}	0.002 ^{ns}	0.000 ^{ns}	711.11 ^{ns}	0.007 ^{ns}	0.002 ^{ns}	0.229 ^{ns}	0.005 ^{ns}	0.000 ^{ns}
Treatment (concentration	13072.22*)	4.011*	21.215*	42.739*	0.055 ^{ns}	0.005 ^{ns}	405.56 ^{ns}	0.298 ^{ns}	6.494*	16.126*	0.017 ^{ns}	0.000 ^{ns}

*: Significant at \leq 5% level of significance, ns: not significant. GP: germination percentage, GR: germination rate, HL: Hypocotyl length, RL: root length, FW: fresh weight, DW: dry weight,

	Variables	Extract	Species	Treatment	SL	RL	GP	GR
Leaf	Extract	1						
	Species	0.000	1					
	Treatment	0.000	0.000	1				
	HL	-0.424**	-0.047	-0.708**	1			
	RL	-0.165	-0.151	-0.823**	0.857**	1		
	GP	-0.634**	-0.035	-0.604**	0.741**	0.648**	1	
	GR	-0.462**	-0.114	-0.755**	0.898**	0.878**	0.918**	1
Root	Extract	1						
	Species	0.000	1					
	Treatment	0.000	0.000	1				
	HL	-0.350*	-0.009	-0.495**	1			
	RL	0.152	0.091	-0.743**	0.707**	1		
	GP	-0.165	0.264	-0.182	0.380*	0.318	1	
	GR	-0.120	0.057	-0.369*	0.667**	0.567**	0.873**	1

Table 4: Correlation analysis of different treatments of A. adenophora on rice varieties.

*Correlation is significant at the \leq 1% level and ** Correlation is significant at the \leq 5% level;

Significant negative correlations were found between extract and HL, GP, GR; treatment and HL, RL, GP, GR; HL and RL, GP, GR; RL and GP, GR; GP and GR (Table 4) in leaf extract. Similarly, in root extract significant negative correlations were observed between extract and HL; treatment and HL, RL; HL and RL, GP, GR; RL and GR; GP and GR. The correlation indicates the phytotoxic effects of the extracts.

Conclusion

This study demonstrated the suppressive effect of leaf and root aqueous extracts of *A. adenophora* on the seed germination, growth of seedling, seedling dry mass and seedling vigor index of rice varieties 6444 and 2245. The inhibitory effect significantly increased with increasing concentration of extracts indicating that the effect of plant extracts depends on their concentration. Comparing data with fresh and dry leaf and root extract, aqueous extract of dry leaves and roots of *A. adenophora* had more potent inhibitory effect on germination percentage, seedling vigor index, seedling growth and biomass in both the varieties of rice. Among the two varieties investigated rice variety 6444 is more resistant to leaf extract

while 2245 variety was more resistant to root extract. Based on the results obtained in our study, it could be concluded that *A. adenophora* caused a phytotoxic effect on test crops by releasing water soluble phenolics, which may serve as possible allelopathic agent to establish its allelopathy. However further studies are required to evaluate the inhibitory effects of *A. adenophora* under field conditions.

Acknowledgement

We are thankful to the Head, Department of Botany for providing necessary facilities. We also thank both the reviewers and concerned editor for their closer look in to the manuscript and valuable suggestions made. DST, New Delhi (CRG/2019/004139) financially supported this research.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

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