



ALLELOPATHIC EFFECTS OF *CHROMOLAENA* AND *LANTANA* LEAF EXTRACT ON MUNG BEAN SEEDS

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ABSTRACT

Among the natural or man guided agro-ecosystems, surrounding plants may interact with the growth and improvement of other species. The compounds (allelochemicals) connected in interspecific chemical interactions (allelopathy) with higher plants are often phytotoxic a herbicidal to other species or even to the species producing them (autotoxicity). The purpose of this investigation is to screen out the phototoxicity of the leaf extracts of *Chromolaena odorata* and *Lantana camara* on mung bean (*Vigna radiata* L. cv. K 851), measured in terms of seed germination, plant growth and some feasible biochemical indices.

Modern study exhibition that seed pretreatment of mung bean with several concentrations [1:1 and 1:2 (w/v)] of *Chromolaena* and *Lantana* leaf extracts for 24 hours duration reduced percentage germination. Levels of DNA and RNA were significantly reduced with concomitant increase of amylase activity in seed samples pretreated with leaf extracts of *Chromolaena* and *Lantana*. Decreased plant potential in the plant extract-pretreated samples was evidenced from the lower level of chlorophyll, DNA and RNA as well as higher level of amylase activity. Leaf extract-induced reduced germinability and influenced activity of amylase enzyme being the important allelopathic indices, it can be concluded that *Chromolaena* and *Lantana* can potentially render allelopathic action on mung bean. Consequently, this study calls for the proper monitoring of *Chromolaena* and *Lantana* and other invasive weeds showing similar behaviour.

KEY WORDS

Allelopathy, Chlorophyll, *Chromolaena*, Germination, *Lantana*, Nucleic acid

1. INTRODUCTION

The interference in the growth of one plant by another can result either from competition which involves the removal of some factors (nutrient, water and light) from the environment, habitat or through chemical (s) released from one plant (donor) that effect to other (receiver) sharing the habitat. The phenomenon known as "allelopathy" is now considered as important as competition for influencing plant growth both in natural and agricultural ecosystem. In natural or man managed

agro ecosystems, neighbouring plants may interact with the growth and development of other species. The term allelopathy signifies that interacting or inhibition of growth [1] both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization residue decomposition and other processes. These interactions are widely known in different groups of plants such as algae, lichens, crops, as well as annual and perennial weeds [2-12]. There is much evidence that allelochemicals liberated from

certain weeds into the soil reduce crop growth [13-20]. Approximately 6700 species, out of about 3,000,000 species of the flowering plants are recorded as weeds in agro ecosystems of the world [21] of these, 76 weed species are categorized as “the World’s Worst Weeds” [22]. Only 15 species of the crops which supply 90% of the world’s food occupy 75% of the world’s tilled land [23]. Most of the food species belong to five families viz., Poaceae, Solanaceae, Convolvulaceae, Euphorbiaceae and Fabaceae and these families also include most of the common weeds. In crop subsystems of agroecosystem, crop species (often exotic) are selectively cultivated but weeds grow themselves in crop fields and interact with the crop species in various ways, including reduction in crop yields. Thus, for economic gain, weed control measures become inevitable which increase labour requirements and expenditure for weed control. Hence, various methods of weed controls such as cultural, mechanical and chemical have been developed. Most of the synthetic herbicides are non-biodegradable and hence cause, soil and water pollution. The development of biodegradable herbicides and biological control method needs an understanding of crop-weed interactions *in vivo* and *in vitro*, especially with reference to chemical interaction (allelopathy), which may help to keep the interfering under reasonable control. Total eradication of weeds for ever is not possible. Therefore, a sound understanding is needed of the behavior of crop and weed plants growing together in association and sharing the common resources (nutrients and water) in agroecosystems. These aspects of the crop weed interference viz., allelospoly and allelopathy are considered here with reference restricted mostly for Indian crops and weeds.

Allelopathy is also an expression of the ecological phenomena which are normal constituents of the terrestrial plants [24, 2]. There are some common indices for assessing allelopathic action of plants or plant parts. These include, among others, germination behavior and other physio-biochemical responses of test species [25, 19, 18, 26, 27]. With considerable evidences adduced during the past few decades demonstrating the presence of inhibitory compounds in a wide variety of plant types and plant parts, the recent upsurge of interest in allelopathy, with major volumes of collected papers and books regularly published [2, 28, 29, 30, 31, 32] has established the topic as one of biological significance. Recently it is focused on

establishing research procedures which may improve the credibility of evaluations of the allelopathic potential of exotic weeds *Eupatorium odoratum* and *Lantana camara* which have become invasive and forms monospecific stands in different ecosystems in West Bengal [27]. *Eupatorium* and *Lantana* are the serious weeds in 47 countries [22] owing to its wide adaptability to different environmental conditions and habitats. Limited research has been done on the allelopathic effect of phytotoxicity of *Eupatorium* and *Lantana* to other plants. There is a general mood of consensus now-a-days that invasive plants displace the local biodiversity through their harmful effects including allelopathy [33, 34, 25, 20]. In fact, allelopathic action of any plants and parts affects germination behavior, seed metabolism and growth performance of target species which in turn may discourage a species from thriving, thus influencing the whole structure in course of time [35].

Therefore, it was presumed that perhaps an allelopathic effect of *Chromolaena* and *Lantana* on mung bean may be responsible for the inhibitory effect on seed germination behavior, seed metabolism and growth performance of target crop. Allelopathic effects may be due to the presence of allelochemicals in *Chromolaena odorata*, like different types of phenolic compounds, alkaloids eupatorine, eupatorene, odoramine etc. in *Lantana camara* like phenolic compounds (protocatechuic acid, gentisic acid, p-hydroxy benzoic acid, vanillic acid, caffeic acid, syringic acids, vanillin, p-coumaric acid, m-coumaric acid, ferulic acid, salicylic acid, o-coumaric acid, t-cinnamic acid, methyl coumarin), triterpenoids (lantadene A, lantadene B, icterogenin reduced lantadene A, reduce lantadene B, lantadene C, oleanolic acid, oleanonic acid, ursolic acid, ursonic acid, 4-epihederagonic acid lantadene D, lancamarone, lantanolic acid, lantic acid, lantanolic acid, betulonic acid, betulinic acid, lantabutulinic acid) essential oils (phellandral, β -cymene, α -phellandrene, dipentene-1- tarpinene, caryophyllene, cadinene, cineol, linalool, geraniol, A-terpimeol, citral) and flavonoids (umuhengerin, 5-hydroxy-6, 6, 3, 4, 5-pentamethoxyflavone), biocides (active principles have not been characterized), Juvenile hormones (active principles have not been characterized), growth hormones (gibberellins GA₃ like substances). They may be interacting various physiological processes. Therefore, studies were conducted to test this hypothesis and laboratory experiments have confirmed this.

The objectives of this paper are to share our experiences in allelopathy research conducted in our University research field and to propose a standard protocol for biological assessments (bioassays) of some allelopathic potential exotic and invasive weeds and crop.

2. MATERIALS AND METHODS:

Fresh, mature leaves (500 g each) of *Chromolaena odorata* (L.) Robins. (Family- Asteraceae) and *Lantana camara* L. (Family-Verbenaceae) were collected from Vidyasagar University campus, Paschim Medinipur, West Bengal, India; were thoroughly homogenized using 300 ml double distilled water. The homogenate was strained using a fine cloth and then centrifuged at 5000 g for 15 minutes. The supernatant was then made up to 500 ml using double distilled water and this was considered 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 double distilled water. And this was taken as the two gradation leaf extract solutions. Fully viable mung bean (*Vigna radiate* L. cv. K-851) seeds in five lots of 25 g each were surface sterilized with 0.1% HgCl₂ solution for 90 seconds. The seed lots were then separately presoaked in the two concentration grade leaf extracts and for control, seeds presoaked in double distilled water for 24 hours and thus allowed the seeds for various biochemical tests. Data on seed germination percentage, DNA and RNA levels and activity of amylase enzyme in seeds were recorded. Chlorophyll, DNA and RNA contents as well as amylase activity were recorded from 10 uniformly growing 30 days old plants raised from each leaf extract treated seeds. The plants were grown in Vidyasagar University research field.

To analyse percentage germination from continuous treatment sets, three groups of 100 fresh seeds (i.e. 300 fresh seeds) were transferred to separate Petri dishes containing filter paper moistened with 10 ml each of leaf extracts and distilled water for control. Germination data were recorded after 120 h of seed soaking following the International Rules of Seed Testing [36].

DNA and RNA levels were analyzed as per the method described by [37] modified by Choudhuri and Chatterjee, [38]. Extraction and estimation of the enzyme amylase was done as per the method described by Khan and Faust [39]. For the assay of this enzyme the blank was taken as zero-time control. The activity of this enzyme was expressed as $[(\Delta A \times T_v)/(t \times v)]$, where ΔA

is the absorbance of the sample after incubation minus the absorbance of the zero time control, T_v is the total volume of the filtrate, t is the time (minutes) of incubation with the substrate and v is the volume of the filtrate taken for incubation [40]. Chlorophyll level was analysed from the leaves of 30 days old plants of each treatment following the method of [41].

All the data were statistically analysed at the treatment and replication levels and least significant difference (LSD) values were calculated at 95% confidence limits [42].

3. RESULTS:

Effect on germination percentage and changes of amylase activity in seed kernels (Table-1).

Data clearly revealed that percentage germination of mung bean seeds were strongly inhibited by continuous treatment with two concentration grades of leaf extracts of *Chromolaena* and *Lantana*. The allelopathic effect of *Chromolaena* leaf extracts was more inhibitory than *Lantana* and the data shows that the more concentrated extracts were more injurious.

Amylase activity was increased in seed samples irrespective of treatments with two concentration grades leaf extracts of *Chromolaena* and *Lantana*.

Effect on changes of DNA and RNA levels in seed kernels (Table-2).

Treatments of the mung bean seeds with leaf extracts of all types could alter gross DNA level of the seeds. Here, both leaf extracts of *Chromolaena* and *Lantana* significantly decreased the DNA content of seeds which clearly established the allelopathic potentiality of *Chromolaena* and *Lantana*.

Leaf extracts of *Chromolaena* and *Lantana* significantly reduced RNA level in seed kernels. Effect on changes of chlorophyll level and amylase activity in leaves of plants (Table-3).

Allelochemicals remarkable reduced the level of chlorophyll in leaves of mung bean plants raised from seeds pretreated with leaf extracts of all concentration. Activity of the enzyme amylase was found to be increase in leaves of mung bean plants. Here, allelopathic effect of *Chromolaena odorata* was more than *Lantana camara* and the more concentrated leaf extractions was more inhibitor.

Effect on changes of DNA and RNA levels in leaves of plants (Table-4).

Data shows that the pretreating agents significantly decreased the DNA content in leaves of mung bean plant.

The same result was noted like DNA level. Here, more concentration grade i.e. 1:1 was showed more inhibitory effect than control. Both leaf extract of *Chromolaena* strongly inhibited the RNA levels of mung bean plants.

4. DISCUSSION:

The present study shows that continuous treatment of mung bean seeds with leaf extracts of *Chromolaena odorata* and *Lantana camara* reduced seed germinability and influenced amylase activity (Table – 1), decreased DNA and RNA levels (Table – 2).

Analysis of germination behavior is considered to be a reliable index of evaluation of allelopathic action [25, 43, 44, 45, 46, 47]. Reduced germinability is important effects of allelopathic action of plants and such action is chiefly exerted by a number of inhibitors of diverse chemical nature [35]. In this investigation the leaf extracts induced inhibition of percentage seed germination was noted significantly higher than control

and these are clear indicative of the allelopathic action of the test material. The relatively high allelopathic potential of *Chromolaena* and *Lantana* were recorded from its stronger germination inhibitory capacity. On the other hand, more, concentrated leaf extracts were more injurious than more diluted extract solutions. More concentrated plant extracts have more inhibitory compounds. Allelopathic action of *Chromolaena* and *Lantana* can also be corroborated from the present data on the leaf extract-induced reduction of DNA and RNA levels as well as increased the activity of amylase enzyme. Various inhibitors present in plants having allelopathic property reduced the overall metabolism of plants or plant parts are reported to be strongly impaired [48, 43]. Results, therefore, point out that both leaf extracts of *Eupatorium odoratum* and *Lantana camara* possesses some chemicals which efficiently rendered allelopathic action on mung bean seeds. Reduced plant growth and slowed rate of plants establishment are also convincing evidence of allelopathic action [48, 25, 9, 49, 11]. Allelopathic potential of *Chromolaena* and *Lantana* plants can reduce plant metabolism like chlorophyll (Table – 3) DNA and RNA (Table – 4) levels as well as increased amylase (Table – 3) activity in leaves.

Table-1. Effect of seed pretreatment with leaf extracts of *Chromolaena* and *Lantana* on percentage germination and amylase activity of mung bean seeds.

Treatments	Germination %	Amylase (unit/h/g fr.wt.)
Control	100.00	11.23
Eupatorium leaf extract (1:1)	28.00	28.30
Eupatorium leaf extract (1:2)	32.00	27.10
Lantana leaf extract (1:1)	46.00	21.50
Lantana leaf extract (1:2)	48.00	19.70
LSD ($P=0.05$)	2.55	0.95

Table-2. Effect of seed pretreatment with leaf extracts of *Chromolaena* and *Lantana* on DNA and RNA level in kernels of mung bean seeds.

Treatments	DNA ($\mu\text{g/g fr.wt.}$)	RNA ($\mu\text{g/g fr.wt.}$)
Control	38.21	161.33
Eupatorium leaf extract (1:1)	15.95	80.21
Eupatorium leaf extract (1:2)	17.22	88.55
Lantana leaf extract (1:1)	22.40	115.29
Lantana leaf extract (1:2)	26.34	121.75
LSD ($P=0.05$)	1.56	6.23

Table-3 Effect of seed pretreatment with leaf extracts of *Chromolaena* and *Lantana* on changes of chlorophyll content and amylase activity in leaves of mung bean seeds.

Treatments	Chlorophyll (mg/g fresh weight)	Amylase (unit/h/g fr.wt.)
Control	1.41	7.85
Eupatorium leaf extract (1:1)	0.70	19.55
Eupatorium leaf extract (1:2)	0.81	16.21
Lantana leaf extract (1:1)	1.05	11.37
Lantana leaf extract (1:2)	1.13	10.21
LSD ($P=0.05$)	0.06	1.55

Table-4 Effect of seed pretreatment with leaf extracts of *Chromolaena* and *Lantana* on changes of DNA and RNA content in leaves of mung bean seeds.

Treatments	DNA ($\mu\text{g/g fr. wt.}$)	RNA ($\mu\text{g/g fr. wt.}$)
Control	35.67	132.62
Eupatorium leaf extract (1:1)	12.22	60.55
Eupatorium leaf extract (1:2)	13.75	67.39
Lantana leaf extract (1:1)	18.99	90.11
Lantana leaf extract (1:2)	21.34	96.27
LSD ($P=0.05$)	1.15	5.76

5. CONCLUSION:

Results, shows that the leaves of *Chromolaena odorata* and *Lantana camara* possesses allelochemicals, which efficiently impart allelopathic action on the present bioassay material. It further reemphasises the fact that fast growing exotic invasive weeds like *Chromolaena* and *Lantana* having growth-suppressing property should be treated as a potential threat to plant biodiversity in a natural ecosystem. Therefore, this study calls for the proper management of *Chromolaena* and *Lantana* or other invasive weeds showing similar behavior.

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