

HERBICIDAL POTENTIAL OF *LANTANA CAMARA* L. ON *LUDWIGIA* SPP IN PADDY SOIL

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ABSTRACT

This study assessed herbicidal potential of *Lantana* (*Lantana camara* L.), on two commonly found paddy field weeds; *Ludwigia decurrens* Walt. and *Ludwigia hyssopifolia* (G. Don) Exell. Methodology involved a laboratory bioassay using 1:5 w/v and 1:10 w/v aqueous *Lantana* dried leaf extracts to assess germination, cotyledon expansion, root and shoot development of *Ludwigia* spp. Green house experiments included paddy soil amendments using 1g, 2g, 4g dried *Lantana* leaf residues in 85g soil and 30g, 60g, 120g residues in 2.5 kg paddy soil respectively to test seedling establishment and growth of *Ludwigia* spp. Toxicity of *Lantana* on rice (*Oryza sativa*) was tested by growing rice cv. 'BG 353' with *L. decurrens* in *Lantana* amended paddy soil. Toxic effects of *Lantana* on naturally occurring micro-biota were also assessed by diffusion method using 1.5 w/v *Lantana* dried leaf extracts on pure cultures of fungi and bacteria isolated from paddy soil. Results indicated that 1:5 w/v was the strongest extract to suppress root and shoot development of seedlings of both weeds. An amount of 4g *Lantana* leaf residue in 85g paddy soil suppressed germination and seedling establishment of *L. hyssopifolia* while higher quantities such as 120g of dried *Lantana* leaf residues in 2.5 paddy soil was required to suppress growth of *L. decurrens*. No potential harmful effects of *Lantana* were observed on growth of rice seedlings and microbiota of paddy soil. Higher quantities such as 120g in 2.5 paddy soil were required to suppress growth of *L. decurrens*.

Key words: Allelopathy, Herbicidal – potential, *Lantana*, *Ludwigia*, Phytotoxicity

INTRODUCTION

Allelochemicals are partly responsible for the impressive success of alien invaders in non-native environments, thus considered as 'chemical weapons' (Hierro and Callaway, 2003, Kim and Lee, 2011, Ni *et al.* 2012). The common *Lantana*, *Lantana camara* L., (family: Verbenaceae), listed among the world's hundred worst invasive alien species (IUCN, 2001) is reported to cause substantial ecological and economic losses to agriculture and biodiversity in many countries (Holm *et al.* 1979, Swarbrick *et al.* 1998, Gooden *et al.* 2009). It contains mono, tri and sesquiterpenes including Lantadene A and B, iridoid and phenyl ethanoid glycosides, flavonoids as allelochemicals (Ghisalberti, 2000) that have shown promising toxic effects on many plant species such as *Morrenia odorata* Lindl. (Achhireddy and Singh, 1984), *Lemna paucicostata* Hegelm (Sutton and Potier 1989), *Eichhornia crassipes*

(Mart). Solms. (Zhang *et al.* 2005), *Commelina benghalensis* L., *Echinochloa colonum* (L.) Link., *Digitaria sanguinalis* (L.) Scop, *Panicum psilopodium* Trin. (Bansal, 1998), *Microcystis aeruginosa* (Kong *et al.* 2006), *Abutilon theophrasti* Medik., *Lepidium virginicum* L. (Mersie and Singh, 1987), *Cyclosorus dentatus* (Forsk.) Ching. (Wadhawani and Bhardwaja, 1981) and *Amaranthus hybridus* L. (Verdeguer *et al.* 2009). Hence, allelopathic properties of plant invaders could be positively manipulated to maintain ecological sustenance of agricultural systems (Prasad, 1998, Qasem and Foy, 2001, Xuan *et al.* 2005, Narwal, 2010). It has been a common indigenous practice to use of *Lantana camara* leaf residues (either alone or together with other plant debris) in traditional farming systems in India (CSIR 1966), Kenya (WAC 1995, Roothaert and Franzel, 2001) and Sri Lanka (Ranganath 1993).

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Such usage also provide opportunities to control populations and manage the spread of alien plant invaders at a minimum cost (Saha *et al.* 2007).

The present work aims to explore further the herbicidal properties of *Lantana camara* (Lantana) by describing toxic effects produced by aqueous leaf extracts and dried leaf residues on seed germination, cotyledon expansion, seedling establishment and growth of two noxious broad leaved paddy field weeds *Ludwigia decurrens* Walt. and *Ludwigia hyssopifolia* (G. Don) Exell., that prevail in wet lowland paddy fields of South East Asia including Sri Lanka (Chandrasena 1988). Investigations also included identification of any potential harmful effects of Lantana on a popular low country grown direct-seeded rice cultivar (*Oryza sativa* cv. 'BG 353') and naturally occurring bacteria and fungi species found in herbicide free paddy soil obtained from fields of Borelesgamuwa area of the Western Province, Sri Lanka.

MATERIALS AND METHODS

Germination and seedling development of *Ludwigia* spp exposed to Lantana leaf extracts

According to preliminary laboratory trials, weights of 200g and 100g of air dried mature Lantana leaves each were soaked in 1000 ml distilled water at 10°C for 7 days and 1:5 (w/v) and 1:10 (w/v) aqueous filterates were obtained. Half of each solution was boiled under a Bunsen gas flame for 5 minutes to identify any changes of the filterate. More than two thousand *L. decurrens* and *L. hyssopifolia* seeds were hand picked from mature capsules, rinsed under running tap water in muslin bags for twenty minutes and blotted dried. Seeds were placed (10 per Petri plate) on dry filter paper (No. 1 Whatmann International, Maidstone UK) lined on 9 cm diameter glass Petri plates. The experiment was conducted according to a completely randomized design with 20 replicates at 30±1 °C under 12h day-length conditions. Seeds were subjected to treatments

(1:10 boiled extract, 1:10 unboiled extract, 1:5 boiled and 1:5 unboiled extracts) by moistening the filter papers daily with an equal volume of appropriate solutions. Seeds treated with distilled water were used as the control. Seed germination (radicle length of ≥ 2 mm) was recorded until no germination was observed for three consecutive days. Lengths of shoots and roots of the germinated seedlings were measured and the number of seedlings with expanded cotyledons was recorded on the 19th day after germination.

Germination and/or seedling emergence of *Ludwigia* spp in paddy soil amended with Lantana leaf residues

Germination of *Ludwigia* seeds in paddy soil containing different quantities of Lantana leaf residues was monitored in a greenhouse at 29±1 °C under 12h day length conditions. Dried Lantana leaf residues weighing 1g, 2g and 4g were each mixed with 85 g of pesticide free paddy soil (sandy loam in texture, pH 5.14 at 1:25 water, Organic matter 1.3%, Oslens's Phosphorous 6.5 ppm) in plastic pots (65 mm in diameter and 35 mm in height) to obtain different soil residue combinations. The ratio of residue: soil has been chosen in previous experiments (Madushani and Ranwala, 2004, Chaturani and Ranwala, 2005). Each pot had 10 weed seeds of either species. Controls contained paddy soil without Lantana residues. Another set of pots containing Lantana residues was kept without seeds of test species to monitor the seedling establishment of the existing seed bank of paddy soil. Pots were arranged in completely randomized design with 10 replicates and kept moist throughout. Emergence of seedlings from introduced seeds of test species and paddy soil seed bank was monitored for 22 days.

Growth of *L. decurrens* seedlings in leaf residue amended paddy soil (with or without rice plants)

The experiment was designed to monitor the growth of *L. decurrens* which was able to establish seedlings in soil containing dried Lan-

tana leaf residues. Here too, the ratio of residue: soil was maintained as same above but higher quantities of materials were used. Thus, Lantana leaf residues weighing 30 g, 60 g and 120 g were each mixed with 2.5 kg of paddy soil in a total of 50 pots (380 mm in diameter and 120 mm in height). The control contained paddy soil without Lantana residues. To each pot, five 4 week old *L. decurrens* seedlings (raised in trays in green house conditions up to an approximately 5cm height) were introduced. Each treatment was replicated ten times. Pots were arranged in completely randomized design and plants were kept moist throughout. Plant height and leaves per plant was recorded for *L. decurrens* seedlings weekly for six weeks (so as to be equivalent with critical period of rice) and the total dry weight was obtained. The same methodology was followed for *L. decurrens* grown in pots (five 4 week old weed seedlings) with one 2 week old rice plant of the same height (raised in trays in green house conditions) belonging to cultivar 'BG 353', one of the popular cultivars used for direct-seeded rice cultivations in wet lowlands of Sri Lanka. Additionally, plant height, leaves per plant and biomass of rice seedlings was also measured at the end of six weeks to identify any toxic effects of Lantana on growth of rice.

Effects of Lantana on bacteria and fungi of paddy soil

For isolation of naturally occurring fungi and bacteria, a dilution series of paddy soil suspensions was prepared and 0.1 mL aliquots of 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5} dilutions were introduced separately to sterile Potato Dextrose Agar (PDA) and Nutrient agar (NA) media supplemented with Tetracyclin (3 μ L/mL) and Chloramphenicol (1 μ L/mL) respectively. The pH was adjusted to 5.14 and incubated at 37°C for 7 days. The fungi were purified at 37°C on sterile PDA slants while bacteria were isolated in standard nutrient broths (Carter & Gregorich, 2007) and separately stored at 37°C. Culture collections of bacte-

rial (*Streptococcus* sp., *Staphylococcus* sp and *Bacillus* sp.) and fungal (*Aspergillus* sp., *Trichoderma* sp. and *Fusarium* sp.) obtained from the Department of Plant Sciences, University of Colombo were used as references. Unboiled Lantana (dried) leaf extract (1: 5 w/v) was introduced to bacterial and fungal cultures containing 5 replicates each. Well diffusion method was used for bacteria cultured on NA as described by Booth (1971). Petri plates were incubated at 37°C for 20 hrs. Disc diffusion method (Murray *et al.* 1995) was used to assess toxicity on soil fungi incubated at 30 \pm 1°C for 72 h. Appearance of clear inhibition zones on NA and PDA plates in the presence of *L. camara* extract was considered as a toxic effect produced by Lantana.

All data sets were separately subjected to Analysis of Variance (ANOVA) using software SPSS (version 10) and the significance among treatments was tested using Least Significant Difference (LSD) at 0.05 probability level.

RESULTS

Effect of Lantana leaf extract on germination and seedling development of *Ludwigia* spp.

Germination, cotyledon expansion, elongation of roots and shoot growth of *Ludwigia* spp. varied between boiled and unboiled Lantana extracts and of the test species.

The Final Germination Percentage (FGP) of *L. decurrens* was significantly increased when seeds were in contact with Lantana leaf extract while germination of *L. hyssopifolia* seeds depended upon the strength of the Lantana extract of the medium. A higher strength (1:5) of Lantana extract significantly ($P < 0.05$) inhibited germination (the boiled extract showing a greater inhibition) and the lower strength (1:10) stimulated germination indicating a higher FGP over the control.

Table 1. Final Germination Percentage of *L. decurrens* and *L. hyssopifolia* seeds and Percentage cotyledon expansion, root and shoot lengths of *L. decurrens* and *L. hyssopifolia* seedlings exposed to aqueous Lantana leaf extract under laboratory conditions. Values are means of 20 replicates. Standard errors are given in parentheses at P<0.5 level.

Treatment (Lantana extract)	Final Germination Per- centage		Percentage of seedlings with expanded cotyledons		Average root length (mm)		Average shoot length(mm)	
	<i>L. decurrens</i>	<i>L. hyssopifolia</i>	<i>L. decurrens</i>	<i>L. hyssopifolia</i>	<i>L. decurrens</i>	<i>L. hyssopifolia</i>	<i>L. decurrens</i>	<i>L. hyssopifolia</i>
Control	29.00 ^{x,p} (±0.40)	68.00 ^{x,p} (±0.37)	24.50 ^{x,p} (±0.32)	64.00 ^{x,p} (±0.87)	03.13 ^{x,p} (±0.03)	23.2 ^{x,p} (±0.21)	03.99 ^{x,p} (±0.003)	37.30 ^{x,p} (±0.31)
1:10 Un- boiled	47.00 ^{y,q} (±0.56)	88.00 ^{y,q} (±0.27)	31.50 ^{x,q} (±0.53)	64.50 ^{x,q} (±0.49)	00.14 ^{y,q} (±0.008)	07.60 ^{y,q} (±0.02)	03.07 ^{y,q} (±0.02)	30.00 ^{y,q} (±0.11)
1:10 Boiled	40.50 ^{y,q} (±0.39)	81.00 ^{y,q} (±0.28)	16.00 ^{x,q} (±0.33)	56.50 ^{x,q} (±0.54)	00.09 ^{y,q} (±0.006)	05.40 ^{y,q} (±0.01)	02.59 ^{y,q} (±0.01)	17.80 ^{y,q} (±0.07)
1:5 Un- boiled	41.00 ^{y,q} (±0.40)	50.00 ^{z,q} (±0.39)	25.00 ^{x,q} (±0.14)	00.50 ^{y,q} (±0.05)	01.05 ^{z,q} (±0.004)	03.10 ^{y,q} (±0.02)	00.00 ^{z,q} (±0.00)	12.40 ^{z,q} (±0.06)
1:5 Boiled	38.00 ^{y,q} (±0.46)	30.50 ^{z,r} (±0.39)	0.00 ^{z,q} (±0.00)	00.50 ^{y,q} (±0.05)	01.21 ^{z,q} (±0.005)	06.00 ^{y,q} (±0.02)	00.00 ^{z,q} (±0.00)	04.90 ^{z,q} (±0.02)

Superscripts ^{x,y,z} and ^{p,q,r} in a column show significance between concentrations and boiled- unboiled nature of the extract respectively at P ≤ 0.05 significant level

Further contact of germinated *Ludwigia* seeds with Lantana extract affected cotyledon expansion of young seedlings. The boiled higher strength (1:5) was much effective in inhibiting expansion of cotyledons in both *Ludwigia* spp while the unboiled extract of the same strength inhibited cotyledon expansion only in *L. hyssopifolia*. Boiling of the Lantana extract had no effect on elongation of roots and shoots. Nevertheless, higher strength of the Lantana extract produced significant suppressive effects on shoot growth than root growth of young *Ludwigia* seedlings (Table 1).

Effect of Lantana leaf residues on seedling emergence of *Ludwigia* spp.

Seedling emergence of *L. decurrens* was not affected due to the addition of Lantana residues to paddy soil while that of *L. hyssopifolia* seeds showed a significant suppression when residues were added over and above 2g per 85g to paddy soil. Seedling emergence of monocotyledon and dicotyledon weeds from the existing seed bank of paddy soil showed a decreasing trend with increasing weights of *L. camara* residues (Table 2).

Table 2. Seedling emergence of *L. decurrens*, *L. hyssopifolia* and associated other weeds exposed to different weights of Lantana residue in soil under glasshouse conditions. Values are means of 20 replicates. Standard errors are given in parentheses at P<0.5 level.

Seedling emergence	Lantana Leaf Residue in paddy soil (g)			
	Control	1	2	4
<i>L. decurrens</i> (%)	40.00 ^x (±0.47)	47.00 ^x (±0.67)	59.00 ^x (±0.91)	57.00 ^x (±0.63)
<i>L. hyssopifolia</i> (%)	90.00 ^x (±0.47)	85.00 ^x (±0.50)	47.00 ^y (±0.76)	11.00 ^z (±0.38)
Monocotyledon seedlings	35.67 ^x (±0.38)	42.83 ^x (±0.42)	29.50 ^y (±0.23)	17.33 ^z (±0.17)
Dicotyledon seedlings	43.50 ^x (±0.47)	27.17 ^x (±0.40)	15.33 ^x (±0.20)	09.17 ^y (±0.15)

Superscripts ^{x,y,z} in a row indicate significance between treatments at P ≤ 0.05 significant level

Effect of Lantana residues on growth of *L. decurrens* seedlings grown with or without rice

Although *L. decurrens* seedlings were able to survive in a paddy soil growing medium containing Lantana leaf residues, their growth, in terms of height, leaf and biomass production was significantly decreased when a higher weight of Lantana residues (60g and 120g) were mixed with paddy soil.

However presence of the rice plants in the growing medium favoured growth of *L. decurrens* rather grown alone. There was no change in leaf production and biomass of rice plants grown with *L. decurrens* in a medium containing different weights of Lantana residues (Table 3, Figures 1 and 2).

Effect of Lantana on bacteria and fungi in paddy soil

Pure cultures of two soil bacteria and three soil fungi were isolated from paddy soil. No clear inhibition zones were observed in any of these species when *L. camara* leaf extract was in contact with the microbes. The observations were same with known bacteria: *Streptococcus* sp., *Staphylococcus* sp. and *Bacillus* sp. and known fungi: *Aspergillus* sp., *Trichoderma* sp. and *Fusarium* sp. Remarkable antimicrobial activity was recorded only by the antibiotic and antifungal solutions (Table 4).

Table 3. Dry weight *L. decurrens* seedlings and growth performance of rice seedlings grown in paddy field soil mixed with different weights of Lantana. Values indicate averages of 6 week old 50 plants for *L. decurrens* and 10 plants for rice. Standard errors are given in parentheses at P < 0.5 level.

	Lantana Leaf Residue in paddy soil (g)			
	Control	30	60	120
Dry weight (g) of <i>L. decurrens</i> seedlings				
grown without rice	0.31 ^x (±0.07)	0.69 ^y (±0.18)	0.02 ^x (±0.02)	0.01 ^x (±0.01)
grown with rice	1.24 ^x (±0.03)	0.76 ^x (±0.01)	0.28 ^y (±0.01)	0.04 ^y (±0.00)
Growth performance of rice seedlings				
Number of leaves/plant	4.10 ^x (±0.18)	4.90 ^x (±0.18)	4.80 ^x (±0.13)	4.80 ^x (±0.13)
Plant height (cm)	31.85 ^x (±1.01)	31.30 ^x (±1.85)	26.20 ^x (±1.09)	29.85 ^x (±0.61)
Total dry weight (g)	1.06 ^x (±0.01)	1.21 ^x (±0.01)	0.90 ^x (±0.01)	0.97 ^x (±0.02)

Superscripts ^{x,y,z} in a row indicate significance between treatments at P ≤ 0.05 significant level

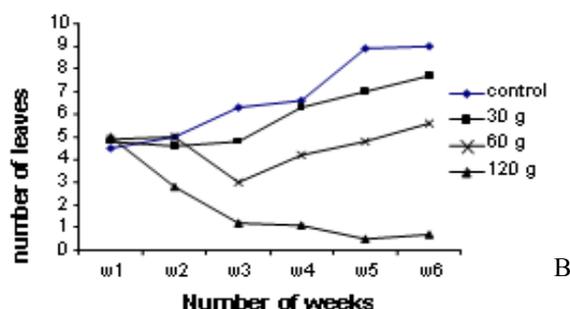
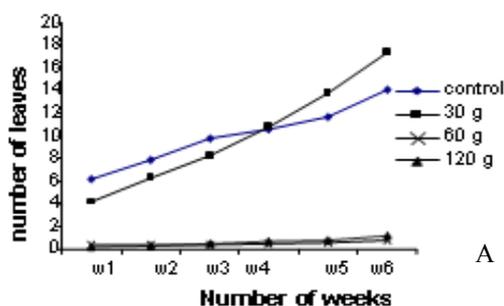


Figure 1. Number of leaves per plant of *L. decurrens* when grown with Lantana leaf residues (A) without rice plants (B) with rice plants. Values indicate averages of 50 plants

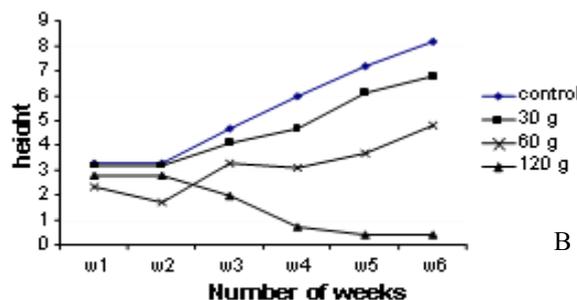
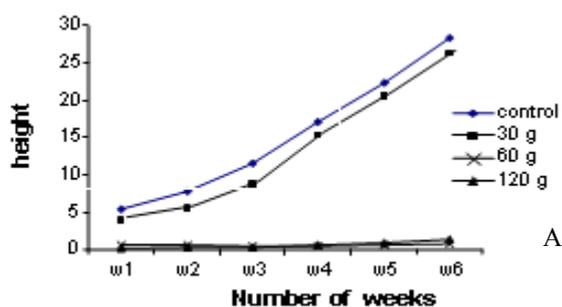


Figure 2. Plant height of *L. decurrens* when grown with Lantana leaf residues (A) without rice plants (B) with rice plants. Values indicate averages of 50 plants.

Table 4: Effect of *L. camara* extracts and antibiotic solution (Amocyclin 1mg/mL) on soil bacteria and antifungal solution (Ketoconazole 2mg/mL) on soil fungi

Soil microflora	Amocyclin (1mg/mL)	Ketoconazole (2 mg/ML)	Extract from undecomposed <i>Lantana</i> residues	
			1:5	1:10
Bacteria species				
Isolate 1	***	NA	-	-
Isolate2	***	NA	-	-
<i>Streptococcus</i> sp	***	NA	-	-
<i>Staphylococcus</i> sp	*	NA	-	-
<i>Bacillus</i> sp.	*	NA	-	-
Fungal species				
Isolate 1	NA	*	-	-
Isolate2	NA	*	-	-
Isolate 3	NA	-	-	-
<i>Aspergillus</i> sp	NA	*	-	-
<i>Tricoderma</i> sp	NA	*	-	-
<i>Fusarium</i> sp	NA	*	-	-

Symbols *** and * indicate high and low sensitivity respectively, - indicates insensitive status and NA, not applicable

DISCUSSION

Growth suppressive responses of the two *Ludwigia* species, *L. hyssopifolia* and *L. decurrens* to aqueous Lantana leaf extracts and dried residues varied significantly with regard their identity and growth stage at which the treatments were imposed. Our results indicated that allelochemicals of Lantana could enhance seed germination of *Ludwigia* spp at 1: 5 and 1:10 w/v concentrations, but able to completely suppress the establishment and growth of *L. hyssopifolia* seedlings via inhibiting elongation of root and shoots. In comparison, the phytotoxic effect of Lantana on *L. decurrens* was more effective at a later stage during seedling establishment and subsequent growth of seedlings. This indicated that *L. hyssopifolia* was much susceptible to allelopathic effects of Lantana than *L. decurrens*. These results may correlate with their differences in germination ecology which in turn depends upon phylogenetic relationships of the genus (Fay and Duke, 1977, Wogu and Ugborgho, 2000), physiological and biochemical properties of the species (Kobayashi, 2004).

Phytotoxins often inhibit cell division and expansion (Tomaszewski and Thimann, 1966), often slowdown or stop photosynthesis and change the amount of chlorophyll and respiration of plants leading to death (Batish *et al.* 2002). Phytotoxic potential of Lantana was more pronounced when Lantana was applied in 'higher amounts' as concentration depended growth suppression has been a characteristic of allelopathic interactions (Ashrafi *et al.* 2008, Inderjit and Nilsen, 2003, Randhawa *et al.* 2002). The present study also supports this idea as a higher 'dose' of Lantana (more concentrated extract and/or more heavier weight of residues per unit weight of paddy soil) was found to be more effective for suppression of seedling growth and development of *Ludwigia* species as well as for the emergence of other associated weed seeds in paddy soil. Further, our results also revealed that boiling of the extract would be more effective in suppressing germination and cotyledon expansion of *Ludwigia* spp. However, the reasons for such an observation cannot be explained without further investigations. Boiling the extract would have also destroyed microorganisms in the extract enabling a clear vi-

sion of the allelopathic effects.

This work was also be in line with previous findings that crude extracts exhibit greater allelopathic effects than that produced by residues, as the visible changes are a result of reactions of different groups of chemical compounds directly leached out from tissues into the testing medium than the ones that are slowly released by decomposition to the environment (Inderjit and Callaway 2003). Compared to laboratory experiments, soil amendment experiments often interfere with chemical, physical and biological properties of soil and their dynamics in spatial and temporal scales (Bhowmik and Inderjith 2003, Inderjit 2001, Kobayashi, 2004). Therefore, visible effects of laboratory bioassays in allelopathy research may or may not always correspond with observations in either green house studies or in field (Inderjit and Nilsen, 2003). Nevertheless, the results of our laboratory study using crude extracts coincided with that of green house experiment to which crushed residues were directly incorporated, especially when higher amounts were used. However, field trials are recommended for recognizing promising results.

CONCLUSION

The study exhibited potential herbicidal effects of *L. camara* on *Ludwigia* spp. in a paddy soil medium containing rice seedlings in their early growth stage equivalent to the critical period. Additionally, it was also shown that *L. camara* does not possess toxic effect towards micro biota of paddy soil. These findings strengthen evidence for herbicidal property of *L. camara* and suggest its possible applications towards development of bio-herbicidal mixtures for organic paddy production in Sri Lanka.

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