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Evaluation of allelopathic effect of *Solanum nigrum* leaf extracts on mung bean seeds

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Abstract

In the present investigation, the allelopathic effect of leaf extracts of *Solanum* sp. was evaluated using mung bean seeds on the basis of some physiological and biochemical parameters. Seed germination behaviour such as percentage germination, T_{50} values (h), TTC stainability of mung bean seeds was analysed. Some biochemical parameters like leaching of free amino acids, soluble carbohydrates, proteins and dehydrogenase enzyme activity were also assessed from mung bean seeds. Results revealed that *Solanum* leaf extracts 1:1 exhibited more inhibitory effects than the other treatments on mung bean seeds.

Key words : Allelopathy, *Solanum*, mung bean, seed germination.

In agricultural systems, the influence of the secondary metabolites released by the whole plants or plant parts on other plants was found either promotive or inhibitory on growth and development of many species[18, 21]. Such type of interactions is predominantly found in wide groups of plants such as algae, lichens, many crop plants and also in some exotic plants[2,3]. Allelopathic chemicals can be present in any part of the plant such as leaves, flowers, roots, fruits, seeds, rhizomes, pollens or stems. They can also be found in the surrounding soil. Allelochemicals are released into the environment by root exudation, leaching from above ground parts and volatilization and by decomposition of plant material[18]. The positive or negative effects of the allelochemicals released by the plants to the neighbouring plants are mainly depending on the interactions between the plant species[4,19]. Such type of behaviour is due to nature of allelochemicals released.

Allelochemicals may interfere with seed germination, root/shoot length or may be the metabolic status of the seeds. Many crop and weed species have been found which possess allelopathic activity on the growth and

development of other plant species[8,22].

In this investigation, an attempt was made to evaluate the allelopathic efficacy of the *Solanum nigrum* leaf extracts on mung bean seeds. Several physiological and biochemical parameters were analysed from the leaf extract treated seed samples. Seed germination behaviour and metabolism of mung seeds were evaluated as these are the important allelopathic indices.

Plant sample collection and preparation of aqueous extract :

For performing the experiments fully grown healthy leaves of *Solanum nigrum* L. were collected from Serampore college campus, Hooghly, West Bengal. Leaves were then thoroughly washed under tap water to remove the adherent dust particles and then with distilled water, air dried and weighed. The weighed leaves (500g) were grounded in mortar and pestle using 300 ml distilled water at room temperature. The homogenates were strained using muslin cloth to remove the debris and centrifuged at 5000g for 15 minutes. The supernatant was then made up to 500 ml using distilled water and this was considered as 1:1 (w/v) proportion stock solutions of the leaf extract. From this stock solution another concentration grade 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 analysis[11,14].

Fully viable mung bean seeds were surface sterilized with 0.1% $HgCl_2$ solution for 90 seconds. The seed lots were then separately pre-soaked in the two concentrations of leaf extracts (1:1 and 1:2) of *S. nigrum* for 24 h and then thoroughly surface-washed with tap water followed by distilled water and dried. Dried seeds were then used as test materials for analysing various physiological and biochemical parameters. Data was collected on seed germination percentage, T_{50} value, TTC stainablity, fresh and dry weight of 10 days old plants raised from such seeds, average length of root and shoot, leaching of free amino acids and soluble carbohydrates, changes in the level of proteins, and dehydrogenase enzyme activities in seeds.

Percentage seed germination data was recorded at every 24 h intervals up to 96 h of seed soaking following the International Rules for Seed Testing[7] (Table-1).

T_{50} values (*i.e.,* time in hour required for 50% germination) for germination of mung bean seeds were recorded following the method described by Coolbear *et al*.,[5] (Table-2).

To analyze TTC stainability, 100 dehusked treated mung bean seeds were allowed to imbibe in 0.5% TTC (2, 3, 5-triphenyl tetrazolium chloride) solution (w/v) in petri dishes for 24 h in dark. The percentage of TTC-stained (red coloured) seeds were calculated from the total number of seeds[6] (Table -2).

Free amino acids and soluble carbohydrate and soluble proteins contents in the seed leachates were analysed after immersing treated 10g seed samples of mung bean in 100 ml distilled water for 24 h. (Table-3).

Table-1. Effect of *Solanum nigrum* leaf extracts [1:1 & 1:2 (w/v) each] on percentage germination of mung bean seeds.

Treatments	Percentage germination after hours (h)				
	0	24	48	72	96
Control (Distilled water)	0	40	100	100	100
Solanum nigrum leaf extract (1:1)	0	0	28.7	50	50
Solanum nigrum leaf extract (1:2)	0	12.2	40.2	75.1	75.1
LSD (P=0.05)	-	-	1.97	4.26	4.26

Table-2. Effect of *Solanum nigrum* leaf extracts [1:1 & 1:2 (w/v) each] on T_{50} values (h) and TTC stainability (%) of mung bean seeds.

Treatments	T_{50} values (h)	TTC stainability (%)
Control (Distilled water)	18	100
Solanum nigrum leaf extract (1:1)	72.8	85
Solanum nigrum leaf extract (1:2)	52	87.5
LSD (P=0.05)	1.56	1.82

Table-3. Effect of *Solanum nigrum* leaf extracts [1:1 & 1:2 (w/v) each] on leaching of free amino acids, soluble carbohydrate and soluble protein contents (mg/g/10ml) of mung bean seeds

Treatments	Free amino acids (mg/g/10ml)	Soluble carbohydrate (mg/g/10ml)	Soluble protein (mg/g/10ml)
Control (Distilled water)	0.124	0.236	0.114
Solanum nigrum leaf extract (1:1)	0.574	0.388	0.849
Solanum nigrum leaf extract (1:2)	0.484	0.316	0.305
LSD (P=0.05)	0.08	0.05	0.05

Table-4: Effect of *Solanum nigrum* leaf extracts [1:1 & 1:2 (w/v) each] on the dehydrogenase activity (OD/g wet wt./5ml) of mung bean seeds.

Treatments	Dehydrogenase activity (OD/g wet wt./5ml)
Control (Distilled water)	0.92
Solanum nigrum leaf extract (1:1)	0.78
Solanum nigrum leaf extract (1:2)	0.90
LSD (P=0.05)	0.08

Table-5. Effect of seed pre-treatment with leaf extract of *Solanum nigrum* L. on shoot length, root length, fresh weight and dry weight of 10 days old mung bean plants.

Treatments	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)
Control (Distilled water)	13.6	5.5	6.50	3.06
Solanum nigrum leaf extract (1:1)	6.5	2.7	3.84	0.602
Solanum nigrum leaf extract (1:2)	9.9	4.2	4.48	1.064
LSD (P=0.05)	0.51	0.18	0.24	0.06

Photographs :

Percentage of seed germination after hours	**Control**	**LE 1:1**	**LE 1:2**
24			
48			
72			
96			

Fig. 1. Effect of *Solanum nigrum* L. leaf extracts on percentage germination of mung bean seeds. (LE= Leaf extract).

Fig. 2. Effect of *Solanum nigrum* leaf extracts on root and shoot length of mung bean seeds.

Free amino acids from seed leachates were quantified following the method of Moore and Stein[13] modified by Bhattacharjee[1]. The quantitative estimation was made by comparing the optical density (O.D.) values from a standard curve prepared from glycine.

Soluble carbohydrates (from seed leachates) were determined following the method of McCready *et al.*,[12] with slight modification. Actual quantity was evaluated from a prepared standard curve with glucose.

From the leachate stock, soluble protein level was determined following the method of Lowry *et al*,[9] with slight modification.

To analyse dehydrogenase activity 20 seeds of each treatment groups 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. This method was adopted after Rudrapal & Basu, 1979 (20) with slight modification (Table-4).

Fresh weight (g) and dry weight (g) were measured from intact seedlings, raised from untreated and treated seeds. Data were recorded from 10 days old, uniformly grown mung bean plant of each treatment group including control. The shoot and root length were recorded separately for different concentrations of both species[14] (Table-5).

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 Sukhatme[17].

Table 1: In case of germination percentage of mung bean seeds, *Solanum* leaf extracts (1:1) showed significantly reduced

percentage germination than the other treatments.

Table 2: T_{50} values (h) were concomitantly higher in 1:1 leaf extract treated mung bean seeds. In case of TTC stainability, seeds treated with leaf extracts of *Solanum* leaf extracts (1:1) showed reduced effect where in case of control seeds show higher TTC stainability.

Table 3: Table 3 shows leaching of free amino acid, soluble carbohydrates and soluble protein contents of mung bean seeds treated with both the leaf extracts, where profound leaching was observed in case of leaf extracts (1:1) treated seeds.

Table 4: Results revealed in table 4 shows dehydrogenase activity of mung bean seeds. Seeds pretreated with leaf extracts of *Solanum* (1:1) shows reduced dehydrogenase activity over control.

Table 5: Table 5 shows growth parameters of mung bean seeds where in case of control seeds, showed higher growth than the other treatments.

The allelopathic effects on seeds have been successfully 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[4,10,14,16].

In the present investigation, *Solanum* leaf extracts was used as experimental material for analysing the allelopathic potential. These plants grew profusely and positively rendering allelopathic action on mung bean seeds. During this investigation, various physiobiochemical approaches were considered as the indices for the evaluation of allelopathic action. There are reports in literature that plant having allelopathic potential can reduce seed germinability, TTC stainability along with some other growth parameters as well as metabolic activity of some plants[11,14,15].

Thus, a conclusion may be drawn from the experimental results that, the inhibitory allelopathic effect was found by the aqueous leaf extracts of *Solanum* on mung bean seeds.

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