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Research Article
Allelopathic Potentials of *Chromolaena odorata* L. on Growth and Biochemical Characteristics of *Salvadora persica*

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Abstract
Background and Objective: Siam weed is a fast-growing perennial, is now regarded as one of the most harmful weeds present on earth due to its highly invasive and allelopathic nature. The present investigation was aimed to evaluate the allelopathic effect of aqueous extract and leaf litter leachate extracts from *Chromolaena odorata* on the growth of mangrove associate plant *Salvadora persica*. **Materials and Methods:** Germination of *Salvodora persica* was performed in Petri dishes under different concentrations (20, 40, 60 and 80%) of leaf aqueous extracts of *Chromolaena odorata* collected from natural habitats of Konkan region of Maharashtra, India. At the end of experiment (10 days), the growth and biochemical characteristics of *S. persica* seedlings were measured. The data were statistically analyzed by one-way ANOVA analysis of variance. **Results:** The effects of extracts on germination percentage, seedling growth and dry biomass were investigated. Higher concentrations of extract (60 and 80%) significantly reduced germination percentage, radicle length, plumule length and dry matter accumulation of the *Salvadora* seedlings as compared to control. The inhibitory effect was concentration dependent and the more pronounced effect noticed in leaf litter leachate extract. A significant correlation was found in different concentrations of extracts used with respect to morphological attributes. The allelopathic effect also noticed in biochemical parameters. The GC MS analysis of leaves revealed the presence of some allelochemical compounds which supported the allelopathic potential of leaf extract. **Conclusion:** Based on the findings, it could be speculated that the delayed germination and low germination rate of the test species after treatment by extracts may be due to the allelochemicals present in the extracts which might release phenolics into the soil and these are probably involved in the growth inhibitory effect of surrounding plant species.

Key words: Fast growing perennial, allelopathic potential, siam weed, biochemical changes, harmful weeds, *Chromolaena odorata*, *Salvadora persica*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Allelopathy is a natural biological phenomenon in which an organism produces some biochemical compounds which influence the growth, survival, reproduction and biological processes of other organisms. These biochemical compounds are known as allelochemicals and can have positive allelopathy (beneficial) or negative allelopathy (detrimental) effects on the targeted organisms. According to Stamp[1], these allelo-chemicals are a set of secondary metabolites, which are not required for metabolic activities such as growth, development and reproduction of the mother organism. These effects are selective and concentration dependent and may have an either inhibitory or stimulatory effect on the growth of subsequent crops or weeds[2-4]. A Large number of chemicals have been identified and found to release from crops through volatilization, leaching, decomposition of crop residues and root exudation. Recently, the focus of role of allelopathy in agriculture is shifted towards effects of leachates from plants, plant extracts and decomposing plant residues. Plant residues generally contain a variety of toxic compounds that are known inhibitors of seed germination or seedling development[5]. Plant leachates are recorded to have suppression of seed germination and vegetative propagules and also early seedling growth[6,7]; the decrease in radicle growth has also been reported by Casado[8]. According to Patrick[9], the degree of damage to the crop is related to the degree of contact of roots to the leachates or residues. Phyto-toxicity from a crop might be from indirect effects of micro-organisms and direct toxic actions[10]. Aqueous extract of some plants reduces seedling growth[11]; root and shoot growth[12]; germination[13] and stimulate mortality of plants[14].

Chromolaena odorata (Linn) R.M. King and H. Robinson (Asteraceae) (syn. *Eupatorium odoratum* L. or *Osmia odorata* L.) known as siam weed is a fast-growing perennial, diffuse and scrambling shrub native to central and southern America, then introduced into the tropical regions of Asia, Africa and the Pacific where it is an invasive weed. The plant grows to 3-7 m in height when grown in the open and it goes by many common names including devil weed, French weed, communist weed, hagonoy, co hoy etc.[15-19]. This invasive weed is introduced to many places, either intentionally as an ornamental plant or accidentally, it is now regarded as one of the most harmful weeds present on earth due to its highly invasive and allelopathic nature[17]. This weed suppresses crops and other plants in its surroundings by competing for nutrients and water, over-shading and allelopathy[20]. The younger leaves of *C. odorata* are toxic due to high levels of nitrate[21].

In a preliminary survey, it was marked that a vast area of land, especially in the roadside of the coastal area of Sawantwadi, Maharashtra are infested with the weed. The literature on the effect of siam weed or its extracts on the growth of other coastal plants is quite scanty. Keeping in mind the above background, the present study was undertaken with the following objectives: (i) To evaluate the allelopathic effects of aqueous extract of leaf extract and leaf litter leachate extract of siam weed on morphological and biochemical parameters, of a coastal plant *Salvadora persica*, (ii) Identification of allelo-chemicals through GC-MS analysis of both extract and (iii) To identify which extracts cause sufficient suppression to growth of the studied species.

MATERIALS AND METHODS

Preparation of aqueous extract: The fresh leaves of Insect-free, disease-free plants of *C. odorata* and fallen matured senescent leaves were collected from the old trees growing in the coastal area near Sawantwadi, Maharashtra, during the months of November-December, 2016 and further analysis was carried out in next 6 months. The leaves were washed thoroughly with distilled water and air-dried at room temperature for 96 h. Both, fresh as well as fallen matured leaves chopped into 1 cm long pieces and were grated with the mechanical grater. The ground plant was soaked in 1 L of water for 24 h. The extracts were then filtered with muslin cloth followed by Whatman filter paper No. 1. This served as the stock solution from which other concentrations (20, 40 and 60%) were prepared by way of dilution.

Bioassay: The seeds of a test plant *Salvadora persica* were first treated with distilled water to remove the stress of debris and sand particles. Before seed germination test, empty and undeveloped seeds were discarded by floating in tap water. To avoid possible inhibition caused by toxins from fungi or bacteria, seeds were surface sterilized with 10:1 distilled water/bleach (commercial NaClO) solution for 5 min and then washed 6-7 times with distilled water. Sixty seeds were divided into three replicates of 20 seeds each were soaked for 4 h in 20, 40, 60 and 80% of leaf extract and leaf litter leachate extract. Control seeds were soaked in 10 mL of distilled water. The seeds were allowed to germinate in 20 cm diameter Petri dishes with a tight-fitting lid and placed in sterilized polyethene bags to prevent further loss of volatiles and kept in a seed germinator and were irrigated with 10 mL of the extract on alternate days. The emergence of a radical approximately 1 mm in diameter was taken as the index of germination. The dry biomass was determined after oven drying at 80°C for 24 h.

Biochemical analysis: Biochemical parameters such as Chlorophylls content were estimated according to Arnon[22], by extracting the chlorophylls in 80% acetone and expressed as mg g^{-1} FW. Soluble protein was extracted from the leaf samples of the test according to the method of Lowry *et al*.[23] and expressed as mg g^{-1} FW. Estimation of proline was done following the method of Bates *et al*.[24]. Proline was extracted in 3% sulfosalicylic acid, estimated by using acid ninhydrin reagent and measuring the absorbency of the toluene chromophore at 520 nm and expressed as µM g^{-1} DW. Total phenol content was assayed according to Swain and Hillis[25] and expressed as µg g^{-1} DW.

GC-MS analysis for phytochemical compounds: Samples were analyzed with a Hewlett-Packard (HP) 6890 gas chromatograph fitted with a Gerstel MPS2 autosampler and coupled to an HP 5973 N mass spectrometer. The carrier gas was helium (BOC gases, Ultra High Purity), flow rate 1.2 mL min^{-1}. The oven temperature was started at 50°C, held at this temperature for 1 min, then increased to 220°C at 10°C min^{-1} and held at this temperature for 10 min. The injector was held at 200°C and the transfer line at 250°C. For quantification of the compounds, mass spectra were recorded in the Selective Ion Monitoring (SIM) mode using NIST library.

Statistical analysis: Statistical analysis, one way ANOVA of data was done using software of SPSS 13.0. The correlation coefficient was determined by pouting data from all treatments and the relationship between seedling growth parameters was examined. Significance between control and treatment was compared at 0.05 probability levels.

RESULTS

Morphological changes: The higher concentration (60 and 80%) of both, fresh leaves as well as leaf litter leachate extract significantly reduced the germination percentage, root length, shoot length and dry biomass and chlorophyll content of *Salvadora* seedlings. At 80% concentration of leaf litter leachate extract, the germination percentage was reduced by 52% and at the same concentration of the fresh leaves extract, it was reduced by 47% (Fig. 1a). The reduced significant

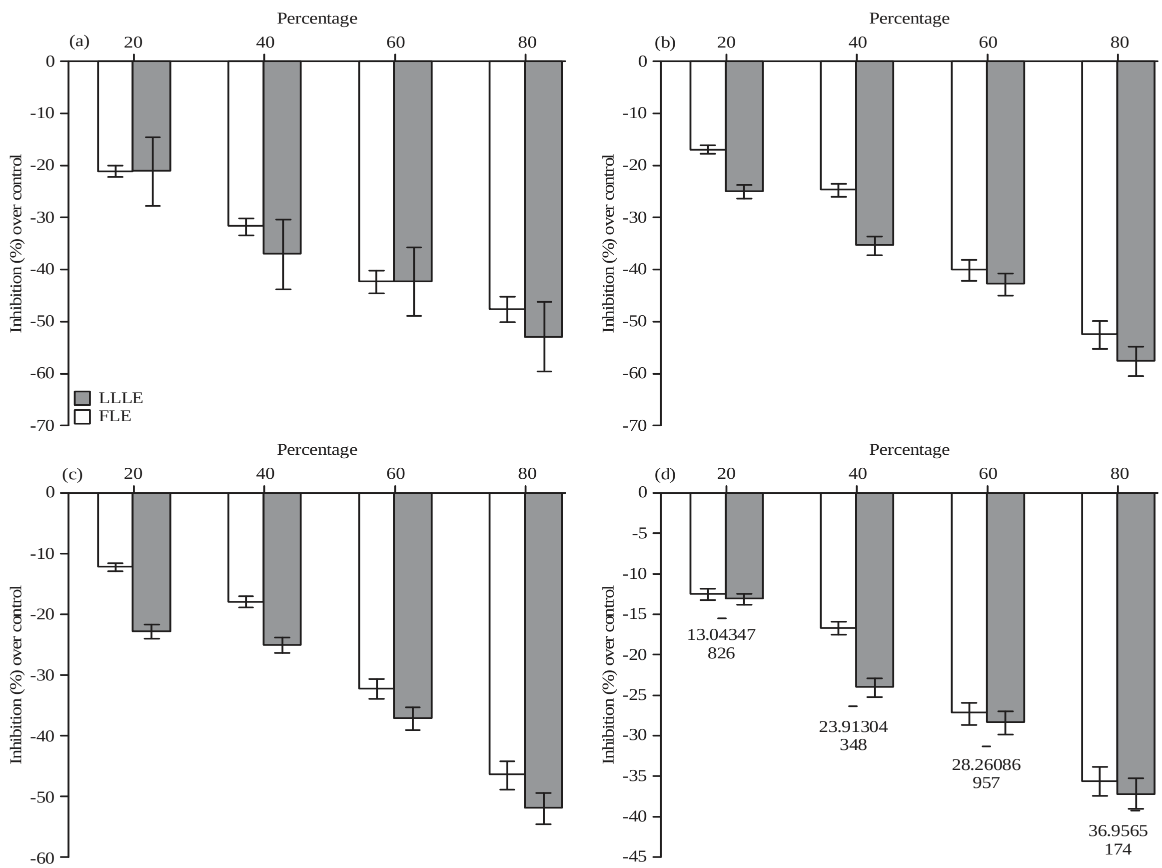

Fig. 1(a-d): Effects of fresh leaf extract (FLE) and leaf litter leachate (LLLE) of *C. odorata* on (a) Germination (%), (b) Seedling growth (shoot length), (c) Root length and (d) Dry matter of *Salvadora persica*

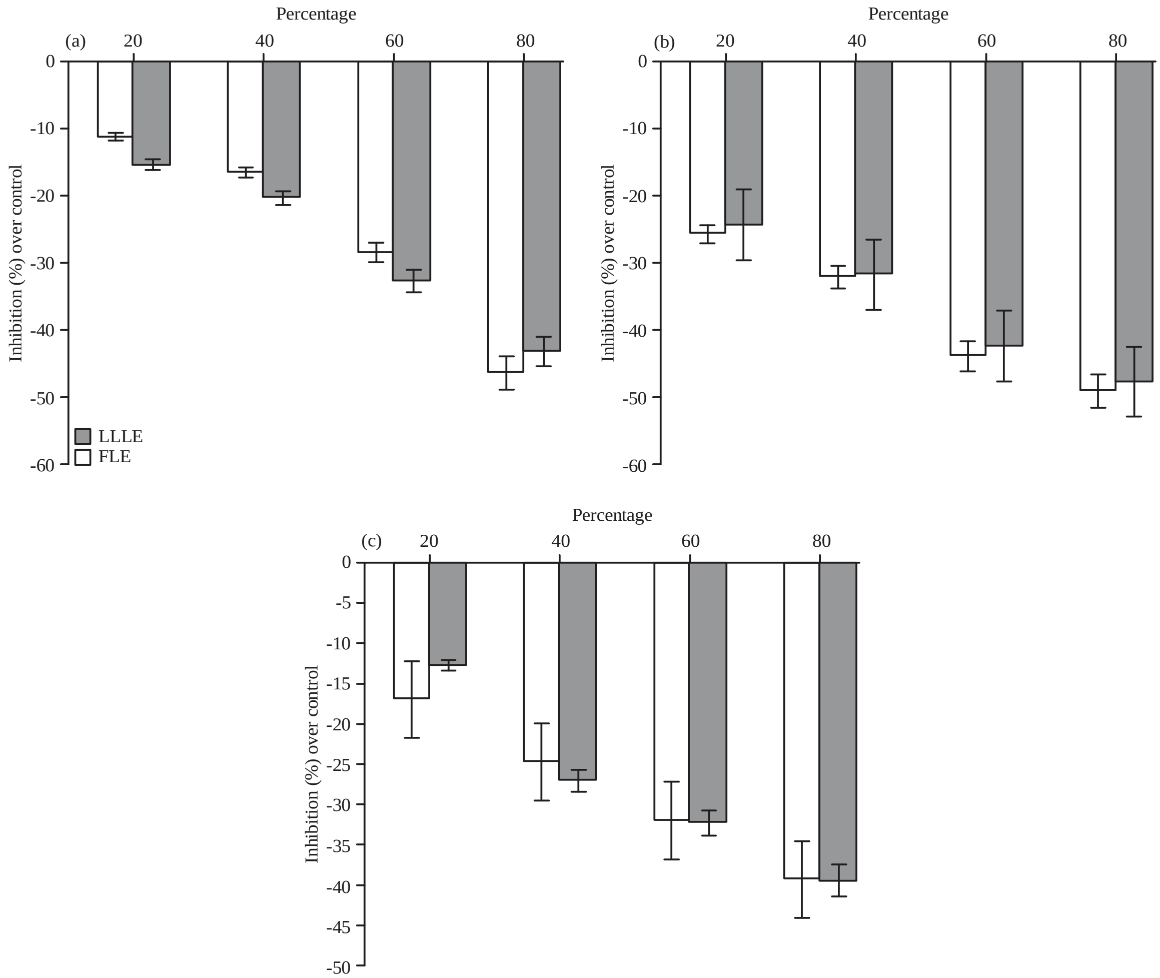

Fig. 2(a-c): Effects of fresh leaf extract (FLE) and leaf litter leachate (LLLE) extract of *C. odorata* on chlorophyll content, (a) Chl a-, (b) Chl b- and (c) Total Chlorophyll of *Salvadora persica*

trend in root and shoot length of *Salvadora* seedling occurred more at 80%. The percentage reduction in shoot length (57.33%) and root length (51.78%) observed at 80% leaf litter leachate extract (Fig. 1b and c). The dry biomass reduction significantly noticed more at 80% of all extract types, but the retardation effect varies with extract type, more in leaf litter leachate extract reduced by 37% followed by fresh leaves extract (Fig. 1d).

Biochemical changes: Both leaf extracts significantly decreased the amount of chlorophyll content. The maximum significant decrease in chlorophyll content was found with leaf litter leachate extract at 80% as compared to control (Fig. 2 a, b and c). The soluble protein content of *Salvadora* was reduced by 50-60% due to the leaf litter leachate extract and fresh leaf extract of *C. odorata* (Fig. 3a). The maximum reduction (32-36%) of total sugar was observed at 80% leachate concentration over control (Fig. 3b). The free proline content in *Salvadora* seedlings increased with increasing extract concentrations. At 80% leachate concentration nearly 26% increase in proline was observed over control (Fig. 3c). The phenol content in *Salvadora* seedling increased with increasing extract concentrations than control (Fig. 3d). The maximum stimulation (37%) of phenol content was observed at 80% leaf litter leachate concentrations (Fig. 3d).

There was a significant reduction ($p<0.05$) between chlorophyll and shoot length at 80%, leaf litter leachate concentration (%). The significant reduction ($p<0.05$) at 80% concentration was also observed in shoot length and phenol (Table 1).

Phytochemical analysis: Mass spectral analysis of bioactive volatile compounds from leaf litter leachate and fresh leaves of *Chromolena odorata* was shown in Table 2. Both the

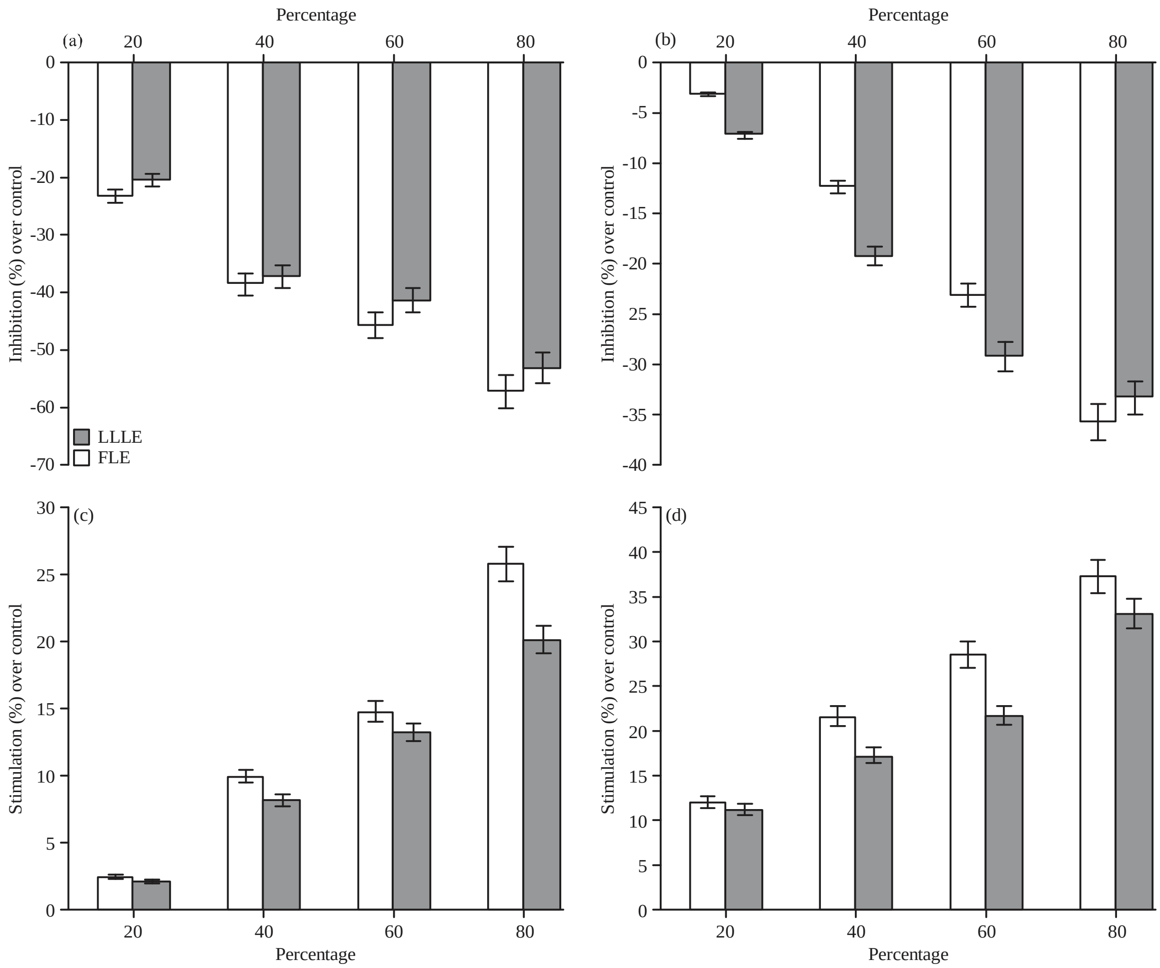

Fig. 3(a-d): Effects of fresh leaf extract (FLE) and leaf litter leachate (LLLE) extract of *C. odorata* on biochemical parameters of *Salvadora persica*, (a) Soluble protein content, (b) Total sugars, (c) Proline and (d) Phenol content

Table 1: Pearson correlation of biochemical parameters of test plants

Leaf litter leachate	Concentration (%)	Parameters level of significance
40	Phenol and chlorophyll	p<0.01
60	Phenol and proline	p<0.01
80	Chlorophyll and shoot length	p<0.05
	Shoot length and dry matter	p<0.01
	Phenol and soluble sugars	p<0.01
	Shoot length and phenol	p<0.05

extracts were rich in bioactive volatile compounds. Among these volatile compounds, some represented the class of monoterpenes, alkenes and alcohols. The major fatty acids found in leaf litter leachate extract were n-hexadecanoic acid, Cyclopentadecanone, Oleic acid, Octadecanal and 1,2-benzenedicarboxylic acid. Whereas, in leaf extract, the major fatty acids were n-hexadecanoic acid, Oleic acid, Octanoic acid, 2-Heptenal and 1,2-benzenedicarboxylic acid.

DISCUSSION

In the present investigation fresh extract and leaf litter leachate extracts of various concentrations of *C. odorata* had varying degrees of inhibition on the germination and growth of *Salvadora* seeds, reflects the allelopathic potential of the plant. According to Tawaha and Turk[26], lowering in germination rate due to allelo-chemical stress may be because of inhibition of water uptake. Cell division and elongation, which are growth prerequisite are known to be inhibited by allelo-chemical. Oudhia[27] in his study reported that the inhibition of seed germination was found to be concentration-dependent. Gulzar and Siddiqui[28] also reported that the aqueous extracts of various concentrations of leaf, fruit and flower of *C. procera* had varying degrees of inhibition on the germination and growth of mustard seeds, reflecting the allelo-pathic potential of the plant.

Table 2: Compounds detected in GC-MS/MS analysis in the leaf litter leachate (LLLE) and fresh leaf extract (FLE) of *Chromolena odorata*

Mol. wt.	Name of compound	LLLE area of peak (%)	FLE area of peak (%)
256	n-Hexadecanoic acid	22.05	18.53
240	Cyclopentadecanone	10.04	0.00
282	Oleic acid	7.20	5.37
268	Octadecanal	6.14	2.10
390	1,2-benzenedicarboxylic acid	5.10	4.14
112	2-Heptenal	3.42	4.47
154	2-Decenal	3.16	3.08
144	Octanoic acid	3.10	4.57
228	Tetradecanoic acid	2.98	0.00
102	2-Hexanol	2.94	0.00
142	Nonanal	2.70	4.18
618	Tetratetracontane	2.49	2.80
338	Tetracosane	2.43	3.25
270	Hexadecanoic acid	2.40	2.48
254	Heptadecane	2.31	0.00
296	Heneicosane	2.27	0.00
268	Oxirane	1.70	0.00
492	Pentatriacontane	1.35	3.60
126	2-octenal	1.31	4.38
282	2-nonadecanone	1.20	2.34
152	2,4-decadienal	0.96	2.63
210	1-pentadecene	0.25	0.96
249	9,12-Octadecadienoic acid	0.00	2.12
182	1-tridecene	0.00	3.76
324	4,8,12,16-tetramethylheptadecan-4-olide	0.00	3.01
158	Dec-2-en-1-ol	0.00	3.45
168	2-undecenal	0.00	3.20
256	Eicosanoic acid	0.00	2.12
100	Hexanal	0.00	2.29
252	1-Octadecene	0.00	0.77
	Total	87.50	89.60

The decrease in the chlorophyll concentration of *Salvadora* in all concentration of both extracts further reflected the reduction or decrease in the total soluble sugar. Reduction in chlorophyll content was previously reported as a result of allelo-chemical stress by several workers[29-31] which could be attributed to the inhibition of chlorophyll biosynthesis and/or the stimulation of chlorophyll[32]. Desai and Gaikwad[33] reported the reduction in chlorophyll content of rice cultivars due to the allelo-chemicals present in the leachate of mangrove *Excoecaria agallocha*. In this comparative study, though all both extract showed significant allelo-pathic potential, the degree of inhibition seemed to be highest in the case of the leaf litter leachate extract of *C. odorata*. According to Tripathi *et al.*[34] the reduction in photosynthetic pigments is due to adverse effect of allelo-chemical stress. They further stated that the reduction in chlorophyll contents observed in all the concentrations might be due to degradation of chlorophyll pigments or other phytochemicals present in leaf leachates. In view Pawar and Chavan[35], reduction in chlorophylls may decrease the photosynthesis and thereby substantially decrease further metabolites like total sugars, proteins and soluble amino acids. The reduction in chlorophyll content by leachates might be due to (i) Degradation of chlorophyll pigments or reduction in their synthesis and (ii) Action of flavonoids, terpenoids or other phytochemicals present in leaf litter leachate[36]. Increase in phenol contents was also responsible for reducing the seedling growth. The increase in phenol contents was correlated with the reduction in seed germination and seedling growth of *Salvadora*. The accumulation of proline content during the allelopathic effect in sorghum was reported by Pawar and Chavan[35]. According to them, the proline accumulation under stress conditions was mainly due to increased synthesis from glutamate. Increased proteolysis in germinating seeds can lead to an increase in free proline content along with other amino acids.

Both the extracts showed some allelo-chemical compounds. Xian *et al.*[37] reported several fatty acids with allelopathic activity. The myristic acid and palmitic acid inhibited the plant growth[38]. Geethambigai and Prabhakaran[39] studied the allelopathic potential of *Cyperus* and *Cynodon* on germination and growth of some rice cultivars and found that the weed *C. dactylon* contains beta-sitosterol, beta-carotene, vitamin C, palmitic acid and triterpenoids. According to Singh *et al.*[40] the most common allelo-chemicals include cinnamic and benzoic acids, flavonoids and terpenes. According to Einhellig[41], these compounds were phytotoxic. In view of John and Sarada[42], phenolic allelo-chemicals inhibited the plant root elongation, cell division, change the cell ultra-structure and subsequently interfered with normal growth and development of the whole plant. Desai and Gaikwad[33] studied the allelopathic effect of a mangrove *Excoecaria agallocha* on rice, they also reported some growth inhibitory allelo-chemicals in their study. Hence, if the plant present nearby agriculture land, they may disturb the stand establishment of cash crops. Thus, there is a need to take serious call on the presence of this plants near the crop filed. Further research can explore the effect of allelochemicals present in *C. odorata* as well as the allelopathic mechanism on other crop plants, through which this phytotoxic plant distracts the neighboring plants.

CONCLUSION

The extract of the weed *C. odorata* inhibited the germination and seedling growth of *Salvadora* due to its phytotoxic effects. The allelo-chemicals present in the weed extracts might be jointly synergistic to seeds germination and seedling growth of finger millet. Similarly, the phytotoxicity of *C. odorata* in this study might be due to the interactions of various groups of fatty acids and phenols. Hence, if present in the field, this weed can disturb the stand establishment of neighbouring plants. There is a need to take a serious notice of the presence of this weed in the crop fields and nearby places. Experiments to investigate its allelopathic behaviour on other crop plants and to better understand the role of this weed plant in structuring cropping in coastal areas.

SIGNIFICANCE STATEMENT

This study elucidate that the aqueous leaf extracts of the siam weed have a wide range of activities and containing allelopathic compounds with strong potential, which may play important role in weed control and could be used as an alternative of chemical compounds.

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