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Phytotoxicity Studies of *Chromolaena* Odorata (L.) R.M. King and H. Robinson. Through Seed Germination Bioassays

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Abstract

Invasive or alien weeds are those which are introduced to new areas intentionally or unintentionally. They are undesirable plants which affect crop production, their quality and quantity, other resource utilization and income generation activities of the humans. *Chromolaena odorata* is one such invasive or alien weed which has invaded Maval region in Pune district. *Chromolaena odorata* has shown gradual increase in its abundance and decrease in natural Phytodiversity and crop diversity over frequent field visits. The present study was therefore undertaken to investigate allelopathic potential of leachates of root, stem, and leaf of *Chromolaena odorata* on common legumes in Maval tehsil like Horsegram, Lentil, and Mothbean in seed germination bioassay experiment. Petri plate bioassay study revealed that lower concentrations of leachates promoted root length, shoot length, Vigour index but higher concentration (20%) of aqueous leachates reduced seed germination in Horsegram, Lentil, and Mothbeans significantly as compared to other concentrations (4%, 8%, 12%, 16% and control). Treatment with higher concentration caused a remarkable increase in the rate of inhibition. Phytochemical analysis showed the presence of Flavonoids, Saponins, Tannins, Alkaloids, Steroids and Terpenoids. The inhibitory effects on seed germination revealed allelopathic potential of weed *Chromolaena odorata*. Toxicity effect was more in 20% concentration of leaf leachates than in root and stem. This may be due to presence of more allelochemicals in leaf than in root and stem.

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Introduction

The genus *Chromolaena* (Asteraceae) has more than 165 species. One of the members of this genus *Chromolaena odorata* (L) R. King and H. Robinson is an obnoxious Neotropical, allelopathic invasive weed which has an influence on the growth and development of other plants in multiple modes of actions depending on bioactive compounds and the sensitivity of target plants.[1] It has considerably extended its distribution to the paleotropics in the last 150 years and is now thoroughly naturalized in the parts of Africa, India, Indonesia, Ceylon, China, Malaysia.[2] It was first introduced in India in Calcutta in 1845[3] from central America. The distribution of *Chromolaena odorata* mostly depends on temperature and moisture availability and hence invades the biodiversity rich regions of India like Eastern Ghats, Western Ghats, the Eastern and North-eastern Himalayas.[4] Presently this weed is infesting agricultural lands and is becoming a dominated weed in many areas. As old as agriculture weeds have substantially adapted characteristics that enable them to grow, flourish, invade and dominate an important part of the natural system.[5] This invasiveness and aggressive growth can be attributed not just to its heavy seed production, but also to its high amount of allelochemicals in the leaves.[6] These Allelochemicals or the phytotoxic secondary metabolites have the potential to mediate interspecific plant –plant interference by reducing or inhibiting establishment, growth and survival of other plants or crops.[7] Many researchers investigated number of such allelochemicals from different parts of *Chromolaena odorata*. They are released in soil by various modes such as leaching, residual decomposing and mulching depending upon the species and environmental conditions. Phytotoxins or allelochemicals like Ceryl alcohol, eupatol, (sesquiterpenes alcohol), Lupeol and B-amyrin (terpene alcohol) Salvagnini (Flavon), flaven,4-5 dihydroxy 3-7 dimethoxy Flavon and odratin and P. anisic acid are present in leaves and different plant parts of *Chromolaena*.[8] Alkaloids, Terpenoids, flavonoids, Saponins, phenols, cardiac glycosides, resins and Anthraquinones are also present in root, stem, and leaf extract of *Chromolaena* weed. Allelochemicals like Tannin, phytosterols, Coumarin, Quinone, Steroids, Acids are also investigated by many researchers[9,10] Most of these allelochemicals are released during germination and early growth stages[1] and hence show significant effect on seed germination in many crops. Investigators carried out experiments with aqueous extracts and leachates of different parts of *Chromolaena* on crops and observed significant reduction in germination percentage, Root and Shoot length, Vigour index, Elongation ratio.[11,12,13,14.] Plenty of research has been carried out but meagre work is reported on Horesgram (*Mycrotyloma uniflorum*), Lentil (*Lens culinaris*) and Mothbean (*Vigna aconotifolia*) in Maval region of Pune district (MS). Hence present study was undertaken to investigate Phytotoxicity of this noxious weed on crops like Horsegram, Lentil, and Mothbean.

Materials and Methods

Collection and Processing of Plant Material

Fresh and healthy *Chromolaena odorata* plants were collected from Kanhe area of Maval (180 44’54.96” N and 730 38’ 27.60” E) in the month of November 2019 and brought to laboratory for seed germination bioassay experiments. All the plant parts were separated and thoroughly cleaned with distilled water, wiped, and then air shade dried at room temperature. Fine powder of root, stem and leaves were obtained by grinding and sieving.

Preparation of Aqueous Leachates

The aqueous leachates were prepared by soaking 20 grams of dried powder of each part (Root, Stem, and Leaves) separately in 100 ml of distilled water at room temperature. After 24 hours each leachate was filtered through muslin cloth. This 20% concentrated leachate was diluted to 4%, 8%, 12%, and 16% by adding distilled water and stored in amber-coloured bottles in refrigerator.

Procurement of Seeds for Bioassay

Local variety of seeds of Horsegram, Lentil, and Mothbean were collected from Tung region of Maval Tehsil. The seeds were cleaned with 0.1 Hgcl2 and washed with distilled water for 3-4 times and used for bioassay.

Seed Germination Bioassay

It was carried out in sterilized petriplates of size 9 inch by placing a moistened germination paper with respective concentration of leachates and 10 seeds of legume were placed equidistantly in every plate. All the sets were arranged in triplicates. The germination papers were moistened regularly with leachates as per the need thereafter for about 8-10 days. Distilled water was used as control. Petri plates of each legume for different plant parts (Root, Stem, and leaves) and for each concentration replicating three times at room temperature 28-30 °C. Experiment was kept till germination was observed and results were recorded. Seed germination parameters such as Germination percentage, Inhibition percentage, Root length, shoot length, Root and Shoot Ratio, Vigour index, Relative Elongation Ratio of Root (RERR), Relative Elongation Ratio of Shoot (RERS), Relative Germination Ratio (RGR) were calculated.[15,16]

Phytochemical Analysis

All the tests were performed by standard physico-chemical methods for detection of various phytochemicals for their presence in the selected invasive weed[17,18]

Statistical Analysis

All the data were statistically analyzed using One-way ANOVA followed by Tukey's multiple comparison assuming equal variance. The data were analyzed using Minitab 17.0 software version.

Results

Effect on Seed Germination

Table No. 1 shows the germination percentage of all test crops seeds. Higher concentrations of aqueous root, stem, and leaf leachates, such as, 16%, and 20%, inhibited seed germination in Horsegram, but a statistically significant decrease in seed germination was seen at 20%. Lower concentrations of aqueous root, stem, and leaf leachates, such as 4% and 8%, promoted seed germination in Horsegram. In leaf leachate, Relative Elongation Ratio of Root (12.54) and Relative Elongation Ratio of Shoot (19.43) was significantly reduced more compared to control than in root and stem leachates. The maximum Relative Germination Ratio (109.86) was observed when Horsegram seeds were exposed to lower concentration (4%) of leachates and minimum RGR (35.79) was noted at higher concentration (20%). When the same lower concentrations (4%,8%) of root, shoot and leaf leachates were applied to Lentil seeds, statistically significant promotion in germination percentage was seen in comparison to the control, and at higher concentrations (16%, and 20%), a significant reduction was seen. In leaf leachate Relative Elongation Ratio of Root (91.92) and Relative Elongation Ratio of Shoot (59.1) were significantly reduced as compared to control as in root and stem leachates. The maximum Relative Germination Ratio (107.4)) was observed when Lentil seeds were exposed to lower concentration (4%) of leachates while the minimum (70.07) was observed when the seeds were exposed to higher concentration (20%) of leachates. When mothbeans were exposed to different concentrations of root, stem, and leaf leachates lesser concentrations (4% and 8%) significantly encouraged seed germination, whereas at higher concentrations (16%, and 20%) statistically significant inhibition in seed germination was observed. Relative Elongation Ratio of Root (41.49) and Relative Elongation Ratio of Shoot (31.35) in leaf leachate were significantly reduced as compared to control as in root and stem leachates. The maximum Relative Germination Ratio (110) was observed when Mothbeans were exposed to lower concentration (4%) of leachates and the minimum RGR (22.98) was observed at higher concentration (20%) of leachates.

Effect on Root Length

When the seeds of Horsegram, Lentil and Mothbean were exposed to root, stem, and leaf leachates of various concentrations of weed *Chromolaena odorata* reduction in root length was observed at higher concentration (20%) as compared to control. Root leachate of lower concentrations caused increase in root length in all crops, but higher concentration (20%) caused statistically significant reduction in root length of Mothbean seeds as compared to control than Horsegram and Lentil. Same results were obtained in case of stem leachates. Higher concentration of stem leachate (20%) reduced root length significantly in Mothbeans while lower concentrations increased root length of all crop seeds (Table 2). On application of various concentrations of leaf leachate, it was observed that root length was reduced in higher concentration 20% significantly in case of Horsegram, Lentil and Mothbeans while lower concentrations promoted increase in root length of all crop seeds. (Table 2).

Table 1: Effect of aqueous leachates of Chromolaena odorata on germination percentage

Treatment	HORSEGRAM			LENTIL			MOTHBEAN		
	RL	SL	LL	RL	SL	LL	RL	SL	LL
Control	90 ^{ABC}	90^{ABC}	90^{AB}	90A^{BC}	90A^{B}	66.67^{BC}	96.67^{AB}	93.33^{A}	86.67^{AB}
4%	97.78^{A}	95.56^{A}	96.67^{A}	100^{A}	97.78^{A}	92.22^{A*}	100^{A}	96.67^{A}	94.44^{A}
8%	92.22^{AB}	88.89^{AB}	92.22^{AB}	88.89^{AB}	94.44^{AB}	92.22^{A*}	88.89^{ABC}	94.44^{A}	86.67^{AB}
12%	85.56^{BC}	85.56^{BC}	83.33^{AB}	80^{BC}	88.89^{AB}	86.67^{AB}	88.11^{BCD}	90^{A}	85.56^{AB}
16%	76.67^{C}	77.78^{C}	73.33^{B}	81.11^{ABC}	82.22^{A}	75.56^{B}	74.44^{CD*}	83.33^{A}	76.67^{B}
20%	60 ^{D*}	66.67^{D*}	32.22^{C*}	68.89^{C}	67.78^{C*}	46.67^{C}	66.67^{D*}	68.89^{A}	20^{C*}

RL-Root leachate SL- stem leachate LL- leaf leachate. Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA.

Table 2: Effect of aqueous leachates of Chromolaena odorata on Root length in cm

Treatment	HORSEGRAM			LENTIL			MOTHBEAN		
	RL	SL	LL	RL	SL	LL	RL	SL	LL
Control	4.711^{AB}	3.656^{B}	4.836^{B}	3.300^{AB}	2.700^{ABC}	2.267^{BC}	3.578^{AB}	5.800^{A}	4.078^{B}
4%	5.411^{A}	5.544^{AB}	7.059^{A*}	4.041^{AB}	4.096^{ABC}	4.307^{A*}	4.570^{A}	4.530^{B}	5.030^{AB}
8%	5.670^{A}	6.896^{A}	7.974^{A*}	5.859^{A}	5.859^{A}	7.974^{A*}	4.052^{A}	5.881^{AB}	5.281^{AB}
12%	5.541^{A}	6.026^{AB}	8.426^{A*}	3.663^{B}	3.663^{B}	8.426^{A*}	4.670^{A}	6.181^{A}	6.189^{A*}
16%	5.363^{A}	5.952^{AB}	7.870^{A*}	5.981^{A}	5.981^{A}	7.870^{A*}	4.070^{A}	4.644^{B}	4.990^{AB}
20%	3.826^{B}	3.826^{B}	1.048^{C*}	3.199^{B}	3.199^{B}	0.744^{C}	1.930^{B}	2.222^{C*}	0.600^{C*}

RL-Root leachate SL- stem leachate LL- leaf leachate. Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA.

Effect on Shoot Length

Application of Root, stem, and leaf leachates of various concentrations to crop seeds like Horsegram, Lentil and Mothbean showed significant reduction in shoot length at higher concentration as compared to control (Table 3). Inhibitory effect increased with increase in concentrations. When Horsegram seeds were exposed to root leachates increase in shoot length was observed at lower concentrations but significant reduction in shoot length was observed at 20% of root leachate than in stem leachate and leaf leachate as compared to control. In case of Lentil seeds increase in shoot length was observed at lower concentrations of all leachates but reduction in shoot length was observed at higher concentration (20%) in all leachates. There was a statistically significant reduction in shoot length of Mothbeans at higher concentration (20%) of leaf leachate than root, stem leachates but shoot length was promoted at lower concentrations of leaf leachate. (Table 3).

Effect on Vigour Index

Seed vigour index of Horsegram, Lentil and Mothbeans under the influence of various concentrations of root, stem and leaf leachates is presented in Table 4. In case of Horsegram seeds vigour index was significantly reduced at higher concentration (20%) of leaf leachate than in root and stem leachates as compared to control. In case of Lentil, vigour index is reduced significantly at higher concentration (20%) of root leachate than in stem and leaf leachates as compared to control while Mothbeans showed statistically significant reduction in all treatments at higher concentration (20%).

Table 3: Effect of aqueous leachates of Chromolaena odorata on Shoot length in cm

Treatment	HORSEGRAM			LENTIL			MOTHBEAN		
	RL	SL	LL	RL	SL	LL	RL	SL	LL
Control	2.178^{B}	2.933^{AB}	2.211^{BC}	3.104^{BC}	3.022^{A}	0.833^{BC}	1.822^{A}	1.188^{AB}	1.200^{A}
4%	2.348^{BC}	1.756^{BCD}	2.359^{B}	4.585^{AB}	3.656^{A}	3.107^{A}*	1.581^{A}	1.185^{AB}	1.156^{A}
8%	2.737^{B}	2.004^{ABC}	5.070^{A}*	4.981^{A}*	4.067^{A}	2.448^{A}	1.251^{A}	1.341^{A}	1.804^{A}
12%	2.785^{B}	1.515^{CD}*	2.511^{B}	3.881^{BC}	4.100^{A}	2.589^{A}*	1.600^{A}	1.378^{A}	1.170^{A}
16%	4.163^{A}*	2.804^{D}*	2.856^{B}	3.974^{A}*	2.678^{A}	2.278^{AB}	1.430^{A}	1.600^{A}	2.141^{A}
20%	1.493^{C}*	0.877^{C}	0.437^{C}	2.256^{C}	2.493^{A}	0.641^{C}	0.466^{B}*	0.763^{B}	0.126^{B}*

RL-Root leachate SL- stem leachate LL- leaf leachate Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA.

Table 4: Effect of aqueous leachates of Chromolaena odorata on Vigour index

Treatment	HORSEGRAM			LENTIL			MOTHBEAN		
	RL	SL	LL	RL	SL	LL	RL	SL	LL
Control	620^{A}	633.0^{A}	657.3^{C}	515.0^{C}	515.0^{ABC}	208.4^{CD}	526AB	649.9^{A}	491.0A
4%	750.1^{A}	705.9^{A}	915.5^{AB}*	881.9^{AB}*	731.1^{AB}	695.3^{A}*	616.3^{A}	548.2^{A}	601.4A
8%	750.9^{A}	730.5^{A}	1048.0^{A}*	971.7^{A}*	856.6^{A}	502.2^{B}*	475.9^{B}	677.5^{A}	654.1A
12%	714.1^{A}	515.8^{A}	908.9^{AB}*	593.5^{BC}	695^{A}	491.2^{B}*	510.5A^{B}	677.7^{A}	669.9A
16%	593.5^{A}	670.7^{A}	810.4^{BC}	849^{AB}*	474.2B^{C}	414.9^{BC}	418.1^{B}	543^{A}	590.3A
20%	350.2^{B}	400.2^{A}	62.1^{D*}	403.8^{C*}	369^{C}	72.3^{D}	165.5C*	189.4^{B*}	56.6^{B}*

RL-Root leachate SL- stem leachate LL- leaf leachate Values followed by same letter(s) within a column did not differ significantly at 5% level of significance with one-way ANOVA.

Table 5: Preliminary Phytochemical screening of aqueous extracts of Chromolaena odorata

Sr. no.	Phytochemical tests	Root	Stem	Leaf
1	Flavonoids	+	+	+
2	Saponins	+	+	+
3	Tannins	+	+	+
4	Glycosides	+	+	+
5	Alkaloids	+	+	+
6	Steroids	+	+	+
7	Terpenoids	+	+	+

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of aqueous extracts of different parts of *Chromolaena odorata* were carried out in lab. It revealed the presence of Flavonoids, Saponins, Tannins, Glycosides, Alkaloids, Steroids and Terpenoids as shown in table no.5. As *Chromolaena odorata* is allelopathic weed many allelochemicals are present in all parts of this weed which affects seed germination in crops. (Table 5).

Discussion

Studies on invasive weeds which are dominant in crop systems were carried out by many investigators on various crops. The effects of *Chromolaena odorata* weed extracts were studied on germination of seeds like Horsegram, lentil and moth beans which are common crops of Maval area. The results revealed that aqueous leachates of different parts of *Chromolaena odorata* have inhibitory effects on seed germination, root length, and shoot length. Such inhibitory effects might be due to presence of allelochemicals in *Chromolaena odorata*. The present study clearly indicated that aqueous leachates of root, stem and leaves of *Chromolaena odorata* showed allelopathic effect on seed germination in crops like Horsegram, Lentil and Mothbean. Allelopathy included both positive (growth promoting) and negative (growth inhibiting) effects.[19,20,21,22] Phytotoxicity was observed at higher concentrations while at lower concentrations the allelochemicals may act as growth regulators.[23] Many researchers have reported that these allelochemicals secreted by different parts of *Chromolaena* were phytotoxic and cause significant effect on seed germination in various crops.[24,25,26] Similar results were reported in case of other weeds and crops.[27,28,29,30,31] Among the three test crops Mothbeans were more affected than Horsegram and Lentil seeds. This finding was related with the findings that crop seeds with smaller size were more susceptible to allelopathic effect of weed.[16] Higher the concentrations of leachates lower was the seed germination. This result is corroborating with the earlier reports indicating that allelopathy is concentration dependent phenomenon.[32,33] More reduction in root length on application of higher concentrations of aqueous leachates of different parts of *Chromolaena* was noted as compared to control while increase in root length was noted at lower concentrations of leachates in all crop seeds. This result showed correlation with previous findings.[34,35,36,37,38,39] In case of shoot length of crops, more inhibitory effect observed due to higher concentrations (16% and 20%) of aqueous leachates, while lower concentrations showed promoting effect on shoot length. Our results have similarity with findings in Maize, Soybean, cotton and in grass.[40,41,42,43] Such type of inhibition of seed germination due to different concentrations of aqueous leachates of weeds were observed by many researchers in other crops like Maize, Cowpea, Sesame, Radish, Mustard, Soybean, Tomato and in Rice.[44,45,46,47,48] Vigour index in Horsegram was significantly promoted when exposed to lower concentrations of leaf leachate than in Lentil and Mothbeans as compared to control while inhibited when exposed to higher concentration of leaf leachates than stem and root leachates as compared to vigour index in Lentil and Mothbeans. Our results are similar to the results of other workers[49,50] The tolerance level of allelopathic activities of various concentrations of different parts of *Chromolaena* in terms of vigour index can be represented as Horsegram>Lentil>Mothbeans. Among the plant parts leaves of *Chromolaena* weed were most inhibitory to the crop seed germination than root and stem. This indicated presence of more allelochemicals in it.

Conclusion

Present investigation throws light on the allelopathic potential of *Chromolaena odorata* on seed germination in agricultural crops like Horsegram, Lentil and Mothbean. The allelopathic effect of aqueous leachates of *Chromolaena* is due to secretion of various allelochemicals present in different parts like root, stem, and leaves. More investigation is needed to find the compounds responsible for such promotive and inhibitory activities in seed germination in Horsegram, Lentil and Mothbean like crops as very meagre work has been done on this aspect. It is also suggested that instead of manual eradication of this weed more efforts should be taken to think on its use for some constructive purpose in sustainable agriculture.

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Conflict of Interest

The authors declare no conflict of interest.

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