Australian Journal of
Crop Science
AJCS

AJCS 7(9):1369-1374 (2013) ISSN:1835-2707

# Allelopathic potential of five Labiatae plant species on barnyard grass (*Echinochloa crus-galli*)

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## Abstract

Uninterrupted application of synthetic herbicides to control barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv. ) makes them resistance against many herbicides with different mode of action. To overcome this problem, many researchers are interested in searching new novel natural compounds in medicinal plants to develop natural herbicides. Plants belongs to the Labiatae family attracted the attention of many researchers in pharmacological interest because of their toxic potential and medicinal properties. However, there is very limited information available for the allelopathy of this family. To explore the allelopathic potential of the aqueous methanol extract of five Labiatae plants: *Leucas aspera* L., *Leonurus sibiricus* L., *Ocimum tenuiflorum* L., *Mentha sylvestris* L. and *Hyptis suaveolens* L. were tested against barnyard grass at four different concentrations (3, 10, 30 and 100 mg DW equivalent extract/mL). The root growth was more sensitive to the plant extracts than the coleoptile growth and the inhibitory activities were concentration dependent. At the concentration of 100 mg DW equivalent extract/mL, *L. aspera* and *H. suaveolens* plant extracts strongly inhibited the seedling growth of barnyard grass. However, at the same concentration the lowest inhibition was observed in case of *O. tenuiflorum* plant extract. Among the plant extracts, the seedling growth of barnyard grass was most susceptible to *L. aspera* plant followed by *H. suaveolens* to confirm 50% coleoptile and root growth inhibition (defined as $I_{50}$), whereas that of barnyard grass was less susceptible to *O. tenuiflorum*. These results suggest that *L. aspera* and *H. suaveolens* possess strong allelopathic potential and therefore, could be used as the good candidates for isolation and identification of allelochemicals to develop environment friendly new natural herbicides to control barnyard grass.

**Keywords:** Allelochemicals; *Hyptis suaveolens*; *Leonurus sibiricus*; *Leucas aspera*; medicinal plants; *Mentha sylvestris*; natural herbicides; paddy weed; *Ocimum tenuiflorum*; sustainable agriculture.
**Abbreviation:** DW – dry weight, $I_{50}$ – concentration required for 50% inhibition, sig. – significance, df – degrees of freedom.

## Introduction

Weeds, the primary constrain for rice production can reduce the rice yields from 44 to 96% in one crop season if the weeds are not control from the crop fields (Ampong-Nyarko and De Datta, 1991). Moreover, on an average 13-30% of crop reproduce is actually lost in the farmers' fields even after adopting conventional weed control techniques due to the weeds that grow after weed control (Swarbrick and Mercado, 1987; Mamun, 1990), though it may vary from country to country. Among the major weeds of rice, barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.) is the most notorious one (Holm et al., 1977). The $C_4$ photosynthetic system of barnyard grass gives it greatest advantage under hot, arid and high light conditions (Patterson, 1985; Vidotto et al., 2007) as well as higher water and nitrogen use efficiency than $C_3$ plants like rice (Ampong-Nyarko and De Datta, 1991). Another special characteristic, 'mimicry' with rice seedlings helps it to escape manual weeding (Barrett, 1983; Gibson et al., 2002). These two special characteristics make barnyard grass more competitive against rice and can reduce rice yields up to 100% (Ampong-Nyarko and De Datta, 1991). To control this notorious weed from the rice fields on an average three million tonnes of herbicide has been used every year in almost all agricultural systems by the farmers (Stephenson, 2000). This over and uninterrupted use of synthetic herbicides in the rice field makes them resistance to some herbicides (Juliano et al., 2010; Beltran et al., 2012) and in the same time creates environmental hazards. To avoid these detrimental effects of synthetic herbicide, research on novel natural plant products have moved from the fringe to the mainstream for the development of ecologically acceptable, environment friendly, cost-effective and relatively safe natural herbicides. Many researchers around the world show their keen interest on medicinal plants for searching new novel compounds as it may provide the clues to new and safe herbicide chemistry (Duke, 1986; Nimbal et al., 1996; Bhowmik and Inderjit, 2003; Li et al., 2009).

In addition, screening of medicinal plants for new natural compounds is easier than other plants (Fujii et al., 2003), possibly due to their existed certain metabolic compounds which was used for curing many diseases of human being. In order to compete with neighboring plant species for light, nutrient and moisture, a number of plants have been reported to release allelochemicals to the surrounding environment through volatilization from the leaves (Petrova, 1977; Oleszek, 1987), leaching from the above ground parts by precipitation (Overland, 1966), decomposition of leaf litter or sloughed root tissues (Guenzi et al., 1967; Hedge and Miller,

**Table 1.** Analysis of Variance (ANOVA) of the percent inhibition of five Labiatae plant species on barnyard grass.

| | | Coleoptile growth | | Root growth | |
|---|---|---|---|---|---|
| Source | df | F | Level of sig. | F | Level of sig. |
| Plant species | 4 | 23.5 | 0.000 | 27.5 | 0.000 |
| Concentrations | 3 | 49.0 | 0.000 | 69.9 | 0.000 |
| Plant species × Concentrations | 12 | 6.2 | 0.000 | 6.3 | 0.000 |

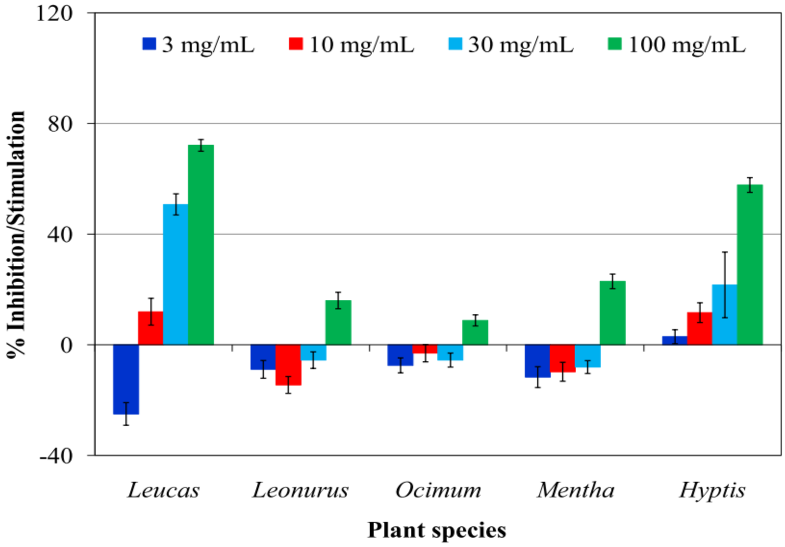

**Fig 1.** Percent inhibition/stimulation of the coleoptile growth of barnyard grass by the aqueous methanol extracts of five Labiatae plant species. Concentrations of tested samples correspond to the extract obtained from 3, 10, 30 and 100 mg dry weight of each plant material. The negative (–) value in the Y-axis indicates stimulation and positive (+) value indicates inhibition of coleoptile growth of barnyard grass by the plant extracts.

1990), microbial transformation from the decayed leaf, stem, leaf litter or roots (Chick and Kielbaso, 1998), through root exudates (Tang and Young, 1982), from pollen of some crop plants (Cruz-Ortega et al., 1988) or other processes in both natural and agricultural systems (Ferguson and Rathinasabapathi, 2009). These allelochemicals could be used as lead for bio-herbicide production (Duke et al., 2000; Vyvyan, 2002). Recently, the efforts have been made by many researchers to identify and isolate those allelochemicals from different parts of the plant and apply them as a tool for sustainable and eco-friendly weed control strategies (Khanh et al., 2005).

Labiatae, a large dicotyledonous family belongs to the Angiosperm order Tubiflorae (Rendle, 1959), also designated as the Lamiaceae or mint family, comprising at least 3500 species in about 180 genera (Lovett and Weerakoon, 1983). The most distinct characteristics of this family are aromatic because of the presence of considerable amounts of volatile oils like terpenes and their oxygenated derivatives in their glandular epidermal hairs, which are commercially significance. Beside these, 175 species of 45 genera of this family are considered as weeds in different parts of the world (Holm et al., 1979). The plants of Labiatae family attracted the attention of many researchers in pharmacological interest because of their toxic potential and medicinal properties (Lovett and Weerakoon, 1983). However, limited information is available about their role in allelopathy (Singh and Pandey, 1982; Almeida et al., 2008; Chatiyanon et al., 2012). The present study was therefore, undertaken to investigate and compare the allelopathic potentiality of five Labiatae plant species which could finally help to find out the good candidates for isolation and identification of allelochemicals for the development of potent natural herbicides to control barnyard grass, the world worst paddy weed.

## Results

The inhibition percent of coleoptile and root growth of barnyard grass by the aqueous methanol extracts of five Labiatae plant species are shown in Figure 1 and 2, and observed a marked inhibition difference in both cases of coleoptile and root growth. Moreover, the inhibition percent of barnyard grass responded differently with the five Labiatae plant extracts. The two-way ANOVA showed that the extracts of five Labiatae plant species and the concentration used in the experiment as well as their interactions has a significant ($p<0.001$) effect (Table 1) on barnyard grass. The seedling growth inhibition of barnyard grass by the plant extracts was concentration dependent (Figure 1 and 2).

### *Effects of plant extracts on the coleoptile growth of barnyard grass*

The aqueous methanol extracts of *L. aspera* and *H. suaveolens* significantly inhibited the coleoptile growth of barnyard grass at concentrations greater than 10 mg DW equivalent extract/mL, but in case of *L. sibiricus*, *O. tenuiflorum* and *M. sylvestris*, the inhibition was observed at 100 mg DW equivalent extract/mL (Figure 1). At concentration 100 mg DW equivalent extract/mL, the inhibition percent of *L. aspera* and *H. suaveolens* extracts on the growth of barnyard grass coleoptiles were 72 and 58, respectively. In contrast, on the same concentration the inhibition of *M. sylvestris*, *L. sibiricus* and *O. tenuiflorum* on the coleoptiles growth were 23, 16 and 9%, respectively.

**Table 2.** $I_{50}$ values of the aqueous methanol extracts of five Labiatae plant species for coleoptile and root growth of the barnyard grass.

| Plant species | $I_{50}$ (mg dry weight equivalent extract/mL) | |
|---|---|---|
| | Coleoptile growth | Root growth |
| *Leucas aspera* | 29.7 | 4.3 |
| *Leonurus sibiricus* | 116.9 | 46.0 |
| *Ocimum tenuiflorum* | 124.0 | 66.2 |
| *Mentha sylvestris* | 111.0 | 56.5 |
| *Hyptis suaveolens* | 76.1 | 13.6 |

Note: The values were determine by a logistic regression analysis after bioassays.

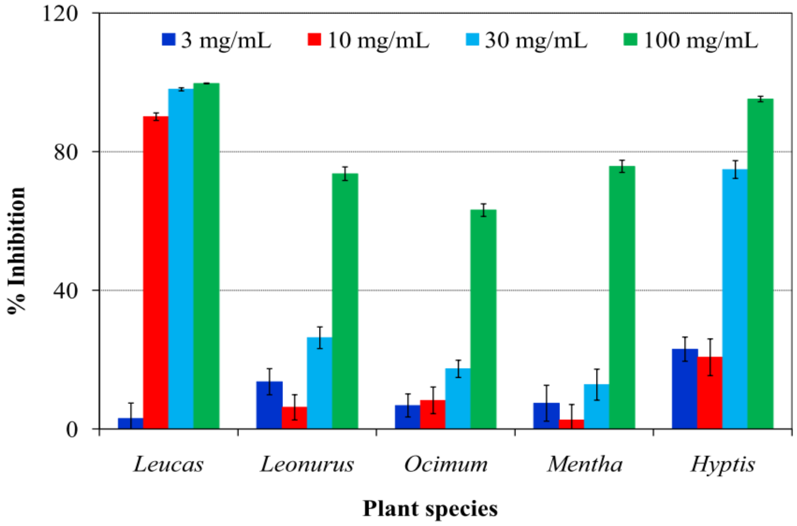

**Fig 2.** Percent inhibition of the root growth of barnyard grass by the aqueous methanol extracts of five Labiatae plant species. Concentrations of tested samples correspond to the extract obtained from 3, 10, 30 and 100 mg dry weight of each plant material.

Furthermore, the aqueous methanol extracts of *L. sibiricus, O. tenuiflorum* and *M. sylvestris* stimulated the coleoptile growth of barnyard grass at concentrations less than 30 mg DW equivalent extract/mL and that of barnyard grass by *L. aspera* at concentration of 3 mg DW equivalent extract/mL. On the other hand, no growth stimulation was observed when barnyard grass seeds were subjected to *H. suaveolens* plant extract.

### *Effects of plant extracts on the root growth of barnyard grass*

The aqueous methanol extracts of five Labiatae plants species significantly ($p<0.001$) inhibited the root growth of barnyard grass at any concentration under studied (Figure 2). At concentration of 30 mg DW equivalent extract/mL, the root growth inhibition of barnyard grass was at 98, 75, 13, 26 and 17% by *L. aspera, H. suaveolens, M. sylvestris, L. sibiricus* and *O. tenuiflorum* plant extracts, respectively. The root growth of barnyard grass was completely (100%) inhibited when applied to *L. aspera* extract at concentration of 100 mg DW equivalent extract/mL, whereas at the same concentration 95, 76, 74 and 63% inhibition were found on root growth by *H. suaveolens, M. sylvestris, L. sibiricus* and *O. tenuiflorum* plant extracts, respectively.

### *Sensitivity of barnyard grass to the five Labiatae plant species*

The $I_{50}$ value of barnyard grass ranges from 4.30 to 124.0 mg DW equivalent extract/mL depending on the plant extracts (Table 2). Moreover, among the plant extracts the coleoptile and root growth of barnyard grass was most sensitive to *L. aspera* followed by *H. suaveolens*, whereas that of barnyard grass was least sensitive to *O. tenuiflorum* (Table 2).

## Discussion

The inhibitory activity of the aqueous methanol extracts of five Labiatae plant species on the seedling growth of barnyard grass increased with the increase of concentration. However, the magnitude of inhibition of barnyard grass by each plant extract was different from others (Figure 1 and 2). This type of growth inhibition by the allelopathic plants extract was also reported by Caussanel (1979); Inderjit and Keating (1999); An et al. (2005); Batlang and Shushu (2007) and Pukclai and Kato-Noguchi (2011). The asymmetrical susceptibility of barnyard grass to five Labiatae plant extracts could be due to inherent differences in various bio-chemicals involved in the process.

In comparison to the coleoptile growth of barnyard grass, its root growth was more sensitive to the aqueous methanol extracts of five Labiatae plant species (Figure 1 and 2). These results are in agreement with the earlier findings of Stachon and Zimdahl (1980); Aliotta et al. (1993); Levizou et al. (2002) and Pukclai and Kato-Noguchi (2011) who reported that the extracts of allelopathic plant had higher root growth inhibition than the coleoptiles. It might be due to the more intensive contact between roots and plant extracts. In addition, the coleoptile growth of seedlings largely depends on cell expansion which is relatively insensitive to the allelochemicals, whereas root growth requires not only cell expansion, but also cell proliferation which is sensitive to the allelochemicals (Nishida et al., 2005). As a result, the root growth exhibits higher inhibition than the coleoptile growth. On the other hand, Salam and Kato-Noguchi (2010) reported that the higher root growth inhibition is mainly because the roots are the first organ to absorb allelochemicals from the environment, and the permeability of allelochemicals into root tissue is higher than the shoot tissue (Nishida et al., 2005).

The growth inhibition of barnyard grass in presence of allelochemicals could be for the reason of lower cell division, elongation and expansion rate which are growth pre-requisites (Rice, 1984; Ortega et al., 1988; Einhellig, 1996; Jacob and Sarada, 2012). Furthermore, allelochemicals inhibit the respiration (Inderjit and Keating, 1999), ion absorption process (Qasem and Hill, 1989), enzyme activity (Sato et al., 1982), synthesis of plant endogenous hormones and protein synthesis (Jacob and Sarada, 2012), alteration of the phytochrome control of germination (Leather and Einhellig, 1988) and thus, results in arrested plant growth (Santosh et al., 2004). Allelochemicals may produce more than one effect of the above on the cellular processes that could be responsible for the reduced seedling growth of barnyard grass. However, the details of the biochemical mechanism through which allelochemicals exert a toxic effect on the growth of any plant species are still not well known (Zhou and Yu, 2006).

The strong inhibitory activity of *L. aspera* and *H. suaveolens* plant extracts on barnyard grass is congruent with the previous findings of Islam and Kato-Noguchi (2012), Chatiyanon et al. (2012) and Kapoor (2011). They also observed higher growth inhibition of *L. aspera* and *H. suaveolens* extracts on other test plant species. Never the less, the stimulatory activity on the coleoptiles growth of barnyard grass by the aqueous methanol extract of *L. sibiricus, O. tenuiflorum* and *M. sylvestris* at concentrations less than 30 mg DW equivalent extract/mL and that of *L. aspera* at concentration 3 mg DW equivalent extract/mL are in line with the findings of many other researchers. They reported that allelochemicals can stimulate the seedlings growth at very low concentrations but inhibit the seedlings growth at high concentrations (Rice, 1984; Lovett et al., 1989; David and Erik, 2000; Liu and Chen, 2011).

It was describe before that every year millions of tonnes of synthetic herbicides has been applied by the farmers only to control the barnyard grass from their paddy fields. The higher amount of herbicide application lead to an increase in production cost as well as severe environmental problems for example, degradation of agricultural land by abolishing soil-biota (Pell et al., 1998), ground water contamination (Aktar et al., 2009); reduction of fisheries (Khan and Law, 2005); development of herbicide resistance weed bio-types (Vyvyan, 2002); destruction of beneficial predators of pests and thereby increased the virulence of many species of agricultural pests (Wilson and Tisdell, 2001). It has also increased the mortality and morbidity of humans. Consequently, there is a crucial need to develop new environmental friendly techniques to control barnyard grass in more efficiently. In this regard our findings might provide some useful hints for the researchers to develop new natural herbicides to control this world worst weed.

## Materials and methods

### *Plant materials*

Whole plants (leaves, stem and roots) of *Leucas aspera* L., *Leonurus sibiricus* L., *Ocimum tenuiflorum* L., *Mentha sylvestris* L. and *Hyptis suaveolens* L. were collected from Bangladesh during the month of March-April, 2012. After collection, plants were washed with tap water to remove the soil and other debris followed by sun drying. The dried plants were then kept in a refrigerator at 2 ºC temperature until extraction.

### *Test plant species*

Barnyard grass was selected as a test plant species for the current experiment because of its worldwide distribution as a worst paddy weed.

### *Extraction procedure*

The whole parts (leaves, stem and roots) of each dried plant material (30 g) were cut into small pieces and extracted with 300 mL of 70 % (v/v) aqueous methanol for 48 h. After filtration using one layer of filter paper (No. 2; Advantec® Toyo Roshi Kaisha, Ltd., Tokyo, Japan), the residue was re-extracted with 300 mL of 100% methanol for 24 h and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40 ºC. All the activities *i.e.* washing, drying, storing, extraction and bioassay were done separately for each individual species throughout the whole research work.

### *Bioassay*

An aliquot of the extract (final assay concentration was 3, 10, 30 and 100 mg DW equivalent extract/mL) of each plant was evaporated to dryness at 40 ºC in *vacuo* by rotary evaporator, dissolved in 5.0 mL of methanol and added to a sheet of filter paper (No. 2) in a 28 mm Petri dish. The methanol was evaporated in a draft chamber then the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween-20 (polyoxyethylene sorbitan monolaurate; Nacalai Tesque, Inc., Kyoto, Japan) which was used for surfactant and did not cause any toxic effects. Ten pre-germinated (germinated in the darkness at 25 ºC for 24 h after overnight soaking) seeds of barnyard grass were arranged on the filter paper in the Petri-dishes. The coleoptile and root lengths of the seedlings were measured at 48 h after incubation in darkness at 25 ºC. Control seeds were sown on the filter paper moistened with 0.6 mL of 0.05% (v/v) aqueous solution of Tween-20 without plant extract. The inhibition percent was calculated using the following equation:

$$Inhibition\,(\%) = [1 - \frac{length\ with\ aqueous\ methanol\ extract}{length\ of\ control}] \times 100$$

### *Statistical analysis*

The bioassay experiment was conducted in a completely randomized design with six replications. Experimental data were analyzed using predictive analytics software (PASW) statistics 17.0 (SPSS Inc., Chicago, Illinois, USA). All measured variables were subjected to two-way analysis of variance (ANOVA) and the differences between the means were compared using the least significant difference (LSD) at a 5% level of probability. The concentration required for 50% inhibition (express as $I_{50}$) of barnyard grass in the assay was calculated from the regression equation of the concentration response curves, using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, California, USA).

## Conclusion

Our results indicate that the aqueous methanol extracts of *L. aspera* and *H. suaveolens* have strong allelopathic potential than *L. sibiricus, O. tenuiflorum* and *M. sylvestris* plant extracts to suppress the seedling growth of barnyard grass. Therefore, *L. aspera* and *H. suaveolens* could be act as potential candidates for further isolation and identification of allelochemicals for the development of new natural herbicides to control barnyard grass from the paddy fields. Moreover, the crude extract and/or residue of *L. aspera* and *H. suaveolens* could also be recommended to apply directly as bio-herbicide.

## Acknowledgements

The authors wish to acknowledge Sabina Yeasmin (Faculty of Agriculture and Environment, The University of Sydney, Australia) and Dr. AKM Aminul Islam (Department of Genetics and Plant Breeding, Banghabandhu Seikh Mujibur Rahman Agricultural University, Bangladesh) for providing the plant materials to the authors. The Plant Protection Wing (Plant Quarantine), Department of Agricultural Extension, Ministry of Agriculture, Government of the Peoples Republic of Bangladesh to give the permission to the authors for bringing the dried plant materials from Bangladesh, and the financial assistance of Japanese Government (*Monbukagakusho*: MEXT) to carry out this research are also thankfully acknowledged.

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