The allelopathic potential of bryophyte extract on seed germination and seedling growth of *Bidens biternata*

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Abstract: The present work embodies the germination behaviour of *Bidens biternata* in response to different bryophyte extracts. The bryophyte species used were: *Targionia hypophylla*, *Marchantia polymorpha*, *Plagiochasma appendiculatum*, *Brachythecium buchananii*., *Leucodon secundus*, *Timmiella anomala* , *Rhodobryum roseum* and *Plagiomnium integram*. The extracts of these bryophytes were prepared in water (aqueous extract), acetone and methanol (lipophilic extracts) in order to dissolve the bio-active principles present in their thalli. In control experiments seed germination was 100% whereas bryophyte extracts had different degrees of inhibitory effect in different solvents. Complete inhibition was recorded in 100 % acetone extracts of most of the bryophyte species and with the decrease in concentration of extracts, germination increased. Like the acetone extracts, seed germination was also retarded in methanolic extract with the increase in concentrations. The 100 percent lipophilic extract of *Targionia*, *Marchantia*, *Plagiochasma*, *Rhodobryum* and *Plagiomnium* were found most effective where the seed germination was completely checked. Initiation of germination was also adversely affected by the increase in concentration of extract in organic solvents but in aqueous extracts germination was not delayed significantly. Total time taken for the completion of germination varied between the bryophyte species, concentrations and the solvents used. In organic solvent extracts, in some cases, germination was completed if water is added periodically. [Nature and Science. 2009;7(6):30-38]. (ISSN: 1545-0740).

Keywords: Bryophyte, extract, germination, initiation, completion, inhibition.

INTRODUCTION

Bryophytes living on variety of habitats represented a group of plants, which were exposed to different environmental and biotic dangers by virtue of their miniature size. Perhaps, for this reason, many of the secondary metabolites especially terpenoids and phenolic compounds of quite numerous chemical structures are synthesised as defense systems (Herout, 1990). These chemical substances effectively deter herbivores and pathogens from attacking them. Possibly, it may be one of the reasons why so many bryophytes grow in more or less in pure turf and cushions or mats. These allelochemicals also known as allomones have an advantage to the plant that produces them by preventing the growth of other plant species that may compete for soil nutrients, CO_2 and sunlight.

Huneck and Schreiber (1972) reported that allelopathic chemicals obtained from bryophytes possess growth regulatory activities. Asakawa et al. (1976 b) suggested that higher plants sometimes do not grow in these places inhabited by certain bryophytes because some liverwort gives off allelopathic compounds. The crude extracts of bryophytes show inhibitory activity against germination, root elongation and second coleoptile growth of rice in husk, wheat, lettuce and radish. Asakawa (1981,) and Asakawa et al. (1979 a, 1982) reported that sesquiterpene lactones isolated from liverworts had plant growth inhibitory effects on the germination and root elongation of rice husks at concentration of 50-200 μg/ml, while sesquiterpene dial, polygodial and diterpene dials, perrottetianal A and B had a weak inhibitory effect (100-500 μg/ml). Contrary to liverworts, little is known about the secondary compounds from mosses. *Leucobryum glaucum* grows in cushions of a diameter up to 30 cm in forests. These cushions store water for a long time during dry periods and yet young seedlings of forest trees are very rarely found. Similarly, the mats of *Sphagnum* are almost free from other plants. These examples indicate some type of allelopathy between bryophytes and other plants. Gavrillova (1970) reported that aqueous extracts of *Polytrichum commune* and *Sphagnum* spp. inhibited the growth of *Pinus* and *Picea* seedlings, but stimulated the growth of *Larix* seedlings.

The present research was carried out to study the allelopathic effect of different bryophyte extracts on the seed germination and early seedling growth behaviour of *Bidens biternata* (Lour.) Merr & Sherff. was examined. The *B. biternata* is a common weed species of the Kumaun Himalayan region which occupy disturbed as well as undisturbed habitats of the region. It produces a large number of seeds with 100% germination capacity which is resulting in rapid spread of this species over a large area. The objective of this experiment was to find natural herbicides for the biological control of *B. biternata* to reduce the risk of manufactured herbicides.

MATERIAL AND METHODS

Collection and identification of bryophytes

Three liverworts viz., *Marchantia polymorpha* L., *Targionia hypophylla*, and *Plagiochasma appendiculatum* Lehm. et Lindeb. and five mosses viz. *Leucodon secundus*. Schwaegr., *Rhodobryum roseum* (Schimp.) Limpr., *Plagiomnium* integrum Hedw., *Timmiella anomala* (De Not.) Limpr. and *Brachythecium buchananii* have been selected to study their effect on germination behaviour of *B. biternata* seeds.

The bryophytes were collected by regular and repeated local field trips at different localities of Nainital. The plants were collected in the first week of August. Fresh plants, devoid of dead tissue and with proper reproductive organs (to aid in identification), were collected. The plants were freed from contaminant parts of other plants, if present, and were carefully scooped out. Plants thus collected were kept in separate polyethylene bags and sealed immediately. The collected plant material was sorted out on the basis of their characters and identified following Kashyap (1929) and Gangulee (1972, 1977, 1980).

Collection of *Bidens biternata* seeds

The seeds of *B. biternata* were collected in July from monospecific stand of *B.biternata* situated at Ayarpatta hill at Nainital. Seeds were packed in polyethylene bags, brought to the laboratory, air dried and healthy seeds were sorted out.

Preparation of bryophyte extract

The bryophyte material was washed to remove adhering soil particles and blotted. As specimens were small and were not collected in large amounts due to conservation viewpoint, extracts were prepared from entire green part of the thallus. Water, methanol and acetone were used as extracting solvents. For preparing extract 5 g fresh material of bryophyte was ground with a pinch of sand in mortar to yield a pulp and dissolve in 50 ml of solvent and shaked in rotary shaker (200 r.p.m.) for 1 h, and filtered with Whatman No. 1 filter paper. Final volume of the extract was made upto 100 cc by adding respective solvent and considered as full concentration (100%). Then this extract was diluted to 70%, 50% and 20% concentration.

Experimental design

For each bryophyte species and for each extract the petri plates were prepared in triplicate. The petri plates were lined with a thin layer of cotton and filter paper and sterilised. In each petri plate 20 seeds of *Bidens* were placed to observe the germination behaviour in various concentrations of bryophyte extracts in different solvents. Control plates were also prepared in the same manner for each test. In each Petri plate 10 cc of extract was poured. For organic solvent after pouring 10 cc extract in Petri plate the solvent was evaporated aseptically at 35 ^{o} C and then 20 seeds were placed and 5 cc of distil water was added. It was assumed that active principles dissolved in organic solvent were absorbed in filter paper. In control experiment of each concentration solvents of same concentration but without bryophyte extract were used. The experiments were done at room temperature (20^{o} – 22^{o} C) and were carried out for 30 days. The seeds were considered germinated if the radical exceeded 3 mm in length.

RESULTS

The allelopathic potential of different bryophytic extracts on germination of *Bidens biternata* is shown Table 1.The germination of *Bidens biternata* seeds was hundred percent in each of the control experiments. However, different degrees of germination were found in various concentrations of bryophytic extracts. The hundred percent lipophilic (acetone and methanol) extracts of the species of *Targionia*, *Plagiochasma*, *Brachythecium*, *Rhodobryum*, *Marchatia* and *Plagiomnium* checked the germination completely, while in the extracts of the remaining species percent germination ranged between 32% (methanolic extract of *Rhodobryum*) and 100% (acetone extract of *Plagiomnium* and methanol extract of *Leucodon* and *Brachythecium*). The 70 and 50 percent methanolic extract of liverworts was as effective in inhibiting the seed germination as 100 percent extract. The methanolic extract of *Brachythecium* had no inhibitory effect on seed germination. Further, 20 percent extract of *Targionia* was effective in checking the seed germination while the extracts of rest of the species were totally ineffective. Irrespective of the concentrations, the water extracts of all bryophyte species except *Rhodobryum* were ineffective in controlling the seed germination.

In controlled conditions, germination was initiated within 24 hours, while it was delayed by 1 (*Marchantia*) to 18 days (*Plagiochasma*) in lipophilic extracts of liverworts. For the extracts of mosses, radicle initiation occurred between 5 and 14 days (Table 2). The methanolic extracts of *Brachythecium* delayed the germination by 5 days, irrespective of the concentration. In aqueous extracts, initiation was delayed from 1 day (*Rhodobryum* 70%) to 8 days (*Marchantia* 100%).

In controlled condition, the germination was completed within 48 h. However, for liverworts in most of the cases, irrespective of concentration, germination was not completed in lipophilic extracts till the culmination of the experiments (Table 3). The exception was *Plagiochasma*, where germination was completed between 3 and 7 days in acetone extract. For mosses, the effect of acetone extracts of *Leucodon*, *Timmiella* and *Brachythecium* was most promising with regard to the inhibition of germination, while in methanolic extract of *Plagiomnium* and *Rhodobryum* germination was not completed. Contrary to this, in the aqueous extracts of bryophytes germination of *B,biternata* seeds was completed between 10 and 4 days (*Marchantia* and *Targionia*) in liverworts and between 3 (*Plagiomnium*) and 9 (*Rhodobryum*) days in mosses.

Table 1. Percent seed germination in *B. biternata* as affected by different bryophytic extracts.

Bryophytic species	Solvent											
	Acetone (%)				Methanol (%)				Water (%)			
	100	70	50	20	100	70	50	20	100	70	50	20
Liverworts												
T.hypophylla	-	-	56	76	-	-	-	-	92	80	60	80
M.polymorpha	88	88	92	96	-	-	-	96	100	100	100	100
P.appendiculatum	-	100	100	100	-	-	80	88	100	100	100	100
Mosses												
L. secundus	-	-	-	84	100	60	100	100	100	100	100	100
T. anomala	56*	64	76	100	32	-	-	100	100	100	100	100
R. roseum	-	100*	100*	100	32	-	-	28	100	100	100	64
P. integrum	100*	44*	72*	100	-	-	-	28	76	84	80	84
B. buchananii	-	-	-	80	100	100	100	100	100	100	100	100

* = After the addition of sufficient water (10 cc)

Table 2. Number of days taken for initiation of seed germination in *B. biternata* as affected by different bryophytic extracts.

Bryophytic species	Solvent used											
	Acetone				Methanol				Water			
	100	70	50	20	100	70	50	20	100	70	50	20
Liverworts												
T.hypophylla	-	-	07	07	-	-	-	-	04	04	03	03
M.polymorpha	11	08	01	01	-	-	01	01	08	02	05	04
P.appendiculatum	-	18	03	03	-	-	04	04	03	03	03	03
Mosses												
L. secundus	-	-	05	05	05	05	05	05	04	04	04	03
T. anomala	-	-	08	08	06	05	05	05	05	04	04	04
R. roseaum	11	11	09	09	07	-	09	09	04	01	04	04
P. integrum	-	09	07	07	-	-	14	08	07	07	07	07
B. buchananii	-	-	-	05	05	05	05	05	04	04	04	03

Table 3. Actual number of days taken for completion of germination in *B. biternata* as affected by different bryophytic extracts.

Bryophytic species	Solvent used											
	Acetone				Methanol				Water			
	100 %	70%	50%	20%	100 %	70%	50%	20%	100 %	70%	50%	20%
Liverworts												
T.hypophylla	-	-	-	-	-	-	-	-	04	07	05	-
M.polymorpha	05	-	-	-	-	-	-	-	10	08	06	-
P.appendiculatum	-	03	03	07	-	-	-	-	07	06	07	-
Mosses												
L. secundus	-	-	-	-	06	06	06	06	06	06	05	06
T. anomala	-	-	-	-	08	-	04	06	06	07	07	06
R. roseaum	-	-	-	05	-	-	-	09	08	09	08	09
P. integrum	-	05	06	04	-	-	-	-	03	04	05	-
B. buchananii	-	-	-	08	06	06	06	06	06	06	05	06

Table 4. Relative performance of seed germination in *Bidens biternata* in different bryophyte extracts as compared to control.

Species	SOLVENTS			
	Concentration of extract (%)	Acetone	Methanol	Water
T.hypophylla	100	-	-	0.92
	70	-	-	0.80
	50	0.56	-	0.60
	20	0.76	-	0.80
M.polymorpha	100	0.88	-	1.00
	70	0.88	-	1.00
	50	0.92	-	1.00
	20	0.96	0.96	1.00
P.appendiculatum	100	-	-	1.00
	70	1.00	-	1.00
	50	1.00	0.80	1.00
	20	1.00	0.88	1.00
L. secundus	100	-	1.00	1.00
	70	-	0.60	1.00
	50	-	1.00	1.00
	20	0.84	1.00	1.00
T. anomala	100	0.56	0.32	1.00
	70	0.64	-	1.00
	50	0.76	-	1.00
	20	1.00	1.00	1.00
R. roseaum	100	-	-	0.84
	70	1.00	-	0.80
	50	1.00	-	0.60
	20	1.00	0.28	0.64
P. integrum	100	1.00	-	0.76
	70	0.44	-	0.84
	50	0.72	-	0.80
	20	1.00	0.28	0.84
B. buchananii	100	-	1.00	1.00
	70	-	1.00	1.00
	50	-	1.00	1.00
	20	0.80	1.00	1.00

Table 5. Seedling size of *Bidens biternata* as compared to control in different bryophytic extracts.

Species	SOLVENTS			
	Concentration of extract (%)	Acetone	Methanol	Water
T.hypophylla	100	-	-	1.09
	70	-	-	1.17
	50	0.46	-	1.01
	20	0.64	-	1.02
M.polymorpha	100	1.09	-	1.27
	70	1.09	-	1.27
	50	1.10	-	1.27
	20	0.81	0.86	1.28
P.appendiculatum	100	-	-	0.98
	70	0.84	-	1.00
	50	0.71	0.87	0.98
	20	0.51	0.84	1.02
L. secundus	100	-	1.24	1.37
	70	-	1.17	1.38
	50	-	1.09	1.29
	20	1.28	1.09	1.29
T. anomala	100	1.11	1.07	1.43
	70	1.12	-	1.50
	50	1.18	-	1.42
	20	1.11	1.24	1.42
R. roseaum	100	-	-	1.24
	70	1.05	-	1.24
	50	1.05	-	1.19
	20	1.21	0.88	1.17
P. integrum	100	0.91	-	1.33
	70	0.87	-	1.10
	50	0.89	-	1.33
	20	0.84	0.85	1.27
B. buchananii	100	-	1.07	1.25
	70	-	1.08	1.56
	50	-	1.08	1.56
	20	1.05	1.08	1.33

DISCUSSION

The present work examines the growth regulatory activities of three liverworts (*T. hypophylla, M. polymorpha* and *P. appendiculatum*) and five mosses (*L. secundus, T. anomala, R. roseum* and *P integrum B. buchananii*) on germination behaviour of *B. biternata* seeds. Irrespective of the concentration, in aqueous extracts of all bryophyte species germination was approximately 100 %. However, in lilophilic (acetone and methanol) extracts germination of *B. biternata* exhibited marked variations and declined with the increase in concentration of lilophilic extracts of bryophyte. This has indicated that allelochemicals (terpenoids and phenols) present in the tissues of bryophytes were not soluble in water. In present study, methanolic extract appeared to be more effective than the acetone extract. The methanolic extract of

Targionia was the most effective where germination was completely inhibited. Chemicals with allelopathic activity are present in many plants and in various organs and have potential as either herbicides or templates for new herbicide classes (Duke et al 2000).

At higher concentration of acetone soluble extracts (100 %) germination percentage was generally low and germination was accomplished only in *Marchantia*, *Timmiella* and *Rhodobryum*. In rest of the extracts, seeds failed to germinate. In *Timmiella*, *Rhodobryum* and *Plagiomnium*, germination was brought out only after the addition of sufficient water at regular intervals. On the other hand, methanolic (100 %) extracts of *Targionia*, *Marchantia* and *Plagiochasma* exhibited complete inhibition. The results indicate that these mosses contain the terpenoids, phenols or other compounds which are more readily soluble in acetone rather than methanol. Asakawa (1990) reported a group of terpenoids isolated from liverworts that exhibited complete inhibition of germination in rice in husk. Exceptional seed germination was observed in 100 percent methanol and acetone extracts of *Timmiella*, *Rhodobryum* and *Plagiomnium* possibly due to the dilution of the inhibitory effect of the active principles by addition of water at regular intervals. Mosses usually lack mono- and sesqui- terpenoids in their tissues but they are rich in steroids and flavonoids (phenolic) and it is likely that these compounds may somehow be responsible for the allelopathic effects observed in the present study. Time taken for the initiation of germination of seeds increased with the decrease in concentration of extracts. In aqueous extracts of all the experimental species, no significant difference was observed in time for the initiation of germination. At higher concentration of lipophilic extract, germination was maximally delayed up to 15 days (*Plagiochasma*, 70 % alcohol extract) compared to the water extract. In *Rhodobryum* and *Plagiomnium* extracts, germination was initiated only after the addition of water.

Total time taken for the completion of germination of seeds varied according to the type of the extracts. Variation in total germination period appears to be independent of the concentration of the moss extract. Some other factors like size of the seeds may be responsible for delaying the completion of germination in certain extracts. Among liverworts, maximum time was taken by *Marchantia* (10 days in 20 percent aqueous extract).

Relative performance of *B. biternata* seed germination in different bryophyte extracts as compared to control was mainly inhibitory (Table 4). Among the extracts of liverworts, maximum inhibition was shown by *Targionia* as it checked the seed germination completely in 70 and 100 percent acetone extracts, 20 to 100 percent inhibition in methanol extract and about 10 percent inhibition in aqueous extract (Table 1). The allelopathic effect of *Targionia* on seed germination may be attributed to the allomones present in their thalli. Sharma (1999) reported about 22 terpenoids in *Targionia hypophylla*, collected from Nainital. Some of the major components are α-pinene, bi- cyclogermacrene, plagiochiline N, Vitranal, eucarvone etc. Also, *Targionia* generally grows as a pure patch in nature. Sometimes few mosses and rarely angiosperms are intermingled with it thus, indicating the presence of volatile allelochemicals in their tissues which appeared to be unfavourable for the growth of other plants in near vicinity of this plant. In addition to *Targionia*, *Brachythecium* and *Leucodon* exhibited complete inhibition in 50, 70 and 100 % acetone extracts. *Rhodobryum* and *Plagiomnium* showed 20 to 40 % inhibition in seed germination in water extracts.

Effect of bryophyte extract (as compared to control) on seedling size is given in Table 5. Irrespective of the concentration, the extracts of liverworts retarded the seedling size more in comparison to the moss extracts. Among all bryophytes, *Plagiochasma* and *Targionia* extracts were most effective in reducing the seedling growth. This may be due to the presence of large number (27) of terpenoids in the tissues of *Plagiochasma appendiculatum* of this locality (Joshi 1999). The major compounds are β –pinene α-elemene, thujopsene, bicyclogermacrene, Cuparene etc. Several modes of action attributed to allelopathic compounds include effect on cell elongation and ultrastructure of roots by inhibition of cell division (Rice 1984). Among solvents, generally the acetone extract was more effective in reducing the seedling growth. However, no significant effect of concentration of extract was observed.

Struggle for space and nutrients for propagation, continuity and university is the most powerful law of nature. In this trend, some plants have allelopathic potential by releasing allelochemicals to their surrounding that have deleterious or beneficial effects on other plants. The compounds are released to the environment by mean of volatilization, leaching, decomposition of residues and root exudation. These compounds inhibited plant growth by affecting many physiological processes. The degree of inhibition depends on their concentration. Results of present experiment indicate that the bryophyte extracts had inhibitory potential in reducing and checking seed germination of *B. biternata* and can be used in biological control of this weed species.

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