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ALLELOPATHIC POTENTIAL OF LEAF LEACHATES OF CHROMOLAENA AND LANTANA ON MUNG BEAN (*VIGNA RADIATA* L. CV. K-851) SEEDS

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ABSTRACT: Chromolaena and Lantana plants were analyzed to evaluate the existence of allelopathic effect using fully viable seeds of mung bean as the bioassay material. The study shows that pretreatment of mung bean seeds with various concentrations [1:1 and 1:2 (w/v)] of Chromolaena and Lantana leaf leachate for 24 h interval reduced the percentage germination. DNA and RNA contents were significantly reduced with a remarkable increase of amylase activity in seed samples pretreated with leaf leachate of Chromolaena and Lantana. The seedling performance was found to be much poor when the seedlings were raised from seeds which underwent pretreatment with the plant leachates. Evidenced from the lower levels of chlorophyll, DNA and RNA as well as a higher level of amylase activity in leaves of seedlings. Leaf leachate causes the reduction of 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. It further reemphasizes the fact that fast-growing exotic invasive weeds like Chromolaena and Lantana having inhibiting property should be treated as a potential threat to plant diversity in a natural ecosystem.

INTRODUCTION: The growth differentiation of plants by other plants could be due to external environmental factors like height, temperature, air water, habitat as same times due to the secretion of chemicals that may act as a growth inhibitor of plants. Here the donor is benefitted, and the receiver is affected. In a recent system of agricultural land and crop management, not only competition but also allelopathy plays as important role in natural and managed maintained ecosystem.

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The term allelopathy 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 [1]. These type of chemical signaling or interactions are widely known in different groups of plants ranging from algae, lichens, and crops as well as annual and perennial weeds [2, 3, 4].

There is a huge number of examples that allelochemicals liberated from certain weeds into the soil reduce crop growth and development cause economic loss [5]. Approximately 6700 species, out of about 3,000,000 species of the flowering plants are classified as weeds in agroecosystems of the whole world [6] of these, 76 weed species are specially categorized as "the World's Worst Weeds" [7].

Only 15 species of the crops which supply 90% of the world's food occupy 75% of the world's agricultural land [8]. Most of the food species belong to five families *viz.*, Poaceae, Solanaceae, Convolvulaceae, Euphorbiaceae and Fabaceae, and all of these families also include most of the common weeds. In crop subsystems of the agroecosystem, crop species (often exotic) are selectively cultivated, but weeds grow themselves in crop fields without any nourishment and interact with the economically beneficial crop species in various ways, including a sharp reduction in crop yields. Thus, for economic gain, weed control measures become inevitable, which increase labor requirements and cost for weed control.

Hence, various multiple methods of weed controls, such as cultural, mechanical, and chemical have been developed and employed rapidly. Most of the synthetic herbicides are non-biodegradable, and hence cause, soil and water pollution remains in the environment for a long time by biomagnifications or other means. The development of biodegradable and environment freely herbicides and biological control method need a better understanding of crop-weed interactions in both *in-vivo* and *in-vitro* conditions especially concerning chemical interaction (allelopathy), which may help to keep the interference under reasonable control. Complete weed eradication 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 agro ecosystems. 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 an ecological phenomenon which are normal constituents of the terrestrial plants [9, 10] and is very common and frequent. These include, among others, germination behavior and other physio-biochemical responses of test species [11, 12, 13, 14, 15, 16]. 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, books, journals regularly published [17, 18, 19] has established the topic as one of biological importance. In recent times it is focused on establishing research procedures which may improve the importance of evaluations of the allelopathic potential of exotic nonnative weeds *Chromolaena odorata* and *Lantana camara* which have become invasive and forms monospecific stands in different ecosystems in West Bengal and surroundings [20]. Chromolaena and Lantana are known to be serious weeds in 47 countries owing to its wide adaptability to different environmental conditions and habitats [7].

A very little pioneer research has been done on the allelopathic effect of phytotoxicity of Chromolaena and Lantana to other plants. There is a general motion nowadays that invasive plants displace the local and natural endemic biodiversity through their detrimental effects, including allelopathy [21, 22]. Allelopathic action of any plants and parts affects germination behavior, seed metabolism and growth performance of target species which in turn may disturb a species from thriving, thus affecting the complete structure in a long time [23].

Allelopathic effects may be due to the presence of allelochemicals in *Chromolaena odorata*, like different types of phenolic compounds, alkaloids eupatorine, eupatoredene, odoratamine, *etc*. In *Lantana camara* 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 acidlantadene D, lancamarome, lantanolic acid, lantic acid, lantanalic acid, betulonioc acid, betulic acid, lantabutulic acid) essential oils (phellandral, β-cymene, α-phell-andrene, dipentene-1- tarpinene, caryophyllene, candinene, cineol, linallool, geraniol, A-terpimeol, citral) and flavonoids (umuhengerin, 5-hydroxy-6, 6, 3, 4, 5-pentametho-xyflavone), biocides (active principles have not been characterized), Juvenile hormones (active primitives have not been characterized), growth hormones (gibberelline GA_3 like substances) are present that may acts as allelochemicals.

They may be interacting with various physiological processes and may hamper several biochemical processes. Therefore, studies were conducted to test this hypothesis, and laboratory experiments have confirmed this. Finally, the experiment concludes and summarises about the allelopathic potency of Chromolaena and Lantana leaf leachates on mung bean seeds that can elucidate a proposal regarding establishment of the standard protocol for biological assessment of the allelopathic potential of some exotic and invasive species which are generally weeds and crops.

MATERIALS AND METHODS: Fresh, mature leaves of *Chromolaena odorata* (L.) R. M. King & H. Rob. (Asteraceae) and *Lantana camara* L. (Verbenaceae) [Authentication number, SK-05, and SK-02, CNH/Tech.II/2019/14, Dated: 28/03/2019] were collected from Vidyasagar University campus, Paschim Medinipur, West Bengal, India; were thoroughly sundried for 15 days. The dry leaves (500 g each) samples of the Chromolaena and Lantana were kept separately in 300 ml double distilled water in 1000 ml beaker for 48 h, and the leachate was decanted in a separate beaker. The total volume of the leachate was then made up to 500 ml volume 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. And this was taken as the two gradation leaf leachate solutions.

Five lots of viable mung bean (*Vigna radiata* L. cv. K-851) seeds of 25g were surface sterilized with 0.1% $HgCl_2$ solution for 90 seconds. The seed lots were then separately presoaked in the two concentration grade leaf leachates and for control. Data on seed germination percentage, DNA and RNA levels, and activity of amylase enzyme in seeds were tested. Chlorophyll, DNA and RNA contents, as well as amylase activity, were recorded from 10 uniformly growing 30 days old plants raised from each leaf leachate treated seeds. The plants were grown in Vidyasagar University research field for these analyses. The percentage of seed germination can be analyzed 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 leachates and distilled water for control. Germination data were recorded after 120 h of seed soaking following the International Rules of Seed Testing [24].

DNA and RNA levels were analyzed as per the method described by Cherry [25] modified by Choudhuri and Chatterjee [26]. Extraction and estimation of the enzyme amylase were done as per the method described by Khan and Faust [27]. For the assay of this enzyme, the blank was taken as zero time control. The activity of this enzyme was expressed as $[(\Delta A \times Tv) / (t \times v)]$, where ΔA is the absorbance of the sample after incubation minus the absorbance of the zero time control, Tv 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 Fick and Qualset [28]. Chlorophyll level was analyzed from the leaves of 30 days old plants of each treatment following the method of Arnon [29]. All the data were statistically analyzed after treatment and replication levels, and least significant difference (LSD) values were calculated at 95% confidence limits Panse and Sukhatme [30].

RESULTS:

Effect on Germination Percentage and Changes of Amylase Activity in Mung Bean Seed Kernels Table 1: Data indicates that percentage germination of mung bean seeds was strongly inhibited by continuous treatment with two concentration grades of leaf leachate of Chromolaena and Lantana. The allelopathic effect of Chromolaena leaf leachate was more inhibitory than Lantana, and the data shows that the more concentrated leachate was more injurious. Amylase activity was increased in seed samples irrespective of treatments with two concentration grades leaf leachate of Chromolaena and Lantana.

Effect on Changes of DNA and RNA Levels in Mung Bean Seed Kernels Table 2: Treatments of the mung bean seeds with leaf leachate of all types could alter gross DNA level of the seeds. Here, both leaf leachate of Chromolaena and Lantana significantly decreased the DNA content of seeds which established the allelopathic potentiality of Chromolaena and Lantana. Leaf leachates of Chromolaena and Lantana significantly reduced RNA level in seed kernels.

Effect on Changes of Chlorophyll Level and Amylase Activity in Leaves of Mung Bean Plants Table 3: Allelochemicals remarkably reduced the level of chlorophyll in leaves of mung bean plants raised from seeds pretreated with leaf leachate of all concentration. The activity of the enzyme amylase was found to increase in leaves of mung bean plants. Here, the allelopathic effect of Chromolaena was more than Lantana, and the more concentrated leaf leachates were the best inhibitor.

Effect on Changes of DNA and RNA Levels in Leaves of Mung Bean Plants Table 4: Data reveals that the pretreating agents significantly decreased the DNA content in leaves of the mung bean plant. The same result was in case of RNA level. Here, more concentration grade, *i.e.* 1:1 was showed more inhibitory effect than control. Both leaf leachate of Chromolaena strongly inhibited the RNA levels of mung bean plants.

TABLE 1: EFFECT OF SEED PRETREATMENT WITH LEAF LEACHATES 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	10.10
Chromolaena leaf leachate (1:1)	35.00	23.5
Chromolaena leaf leachate (1:2)	40.00	22.9
Lantana leaf leachate (1:1)	52.00	14.1
Lantana leaf leachate (1:2)	60.00	11.0
LSD (P=0.05)	3.45	0.91

TABLE 2: EFFECT OF SEED PRETREATMENT WITH LEAF LEACHATES OF CHROMOLAENA AND LANTANA ON DNA AND RNA LEVEL IN KERNELS OF MUNG BEAN SEEDS

Treatments	DNA (μg/g fr.wt.)	RNA (μg/g fr.wt.)
Control	40.32	156.81
Chromolaena leaf leachate (1:1)	21.54	96.32
Chromolaena leaf leachate (1:2)	25.73	105.81
Lantana leaf leachate (1:1)	30.15	129.33
Lantana leaf leachate (1:2)	34.61	135.71
LSD (P=*0.05*)	2.09	8.73

TABLE 3: EFFECT OF SEED PRETREATMENT WITH LEAF LEACHATES OF CHROMOLAENA AND LANTANA ON CHANGES OF CHLOROPHYLL CONTENT AND AMYLASE ACTIVITY IN LEAVES OF MUNG BEAN PLANTS

Treatments	Chlorophyll (mg/g fr.wt.)	Amylase (unit/h/g fr.wt.)
Control	1.35	7.41
Chromolaena leaf leachate (1:1)	0.88	14.66
Chromolaena leaf leachate (1:2)	0.97	12.89
Lantana leaf leachate (1:1)	1.20	9.19
Lantana leaf leachate (1:2)	1.28	8.22
LSD (P=*0.05*)	0.08	1.36

TABLE 4: EFFECT OF SEED PRETREATMENT WITH LEAF LEACHATES OF CHROMOLAENA AND LANTANA ON CHANGES OF DNA AND RNA CONTENT IN LEAVES OF MUNG BEAN PLANTS

Treatments	DNA (μg/g fr. wt.)	RNA (μg/g fr. wt.)
Control	33.56	125.37
Chromolaena leaf leachate (1:1)	18.15	76.71
Chromolaena leaf leachate (1:2)	21.20	87.22
Lantana leaf leachate (1:1)	25.71	110.25
Lantana leaf leachate (1:2)	28.19	118.75
LSD (P=*0.05*)	1.69	6.62

DISCUSSION: At present, the investigation shows that random treatment of mung bean seeds with leaf leachate of Chromolaena and Lantana sharply reduced seed germinability and also influenced amylase activity **Table 1**, decreased DNA and RNA levels **Table 2**.

Germination behavior of seed is considered to be a reliable index of evaluation of allelopathic action [31, 32]. Reduced germinability is a very important effect of allelopathic action of plants, and such action is chiefly exerted by several inhibitors of diverse chemical nature [33].

In this investigation, the leaf leachate induced inhibition of the percentage of seed germination was noted significantly higher than control, and there is clear indications of the allelopathic action of the test materials. The relatively high allelopathic potential of Chromolaena and Lantana were recorded from its stronger germination inhibitory capacity. On the other hand, more concentrated leaf leachate was more injurious than more diluted leachate solutions. More concentrated plant leachate has more inhibitory compounds. Allelopathic action of Chromolaena and Lantana can also be correlated from the present data on the leaf leachate-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, and they are reported to be strongly impaired [34, 35].

Results, therefore, point out that both leaf leachate of Eupatorium and Lantana 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 [36, 37]. The leaf leachates of Chromolaena and Lantana plants show allelopathic potency, which can reduce plant metabolism like chlorophyll **Table 3** DNA and RNA **Table 4** contents as well as increases the amylase **Table 3** activity.

CONCLUSION: The leaves of Chromolaena and Lantana possesses allelochemicals, which efficiently impair allelopathic action on the present bioassay material. It further reemphasizes 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, the present study calls for the proper management of Chromolaena and Lantana or other invasive weeds showing similar activity. So, it is high time that more investigation should be done regarding the invasion of plants like weed species; *Chromolaena odorata* and *Lantana camara* and their irreversible detrimental effect on economically important plants like mung bean plants.

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CONFLICT OF INTEREST: Nil

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