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Research Paper

Allelopathic effects of different extracts of honeyweed (*Leonurus siribicus*) on seeds germination and seedlings growth of some selected vegetables

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ABSTRACT

This study investigate the allelopathic effect of Honey weed (*Leonurus siribicus* L.) on some selected vegetable seeds and their seedlings growth. The selected vegetable seeds and seedlings were Brinjal (*Solanum melongena*), Okra (*Abelmoschus esculentus*), Amaranth (*Amaranthus tricolor*) and Cucumber (*Cucumis sativus*). The aqueous, ethanol and acetone extracts of *L. siribicus* were significantly decreased and inhibited germination percentage of selected vegetable seeds. The extracts were also retarded the root and shoot growth of the same in compare to control. This study reveals that different extracts of *Leonurus siribicus* may contain allelochemicals which are responsible for decreasing germination and shoot and root growth retardation. Further study is needed to determine the effective allelochemicals from different extracts of *L. siribicus* for controlling weed in practicing organic agriculture.

Keywords: Vegetable seeds; Germination; Extract; Seedling growth.

INTRODUCTION

Plants live together in communities composed of one or more species. Some plants may inhibit germination, emergence and subsequent growth of other plants by exuding toxic substances (Benyas, et al., 2010). These substances are called allelopathic chemicals or allelochemicals and the process is called allelopathy (Rice, 1984). Allelopathic chemicals may be distributed broadly among organs such as seeds, flowers, pollen, leaves, stems, and roots, or sometimes found in just one or two of such organs (Zeng, et al., 2008). When these allelochemicals are taken up by germinating seeds of the same or of other plant species there may be some degrees of germination and emergence inhibition or growth injury (Bochow, 1965). However, several allelochemicals causing germination and/or growth inhibitions have been isolated from plant tissues and soils, which are usually secondary plant products or waste products of the main metabolic pathways of plants (Whittaker and Feeny, 1977; Hall and Henderlong, 1989; Chon and Kim, 2002; Ashrafi, et al., 2007). These isolated metabolites can inhibit or delay germination and also inhibit or stimulate the growth of roots and shoots of neighboring plants (Ninkovic, 2003). Generally, interactions between plants are called interference and include positive, negative, and neutral effects on each other (Olofsdotter, 2001; Inderjit, et al., 2001; Golisz, et al., 2007). Interference has two components: competition and allelopathy. Competition between weeds and crops occurs when some factors, such as water, nutrients, or sunlight is insufficient to meet the needs of both the weed and the desired plant (Gibson and Liebman, 2003; Kadioglu and Yanar, 2004; Tanveer, 2010). Weeds can also affect a crop's growth by releasing allelochemicals into the growing environment. All plant parts of the weed including leaf, stem, root, and fruit have allelopathic potential. However, various parts of weeds show different behavior in exerting their allelopathic effects on crops. Weeds also exert allelopathic effects on crop seed germination and growth by releasing water-soluble compounds into the soil (Olofsdotter, et al., 1999; Zuo, et al., 2008). Many phytotoxic chemical substances are known to be exuded by plants to suppress emergence or growth of the other plants. As the knowledge on these substances advances, these may be used as herbicide, which will be very beneficial for environment (Edrisi and Farahbakhsh, 2011).

One of the weeds, *Leonurus siribicus* L. (Family-*Lamiaceae*), commonly called Honeyweed or Siberian motherwort, is an annual or biennial herbaceous plant with upright stems that grow from 20 to 80 cm tall native to central and Southwest Asia (Sheng Ming, et al., 2006). This plant has long petioled basal leaves that are ovate-cordate in shape. The leaves have toothed margins and are incised with deeply cut lobes. Typically, one or a few flowering stems are produced from short tap-roots. This weed species is also very common in Bangladesh. They grow frequently in crop fields here in Bangladesh. It is regarded as medicinal plant especially used for the treatment of gynecologic disorders such as menorrhagia, menostasia, and other irregular menstruation (Sheng Ming, et al., 2006). Various research works have been conducted with its medicinal value but research data are not available on its allelopathic effect.It is important note that it is the first work about allelopathic effects of honeyweed to date in Bangladesh. In a study conducted by Sheng Ming, et al. (2006), it has been found that *L. siribicu*s contains various phenolic compounds like 4-hydroxythiophenol, syringic acid, apigenin, genkwanin, isoquercitrin, rutin, 4-hydroxybenzoic acid etc. Syringic acid and 4-hydroxybenzoic acid are closely related to allelopathic effect on some plants (Sasikumar, et al., 2001). Thus, this study was conducted in order to investigate the allelopathic effect of *L. siribicus* on some selected vegetable plants.

MATERIALS AND METHODS

Experimental Site**:** The experiment was conducted at research laboratory, Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh, during April 2012- June 2012.

Collection of Leonurus siribicus and vegetable seeds**:** Aerial parts of *L. siribicus* were collected from different agricultural fields of Santhia and Atghoria Upazila under Pabna district. Brinjal (*Solanum melongena*), Okra (*Abelmoschus esculentus*), Amaranth (*Amaranthus tricolor*) and Cucumber (*Cucumis sativus*) seeds were selected due to their short growth period and available in sub-tropical countries. The seeds of these vegetables were collected from the Dinajpur local seed market. The purity and germination percentages of these seeds were 95% and 90%, respectively.

Preparation of aqueous extracts of Leonurus siribicus: About 50g of fresh aerial parts of *Leonurus siribicus* were cut into smaller pieces and blended by blender and taken into a 1000ml reagent bottle with water upto the mark. It was kept for 72 hours at room temperature with regular interval of stirring. After 72h, the resulting brownish and dark solution were filtered through three layers of very fine cloth and finally by filter paper. Then the filtered suspension was dried by BUCHI Rota vapor R-114 connected with BUCHI water bath B-480 at 100°C. The dried extract was weighed by digital balance. 5% solution from this extract was prepared with distilled water for treating the seeds of vegetable crops.

***Preparation of acetone extract and ethanol extract of Leonurus siribicus*:** 10Kg of aerial parts of *L. siribicus* were cleaned and cut into small pieces. The small pieces of aerial parts were dried in sun light for 7 days followed by oven at 70°C for 48h. The dried stem was grinded by Grinding Machine and obtained about 4kg powder. 2Kg powder was dissolved in 5L absolute acetone (96%) and another 2Kg in absolute ethanol (98%) and incubated for 72h for a suspension. These suspensions were filtered with thin, clean cloth and then by filter paper. Then suspensions were dried by BUCHI Rota vapor R-114 connected with BUCHI water bath B-480 at 50°C and 70°C, respectively. The dried extracts were weighed by Digital balance. 5% solution of each extracts was prepared with water for treating the seeds of vegetable crops. The individual plant extracts were investigated as following sequential treatments:

A- Control (Use distilled water) (T_c); B- Aqueous extract of *Leonurus siribicus* (T_1); C- Ethanol extract of *Leonurus siribicus* (T_2); D- Acetone extract of *Leonurus siribicus* (T_3)

***Set up for the investigation of vegetable crop seeds*:** 48 petridishes were cleaned and divided into 4 groups for 4 vegetables seeds groups and two sheets filter papers were placed on it. For each vegetable seeds, petridishes were divided into four treatments with three replications separately (Fig 1). 15ml of each aqueous, ethanol, acetone extract and distilled water were treated in each petridishes according to experimental design. After that, 25 seeds of each vegetable crop were kept in each petridish. The petridishes were placed in natural diffused light under laboratory conditions at 29±2°C temperature and relative humidity of 85±5%. Five ml of water was applied per day per Petri-dish to keep constant moisture. The percent germination, shoot length, root length were recorded accordingly with a regular interval of three days.

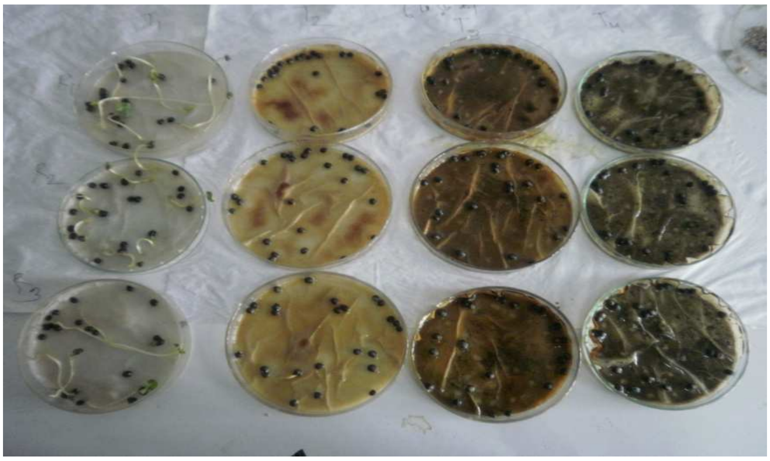

Figure-1: Seeds set up for the investigation

Data collection and statistical analysis: The data regarding each vegetable seed group were recorded three times starting from just after first germination maintaining a regular interval of 3 days. Data were analyzed by using MSTAT-C computer program following Duncan's Multiple Range Test with 5% level of significance.

RESULTS

Tables 1, 2 and 3 showed the allelopathic effect of different extracts of *L. siribicus* on germination and growth of selected vegetable crops in comparison to control.

Effects of *Leonurus siribicus* extracts on Brinjal:

Percent germination: The percent germination was counted in 8^{th}, 11^{th} and 14^{th} days presented in Table 1. In the 8^{th} day, the highest germination was found in T_c (8%) followed by T_3 (4%). No germination recorded in both T_1 and T_2. In 11^{th} day, highest germination was found in T_c (26.67%) followed by T_1 and T_3. Lowest germination percentage was recorded in T_2 (2.67%). In 14^{th} day, the highest germination was found in T_c (38.67%) and the lowest germination was recorded in T_2 (6.67%).

***Shoot length*:** The growth of shoot length of Brinjal was significantly inhibited by different extracts of *Leonurus siribicus* (Table 2). After 7 days after sowing (DAS), T_c showed the highest shoot length (0.20cm) followed by T_3 (0.17 cm) whereas the lowest shoot length (0.00cm) was recorded in T_1 and T_2. The highest shoot length of brinjal seedling was found in T_c i.e. control treatment (2.40cm) at 10 DAS and lowest value was found in T_2 (0.20cm). T_1 (0.30cm), T_2 (0.20cm) and T_3 (0.27cm) are not statistically different but significantly differ from T_c (2.40). At 13 DAS, the highest shoot length was recorded in T_c (2.87 cm) and the lowest was found in T_3 (0.30cm). Here also only T_c is statistically different from the other three.

Root length: Root length of brinjal was significantly influenced by different extracts of *L. siribicus* (Table 3). After 7 DAS, control treatment (T_c) showed the highest root length (0.27cm) which was statistically similar to T_3 treatments, whereas the lowest root length (0.00cm) was recorded in both T_1 and T_2 treatments. The highest and lowest root length of brinjal seedling was found in T_c i.e. control treatment (3.10cm) and T_3 (0.27cm), respectively at 10 DAS. Here T_c differs significantly from others. At 13 DAS, the highest root length was also recorded in T_c (3.57cm) and the lowest was found in T_3 (0.33cm). T_1, T_2 and T_3 were statistically similar but different from T_c.

Effects of *Leonurus siribicus* extracts on Okra

Percent germination: The highest germination percentage was counted in 2^{nd}, 5^{th} and 8^{th} days presented in (Table 1). In the 2^{nd} day, the highest germination was found in T_c (38.67%). No germination was recorded in T_1, $T_2$and T_3. In 5^{th} day, the highest germination was found in T_c (54.67%) followed by T_1 (2.67%). No germination percentage was recorded in both T_2 and T_3. In 8^{th} day, the highest germination was found in T_c (61.33%) followed by T_1 (2.67%) and no germination was recorded in both T_2 and T_3.

Shoot length: Shoot length of okra was significantly influenced by different extracts (Table 2). At 1 day after sowing (DAS), T_c showed the highest shoot length (1.27cm). The lowest shoot length (0.00cm) was recorded in T_1, T_2 and T_3, respectively. The highest shoot length of okra seedling was found in T_c i.e. control treatment (7.30cm) at 4 DAS and lowest value was found in T_1, T_2 and T_3. Here only T_c is statistically different from other three. At 7 DAS, the highest shoot length was recorded in T_c

(11.17cm) and the lowest was found in T_1 (0.83cm). Here also only T_c is statistically different from the other three.

Root length: Root length of okra was significantly influenced by different extracts of *L. siribicus* (Table 3). At 1 DAS, control treatment (T_c) showed the highest root length (2.03cm) whereas the lowest root length (0.00cm) was recorded in T_1, T_2 and T_3 treatments. The highest root length of okra seedling was found in T_c i.e. control treatment (3.30cm) and lowest value (0.00cm) was found in T_1, T_2 and T_3 at 4 DAS. Here T_c was statistically different from others. At 7 DAS, highest root length was also recorded in T_c (5.00cm) followed by T_1 (0.40cm) and the lowest value (0.00cm) was found in both T_2 and T_3. T_1, T_2 and T_3 are statistically similar but different from T_c.

Effects of *Leonurus siribicus* extracts on Amaranth

Percent germination: The highest germination percentage was counted in 6^{th}, 9^{th} and 12^{th} days presented in (Table 1). In the 6^{th} day, the highest germination was found in T_c (32.00%). No germination was recorded in T_1, $T_2$and T_3. In 9^{th} day, the highest germination was found in T_c (37.33%) followed by T_1 (2.67%). No germination was recorded in both T_2 and T_3. In 12^{th} day, the highest germination was found in T_c (41.33%) followed by T_1(6.67%) and no germination was recorded in both T_2 and T_3.

Shoot length**:** Shoot length of amaranth was significantly influenced by different extracts of *L. siribicus* (Table 2). At 5 days after sowing (DAS), T_c showed highest shoot length (1.97cm). The lowest shoot length (0.00 cm) was recorded in T_1, T_2 and T_3. The highest shoot length of amaranth seedling was found in T_c i.e. control treatment (2.77cm) at 8 DAS followed by T_1 (0.10) and lowest value (0.00cm) was found in both T_2 and T_3. Here only T_c is statistically different from other three. At 11 DAS, the highest shoot length was recorded in T_c (2.67cm) followed by T_1 (0.27cm). No shoot development was found in both T_2 and T_3. Here also only T_c is statistically different from the other three.

Root length**:** Root length of amaranth was significantly influenced by different extracts of *L. siribicus* (Table 3). At 5 DAS, control treatment (T_c) showed the highest root length (2.37cm) whereas the lowest root length (0.00cm) was recorded in T_1, T_2 and T_3 treatments. The highest root length of amaranth seedling was found in T_c i.e. control treatment (2.40cm) followed by T_1 (0.13cm) and lowest value (0.00cm) was found in both T_2 and T_3 at 8 DAS. Here T_c was statistically different from others. At 11 DAS, the highest root length was also recorded in T_c (2.23cm) followed by T_1 (0.73cm) and the lowest value (0.00cm) was found in both T_2 and T_3. T_1, T_2 and T_3 are statistically similar but different from T_c.

Effects of *Leonurus siribicus* extracts on Cucumber

Percent germination: The highest germination percentage was counted in 2^{nd}, 5^{th} and 8^{th} days presented in (Table 1). In the 2^{nd} day, the highest germination was found in T_c (58.67%) followed by T_3 (2.67%). The lowest germination (1.33%) was recorded in both T_1, T_2. In 5^{th} day, the highest germination was found in T_c (69.33%) followed by T_3 (4.00%). The lowest germination percentage (2.67%) was recorded in both T_1 and T_2. In 8^{th} day, the highest germination was found in T_c (78.00%) followed by T_3(4.00%) and lowest germination (2.67%) was recorded in both T_1 and T_2.

Shoot length**:** Shoot length of cucumber was significantly influenced by different extracts of *Leonurus siribicus* (Table 2). At 1 day after sowing (DAS), T_c showed the highest shoot length (2.33cm) followed by T_3 (0.67cm). The lowest shoot length (0.60cm) was recorded in both T_1 and T_2. The highest shoot length of cucumber seedling was found in T_c i.e. control treatment (4.37cm) at 4 DAS followed by T_2 (1.20cm) and lowest value (0.90cm) was found in T_3. Here only T_c is statistically different from other three. At 7 DAS, the highest shoot length was recorded in T_c (11.67cm) followed by T_2 (1.20cm). The lowest shoot development was found in T_3 (0.93cm). Here also only T_c is statistically different from the other three.

***Root length*:** Root length of cucumber was significantly influenced by different extracts of *Leonurus siribicus* (Table 3). At 1 DAS, control treatment (T_c) showed the highest root length (3.27cm) followed by T_3 (0.13cm) whereas the lowest root length (0.07cm) was recorded in both T_1 and T_2 treatments. The highest root length of cucumber seedling was found in T_c i.e. control treatment (4.03cm) followed by T_3 (0.43cm) and lowest value (0.13cm) was found in both T_1 at 4 DAS. Here T_c was statistically different from others. At 7 DAS, the highest root length was also recorded in T_c (5.47 cm) followed by T_3 (0.57 cm) and the lowest value (0.13 cm) was found in both T_1 and T_2. T_1, T_2 and T_3 are statistically similar but different from T_c.

DISCUSSION

Here we investigate the allelopathic effects of honey weed extracts on some vegetables seed. There are many data about medicinal effects of honey weed but to the best of our knowledge it is the first work of allelopathic effects of honey weed on vegetables seed to date. Different extracts (aqueous, ethanol and acetone) were applied to investigate the percent germination, shoot length and root length of brinjal, okra, amaranth and cucumber. Different extracts significantly reduced percent germination and inhibited growth of shoot and root length of brinjal, okra, amaranth and cucumber than the control. Possibly all extracts of *Leonurus siribicus* may contain some growth inhibitors or allelochemicals or other bioactive substances, which may responsible for stunting the shoot length of cucumber (Sasikumar, et al., 2001; Sheng Ming, et al., 2006). Different extracts may be contained some toxic substances (allelochemicals) that inhibited germination, root and shoot growth (Benyas, et al., 2010). When these allolochemicals taken up by germinating seeds subsequently inhibited germination percentage, shoot and root growth (Bochow, 1965). Many experiments have been conducted to find out the effects of different plant extracts on germination, shoot and root growth. *Tinospora cordifolia*, a wild medicinal plant was applied to investigate the effects of that plant extract on germination, shoot and root growth (Akter, et al., 2012). They found that different extracts significantly reduced germination %, root and shoot growth of radish, swamp cabbage and lady's finger. Roy et al., 2012 also reported that the seed and seedling growth of swamp cabbage and okra in response to aqueous extract of 4 herbal plant leaves. These 4 extracts significantly reduced the germination, shoot and root growth.

CONCLUSIONS

Active allelochemical(s) present in the aerial parts of *Leonurus siribicus* were soluble in all solvents used. Thus, aqueous, ethanol and acetone extract of the aerial part of *Leonurus siribicus* was shown to inhibit the germination and retarded the subsequent growth of selected vegetables. Its presence in crop field will drastically reduce the crop yield of studied vegetables and it might have similar effect on other field crops. There may have an option of using allelochemical(s) present in *Leonurus siribicus* as herbicide for ensuring better performances in organic agriculture.

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Table- 1: **Effects of different extracts of *Leonurus siribicus* on percent germination of Brinjal, Okra, Amaranth and Cucumber seeds.**

Treatments	% Germination											
	Brinjal			Okra			Amaranth			Cucumber		
	8th day	11th day	14th day	2nd day	5th day	8th day	6th day	9th day	12th day	2nd day	5th day	8th day
Control (T_c)	8.00 a	26.67 a	38.67 a	38.67 a	54.67 a	61.33 a	32.00 a	37.33 a	41.33 a	58.67 a	69.33 a	76.00 a
Aqueous Extract (T_1)	0.00 b	8.00 b	12.00 b	0.00 b	2.67 b	2.67 b	0.00 b	2.67 b	6.67 b	1.33 b	2.67 b	2.67 b
Ethanol Extract (T_2)	0.00 b	2.67 b	6.67 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	1.33 b	2.67 b	2.67 b
Acetone Extract (T_3)	4.00 ab	8.00 b	8.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	2.67 b	4.00 b	4.00 b

- Data expressed in mean value; Different letter in a column differ significantly at 5% level of significance (as per DMRT).

Table- 2: **Effects of different extracts of *Leonurus siribicus* on shoot length of Brinjal, Okra, Amaranth and Cucumber seeds.**

Treatments	Shoot length (cm)											
	Brinjal			Okra			Amaranth			Cucumber		
	8th day	11th day	14th day	2nd day	5th day	8th day	6th day	9th day	12th day	2nd day	5th day	8th day
Control (T_c)	0.20 a	2.40 a	2.87 a	1.27 a	7.30 a	11.17 a	1.97 a	2.77 a	2.67 a	2.33 a	4.37 a	11.67 a
Aqueous Extract (T_1)	0.00 b	0.30 b	1.50 ab	0.00 b	0.00 b	0.83 b	0.00 b	0.10 b	0.27 b	0.60 a	1.13 b	1.13 b
Ethanol Extract (T_2)	0.00 b	0.20 b	0.87 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.60 a	1.20 b	1.20 b
Acetone Extract (T_3)	0.17 a	0.27 b	0.30 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.67 a	0.90 b	0.93 b

- Data expressed in mean value; Different letter in a column differ significantly at 5% level of significance (as per DMRT).

Table -3: **Effects of different extracts of *Leonurus siribicus* on root length of Brinjal, Okra, Amaranth and Cucumber seeds.**

Treatments	Root length (cm)											
	Brinjal			Okra			Amaranth			Cucumber		
	8th day	11th day	14th day	2nd day	5th day	8th day	6th day	9th day	12th day	2nd day	5th day	8th day
Control (T_c)	0.27 a	3.10 a	3.57 a	2.03 a	3.30 a	5.00 a	2.37 a	2.40 a	2.23 a	3.27 a	4.03 a	5.47 a
Aqueous Extract (T_1)	0.00 b	0.37 b	1.20 b	0.00 b	0.00 b	0.40 b	0.00 b	0.13 b	0.73 b	0.07 b	0.13 b	0.13 b
Ethanol Extract (T_2)	0.00 b	0.57 b	0.97 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.07 b	0.17 b	0.13 b
Acetone Extract (T_3)	0.17 a	0.27 b	0.33 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.13 b	0.43 b	0.57 b

- Data expressed in mean value; Different letter in a column differ significantly at 5% level of significance (as per DMRT).

