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Evaluation of Allelopathic Activity of Aqueous Leaf Extracts of *Alternanthera brasiliana* (L.) Kuntze on Growth Parameters of *Vigna radiata* seeds

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Abstract

Alternanthera brasiliana is a perennial and evergreen herb which is native to sub-tropical and tropical regions of Australia, India and South America. This study describes the phytochemicals present in aqueous extract of *A. brasiliana* leaf, and also the allelopathic activity of aqueous leaf extracts on seeds of *Vigna radiata*. Allelopathic activity of aqueous extracts of *A. brasiliana* against *V. radiata* seeds was tested by using seed germination bioassay. The study revealed that the aqueous extract of *A. brasiliana* showed an inhibitory effect on the growth of *V. radiata* seeds in a concentration dependent way. The leaf extracts of *A. brasiliana* showed negative allelopathic activity due to the presence of several phytochemicals, by inhibiting the plant growth parameters.

Keywords: *Alternanthera*; Allelochemicals; Allelopathy; Germination; Inhibition

Introduction

Several species of *Alternanthera* are widely grown for their ornamental leaves which are of green and yellow, or bronze and green, or red and pinkish brown. Five species have been recorded from India. It is found throughout hotter parts of India, ascending to an altitude of 1,200 m in Himalayas; also cultivated as a pot- herb (Marg, 2005). It is found especially around tank and ponds (Khare, 2007). The genus name *'Alternanthera'* is a combination of 'alternans' which is Latin for alternating and 'anthera' which is Greek for anthers, indicating how alternate anthers of plants from this genus are sterile. Species epithet *'brasiliana'* refers to the Brazilian origin of the species form. *A. brasiliana* being known as "penicilina" or terramicina, widely used by rural communities as medicinal agent to cure different disease, such as inflammation, and dolorous or infection processes, wound healing, analgesic, antitumor activity, immunomodulator and lymphocyte proliferation. *A. brasiliana* produce different compounds with possible analgesic action under the influence of different kind of lights (Macedo, 2004).

It is used against cough and diarrhoea in Brazilian popular medicine (Brochado, 2003). *A. brasiliana* leaf is found possess antioxidant properties and is rich in elements such as carbon, hydrogen, nitrogen and sulfur; calcium, potassium, magnesium and phosphorus; copper, zinc and sodium are present in appreciable levels (Chandran, 2017).

The beneficial or harmful effect of one plant on another by the release of biochemicals called allelochemicals is termed as allelopathy. Allelochemicals are released from the plant parts by volatilization, residue decomposition, leaching, root exudation and by other processes in natural and agricultural systems. The allelopathic effect of a weed on a crop can be ascertained by reduced germination and growth of the latter, a technique known as plant bioassay. The extent of allelopathic inhibition on germination and seedling growth of crops varies from weed species to weed species (Hamayun, 2005) and its plant parts (Economou, 2002: Aziz, 2008). Most weed species have inhibitory effects on crops; yet some weed species also exhibit stimulatory effects (Kadioglu, 2005).

Phenolic compounds such as flavonoids, phenols, and tannins attributes to the allelopathic effect of selected medicinal plants. The effect may be due to synergistic effect rather than single constituent. These phenolic compounds interfere with the functions of respiratory enzymes in seed germination and thereby cause inhibitory effects on its germination (Muscolo, 2001), alteration in the activities of the growth hormone gibberellic acid which is responsible for the stimulation of seed germination (Olofsdotter, 2001). In the present study, an attempt was made to analyse the phytochemical constituents present and allelopathic activity of *A. brasiliana* leaf extracts against *V. radiata* seeds.

Materials and Methods

Collection and identification of plant

A. brasiliana (L.) O. Kuntze was collected from S.D.V. College of Arts and Applied Science College campus, Alappuzha, Kerala, India and Dr. Shaji P.K., Scientist, Environmental Resources Research Centre (ERRC), Thiruvananthapuram, Kerala State, India, identified and authenticated the plant. The shade dried leaf samples were cleaned, washed, dried and pulverized to coarse powder using an electric grinder and stored for further studies.

In vitro allelopathic activity

Plant extract preparation

The dried *A. brasiliana* leaf powder (100 g) was weighed and extracted in sterile distilled water for 24 hours in a reagent bottle at room temperature and was then filtered using Whatman filter paper No. 1. The pH of the extract was adjusted to 7 and was further diluted with sterile distilled water to 20, 40, 60, 80 and 100 %. The 20 % concentration was prepared by mixing 20 ml of the crude extract with 80 ml of sterile distilled water. Similarly, 40 %, 60% and 80 % concentrations were prepared by mixing 40 ml of extract with 60 ml of distilled water, 60 ml extract with 40 ml water, 80 ml extract with 20 ml water respectively. The 100 % concentration was prepared by taking 100 ml crude extract with our adding any distilled water. These concentrations are used for further analysis.

Phytochemical Analysis

The phytochemical analysis of aqueous extract of *A. brasiliana* was done by using standard methods (Sofowara, 1993; Trease, 1989; Harborne, 1973).

Plant material and surface sterilization

Seeds of *V. radiata* were purchased from local market and surface sterilized in 0.1% mercuric chloride and washed thoroughly with sterile distilled water thrice.

Seed germination bioassay

Ten *V. radiata* seeds were placed separately in different petri plates and were irrigated solely with *A. brasiliana* aqueous extracts of concentrations 20, 40, 60, 80 and 100% along with control plate (irrigated with sterile distilled water). For each treatment, three different replicates were tested with 10 seeds each and the average values were reported. 1ml of each of aqueous extracts was added to petri dish laid with filter paper every day to avoid drying out of filter paper during the course of experiment. The petri plates were kept in a germinator (25±3˚C, 70% humidity and 12 h photoperiod) for 7 days.

$G\% = (a/b)\ 100$

Where, *a* is a proportion of germinant and *b* the total number seeds germinated in control.

Seed germination index

Ten seeds were arranged in 9 cm petri dishes lined with Whatman No. 1 filter paper with a day temperature of 25-33°C and a night temperature of 20-25°C. Aliquots of 1 ml of *A. brasiliana* extract were daily added to the three replicates. The percentage germination of the plumule was recorded for 7 days. Seed germination index (SGI) was calculated as per the formula (Scott, 1984) :

$$SGI=\sum Ti\ Ni/S$$

Where "*Ti*" is the number of days after sowing, "*Ni*" is the number of seeds germinated on day "*I*", and "*S*" is the total number of seeds planted

Phytotoxicity

The phytotoxicity (PT) of *A. brasiliana* was expressed as percentage of the germination at different concentrations compared with control, higher values indicating lower toxicity (Cayuela, 2007).

$$PT = [1-(allelopathic/control)]/100$$

Speed of germination

Speed of germination was calculated by formula

$$S=[N_1/1 + N_2/2\ldots] \times 100$$

Where N_i/i is the ratio between number of seeds germinated per day

Plant biomass determination

Plant biomass was checked every day during the course of seven days of experimentation. The increase in plant biomass was recorded by subtracting the weights of two consecutive days for each concentration of three replicates and the average values are expressed in grams (g).

Results

Phytochemical Analysis

The qualitative phytochemical evaluation of aqueous leaf extracts of *A. brasiliana* indicated the presence of carbohydrates, glycosides, saponins, phenols, tannins, and terpenoids.

In vitro allelopathic activity

The allelopathic activity of aqueous leaf extract of *A. brasiliana* was studied against seeds of *V. radiata* and different seed germination parameters observed are given below.

Seed germination bioassay

Germination can be described as the process of reactivation of metabolic machinery of the seed resulting in the emergence of radicle and plumule. The rate of germination indicates how many seeds of a particular plant species are likely to germinate on a specific period of time and usually expressed in percentage. The seed germination percentage and percentage of growth inhibition on the seventh day for different concentrations are given in table 1.

Table 1: Effect of aqueous extract on seed germination on 7th day

Concentration of extract (%)	Number of seeds sown	Number of seeds germinated	Percentage of germination	Percentage of inhibition
Control	10	10 ±0	100	0
100	10	7.66 ±0.57	76.6	23.4
80	10	8±0	80	20
60	10	8.66±0.57	86.6	13.4
40	10	9±0	90	10
20	10	9.33±1.15	93.3	6.7

From table 1 and 2, it is evident that the percentage of germination decreases with increase in concentration of the extract. Also, the percentage of inhibition increases when the concentration of extract increases. From this we can say that the aqueous leaf extract of *A. brasiliana* shows negative allelopathy against *V. radiata* seed germination.

Table 2: Seed germination percentage of *V. radiata*

Days	Control (water)	*A. brasiliana* leaf extract concentrations (%)				
		100	80	60	40	20
1	100	73.3	73.3	76.6	76.6	86.6
2	100	73.3	76.6	76.6	80	93.3
3	100	76.6	76.6	80	80	93.3
4	100	76.6	80	80	80	93.3
5	100	76.6	80	80	86.6	93.3
6	100	76.6	80	86.6	90	93.3
7	100	76.6	80	86.6	90	93.3

Seed germination index of *V. radiata*

Seed germination index of *V. radiata* gradually increased as the number of days increased in the same concentration. Control showed the highest values for seed germination indices each day. The seed germination indexes for other concentrations are given in table 3.

Table 3: Seed germination index of *V. radiata*

Concentration (%)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	1	2	3	4	5	6	7
100	0.74	1.47	2.30	3.07	3.84	4.60	5.4
80	0.73	1.53	2.29	3.2	4	4.8	5.6
60	0.77	1.54	2.4	2.4	4.0	5.1	6.0
40	0.77	1.6	2.4	2.4	4.3	5.4	6.3
20	0.87	1.87	2.8	3.7	4.6	5.5	6.5

Speed of germination and phytotoxicity

In control plate, the speed of germination was found to be 2091. *V. radiata* showed the lowest speed of germination of 1936.5 in 100 % extract concentration. It was also noted that when the concentration of extract increased, the speed of germination decreased. The highest speed of germination among the different extract concentrations was found in 20% concentration. The results are given in table 4. From the results, it was clearly evident that 100 % concentration of *A. brasiliana* extracts showed decrease in speed of germination and also low phytotoxicity. So, this can be considered as negative allelopathy.

At 100 % concentration, *V. radiata* showed a phytotoxicity of 6.7 and at 20 % concentration, it showed a phytotoxicity of 23.4. It is very clear from the experiment that when the concentration of the extract increased, the phytotoxicity decreased considerably. The phytotoxic values obtained for different concentrations of plant extract for *V. radiata* are shown in table 4.

Table 4: Speed of germination and phytotoxicity

Concentration	Speed of germination	Phytotoxicity
Control	2091	0
100	1936.5	6.7
80	1978	10
60	2042	13.4
40	2083	20
20	2350	23.4

Plant biomass

Plant biomass of *V. radiata* decreased in a concentration dependent manner and the control showed the highest biomass. As the days increased, the biomass also increased. On the 7th day, control showed a value of 4.67 g, 20% showed a value of 4.64 g and 100% showed a value of 4.35 g. The corresponding biomass values obtained for other concentrations are given in table 5.

Table 5: Plant biomass (g) of *V. radiata*

Days	Concentration of *A. brasiliana* leaf extract					
	Control	100	80	60	40	20
1	1.67	0.35	0.50	0.53	0.97	1.09
2	1.79	0.78	0.84	0.88	1.01	1.11
3	1.85	0.85	0.90	1.04	1.21	1.23
4	1.97	1.20	1.25	1.32	1.40	1.54
5	2.79	2.03	2.13	2.20	2.32	2.47
6	3.95	3.03	3.07	3.13	3.20	3.54
7	4.67	4.35	4.50	4.53	4.58	4.64

Discussion

Phytochemicals are also called as secondary metabolites; usually occur in complex mixtures in different plant organs and stages of development (Wink, 2004). Alkaloids, phenolic compounds, saponins, flavonoids, phytosterols, tannins and carbohydrates were present in *A. braziliana* (Kannan, 2014). The release of growth retardatory substances into the soil by *Alternanthera* and its accumulation in bioactive concentrations adversely affected the growth of rice seedlings (Mehmood, 2014). The delay in seed emergence and reduction in seedling growth of rice with *A. philoxeroides* residues compared with those of *Alternanthera sessilis* could be attributed to the presence of more marked inhibitory effect of allelochemicals. It was also suggested that the phytochemicals present in the leaves produce water soluble phytotoxins which could inhibit the seed germination. Kadioglu, 2005, reported an increase in seedling growth of rice at 1% residue concentration of *A. sessilis* and *A. philoxeroides* and this could be attributed to the presence of allelochemicals which had stimulatory effects.

Similarly in the present study, 100 % aqueous extract exhibited negative allelopathy and showed decrease in all growth parameters of *V. radiata* seeds. The allelopathic activity reported for *Lolium rigidum* extracts exhibited positive response and it was correlated with the total phenolic content in the tissue of the wheat cultivars (Wu, 1998). Phenolic compounds such as flavonoids, tannins and phenols were previously reported as the most commonly and widely distributed water soluble alleochemicals. They are released to soil by volatilization process and mix easily in the soil nutrients which may be the reason for their higher allelopathic activity (Rice, 1984).

Conclusion

From the present study, it is evident that *A. brasiliana* contains many bioactive compounds such as alkaloids, phenolic groups, flavonoids, saponins, tannins, phytosterols and carbohydrates. The findings suggest a negative allelopathic effect of aqueous leaf extract of *A. brasiliana* on *V. radiata* seed germination. The aqueous extract exhibited inhibitory effect on the growth parameters such as seed germination index, phytotoxicity and the speed of germination. Further analysis is needed to find out the actual reason for negative allelopathy.

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