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Seed potentiation of lentil by herbal manipulation

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Abstract
Effect of *Gleichenia* leaves on lentil (*Lens culinaris*) seeds was evaluated by physiological and biochemical approaches. Some reliable indices were taken as the major findings of the experimental plant species. It was concomitant from the detailed experimental results that the concentrations of leaf extracts (1:1 and 1:2) exert positive allelopathic effect. The plant extracts (1:1 and 1:2) of *Gleichenia* reduced the percentage germinability of lentil seeds. The plant extract-induced biochemical changes associated with enhanced leaching of free amino acids and soluble carbohydrate contents followed by reduced insoluble carbohydrates, RNA contents and dehydrogenase enzyme activity from the seed species were also analysed.

Keywords: herbal manipulation, *Gleichenia*, lentil, seed germination, leaf extract

Introduction
In natural or man managed agroecosystems, neighbouring plant sp. may interact with the growth and development of other species. Weeds cause a number of harms in agroecosystems. They are unwanted plants which interfere with agricultural operations, compete with crop plants for light, water, nutrients and space and also reduce crop growth and yield through the release of phytotoxins as leachates, exudates, volatiles and de-composition products (Rice, 1984) [1]. Allelopathy may be explained as "Any process involving secondary metabolites produced by plants, microorganisms, viruses, fungi that influence the growth and development of agricultural and biochemical systems (including animals), including positive and negative effects". It signifies that interacting or inhibition of growth both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization residue decomposition and other processes (Molish,1937) [2]. These interactions are widely known in different groups of plants such as algae, lichens, crops, as well as annual and perennial weeds (Chatterjee *et al.*, 2012; Ojha *et al*., 2013) [3, 4].
In Darjeeling hills, West Bengal, India an abundance of *Gleichenia* sp. found growing on the road side as well as on the hilly area. There are some common indices for evaluating the allelopathic action of plants and plant parts; these include germination behaviour and other physiobiochemical parameters for responses of test species (Dogra *et al.*, 2011) [5].
In the present investigation an attempt was made to assess the herbal potential of *Gleichenia* leaf extracts by using lentil seeds as bioassay materials.

Materials and Methods
Experiments of the present investigation were carried out with fully viable healthy seeds of lentil as the test material. Healthy mature leaves of *Gleichenia* sp. were collected from Darjeeling Govt. College Campus, Darjeeling, W.B. Leaves were detached and washed with distilled water to remove the adherent dust particles. Leaves of *Gleichenia* (500 g) were thoroughly homogenized by mortar and pestle using 250 ml distilled water. The homogenate was strained using fine cloth and then centrifuged at 4000*g* for 10 minutes. The supernatant was then made up to 500 ml using distilled water and this were considered as 1:1 (w/v) proportion stock solution of leaf extract. From this stock solution another concentration grade in the proportion of 1:2 (w/v) was prepared using distilled water and thus two concentrations of solution were prepared. These two concentration grades of leaf extracts were used for experimental purposes (Maity *et al.*, 2015) [6].
Fully viable 200 g of lentil seeds were surface sterilized with 0.1% $HgCl_2$ solution for 90 seconds. The seed lots were then separately pre-soaked in this two concentration grades of leaf extracts for 12 hours. From the treated seed samples germination behaviour (percentage and T_{50} of seed germination), leaching of free amino acids, soluble carbohydrates, insoluble carbohydrates, RNA contents and dehydrogenase activity were recorded.

To find out the seed germination, seeds were pre-soaked with leaf extracts (1:1) and (1:2) and distilled water (control) for 24 hours. To analyse percentage of seed germination, the individual 100 seed samples of each treatments were transferred to Petri dishes containing filter paper moistened with distilled water. Seed germination data recorded at every 48 hours intervals up to 96 hours of seed soaking following the Rules of ISTA, 1976 [7].

T_{50} values of seeds were recorded after 96h of seed soaking. The time (hours) taken for 50% germination of seeds (T_{50}) was determined following standard method (Coolbear *et al*., 1984) [8].

Estimation of soluble carbohydrates from seed leachates was determined following the method of McCready *et al.* with slight modification (McCready *et al.*, 1950) [9]. Free amino acid levels from the seed leachates of each treatment were analysed following the method of Moore and Stein,1948 modified by Bhattacharjee, 1984 [10, 11]. Dehydrogenase activity was adopted as per the method described by Rudrapal & Basu, 1979 [12]. RNA contents from the seed kernels was analysed as per the method of Cherry, 1962 modified by Choudhuri and Chattarjee, 1970 [13, 14].

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, 1967 [15].

Results and Discussion

The effect of different concentrations of aqueous leaf extracts from leaves of *Gleichenia* sp. was found inhibitory to various parameter *viz.* seed germination behaviour and metabolism of lentil seeds.

Table 1: In case of seed germination percentage, the leaf extract (1:2) significantly reduced percentage germination over control.T_{50} values (h) were concomitantly higher in both the *Gleichenia* leaf extract treated lentil seeds.

Table 2: In case of leaching of soluble carbohydrates of both the seeds treated with leaf extracts shows high amount of leaching in both lentil seeds. Free amino acid leaching of the experimental seeds treated with leaf extracts (1:2) shows less leaching over control.

Table 3: Total content of insoluble carbohydrates of lentil seeds were reduced significantly when treated with leaf extract (1:2) of *Gleichenia* over control. Total RNA contents reduced in lentil seeds after treated with the leaf extracts.

Table 4: Total content of dehydrogenase enzyme of lentil seeds were reduced significantly when treated with leaf extract (1:2) of *Gleichenia* over control.

Table 1: Effect of seed pretreatments with *Gleichenia* leaf extracts (1:1 and 1:2) on percentage (%) germination and T_{50} values of lentil seeds.

Treatments (W/V)	Percentage germination (%)			T_{50} Values (h)
	0	48	96	
Control (dH_20)	0	100	100	24.00
Leaf extracts (1:1)	0	85.2	87.4	28.16
Leaf extracts (1:2)	0	82.5	84.6	29.09
LSD (P=0.05)	-	1.06	1.29	0.66

Table 2: Effect of seed pretreatments with *Gleichenia* leaf extracts (1:1 and 1:2) on leaching of free amino acid contents (µg/ml) and soluble carbohydrates (µg/ml) of lentil seeds.

Treatments (W/V)	Amino acid	Soluble carbohydrates
Control (dH_2O)	227.08	341.79
Leaf extracts (1:1)	203.55	397.67
Leaf extracts (1:2)	186.50	418.26
LSD(P=0.05)	12.50	16.45

Table 3: Effect of seed pretreatments with *Gleichenia* leaf extracts (1:1 and 1:2) on insoluble carbohydrates contents (µg/ml) and RNA contents (µg/ml) of lentil seeds.

Treatments (W/V)	Insoluble carbohydrates	RNA contents
Control (dH_2O)	47.4	54.8
Leaf extracts (1:1)	32.6	48.6
Leaf extracts (1:2)	29.5	46.4
LSD (P=0.05)	1.85	1.96

Table 4: Effect of seed pretreatments with *Gleichenia* leaf extracts (1:1 and 1:2) on total dehydrogenase enzyme (unit/min/g/fr. wt.) contents of lentil seeds.

Treatments (W/V)	Total Dehydrogenase		
	0	48	96
Control (dH_20)	0	100	100
Leaf extracts (1:1)	0	92.50	80.12
Leaf extracts (1:2)	0	82.5	75.50
LSD (P=0.05)	-	4.20	3.29

The herbal effect on experimental seeds have successfully established in many investigations. Various exotic plant species shows their specific bioactive compounds in natural plant communities have strong effects by plant-plant interaction by releasing specific phytochemicals [16]. Seed germination behaviour of lentil is slowed down by *Gleichenia* leaf extracts. This result is also in conformity with reported observation of many workers (Das, 2008) [17].

Thus, a conclusion can be drawn from this entire investigation that *Gleichenia* leaf extracts (1:2) shows more positive allelopathic effects in respect to the concentration (1:1) on the experimental bioassay material lentil seeds.

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