BAOJ Biotechnology

Pratap Chandran R, et al. BAOJ Biotech 2018, 4: 1
4: 033

Research Article

Allelopathic Activity of Leaf Extracts of Mimosa Pudica on Growth Parameters of Brassica Juncea Seeds

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Abstract

Mimosa pudica L. commonly known as touch me not plant is semi erect sub shrub of tropical America, Australia and also found in India. The focus of the study was to evaluate the allelopathic activity of aqueous leaf extract of *M. pudica* against different germination parameters of *Brassica juncea* seeds. The experiment was carried out in petridishes with ten *B. juncea* seeds and were irrigated separately with different concentrations of plant extracts. The control plants were irrigated with sterile distilled water. The germination parameters such as seed germination percentage, seed germination index, average radicle and plumule length, seed vigour index, phytotoxicity, speed of germination and plant biomass were studied. *B. juncea* seeds showed an inhibition of seed germination of about 26.7% and the germination index was 5.131 after treating with *M. pudica* leaf extract (100%) after 7 days of experimentation. The average radicle and plumule length observed after 7th day was 4.12 and 4.89 cm and the control values were 5.04 and 6.29 respectively. The control showed a speed of germination (2091) where as the 100% extract showed a value of 633. Similarly, the phytotoxicity values were very high in the case of 100% extract concentration (26.7) and the control did not show any phytotoxicity. The plant biomass in plate with 100% extract concentration was 6.97 g and 7.6 g in the control plate. The present work reports the inhibitory effect of *M. pudica* on all the observed growth parameters of B*rassica juncea* seeds in a concentration dependent manner.

Keywords: Allelopathic Activity; Aqueous Extract; *B. Juncea*; Germination; *Mimosa Pudica*

Introduction

Mimosa pudica L. is a creeping annual or perennial herb and is most commonly reported as a weed of pastures, lawns, and several crops, including maize, sugarcane, rubber, tea, sorghum, soybeans, and upland rice [1]. From the whole plant of *M. pudica* terpenoids, flavonoids, glycosides, alkaloids, quinines, phenols, tannins, saponins, and coumarins were characterized [2]. The leaves of this plant possess sedative and tonic properties and has been used in various treatments such as diarrhea, dysentery and various urinogenital infections. *M. pudica* is also found to possess antibacterial, antivenom, antifertility, anticonvulsant, antidepressant, and aphrodisiac activities, and is used for piles, dysentery, sinus and wounds [3]. *M. pudica* is an aggressive weed which suppresses the growth of other plants.

In agro ecosystems, allelochemicals have detrimental effects on the growth of associated and next-season crops. In addition to this, weeds can exhibit allelopathy against crop plants [4]. In forest ecosystems, allelochemicals produced by invasive plants can inhibit the growth of competing vegetation through direct or indirect means, thereby providing the invader with a competitive advantage [5,6]. *M. pudica* is an invasive weed and it is also included in the global invasive species

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Sub Date: July 03rd, 2018, **Acc Date:** July 23rd, 2018, **Pub Date:** July 24th, 2018

Citation: Pratap Chandran R, Deepak V, Krishna S, Fathima S, Ameena T, Raj J (2018) Allelopathic Activity of Leaf Extracts of Mimosa Pudica on Growth Parameters of Brassica Juncea Seeds. BAOJ Biotech 4: 033.

database [7]. The presence of allelochemicals in large quantities were reported in leaves, pods and in seeds of *M. pudica* [2]. This prompted us to study the allelopathic effect of aqueous leaf extract of *M. pudica* against *B. juncea* seeds which are small and easy to germinate and this make it suitable for in vitro analysis. The main aim of the study was to find the detrimental effects of allelochemicals released by *M. pudica* and the effects that it cause to the different germinaton parameters of *B. juncea* seeds. The results obtained clearly depicted the inhibitory action of *M. pudica* leaf extracts on germination of *B. juncea* seeds.

Materials and Methods

Collection and Identification of Plant

M. pudica was collected during December 2017 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 and the voucher specimen number 7409 deposited in ERRC. The shade dried leaf samples were cleaned, washed, dried, pulverized to coarse powder using an electric grinder and stored in air tight container for further studies.

Preparation of Plant Extract

One hundred gram of dried leaf powder of *M. pudica* were weighed and extracted in sterile distilled water. The leaves were shade dried to reduce water content and this makes it easy to prepare different dilutions. In the shade dried plant powder, there will not be any further enzymatic or metabolic alteration of natural plant product would become possible. All compounds can be recovered in a natural un altered form, while green leaves due to presence of chloroplast and active metabolic and protein synthesizing machinery there remains a possibility of formation of new compounds or intermediates or secondary metabolites in responses to light and other factors. The powder was kept in sterile distilled water for 24 hours in a reagent bottle at room temperature and then filtered using Whatman No 1 filter paper. The pH of the extract was adjusted to 7. This extract was further diluted to 20, 40, 60, 80 and 100 % and used for further in vitro allelopathic studies.

Plant Material and Surface Sterilization

Seeds of *B. juncea* were purchased from local market and surface sterilized using 0.1% mercuric chloride and washed thoroughly with sterile distilled water.

Seed Germination Assay

Ten seeds of *B. juncea* were placed separately in 9 cm petri plates for in vitro germination studies. Different concentrations of *M. pudica* aqueous leaf extracts were irrigated solely with concentrations of 20, 40, 60, 80 and 100 % along with control plate (with sterile distilled water). For each treatment, three different replicates were tested with 10 seeds each. 5 ml of each aqueous leaf 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 at (25±3˚C, 70% humidity and 12 h photoperiod) for 7 days. Germinated seeds (considered when radicle emerges ≥ 1mm) were daily counted for 7 days. To assess the rate of germination, final germination percentage (G %) was calculating from formulas:

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

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

Seed Germination Index

Ten *B. juncea* seeds were arranged in 9 cm petri dishes lined with Whatman No. 1 filter paper under normal laboratory conditions, with a day temperature of 25-33°C and a night temperature of 20-25°C. Aliquots of 1 ml of each dilution of M. pudica extracts were added daily to three replicates. The percentage germination of the plumule was recorded every day. The seed germination index (SGI) was calculated according to Scott et al. [8] as follow:

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

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

Seed Vigour Index

Seed vigour is an important quality parameter which needs to be assessed to supplement germination and viability test to gain insight into the performance of a seed in the field or in storage. Seed vigour is defined as the sum total of those properties of the seed which determine the level of activity and performance of the seed during germination and seedling emergence.

Seed vigor index (SVI) was calculated by using formula

SVI = Germination percentage × seedling length

Phytotoxicity

The phytotoxicity of the target species were expressed as a percentage of the germination at different concentrations with respect to control, higher values indicating lower toxicity [9].

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 numbers of seeds germinated per day.

Plant Biomass Determination

Plant biomass was noted during the course of seven days of experimentation. The increase in plant biomass was recorded by subtracting the weights of the two plates of consecutive days for each concentration

Results

Germination is the growth of a plant contained within a seed resulting in the formation of the seedlings, and also the process of reactivation of metabolic machinery of the seed resulting in the emergence of radicle and plumule. The germination rate describes how many seeds of a particular plant species, variety are likely to germinate over a specified duration. It is a measure of germination time course and is expressed in percentage. Table 1 shows the seed germination percentage of the *B. juncea.* Seed germination percentage of *B. juncea* seeds during the seven days are given in Table 2. 100 % germination was observed in control seeds after third day and the lowest (73 %) was observed in 100 % plant extract concentration on the seventh day.

Table: 1. Seed germination and inhibition percentage on seventh day.

Concentration of extract (%)	Number of seeds sown	Number of seeds germinated	Percentage of germination	Percentage of inhibition
Control	10	10	100	0
20	10	8.66	86.6	13.4
40	10	9.33	93.3	6.7
60	10	9.66	96.6	3.4
80	10	7.66	76.6	23.4
100	10	7.33	73.3	26.7

Table: 2. Seed germination percentage of *B. juncea*

Days	Control	*M. pudica* leaf extract concentration %				
		20	40	60	80	100
1	40	30	30.33	0	0	0
2	80	73.3	53.33	13.33	0	0
3	100	76.6	80	70	46.66	23.33
4	100	76.6	83.33	80	56.66	43.33
5	100	80	93.33	86.66	73.33	66.66
6	100	83.33	93.33	93.33	76.66	73.33
7	100	86.66	93.33	93.33	76.66	73.33

The seed germination index from day 1 to 7 is given in the table 3. Seed germination index increases as the number of days increases in the same concentration. The control showed the highest value for the seed germination index each day. Concentrations 60, 80, 100 showed zero germination on the day 1 (Table 3).

Table: 3. Seed germination index of *B. juncea*

Concentration of extract (%)	Days						
	1	2	3	4	5	6	7
Control	0.4	1.6	3	4.1	5.3	6	7.13
20	0.3	1.4	2.298	3.064	4	4.998	6.062
40	0.033	1.066	2.4	3.33	4.66	5.59	6.31
60	0	0.266	2.1	3.2	4.5	5.6	6.76
80	0	0	1.39	2.26	3.66	4.59	5.36
100	0	0	0.699	1.73	3.33	4.2	5.131

table: 4. Average radicle length (cm) of *B. juncea* seeds

The average radicle length of *B. juncea* on each day under the different concentrations of the aqueous leaf extract were recorded. The control attained a radicle length of 5.04 cm on the seventh day. The concentration 80 and 100 showed the highest length retardation of 0.73 cm and 0.92 cm in radicle length respectively, when compared with the control after the seventh day of the experiment. The average radicle length of other concentrations and the average values obtained up to seventh days are given in table 4. The radicle length has come down when the concentration of the extract was increased.

Table: 4. Average radicle length (cm) of *B. juncea* seeds

Plant	Days	Control (water)	*M. pudica* leaf extract concentrations (%)				
			20	40	60	80	100
B. juncea	1	0.77	0.71	0.72	0	0	0
	2	1.02	0.99	0.97	0.68	0	0
	3	1.20	0.94	0.99	0.89	0.88	0.83
	4	1.89	1.57	1.34	1.26	1.17	1.11
	5	2.96	2.88	2.59	2.48	2.31	2.19
	6	4.27	4.11	4.19	4.08	3.86	3.77
	7	5.04	4.96	4.88	4.63	4.31	4.12

Table: 5. Average plumule length (cm) of *B. juncea* seeds

The average plumule length of the *B. juncea* on each day under different concentrations of the aqueous leaf extracts was recorded. The control showed a plumule length of 6.29 cm on the seventh day of the experiment. The concentration 80 % and 100 % showed the highest plumule length retardation of 1.19 cm and 1.4 cm respectively. The concentration 20 % showed the lowest plumule length retardation of 0.12 cm after the seventh day of the experiment. The average plumule length values are given in the table 5.

Table: 5. Average plumule length (cm) of *B. juncea* seeds

Plant	Days	Control (water)	*M. pudica* leaf extract concentrations (%)				
			20	40	60	80	100
B.juncea	1	0	0	0	0	0	0
	2	1.64	1.57	1.42	1.29	0	0
	3	2.29	2.23	2.07	1.77	1.10	1.01
	4	2.79	2.64	2.24	1.99	1.29	1.10
	5	4.01	3.95	3.98	3.86	3.24	2.71
	6	4.98	4.87	4.74	4.54	4.48	3.56
	7	6.29	6.07	5.76	5.29	5.1	4.89

The seed vigour index of the *B. juncea* on each day under different concentration of the extract was calculated and given in table 6. The control showed the highest value of 504, whereas the 100% concentration showed 302.11 after the seventh day of the experiment. Higher the index value, greater is the activity of the seed during germination.

Table: 6. Seed vigour index of *B. juncea*

Plant	Days	Control (water)	*M. pudica* leaf extracts concentrations (%)				
			20	40	60	80	100
B. juncea	1	30.8	21.3	21.83	0	0	0
	2	81.6	72.56	51.73	9.06	0	0
	3	120	72.00	79.20	62.30	41.06	19.36
	4	189	120.26	111.66	100.8	66.29	48.09
	5	296	230.4	241.72	214.91	169.39	145.8
	6	427	342.48	391.05	380.78	295.9	276.45
	7	504	429.83	455..45	432.11	330.14	302.11

Table: 7. Phytotoxicity and speed of germination of *B. juncea* seeds
The 100% concentration of extract showed the phytotoxicity of 26.7. When the concentration of the plant extract increased, the value of the phytotoxicity also increased. The phytotoxic values obtained for the different concentrations are given in the table 7.

B. juncea showed the highest speed of germination of 2091 in control. It is also noted that as the concentration of extract increased, the speed of germination decreased. In 100 % leaf extract *B. juncea* showed a concentration value of 633. The values obtained for speed of germination at different concentrations are given in table 7.

Table: 7. Phytotoxicity and speed of germination of *B. juncea* seeds

Concentrations	Phytotoxicity	Speed of germination
Control	0	2091
20	13.4	1641
40	16.7	1363
60	19.4	1092
80	23.4	773
100	26.7	633

The biomass of *B. juncea* was recorded on each day under different concentrations. The highest amount of plant biomass produced was 7.64 g in control on the seventh day and the lowest was 6.97 in 100 % extract concentration. The values of plant biomass in different concentrations from day 1 to day 7 are given in the table 8.

Table: 8. Plant biomass of *B. juncea*

Days	Concentration of *M. pudica* leaf extract					
	Control	20	40	60	80	100
1	4.21	4.08	3.95	3.60	3.46	3.33
2	4.96	4.78	4.57	4.41	4.39	4.19
3	5.01	4.98	4.80	4.64	4.52	4.43
4	6.28	6.09	5.90	5.84	5.70	5.63
5	6.42	6.34	6.18	6.01	5.81	5.66
6	6.93	6.77	6.51	6.31	6.17	6.02
7	7.64	7.50	7.35	7.21	7.08	6.97

Discussion

In this study, different concentrations such as 20, 40, 60, 80 and 100 % aqueous leaf extract of *M. pudica* were used against *B. juncea* seeds for the analysis of in vitro allelopathy and in our knowledge this is the first report. The present study reports the negative allelopathic effect (reduction of plant growth parameter) of aqueous leaf extract against the test plant. The control showed the 100% seed germination on the third day, whereas the 100 % extract showed only 73.33 on the seventh day. The germination rate describes number of seeds of a particular plant species likely to germinate over a given time. It is a measure of germination time and is usually expressed in percentage. The aqueous extract of the *M. pudica* showed inhibitory effect on the growth of *B. juncea* seed in a concentration dependent manner. Seed germination index increased from day one today seven in each dilution.

The average radicle and plumule length decreased from the lower concentration to higher extract concentration in different dilutions. The concentration 80 and 100 % showed inhibition of 0.73 cm and 0.92cm in radicle length when compared to the control, while the concentration 20 % showed the lowest inhibition of 0.08 cm after the seventh day of the experiment. The extract concentrations 80 and 100 % showed a difference of 1.19 cm and 1.4 cm respectively in plumule length. The concentration 20 % showed the lowest inhibition of 0.12 cm after the seventh day of the experiment.

Seed vigour is the quality parameter which is to be assessed to supplement germination and viability tests to gain insight into the performance of a seed in the field or in storage. It is also the sum total of the properties that determine the activity of seeds of germination in a wide range of environments [10]. The seed vigour index of the *B. juncea*

showed an increase in the value as the number of days increased and for the same time the value decreased as the concentration of the leaf extract increased. The control showed a value of 504 and the lowest value of 302.11 was noted for the 100 % concentration on the seventh day. Vigour can be considered as the potential performance of viable seeds in agricultural practice and this is determined by the complex interaction between genetic and environmental components [11,12].

The concentration of 100 % showed the phytotoxicity of 26.7. When the concentration of the plant extract increased, the value of the phytotoxicity also increased. The 20 % concentration of showed the least value. *B. juncea* showed the highest speed of germination of 2091 in control. It is also noted that the speed of germination decreased as the concentration of the extract increased. The lowest speed of germination was recorded for 100 % concentration of with a value of 1641. Overall, the aqueous leaf extract of *M. pudica* affected all the growth parameters of *B. juncea* seeds. Mimosine is an important allelochemical found in Mimosa spp. reported to be responsible for the strong allelopathic activity by suppressing the growth of plants and plant fungi [13] and it exists in large quantities in leaves, pods and seeds of tropical legumes of the genus *Leucaena.*

Conclusion

The present study revealed that the aqueous leaf extract of *M. pudica* showed inhibitory effect on the growth of *B. juncea* seeds in a concentration dependent manner. The extract affected all the germination parameters of *B. juncea* seeds. Based on the present observations, the aqueous leaf extract of *M. pudica* contains phytotoxic materials and this could be used as an alternative to toxic synthetic herbicides.

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