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Allelopathic potential of a noxious weed on mung bean

Parthapratim Maiti*
Department of Botany, Midnapore College, Midnapore-721 101, West Bengal, India.

Ram Kumar Bhakat
Department of Botany and Forestry, Vidyasagar University, Midnapore-721 102, West Bengal, India.

Alok Bhattacharjee
Centre for Advanced study, Department of Botany, Burdwan University-713 104, Burdwan, West Bengal, India

ABSTRACT

Eupatorium odoratum have invaded the waste lands of South West Bengal, India. A field study indicated a gradual and also significant increase in *Eupatorium odoratum* accompanied with significant decrease in other coexisting species. Considering the above in mind, a study was undertaken to evaluate the existence of inhibitory effect of leaf extracts and leaf leachates noxious weed *Eupatorium odoratum* using fully viable seeds of mung bean (*Vigna radiata*) as the bioassay material. The study showed the reduced the percentage germination and TTC stainability along with extended T50 values of mung bean seeds. The levels of protein, DNA and RNA, activities of dehydrogenase and catalase enzymes were significantly retarded in pretreated seed samples. Amino acid and sugar levels were increased in the leachates of seeds pretreated with leaf extracts and leaf leachates. Thus, from the overall results it can be concluded that various inhibitors present in *E. odoratum* can impart strong inhibitory effect on mung bean. The study suggests that the leaves of *E. odoratum* possess phytotoxic or allelopathic chemicals which potentially rendered the inhibitory action on mung bean seeds.

Keywords: Allelopathy, catalase, DNA, *Eupatorium odoratum*, protein, RNA.

*Corresponding author
E-mail: ppmaiti@yahoo.com
Phone: +91 9474000791

INTRODUCTION

In natural or man managed agroecosystems, neighboring plant species 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 decomposition products (Rice 1984). Due to their interference with crops, they reduce crop productivity leading to huge losses on a global scale.

About 240 weed species are reported to be phytotoxic and interfere with the growth and production of crops (Qasem and Foy 2001). Phytotoxic secondary metabolites have the potential to mediate interspecific plant-plant interference by reducing or inhibiting competitor establishment, growth and survival. The endogenous chemical-induced inhibition of one plant species by another represents a form of chemical warfare between plants competing for limited light, water and nutrient resources. Many phytotoxic allelochemicals have been isolated, identified, and found to influence a number of physiological reactions. Mode of action of some allelochemicals have been described and these render biotic stress known as allelochemical stress, which can have an indirect or direct effect on receiver plant (Cruz-Ortega et al. 2002, Singh et al. 2013).

These compounds have been shown to affect many different cellular processes in target plant species, including disruption of membrane permeability, ion uptake, inhibition of electron transport in both photosynthesis and the respiratory chain, cause damage to DNA and protein, alterations of some enzymatic activities and ultimately lead to programmed cell death (Ding et al. 2007). Although much studies in this direction are being done recently, in India this aspect has attracted very little attention. Considering the above in mind, the aim of the present study is to screen out the inhibitory effect of an exotic weed *E. odoratum* which has become invasive and forms monospecific thickets in roadsides, forest margins and crop field edges in West Bengal (Bhakat and Maiti 2003).

Eupatorium odoratum leaves contains phytotoxins or allelochemicals (inhibitors) like ceryl alcohol, eupatol (a sesquiterpene alcohol), lupeol and β-amyrin (terpene alcohol), salvigenin (flavone), isosakuranetin (flavan), isosakuranetin mono-methyl ether, 4,5-dihydroxy-3,7-dimethoxy flavone (flavonones) and odoratin (chalcone) and *p*-anisic acid in its different plant parts (Ambika 2002) and these chemicals interfere with various physiological and biochemical processes of seed germination, root elongation, plant growth as well as various metabolic activities of many species.

Most of the reports (Bhakat et al. 2006, Amoo et al. 2008, Bhakat and Maiti 2012) are based on the preliminary investigations on some putative phytotoxins-induced changes in germination behaviour and growth parameters of crops. However least attention was given to correlate the changes of growth behaviour with the metabolic status of the test plants.

Thus, in this investigation, an attempt was made to focus on this aspect, and for analysing this, we selected *E. odoratum* as a donor weed and mung bean as the target species. Accordingly, this study was designed to determine the phytotoxic or allelopathic influence of different concentrations of leaf extracts and leaf leachates of *E. odoratum* on the correlative changes of growth and metabolism of mung bean seeds.

MATERIAL AND METHODS

The experments were done with seeds of *Vigna radiata* L. cv. K851 (Fabaceae), procured from local seed market of Midnapore town. Healthy leaves of *E. odoratum* L. (Asteraceae) were collected from actively growing populations in Midnapore (21°36' and 22°5' north latitude and between 86°33' and 88°11' east longitude) and its suburbs during the June-August, 2012. The leaves were detached and washed with distilled water to remove the adherent dust particles.

Fresh, mature and healthy leaves (500 g) of *E. odoratum* were thoroughly homogenized using 300 ml double distilled water. The homogenate was strained using a fine cloth and thereafter it was stirred manually for 2.0 min and then filtered through Whatman No. 1 filter paper. The filtrate was made up to 500 ml using double distilled water and this aqueous leaf extract was considered stock solution of 1:1 (w/v) ratio. From this stock solution, 3 concentrations [1:1, 1:2 and 1:3 (w/v)] were prepared using double distilled water, and distilled water was used as control.

Another lot of shade dried 500 g leaf samples of *E. odoratum* was kept immersed in 300 ml double distilled water at room temperature (27 ºC) for 48 h. Thereafter, it was stirred manually for 2.0 min and filtered through Whatman No. 1 filter paper. The total volume of the leachate was made up to 500 ml using double distilled water and this was considered as the 1:1 (w/v) proportion of leaf leachate. From this stock solution, three concentrations [1:1, 1:2 and 1:3 (w/v)] were prepared using double distilled water, and distilled water was used as control.

Mung bean seeds were surface sterilized with 0.1% $HgCl_2$ solution for 90 sec. The seed lots were then separately pre-soaked in the three concentrations of leaf extracts or leaf leachates of *E. odoratum* for 24 h and then thoroughly surface-washed with tap water followed by distilled water. Data on seed germination percentage, T50 value, TTC stainabilty, leaching of free amino acids and soluble carbohydrates, changes in the level of proteins, DNA and RNA, and activities of dehydrogenase and catalase in seeds were recorded.

Germination was recorded 7 days after seed soaking following ISTA rules (1976). The time required for 50% seed germination (T_{50}) was determined by the method of Coolbear et al. (1984). To analyse TTC stainability, 100 dehusked 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 (Halder 1981).

Free amino acids and soluble carbohydrate contents in the seed leachates were analysed after immersing 10 seed samples of mung bean in 10 ml distilled water for 24 h. In these leachates, free amino acids and soluble carbohydrate were quantified as per method of Moore and Stein (1948) and McCready et al. (1950) respectively. To analyse protein from seed kernels we used method of Lowry et al. (1951). Extraction of nucleic acids (DNA and RNA) was done from 100 mg seed kernel as per method of Biswas and Choudhuri (1978). Both DNA and RNA were analysed as per the method of Cherry (1962) modified by Choudhuri and Chatterjee (1970).

The activity of total dehydrogenases of intact seeds was analysed by the reaction of tetrazolium chloride according to the method of Rudrapal and Basu (1979). Extraction and estimation of Catalase enzyme activity was determined following the method of Snell and Snell (1971) modified by Biswas and Choudhuri (1970). In each enzyme assay, value at zero time was taken as blank and the activity of each enzyme was expressed as $[(\Delta A \times Tv)/(t \times \mathbf{v}) \times g$ fr. wt. of tissue], where ΔA is the OD value of blank OD minus sample OD, Tv is the total volume of the filtrate, **t** is the time (hour) of incubation with the substrate and **v** is the volume of filtrate taken for incubation (Fick and Qualset 1975).

All the data were statistically analyzed at the treatment and replication levels (Panse and Sukhatme 1967). In table LSD (least significant difference) values (at 5% level) were incorporated.

RESULTS AND DISCUSSION

The phytotoxic effects of different concentrations of aqueous extracts and leachates from leaves of *E. odoratum* were inhibitory to all parameters *viz.*, seed germination to metabolism of mung bean seeds (Tables 1-4).

Table 1. Effect of seed treatment with leaf extracts and leaf leachates of *Eupatorium odoratum* for 24 h on percentage germination, time (h) to 50% germination (T_{50}) and TTC-stainability of mung bean seeds.

Treatments	Germination (%)	T50	TTC-Stainability (%)
Control	100.00	8.00	100.00
Leaf extract (1:1)	23.33	NA	60.49
Leaf extract (1:2)	46.66	NA	68.30
Leaf extract (1:3)	65.00	36.33	94.00
Leaf leachate (1:1)	71.33	32.25	85.33
Leaf leachate (1:2)	78.33	46.54	89.46
Leaf leachate (1:3)	85.66	51.25	96.50
LSD (P=0.05)	4.01	1.16	5.48

NA: Nonattainment of 50% germination.

Table 2. Effects of seed pre-treatment with leaf extracts and leaf leachates of *Eupatorium odoratum* on the levels of amino acids and soluble carbohydrates analysed from seed leachates of mung bean.

Treatments	soluble carbohydrates (mg/g/10 ml)	Amino acids (mg/g/10 ml)
Control	2.02	0.66
Leaf extract (1:1)	4.24	1.65
Leaf extract (1:2)	3.56	1.12
Leaf extract (1:3)	3.01	0.91
Leaf leachate (1:1)	4.00	1.03
Leaf leachate (1:2)	3.40	0.82
Leaf leachate (1:3)	2.75	0.75
LSD (P=0.05)	0.20	0.07

Table 3. Effects of seed pre-treatment with leaf extracts and leaf leachates of *Eupatorium odoratum* on the levels of proteins, DNA, RNA from seed kernels of mung bean.

Treatments	Proteins (mg/g wet wt.)	DNA (µg/g wet wt.)	RNA (µg/g wet wt.)
Control	116.47	106.44	809.46
Leaf extract (1:1)	35.41	63.02	574.87
Leaf extract (1:2)	40.25	68.99	622.55
Leaf extract (1:3)	48.90	78.40	687.41
Leaf leachate (1:1)	37.55	71.58	600.51
Leaf leachate (1:2)	50.48	89.63	658.44
Leaf leachate (1:3)	65.10	94.22	784.15
LSD (P=0.05)	4.11	5.58	41.65

Table 4. Effects of seed pre-treatment with leaf extracts and leaf leachates of *Eupatorium odoratum* on activities of dehydrogenase and catalase in mung bean seeds.

Treatments	Catalase (unit/h/g wet wt.)	Dehydrogenase (ΔOD/10 ml)
Control	104.26	0.88
Leaf extract (1:1)	56.33	0.21
Leaf extract (1:2)	62.34	0.44
Leaf extract (1:3)	75.84	0.58
Leaf leachate (1:1)	65.14	0.55
Leaf leachate (1:2)	74.79	0.68
Leaf leachate (1:3)	84.15	0.76
LSD (P=0.05)	5.47	0.03

Results revealed that leaf extracts and leaf leachates of *E. odoratum* caused significant inhibition on the germination behaviour of mung bean seeds

(Table 1). This study clearly demonstrated the suppressive effect of *E. odoratum* leaf extracts and leaf leachates on the germination of mung bean seeds. Data further showed that after the treatment of the seeds with leaf extracts and leaf leachates percentage of seed germination and TTC stainability were decreased, whereas the time required for 50% germination (T_{50}) of the seeds was increased (Table 1). The leaf extracts and leaf leachate of high concentration i.e., 1:1 concentration grade treated mung bean seeds could not attain 50% germination. All concentrations of *E. odoratum* leaf extracts and leaf leachates reduced germinability and caused slower rate of germination which are considered to be the important visible and reliable indices for the evaluation of phytotoxic effect. Bhakat et al. (2006) and Maiti et al. (2008) also observed that many bioassay species lose their ability to germinate normally as a result of reduced seed viability. Maximum inhibition was observed in mung bean seeds when pretreated with 1:1 concentration of plant extracts and leachates. This indicates that inhibitory effects of the leaf extracts and leaf leachates were concentration-dependent.

The germination potency of pretreated seeds of mung bean with leaf extracts and leaf leachates can also be determined from the percentage of TTC staining (Table 1). Results showed that the TTC stainability declined steadily with increased concentrations of the plant extracts and leachates. The results are in agreement with those found by (Bhattacharjee et al. 2003, Bhakat et al. 2006, Sodaeizadeh et al. 2009, Anjum et al. 2010, Maiti et al. 2010).

The leaching of amino acids and soluble carbohydrates (Table 2) was higher, when mung bean seeds were treated with leaf extracts and leaf leachates and the magnitude of leaching was less in leaf leachate treatments. Higher the concentrations of leaf extracts and leaf leachates, more was the accumulation of amino acids and soluble carbohydrates in the pre-treated seed samples. Along with the changes associated with reduction of protein, DNA and RNA from pretreated seeds a proportional shift in metabolism of the germinating mung bean seeds was observed in seed kernels and the allelopathic action of the leaf extracts and leaf leachates possibly played a significant role in the deterioration of the germinating seeds. The decline of protein, DNA and RNA (Table 3) as well as activities of the enzymes dehydrogenase and catalase (Table 4) declined in the treated seeds with leaf extracts and leaf leachates for 24 h possibly indicate the allelopathic influence of *E. odoratum*. However, the rates of decrease in activities occurred rapidly in seeds pre-treated with leaf extracts and leaf leachates of *E. odoratum*. The results revealed that higher the concentrations of the leaf extracts and leaf leachates, lower were the enzyme activities (Maiti et al. 2008). The effect of *E. odoratum* leaf extracts was found more inhibitory than that of leaf leachates and the data shows that the more concentrated extracts were more injurious (Maiti et al. 2010). Leaf extracts and leaf leachates of *E. odoratum* containing putative allelochemicals rendered harmful effect on plant growth even under stressful condition. Allelochemical stress drastically inhibited seed germination and metabolism of mung bean. A drastic reduction was encountered when data were recorded after experiencing the inhibitory action rendered by the plant extracts and leachates. Allelochemical stress as imposed by seed pretreatment with leaf extracts and leaf leachates of *E. odoratum* caused an increase in amino acid and soluble carbohydrate and the reduction of insoluble carbohydrate levels in seed kernels of mung bean.

Reports exist in the literature that impairment of seed germination and seedling vigour might be due to imbalance in metabolism and metabolite transport, regulated by various enzyme activities from seeds (Padhy et al. 2000, Maiti et al. 2008). Leaf extracts and leaf leachates mediated stress damage of cell membrane structure might be other factors that augment phytotoxicity to pretreated seeds.

Thus, on the basis of the experimental results on germination behaviour and biochemical changes in seeds pretreated with leaf extracts and leaf leachates it can be assumed that the leaf extracts and leaf leachates of *E. odoratum* rendered adverse effects on mung bean seeds with respect to the physiology and biochemistry of seed germination. Various inhibitors present in plants having phytotoxic property reduced the overall metabolism of plants. Therefore, results showed that both leaf extracts and leaf leachates of *E. odoratum* possess some chemicals which efficiently rendered phytotoxic or allelopathic action on mung bean seeds. The interaction of these chemicals with enzymatic activities might have regulated the energy metabolism and thus consequently resulted in impairment of germination behaviour and metabolism of the test seed samples (Maiti et al. 2010). Thus in the near future this phenomenon would reduce the availability of forest areas and natural resources as well as agricultural products on which people depend. This is a serious concern for biodiversity conservation and human society.

CONCLUSION

Thus, from the overall experimental analysis it can be concluded that various inhibitors present in

E. odoratum can impart strong inhibitory effect on mung bean. The study suggests that the leaves of *E. odoratum* possess phytotoxic or allelopathic chemicals which potentially rendered the inhibitory action on mung bean seeds. Keeping the above in mind, the inhibitory and growth suppressing allelopathic property of *E. odoratum* should be treated as a potential threat to plant diversity, both in natural and man-made ecosystems. The identification of potential allelopathic plant *E. odoratum* can be further subject to isolation and characterization of allelochemicals which can helpful in development of eco-friendly green herbicide.

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