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RESEARCH ARTICLE

Herbicidal efficacy of *Excoecaria agallocha* L., a mangrove plant on growth and development of barnyard grass (*Echinochloa colona* L.)

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Abstract

Barnyard grass (*Echinochloa colona* L.) is one of the noxious weed in rice field which significantly affects the growth and development of rice from germination stage to harvest stage. Various concentrations (1, 2.5, 5, 10, 15 and 20g/l) of aqueous extracts prepared from shade dried leaves of (*Excoecaria agallocha* L.) a milky mangrove and determined their herbicidal efficacy against the germination, growth and biochemical changes of barnyard grass. The green house experimental results showed that the germination of weed seed was gradually delayed when concentration increased from lower to higher level and higher degree of inhibition was noticed when it exposed to 15 and 20g/l) extract concentration. Generally, the radical length was more suppressed than plumule length against the extract treatments.

INTRODUCTION

Weed may be defined as plants with little economic value and possessing the potential to colonize disturbed habitats of those modified by human activities. Weed causes a number of harms in agro ecosystem, due to their interference with reduce crop yields leading to huge losses on a global scale. Allelopathic interference mechanisms are particularly difficult to separate from field condition. Plant interference can be defined as any physical or chemical mechanism that results in the reduction of plant growth over times due to the presence of other plant (Leslie A. Weston and Duke 2003). Competition is usually described on the process whereby plants interfere with the growth of neighboring plants by utilization or competition for growth-limiting resources (light, space, nutrients and moisture). The costs of weed eradication are also enormous. (Macias et al., 2004).With the discovery of synthetic herbicide in the early 1930s, there was shift in the weed management practices toward high input and target oriented ones, but the use of herbicides has proved an increasing incidence to resistance to herbicide in weeds.

The intention of any herbicide treatment is to reduce the abundance of weeds to below some economically acceptable threshold, judged on the basis of the amount of damage that can be tolerated to crops. However, if herbicides are not used properly, damage may be caused to crop plant, mammals and birds. There are also concerns about the toxicity of some herbicides, which may affect people using these chemicals during the course of their occupation, people indirectly exposed through drift or residue on food and wildlife.

By these reasons, allelopathy is an emerging tool to replace the synthetic herbicides for ecofriendly weed management process in the sustainable agriculture. Hence, the present investigation was aimed to evaluate the herbicidal efficacy of *Excoecaria agallocha* ; a milky mangrove species of Pitchavaram mangrove forest, Tamil Nadu, India on barnyard grass (*E. colona* L.).

2. Materials and Methods

Experiments were carried out during the month of January 2013 to June 2013 at Botanical Garden, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu. Physico-chemical properties of experimental soil has been described in table no. 2.

2.1 Plant sample collection

Leaves of *Excoecaria agallocha* L. were collected from Pitchavaram mangrove forest and viable seeds of Barnyard grass (*Echinochloa colona* L.) were collected from the post harvest filed of rice of Annamalai Nagar, Tamilnadu and stored in polyethene bags in dry and dark place until further use.

2.2Plant extract preparation

E.agallocha leaves were thoroughly washed in the tap water by 2 to 3 times followed by 0.1% Mercuric Chloride ($HgCl_2$) for maximum sterilization. Thereafter, leaves were chopped into small pieces and dried in to the hot air oven at 67°c for about 48 hours than grinded with the help of ordinary grinder until a powder form is formed. Various concentrations of aqueous leaf extracts prepared at the level of extract i.e. C (T_0). 1(T_1), 2.5 (T_2), 5 (T_3), 10 (T_4), 15(T_5) and 20 (T_6)

2.3 Bioassay for germination percentage:

Rectangular plastic tray (45cm×30cm×12cm) containing 3.5 kilogram of normal garden soil (description of soil is mentioned in table no 2) used as a medium for the bioassay experiments. The 100 viable seeds of Barnyard grass were taken and sterilized for two minutes in 1% Sodium hypochlorite (NaClO) solution for 25 min than thoroughly washed with tap water and sown.

Each tray was irrigated uniformly by different concentrations of aqueous extracts C (T_0). 1g/l (T_1), 2.5 g/l (T_2), 5 g/l (T_3), 10 g/l (T_4), 15 g/l (T_5) and 20 g/l (T_6) and the tap water was used as control. Each experiment was carried out with five replicates and extracts/water was irrigated to the tray in alternative day's upto 40th day and germination percentage was recorded on 10th day after seed sown (DAS).

2.4 Bioassay for seedling growth (physical and chemical parameter)

Weed seedlings were uprooted, washed thoroughly and used for analysis of shoot and root length, fresh weight, dry weight, chlorophyll, sugar and protein contents on 15, 30 and 45 DAS. The mean data was statistically analyzed by ANOVA followed by Tukey's Multiple Range Test at $P < 0.05\%$ level.

2.5. Preliminary Phytochemical analysis of E.agallocha. (Suganya et al. 2012)

Pet. Ether, n-hexane, chloroform, ethyl acetate, ethanol and methanolic extraction of E.agallocha leaf was investigated for the presence of phytochemical constituents such as Alkaloids, Flavonoids, Saponins, Cardiac glycosides, Glycosides, Saponins glycosides and Tannins (Table-1). The result showed the presence of cardiac glycosides and glycosides in pet. Ether; glycosides, steroids and tannins in hexane; alkaloid, flavonoid, Saponins, Saponins glycosides, steroids and tannins in chloroform; alkaloid, cardiac glycosides, flavonoid, glycosides, Saponins, Saponins glycosides and steroids in ethyl acetate, cardiac glycosides, Saponins, Saponins glycosides and tannins in ethanol; alkaloid, cardiac glycosides, flavonoid, glycosides, Saponins glycosides, steroids and tannins in methanol.

3. Result and Discussion

3.1. Germination percentage

The bioassay results revealed that the germination was gradually reduced with increasing the extract concentrations over control (Table-3). At T_5 (15g/l) and T_6 (20g/l) aqueous extract concentration, *E. agallocha* exhibited significant phytotoxic effects on the seed germination of *E. colona*. These results are coincides with Bhagvathy and Xavier (2007) who noticed that aqueous extracts of *Eucalyptus* leaves decreased the sorghum seeds germination percentage as the concentration of extract increased. Prashad and Pridarshani (2006) also stated that aqueous leaf and flower extracts of *Parthenium hysterophorus* significantly reduced the seed germination, seedling survival, hypocotyl and cotyledon area of turnip which is strong support of present findings. Significant germination percentage reduction (81% at 10^{th} DAS) was recorded at T_6 than other concentration. Similar findings were reported by Xiangju Li et al. (2005) in which, the aqueous extracts of wheat plants collected at seedling stage of all the cultivars noticeably reduced the germination of crab grass. Dengre and Singh (2007), who stated that 10% leachates of *Ageratum canzyoides* caused maximum inhibition in root length followed by *Parthenium hysterophorus* (27.4) and *P. plebeium* (18.6%). The reduction in the germination percentage of weed seed may be caused by adverse effect on α and β amylase when treated with E.agallocha extract. It can also possible to change cell wall permeability of seed due to phytotoxic effect of E.agallocha.

3.2 Seedling growth and weed biomass of barnyard grass (Fig – 4).

At T_6 treatment, shoot length, root length, fresh weight and dry weight of weed seedling noticeably decreased. These findings are favored by Fatunbi et al., (2009), in which they observed that leaf extracts of

Acacia mearnsi exhibit the profound suppression of seed germination, radicle elongation, the seedling emergence in the soil and biomass production during the early growth of sorghum at higher extract concentration. Barkatullah et al., (2010) reported that various allelochemicals form aqueous extract of *Dodonaea viscosa* leaves, bark and flower which exhibited allelopathic stress against the germination seedling growth, fresh and dry weight of maize. Result shown that at higher concentration (20g/l) of aqueous extract, the inhibition percentage of root was recorded as 74%, 81% and 76% over the control at 15^{th} DAS, 30^{th} DAS and 45^{th} DAS respectively. Similar result was also observed by Holithi et al., (2008) who noticed that the water extract of the cucumber plants inhibited the germination, growth of the shoots and roots and fresh weight of barnyard grass under petri dish conditions. The reduction percentage on shoot length was recorded as high as 74%, 79% and 70% over the control at 15^{th} DAS, 30^{th} DAS and 45^{th} DAS respectively. The inhibition was more pronounced in root system than shoot system since root is the first receptor for all the allelochemicals. The degree of reduction percentage is directly proportional to the strength of extract. The reduction in shoot length and root length may be due to adverse effect of allelochemicals which are present in aqueous extract of *E. agallocha*. It is also possible to effect on certain growth hormones which are very necessary for plant growth. At 20% extract concentration, fresh weight was drastically reduced upto 76%, 81% and 70% over the control at 15^{th} DAS, 30^{th} DAS and 45^{th} DAS respectively. In case of dry weight, the percentage of reduction was 75%, 79% and 67% compare to control at 15^{th} DAS, 30^{th} DAS and 45^{th} DAS respectively. Reduction in fresh and dry biomass of barnyard grass may cause by the significant reduction in chlorophyll and sugar content of weed when treated with aqueous extract of *E.agallocha*.

3.3 Chlorophyll and sugar contents of barnyard grass.

Herbicidal effect of *E. agallocha* on changes in chlorophyll and sugar contents of *E. colona* are represented in table 5. The results revealed that, all the concentrations have exerted the toxic effect to reduce the chlorophyll and sugar contents of the weed seedlings. At low concentration, reduction was insignificant but at higher level, the phytotoxic effect was well prominent.

At 20g/l concentration of *E.agallocha* on inhibition percentage of total chlorophyll contents was 67%, 77% and 62% over the control on 15^{th} DAS, 30^{th} DAS and 45^{th} DAS respectively. This finding was supported by Bhagvathy and Xavier (2007), who noticed that the chlorophyll ‘a’, chlorophyll ‘b,’ total chlorophyll and carotenoid contents of the sorghum plants were reduced due to application of leaf extracts of *Eucalyptus* on reference with control plants. Bouchagir et al., (2008) reported that aqueous extract of Bermuda grass significantly reduced the amount of chlorophyll in cotton plants. Obtained results are also supported by Viles and Reese (1996), who reported that the chlorophyll contents in the seedlings of *Lactuca sativa, Panicum virgatum, Sobrobolus heterolepis*, decreased when treated with shoot extract of *Echinacea angustifolia*. The percentage of total sugar content inhibition was 71%, 78% and 72% at 20g/l extract concentration over the control on 15^{th} DAS, 30^{th} DAS and 45^{th} DAS respectively. Similar findings were observed by Prasad et al., (1999) who noticed that the application of aerial and root biomass of *Rhamnus virgatus* trees significantly decreased the sugar and starch contents in turnip as compared to control. Such type of sugar reduction may be due to the allelochemicals which may alter the leaf diffusibility, transpiration rate and stomatal aperture in bean and sunflower and these might also affect photosynthesis and directly carbohydrates amount of bean and sunflower (Polova and Vicherkova (1986) and Vicherkov and Polova (1986).

3.4 Amino acid and protein content of barnyard grass.

Table 6 shows the result of amino acid and protein content of *E. colona* treated with various concentrations of aqueous leaf extract of *E.agallocha*. The inhibition level on amino acid was upto 71%, 72% and 61% over the control at 15^{th} DAS, 30^{th} DAS and 45^{th} DAS respectively. These results are favored by Bhagvathy and Xavier (2007) who found that sorghum plants treated with *Eucalyptus* leaf extract had strong inhibitory effect on the protein content. Generally allelochemicals caused a gradual decrease in the content of total proteins when increased the extract concentration on weed species. The retardation of amino acid and protein content was directly proportional to the extract concentration. At 20g/l extract concentration, the percentage of inhibition on protein content was recorded as 80%, 83% and 68% over the control at 15^{th} DAS, 30^{th} DAS and 45^{th} DAS respectively. At the lower extract concentrations protein showed insignificant retardation. Allelochemicals can also effect the adverse effects on protein synthesis by disturbing translation process or they can also effects the all the intermediate enzymes which are very necessary for translation process.

Table 1. Preliminary Phytochemical analysis of *Excoecaria agallocha* L. (Suganya et al., 2012)

Phytochemical	**Organic solvent**					
	Pet. Ether	n-Hexane	Chloroform	Ethyl acetate	Ethanol	Methanol
Alkaloid	-	-	+	+	-	+
Cardic glycosides	+	-	-	+	+	+
Flavonoid	-	-	+	+	-	+
Glycosides	+	+	-	+	-	+
Saponins	-	-	+	+	+	-
Saponins glycosides	-	-	+	+	+	+
Steroids	-	+	+	+	-	+
Tannins	-	+	+	-	+	+

(+) symbol represent availability of Phytochemical and (-) symbol represents lacking of Phytochemical.

Table 2. Physiological properties of soil of Experimental pots (pre- treatment and post – treatment)

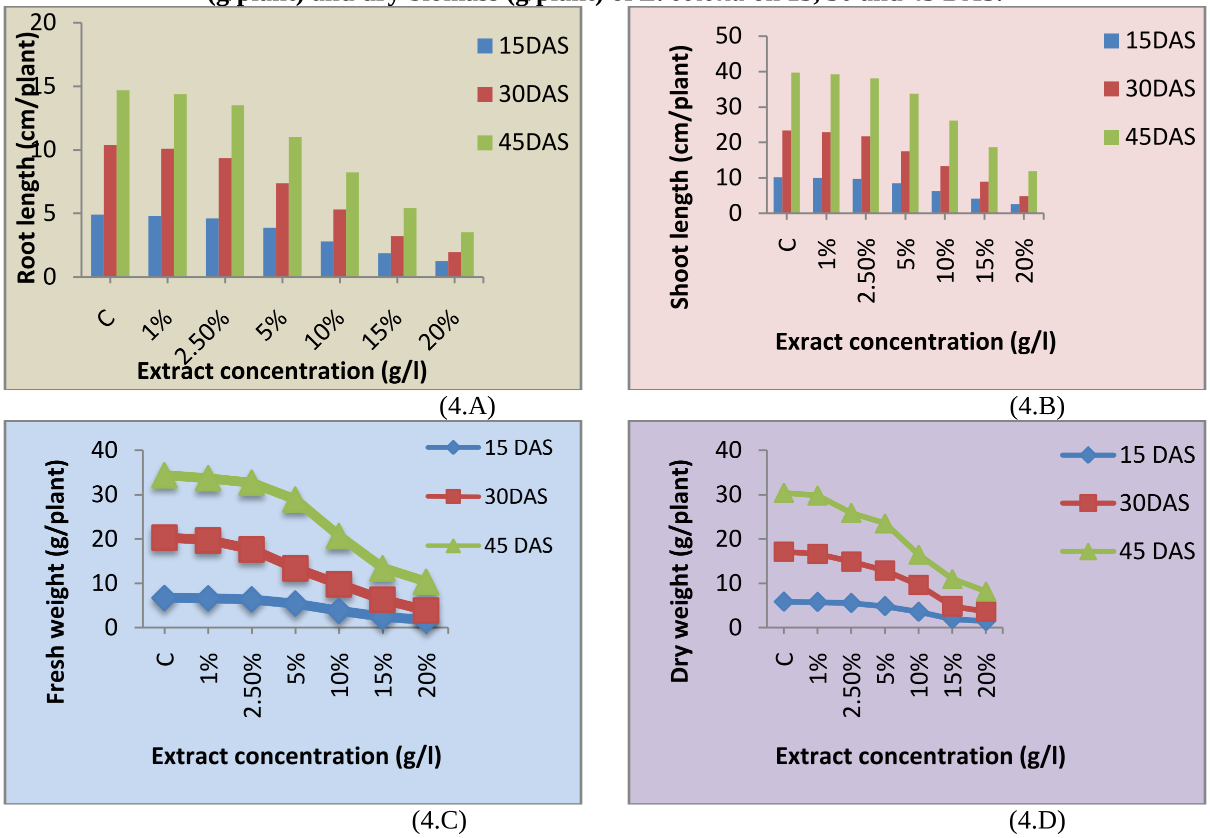
	Mature leave extract treatment on 30 DAS	
	Barnyard grass	
Parameters	Pre-treatment	Post- treatment
Texture	Light clay	Light clay
Sand (%)	61.3	54.2
Silt (%)	29.9	24.5
Clay (%)	21.7	22.1
pH	7.6	7.9
EC(ds/m)	1.25	.233
Organic carbon (%)	.25	.74
Total nitrogen (%)	.89	0.32
Available P (%)	.20	.05

Table 3- Effect of aqueous extract of *E.agallocha* leaves on germination percentage of E. colona L. on 10 DAS.

Extract Concentration	Germination percentage
C (T_0)	90a
1g/l(T_1)	86.13a
2.5 g/l (T_2)	81.32b
5 g/l (T_3)	74.43c
10 g/l (T_4)	54.48d
15 g/l (T_5)	28.62e
20 g/l (T_6)	16.89f

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 4, C, control; 1, 2.5, 5, 10, 15 and 20 denote the aqueous concentration of E.agallocha.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) (P < 0.05).

Fig. 4. Effect of aqueous extract of *E.agallocha* leave on Plant length (cm/plant) and weed fresh weight (g/plant) and dry biomass (g/plant) of *E. colona* on 15, 30 and 45 DAS.



- 4. A- Root length, 4.B- Shoot length, 4.C-Fresh weight and 4.D-Dry weight.
- Mean values ± S. D., n = 5, C, control; 1, 2.5, 5, 10, 15 and 20 denote the aqueous concentration of *E.agallocha*.

Table 5- Effect of aqueous extract of *E.agallocha* leaves on total chlorophyll content (µg/g of plant FW) and total sugar content (µg/g of plant FW) of *E. colona* on 15, 30 and 45 DAS.

Extract concentration	*E. colona*					
	Chlorophyll content			Sugar content		
	15 DAS	30DAS	45 DAS	15 DAS	30DAS	45 DAS
C (T_0)	0.772a	0.743a	0.601a	2.553a	2.410a	1.491a
1g/l(T_1)	0.764a	0.717a	0.594a	2.501a	2.289b	1.416a
2.5 g/l (T_2)	0.748b	0.708b	0.576b	2.425b	2.144b	1.341b
5 g/l (T_3)	0.687c	0.665c	0.528c	2.221c	1.831c	1.992c
10 g/l (T_4)	0.524d	0.505d	0.420d	1.736d	1.420d	0.544d
15 g/l (T_5)	0.308e	0.278e	0.234e	0.868e	0.819e	0.346e
20 g/l (T_6)	0.254f	0.193f	0.128f	0.740f	0.530f	0.197f

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 4, C, control; 1, 2.5, 5, 10, 15 and 20 denote the aqueous concentration of E.agallocha.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) ($P < 0.05$).

Table 6- Effect of aqueous extract of *E.agallocha* leaves on amino acid content (µg/g of plant FW) and protein content (µg/g of plant FW) of *E. colona* on 15, 30 and 45 DAS.

Extract concentration	*E. colona*					
	Amino acid			Protein		
	15 DAS	30DAS	45 DAS	15 DAS	30DAS	45 DAS
C (T_0)	1.239a	1.317a	1.429a	0.537a	0.619a	0.893a
1g/l(T_1)	1.226a	1.277a	1.414a	0.531a	0.606a	0.884a
2.5 g/l (T_2)	1.189b	1.119b	1.371b	0.515b	0.538b	0.778b
5 g/l (T_3)	1.003c	1.040c	1.243c	0.418c	0.420c	0.704c
10 g/l (T_4)	0.792d	0.711d	0.943d	0.327d	0.290d	0.581d
15 g/l (T_5)	0.458e	0.434e	0.571e	0.214e	0.191e	0.442e
20 g/l (T_6)	0.359f	0.368f	0.557f	0.107f	0.105f	0.270f

- Mean values followed by the same letters within each column are not significantly different according to LSD (P =0.05) n = 4, C, control; 1, 2.5, 5, 10, 15 and 20 denote the aqueous concentration of *E.agallocha*.
- Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) ($P < 0.05$).

CONCLUSION

From the present study, it can be concluded that the aqueous extract of *E.agallocha* L. had strong herbicidal effect on E. colona. The data revealed that E.agallocha could be one of Phyto source for developing bio herbicides to controlling such type of noxious weeds. However the detailed study on identification and action of phytochemical on weed species and its associate crops under field ecosystem is required to develop a novel bioherbicide from the botanical source in future.

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