Pak. J. Bot., 46(5): 1693-1701, 2014.

ROOT EXUDATES AND LEAF LEACHATES OF 19 MEDICINAL PLANTS OF PAKISTAN EXHIBIT ALLELOPATHIC POTENTIAL

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Abstract

Laboratory experiments were conducted to evaluate the allelopathic potential of root exudates and leaf leachates of 19 medicinal plants commonly used in Pakistan by plant box and sandwich methods, respectively. In sandwich method, lettuce seedlings were grown with the dry leaf leachates of the selected plant species in a growing media at the rate of 5, 10 and 50 mg dish^{-1} in a completely randomized design with three replications. Their effects on hypocotyl and radicle growth of the lettuce were recorded as a percentage of untreated control. Data was subjected for analysis of variance and treatment means were compared by Tukey's HSD test at $p<0.05$. Results indicated that allelopathic effects of the leaf leachates of all selected plant species on the hypocotyl and radicle elongations of the lettuce varied significantly in all concentrations used in experiments. The hypocotyl growth of the lettuce seedlings was affected from promotion (−6.71% inhibition) caused by *Phlaris minor* to inhibition (78.40%) by *Withania somnifera*. Both species suppressed the radicle length from 33.69–93.30%. Leachates of *W. somnifera* and *Sarcococca saligna* exhibited strong inhibitory results in a concentration dependant manner. After the growth period of 50 days, root exudates of *S. saligna* appeared most detrimental (78.00% inhibition) to radicle growth of the lettuce seedling followed by *W. somnifera* (75.00%) when tested by plant box method. The results presented can be utilized as benchmark information for further joint research on the elucidation of chemicals involved in the allelopathy in nature and in the development of new and potent bioherbicides to combat environmental risk.

Key words: Allelopathy, Medicinal plants, Laboratory screening, Plant box method, Sandwich method, Weed control.

Introduction

Weeds cause many losses due to their interference in agro–ecosystems and have been remained the most troubling agent for the farmers in maximizing their agricultural productivity. The pervasive use of synthetic chemicals for weed control has resulted in herbicide–resistant weeds along with ecological and human health deteriorations. Concerns are increasing day–by–day and world's attention is focused on non–herbicidal innovations to control weeds and thus cutting down the ultimate dependence on harmful synthetic herbicides (Dayan *et al.,* 2009). The phenomenon of allelopathy has emerged as one of the potential alternative tools for integrated weed management in this regard (Albuquerque *et al.,* 2010). Allelopathy encompasses any direct or indirect (stimulatory or inhibitory) impact of one plant on another plant by the exudation of chemicals through different plant parts into the environment (Rice, 1984). These biologically active chemicals or natural products are secondary metabolites, called allelochemicals, act vitally in regularizing the structure of plant communities (Whittaker & Feeny 1971).

Several ways have been suggested to incorporate allelopathy in weed management system including toxic allelopathic extracts (Cheema, 2005); allelopathic cover, smoother, rotational and companion crop (Milchunas *et al*., 2011); mulch or crop residue (Matloob *et al.,* 2010) allelochemicals as a natural herbicides (Dayan *et al.,* 2009; Syed *et al*., 2014) and finally breeding crops cultivars with allelopathic traits (Ibrahim *et al.,* 2013). Allelopathy, therefore, has showed promising results to have great potential in all these prospects (Fujii, 2003; Duke, 2010; Schulz *et al.,* 2013).

Many crop and weed species have been observed for their allelopathic activity However, the exploitation of medicinal plants for their plant growth inhibitory activity is of recent interest. Fujii *et al*., (2003) screened 239; Gilani *et al*., (2010) 81 and Shinwari *et al*., (2013) 38 medicinal plant species for their allelopathic activity against lettuce seedling and outlined the possible candidates among them to be used for further isolation and identification of allelochemicals. Pakistan has a diverse range of climatic and phytogeographic conditions resulting in diverse flora containing several medicinal plant species. About 4,950 plants species have their existence in Pakistan and 300 species of them have been identified as medicinal plants (Shinwari *et al.,* 2002). These species can be exploited for the possible source of allelochemicals that would help in reducing the over–reliance on synthetic chemicals and thereby leading towards a sustainable safe and agriculture system.

To investigate the possible involvement of allelopathy, laboratory bioassays are the first step used. Therefore, allelopathic phenomenon amongst plants can be exploited by using different laboratory screening bioassays (Fujii *et al*., 1991, 2003). Unfortunately, there are only limited numbers of reports conducted on this aspect of medicinal resources of plants.

In the present study, 19 commonly used medicinal plant species having pharmaceutical importance in Pakistan were investigated for their allelopathic potential by using several laboratory techniques. The objectives of the present study were (i) to evaluate the allelopathic potential of leaf leachates and root exudates of selected medicinal plants species, (ii) to rank them in the order of their activity, and (iii) to suggest the most active species for isolation and identification of putative allelochemicals present in it.

Materials and Methods

Donor plants: Nineteen plant species (Fig. 1) were used as donor plants. Their seeds were collected from the fields of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan (PMAS–AAUR) and National Agriculture Research Center, Islamabad. Seeds of these plants were grown in a greenhouse in Japan for 50 days until each plant gained 1–2 g fresh weight. Then, they were used in a bioassay for allelopathic effect of root exudates by the plant–box method (Fujii, 1994; Shiraishi *et al*., 2002, 2005).

Receptor plant: Seeds of *Lactuca sativa*, L. *cv.* Great Lakes 366 (lettuce) bought from Takii Seed Co. Ltd., Kyoto, Japan, were used as receptor plant material.

Bioassay for root exudates from the selected plant species by the plant box method: The root exudates from the donor species were assayed for their allelopathic potential by the plant box method. The roots of these plants (donor plants) were placed in a nylon gauze tube. Thereafter, these tubes were laid in a corner of a plant box (width=60 × length = 60 × height=95; all in millimeters). An untreated control was prepared in another plant box without a donor plant. Medium was prepared by using 0.75% (w/v) Agar (Wako Pure Chemical Industries, Osaka), cooled (~36°C), and poured to boxes. Thirty three seeds of lettuce were then positioned on the cooled agar gel in every box. In a growth chamber, the plants were incubated at 25°C for five days, for 12 hours in the daylight. Three replications were put up for each donor plant.

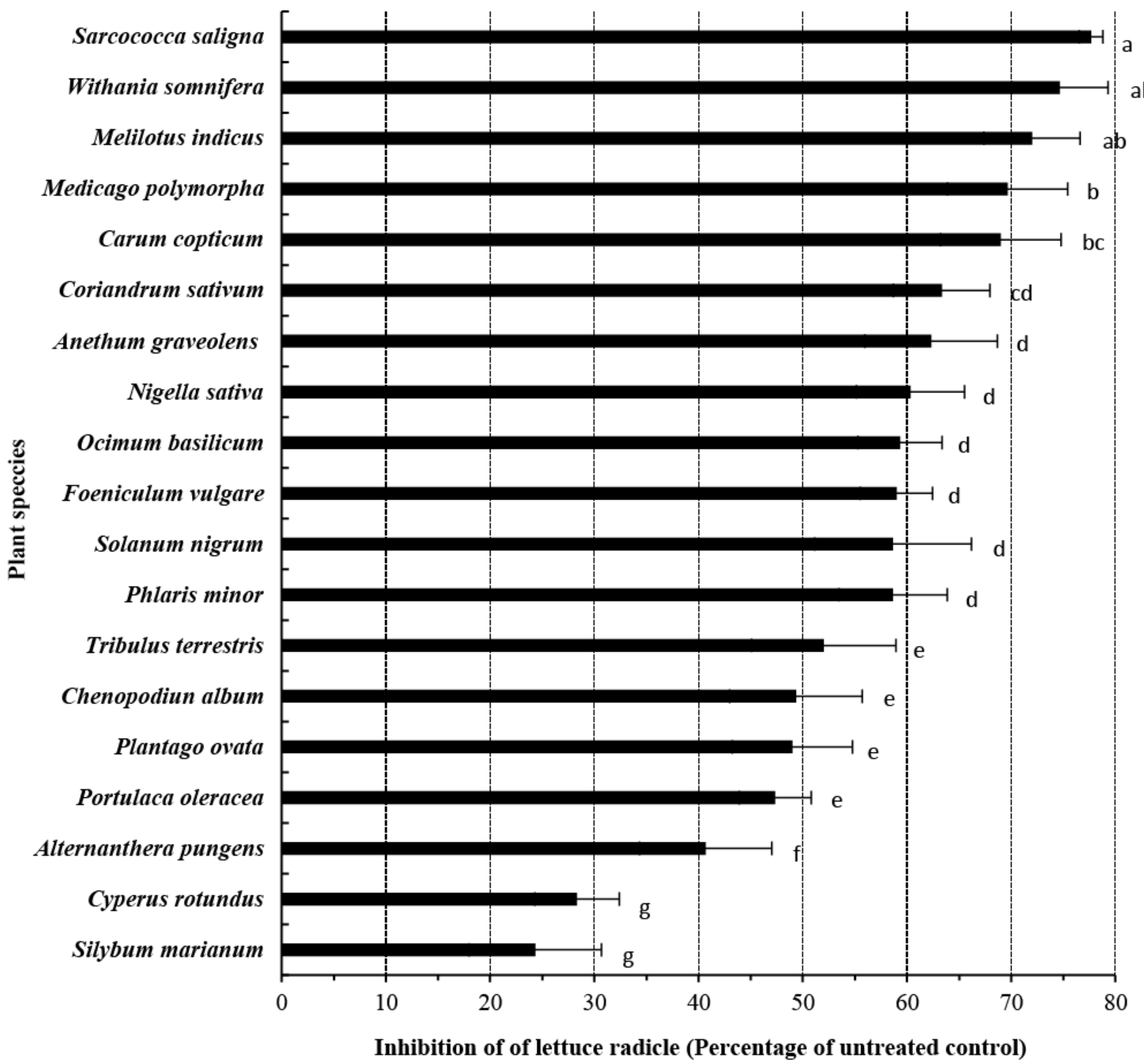

Fig. 1. Inhibitory activities of root exudates of 19 different plant species on the radicle length of lettuce seedling. Length of each bar represents the inhibition of radicle length of lettuce seedlings by root exudates of a donor species as a percentage of untreated control. Each bar in a graph is a mean of three replicates with Standard error. Bar sharing the same letters are not significant at $p<0.05$ according to Tukey's HSD test.

Collection of data: Regression was used to estimate intercept *(a)* for the radicle lengths of the receptor (lettuce), and their distances from the root of the donor plant were calculated. For the control, a second intercept *(a_0)* was determined. To do this, the lengths of radicle of the lettuce seedlings and their distances from the corner of the plant box were used. To know whether the root exudates have allelopathic effect or not, inhibition (%) was calculated and compared to the radical length in control by using the formula given below:

$$\text{Inhibition (\%)} = (1 - a / a_0) \times 100 \qquad \text{(Eq. 1)}$$

Bioassay for leaf leachates from the selected plant species by sandwich method: The sandwich method, developed by Fujii (1994), was used to inspect the allelopathic effects of leachates (from dry leaves). Fully expended leaves of selected plants species leaves were rinsed with tap water and then oven dried (60°C) for 24 h and finally stocked in polythene zip bags until used. A six–well multidish plate (Nalge Nunc International, Tokyo) was used to sandwich the dried leaves in agar substrate. About 5 mL of autoclaved agar was added in the wells of these dishes, containing 5 mg, 10 mg, and 50 mg of dried leaves. Thereafter, another 5mL of agar was poured. Three replicates for every treatment was done. As the control, an agar substrate without having any plant leave was employed. In the next step, 5 seeds of lettuce were latticed on the gelled agar present in all wells of the dish. Three days after incubation (25°C) in dark, the radicle and hypocotyl length of five seeds, in each well, was measured in millimeters by using graduated graph and their means were calculated. To know whether leaf leachates have allelopathic activity or not growth/inhibition of the lettuce seedling was compared with untreated control by employing the following formula:

$$\text{Growth (\%)} = \frac{\text{Average length of } \frac{\text{radicle}}{\square} \text{hypocotyl at particular treatment}}{\text{Average lenght of } \frac{\text{radicle}}{\text{hypocotyl}} \text{at control}} \times 100 \qquad \text{(Eq. 2)}$$

Inhibition (%) was calculated by using the following formula:

$$\text{Inhibition (\%)} = 100 - \text{Growth (\%)} \qquad \text{(Eq. 3)}$$

Ranking and categorization of plant species into different inhibition level: To evaluate the mean inhibition (%) of each screened species, inhibition percentage at each of its applied concentration was averaged (mean value). Plants were then ranked in decreasing order of their inhibitory activity separately for hypocotyl and radicle length. Based on mean inhibition value, plants were classed into four levels: 80–70%, 69–60%, 59–50%, and less than 50%, assigned as first, second, third, fourth most effective levels causing inhibition of hypocotyl elongation of lettuce . Similarly, they were classed into six levels: more than 90%, 89–80%, 79–70%, 69–60%, 59–50 % and less than 50% assigned as the first, second, third, fourth, fifth, and sixth most effective levels causing inhibition of radicle elongation of lettuce (Hong *et al.,* 2003). Numbers of screened plant species falling each category were separated to estimate their inhibition level.

Statistical analysis: Data regarding the percentage growth of lettuce seedlings by the donor plants was subjected for the analysis of variance (ANOVA) using software STATISTIXS 8.1 (Analytical Software©, 2005). Completely randomized design (CRD) was employed and means were compared by Tukey's HSD (Honest significant Difference) test at $p<0.05$.

Results and Discussion

Allelopathic potential of the root exudates of selected plants by plant box method: The root exudates of the different donor species significantly (<0.05 by Tukey's honestly significant difference test) inhibited the radicle length of lettuce seedlings when assessed by the plant–box method (Fig. 1). Among all the tested plants, the highest inhibition of lettuce radicle length was caused by the root exudates of the *S. saligna*. The effect of root exudates of *S. saligna*, *W. somnifera* and *M. indica* on the radicle length of lettuce seedlings was found non-significant. The three plants inhibited the radicle length by 78, 75 and 72% compared to control. The lowest activity was recorded for exudates released by the roots of *S. marianum* with the minimum suppression of 24% of the radicle elongation.

Figures 2-5 show the relationship between the distance from the root and the radicle elongation of lettuce as a test plant. The allelopathic behavior of root exudates of a donor plant can be described in terms of intercept, slop, regression line, and regression coefficient. An intercept value shows the relative inhibitory activity in a regression equation. The higher value of intercept for control (38.9) and *S. marianum* (30.7) showed their weaker allelopathic potential (Figs. 2-3). Whereas, a small intercept values (10.0 and 8.5) exhibited by *W. somnifera* (Fig. 4) and *S. saligna* (Fig. 5) presented the stronger allelopathic phenomenon. The slope of linear equations predicted the movement rate of phytotoxic substance (Figs. 2-5). The regression equation with a small intercept and with a small slope value suggests the strong inhibitory activity of a plant. According to Shiraishi *et al*., (2002), a very few allelopathic species satisfy these two conditions. In the present study, the regression lines showed that value of radicle length of lettuce seedlings was very low (Figs. 4 and 5) near the root zone for the root exudates active plants (*S. saligna* and *W. somnifera);* whereas, it was higher for *S. marianum* (Fig. 3). A straight line was observed for untreated control (Fig. 2) showing that radicle length remained almost the same, irrespective of the distance from one corner to the other corner of the plant box. The linear regression coefficient (R^2) was found higher for *S. saligna and W. somnifera* (0.93 and 0.78, respectively) suggesting a strong relationship between root distance and seedling length. The decreased length of radicle was due to allelopathic exudates released by the donor plants. In addition, Figs. 4 and 5 also explained that less than 50 mm of radicle length was found near the roots of allelopathically active donor plants, when distances from the roots of these plants were almost less than 30 mm. It suggested the root distance as one of the important factors in effectiveness of allelopathic plant to control the growth of other plants.

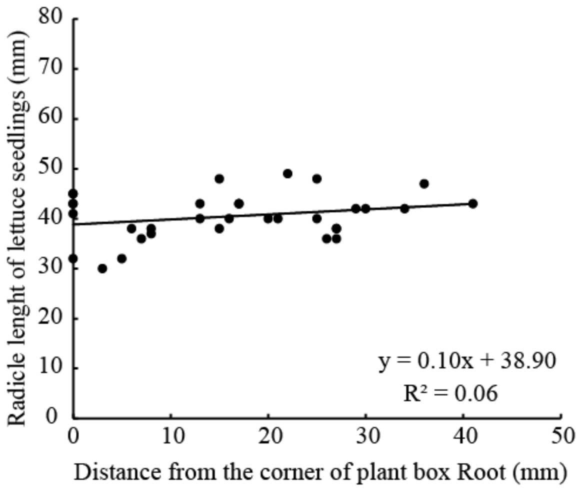

Fig. 2. Radicle elongation of lettuce seedling grown in plant box containing agar with no donor plant as the untreated control.

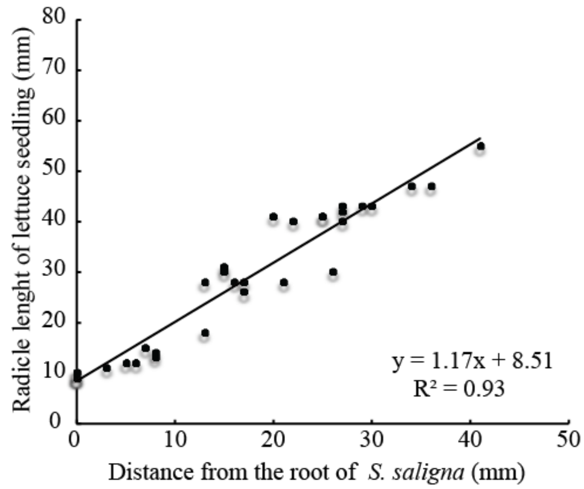

Fig. 5. Radicle elongation of lettuce seedling grown in plant box containing agar with *S. saligna* as a donor plant.

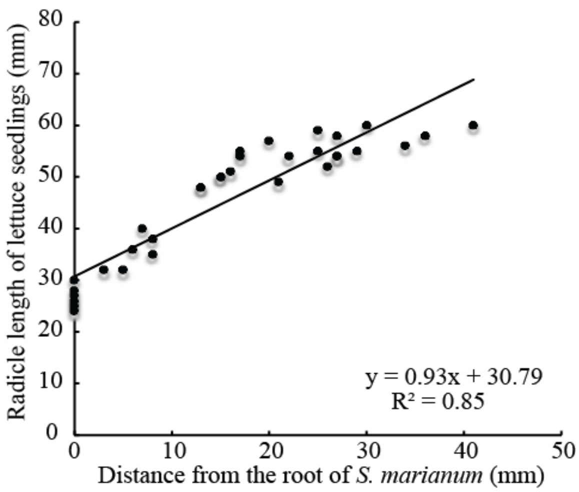

Fig. 3. Radicle elongation of lettuce seedling grown in plant box containing agar with *S. marianum* as a donor plant.

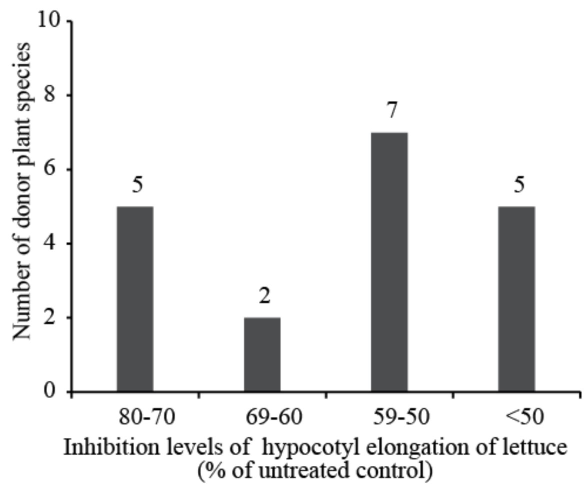

Fig. 6. Frequency distribution of plant species for allelopathic activity against lettuce hypocotyl as determined by sandwich method.

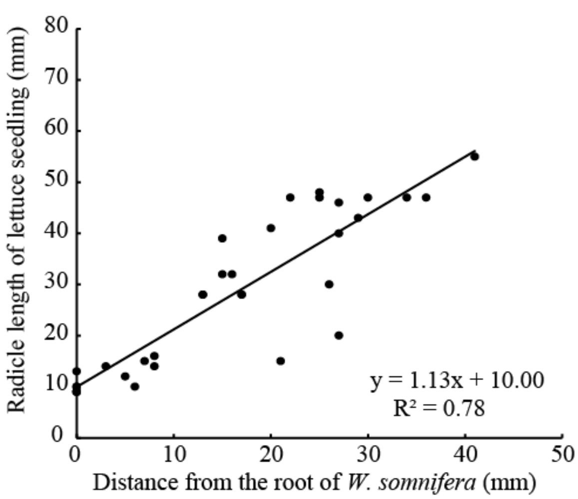

Fig. 4. Radicle elongation of lettuce seedling grown in plant box containing agar with *W. somnifera* as a donor plant.

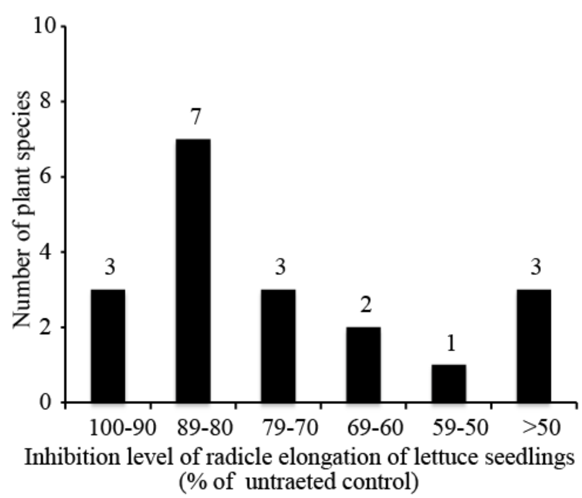

Fig. 7. Frequency distribution of plant species for allelopathic activity against lettuce radicle as determined by sandwich method.

Allelopathy of plants leachates, extracts and exudates are well documented but there are a few reports that used the intact plant like used in plant box method. Results of the present studies indicated that *S. saligna*, a plant from *Buxaceae* family, remained at the top in terms of its inhibitory activity against the lettuce seedlings. The family is a rich constituent of alkaloids, which is a famous class of compounds for its cholinesterase inhibition, antibacterial and antileishmanial activities (Devkota*,* 2008). Studies revealed that other than pharmacological activities, alkaloids in a plant also act as a defense compound against other plants, insects, bacteria and vertebrates (Iqbal *et al.,* 2002; Wink *at el.,* 1998). Therefore, the strongest allelopathic inhibition caused by the root exudates of *S. saligna* may be attributed to the presence of alkaloids, biologically active compounds present in it.

Among the tested species, *W. somnifera* and *S. nigrum* belonging to family *Solonaceae,* exhibited the relatively higher inhibition of radicle seedlings than other species. The allelopathic potential of many members of this family like datura, egg plant, red pepper, sweet pepper, tobacco species, and tomato is well established (Zhang *et al.,* 2005; Kato–Naguchi & Tanaka, 2003; Stella, 1987). These evidences are in support that in the present study the tested plant species from the same family inhibited the growth of lettuce radicle due to their allelopathic potential.

Leguminous plants, *M. indica* and *M. polypormha,* also demonstrated their higher values of inhibition (Fig. 1). Velvet bean from the same family has been extensively studied for the exploitation of allelochemicals and major compound isolated as allelochemicals was L–DOPA (Fujii *et al.,* 1990). Numerous Leguminous cover crop, have been recommended for weed control because of their allelopathic properties. Hairy vetch, a Leguminous crop, used as a food and cover crop in USA and Japan, has been reported for its allelopathic potential due to presence of Cyanamide as a new allelochemicals present in it (Kamo *et al.,* 2006). However, the lower allelopathic potential of *C. rotuntus* has also been reported by Humayun *et al*., (2005) while studying the allelopathic effect of tested species on *Zea mays*.

Allelopathic potential of leaf leachates of selected plants species by sandwich method: Both hypocotyl and radicle elongations of lettuce seedlings were affected by the leaf leachates of different plant species ranging from almost complete inhibition of radicle at higher leaf concentration to the stimulation of hypocotyl at the lower one (Tables 1 & 2). The applied plants inhibited the radicle length more than that of the hypocotyl of the test plant.

Hypocotyl growth: Results present in Table 1 showed that when the lettuce seedlings were grown with 5 mg dry leaves of selected plant species, the highest inhibition of hypocotyl was recorded for *M. polymorpha* (70.67%) followed by the three species; *M. indica, S. saligna,* and *W. somnifera* that inhibited the hypocotyl elongation up to 67%. *A. pungens* and *N. sativa,* though did not show any significant difference between them but, caused a pronounced reduction in hypocotyl length when compared to control. Leaf leachates of all other species exhibited low allelopathic activity, *i.e.* <50% inhibition, except for the *P. minor* and *P. olerecea* that promoted the growth of hypocotyl by128.07 and 116.7% respectively.

When the selected plants species were screened for their allelopathic potential at moderate leaf concentration of 10 mg dish^{-1}, *A. pungens and M. polymorpha* exhibited the maximum inhibition (≈77% inhibition) of hypocotyl. Marked reduction of 74.73% and 73.23% was recorded for the two species: *A. graveolens* and *M. indica* compared with the control. A set of three species, *M. indica, S. saligna,* and *W. somnifera***,** showed non–significant pattern for hypocotyl inhibition among them but were still significant compared to control (Table 1). In contrast to all other species, stimulation in hypocotyl growth of lettuce was observed for *P. minor and P. oleracea*; 129.20 and 100.30% growth, respectively, the value exceeded to control for which growth was considered 100%.

Contrary to leaf concentration of 5 and 10 mg dish^{-1}, none of the plant species was showed any signs of hypocotyl growth promotion when 50 mg dish^{-1} of leaf foliage was incorporated. Significant results were obtained when species were investigated for their growth inhibitory activity on hypocotyl growth of lettuce at this concentration ($p<0.05$). However, many species exhibited similar level of activity. Five species; *C. copticum, C. sativum, M. indica, S. saligna,* and *W. somnifera,* inhibited the hypocotyl growth to 96% that is almost a complete inhibition unlike untreated control. The inhibition magnitude was observed not less than 50% for all other remaining species with the exception of C. *rotundus, P. minor* and *P. olerecea*, the three species presenting the least inhibition of lettuce hypocotyl in the results. The inhibitory percentage for these species was recorded as 27.20, 37.13, and 19.43%, respectively.

The inhibition or stimulation of seedlings growth was mainly due to the presence of allelochemicals. The differential allelopathic behavior of plants understudy, in terms of inhibition or stimulation of the growth parameter, reflects the presence of different allelochemicals in different amounts. Similar trends have been reported by Chon *et al.,* (200~~3~~5). Different allelopathic behavior was observed for species at all the three applied leaf concentrations. A proportionate increase in inhibition percentage for the hypocotyl elongation was observed for *M. indica, S. saligna* and *W. somnifera* when the amount of leaf foliage was increased from 5 to 10 (two folds) and then from 10 to 50 mg dish^{-1} (five folds concentration) almost same folds in reduction were recorded. The findings are consistent with Iqbal *et al*., (2006), who reported that decrease in growth of radicle and hypocotyl, was indirectly proportional with applied concentrations of aqueous extract of *Lyrocus radiata* Herb. A similar concentration–dependent inhibition patterns of test plant species has also been reported by others (An *et al*., 2005; Batlang & Shushu, 2007). On the other hand, species like *C. copticum, C. sativum, C. album,* and *F. vulgare* showed a sudden sharp rise at the higher concentration. Gilani *et al.* (2010), also demonstrated such undistributed pattern of inhibition in hypocotyl growth by *Xanthium sturmonium* L. Contrary to all species, *P. minor* and *P. oleracea* promoted the hypocotyl growth at leaf leachates of 5 and 10 mg dish^{-1}.

Table 1. Allelopathic effect {growth (inhibition)} of plant leaf leachates (% of control) and their concentrations on lettuce hypocotyl as determined by sandwich method.

Plant species	Treatments (mg/dish)			Means inhibition	Rank*
	5	10	50		
Alternanthera pungens	43.00 k (57.00)	22.60 l (77.40)	14.83 i (85.17)	73.20	**5**
Anethum graveolens	57.00 j (43.00)	25.23 k (74.77)	12.63 j (87.37)	68.37	**6**
Carum copticum	65.30 h (34.70)	70.20 e (29.80)	3.93 l (96.07)	53.53	**11**
Chenopodiun album	67.10 gh (32.90)	71.07 e (28.93)	10.23 k (89.77)	50.53	**13**
Coriandrum sativum	62.90 i (37.10)	70.00 e (30.00)	3.53 l (96.47)	54.53	**10**
Cyprus rotundus	94.70 c (5.30)	96.30 c (3.70)	72.80 b (27.20)	12.06	**17**
Foeniculum vulgare	84.10 e (15.90)	82.27 d (17.73)	31.90 e (68.10)	33.92	**16**
Medicago polymorpha	29.30 m (70.70)	22.97 l (77.03)	14.10 ij (85.90)	77.87	**4**
Melilotus indica	34.03 l (65.97)	26.77 jk (73.23)	4.47 l (95.53)	78.24	**2**
Nigella sativa	41.37 k (58.63)	44.30 g (55.70)	22.03 g (77.97)	64.10	**7**
Ocimum basilicum	60.90 i (39.10)	52.57 f (47.43)	8.97 k (91.03)	59.19	**8**
Phlaris minor	128.07 a (-28.07)	129.20 a (-29.20)	62.87 c (37.13)	-6.71	**19**
Plantago ovata	68.77 g (31.23)	36.53 i (63.47)	20.20 h (79.80)	58.17	**9**
Portulaca oleracea	116.87 b (-16.87)	100.30 b (-0.30)	80.57 a (19.43)	0.75	**18**
Sarcococca saligna	33.00 l (67.00)	28.93 j (71.07)	3.87 l (96.13)	78.07	**3**
Silybum marianum	75.87 f (24.13)	42.87 g (57.13)	24.47 f (75.53)	52.26	**12**
Solanum nigrum	87.87 d (12.13)	39.93 h (60.07)	40.27 d (59.73)	43.98	**15**
Tribulus terrestris	66.90 gh (33.10)	51.17 f (48.83)	31.87 e (68.13)	50.02	**14**
Withania somnifera	32.90 l (67.10)	28.27 j (71.73)	3.63 l (96.37)	78.40	**1**
LSD 0.05	**2.10**	**2.09**	**1.63**		

Means sharing same letter in a column are insignificant at $p<0.05$ according to Tukey's HSD test
Values given in parenthesis are inhibition percentage over control.
*Plants were ranked in decreasing order of their inhibitory activity

Table 2. Allelopathic effect {growth (inhibition)} of plant leaf leachates (% of control) and their concentrations on lettuce radicle as determined by sandwich method.

Plant species	Treatments (mg/dish)			Means inhibition	Rank*
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Means sharing same letter in a column are not significant at $p<0.05$ according to Tukey's HSD test
Values given in parenthesis are inhibition percentage over control
*Plants were ranked in order of their inhibitory activity

When plants were ranked based on inhibition value of hypocotyl length of lettuce seedlings, *W. somnifera* was ranked on the top followed by *M. indica* and *S. saligna;* which were found to be the second and third most effective plants; whereas, *P. minor* was the only species that caused the hypocotyl extension and was ranked at the bottom among all the tested plants. Thereafter, plants were classed into different inhibitory levels, five plant species including W*. somnifera, M. indica, S. saligna, M. polymorpha,* and *A. pungens,* consisted the first most–active group that reduced the hypocotyl growth to >70%; whereas, five species ranked 15–19 (Table 1) showed the inhibitory activity <50% (Fig. 6).

Radicle growth: Radicle elongation is the most sensitive growth variable measured in allelopathic investigations. The leaf leachates of all the donor plant species inhibited the radicle elongation of lettuce significantly ($p<0.05$ at their all three used concentrations; Table 2). The allelopathic effects (growth/inhibition) of plant species were seemed to depend on applied leaves concentration in terms of radicle elongation. The maximum values of elongation (*i.e*., the minimum percentage inhibition) were recorded at lower leave concentration (5 mg dish^{-1}); whereas, those of the minimum elongation (the maximum % inhibition) were found at higher leaves concentration (50 mg dish^{-1}) compared to control. At the lower concentration of leaf (5 mg dish^{-1}), *S. saligna* and *W. somnifera*, showed insignificant ($p<0.05$) results between them causing the highest inhibition of radicle (88.77 and 88.90%, respectively). The inhibitory trend was followed by *M. indica* and *N. sativa,* (both were statistically at par) that exhibited 84.27 and 83.90 % inhibition. A concomitant reduction (71.63%) of radicle growth of lettuce seedling was observed for the leachates of *Tribullus terrestris.* All other plant species inhibited the radicle elongation of less than 70% at this concentration. However, *Phlaris minor* did not show any remarkable inhibition and caused almost the same elongation of 98% of control. Even at lower leave concentration different plants species inhibited the radicle elongation of lettuce, ranging from 2−88%, suggesting that tested plant species had different allelopathic potential (Table 2). The findings are in agreement with Oolfsdotter *et al*., (2002), who proposed the criteria for the plant or its debris to be an allelopathic; it must be able to inhabitable the growth or growth functions of other plants at different concentrations.

At moderate leaf concentration of 10 mg dish^{-1}, plant species exhibited better allelopathic inhibition. Leaf leachates of *S. saligna* and *W. somnifera* resulted in a stronger inhibition of lettuce radicle (93.73 and 94.70%). It was followed by *N. sativa* that showed inhibitory magnitude of 88.73% against lettuce radicle. Next to them were the other four species including *A. pungens, A. graveolens, M. indica* and *S. nigrum,* which were found to produce statistically similar results but still significant to other species when compared to the control (Table 2). The least inhibitory activity (29.50%) was recorded for the *Plantago ovata* that could not reduce the radicle growth to an appreciable level as compared to other species and the control as well. It was found that 10 mg dish^{-1} leave concentration was enough to inhibit the radicle elongation up to > 90% as could do by *S. saligna* and *W. somnifera*. The similar results were reported by Morikawa *et al.,* (2012), who screened 170 *Peruvian* species by sandwich method at the same concentration and found radicle inhibition of >90% for some plant species.

When those plant species that produced significant results at $p<0.05$ were evaluated for their allelopathic potential at higher concentration of 50 mg dish^{-1} compared to the control then it was observed that almost all the species showed stronger inhibitory activity against radicle elongation. The maximum value for inhibition approached to >96%, whereas the minimum was recorded as 68%, ***i.e.***, even remarkable in this regard. Eight plant species showed significantly higher rate of reduction (>96%), when compared to control (Table 2). It included *S. saligna* followed by *W. somnifera, A. graveolense, C. copticum* and *C. sativum, and O. basilicum*. *M. indica* remained the second most active plant to cause the substantial reduction (94.57%) of radicle elongation of lettuce seedling. Additionally, the statistical differences for the allelopathic inhibition of radicle length among other four species; *A. Pungense, P. oleracea, S. marianum* and *T. terrestris* were found non–significant that retarded it up to 93% but difference was still significant over the control. Pronounced inhibition (≈91%) was produced by *C. album* and *S. nigrum.* However, all other remaining species inhibited the radicle elongation of <90% (Table 2), that, in fact, is a great level of inhibition. Among all screened species, the least inhibition (68%), at this applied concentration, was recorded for the *P. minor,* the plant that showed the weakest inhibition at less leave concentration used in the experiment. The results are in accordance with Fujii **et al.** (2003), who investigated a few other plants with allelopathic potential responded from >80−0.3% when tested for their allelopathic potential at leaf litter of 50 or 10 mg dish^{-1} in the sandwich method. It explained that the toxicity level of species, if have allelopathic potential, increases at higher concentrations (Syed *et al.,* 2014). The species responses were seemed to be concentration dependant. Gilani *et al*., (2002 & 2003) also demonstrated in other plants that inhibitory activity of leaf litter increased with increasing their applied concentrations. There was great reduction radicle elongation of lettuce from 2−68% as concentration of applied leaf of *P. minor* was increased from 5−50mg dish^{-1} (Table 2). Similarly, *P. ovata* showed an increase in the inhibition from 8−70% at higher concentration. On the other hand, trends in allelopathic activity of two plant species; *W. somnifera* and *S. saligna,* was irrespective of incorporated leaf material. They were highly effective even when screened at the lower leave concentration, both inhibiting the radicle elongation ≈ 81%. Shiraishi *et al*., (2005) reported the vigorous inhibitory activities of *Oxlais brasiliensis* and *Lycoris radiate* on radicle growth were independent of applied concentrations.

When plants were ranked according to their mean inhibition value of lettuce radicle seedlings, *W. somnifera* was ranked the first followed by *S. saligna* and then *M. polymorpha.* Three of them showed >90% suppression radicle elongation of lettuce and thus fell in the first level of inhibition, which is the strongest one (Fig. 7). The results were supported by Gilani *et al.* (2010), who explored the allelopathic potential of 81 medicinal plant species and reported the high level of inhibition of radicle elongation of lettuce by two species, *viz*., *W. somnifera* and *W. coagulans.* Recently, the foliage leachates of *S. saligna* have been investigated to affect the different growth variable of *Abies pindrow* Spach (Ombir & Vidya, 2012). This highly adverse rate of reduction by the above–mentioned species gives strong clue for the presence of allelochemicals.

Among 19 screened species, 7 species; *N. sativa*, *M. indica, A.pungens*, *A. graveolens*, *S. nigrum*, *T. terrestris, and S. nigrum,* comprised the second most effective group of the allopathic inhibition (80–89%). Most of highly potential plants, when evaluated for their allelopathic activity, showed mean inhibition in the range (Fujii *et al.,* 2003; Hong *et al.,* 2003; Gilani *et al.,* 2010). However, *C. rotundus, P. ovate, and P. minor*, three species, in order of their inhibitory ranking, constituting the least active group of plant species that inhibited less than 50% growth of radicle of lettuce.

Conclusion

Screening tests provided the clear clue for the presence of allelochemicals in the root exudates and leaf leachates of screened species. The interaction between these chemicals and test species (lettuce) created unfavorable conditions for its growth and caused reduction in radicle and hypocotyl elongation. The leaf leachates of screened medicinal plants were found more allelopathic than their root exudates. The reasons for higher allelopathic activity of leaf leachates could be due to (i) the presence of higher amounts of the same allelochemicals in them, (ii) the leachates and exudates might have contained some other active substance(s) (iii) if each organ had the same substances then the quantity released may be different. The ranking of screened plants, in order of their inhibitory activity, gives information for strong allelopathic potential of *W. somnifera* and *S. saligna* and suggests their selection for the isolation and identification of patent allelochemicals in them. These plants can be exploited for elucidation of potent allelochemicals present in the active plants.

The present study was conducted using agar gel media. Further studies by using their water soluble extracts and under green house and field conditions is recommended to evaluate their more detailed inhibitory behavior. Incorporating the medicinal plants with strong allelopathic activity in an agro-ecosystem would be an effective weed control strategy.

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(Received for publication 15 April 2013)