

Evaluation of allelopathic potential of *Globba* leaf extracts on germination behaviour and metabolism of lentil seeds

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Abstract

Many plants interact to their native plants in term of allelopathic activity. It may be promotive or inhibitory. Various allelochemical compounds from plant or plant parts have an interaction to neighboring plants or organisms. In the present investigation, the allelopathic effect of *Globbaracemosaleaf* (aqueous) extracts on lentil seeds was evaluated. Seed germination behaviour such as percentage germination, T_{50} values, TTC stainability of lentil seeds was analysed. Some biochemical parameters like leaching of free amino acids, soluble carbohydrates and dehydrogenase enzyme activity were assessed from lentil seeds. Leaf extracts pretreated seeds show inhibitory germination rate along with higher T_{50} values over control (distilled water). Leaching of free amino acids and soluble carbohydrates are also higher in *Globba* leaf extracts treated seeds. Dehydrogenase enzyme activity shows lower in case of 1:1 leaf extract treated lentil seeds. Thus, a conclusion can be drawn from the experimental results that *Globba* leaf extracts exert positive allelopathic effect on lentil seeds.

The term 'Allelopathy' was coined by Molisch¹³, after that Rice¹⁹, defined allelopathy as "any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment". Later in 1996, the allelopathy was explained as positive and negative influences of secondary metabolites produced by plants on growth and development of agricultural systems²³. Allelochemicals may show promotive or inhibitory, depending upon the interaction with the plant species^{5,20}.

Several allelopathic compounds have been isolated and identified from various plant parts worldwide and many biologically active allelochemicals may potentially use as pharmaceutical purposes⁶. The study of allelopathy is important to ecological and agricultural point of view due to the regulatory impact of the allelochemicals³. The inhibitory allelopathic effect of plant extracts with special reference to various physiological and biochemical changes on seeds have been established by many workers^{2,9,16,17}.

As allelopathic effect was reported in many species of Zingiberaceae family, *Globba* plant leaf extract was used for the evaluation of allelopathic interaction on other plants and lentil seeds was used as a bioassay material for the experimental purposes. In the present investigation, to assess the allelopathic potential of leaf extracts of *Globba* on lentil seeds, some specific physiological and biochemical parameters were analysed.

Experiments of the present investigation were carried out with fully viable healthy seeds of lentil as the bioassay material. Healthy mature leaves of *Globba racemosa* were collected from Darjeeling hills, West Bengal, where the plant population growing profusely at an altitude about 6600 ft. Leaves were detached and washed with distilled water to remove the adherent dust particles. Mature leaves of *Globba* (500g) were thoroughly homogenized by mortar and pestle using 300 ml distilled water. The homogenates were strained using fine cloth and then centrifuged at 5000g for 15 minutes. Both the supernatants were then made up to 500 ml using distilled water and these were considered as 1:1 (w/v) proportion stock solutions of the leaf extracts. From these stock solutions another concentration grades of leaves in the proportion of 1:2 (w/v) were prepared using distilled water and thus two concentration graded solutions of the leaf extracts were prepared. These two concentration grades of leaf extracts were used for allelopathic analysis¹⁰.

Fully viable 200 g lentil seeds were surface sterilized with 0.1% HgCl₂ solution for 90 seconds. The seed lots were then separately pre-soaked in these two concentration grades

of *Globba* leaf extracts for 12 hours. From the treated seed samples germination behaviour (percentage and T₅₀ values of seed germination), TTC stainability, free amino acid and soluble carbohydrate contents (from seed leachates) along with dehydrogenase activity were recorded.

Percentage seed germination data recorded at every 24 hours intervals up to 72 hours of seed soaking following the International Rules for Seed Testing⁸ (Table-1).

T₅₀ values (time in hour required for 50% germination) of germination of lentil seeds were determined following the method described by Coolbear *et al.*⁴ (Table-2).

The percentage TTC-stained (red coloured) seeds were calculated from the total number of seeds of each treatment. This method was adopted essentially after Halder⁷ (Table-3).

Free amino acids (from the leachate stock) were quantified following the method of Moore and Stein¹⁵ modified by Bhattacharjee¹. The quantitative estimation was made by comparing the optical density (O.D.) values from a standard curve prepared from glycine (Table-4).

Soluble carbohydrates (from seed leachates) were determined following the method of McCready *et al.*¹¹ with slight modification. Actual quantity was evaluated from a previously prepared standard curve with glucose.

To analyse dehydrogenase activity 20 seeds of each treatment were imbibed in 0.5%

TTC (2, 3, 5-triphenyl tetrazolium chloride) solution (w/v) in test tube and incubated for 12 hours in dark. The hydrogen atom released by the total dehydrogenase which are involved in the respiration process of living tissue reduce tetrazolium to red coloured formazan¹⁴. This formazan, produced after incubation was extracted with 5 ml of absolute alcohol and OD values of the solution were recorded at

520nm. This method was adopted after Rudrapal & Basu²² with slight modification.

Statistical analysis of the data was done in terms of least significant difference (LSD) which was calculated at 95% confidence limits and as per the method of Panse and Sukhatme¹⁸.

Table-1. Effect of *Globba* leaf extracts [1:1 & 1:2 (v/v) each] on percentage germination of lentil seeds

Treatments	Percentage germination after hours (h)			
	0	24	48	72
Control (Distilled water)	0	76.52	88.43	100
<i>Globba</i> leaf extracts 1:1	0	52.80	68.22	80.63
<i>Globba</i> leaf extracts 1:2	0	62.32	76.45	86.14
LSD (P=0.05)	-	4.55	5.82	5.60

Table-2. Effect of *Globba* leaf extracts [1:1 & 1:2 (v/v) each] on T₅₀ Values (h) and TTC stainability (%) of lentil seeds.

Treatments	T ₅₀ Values (h)	TTC stainability (%)
Control (Distilled water)	18.35	100
<i>Globba</i> leaf extracts 1:1	23.80	74.22
<i>Globba</i> leaf extracts 1:2	20.47	80.46
LSD (P=0.05)	1.03	4.96

Table-3. Effect of *Globba* leaf extracts [1:1 & 1:2 (v/v) each] on leaching of free amino acid and soluble carbohydrate contents (mg/g/10ml) of lentil seeds.

Treatments	Free amino acids (mg/g/10ml)	Soluble carbohydrates (mg/g/10ml)
Control (Distilled water)	8.20	16.35
<i>Globba</i> leaf extracts 1:1	12.62	24.22
<i>Globba</i> leaf extracts 1:2	10.86	21.75
LSD (P=0.05)	1.09	1.92

Table-4: Effect of *Globba* leaf extracts [1:1 & 1:2 (v/v) each] on the dehydrogenase activity (Δ OD/g wet wt./5ml) of lentil seeds

Treatments		dehydrogenase activity (Δ OD/g wet wt./5ml)
Control (Distilled water)		1.62
<i>Globba</i> leaf extracts	1:1	0.86
	1:2	1.25
LSD (P=0.05)		0.22

Photographs:



Globba racemosa leaf (left) and lentil seeds were treated with *Globba* leaf extracts (1:1 and 1:2) and distilled water (right)

In case of germination percentage of lentil seeds, *Globba* leaf extracts (1:1) treated seeds show significantly reduced percentage germination than the other treatments (Table 1). T_{50} values (h) were concomitantly higher in *Globba* leaf extracts treated seeds over control (Table-2). In case of TTC stainability of lentil seeds treated with leaf extracts of *Globba* leaf extracts (1:1) showed reduced stainability percentage (Table-2). Table-3 shows free amino acid leaching of lentil seeds

treated with the leaf extracts; profuse leaching of amino acid was found in case of *Globba* leaf extracts (1:1) treated seeds. In case of leaching of soluble carbohydrates of lentil seeds treated with leaf extracts; higher amount of leaching in *Globba* treated seeds was found over control (Table-3). Results revealed in table 4 shows dehydrogenase activity of lentil seeds. Seeds pretreated with leaf extracts of *Globba* (1:1) shows reduced dehydrogenase activity over control. The allelopathic effects on seeds

have successfully been established in many investigations. Various exotic plant species shows their specific allelochemicals in natural plant communities have strong allelopathic effects by plant-plant interaction by releasing specific allelochemicals^{9,16,21}.

There are reports in literature that plants having allelopathic potential can reduce seed germinability, TTC stainability, seedling growth as well as metabolic activity of some plants^{16,22}. My results are also in conformity with the reported observations of some previous workers.

Thus, it may be concluded from the experimental results that, the different concentrations of aqueous leaf extracts of *Globba* were found inhibitory to various parameters on lentil seeds over control and shows positive allelopathic effect.

Future vision: Further research may be encouraged to identify the specific allelochemical compounds from the leaf extracts along with the use of such chemicals as a potent bioherbicide purposes in a biotechnological approach.

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