



## Allelopathic potential of mangrove species *Excoecaria agallocha* L. on growth responses of some agricultural crops

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*Excoecaria agallocha* L. also known as milky mangrove and 'Blind-Your-Eyes'-belonging to Euphorbiaceae family commonly grown in the mangrove forest of Pitchavaram, Tamil Nadu, south India was tested for its allelopathic potential against seed germination and early seedling growth of green gram (*Vigna radiata* (L.)R. Wilczek.), Black gram (*Vigna mungo* (L.)Hepper), groundnut (*Arachis hypogaea* L.), pearl millet (*Pennisetum typhoides* St. & Hub.) and finger millet (*Eleusine coracana* Gaertn.). Green house experimental studies were conducted in plastic pot with normal field soil for the evaluation of germination and growth attributes of crop plants by applying various concentrations (2,5,10,15,20 and 25%) of leaf extracts of *E. agallocha*. The results showed that the germination percentage was gradually decreased, seedling lengths also affected in all the five crops from lower concentration to higher concentrations of leaf extracts. The fresh and dry weights, Chlorophyll, amino acid and protein contents of crop seedlings were decreasing with increasing concentration of leaf extracts. The inhibitory effect was concentration dependent. The results clearly indicated that the leaf extracts of *E. agallocha* exhibited strong inhibitory effects on the germination and growth parameters of millets than pulses.

**KeyWords:** Allelopathy, *Excoecaria agallocha*, green gram, black gram, groundnut, pearl millet, finger millet.

## INTRODUCTION

Allelopathy is an important mechanism of plant interference by the addition of plant-produced phytotoxins to the plant environment. Many of the phytotoxic substances suspected of causing germination and growth inhibition have been identified from plant tissues and soil. These substances are termed allelochemicals or allelochemicals (Whittaker and Fenney, 1971).

Plants produce a large variety of secondary metabolites like phenols, tannins, terpenoids, alkaloids, polyacetylenes, fatty acids and steroids, which have an allelopathic effect on the growth and development of the same plant or neighbouring plants. Considerable knowledge has been obtained concerning the chemicals involved in allelopathy (Narwal, 1994). Patrick *et al.*, (1963) reported that depending on the decomposing conditions, substances of plant parts are highly toxic and nontoxic or stimulatory to other plant growth.

Plant parts like roots, rhizomes, stems; leaves, flowers/inflorescence, pollens, fruits and seeds are known to contain allelochemicals (Rice, 1984). Evidence for allelopathy has accumulated in the literature over many years and many kinds of allelochemicals have been identified, isolated and characterized from various plants, (Putnum, 1988; Gross, 1994; Seligler, 1996; Oyun, 2006; Bouchagier *et al.*, 2008; Borella and Lindamir, 2009 and Swapnal and Siddique, 2010).

Allelopathy includes both promoting and inhibitory activities and is a concentration dependent phenomenon (Daniel, 1999). Rajangam (1986), reported that the aqueous extracts of mangrove species *Avicennia marina* influenced on germination and growth of paddy. Rajangam and Arumugam recorded the inhibitory effects of *Excoecaria agallocha* plant extracts on rice seedlings. Li Jing *et al.*, (2010) reported that *Sonneratia apetala* potentially control *Spartina alterniflora* a weed by using allelopathy as a tool. However, the little information is available concerning the allelopathic studies in mangrove species with crop plants. Hence the present investigation has been aimed to evaluate the allelopathic potential of *Excoecaria agallocha* L. a mangrove species of Pitchavaram on growth and development of green gram (*Vigna radiata*

(L.)R.Wilczek.), Black gram (*Vigna mungo* (L.)Hepper), groundnut (*Arachis hypogaea* L.), pearl millet (*Pennisetum typhoides* St.& Hub.) and finger millet (*Eleusine coracana* Gaertn.). *Excoecaria agallocha* L. belonging to Euphorbiaceae family also known as milky mangrove, blind-your-eye mangrove and river poison tree, is poisonous. Contact with skin can cause irritation and rapid blistering; contact with eyes will result in temporary blindness.

## MATERIALS AND METHODS

Matured and fresh leaves of *Excoecaria agallocha* L. collected from Pitchavaram mangrove forest (11° 27'N 79° 47'E), is located 12km north east of Chidambaram, Cuddalore District, Tamil Nadu, India. Collected leaves were washed thoroughly and allowed to dry under shade for 20-25 days then made into fine powder. 1kg of powder was soaked in 2lit. distilled water and kept for 48 hours at room temperature with occasional shaking. The infusion was decanted and filtered through three layers of Whatman No.1 filter paper. From this (100%), different concentrations (2, 5, 10, 15, 20 and 25%) were prepared using distilled water for further studies.

**Table-1. Allelopathic Potential of *E.agallocha* on germination percentage of green gram, black gram, groundnut, pearl millet and finger millet (P < 0.05 Level).**

Extract Con. (%)	<i>Green gram</i>	<i>Black gram</i>	<i>Goundnut</i>	<i>Pearl millet</i>	<i>Finger millet</i>
Control	100	100.	98	95	94
2%	92 ( -8)	95 ( -5)	96.0 ( -2.04 )	90 (-5.2)	89 (-5.3)
5%	86 ( -14.0)	92 ( - 8.0 )	94.0 (-4.08)	82 (-13.7)	80 (-14.9)
10%	78 ( -22.0)	85 (-15.0)	87.0 (-11.2)	71 (-25.3)	69 (-26.6)
15%	71 ( -29.0)	79 ( - 21.0)	78.0 (-20.4)	63 (-33.7)	58 (-38.2)
20%	65 ( -35.0)	65 (-35.0)	71.0 (-27.5)	55 (-42.1)	51 (-45.7)
25%	52 ( -48.0)	59 (-41.0)	64.0 ( - 34.0 )	44. (-53.7)	42 (-55.3)
Variance	272.9048	238.1429	175.6667	350.9524	386.6667
Source of Variation	Pulses			Millets	
F	Treatments 101.87	crops 11.042		Treatments crops 644.41	18.06

The data in parantheses indicates % of decrease(-) over control.

**Table-2.Effect of aqueous leaf extract of *E.agallocha* on Seedling length (cm/plant) of green gram, black gram, groundnut, pearl millet and fingermillet (P < 0.05 Level).**

Extact Con.( %)	Greengram		Blackgram		Groundnut		Pearlmillet		Fingermillet	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
C	5.5	8.6	6.4	8.3	6.6	8.0	6.2	7.6	5.3	7.2
2	5.3 (-3.6)	8.4 (-2.3)	6.2 (-3.12)	8.2 (-1.2)	6.5 (-1.5)	7.8 (-2.5)	5.8 (-6.45)	7.4 (-2.6)	4.9 (-7.5)	7.0 (-2.8)
5	5.0 (-9.1)	8.1 (-5.8)	6.2 (-6.2)	8.0 (-2.4)	6.2 (-6.0)	7.5 (-6.25)	5.3 (-14.5)	7.1 (-6.6)	4.5 (-15.1)	6.7 (-6.9)
10	4.7 (-14.5)	7.8 (-11.9)	5.7 (-10.9)	7.5 (-8.5)	5.9 (-10.6)	7.2 (-10.0)	4.9 (-20.9)	6.7 (-11.8)	4.0 (-24.5)	6.1 (-15.3)
15	4.2 (-23.6)	7.2 (-16.2)	5.3 (-17.2)	6.9 (-15.8)	5.5 (-16.6)	6.9 (-13.7)	4.5 (-27.5)	6.2 (-18.4)	3.6 (-32.1)	5.6 (-22.2)
20	3.8 (-30.9)	6.7 (-22.1)	4.9 (-23.4)	6.5 (-21.7)	5.1 (-22.2)	6.4 (-20.0)	4.1 (-33.9)	5.4 (-28.9)	3.3 (-37.7)	5.1 (-29.1)
25	3.3 (-40.0)	6.1 (-29.1)	4.4 (-31.2)	6.0 (-27.7)	4.7 (-28.7)	5.9 (-26.2)	3.6 (-41.9)	4.9 (-35.5)	2.9 (-45.2)	4.5 (-37.5)
Variance	0.65619	0.85619	0.52952	0.80285	0.51476	0.57333	0.85805	1.04574	0.76231	1.02574
Source of variation	Pulses- Root Treatments Crops		Pulses- Shoot Treatments Crops		Crops		Millets- Root Treatments Crops		Millets-Shoot Treatments Crops	
F	508.14	919.00	179.47	29.76	522.53	803.30	321.22	106.77		

The data in parantheses indicates % of decrease(-) over control.

**Table-3.Effect of aqueous leaf extract of *E.agallocha* on Fresh Weight (FW) and Dry Weight(DW) (g/plant)of green gram.black gram,groundnut, pearl millet and fingermillet (P < 0.05 Level).**

Extract Con.(%)	Greengram		Blackgram		Groundnut		Pearlmillet		Fingermillet	
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
C	0.95	0.32	1.06	0.39	3.2	1.7	0.81	0.28	0.77	0.24
2	0.93	0.31	( - 1.8)	( - 2.5)	3.1	1.6	0.78	0.26	0.75	0.23
5	( -9.5)	12.5)	( - 6.8)	( - 10.2)	( -6.2)	11.6)	( -3.7)	( -7.1)	( -2.5)	( -11.5)
10	0.75	0.25	0.83	0.32	2.7	1.45	0.65			
15	( - 21.0)	( - 21.8)	( - 18.6)	( - 17.9)	( - 15.6)	( - 14.7)	( - 19.7)	( -28.6)	( -23.3)	( -30.7)
20	0.63	0.21	0.72	0.28	2.5	1.2	0.55			
25	( - 33.7)	( - 34.3)	( - 29.4)	( - 28.2)	( - 19.3)	( - 29.4)	( - 22.1)	( -39.3)	( -37.6)	( -42.3)
Varian	0.51	0.19	0.61	0.24	2.2	1.0	0.39			
ce	( - 46.3)	( - 40.6)	( - 40.2)	( - 38.4)	( - 31.2)	( - 41.1)	( - 51.8)	( -42.8)	( -51.9)	( -50.0)
Source	0.48	0.16	0.55	0.21	1.9	0.95	0.33			
of	( - 49.5)	( - 50.0)	( - 46.1)	( - 46.1)	( - 40.6)	( - 44.1)	( - 59.2)	( -50.0)	( -61.0)	( -53.8)
varianc	0.377	0.003	0.041	0.004	0.236	0.087	0.035	0.00272		0.00224
e	67	76	65	8	19	02	46	9	0.034257	8
F	9.17	292.83	3.379	149.35			442.66	38.22	159.	74.76

The data in parantheses indicates % of decrease(-) over control.

**Table-4.Effect of aqueous leaf extract of *E.agallocha* on total Chlorophyll,Aminoacid and Protein contents(mg/g.fr.wt.) of green gram.black gram and groundnut, (P < 0.05 Level).**

Extract Con. (%)	Green gram			Blackgram			Groundnut		
	Total Chl.	Ami no acid	Protei n	Total Chl.	Amin o acid	Protei n	Total Chl.	Amino acid	Protei n
C	1.05	3.11	9.12	1.25	3.23	9.45	1.45	4.56	12.45
2	1.00	3.05	9.05	1.12	3.18	9.38	1.38	4.49	12.33
	(-16.6)	(-1.9)	(-0.7)	(-10.4)	(-1.5)	(-0.7)	(-4.8)	(-1.5)	(-0.9)
5	0.950	2.95	8.85	1.00	3.12	9.22	1.19	4.32	12.15
	(-20.8)	(-5.1)	(-2.9)	(-20.0)	(-3.4)	(-2.4)	(-17.9)	(-4.8)	(-2.4)
10	0.915	2.82	8.62	0.960	2.95	9.00	1.12	4.20	11.93
	(-23.7)	(-9.3)	(-5.5)	(-23.2)	(-8.6)	(-4.7)	(-22.7)	(-7.8)	(-4.2)
		2.63	8.15		2.79				
15	0.829	(-	(-	0.869	(-	8.72	1.02	3.95	11.75
	(-30.9)	15.4)	10.6)	(-30.5)	13.6)	(-7.7)	(-29.6)	(-13.3)	(-5.6)
		2.49	7.81		2.65				11.13
20	0.785	(-	(-	0.822	(-	8.51	0.985	3.82	(-
	(-34.2)	19.9)	14.2)	(-34.2)	17.9)	(-9.9)	(-32.0)	(-16.2)	10.6)
		2.32	6.95		2.51	7.84			10.14
25	0.712	(-	(-	0.785	(-	(-	0.960	3.63	(-
	(-40.7)	25.0)	23.8)	(-37.2)	22.3)	17.0)	(-33.7)	(-20.4)	18.5)
		0.088	0.615	0.0280	0.077	0.324	0.0375	0.1222	0.662
Variance	0.01472	22	99	3	08	79	3	4	64
Source of variance	Total Chl. Treatments Crops			Amino acid Treatments Crops			Protein Treatments Crops		
F	36.277			62.217			2352.10		
							168.903		
							69.290		
							1003.02		

**Table-5.Effect of aqueous leaf extract of *E.agallocha* on total Chlorophyll,Aminoacid and Protein contents (mg/g.fr.wt.)of pearl millet and fingermillet (P < 0.05 Level)**

Extract Con. (%)	Pearlmillet			Fingermillet		
	Total Chl.	Amino acid	Protein	Total Chl.	Amino acid	Protein
C	0.92	3.05	8.23	0.87	3.02	8.12
2	0.854 (-7.2)	3.00 (-1.6)	8.12 (-1.3)	0.821 (-5.6)	2.93 (-2.9)	7.82 (-3.7)
5	0.832 (-9.5)	2.85 (-6.5)	7.65 (-7.0)	0.765 (-7.65)	2.72 (-9.9)	7.55 (-7.0)
10	0.819 (-11.0)	2.62 (-14.1)	7.43 (-9.7)	0.652 (-25.1)	2.53 (-16.2)	7.31 (-10.1)
15	0.737 (-19.8)	2.41 (-20.9)	7.12 (-13.5)	0.585 (-32.7)	2.34 (-22.5)	6.84 (-15.6)
20	0.602 (-34.5)	2.23 (-26.9)	6.83 (-17.0)	0.512 (-41.1)	2.12 (-29.8)	6.45 (-20.6)
25	0.538 (-41.5)	2.02 (-33.8)	6.15 (-25.3)	0.438 (-49.6)	1.95 (-35.4)	5.85 (-28.0)
Variance Source of Variation	0.019639	0.15579	2 0.16256	0.026308	0.469187	0.54324
F	Treatments 35.439	Crops 24.601	Treatments 606.7636	Crops 44.304	Protein Treatment s 161.9442	Crops 29.377

The data in parantheses indicates % of decrease(-) over control.

Certified seeds of green gram (*Vigna radiata* (L.)R.Wilczek.)Cv.VBN-1,Black gram (*Vigna mungo* (L.)Hepper.)Cv.T-9,groundnut (*Arachis hypogaea* L.)Cv.VRI-2,pearlmillet(*Pennisetum typhoides* St.&Hub.)Cv.K-3 and fingermillet (*Eleusine coracana* Gaertn.) Cv.CO-11.were used in germination studies in the experiment. The selected seeds were surface sterilized with 0.2g HgCl<sub>2</sub> solution for 2 min. and then washed thoroughly with distilled water. For the germination study, 25 seeds were sown in plastic pots (12cm dia.) with 700g normal field soil under the green house. The treatments were replicated five times.On the first day, different concentrations of equal amount of aqueous extracts of *E. agallocha* was irrigated in each

treatment (2,5,10,15,20 and 25%) in the Plastic pots. Distilled water served as control. Afterwards the seeds were allowed to germinate in the green house till 12 days after the seed was sown. The leaf extracts /distilled water was irrigated per pot on 3, 6, 9 and 11<sup>th</sup> day after the seeds were sown. The emergence of radicle was taken as criterion for the germination of seeds. The growth parameters and bio chemical constituents like Chlorophyll (Arnon,1949), aminoacids (Moore and Stein,1948) and proteins (Lowry *et al.*, 1951) were analyzed on 12 day old seedlings of all the test crops. The obtained mean data were analyzed statistically (ANOVA) to find out the significance (P< 0.05 Level) of the treatments on the crops.

## RESULTS AND DISCUSSION

The results of the germination percentage, seedling growth and fresh and dry weight of green gram,blackgram,groundnut, pearlmillet and fingermillet seeds treated with aqueous leaf extracts of *E.agallocha* are given in Tables-1,2&3. In comparison with the control, aqueous leaf extracts of *E.agallocha* showed inhibitory effects on seed germination percentage, root and shoot length, fresh and dry weight of all the five test crops. The degree of inhibition increased with increasing extract concentration. The highest extract concentration (25%) showed significant inhibitory effects on all growth parameters measured (P<0.05). Among the five test crops studied, the greatest reduction in germination percentage and seedling length, biomass production was noticed in fingermillet. The least inhibition percentage of seed germination and other growth parameters were observed in groundnut than other four crops. The order of inhibition percentage was fingermillet>Pearlmillet>greengram>blackgram>groundnut. Similar findings were previously noticed by Rajangam and Arumugam (1999). They found that the aqueous leaf extract of *E.agallocha* inhibited seed germination and plumule and radicle elongation of rice. Similar allelopathic inhibition of *Eucalyptus*, have been observed in pearlmillet (Pathy *et al.*, 2000). Parvin *et al.*(2011) concluded that the aqueous leaf extracts and roots of *Albizia lebbeck* had highest allelopathic effects on germination, growth and development of mungbean and soybean . The study of Rejila and Vijayakumar,(2011) revealed that aqueous leaf extract of *Jatropha curcas* inhibit the growth of *Capsicum annum*. The present results are in coincide with the studies on *Acacia nilotica* on cereals and legume crops(Duhan, and Lakshminarayanan,1995).

Allelopathic activity depended on the concentration of the extracts, target species, and the plant tissues from which the chemicals were extracted. Increasing inhibitory rates with increasing concentration was in accordance with previous reports (Ismail and Chong 2002; Singh *et al.* 2003; Batish *et al.* 2006). Phytotoxic effects may be caused by more than one chemical component present in the leaves and the crop species react differently to these compounds and the inhibition might have been presence of allelochemicals in the plant extracts (Chaturvedi and Jha,1992).Swaminathan *et al.*, (1989) reported that the potential compounds which are able to induce inhibitory effect on seed germination are identified as phenolic acids. Allelopathic agents may act in many ways directly and indirectly to modify plant growth. They may inhibit cell division and cell elongation, inhibit hormonal relations, modify mineral uptake, retard photosynthesis, inhibit protein synthesis, change permeability of membranes, inhibit specific enzymes and affect respiration and stomatal opening (Rice, 1974 and Lodhi, 1976). Membrane plays as key role in the ability of a plant cell to withstand stress of plants.Allelochemicals can damage cell membrane through direct interact with its constituent or an impairment of some metabolic function necessary to the maintenance of membrane function (Yebing Che *et al.*, 2009).The total chlorophyll, aminoacid and protein contents were significantly reduced in all the five test crops treated with leaf extract of *E.agallocha* (tables-4&5). Among the pulses, groundnut showed lesser inhibition on the biochemical constituents than black gram and green gram.

Between the millets, the maximum inhibitory effect was observed in finger millet than pearl millet. The results showed that the percentage of inhibition on the total chlorophyll, proteins and amino acid contents of test crops increase with increasing the leaf extract concentrations of *E. agallocha*. Similar to this result, there was a reduction on total chlorophyll contents in rice seedlings treated with aqueous leaf extract of *E. agallocha* (Rajangam and Arumugam, 1999) and another mangrove *Avicennia marina* on rice seedlings (Rajangam, 1986). The present results also coincides with Padthy *et al.*, (2000), where the decrease of chlorophyll pigments, protein, carbohydrate and nucleic acid contents of shoot and roots of finger millet by leaf leachates and extract treatments of Eucalyptus. Yang *et al.*, (2006) reported that the reduction of Chlorophyll pigments in rice seedlings was caused by leachates of *Ageratina adenophora* and in groundnut by the extracts treatment of bamboo (Eyine *et al.*, 1989). The application of aqueous leaf extracts of *E. agallocha* influenced negatively on the metabolism of seed germination and seedling growth of pulses and millets. The known targets of the allelochemicals affect the cell division, production of plant hormones and their stability and permeability of membrane, mineral uptake, pigment synthesis, photosynthesis, respiration, amino acid synthesis, nitrogen fixation, specific enzyme activities and conduction tissue (Wink *et al.*, 1998, Rizvi *et al.*, 1992).

Allelopathic inhibition is complex and can involve the interaction of different classes of chemicals like phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, carbohydrates, and amino acids, with mixtures of different compounds sometimes having a greater allelopathic effect than individual compounds alone (James and Bala, 2003). The phenolic acids identified from the leaves of *E. agallocha* are  $\beta$ -hydroxybenzoic, syringic, vanillic, protocatechuic, caffeic and chlorogenic acids (Rajangam and Arumugam, 1999). Phenolic acids have already been reported to be inhibitors on germination and plant growth. The inhibitory effect of phenolic acids on plant growth were noticed by many researchers.  $\beta$ -hydroxybenzoic acid and vanillic acid (Grummer and Beyer, 1960; Whitehead, 1964; Chandramohan *et al.*, 1973), vanillic acid (Leela, 1981), Syringic acid (Guenzi and McCalla, 1966), protocatechuic, vanillic and  $\beta$ -hydroxybenzoic acids (Winter, 1961; Chou and Muller, 1972), chlorogenic acid and caffeic acid (del Moral and Muller, 1970; Al-Mousawi and Al-Naib, 1975; Lodhi, 1975; Kanchan and Jayachandra, 1980). These studies are in conformity with the present findings. Phytotoxicity of allelochemicals present in the leaf extracts of *E. agallocha* might be caused by their synergistic activity on the retardation of growth and biochemical constituents of test seedlings rather than single chemical. The potential of allelopathic inhibition exhibited by *E. agallocha* was more on millets than pulses and the degree of inhibition was dependent on concentration of extracts. Further, the identification of specific inhibiting allelochemical screening studies and wider range of bioassay for pre and post-emergent experiments are required with other crop and weeds to develop a potential natural herbicides from *E. agallocha* in future.

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