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Research Article
Allelopathic Effect of *Excoecaria agallocha* L. Mangrove Leaf Leachate on Germination and Growth Behavior of *Eleusine coracana* (Finger Millet)

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Abstract
Background and Objective: Allelochemicals extracted from plant either through leaf or root can affect growth and productivity of surrounding plants and crops. The purpose of this study was to determine the morphological, physiological and yield responses of finger millet growing near the mangrove *Excoecaria agallocha* L. in coastal areas. **Materials and Methods:** The study was conducted in the laboratory from November, 2016 to January, 2017 and used Randomized Block Design (RBD) with two factors, different concentrations (25, 50, 75 and 100%) of leaf leachates of mangrove *Excoecaria agallocha* and two finger millet varieties (brown and white). Germination, shoot length, seedling dry matter, vigour index were recorded at 10 days after sowing in all the test crops. The photosynthetic pigments, soluble proteins, osmolyte proline and phenol content were also estimated. Further gas chromatographic analysis was also done for the identification of allelochemicals. Correlation coefficient was determined by plotting data from all treatments and the relation amongst seedling growth parameters and seedling vigour index was examined. Significance between control and treatment was compared at 0.05 probability level. **Results:** Results showed that *E. agallocha* leaf leachate extracts have significant effect ($p<0.001$) on the entire seedling growth and highest inhibition was observed at 75 and 100% concentration. The correlation study revealed that the vigour index was significantly correlated with shoot length ($p<0.05$) and dry matter ($p<0.01$) for 100% leaf litter leachate concentration **Conclusion:** It is concluded that the inhibitory effect of the test species on seed germination and seedlings of finger millet cultivars may be related to the presence of allelochemicals including fatty acids, flavonoids and phenolic acids.

Key words: Crop physiology, physiological attributes, growth, development, allelopathic response, *Excoecaria agallocha,* finger millet

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Allelopathy is fascinating and perplexing subject that concerns with the interaction of plants as influenced by the chemical substances that they release into the environment[1-2]. Allelopathy is a mechanism in which chemicals produced by some plant species may increase or decrease the associated plant growth[3]. Such positive or negative effects are due to release of active biomolecules commonly called as "Allelochemicals"[4]. Allelopathic effect of one plant on the growth and development of the same plant or neighboring plants is due to production of a large variety of secondary metabolites. Allelochemicals usually are secondary metabolites, which are produced as byproducts during different physiological processes in plants[5,6]. As a result of litter decomposition a huge number of phyto compounds gets released into the environment. The multiple effects resulting from allelochemicals include effect on cell division, production of plant hormones, membrane permeability, germination of pollen grains, mineral uptake, movement of stomata, pigment synthesis, photosynthesis, respiration, protein synthesis, nitrogen fixation and specific enzyme activities[7,8]. Allelopathic compounds generally occur in natural plant communities and are suggested to be one mechanism by which weeds interfere with crop growth[9]. Several weed species are reported to have allelochemicals that affect germination and growth of crops due to toxicity[10]. The phytotoxic allelochemicals liberated from plants can exert stress which indirectly or directly affects the receiver plants[11]. Plant parts are the source of allelochemicals[12]. Large number of compounds are released by plants in their surroundings which are having either deleterious or beneficial role in an environment[13]. Allelopathy is expected to be an important mechanism in the plant invasion process because the lack of co-evolved tolerance of resistant vegetation to new chemicals produced by the invader could allow these newly arrived species to dominant natural plant communities[14].

Excoecaria agallocha L. is a member of Euphorbiaceae family also known as 'milky mangrove', 'blind-your-eye mangrove' and 'river poison tree'. Contact with skin can cause irritation and rapid blistering; contact with eyes will result in temporary blindness. This plant grows along the banks of cultivated field in konkan region of Maharashtra. Li *et al.*[15] studied allelopathic effect of *Sonneratia apetala* on a weed *Spartina alterniflora.* Allelopathic influence of *Excoecaria agallocha* L. on seed germination and seedling growth of some pulses and millets was studied by Kavitha *et al.*[16]. The widespread and persistent occurrence of this mangrove plant near coastal fields and especially around paddy and ragi crop fields makes it suspicious to cause some adverse effect on these crops through allelopathic interaction. Therefore there is always a threat that it may become a major problem of our cropping system. Keeping in view these facts, this study was planned to evaluate the phytotoxic effect of *E. agallocha* on germination, seedling growth, dry biomass, total chlorophyll content and biochemical parameters of *E. coracana*.

MATERIALS AND METHODS

Collection and preparation of aqueous leachate from leaf litter: Leaf litter of mangrove *E. agallocha* were collected from old trees growing on the banks of paddy fields in village Malvan, Konkan, Maharashtra. The study was conducted in the laboratory from November, 2016 to January, 2017 and used Randomized Block Design (RBD) with two factors, different concentrations (25, 50, 75 and 100%) of leaf leachates of mangrove *Excoecaria agallocha* and two finger millet varieties (brown and white). Samples were recorded by collecting recently fallen leaf litter (identified by its yellow or light brown color). The leaves were sun dried and cut into 3-6 cm pieces. The leaf litter leachates were prepared in our Laboratory, by soaking 200 g leaf litter in 200 mL double distilled water in a flask for 24 h at room temperature ($32\pm2°C$). The leaf litter leachates were filtered first through cheese cloth and then with Whatman No. 1 paper. The leaf litter leachate was diluted with distilled water to 25, 50 and 75 (v/v) concentrations and distilled water was used as control.

Bioassay: Twenty five seeds of each finger millet (*Eleusine coracana*) variety viz white and brown were chosen as test plant and pre-soaked overnight in distilled water. Before seed germination test, empty and undeveloped seeds were discarded by floating in tap water. The selected seeds of finger millet were thoroughly washed with tap water to remove dust for 5 min. 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 25, 50, 75 and 100% of leaf leachates. 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 polythene bags to prevent further loss of volatiles and kept in a seed germinator and were irrigated with

Table 1: Effect of leaf leachates of *E. agallocha* on seed germination and seedling growth of finger millet cultivars at 10 days after sowing

Concentration of leaf leachates	Germination (%)		Shoot length (cm)		Root length (cm)		Dry matter (g plant^{-1})		Vigour index	
	White	Brown	White	Brown	White	Brown	White	Brown	White	Brown
Control (DW)	90	95	6.3	6.8	4.7	5.6	0.45	0.46	991.8	996.5
25%	85	75	5.6	5.1	3.9	4.6	0.39	0.40	596.3	624.8
50%	70	65	5.0	4.4	3.4	4.2	0.36	0.35	498.2	541.3
75%	60	55	3.8	3.9	2.9	3.8	0.32	0.33	365.7	404.5
100%	55	50	2.7	2.9	2.4	2.7	0.27	0.29	302.4	337.5
CD at 5%	1.6	1.4	0.12	0.16	0.12	0.14	0.02	0.02	56.9	57.8

Table 2: Pearson correlation of biochemical parameters of test plants

Leaf litter leachate concentration (%)	Parameters	Level of significance
50	Phenol and chlorophyll	p<0.001
75	Phenol and proline	p<0.01
100	Vigor index and shoot length	p<0.05
	Vigor index and dry matter	p<0.001
	Phenol and soluble sugars	p<0.01
	Vigor index and phenol	p<0.05

10 mL leachate on alternate days. Germination, shoot length, seedling dry matter, vigour index were recorded at 10 days after sowing in all the test crops.

Biochemical analysis: Biochemical parameters such as chlorophyll contents were estimated according to Aron[17], by extracting the chlorophyll 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.*[18] and expressed as mg g^{-1} FW. Estimation of proline was done following the method of Bates *et al.*[19]. 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 [20] and expressed as μg g^{-1} DW.

GC-MS analysis for phytochemical compounds: Samples were analysed with a Hewlett-Packard (HP) 6890 gas chromatograph fitted with a Gerstel MPS2 auto sampler and coupled to a 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° 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 of data was done using software of SPSS 13.0. Nivas and Gaikwad[13] correlation coefficient was determined by pouting data from all treatments and the relation amongst seedling growth parameters and seedling vigour index was examined. Significance between control and treatment was compared at 0.05 probability levels.

RESULTS

Germination and seedling growth: The increase in leaf litter leachate concentrations reduced the seeds germination (%) (Table 1). The higher concentrations of leachate (75,100%) drastically reduced the germination . The germination in both accessions of finger millets were inhibited by leaf litter leachates over control and the inhibition followed the order: white>brown. The inhibition was more at higher concentration in white variety over control. Similar to germination, the shoot height in both finger millet accessions were inhibited due to increase in leaf litter leachate concentrations. The maximum suppression in shoot height was recorded in variety white variety at higher leachate concentrations, over control. Similarly in brown variety the highest decrease in shoot height was noticed at 100% leachate concentration. The roots of white and brown finger millet varieties appeared more sensitive to the *E. agallocha* leaf litter leachate and the suppression was pronounced over

control, which was further reflected in accumulation of dry matter at 100% concentration. The reduction was also recorded in vigour index, with the increasing leachate concentrations. The leaf litter leachate significantly (p<0.05) reduced the vigour index in all rice varieties. The highest reduction in vigour index was recorded in both varieties, with higher doses of leachate concentrations over control (Table 1). The correlation study revealed that the vigour index was significantly correlated with shoot length (p<0.05) and dry matter (p<0.01) for 100% leaf litter leachate concentration (Table 2).

Photosynthetic pigments and biochemical parameters: The chlorophyll contents were reduced significantly (p<0.05) in all treatments than control (Table 3). Moreover, the reduction of chlorophyll-b was greater than chlorophyll-a in both finger

Table 3: Effect of leaf leachates of *E. agallocha* on photosynthetic pigments of two finger millet cultivars at 10 days after sowing

	Chlorophyll a (mg g^{-1} FW)		Chlorophyll b (mg g^{-1} FW)		Total chlorophyll (mg g^{-1} FW)	
Concentration of leaf leachates	White	Brown	White	Brown	White	Brown
Control (DW)	1.52	1.63	0.94	0.95	2.61	2.68
25%	1.35	1.38	0.70	0.72	2.17	2.34
50%	1.27	1.30	0.64	0.65	1.97	1.96
75%	1.09	1.10	0.53	0.55	1.78	1.82
100%	0.82	0.93	0.48	0.50	1.59	1.63
CD at 5%	0.03	0.02	0.04	0.06	0.12	0.14

FW: Fresh weight, DW: Dry weight

Table 4: Effect of *E. agallocha* leaf leachates on soluble protein, proline and phenol content of two finger millet cultivars at 10 days after sowing

	Soluble protein (mg g^{-1} FW)		Proline (μM g^{-1} DW)		Phenol (μg g^{-1} DW)	
Concentration of Leaf leachates	White	Brown	White	Brown	White	Brown
Control (DW)	9.96	10.87	32.30	36.50	122.45	134.2
25%	7.65	8.65	40.12	46.25	137.20	149.3
50%	6.13	6.84	58.32	63.78	225.80	246.3
75%	5.73	5.96	60.20	72.60	379.80	385.7
100%	4.29	5.12	68.70	83.74	409.10	412.6
CD at 5%	0.43	0.32	1.80	2.30	2.10	1.8

DW: Dry weight, FW: Fresh weight

Table 5: Chemical composition of leaf extract

Compounds	Composition (%)
Stigmasterol	2.6
β-sitosterol	3.1
Lupeol	7.8
Phytol	23.4
Squalene	3.2
Stigma 4 en 3 one	8.3
9,12-octadecadienoic acid (z,z) octyl ester	1.6
1,2,3-benzenetriol	3.1
Lauric acid	16.32
Myristic acid	7.81
Palmitic acid	23.8
Arachidic acid	2.7
Behenic acid	0.82
Oleic acid	2.7
Stearic acid	1.3
Linoleic acid	2.1

millet test varieties at higher leachate concentrations (75 and 100%). The decrease in the chlorophyll concentration of finger millet varieties in all concentration of leachate further reflects the reduction or decrease in the total soluble sugar. In this study maximum reduction of total sugar was observe at 100% leachate concentration in all three rice varieties over control (Table 4). The free proline content in seedlings of both varieties increased with increasing leachate concentrations (Table 4). About 68% of proline was increased over control by 100% leachate concentration in brown variety. The phenol content in both finger millet seedling increased with increasing leachates concentrations than control (Table 4). The correlation study revealed that vigour index positively related ($p<0.05$) with phenol content at 100% leachates concentration (Table 2).

Phytochemical analysis: Mass spectral analysis of bioactive volatile compounds of *E. agallocha* leaves is shown in Table 5. It is clear from the table that the leaf leachate is rich in bioactive compounds. The major compound stigma 4 en 3 one and 9,12-octadecadienoic acid (z,z) octyl ester, squalene while other compounds are stigmasterol, β-sitosterol, 1,2,3-benzenetriol and phytol.

DISCUSSION

Among the concentrations, maximum inhibition was observed in 100%, followed by 75%. Similar observation in reduction of seed germination also reported in different crops[21]. An indirect relation between lower germination rate and allelopathic inhibition may be the result of inhibition of water uptake[22]. The inhibition of germination is dependent on the concentration of the extract; perhaps it may be due to the entry of water soluble allelochemicals into the seed, which retards the germination and growth[23]. Likewise, the results of present investigation on allelopathic effect of *E. agallocha* results are in accordance with previous studies of Nivas and Gaikwad[13] and Kavitha *et al.*[16] who observed allelopathic effect of *E. agallocha* on some pulses and millets. The allelopathic effect of different leaf leachate concentration on shoot length have been summarized in the Table 2. Shoot length was found to be suppressed significantly ($p<0.001$) in white variety with all treatments exhibiting concentration dependent. Nevertheless, the root length of both finger millet varieties were greatly inhibited with the increasing of concentration of leachates. The inhibitory effect was much more pronounced in brown variety at higher concentration. Among the studied varieties white exhibited more sensitive response. The inhibition shoot length by *E. agallocha* may be due to the presence of some phenolic compounds. These phenolic compounds might have interfered with the phosphorylation pathway or inhibiting the activation of Mg and ATPase activity or might be due to decreased synthesis of total carbohydrates, proteins and nucleic acids (DNA and RNA) or interference in cell division, mineral uptake and biosynthetic processes[24]. Also, allelochemicals inhibit absorption of ions[25] hence, resulted in arrested growth[26]. The results obtained in the present study were correlate with Beres and Kazinczi[25] in field crops, Sasikumar *et al.*[26] in pulse crops and Kavitha *et al.*[16] in pulses and millets, who noticed that root and shoot growth was inversely correlated to the concentration of the leachate solution. In the present study also increase in concentration retarded the growth of both the root and shoot and eventually affecting the overall length of the seedling. Similarly, the vigour index was also reduced with corresponding increase in concentration. Djanaguiraman *et al.*[27] found a similar type of result, that *E. globules* reduced the vigour index in green gram, black gram and cowpea. A similar inhibitory effect of *Digera muricata* on *Sorghum* was reported by Karthiyayini *et al.*[28]. Rashid *et al.*[29], reported impaired growth of lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*) seeds (root and shoot length and fresh weight) by the allelopathic potential of leaf and root leachates of kudzu (*Pueraria lobata*). Tanveer *et al.*[30] also reported that minimum GI and germination percentage of rice seeds were observed when such seeds were treated with leaf leachates of common cocklebur (*Xanthium strumarium)*. Sahoo *et al.*[31] reported the reduction in dry weight of chilli, soybean, maize, rice and lady's finger at higher concentrations of aqueous leaf extract from *Mangifera indica* L.

The reduction in vigour index in the studied Finger millet varieties may be due to reduced germination and shoot length as vigour index is the product of germination and seedling length. Dry matter per plant directly affects the final yield. *Excoecaria agallocha* leaf leachates decreased the dry matter in all the tested finger millet varieties and the magnitude of reduction was maximum in white variety. The magnitude of reduction in both varieties was concentration dependant. The reduction of biomass was correlated with reduced seedling growth. The reduction in biomass may be due to stunted and reduced seedlings growth[24]. These indicate, among the test varieties brown was comparatively tolerant to growth suppressed due to *E. agallocha* leaf leachates.

In white variety chlorophyll a and b was reduced by 50% and total chlorophyll by 70% in 100% concentrated leaf leachates. Reduction in photosynthetic pigments due to adverse effect allelochemical stress was previously reported[22]. The results of the present study are in accordance with the finding of Singh and Rao[32]. Siddiqui[33] reported reduction in chlorophyll content of *Vigna mungo* due to the allelochemicals present in leachate of black pepper which possibly target enzymes responsible for the conversion of porphyrin precursors. 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[22]. The more reduction of chlorophyll b than chlorophyll a, indicates its susceptibility to stress[18]. Reduction in chlorophylls may decrease the photosynthesis and thereby substantially decrease further metabolites like total sugars, proteins and soluble amino acids[34]. Oyerinde *et al.*[35] revealed the decrease in chlorophyll a, chlorophyll b and total chlorophyll accumulation in young plants of maize after being treated with fresh shoot aqueous extract of *Tithonia diversifolia* which is a weed plant known to possess allelopathic characteristics. Hussain and Reigosa[36] reported similar results regarding the effects of allelochemicals on chlorophyll content and photosynthesis process in plants.

Highest accumulation of proline was observed in white var. (87.46 μM g^{-1} DW) in comparison to brown variety (83.74 μM g^{-1} DW) at higher concentration (100%) of leaf leachates. The similar type of result in increase in proline content in sorghum was reported by Pawar and Chavan[34] also observed. The proline accumulation under stress conditions is mainly due to increased synthesis from glutamate. Increased proteolysis in germinating seeds can lead to increase in free proline content along with other amino acid[34].

The maximum phenol content was observed in white variety (438.9 μg g^{-1} DW) at 100% concentration. Increase in phenol contents was also responsible for reducing the seedling growth. The increase in phenol contents was correlated with reduction in seed germination and seedling growth of white variety. The maximum phenol contents in concentrated leaf leachates (100%) suggested that the concentration of phytotoxic allelochemicals inhibitory to growth.

According to spectral data, the identified compounds; stigma 4 en 3 one and 9,12-octadecadienoic acid (z,z) octyl ester are active phyto components against pathogens. Xian *et al.*[37] reported several fatty acids with allelopathic activity. The myristic acid and palmitic acid inhibits 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. The allelochemicals present in the weed extracts might be jointly synergistic to seeds germination and seedling growth of finger millet. Similarly the phytotoxicity of *E. agallocha* in this study might be due to the interactions of various groups of fatty acids and phenols.

Taking this in to account, it is necessary to ascertain whether the results observed for fallen senescent leaves are same for recently fallen leaves and whether they vary with seasonal conditions. Experiments to investigate its allelopathic behavior on other crop plants and to better understand the role of this mangrove plant in structuring cropping in coastal areas.

CONCLUSION AND FUTURE RECOMMENDATIONS

The results suggest that *E. agallocha* leaf leachate extract contain secondary metabolites that exhibit allelopathic effects at early stages of growth of finger millet plants under laboratory conditions. The growth inhibitory effect was stronger. Hence, if present in field, this plant can disturb the stand establishment of crop plant. There is a need to take a serious notice of the presence of this plant in the crop fields and nearby places. Further research can explore the allelochemicals present in *E. agallocha* as well as the complex allelopathic mechanisms through which this phytotoxic plant disturbs the neighboring plants.

SIGNIFICANCE STATEMENTS

This study discovers the possible inhibitory effect of water soluble substances present within the leaves of mangrove species. This study will help the local inhabitant and researcher to understand the allelopathic mechanism of mangrove *E. agallocha* for the lowering of growth and productivity of crop plants. Further, potential allelochemicals must be characterized as they can provide new and cheap synthetic analogues of natural products having greater selectivity, stability and efficacy to control weeds and pests. They should also undergo toxicity testing to confirm their safety on non-target species.

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