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EVALUTION OF ALLELOPATHIC POTENTIAL OF AN OBNOXIOUS WEED USING MUNG BEAN AS A BIOASSAY MATERIAL

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ABSTRACT
The effect of leaf extracts and leaf leachates of *Lantana camara* L. was studied on the germination behaviour, growth and metabolism of *Vigna radiata* cv. K851 (mung bean). Percentage seed germination, speed of germination, seed viability and field emergence capacity was significantly reduced along with enhancement of T_{50} values of the test species by both leaf extracts and leaf leachates of donor plant. The inhibition of germination behaviour was associated with decreased level of protein as well as activities of catalase and dehydrogenase enzymes, with concomitant increase of amino acids, soluble carbohydrates and amylase activity of mung bean. Both the leaf extracts and leaf leachates showed pronounced inhibition of root, shoot and internode lengths, number of leaves, fresh and dry weight per plant of the test species. The inhibitory effect was strictly concentration-dependant. The inhibition of plant growth was correlated with the decrease in chlorophyll content, level of proteins and the activity of catalase enzyme in leaves. The effect of leaf extracts was found to be pronounced that of leaf leachates. The results of this investigation are suggestive of possible using of the leaf extracts and leaf leachates of *L. camara* as potential agents for formulation of new herbicides and also proper management of *L. camara* as well as other exotic weeds having harmful phytotoxic effects.

KEY WORDS*:* allelopathy, amylase, catalase chlorophyll, dehydrogenase, plant growth, proteins, seed germination, viability.

INTRODUCTION

Phytotoxic secondary metabolites have the potential to mediate interspecific plant-plant interference by reducing or inhibiting competitor's 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 (Bais *et al*. 2003). Allelopathy is believed to be involved in many natural and manipulated ecosystems and play a role in evolution of plant communities (Ridenour and Callaway 2001, Inderjit and Duke 2003). 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 (Inderjit and Duke 2003, Weir *et al.* 2004), and this biotic stress known as allelochemical stress, can have an indirect or direct effect on receiver plant (Cruz-Ortega *et al*. 2002). These allelochemicals have been shown to affect many cellular processes in target plant species, including disruption of membrane permeability (Galindo *et al*. 1999), ion uptake (Lehman and Blum 1999), inhibition of electron transport in both photosynthesis and the respiratory chain (Calera *et al*. 1995, Penuelas *et al*. 1996, Abrahim *et al*. 2000), cause damage to DNA and protein, alterations of some enzymatic activities (Anaya and Pelay-Benavides 1997, Cruz-Ortega *et al*. 1998) and ultimately lead to programmed cell death (Ding *et al*. 2007). Thus, phytochemicals can act as potential instruments for interference and can influence the pattern of vegetation, weed growth and crop productivity (Rice 1948; Foy and Inderjit 2001).

Recently, we have undertaken a comprehensive study on evaluation of allelopathic effect of an abundantly grown exotic weed *L. camara*. *L. camara* leaves contain allelochemicals like phenolic compounds, mono- and sesquiterpenes, triterpenes, triterpenoids, quinines, essential oils, flavonoids, biocides etc. (Raghavan 1976) and these chemicals interfere with various physiobiochemical processes of seed germination, root elongation, plant growth as well as various metabolic activities of many species (Maiti *et al*. 2008). Most of the reports (Chaudhury and Agarwal 2002, Maiti *et al* 2008) are based on the preliminary investigations on some putative allelochemical-induced changes in germination behaviour and growth parameters of target species. However, least attention was given to correlate the changes of growth behaviour with the metabolic status of the test plants.

Thus, in this investigation, experiments were designed to determine the phytotoxic potential of different concentrations of extracts and leachates of *L. camara* leaves and to analyse the correlative changes of growth and metabolism of mung bean.

MATERIALS AND METHODS

Experiments of the present investigation were carried out with fully viable healthy seeds of mung bean (*Vigna radiata* L. cv. K851) as bioassay material. Healthy leaves were collected from actively growing populations of

Lantana camara L. (Verbenaceae) in Midnapore (situated between 21°36/ and 22°5/ north latitude and between 86°33/ and 88°11/ east longitude) and its suburbs during 2005- 2007. The leaves were detached and washed with distilled water to remove the adherent dust particles.

Fresh, mature and healthy leaves (500 g) of this species were thoroughly homogenized using 300 ml double distilled water. The homogenate was strained using a fine cloth and thereafter it was stirred manually for two minutes and then filtrated with the help of Whatman No. 1 filter paper. The filtrate solution was then made up to 500 ml using double distilled water and this was considered 1:1 (w/v) proportion stock solution of the aqueous leaf extract. From this stock solution another concentration of 1:2 (w/v) was prepared using double distilled water. Thus, two concentration grades (1:1 and 1:2) of *L. camara* leaf extracts were used as the test samples for allelopathic studies.

Another lot of shed dried 500 g leaf samples of *L. camara* was kept immersed in 300 ml double distilled water in 1000 ml beaker and kept at room temperature (27°C) for 48 h. Thereafter it was stirred manually for two minutes and filtered through Whatman No. 1 filter paper to prepare aqueous leachate and the leachate was decanted in a separate beaker. The total volume of the leachate was then made up to 500 ml using double distilled water and this was taken as the 1:1 (w/v) proportion of leaf leachate. From this stock solution another concentration grade in the proportion of 1:2 (w/v) was prepared using double distilled water. Thus, two concentration grades (1:1 and 1:2) of leaf leachates were prepared.

Mung bean seeds were surface sterilized with 0.1% $HgCl_2$ solution for 90 sec. The seed lots were then separately presoaked in the three concentrations of leaf extracts or leaf leachates of *L. camara* for 24 h and then thoroughly surface-washed with tap water followed by distilled water. Physiological and biochemical tests were then performed using the pretreated seeds. Data on seed germination percentage, T_{50} value, TTC stinabilty, speed of germination, amino asids, soluble carbohydrates, proteins and activities of dehydrogenase and catalase enzymes 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 as per the method of Coolbear *et al*. (1984). Speed of seed germination was recorded by analysing germination (%) of seed lots of each treatment at 24-h intervals in laboratory up to 120 h after seed soaking as per ISTA (1996). To determine field emergence capacity of seeds, 100 sprouted seeds were sown in the field and after 240 h plumule emerged above the soil surface were counted as field emergence index of germinating seeds (Halder 1981). 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).

Amino acid and soluble carbohydrate levels were analysed from seed kernels presoaked with two concentrations of leaf extracts and leaf leachates or distilled water (control). Extraction and estimation were made as per the method of Moore and Stein (1948) modified by Bhattacharjee (1984) and McCready *et al.* (1950) respectively. Protein was analysed from the seed kernels following the method of Lowry *et al*. (1951). 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 was determined following the method of Snell and Snell (1971) modified by Biswas and Choudhuri (1978). Amylase activity was estimated following the method described by Khan and Faust (1967).

The field experiment was conducted at the research field of Vidyasagar University Farm, Midnapore during the years 2005 and 2007. Data on root, shoot and internodal lengths, number of leaves per plant, fresh and dry weight of plants, protein and chlorophyll contents as well as activity of catalase enzyme in leaves were recorded. Samples for proteins were taken from chlorophyll-free leaf samples as per the method of Kar and Mishra (1976). Methods of extraction and estimation of proteins as well as the activity of catalase enzyme have been already been described. The chlorophyll content was estimated following Arnon's principle (1949). The sample for determining the enzyme activity was taken from uniformly grown leaves of plants, raised from leaf extracts and leaf leachates containing possible allelochemical pretreated seeds at 30-days-old of plant growth.

In each case of enzyme assay, value at zero time was taken as blank, and the activity of each enzyme was expressed as [(**ΔA**×**Tv**)/(**t** × **v**) × **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 in this investigation were statistically analysed at the treatment and replication levels (Panse and Sukhatme 1967). In table LSD (least significant difference) values (at 5% level) were incorporated. In figures SEM (standard error of means) values were used.

RESULTS AND DISCUSSION

The allelopathic effects of different concentrations of aqueous leaf extracts and leaf leachates from leaves of *L. camara* were inhibitory to all parameters *viz.*, seed germination to metabolism of mung bean seeds (Tables 1-5, Figures 1-2). Results revealed that leaf extracts and leaf leachates of *L. camara* caused significant inhibition on the germination behaviour of mung bean seeds (Table 1). This study clearly demonstrated the suppressive effect of *L. camara* 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. All concentrations of *L. camara* 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 allelopathic effect. Similar types of observations were also noted in *Casuarina equisitifolia*, *Ipomoea pes-caprae* (Bhattacharjee *et al*. 2003), and

Eupatorium odoratum (Bhakat *et al*. 2006) leaf extracts and leachates. 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 potential 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 (Table 1) 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) and Maiti *et al* (2008).

It is evident from the present study that the speed of germination (Figure 1) and field emergence capacity (Figure 2) were adversely affected in the seeds pretreated with the test leaf extracts and leaf leachates of *L. camara* in comparison to control. However, this decreasing drift of speed of germination and field emergence was found to be much more prominent in treated sets and the effect was fully concentration dependant. Leaf extract-and leaf leachate-mediated damage of cell membrane structure might be other factors that augment phytotoxicity to pretreated seeds.

Along with the changes associated with reduction of protein and insoluble carbohydrate 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 proteins (Table 2) as well as activities of the enzymes dehydrogenase and catalase (Table 2) declined in the treated seed samples with leaf extracts and leaf leachates for 24 h possibly indicate the allelopathic influence of *L. camara*.

Thus, it can be assumed that the leaf extracts of *L. camara* rendered adverse effects on mung bean seeds with respect to the physiology and biochemistry of seed germination. Earlier reports suggest that relatively permeable membranes of the tonoplast and plasmalemma which normally retain solutes within the cell, lose their integrity and hence, do not act as retentive barrier (Padhy *et al*. 2000). Here the membrane structures might be impaired by the leaf extract and leaf leachate phytotoxins. Possible damage of plasma membrane as a result of seed pretreatment with the leaf extracts and leaf leachates of *L. camara* can be substantiated from the higher leaching of amino acids and soluble carbohydrates from the water imbibed seeds of the present study (Table 3). 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). Along with the changes in leaching of soluble substances 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. Results clearly showed that the levels of proteins as well as activities of the enzymes dehydrogenase and catalase (Table 2) declined in the treated seed samples with leaf extracts and leaf leachates for 24 h. The levels of amino acids and soluble carbohydrates (Table 3) as well as activity of amylase (Table 3) significantly increased in the pretreated seed samples than control ones. The similar type of observation is in agreement with earlier reports (Bhakat *et al* 2006; Maiti *et al* 2008).

The allelopathic potential of *L. camara* can further be substantiated from plant growth and metabolism of mung bean plants. The plant growth performance includes root length, shoot length, internodal length, leaf number, fresh weight and dry weight (Table 5). The biochemical changes include: protein, chlorophyll content as well as activity of catalase enzyme. The growth parameters were significantly reduced in seedlings which were raised from seeds pretreated with leaf extracts and leaf leachates of each concentration. A drastic reduction of proteins and chlorophyll as well as catalase (Table 4) was clearly recorded.

There are numerous reports that dehydrogenase, catalase, amylase and peroxidase enzymes seem to play a vital role during germination and growth (Bhattacharjee *et al.* 2003, Bhakat *et al.* 2006, Isfan and Shariati 2007; Amoo et al. 2008; Maiti *et al* 2008). These are greatly influenced by some putative allelochemicals present in the leaf extracts and leaf leachates of *L. camara*. In our observation these modified physiological processes inhibited and delayed the germination as well as growth of mung bean under the influence of allelochemicals present in leaf extracts and leaf leachates.

This investigation thus concludes that by virtue of its strong inhibitory effect, the invasive and exotic weed *L. camara* has the potential to interrupt regeneration processes of mung bean and other species by decreasing germination, reducing early growth rates, metabolism and selectively increasing mortality. Therefore, as the density of *L. camara* in natural and agricultural ecosystems increases, species richness and crop productivity are likely to be decreased. This study indicates the potential to use allelopathic species suppress the growth of other weeds. It can further be used as a tool to formulate new eco-friendly bio-herbicides to control weeds in agro-ecosystems and natural ecosystems.

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TABLE 1. Effect of seed pretreatment with leaf extracts and leaf leachates of *Lantana* on percentage germination (%), time (h) for 50% germination (T_{50}), TTC stainability (%) and field emergence capacity of mung bean seeds.

Treatment	Germination (%)	T_{50}	TTC stainability (%)	Field emergence capacity (%)
Control	98.0	14.1	100.0	89
Leaf extract(1:1)	86.0	16.9	95.0	66
Leaf extract(1:2)	92.0	15.2	98.1	72
Leaf leachate(1:1)	63.0	58.1	81.7	56
Leaf leachate(1:2)	64.0	38.2	85.3	61
LSD=(P=0.05)	5.77	1.50	4.47	5.61

TABLE 2. Effect of seed pretreatment with leaf extracts and leaf leachates of *Lantana* on the level of proteins and activities of catalase and dehydrogenase in mung bean seeds.

Treatment	Proteins mg / g wet wt.	Catalase Unit/min/g wet. wt.	Dehydrogenase OD/10 seeds/5ml
Control	17.60	6.00	0.20
Leaf extract (1:1)	11.20	5.05	0.15
Leaf extract (1:2)	11.80	5.80	0.17
Leaf leachate (1:1)	09.20	2.42	0.07
Leaf leachate (1:2)	10.20	2.90	0.10
LSD (P = 0.05)	0.91	0.24	0.05

TABLE 3. Effect of seed pretreatment with leaf extracts and leaf leachates of *Lantana* on the level of soluble carbohydrates, amino acids and activity of amylase in mung bean seeds.

Treatment	Soluble carbohydrates mg/g wet wt.	Amino acids mg/g wet wt.	Amylase Unit/min/g wet wt.
Control	3.00	1.40	1.00
Leaf extract (1:1)	4.89	4.10	2.00
Leaf extract (1:2)	3.25	2.40	1.70
Leaf leachate (1:1)	8.25	5.45	4.42
Leaf leachate (1:2)	6.30	4.95	3.90
LSD (P = 0.05)	0.31	0.14	0.11

TABLE 4. Effect of seed pretreatment with leaf extracts and leaf leachates of *Lantana* on chlorophyll content, level of proteins and activity of catalase in leaves of 30 days old mung bean plant.

Treatments	Chlorophyll (mg/g wet wt.)	Protein (mg/g wet wt.)	Catalase (unit/min/g wet wt.)
Control	1.68	5.60	5.80
Leaf extract (1:1)	0.81	4.60	4.96
Leaf extract (1:2)	0.86	4.96	5.27
Leaf leachate (1:1)	0.45	3.52	3.44
Leaf leachate (1:2)	0.57	4.00	4.31
LSD (P=0.05)	0.06	0.36	0.35

TABLE 5. Effect of seed pretreatment with leaf extracts and leaf leachates of *Lantana* on root length, shoot length, internodal length, number of leaves per plant, fresh weight, dry weight of 30 days old mung bean plant.

Treatments	Root Length (cm)	Shoot Length (cm)	Internodal length (cm)	No. of leaves per plant	Fresh weight (g)	Dry weight (g)
Control	36.25	75.75	6.75	24.25	147.50	51.25
Leaf extract (1:1)	25.53	65.75	4.44	14.66	88.75	26.33
Leaf extract (1:2)	29.01	67.00	6.03	19.75	90.00	32.80
Leaf leachate (1:1)	20.05	54.50	3.42	11.50	68.22	20.52
Leaf leachate (1:2)	22.00	61.25	3.70	12.50	75.01	23.33
LSD (P=0.05)	1.98	5.51	0.34	1.16	6.75	2.06

FIGURE 1. Effect of seed pretreatment with leaf extracts of *Lantana* on speed of germination of mung bean seed.

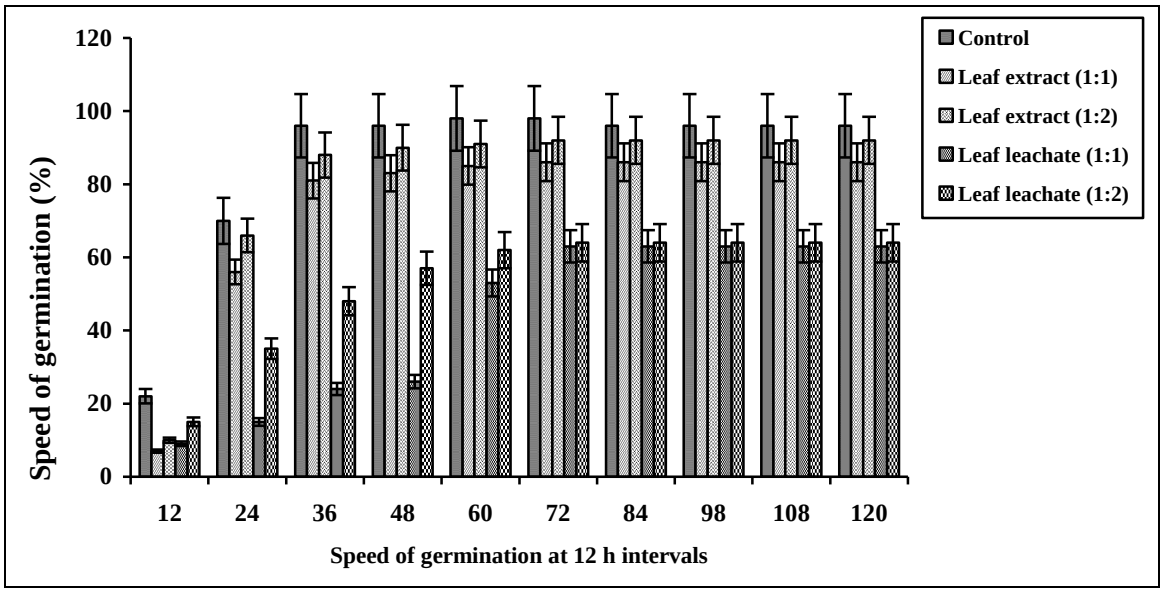

FIGURE 2. Effect of seed pretreatment with leaf extracts of *Lantana* on changes of field emergence capacity of mung bean seeds.