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Allelopathic Potentiality of Medicinal Plant *Leucas aspera*

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Abstract: Excessive use of herbicide in the crop field threatens the environment by destroying flora and fauna. Allelopathic substances could be act as substitutes of chemical herbicides to suppress the target plants. *Leucas aspera* (Willd.) Linn., a herbaceous plant has already provided tangible evidence of its potential with remedies for different sorts of medical problems, but still now, not a single work is reported related to its allelopathic activity. Hence, to explore the allelopathy of *L. aspera*, the aqueous methanol extracts of this plant at four different concentrations (3, 10, 30 and 100 mg dry weight equivalent extract mLG^{1}) were tested against seven test plant species, namely cress (*Lepidum sativum* L.), alfalfa (*Medicago sativa* L.), lettuce (*Lactuca sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crusgalli* L.), jungle rice (*Echinochloa colonum* L. Link) and timothy (*Phleum pratense* L.). The aqueous methanol extract of *L. aspera* significantly inhibited the seedlings growth of all the test plant species at different inhibition levels. The root growth was more sensitive than the shoot growth to the plant extract and the inhibitory activity was concentration dependent. Comparing the extract concentrations required for 50% inhibition, the seedlings growth of timothy was most sensitive to the extract, whereas jungle rice was less sensitive. These results indicated that *L. aspera* may contain growth inhibitory substances and possess allelopathic activity. Therefore, *L. aspera* could be used as a potential candidate for isolation and identification of allelochemicals, which can lead to the development of ecologically acceptable bio-herbicides used for sustainable agriculture.

Key words: Allelochemicals % Growth inhibitor % Methanol extract % Sustainable agriculture % Bio-herbicides

INTRODUCTION

Weeds pose a serious problem for crop production even more than other pests worldwide. In Asia and other continents, around 33-53% crop produce is damaged if weeds are not controlled from the crop fields [1-3]. In spite of advances made in weed control measure, weeds continue to cause serious crop losses every year [4]. On an average, 13-30% of crop reproduce is actually lost in the farmers fields even after adopting traditional weed control techniques [1, 5, 6].

Since the introduction of first commercial herbicide 2, 4–D (2, 4–dichlorophenoxyacetic acid) in 1940s the farmers of different agricultural countries uses thousand tonnes of herbicide per year in order to control weeds. The higher amount of herbicide application lead to an increase in production cost as well as severe environmental problems, like ground water contamination, decline of the number of beneficial flora and fauna and also creates human health hazards directly or indirectly. In addition, uninterrupted use of same herbicides in crop fields makes the weeds resistant against herbicide [7, 8]. To avoid the detrimental effects of herbicide, the researchers of the different corners of the world are now searching for novel natural plant products to develop bio-herbicides. Numerous weeds and crop plants have been reported to possess allelopathic substances in order to compete with neighbouring plant species. Active allelochemicals are found in different parts of the plants like leaves, roots, stem, pollens, flowers, fruits and seeds [9]. These allelochemicals could be used as lead for herbicide production. Recently, the efforts have been made to identify and isolate these allelopathic properties and apply them as a tool for sustainable and eco-friendly weed control strategies [10].

Leucas aspera (Willd.) Linn. (Labiatae), is a small herbaceous, erect plant with a free blooming nature and flowering in the months of March to October. It is pungently aromatic herb and grows abundantly in the high land crop fields, roadsides, homesteads and fallow

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lands of the wide area of south Asia. The decoction of the whole plant is traditionally used for analgesic-antipyretic, anti-rheumatic, anti-inflammatory, antibacterial and antifungal treatment and its paste is applied topically to inflamed areas [11, 12]. It is also used as a medicine for coughs, colds, painful swellings and chronic skin eruptions [13], cobra venom poisoning [14, 15], tooth infections [16] and mosquito repellent [17] by the rural people and possesses wound healing properties [18]. Different types of chemicals such as glucosides, tannins, saponins, sterols, oleic acid, linoleic acid, linolenic acid, palmitic acid, stearic acid, oleanolic acid, ursolic acid, nicotin etc. have already been isolated from the leaves, roots, flower and seeds of this plant [12, 18-20]. To the best of our knowledge, no complete study has directly examined the allelopathic activity of *L. aspera* on weeds, despite of its long history as medicinal values and identification of different novel chemicals. Therefore, research work with *L. aspera* to investigate the allelopathic potentiality, warrant due attention.

MATERIALS AND METHODS

Plant Materials: Whole plants (leaves, stem and roots) of *L. aspera* were collected from Bangladesh during the month of July-August, 2011. After collection, plants were washed with tap water to remove the soil and other debris, dried under shed in order to avoid direct sunlight. Then the dried plants were kept in a refrigerator at 2°C temperature until extraction.

Test Plant Species: Seven test plant species, cress (*Lepidum sativum* L.), alfalfa (*Medicago sativa* L.), lettuce (*Lactuca sativa* L.), Italian ryegrass (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crusgalli* L.), jungle rice (*Echinochloa colonum L. Link*) and timothy (*Phleum pratense* L.) were selected for the present experiment. Among these seven test species, the first three are dicotyledonous and the rest are monocotyledonous. Cress, alfalfa, lettuce and timothy were chosen due to their known seedling growth, whereas Italian ryegrass, barnyard grass and jungle rice were chosen because they are most common weeds in the crop fields and distributed throughout the world.

Extraction of *L. aspera*: Whole plants (leaves, stem and roots) of dried *L. aspera* (100 g) were cut into small pieces and extracted with 1400 mL of 70 % (v/v) aqueous methanol for 48 h. After filtration using one layer of filter paper (No. 2; Toyo Ltd., Tokyo, Japan), the residue was re-extracted with 1400 mL of 100% methanol for 24 h and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40°C.

Bioassay: An aliquot of the extract (final assay concentration was 3, 10, 30 and 100 mg dry weight equivalent extract mLG^{1}) was evaporated to dryness at 40°C in *vacuo* by rotary evaporator, dissolved in 3.0 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 Ltd., Kyoto) Japan which was used for surfactant and did not cause any lethal effects. Ten seeds of cress, alfalfa, lettuce and ten germinated seeds of Italian ryegrass, barnyard grass, jungle rice and timothy were arranged on the filter paper in Petri-dishes. Italian ryegrass and barnyard grass were germinated in the darkness at 25°C for 24 h, jungle rice was germinated in the darkness at 25°C for 48 h and timothy was germinated in the darkness at 25°C for 72 h, after overnight soaking in each cases. The shoot and root lengths of the seedlings were measured at 48 h after incubation in darkness at 25°C. 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 percentage length of seedlings was then determined by reference to the length of control seedlings. The bioassay experiment was conducted with six replications. The inhibition percentage was calculated using the following equation:

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

The concentrations required for 50% inhibition (express as I_{50}) of the test plant species in the assay was calculated from the regression equation of the concentration response curves.

Statistical Analysis: Experimental data were analyzed using statistical software SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA) and GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, California, USA).

RESULTS

Effect of Aqueous Methanol Extract of *L. aspera* on the Shoot Growth of Test Plant Species: The aqueous methanol extract of *L. aspera* significantly inhibited the shoot growth of all the test plant species (Fig. 1).

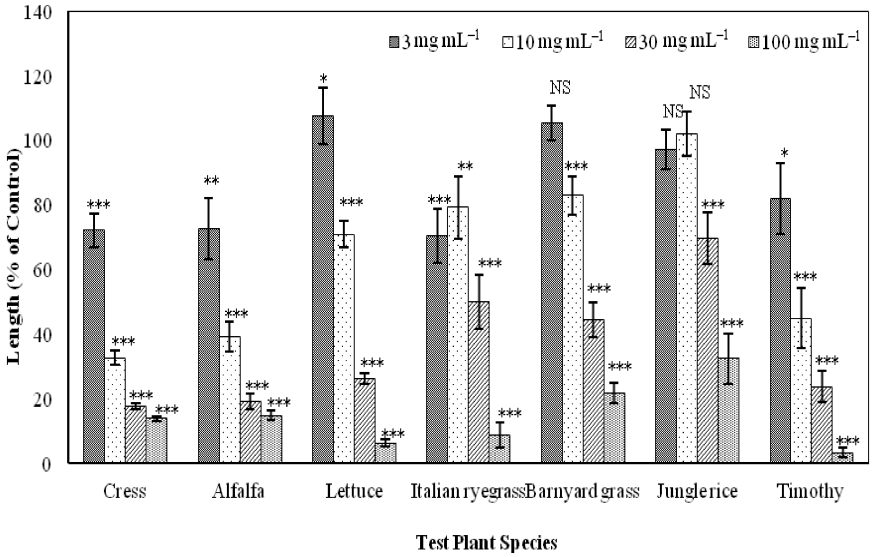

Fig. 1: Effects of aqueous methanol extract of *L. aspera* on shoot growth of the test plant species. Concentrations of tested samples corresponded to the extract obtained from 3, 10, 30 and 100 mg dry weight of *L. aspera*. Means±SE from three independent experiments with 10 seedlings for each determination are shown. Asterisks indicate a significant difference between control and treatment *p<0.05, **p<0.01 and ***p<0.001

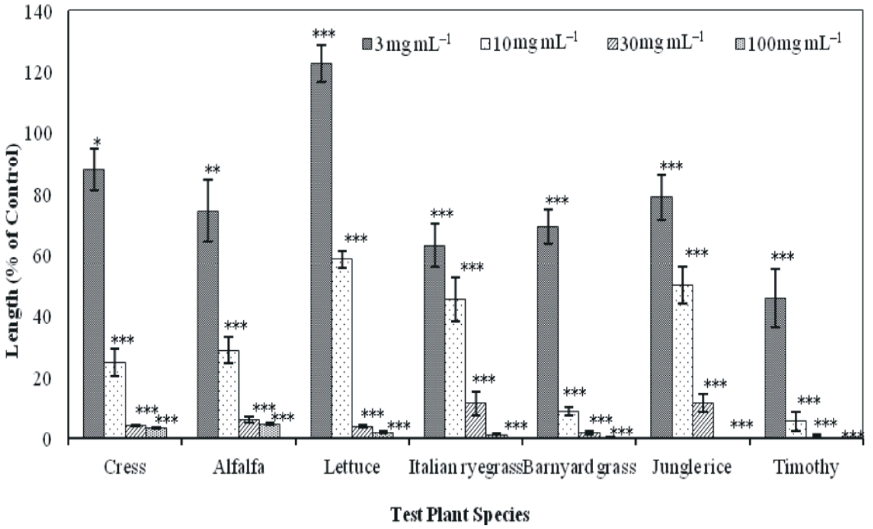

Fig. 2: Effects of aqueous methanol extract of *L. aspera* on root growth of the test plant species. Concentrations of tested samples corresponded to the extract obtained from 3, 10, 30 and 100 mg dry weight of *L. aspera*. Means±SE from three independent experiments with 10 seedlings for each determination are shown. Asterisks indicate a significant difference between control and treatment *p<0.05, **p<0.01 and ***p<0.001.

The extract obtained from 30 mg dry weight of the *L. aspera* plant inhibited the cress, alfalfa, lettuce, timothy, barnyard grass, Italian ryegrass and jungle rice by 81, 73, 73, 73, 54, 45 and 31%, respectively. Percent inhibition increased with the increase of the extract concentrations. The shoot growth of timothy and lettuce seedlings showed more than 90% inhibition at a concentration of 100 mg dry weight equivalent extract mLG^{1}, followed by Italian ryegrass, cress, alfalfa, barnyard grass and jungle rice at 88, 85, 81, 76 and 68%, respectively. On the other hand, the shoot growth of lettuce was significantly stimulated by the extract of *L. aspera* at the concentration of 3 mg dry weight equivalent extract mLG^{1} (Fig. 1). Considering the concentration required for 50% inhibition (I_{50}), it was revealed that the shoot growth of cress seedlings was

Table 1: I_{50} values of aqueous methanol extract of *L. aspera* for shoots and roots of the test plant species

	I_{50} (mg dry weight equivalent extract mLG^{1})	
Test Plant Species	Shoot	Root
Cress	7.0	7.0
Alfalfa	11.0	10.0
Lettuce	18.0	10.0
Italian ryegrass	38.0	7.0
Barnyard grass	37.0	6.0
Jungle rice	60.0	15.0
Timothy	10.0	3.0

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

most sensitive to the extracts than the other test species (Table 1). On the other hand, the shoot growth of jungle rice was less sensitive to the aqueous methanol extracts.

Effect of Aqueous Methanol Extract of *L. aspera* on the Root Growth of Test Plant Species: Root growth of both monocotyledonous and dicotyledonous test plant species were significantly inhibited by the aqueous methanol extracts of *L. aspera* plant (Fig. 2). At a concentration of 30 mg dry weight equivalent extract of *L. aspera* plants mLG^{1}, the root growth of timothy, barnyard grass, lettuce, cress, alfalfa, Italian ryegrass and jungle rice were inhibited by 99, 98, 96, 95, 91, 87 and 86%, respectively. When the test plant species were exposed to the concentration of 100 mg dry weight equivalent extract mLG^{1} the root growth of timothy, jungle rice, barnyard grass seedlings were completely (100%) inhibited and the root growth of Italian ryegrass, lettuce, cress and alfalfa seedlings were inhibited by 99, 98, 96 and 93%, respectively. In contrast, the root growth of lettuce was significantly stimulated by the extract of *L. aspera* at the concentration of 3 mg dry weight equivalent extract mLG^{1} (Fig. 2). Considering I_{50} value (Table 1), the root growth of timothy was most sensitive to the *L. aspera* extracts than the other test plant species, whereas jungle rice was less sensitive to the extracts.

DISCUSSION

The seedlings growth of both the dicotyledonous and monocotyledonous plants species was significantly inhibited by most of the concentration of aqueous methanol extract of *L. aspera* (Fig. 1 and 2). Such inhibition on the shoot and root growth of the test plant species might be due to the presence of allelochemicals in the *L. aspera* plant extract and the percent inhibition of the test plant species showed the similar trend with the increase of concentration of the extracts. These types of growth inhibition by the allelopathic plants extract was also reported by Caussanel [21], Inderjit and Keating [22], An *et al*. [23], Batlang and Shushu [24], Pukclai and Kato-Noguchi [25].

The shoot and root growth inhibition of the test plant species, observed in this experiment might be due to the allelopathic effect of *L. aspera* as there was no intra species competition for light, nutrient, space and moisture. The seedlings of each test plant species used in this experiment were grown in a single Petri-dish without supplying any nutrient solution. The young seedlings only get nutrients from their reserved food material into the seeds. Furthermore, light is dispensable for the young seedlings at development stage [26]. From this viewpoint, it is clear that the seedlings growth inhibition of the test plant species is mainly due to the allelopathic reaction rather than by competitive interference [25, 27].

The growth inhibition of the test plant species, in the presence of allelochemicals could be for the reason of lower cell division, elongation and expansion rate which are growth pre-requisites [28-30]. The chemical agents that inhibit cell division can act in two ways: (i) by affecting the synthesis or the structure of DNA-RNA and (ii) by inhibiting the energy production necessary for the process of mitosis [31]. Both processes are important for the cell division and interferences with them generally cause inhibition of the whole process. Moreover, allelochemicals inhibit the respiration [22], ion absorption process [32], enzyme activity [33], alteration of the phytochrome control of germination [34] and thus, results in arrested plant growth [35]. Allelochemicals may produce more than one effect of the above on the cellular processes that are responsible for reduced plant growth. However, the details of the biochemical mechanism through which allelochemicals exert a toxic effect on the growth of plants are still not well known [36].

Comparing the inhibition of both shoot and root growth of all the test plant species, the aqueous methanol extract of *L. aspera* had higher root growth inhibition than that of the shoot growth (Fig. 1 and 2). These results are in agreement with the earlier findings of many researchers working with other plant materials. Levizou *et al*. [37] found low mitotic division in root apex resulted in higher root inhibition of lettuce when treated with *Dittrichia viscose* leaf extracts. Parallel findings were also reported by Stachon and Zimdahl [38], Aliotta et al. [39], Salam and Kato-Noguchi [40], Pukclai and Kato-Noguchi [25]. The

higher root growth inhibition is mainly because the roots are the first organ to absorb allelochemicals from the environment [41] and the permeability of allelochemicals into root tissue is higher than the shoot tissue [42].

Among the test plant species timothy was the most sensitive plant followed by cress to the extract concentration to show 50% shoot-root growth inhibition, whereas jungle rice was less sensitive to the extract of *L. aspera* (Table 1). These findings indicate that inhibitory effects of *L. aspera* on different test plant species are not identical. This asymmetrical susceptibility to different extracts could be due to inherent differences in various bio-chemicals involved in the process. The species specificity of phytotoxins has also been established for other allelopathic plants species [43]. It is important to note that *L. aspera* had strong inhibitory effect on the growth of two noxious paddy weeds, namely barnyard grass and jungle rice (Fig. 2) at higher concentration, which denotes some promising outcome in near future to control these deleterious weeds species in a sustainable way. Besides this, it was also revealed that the seedlings growth of lettuce was significantly stimulated by the aqueous methanol extract of *L. aspera* at the lowest concentration. Many studies have already proved that allelochemicals can stimulate the seedlings growth at low concentrations but inhibit the seedlings growth at high concentrations [28, 44-46].

CONCLUSIONS

Our results indicated that aqueous methanol extract of *L. aspera* exhibits strong phytotoxicity and possesses allelochemicals to suppress other plant species including barnyard grass and jungle rice, two noxious paddy weeds over the whole world. These results suggest that *L. aspera* could be act as important source of natural herbicides to control major weeds of different crop fields. Further isolation and identification of the allelochemicals from aqueous methanol extract of *L. aspera* is in progress. The detection of these compounds might provide chemical basis for the development of bio-herbicides for environment friendly sustainable crop production systems.

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