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Root and Leaf Extract Allelopathic Effect of *Limnocharis flava* on Seed Germination and Growth of Rice

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Abstract: *Limnocharis flava* is an invasive plant in Sri Lanka that highly invading in paddy fields and riparian ecosystems. In present study we assess the allelopathic potential of leaves and roots of *Limnocharis flava* with the *Oryza sativa* seedlings to evaluate the allelopathic potential of *L. flava* on the growth of *O. sativa*. Simple germination assay with randomized block design was occupied as experimental method. High concentrated leaf extract significantly inhibit the seed germination with reducing radicle and hypocotyl elongation. Growth retardation observed with the increasing leaf extract concentration. However root extract concentration increment was not significant compared to the *L. flava* leaf solutions. This result implies that *L. flava* leaf extract has high allelopathic potential and it may negatively affect rice seedling growth under field conditions.

Key words: Allelopathy · Limnocharis flava · Oryza sativa · Hypocotyl · Radicle

INTRODUCTION

Invasive species can be recognized as non native species that succeed and highly competitive and ultimately dominate in that particular ecosystem. Invasive success is more complex phenomena and no single trait can completely explain or predict the invasive ability of plant [1]. Allelopathy is one of the most discussed traits of invasive plant success [2, 3]. Allelopathy explained as any direct or indirect harmful or beneficial effect of one plant on another by producing chemical compounds that are released in to environment [4, 5]. Invasive plants having allelopathic potential, dominate the ecosystem by affecting germination and growth of recipient plant species. Allelochemicals produced in plants vary with the plant organs. More often allelochemicals are synthesized in plant organs like stems, leaves, roots and fruits [6]. Different tissue extracts of invasive plants have differential impacts [7].

Limnocharis flava, also known as yellow velvet leaf is a perennial aquatic herb, a member of the Limnocharitaceae family. Plant is emergent and native to the Central America. It is a noxious weed in irrigation channels in Indonesia and currently invaded in Sri Lanka and most of the south East Asian countries [8]. Most paddy fields in Sri Lanka are highly invading by this species and higher reproduction through seeds and vegetative fragments further enhance the invasion. However there was no clear knowledge regarding allelopathic potential of *L. flava* as a possible invasion trait. Allelopathy can be evaluated using various experimental methods [9]. Field studies and germination assays were more often used method for evaluate phytotoxicty. Assay experiments were arranged as extracted allelochemicals in petri dishes occupied with paper substrate [10]. Furthermore, simple germination assays provide beneficial information on possible allelochemicals before start more realistic studies [11].

The objective of this study is to assess the effect of aqueous extract of leaves and roots of *L. flava* on seed germination and seedling growth of *Oryza sativa* for evaluating allelopathic potential. Initial simple growth and germination method was occupied to identify differential effect between four aqueous extracts of *L. flava* with a control treatment.

MATERIALS and METHODS

The study was conducted at the Faculty of Engineering, University of Ruhuna at Galle (6° 4′ 4.72″ N, 80° 11′ 11.5″ E). *Limnocharis flava* plants were harvested from a nearby invaded paddy field and separated into

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leaves and roots by using sharp knife. Plant parts were washed several times by using distilled water to remove debris and dirt. They were shed dried and used to prepare aqueous extract. Each component manually blended by using mortar and pestle. 25 g and 50 g of root and leaf material separately dissolved in to 500 ml of distilled water by 24 hours of continuous incubating in water bath (45°C). At the end of incubation period, the solutions were filtered through Whatman no. 1 filter paper and pH value was determined by using pH meter. Final extract was stored at 5°C. Homogeneously developed rice seeds were sterilized in 1% Hydrogen peroxide (H_2O_2) solution for 5 minutes. Then seeds were washed several times by using distilled water to remove residual sterilizer. Pretreated rice seeds soaked in distilled water for 3 hours and then separately soaked in 25g/500ml of low leaf concentration, 50g/500 ml of high leaf concentration, 25g/500 ml of low root concentration, 50g/500 ml of high root solution and control as 500 ml of double distilled water for 4 hours. Ten rice seeds were placed on filter paper in a petri dish and saturated with appropriate 5ml volume of each extract and 5ml distilled water as control. All replicates were arranged as randomized block design and total number of replicates was 20 (four replicates for each treatment). Finally petri dishes were stored in room temperature (28 ±2°C) for incubation. Each petri dish was moistened every other day by using assigned solution. Seed germination percentage was recorded every other day till reached to the maximum germination level. After 72 hrs *O. sativa* had reached the highest germination rate. In final germination level all seeds were separated from the petri dishes and hypocotyl length and radical length were recorded by using millimeter ruler. Number of adventitious roots, were counted by naked eye and shoot dry weight, root dry weight observed by oven drying plant material at 70°C for 24h.

Statistical Analysis: Statistical analysis were performed by using SPSS version 16.0 (SPSS, Chicago, IL,USA). One factor analysis of variance (ANOVA) was performed for all corresponding variables: root dry weight, shoot dry weight, root to shoot ratio etc, for one fixed factor (aqueous extract). The raw data were used to compute the mean± standard deviation. The homogeneity of variance test check for equality of variances were performed on the data sets to verify the assumption of homogeneity of variances prior to performing a one way ANOVA to check for significant differences ($P<0.05$). When plant aqueous extract, had significant effect with any variable mean comparison done by through using Tukey's post hoc tests. All the *p* values were considered to be significant at $P \leq 0.05$.

RESULTS

Oryza sativa seedlings showed clear variations in germinations and growth responses to five different aqueous solutions. Observed pH values was 6.65 for 50g/ high leaf extract,6.22 for 25g/leaf extract, 6.39 for 50g/root extract, 6.14 for 25g/root extract and 7.0 for the control. Radicle length was significantly high ($p<0.05$) in control treatment and minimum length recorded in 50g/high leaf concentration. Compare to the control both *L. flava* root and leaf extract significantly influenced on root elongation. However between high and low concentration root extracts effect is not significant (Fig.1a). Under five concentrations, hypocotyl length, significantly lower ($p<0.05$) at high leaf concentration and maximum hypocotyl length of 28.55±5.44 observed under control condition (Fig.1b).

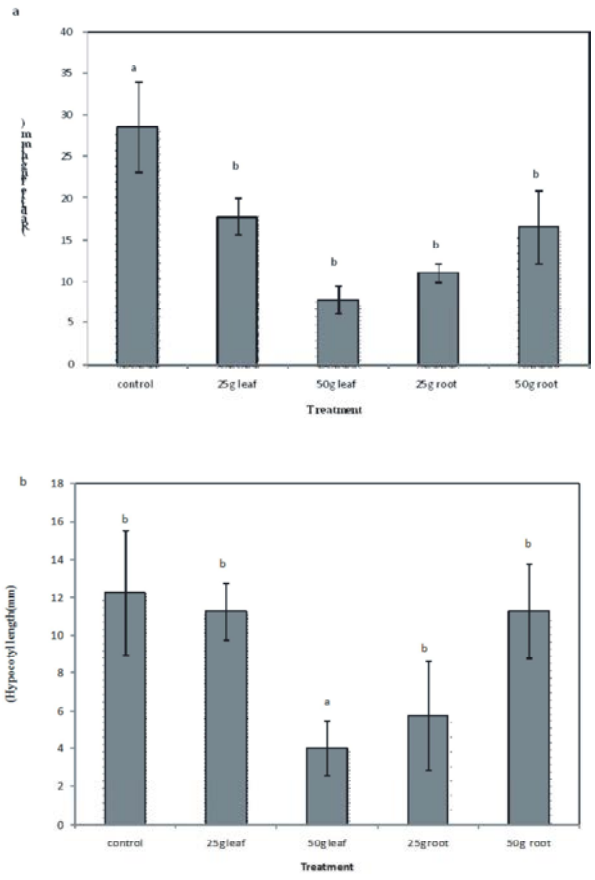

Fig. 1: a Radicle length, b Hypocotyl length of Oryza sativa seedlings exposed to different concentration of L flava plant extracts on± SD, n=10. Different letters mark significant differences, ($p<0.05$)

Table 1: No of adventitious roots, root dry weight, shoot dry weight, germination percentage under different *L. flava* aqueous extracts. Data are mean± SD, n=6 and * represent the significance level at 0.05.

variable	Control	50g Root	25g Leaf	25g root	50g Leaf	
No of adventitious roots	16.07±2.28	5.17±1.48	10.67±2.03	5.57±2.32	4.30±0.87	*
Root dry weight(mg)	1.0±0.2	1.7±1.8	1.2±0.2	0.8±0.07	0.5±0.3	-
Shoot dry weight(mg)	1.0±0.1	0.5±0.1	0.7±0.2	0.4±0.1	0.3±0.1	*
Germination%	92.5±9.57	75.0±12.90	67.5±12.58	77.5±5.00	45.0±12.90	*

The highest number of adventitious roots was observed under control condition and was significantly lower ($p<0.05$) at 50g/high leaf concentration. Maximum seed germination rate was found in control experiment with 92.5±9.57 (48 hours) and maximum seed inhibition was at 50g/high leaf concentration exposed condition (Table 1).

Shoot dry weight significantly (ANOVA, $F_{(4,19)}=1.651$, $p<0.05$) affected by the *L. flava* aqueous extracts. Dry weight was minimum 0.39 mg±0.13mg, at 50g/leaf extract and it was 61% reduction than seedlings grown in control experiment (Table 1).

However separate five aqueous treatments was not significantly (ANOVA, $F_{(4,19)}=2.484$, $p>0.05$) effect on the root dry weight (Table 1).

DISCUSSION

In this study seedling growth is more prominently affected by the high concentration of leaf extract than root extract. This character more related with the earlier findings, like *Lonicera maackii* [12] for herbaceous species and *Excoecaria agallocha* [13] on rice that explain inhibitory and allelopathic potential highly depend on foliar extract. Up to 48 hrs seed germination delayed by the high concentrated leaf extract and it was 51% compared to the control treatment and higher than to the all other treatments. This germination delay and inhibition may be related with alteration of gibberelic acid production [14]. Rice seedling germination was delayed by the leaf extract and after 72 hrs all seeds were germinated in all treatments. This implies seedling growth is more sensitive to the extracts than seed germination. Allelochemicals reaction with seed germination and seedling growth may work with the different mechanisms. Corresponding variation also found in other studies. Seedling growth of three pasture species [15] showed more sensitiveness to allelochemicals compare to seed germination. Biomass allocation for the shoot and root are essential for maintain good crop vigour and it is component of shoot and root dry matter. However our study indicates that *Oryza sativa* shoot dry weight only significantly affected by allelopathic activity of recipient plant. Decrease in dry matter content may be related with the reduction of shoot length. This results inline with other study, that plant residues and extracts of yellow nut sedge caused significant dry matter reduction in corn and soybean [16]. Compared to the control, dry matter reduction in 50g high leaf concentration exposed seedlings was 70%. Both Root elongation and hypocotyl elongation negatively affected by the high concentration of leaf extract but effect for root is more critical than to hypocotyl. Compare to the all other treatments adventitious root formation significantly low in high leaf concentration. Early root formation is very much important for establishment of an optimum seedling stand in rice cultivation. As a major agricultural crop early emergence and good root system will facilitate plant to get better anchorage and improve nutrient absorption capacity [17, 18]. Reduction of adventitious root formation can leads a reduction in nutrient uptake of the plant and overall growth performance and yield may negatively affect. The overall potential of *L. flava* leaf extracts to inhibit the adventitious root formation and root development of rice seedlings is an important phenomena that provide the dominance of this species within infested paddy cultivation. Invasive plants, various plant components are contributed for allelopathic activity. From all plant parts leaf is the most metabolically active organ with higher concentration [19].Considering the concentration effect of aqueous extract, leaf concentration increment, only show significant effect to seedling growth of rice. This finding consisted with the earlier findings like *Prosofis juliflora* [20] that explain high concentration of leaf extract reduce the seedling growth of wheat. We hypothesize this phenomena by compounds include in *L. flava* leaf extracts more water soluble and toxic than to root extract compounds. Past studies explained that lower ph values are inhibit the rice germination and growth [21], however all our treatments pH values near 7.0 and revealed that each treatment pH level not effect for seedling growth or germination. The bioassay data presented by this study represent only what plant components are more allelopathic for *Oryza sativa* as

preliminary study, for *L.flava* leaf age and recipient plant growth stage are factors more worthwhile for consider future studies. Although the composition of the leaf and root extracts of *L. flava* not known, for this more specific chemical assessment are important for identify potential allelochemicals include in *L. flava* leafs as future studies. Finally, our study indicated that *L. flava* leaf extracts appeared to have more negative effect on seedling growth than root extracts. Increasing concentration of leaf extract significantly reduced the *Oryza sativa* seedling growth and the adventitious root formation, radicle elongation and hypocotyl elongation. Further in high invasion, *L. flava* leaf senescence can cause growth inhibition by competing with cultivated paddy rice. Knowing and identifying active compound in leaf litter more beneficial for restore the rice growth condition in field level.

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