0971-4693/94 US $ 5.00
Allelopathy Journal 32 (2): 223-232 (2013) *International Allelopathy Foundation* 2013
Tables: 4, Figs: 7

Allelopathic activity of medicinal plants and weeds from Pakistan

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(Received in revised form: August 24, 2013)

ABSTRACT

The phytotoxicity of 20-medicinal plants and weed species from Pakistan was evaluated. Effects of root exudates and dried leaves of test plant species on *Lactuca sativa* (lettuce) were examined. The phytotoxicity varied with the mode of release of phytotoxins, whether released by root exudates, volatilization or residues. In Plant box method, the *Tagetes minuta* showed strongest inhibitory activity followed by *Setaria verticillata* and *Mirabilis jalapa*) among the species investigated. In Sandwich method, maximum radical growth inhibition occurred in *Pyrus pashia* followed by *Solanum surattense* and *Solanum villosum*. While in Dish-pack method, maximum radical growth inhibition was in *Tagetes minuta* followed by *Prosopis juliflora* and *Lantana camara.* These results may provide benchmark information for further research on the elucidation of chemicals involved in phytotoxicity and might be helpful to develop new and potent bioactive chemicals from natural products.

Keywords: Dish-pack method, germination, lettuce, medicinal plants, phytotoxicity, plant box method, root exudates, sandwich method, seedling growth, weeds

INTRODUCTION

Allelopathy has been defined as any direct or indirect effects of one plant, including micro-organisms, on another through the release of chemical compounds into the environment. It may also include the subsequent influence on growth and development of nearby plants through both inhibitory and stimulatory biochemical interactions. These chemicals inhibits the growth of a species at a certain concentration and may also stimulate the growth of same species or another at lower concentration (12, 13, 17). Chemicals that are potentially allelopathic are present in most plants and tissues (11). Under particular conditions, these chemicals are in effective quantities and for a duration that affects a nearby plant released into the environment through root exudation, leaching, volatilization and residue decomposition (2). These processes of allelopathy are influenced by environmental factors and cannot be considered in isolation (3, 14). Recent interest in allelopathy has focussed on exploiting it to control weeds in forestry and agriculture. Hence, allelopathic crop accessions and natural vegetation have been screened to control weeds (10).

The allelopathic activity of 160 medicinal plants from Japan have been evaluated

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by the Sandwich method (15). In another study, sorghum and soybean (*Glycine max* (L.) Merr.) seedling bioassays were used to demonstrate inhibition to *Kochia scoparia* (L.) Schrad., *Helianthus tuberosus* L., *Xanthium strumarium* L., *Ambrosia trifida* L., and *Rumexcrispus* L. (3). Certain medicinal plant species, including *Tagetes minuta, Mammea americana, Heliopsis longipes, Artemisia tridentate* and *Acorus calamus*, have been explored for secondary metabolites that could be used as commercial insecticides (9).

Several methods have been used to evaluate the allelopathic activity. For example, 70 Japanese plant species have been surveyed for allelopathy by using a water extraction method (8). About 78 medicinal species have been screened for their allelopathic potential by using the solvent (methanol and water) extraction method (4). Recently, 239 medicinal plant species were screened for allelopathic activity by using the newly developed Sandwich method. These methods can be performed in a short time to evaluate the allelopathy of leaf litter leachate of large number of samples in laboratory (7). The Plant box method is a bioassay to screen for allelopathy by means of root exudates (6). Leaf litter leachates of 160 medicinal plants have been evaluated by the Sandwich method. Maximum inhibition activity was found in *Eucalyptus citriodora*, followed by *Clematis terniflora*, *Chinchona sp.*, *Spiraea nipponica* var. *tosaensis*, *Allamanda neriifolia*, *Hibiscus syriacus*, *Tylophora tanakae*, *Melastoma sanguineum*, *Melia azedarach* and *Duranta plumieri*. (15). These results hypothesized and suggest that weeds and medicinal plants may have strong allelochemicals. Medicinal plants are a global resource of medicines and are extremely important for human health and the pharmaceutical industry. However, adverse effects of allelochemicals from medicinal plants and crops may increase the phytotoxicity in soil, that may reduce the production potential of agricultural fields and managed forest systems. Before large-scale cultivation of any medicinal plant is started in an established agricultural field, the plant should be evaluated allelopathically because the chemical effects of prior plants, residues, associated plants and even auto-toxicity may negatively affect the cropping system. This study aimed to prepare the national baseline data on the allelochemical effects of medicinal plants and weeds. This is unique because it is based on the evaluation of allelopathic potential of testing species according to their three different modes of action i.e, (i). Plant box for root exudates, (ii). Sandwhich for leaf leachate and (iii). Dish pack for volatiles. It is not just the comparison of three methods, but this data may be used for further research and be integrated with a national and international database on allelochemicals found in nature.

MATERIAL AND METHODS

Plant Materials

Seeds of 20 plant species include 15 medicinal species like *Acacia modesta*, *Achellia millefolium, Mirabilis jalapa, Plantago major, Pyrus pashia, Rosa brononii, Rubus ellipticus, Rumex nepalensis, Senecio chrysanthemoides, Setaria verticillata, Skimmia laureola, Solanum surattense, Solanum villosum, Tagetes minuta, Verbascum thapsus* and weed species *Cassia occidentalis, Lantana camara, Parthenium hysterophorus, Prosopis glandulosa* and *Prosopis juliflora.* were collected from different parts of Pakistan (Table 1). Lettuce (*Lactuca sativa* L.) was used as a responding plant in the bioassays due to its germination reliability.

Table 1. Test plant spp. Botanical and English names and collection sites

S. No	Botanical Name	English Name	Collection site
		Medicinal Plants	
1.	*Acacia modesta*	Black Sally, Blackwood	Margalla Hills- Islamabad
2.	*Achellia millefolium*	*Yarrow* milfoil,	Mushkpuri- Abbottabad
3.	*Mirabilis jalapa*	4 O'clock plant	Salgran- Islamabad
4.	*Plantago major*	Greater Plantain	Nathia gali- Abbottabad
5.	*Pyrus pashia*	Wild Himalayan Pear	Murree-Rawalpindi
6.	*Rosa brunonii*	Himalayan musk rose	Dunga gali- Abbottabad
7.	*Rubus ellipticus*	Yellow raspberry	Koza gali- Abbottabad
8.	*Rumex nepalensis*	Dock	Ayubia- Abbottabad
9.	*Senecio chrysanthemoides*	Senecio	Dunga gali- Abbottabad
10.	*Setaria verticillata*	Bur bristle grass	Dunga gali- Abbottabad
11.	*Skimmia laureola*	Skimmia	Mushkpuri- Abbottabad
12.	*Solanum surattense*	Yellow-Berry Nightshade	Margalla Hills -Islamabad
13.	*Solanum villosum*	Red Nightshade	Margalla Hills- Islamabad
14.	*Tagetes minuta*	Marigold	Margalla Hills- Islamabad
15.	*Verbascum thapsus*	Common Mullen	Margalla Hills-Islamabad
		Weed species	
16.	*Cassia occidentalis*	Negro coffee	Margalla Hills- Islamabad
17.	*Lantana camara*	Red sage, yellow sage	Margalla Hills- Islamabad
18.	*Parthenium hysterophorus*	Ragweed, Feverfew	Margalla Hills- Islamabad
19.	*Prosopis glandulosa*	Mesquite	Khushhal garh-Kohat
20.	*Prosopis juliflora*	Velvet mesquite	Khushhal garh- Kohat

1. Plant-Box Method: Seedlings of test species were grown in sandy soil in vinyl pots (5.5 cm dia and 6 cm deep) at one seedling per pot in a greenhouse in four replications. Out of these only 3-most healthy seedlings per species were selected for further experimentation. To evaluate the allelopathic potential of root exudates, three seedlings of each species (aged 14 or 21 or 28 days after germination) were selected for bioassay in plant-box (Fig. 1). Within the plant box apparatus, a total of 33 lettuce seeds were planted at 21 different distances (10 mm from each other) from the sample plant roots (Fig. 2), were sown on the agar medium for each of the three replicates per species . The plant-boxes were kept in the incubator [25°C/20°C under 200 $\mu molm^{-2} s^{-1}$ light intensity with a 12h/12h photoperiod] for 5-days. Shoot and root length of each of 33 lettuce seedlings grown against each of the 20 tested species were measured and seed germination (%) against each species were recorded (Table 2; Fig. 3) (6, 16). Then roots of sample plants were carefully removed from the plant-box and gently washed with water. The seedlings were separated in to roots and shoot and dried at 60-70°C for 24 h in oven, and weighed separately. This dried material was then used for the Sandwich and dish pack methods.

2. Sandwich Method: A six-well plate was used for each of 20 species. Three wells had 10 mg of dried leaves, and three wells had 50 mg of dried leaves (Fig. 4), for three replicates of each weight. Two aliquots of 5ml agar were added to each well to form two layers (the second layer acted as a buffer zone between the sample and the lettuce seeds) on the dried leaves. Five equally spaced lettuce seeds were placed on top of the agar in each well and

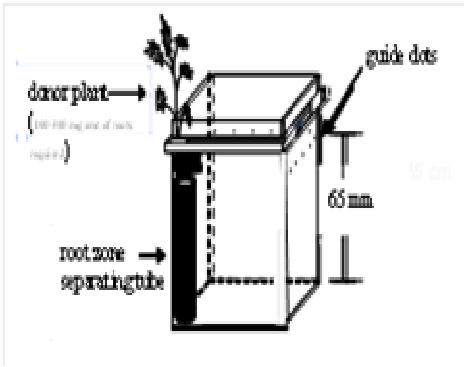

Figure 1. Plant Box Method (Lateral View)

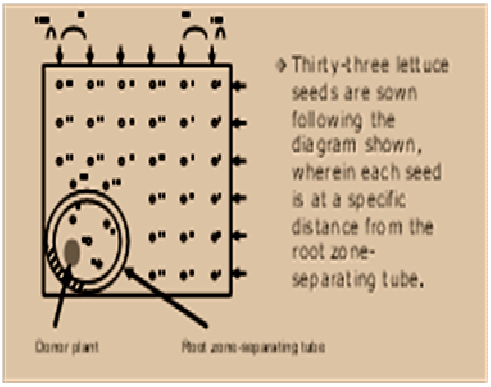

Figure 2. Plant Box method (Top View)

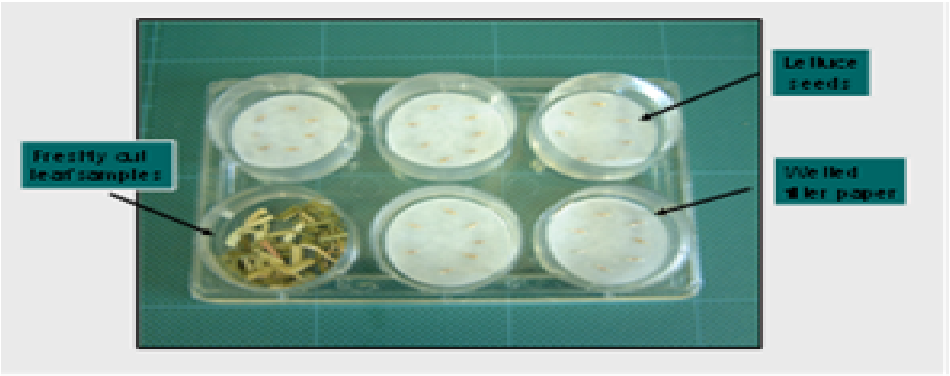

Figure 3. Dish Pack Method

each plate was sealed with plastic tape. The plates were incubated in dark for three days at 25°C. The radicle and hypocotyl lengths of germinated lettuce seeds were measured and seed germination (%) against each species were recorded (Table 3) (7, 16)

3. Dish pack Method: Dishes with six wells (also used for the Sandwich test) were used for the dish pack method (Fig. 3). The lower left well of each plate was filled with 100 mg of dried sample leaves. The sample leaves were cut into pieces of $2mm^2$ to enhance the release of volatiles and then 0.7 ml distilled water was added to the well. A piece of filter paper (Grade1, 33 mm) was placed in each of the other five wells along with seven lettuce seeds and 0.7ml distilled water. The dishes were covered with aluminum foil and sealed with tape. In control dishes, the lower left sample well was left empty. Dishes were incubated in dark for 3-days at 25ºC. The radicle and hypocotyls length of five lettuce seedlings from each well were measured and seed germination (%) against each species were recorded (Table 4; Fig:6,7) (5, 16)

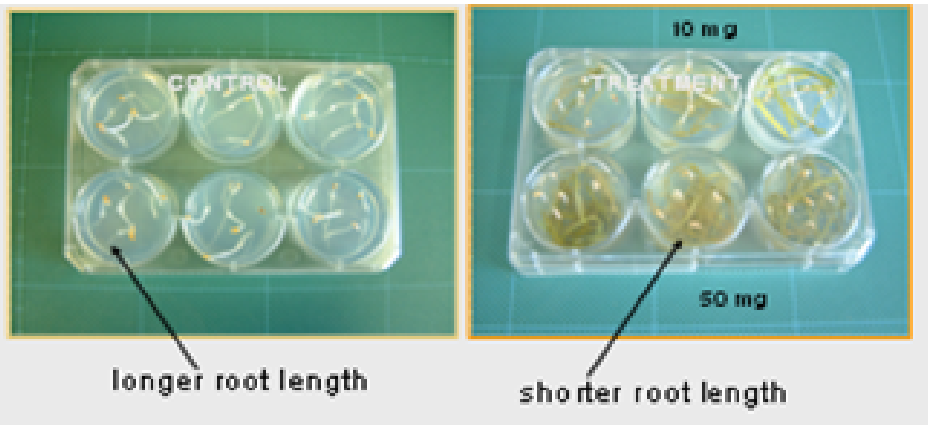

Figure 4. Sandwich Method; Control plate (left) treatment plate (right)

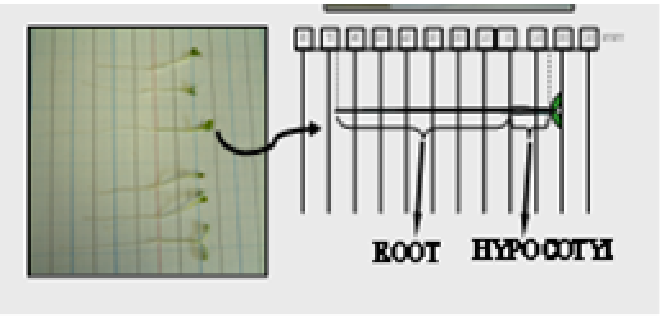

Figure 5: Measurement Technique

Statistical Analysis: ANOVA was applied to evaluate the significance of methods and concentration on the growth of root and hypocotyls. The least significant differences test was used to compare the means of treatments. The data were analyzed using Microsoft Excel 2007 (1, 6).

RESULTS AND DISCUSSION

Results showed that most of the medicinal plants were inhibitory to lettuce seedlings. In plant box method *Tagetes minuta* has shown strongest inhibitory activity followed by *Setaria verticillata* and *Mirabilis jalapa* among the medicinal species under investigation (Table 2; Fig.7).

By sandwich method maximum radical growth inhibition was observed against *Pyrus pashia* followed by *Solanum surattense* and *Solanum villosum* (Table 3). While applying dish-pack method maximum radical growth inhibition was found against *Tagetes minuta* followed by *Skimmia laureola* and *Acacia modesta* (Table 4; Fig. 7). While among weed species maximum redicle growth inhibition was found against *Prosopis juliflora* followed by

Table 2. Phytotoxic effect of root exudates of test species on growth of lettuce seedling by Plant Box method

Plant Species	Lettuce seedlings length *	
	Radicle	Hypocotyl
Control	57.2	8.7
Weed species		
Cassia occidentalis	43.3	7.5
Lantana camara	46.4	13.6
Parthenium hysterophorus	45.8	13.1
Prosopis glandulosa	44.9	10.2
Prosopis juliflora	58.1	11.5
Medicinal Plants		
Acacia modesta	50.0	8.3
Achellia millefolium	41.5	5.6
Mirabilis jalapa	25.6	10.7
Plantago major	52.8	8.4
Pyrus pashia	53.4	11.8
Rosa brononii	57.1	12.6
Rubus ellipticus	45.1	13.4
Rumex nepalensis	57.6	14.6
Senecio chrysanthemoides	48.7	8.7
Setaria verticillata	42.5	12.1
Skimmia laureola	59.8	9.8
Solanum surattense	53.8	16.0
Solanum villosum	41.6	15.6
Tagetes minuta	0	0
Verbascum thapsus	46.7	8.4
LSD ≤ 0.05	3.8	0.6

* Average of replicates.

Parthenium hysterophorus and *Prosopis glandulosa* under plant box method (Table 2; Fig.6). By sandwich method maximum radical growth inhibition in weeds was detected against *Parthenium hysterophorus* followed by *Lantana camara* and *Prosopis glandulosa* (Table 3; Fig.6). After application of dish-pack method maximum radical growth inhibition in weeds was found against *Prosopis juliflora* followed by *Lantana camara* and *Prosopis glandulosa* (Table 4; Fig.6). In another investigation, three dimensional assessments have been made to evaluate allelopathic potential of 38 shared invasive plants of Japan and Pakistan originated from 5 continents round the globe. This investigation resulted *Melilotus officinalis* to be the most noxious species among all due to maximum inhibition effect shown on the radical growth of *Lactuca sativa* followed by *Melilotus alba, Datura stramonium* and *Mirabilis jalapa*. While *Rumex crispus, Plantago lanceolata, Rumex conglomerates* and *Trifolium pratense* have appeared with minimum inhibition effect (16). Over all, within Asteraceae, using the sandwich method, *Achillea millefolium* showed the strongest inhibitory effect after *Tagetes minuta* followed by *Parthenium hysterophorus*. Among leguminous plant species, *Prosopis glandulosa*, followed by *Acacia modesta* showed the strongest inhibition. *Mirabilis jalapa* (Nyctaginaceae) also showed strong

Table 3. Phytotoxic effect of volatile compounds released from residues of test species on growth of lettuce seedling by Sandwich method

Plant Species	Length (mm)*			
	Radicle	Hypocotyl	Radicle	Hypocotyl
	Residues (10 mg)		Residues (50 mg)	
Control	35.7	19.9	34.6	19.9
Weed species				
Cassia occidentalis	15.0	12.7	7.8	10.3
Lantana camara	22.9	20.6	6.9	8.7
Parthenium hysterophorus	16.0	15.3	5.3	12.2
Prosopis glandulosa	11.9	20.4	5.7	10.4
Prosopis juliflora	17.7	22.0	8.2	15.8
Medicinal Plants				
Acacia modesta	13.7	14.1	5.7	9.3
Achellia millefolium	9.2	7.2	3.4	4.0
Mirabilis jalapa	15.4	19.7	4.1	5.1
Plantago major	18.3	19.9	7.3	9.7
Pyrus pashia	7.8	8.7	1.7	3.0
Rosa brononii	28.1	26.9	19.3	20.4
Rubus ellipticus	27.0	29.0	17.0	20.4
Rumex nepalensis	22.9	26.0	9.9	12.8
Senecio chrysanthemoides	12.1	19.7	5.6	9.8
Setaria verticillata	16.6	19.3	5.0	7.7
Skimmia laureola	6.6	9.9	4.0	2.7
Solanum surattense	9.2	9.1	3.1	4.4
Solanum villosum	8.4	13.0	3.7	10.1
Tagetes minuta	13.6	6.4	4.4	2.1
Verbascum thapsus	27.3	23.6	13.9	11.7
LSD ≤ 0.05	3.8	3.3	2.3	2.9

* Average of replicates.

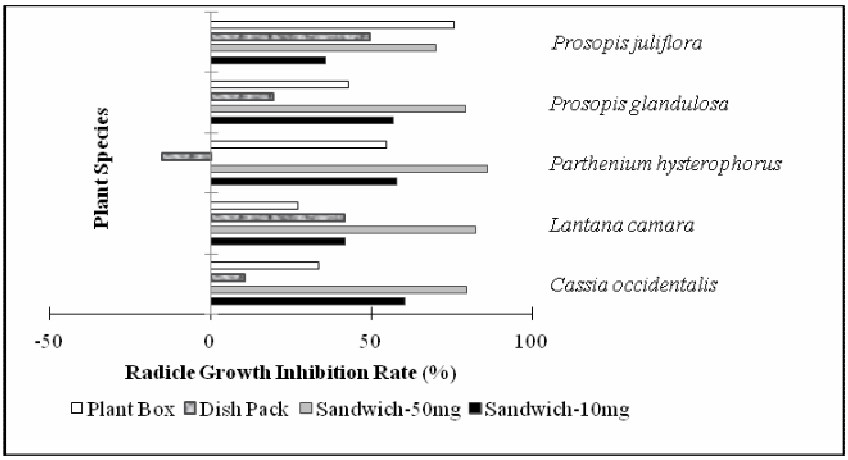

Figure 6. Radicle growth inhibition percentage of weed species against lettuce

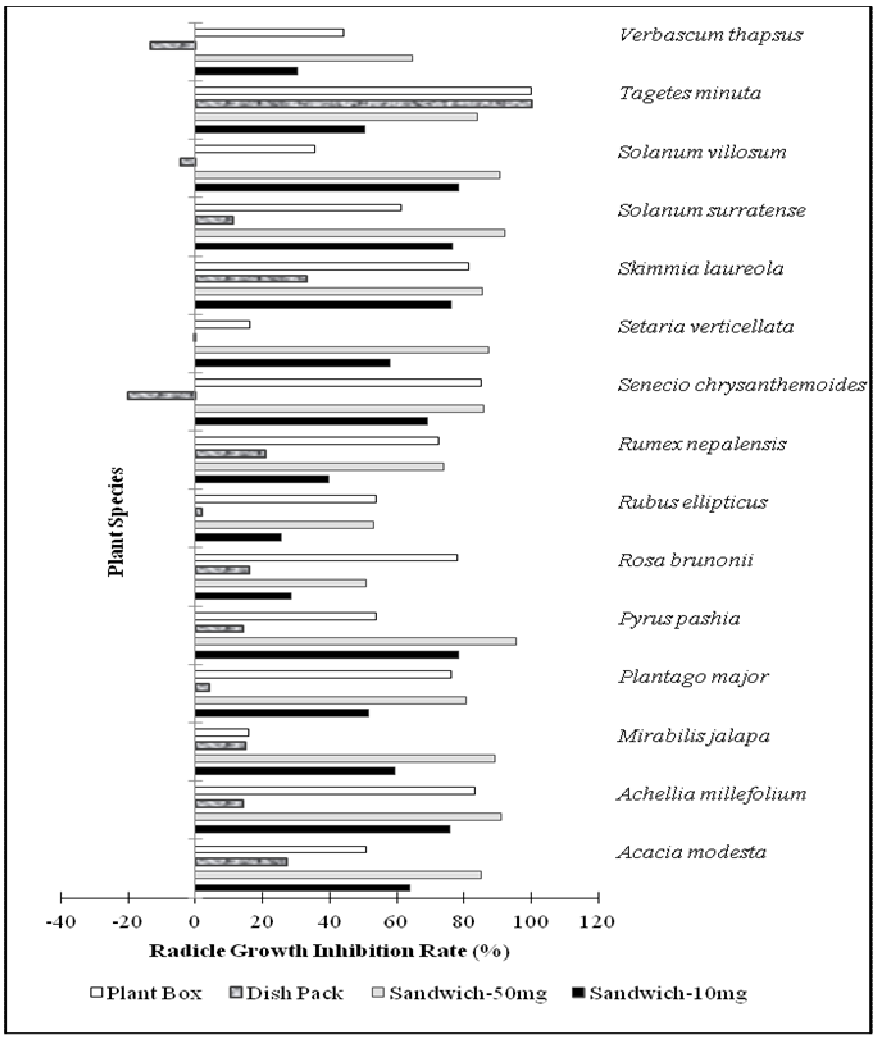

Figure 7. Radicle growth inhibition percentage of medicinal species against lettuce

inhibition from leaf litter and root exudates. Among solanaceous taxa, *Solanum surattense* showed the strongest inhibition through leaf litter exudates, followed by *Solanum villosum* and *Hyoscyamus niger*. Other species that demonstrated inhibition through the sandwich test were *Setaria verticillata* (Poaceae), *Pyrus pashia* (Rosaceae), followed by *Skimmia laureola* (Rutaceae), *Lantana camara* (Verbenaceae) and *Plantago major* (Plantaginaceae) (Table 2, 3, 4 Fig. 6,7). The reason beyond the variations among the bioassay methods applied is actually the three dimensional assessment of phytotoxity of the testing plant species according to their different mode of action. Hence it has been observedthat the phytotoxicity is varied according to the method of the release of the phytotoxins whether it released by root exudates, volatilization or residues.

Table 4. Phytotoxic effect of residues of test species on growth of lettuce seedling by Dish Pack method

Plant Species	Length (mm)*	
	Radicle	Hypocotyl
Control	35.7	19.9
Weed species		
Cassia occidentalis	15.0	12.7
Lantana camara	22.9	20.6
Parthenium hysterophorus	16.0	15.3
Prosopis glandulosa	11.9	20.4
Prosopis juliflora	17.7	22.0
Medicinal Plants		
Acacia modesta	13.7	14.1
Achellia millefolium	9.2	7.2
Mirabilis jalapa	15.4	19.7
Plantago major	18.3	19.9
Pyrus pashia	7.8	8.7
Rosa brononii	28.1	26.9
Rubus ellipticus	27.0	29.0
Rumex nepalensis	22.9	26.0
Senecio hrysanthemoides	12.1	19.7
Setaria verticillata	16.6	19.3
Skimmia laureola	6.6	9.9
Solanum surattense	9.2	9.1
Solanum villosum	8.4	13.0
Tagetes minuta	13.6	6.4
Verbascum thapsus	27.3	23.6
LSD ≤ 0.05	3.8	3.3

* Average of replicates.

CONCLUSIONS

The results may serve as benchmark information for further research on the elucidation of chemicals involved in phytotoxicity and might be helpful in the development of new and potent bioactive chemicals against pests.

ACKNOWLEDGEMENTS

The authors are very much thankful to Higher Education Commission of Pakistan and Pakistan Museum of Natural History Islamabad, and Ministry of Agriculture, Forestry and Fisheries of Japan for financial support of this research endeavor.

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