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ASSESSMENT OF ALLELOPATHIC POTENTIAL OF *CHROMOLAENA ODORATA* (L.) KING AND ROBINSON BY PHYSIOBIOCHEMICAL APPROACH

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ABSTRACT: Use of herbicides and allied chemicals for crop protection negatively influence health, productivity and quality of crops. Hence, in recent years scientists are in favour of bioherbicide formulation which is a potent approach for crop health management. Keeping this in mind an experiment was designed to identify plants having allelopathic potential which are considered as ideal candidates for ecofriendly bioherbicide formulation. In this communication the assessment of allelopathic efficacy of an invasive and exotic weed *Chromolaena odorata* was done by physiobiochemical approach using viable seeds of grass pea (*Lathyrus sativus*) as a bioassay material. Pretreatment with different concentrations of leaf extracts and leaf leachates of *Chromolaena* reduced percentage germination and remarkably extended the time required for 50% germination (T_{50}) of the treated seed samples. Significant reduction of fresh weight and dry weight as well as root and shoot length of 30 days old *Lathyrus* plants were recorded in plant samples, raised from pretreated seeds. The leaf extracts and leaf leachates of *Chromolaena* significantly increased leaching of soluble carbohydrates and free amino acids from *Lathyrus* seeds. Conversely, both protein and chlorophyll contents were reduced in the leaves of 30 days old *Lathyrus* plants, raised from *Lathyrus* seed samples pretreated with the extracts and leachates of *Chromolaena* leaves and the effect was found to be concentration dependent. The catalase and peroxidase activities of grass pea seeds were found decreased after treatment with the different concentrations of *Chromolaena* leaf extract and leaf leachate.

KEYWORDS: Allelopathic potential, *Chromolaena odorata,* physiobiochemical parameters, *Lathyrus sativus,* bioassay material.

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1.INTRODUCTION

International Allelopathy Society defined allelopathy as "Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects"[1].Chemicals released from plants and imposing allelopathic influences are called allelochemicals, most of which are secondary metabolites. There is a general mode of consensus now-a-days that invasive plants displace the local biodiversity through their harmful effects including allelopathy [2,3]. In fact, allelopathic action of any plant or plant parts negatively affects seed germination behavior, seed metabolism and growth performance of target species and influences the ecological diversity of a certain place [4,5,6].*Chromolaena odorata* belonging to the family Asteraceae is native to Central and South America and is now widely distributed in most tropical and subtropical regions of Africa, Asia, Australia and the West Pacific islands [7]. This perennial shrub is one of the world's 100 worst invasive alien species that threatens agriculture and the environment in Central and Western Africa, tropical America, India, Philippines, southern China, South Africa, eastern Indonesia, and Australia [8,9]. *C. odorata* can quickly establish and often form a dense population due its high reproductive capacity, high relative growth rate, through symbiosis with VAM and outcompeting with native plants for light and nutrition [10,11,12,13]. Phenols and alkaloids are the major allelochemicals found in plants that exert the inhibitory effect on seed germination and early seedling growth [14]. There are numerous reports that *C. odorata* contains such allelochemicals like phenols, flavonoids, tannins, alkaloids, steroids etc. [15,16,17].*C. odorata* grows profusely in their natural habitat and can threaten local native species diversity. Keeping this in mind an attempt was made to assess the allelopathic potential of *C. odorata* by some reliable physiological and biochemical indices. Thus, the prime objective of the present study was to determine the influence of the leaf extracts and leaf leachates of *C. odorata* on rendering the inhibitory action on seed germination behavior, seedling growth and metabolism of the plant by virtue of having its allelopathic property.

2. MATERIALS AND METHODS

Mature and healthy leaves of *Chromolaena odorata* (L.) King and Robinson were collected from the Burdwan University campus. Healthy viable grass pea (*Lathyrus sativus*) seeds were taken as a bioassay material. Mature leaves of the plant were washed with distilled water to remove the adherent dust particles and thoroughly dried using blotting paper. Leaf extract preparations of *Chromolaena* were made from fresh leaves by thorough homogenization using 50g leaf samples with 300 ml double distilled water. The homogenate was strained using fine cloth and thereafter it was stirred manually for two minutes and then filtered through Whatman No. 1 filter paper. Then the total volume was made up to 500ml using double distilled water. This was considered as 10% solution for leaf extract sample or stock solution and from this 5% solution was prepared by dilution

using double distilled water. Leaf leachate preparation of *Chromolaena* was made by using another lot of shed- dried 50g leaf samples and this was kept immersed in 300 ml double distilled water for 48 h. Thereafter it was stirred manually for two minutes and then filtered through Whatman No. 1 filter paper. The total volume of leachate was made up to 500ml using double distilled water. This was considered as 10% solution or stock solution for leaf leachate sample and from this 5% solution was prepared by dilution using double distilled water. Grass pea seeds were surface sterilized with 0.1% $HgCl_2$ for 90 seconds. The seeds were then separately presoaked in 10% and 5% *Chromolaena* leaf extracts and leaf leachates for 8 hours. Then washed the seed samples thoroughly with distilled water and dried back to their original weight in intense sunlight. Dried seeds were then used as test materials for analyzing various physiological and biochemical parameters. Data on germination percentage, T_{50} (time required for 50% germination in hours) values of seeds, fresh and dry weight of 30 days old plants raised from such seeds, average length of root and shoot, leaching of free amino acid and soluble carbohydrate from seed as well as chlorophyll and protein contents in leaves of 30 days old plants were recorded. Germination data were recorded after 10 days of seed soaking following the rules of ISTA, 1976 [18]. The time (hours) for 50% germination of seeds (T_{50}) was determined following the method described by Coolbear *et al*., 1984 [19].Fresh weight (g) and dry weight (g) were measured from intact seedlings, raised from untreated and treated seeds. Data were recorded from 30 days old, uniformly grown grass pea plant of each treatment including control. The shoot and root length were recorded separately for different concentrations of both species.For free amino acid and soluble carbohydrate analysis from grass pea seeds, 10 seed samples of each treatment were dipped in 10 ml of double distilled water for 24 hours. The seeds were then removed from water after the stipulated period, the seed leachates were then stirred well, decanted off and the tests were performed. From leachate stock, free amino acid level was quantified following the method of Moore and Stein, 1948 [20] modified by Bhattacharjee, 1984 [21]. Soluble carbohydrate of seed leachate was determined following the method of McCready *et al*., 1950 [22] with slight modification. For chlorophyll estimation leaf tissues (100 mg) were immersed in 5 ml methanol and kept in a refrigerator for 24 hour. The supernatant was decanted off and leaf samples were rinsed repeatedly with a little volume of methanol until they were completely free from green colour. Thus, the final volume of methanol was made exactly up to 10 ml and the intensity of the green colour was measured at 650 nm in a spectrophotometer. The chlorophyll content was estimated following Arnon's principle, 1949 [23]. Protein level was analysed from the same leaf samples after removal of chlorophyll following the method of Lowry *et al*., 1951 [24]. Extraction and estimation of catalase activity was determined from the pretreated *Lathyrus* seeds following the method of Snell and Snell, 1971 [25] modified by Biswas and Choudhuri, 1978 [26]. The activity of peroxidase was also estimated from pretreated *Lathyrus* seeds following the method of Kar and Mishra, 1976 [27]. In each 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 [28].

Each experiment was done in three replicates and the experimental results were expressed as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Seed germinability and T_{50} values (Table-1)

It appears from the data that the percentage germination and T_{50}values of *Lathyrus* were inversely correlated. Seed germinability was recorded to be significantly reduced in all the samples pretreated with leaf extracts and leaf leachates in contrast to control one. On the other hand, T_{50} values were found higher irrespective of samples analyzed when compared with the control one. And this inhibitory trend was noted more prominent at higher concentrations of the leaf extracts used.

Table 1: Effect of seed pretreatment with leaf extracts and leaf leachates of *Chromolaena* on the final percentage (%) of germination and T_{50} values (time required for 50% germination) of *Lathyrus* seeds

Treatments	Percentage (%) of seed germination after 240 hours	T_{50} values of germination (hours)
Control	98.89 ± 1.92	13.67 ± 0.58
Leaf extract (5%)	40.74 ± 5.59	N.A.
Leaf extract (10%)	32.59 ± 5.59	N.A.
Leaf leachate (5%)	51.48 ± 6.51	95.67 ± 4.16
Leaf leachate (10%)	42.96 ± 6.70	N.A.

N.A. = Nonattainment of 50% germination, values were expressed as Mean± SD (n=3)

3.2 Shoot length and root length (Table-2)

With respect to growth analysis study root length and shoot length of 30 days old *Lathyrus* plants were found to be reduced in all leaf extract and leaf leachate-treated samples when compared with control one. The data clearly revealed that treatment-induced inhibitory effect was strictly concentration-dependent and the magnitude of inhibitory effect was remarkable in leaf extract-treated samples in comparison to the leaf leachates.

Table 2: Effect of seed pretreatment with leaf extracts and leaf leachates of *Chromolaena* on the shoot and root length (cm) of 30 days old *Lathyrus* plants

Treatments	Shoot length (cm)	Root length (cm)
Control	24.48 ± 1.89	15.57 ± 1.12
Leaf extract (5%)	14.77 ± 0.40	09.94 ± 0.27
Leaf extract (10%)	13.39 ± 0.49	08.62 ± 0.22
Leaf leachate (5%)	18.03 ± 0.65	11.49 ± 0.30
Leaf leachate (10%)	16.52± 0.45	10.25 ± 0.21

Values were expressed as Mean± SD (n=3)

3.3 Fresh weight and dry weight (Table-3)

As regards the changes of fresh and dry weight study both leaf extracts and leaf leachates of *Chromolaena* were significantly inhibit the fresh and dry weight of the experimental plant *Lathyrus* and magnitude of inhibition was recorded to be maximum in leaf extract-treated samples. Again, the higher concentration of leaf extract of the *Chromolaena* exerted more inhibitory effect in 30 days old test plant.

Table 3: Effect of seed pretreatment with leaf extracts and leaf leachates of *Chromolaena* on changes of fresh weight and dry weight of 30 days old *Lathyrus* plants

Treatments	Fresh weight (g)	Dry weight (g)
Control	6.58 ± 0.48	1.06 ± 0.880
Leaf extract (5%)	3.84 ± 0.10	0.602 ± 0.016
Leaf extract (10%)	3.27 ± 0.11	0.496 ± 0.014
Leaf leachate (5%)	5.18 ± 0.23	0.833 ± 0.032
Leaf leachate (10%)	4.48 ± 0.13	0.694± 0.012

Values were expressed as Mean± SD (n=3)

3.4 Leaching of soluble carbohydrate and free amino acids (Table-4)

Leaching of amino acids and soluble carbohydrates was higher, when *Lathyrus* seeds were treated with leaf extracts and the magnitude of leaching was less in leaf leachate treatments. Higher the concentrations of leaf extracts and leaf leachates, more was the leaching of amino acids and soluble carbohydrates in the treated samples, thus such changes were found concentrations dependent.

Table 4: Effect of seed pretreatment with leaf extracts and leaf leachates of *Chromolaena* on leaching of soluble carbohydrate and free amino acid of *Lathyrus* seeds

Treatments	Soluble carbohydrates (mg g^{-1} 10ml^{-1})	Free amino acids (mg g^{-1} 10ml^{-1})
Control	4.11 ± 0.27	2.33 ± 0.16
Leaf extract (5%)	7.18 ± 0.73	5.06 ± 0.59
Leaf extract (10%)	7.82 ± 0.71	5.56 ± 0.60
Leaf leachate (5%)	5.82 ± 0.55	3.58 ± 0.39
Leaf leachate (10%)	6.32 ± 0.62	4.02± 0.44

Values were expressed as Mean± SD (n=3)

3.5 Chlorophyll and protein content (Table-5)

Data incorporated in table-5 revealed that both chlorophyll and protein contents were remarkably reduced in leaves of *Lathyrus* plants, raised from seeds pretreated with leaf extracts and leaf leachates of *Chromolaena*. The inhibitory effect was comparatively lower in lower concentrations

of the leaf leachates than leaf extracts.

Table 5: Effect of seed pretreatment with leaf extracts and leaf leachates of *Chromolaena* on changes of chlorophyll and protein contents in leaves of 30 days old *Lathyrus* plants

Treatments	Chlorophyll (mg g^{-1}fr. Wt.)	Protein (mg g^{-1} fr. Wt.)
Control	4.75 ± 0.24	23.68 ± 1.90
Leaf extract (5%)	3.77 ± 0.11	13.27 ± 0.32
Leaf extract (10%)	3.59 ± 0.11	11.45 ± 0.37
Leaf leachate (5%)	4.09 ± 0.18	15.12 ± 0.49
Leaf leachate (10%)	3.94 ± 0.13	13.45 ± 0.22

Values were expressed as Mean± SD (n=3)

3.6 Catalase and peroxidase activities (Table-6)

Activities of catalase and peroxidase declined in seed kernels which underwent pretreatment with the leaf extracts and leaf leachates of *Chromolaena*. However, the rate of decreasing the enzyme activities was found highest in seeds which underwent pretreatment with higher concentration of leaf extracts.

Table 6: Effect of seed pretreatment with leaf extracts and leaf leachates of *Chromolaena* on activities of catalase and peroxidase in *Lathyrus* seeds

Treatments	Catalase (unit h^{-1} g^{-1} fr. wt.)	Peroxidase (unit h^{-1} g^{-1} fr. wt.)
Control	92.45 ± 7.07	59.17 ± 4.51
Leaf extract (5%)	51.22 ± 0.92	34.79 ± 0.86
Leaf extract (10%)	46.71 ± 1.22	31.94 ± 1.00
Leaf leachate (5%)	60.41 ± 1.25	42.47 ± 1.54
Leaf leachate (10%)	55.29 ± 0.94	39.25± 1.29

Values were expressed as Mean± SD (n=3)

Results of this investigation can be justifiably discussed and a concrete conclusion can be drawn from this study. In a nut shell results reveal that leaf extracts and leaf leachates of *Chromolaena* irrespective of their concentrations, decreased percent germination along with enhanced T_{50} values (Table 1) of grass pea seeds, reduced root and shoot length (Table 2), as well as fresh weight and dry weight (Table 3) of plants. The treatments also substantially increased leaching of amino acids and soluble carbohydrates from seeds (Table 4). Such changes were also associated with the treatment-induced decrease of chlorophyll and protein (Table 5) contents in leaves of *Lathyrus* plants along with reduction of catalase and peroxidase activities in seed kernel in treated samples (Table 6).Analyses of germination behavior of seed, growth performance of seedlings are considered as reliable indices

for evaluation of allelopathic potential [29, 30, 31, 32, 33]. In the present investigation, results were thus in conformity with the reported observations of many previous workers in this field of study. Reduced seed germinability and slower rate of germination are considered as two important indicators of allelopathic action of plants and such action is exerted by chemical substances which are mostly secondary metabolites [34]. In fact, many allelochemicals including phenolic substances impair anabolic activities with concomitant enhancement of catabolic activities in plants [35, 36, 37]. The regulation of these activities was mainly initiated by hampering a number of biosynthetic pathways which retain the normal functional life of plants. In this study the test plant (*C. odorata*)-induced modulation of germination behaviour, growth and metabolism of the bioassay material (*L. sativus*) is indicative of the existence of allelopathic property in the former plant sample. In fact, chlorophylls, proteins, nucleic acids are considered as vital macromolecules, which play a significant role for maintaining the normal vigour status and standard health of plants. In this investigation plant extract-induced reduction of the macromolecules is indicative of the fact that some inhibitory chemicals/allelochemicals might have played a crucial role for down regulating the biosynthetic process and / or enhanced detrimental activities by stimulating catabolic enzymes of various plants. Allelochemical- induced reduction of a number of vital macromolecules including beneficial enzymes are well documented in the literature. [38, 39, 40]. In this investigation, results indicate that the leaf extracts and leaf leachates of *Chromolaena* possess some strong inhibitory putative chemicals which can render negative allelopathic action as evidenced from impairment of a range of physiobiochemical phenomena in plants including seeds of the bioassay material *Lathyrus sativus*. Thus, the analyses of data generated in our experiment are considered to be reliable enough for assessment of allelopathic potential in *Chromolaena odorata.*

4. CONCLUSION

The application of aqueous leaf extracts and leachates of *Chromolaena* showed negative effect on grass pea, with a reduction in seed germination, root and shoot length, fresh and dry weight of plants, chlorophyll and protein contents from leaves of 30 days old plants and enzyme activities of seeds. Conversely, promotive effect was found in leaching of sugar and free amino acid from seed as well as in case of T_{50} values. A conclusion can be drawn from this study that the plant *Chromolaena odorata* exerts some allelopathic effect on grass pea and hence the plant may be selected as a potential candidate for recommendation of bioherbicide formulation in future. Thus, this study has some value from agricultural point of view for possible establishment of ecofriendly and cost- effective bioherbicides for resource- poor farmers of our country.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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