Pak. J. Bot., 45(S1): 467-474, January 2013.

ALLELOPATHIC EVALUATION OF SHARED INVASIVE PLANTS AND WEEDS OF PAKISTAN AND JAPAN FOR ENVIRONMENTAL RISK ASSESSMENT

MUHAMMAD IBRAR SHINWARI[1], MARYUM IBRAR SHINWARI[2] AND YOSHIHARU FUJII[3]

[1]*Department of Environmental Science, International Islamic University, Islamabad,*
[2]*Pakistan Scientific & Technological Information Centre, Islamabad*
[3]*National Institute of Agro-Environmental Science, Tsukuba, Ibaraki, Japan*
[*]*Corresponding author e-mail: drmishinwari@yahoo.com*

Abstract

In an investigation, three dimensional assessments have been made to evaluate allelopathic potential of 38 shared invasive plants and weeds of Pakistan and Japan originated from 5 continents, carried out at Plant Chemical Ecology Laboratory at National Institute of Agro-Environmental Science, Japan from January 2008 to March 2009. The plant species under investigation have been tested by subjecting their root exudates, leaf litter and volatiles through application of Plant box, Sandwich and Dish pack methods. The results obtained from all 3 different methods have been statistically analyzed and the mean, standard deviation (SD) and SD variance (SDV) has been calculated to determine the inhibition pattern of radicals growth of the lettuce seedlings for environmental risk assessment. According to results, as a whole *Melilotus officinalis* appeared 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 shown minimum inhibition effect. The results presented can be utilized as benchmark information for further joint research on the elucidation of chemicals involved in the allelopathy in nature. The information obtained can also be helpful in the development of new and potent bioactive chemicals from natural products to combat environmental risk.

Introduction

Flora of Pakistan includes about 6000 species with only 3 endemic genera (Douepia in Brassicaceae, Stewartiella in Apiaceae and Decalepidanthus in Boraginaceae). In all, there are some 203 endemic species, constituting about 4% of the flora (Ali, 1978 ; Shinwari & Qaisar, 2011). Many of these endemic species are found in the mountainous regions of northern Pakistan (Shinwari & Gilani, 2003), particularly in the Chitral and Kashmir districts, and in northern Baluchistan. Not with standing, these regions are considered to be relatively poorly known and likely to be sources of new species (Chaudhri, 1977; Frodin, 1984). Plant species richness in the Palas Valley in Pakistan indicates a strong relationship with altitude (Saqib, *et al.,* 2011). It has been conservatively estimated that 580-650 plant species (c. 12% of the flora) are threatened or endangered but also been suggested that this number would increase (Nasir, 1991). He cited habitat destruction, over-exploitation of economic plants, introduction of alien species, and pollution as the major causes for this threat. Pakistan has a long history of introduction of foreign plant and animal species. The main objective of introduction of exotic species was to fill the gap between supply and demand of timber, fuel wood and fodder. In total Pakistan has about 700 alien species (Hussain & Zarif, 2003). Introduction of invasive alien species (IAS) is one of the threats to biodiversity that could even lead to the complete elimination of the native species. Pakistan is a sub-tropical country with diverse biological and ecological features. Agriculture being the most affected component of the biological invasions accounts for the largest sector of the national economy contributing about 25% of the GDP and providing employment to more than half of the labor force. In the context of invasive species there is no comprehensive information about their inventory and their impact on agro-ecosystem (Shinwari *et al.,* 2008b)

The flora of Japan is marked by a large variety of species. There are about 4,500 native plant species in Japan (3,950 angiosperms, 40 gymnosperms, 500 ferns). Some 1,600 angiosperms and gymnosperms are indigenous to Japan. The large number of plants reflects the great diversity of climate that characterizes the Japanese archipelago, which stretches some 3,500 kilometers (2,175 miles) from north to south. The most remarkable climatic features are the wide range of temperatures and significant rainfall, both of which make for a rich abundance of flora. The climate also accounts for the fact that almost 70% of Japan is covered by forest. Foliage changes color from season to season. Plants are distributed in five zones, all of which lie in the East Asian temperate zone (Fig. 1).

In spite of different histories of human occupation and land use, Japan and Pakistan host many of the well as familiar ornamental plants and herbs. There is a similarity of same exotic plant species in both countries. It has been noted over a score of taxa in the family *Asteraceae* common to eastern Asia and Pakistan and mentioned that many other plants can be included. In this paper selected comparison has been made of the invasive flora of Pakistan spread over different latitudes, hemispheres and the range of climates on northern part of Pakistan and similar to those in Japan, from warm temperate to cool temperate, although rainfall is summer-dominant at mid-latitudes (around 35°) and winters are colder in Japan especially at high-latitude (Michael, 2001). Western mountainous area in Pakistan, immediately west of the Himalaya Range, the climate becomes drier than Japan and increases in aridity to the west. Particularly in the current study moist temperate Himalayan Pakistan of Sino-Japanese phyto-geography has been focused as of altitude 2000 to 3000 meters.

Produced by the Cartographic Research Lab
University of Alabama

Fig. 1. Map representing the investigation sites.

In view of these contrasting histories and agricultural practices, it is remarkable that 170 invading plants are common to both Japan and Pakistan. Understanding the reasons for this and why some exotic plants are exclusive to one country may contribute to the development of invasion theory (Ali, 1978; Asai, 1993; Dalton, 1875; Enomoto, 1999; Henderson, 2001; Ito & Morita, 1999; Kasahara, 1976; Maekawa, 1943; Shimizu, *et al.*, 2001 & Shinwari, *et al.*, 2008b).

Allelopathy is a component of most natural communities and agro-ecosystems. This phenomenon arises because growth inhibiting or stimulating, plant- and microbial produced chemicals are released into the environment. It is a real fact that the adverse effects from allelochemicals arising from various invasive plants (Gilani *et al*., 2010) and weeds may reduce production in agricultural fields and managed forest systems. Hence there is a strong need to evaluate the chemical effects of prior plants, residues, associated plants and even auto-toxic potential that may occur in plants or cropping systems. Invading plant species (mostly regarded as weeds) common to Pakistan and Japan that are exotic to both countries generally occur over more than 4 degrees of latitude, may occur in more than one habitat type and are often locally abundant in at least one habitat in both countries.

The prime objective of this study was to carried out 3 dimensional assessment of allelopathic potential of the shared invasive plants of Pakistan and Japan and to establish and share baseline data on the allelochemical effects of selected invasive plants may threatened environment in future. This data can 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 38 shared invasive plants and weed species have been collected from various localities of Pakistan (Lesser Himalayas) and Japan (Tsukuba Mountain). Three dimensional assessments of allelopathic potential of these species have been carried out through analysis of their root exudates, leaf litter and volatiles applying Plant Box, Sandwich and Dish pack methods. Lettuce (*Lactuca sativa* L.) was used as a test plant material in the bioassay because of its reliability for germination.

Plant-Box method: In a brief, the seedlings of the species under investigation were grown in sandy soil in vinyl pots (5.5 cm diameter and 6 cm deep) in a green house in 4 replications. To evaluate the allelopathic activity of root exudates, seedlings aging 14, 21, 28 days after germination were used for bioassay in the plant-box. A total of 33 Lettuce seeds (at 21-different distances from the sample plant roots in the plant box) of each 38 species were sown vertically into the agar medium in three replications. The plant-boxes were kept at 25-20ºC under 200µmol m^{-2} s^{-1} light intensity with a 12h/12h photoperiod for 5 days in an incubator. The length of root (radical) of all the tested species were measured and the germination percentage of the seeds was recorded. The roots of the sample plants seedlings used were carefully removed from the plant-box and gently washed with water after conducting the plant-box experiments. Root and shoots were then cut-off from each seedlings and placed in separate paper bags followed by drying at 60-70ºC for 24 hours in an oven, and, finally, the dried roots and shoots were weighed separately. This dried material has been stored and placed in a paper bag separately for the Sandwich and Dish pack experimental tests (Fujii *et al.*, 2007).

Sandwich method: Leaves of the sample plants dried at 60ºC-70ºC for 24 hours in an oven were subjected to analysis of their allelopathic effects using the Sandwich method. Lettuce (*Lactuca sativa* L.) was used as a test plant material in the bioassay because of its reliability for germination. Three replications each of 10 and 50mg oven dried leaves were placed in each well of the six-well multi-dish plastic plate of each sample. Added 5ml plus 5ml agar in two layers on the dried leaves. Placed 5 lettuce seeds vertically and covered with plastic tape labeled appropriately for incubation in dark for 3 days at 25ºC. Length of the radical was measured and the germination percentage of the seeds was recorded (Fujii *et al.*, 2003) .

Dish Pack method: Leaves of the sample plants dried at 60-70ºC for 24 hours in an oven were subjected to analysis of their allelopathic effects using the Dish pack method. Lettuce (*Lactuca sativa* L.) was used as a test plant material in the bioassay because of its reliability for germination. Multi dishes with 6 wells (as used in case of Sandwich test) with 100mg of the dried sample leaves each placed into the lower left well is used to conduct this method. The sample leaves were cut of about 2mm square with scissors to enhance the release of volatiles from the leaves. Then 0.7ml of distilled water is added depending on the approach. A filter paper (Grade: 1 Size: 33mm) was placed in each other 5 wells with 7 lettuce seeds and 0.7ml of distilled water. Then dishes were covered with aluminum file after sealed from sides with adhesive tape. In the control dish sample well was left blank while other process was repeated same as with that of each sample dish. Appropriately labeled dishes were kept for incubation in dark for 3 days at 25ºC. Length of the radicals of the 5 seedlings out of 7 was measured and the germination percentage of the seeds has been recorded (Fujii *et al.*, 2005).

Statistical analysis: Each experiment was conducted three times and presented as the mean of three replicates. For each plant species in all three methods; the mean and standard deviation (SD) and evaluated the SD variance (SDV has been calculated to determine the inhibition pattern of radicals of the lettuce seedlings (Fujii *et al.*, 2007). The data was analyzed using Microsoft Excel 2007.

Results and Discussion

In total, 38 commonly invading plants and weeds (31 genera and 15 families; of 2 shrubs and 36 herbs) of Pakistan and Japan originated from 5 continents mostly from Europe or Eurasia have been evaluated for their allelopathic effect (Tables 1 & 2).

Invasive Alien Species (IAS) is one of the most significant drivers of environmental change globally. In the United States, IAS now rank second to habitat conversion as a cause of species endangerment and extinction. Even the best-protected natural areas are not immune to the invasion of alien species. A single ecosystem can suffer numerous invasions, with resultant changes in its structure, function, and ability to provide natural resources. Much of the developing world is just beginning to observe significant impacts of IAS in their ecosystems. In contrast, some ecosystems in the developed world, such as the eastern forests of the United States, have been suffering losses from IAS for centuries (near-extinction of American chestnut, *Castanea dentate*, as a result of root rot, *Phytophthora cinnamomi*, and blight, *Cryphonectira parasitica*). This is due in large part to the long history of trade and transport between regions with similar climate – such as between the eastern U.S. and Europe (Pallewatta *et al.*, 2003). In the present study, most of the invasive plants and weeds have originated from Europe and Eurasia followed by Africa and North America (Fig. 2) IAS can also take a heavy financial toll on governments, industries, and private citizens. A recent study estimates that IAS cost the U.S. more than US $100 billion a year (Pimentel *et al.*, 2000) and at least this much in 6 other countries combined (Pimentel *et al.*, 2001).

Table 1. Origin of shared invasive plants and weeds of Pakistan and Japan.

No.	Botanical Name	Family	Habit	Origin
1	*Achillea millefolium** L.	*Asteraceae*	Herb	USA
2	*Agrostis stolonifera* L.	*Poaceae*	Herb	Europe
3	*Amaranthus spinosus** L.	*Amaranthaceae*	Herb	Tropical America
4	*Avena fatua** L.	*Poaceae*	Herb	Eurasia
5	*Cichorium intybus* L.	*Asteraceae*	Herb	Eurasia
6	*Conium maculatum* L.	*Apiaceae*	Herb	Europe
7	*Dactylis glomerata** L.	*Poaceae*	Herb	Europe
8	*Datura stramonium** L.	*Solanaceae*	Herb	Central USA
9	*Echium plantagineum* L.*	*Boraginaceae*	Herb	Europe
10	*Eleusine indica* (L.) Gaert n.	*Poaceae*	Herb	Africa
11	*Festuca arundinacea* Schreb.,	*Poaceae*	Herb	Europe
12	*Lantana camara* L.	*Verbenceae*	Shrub	USA
13	*Leucanthemum vulgare** Lam.	*Asteraceae*	Herb	Europe
14	*Lolium multiflorum* Lam.	*Poaceae*	Herb	Europe
15	*Lolium rigidum* Gaudin	*Poaceae*	Herb	Europe
16	*Lotus corniculatus** L.	*Papilionaceae*	Herb	Europe
17	*Malva parviflora** L.	*Malvaceae*	Herb	South Europe
18	*Medicago lupulina** L.	*Papilionaceae*	Herb	Eurasia
19	*Medicago sativa** L.	*Papilionaceae*	Herb	N. Africa
20	*Melilotus albus* Medik.	*Papilionaceae*	Herb	Europe
21	*Melilotus officinalis* (L.) Lam.	*Papilionaceae*	Herb	Eurasia
22	*Mirabilis jalapa* L.	*Nyctaginaceae*	Herb	Tropical/S. America
23	*Papaver dubium* L.	*Papavaceae*	Herb	N. Africa
24	*Parthenium hysterophorus* L.	*Asteraceae*	Herb	Mexico, USA
25	*Phalaris canariensis* L.	*Poaceae*	Herb	Mediterranean
26	*Phalaris minor* Retz.	*Poaceae*	Herb	Mediterranean
27	*Plantago lanceolata* L.*	*Plataginaceae*	Herb	Europe
28	*Plantago major* L.	*Plataginaceae*	Herb	Eurasia
29	*Poa pratensis* L.	*Poaceae*	Herb	Eurasia, N. Africa
30	*Rubus fruticosus* L.	*Rosaceae*	Shrub	Armenia, USSR
31	*Rumex conglomeratus** Murray	*Polygonaceae*	Herb	Europe
32	*Rumex crispus** L.	*Polygonaceae*	Herb	Europe
33	*Sida spinosa* L.	*Malvaceae*	Herb	North America
34	*Trifolium dubium** Sibth.	*Papilionaceae*	Herb	Western Eurasia
35	*Trifolium pratense** L.	*Papilionaceae*	Herb	Mediterranean
36	*Verbascum thapsus** L.	*Scrophulariaceae*	Herb	Eurasia
37	*Veronica persica** Poir.	*Scrophulariaceae*	Herb	Europe
38	*Vulpia myuros** (L.) C.C.Gmel.	*Poaceae*	Herb	Eurasia

Sources: (Ali, 1978, Asai, 1993, Dalton, 1875, Enomoto, 1999, Henderson, 2001, Ito & Morita, 1999, Kasahara, 1976, Maekawa, 1943, Shimizu, *et al*., 2001, Shinwari, *et al*., 2008b) Note: *Species that are widespread in both countries are marked with an asterisk.

Table 2. Three dimensional assessment by calculation of percentage growth inhibition of radicals of shared invasive plants and weeds of Pakistan & Japan through Sandwich (SW), Dish Pack (DP) and Plant Box (PB) methods.

No.	Botanical name	Family	SW	DP	PB	Criteria	Ranking
1	*Achellia millefolium* L.	*Asteraceae*	91	14	48	*	
2	*Agrostis stolonifera* L.	*Poaceae*	59	-6	63		
3	*Amaranthus spinosus* L.	*Amaranthaceae*	58	14	36		
4	*Avena fatua* Linn.	*Poaceae*	68	-1	84	*	
5	*Cichorium intybus* L.	*Asteraceae*	26	9	53		
6	*Conium maculatum* L.	*Umbelliferae*	67	15	75		
7	*Dactylis glomerata* L.	*Poaceae*	61	14	68		
8	*Datura stramonium* L.	*Solanaceae*	67	37	90	**	**3rd**
9	*Echium plantagineum* L.	*Boraginaceae*	72	-29	57		
10	*Eleusine indica* (Linn.) Gaertn.	*Poaceae*	81	-35	78	*	
11	*Festuca arundinacea* Schreb.	*Poaceae*	65	-10	39		
12	*Lantana camara* L.	*Verbenceae*	82	41	70	*	
13	*Leucanthemum vulgare* Lam.	*Asteraceae*	65	-13	39		
14	*Lolium multiflorum* Lam.	*Poaceae*	76	16	44		
15	*Lolium rigidum* Gaud.	*Poaceae*	71	-14	19		
16	Lotus corniculatus L.	*Papilionaceae*	60	82	39	*	
17	*Malva parviflora* L.	*Malvaceae*	88	12	66	*	
18	*Medicago lupulina* L.	*Papilionaceae*	55	12	72		
19	*Medicago sativa* L.	*Papilionaceae*	72	9	61		
20	*Melilotus alba* Desr. in Lam.	*Papilionaceae*	87	39	71	**	**2nd**
21	*Melilotus officinalis* (Linn.) Pall.	*Papilionaceae*	98	38	81	***	**1st**
22	*Mirabilis jalapa* L.	*Nyctaginaceae*	89	15	84	**	**4th**
23	*Papaver dubium* L.	*Papaveraceae*	56	2	75		
24	*Parthenium hysterophorus* L.	*Asteraceae*	86	-15	45	*	
25	*Phalaris canariensis* L.	*Poaceae*	50	6	66		
26	*Phalaris minor* Retz.	*Poaceae*	74	14	61		
27	*Plantago lanceolata* L.	*Plantaginaceae*	24	-1	25		
28	*Plantago major* L.	*Plantaginaceae*	81	4	44		
29	*Poa pratensis* L.	*Poaceae*	47	-22	45		
30	*Rubus fruticosus* L.	*Rosaceae*	76	10	33		
31	*Rumex conglomeratus* Murr.	*Polygonaceae*	27	8	15		
32	*Rumex crispus* L.	*Polygonaceae*	28	-47	30		
33	*Sida spinosa* L.	*Malvaceae*	42	-21	54		
34	*Trifolium dubium* Sibth.	*Papilionaceae*	48	18	79	*	
35	*Trifolium pratense* L.	*Papilionaceae*	44	-10	35		
36	*Verbascum thapsus* L.	*Scrophulariaceae*	65	-13	67		
37	*Veronica persica* Poiret	*Scrophulariaceae*	61	-17	54		
38	*Vulpia myuros* (Linn.) C.C.Gmel.	*Poaceae*	82	19	70		
	Mean		64.44	5.10	56.18		
	Standard deviation		19.09	23.86	19.49		
	Mean +1 Standard deviation		83.54	28.97	75.67		

Note: *indicates stronger inhibitory activity greater than the mean +1 standard deviation; **,*** indicates increasingly strong inhibitory activity

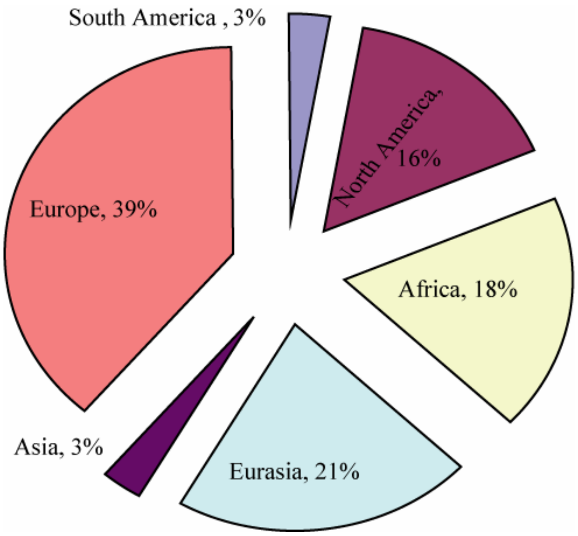

Fig. 2. Origin based continental contribution.

Allelopathic evaluation is an emerging research discipline that can be applied as an important tool in the assessment of environmental threats due to bio-invasion in different parts of the world. Several studies have been made in this respect. Efforts are underway on global level to develop a database of allelopathic plants to control impact of bio-invasion. For instance, Leaf litter of 239 medicinal plants collected from Japan has been screened out for allelopathic activity evaluation (Fujii *et al.*, 2003). The allelopathic activity potential of 160 medicinal plants from Japan has been evaluated by applying Sandwich method on leaf litter (Shinwari *et al.*, 2008a). In Pakistan, preliminary studies have enlisted 700 alien species of vascular plants, and 4500 indigenous species (Hussain & Zarif, 2003). Of these, six can be regarded as high-impact invasive i.e., *Broussonetia papyrifera, Prosopis juliflora, Eichhornia crassipes, Salvinia molesta, Parthenium hysterophorus,* and *Lantana camara*. For example, in a recent report, it has also been described that *Parthenium hysterophorus* L., is a noxious annual weed rapidly spreading across the non-cropped areas of the Khyber Pakhtunkhwa (KP) province and elsewhere in Pakistan and found highly sensitive to amino acid synthesis and photosynthesis inhibitors compared to herbicides with other modes of action. (Khan, *et al.,* 2012). There is no legislation that deals specifically with IAS. Moreover there is a lack of awareness about this serious issue among the general public as well as the policy makers. As a result, most of the plantation campaigns in the country still involve exotic species like *Eucalyptus* species and *Ailanthus altissima*. Most of the invasions are concentrated in the disturbed areas e.g., Agricultural waste lands, proximity of settlements and along roads. Out of 700 alien species in Pakistan nearly 100 have become naturalized and 20 have become invasive so far. It characterizes 15 major invasive alien species (IAS) which are allelopathic as well (Shinwari, *et al.*, 2008b). Limited financial resources demand the need for setting priorities as control and eradication methods for IAS are technically difficult and expensive for the country like Pakistan. Exploitation mechanisms and preventive methods should be the main focus in this regard. More research is needed to understand the invasion process and to evaluate the impact of invasive species on the environment and the agro-ecosystem (Shinwari *et al.*, 2008b). Threats to rainfed and canal irrigated agro-ecosystems of the Punjab in Pakistan by weed infestation has been investigated recently. It has been found that weeds of various localities mostly varied with the mode of irrigation. *Fumaria parviflora* and *Asphodelus tenuifolius* have been observed frequently in Rainfed areas while rarely represented or almost absent in canal irrigated areas. *Carthamus oxayacantha* was also observed at some sites there. The infrequent weeds reported were grasses like *Cynodon dactylon* and *Cyperus rotundus*. It has been noted that none of the weed found crossing the limits of occasional frequency. However, *Anagalus arvensis, Convolvulus arvensis, Chenopodium* sp., *Cynodon dactylon, Melilotus alba, Lepidium sativum, Lathyrus aphaca, Medicago denticulate* and *Rumex dentatus* have been found frequent in canal irrigated areas. *Phalaris minor* and *Avena fatua* have found dense in many areas (Hussain *et al.*, 2012). Evidence has been presented that *Centaurea maculosa* (spotted knapweed), an invasive species in the western United States, replaced native plant species by exuding the phytotoxin (–)-catechin from its roots. The results revealed the inhibition of native species' growth and germination in field soils at natural concentrations of (–)-catechin. In susceptible species such as *Arabidopsis thaliana*, the allelochemical triggers a wave of reactive oxygen species (ROS) initiated at the root meristem, which leads to a Ca^{2+} signaling cascade triggering genome-wide changes in gene expression and, ultimately, death of the root system. The outcome of this study support a "novel weapons hypothesis" for invasive success (Bais *et al.*, 2003).

Previously allelopathic effects of various weed plant species have been identified by different workers for example; sorghum (*Sorghum bicolor*) has been reported to have a good weed killing potential (Putnam *et al.*, 1983), although it is also auto-toxic and may be rotated with other crops to increase yield (Leon, 1976). Several methods have been used to evaluate allelopathic activity. For example, 70 Japanese plant species have been surveyed for allelopathy by using a water extraction method (Fujii *et al.*, 1990). About 78 medicinal species have been screened for their allelopathic potential by using the solvent (methanol and water) extraction method (Fujii *et al.*, 1991). However, bioassay screening methods used in the current study are found effective and less time consuming. According to the present results, *Melilotus officinalis* appeared to be the most noxious species among all due to maximum inhibitory 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 shown minimum inhibitory effect (Table 2).

Allelopathic potential of *Medicago sativa* L. cv. Alfaking (*alfalfa*), *Vicia rillosa* Roth. (hairy vetch) and *Melilotus officinalis* Desr. (yellow sweet clover) was determined on seed germination and seedling growth of *Echinochloa crusgalli* L. , Beauv. (barnyard grass), *Poa annua* L. (annual bluegrass) and *Veronica persica* Poir. It has been observed that application of *Melilotus officinalis* dry powder inhibited the emergence and growth of *Veronica persica* Poir. The powdered biomass of *Melilotus officinalis* caused so drastic inhibition in weeds growth, that it has been recommended as natural herbicide for weed control in crops through intercropping, rotation or soil mulching.(Wu *et al.*, 2010) It has been reported that some cover crops such as *Secale cereale* (rye), *Avena sativa* (oat), *Brassica* spp. (barley, mustard), and *Melilotus officinalis* (sweetclover) contain allelopathic compounds that inhibit weed germination and growth (Weston, 1996). The allelopathic effect of water extracts of five legume forages, i.e., *Vicia villosa, Medicago sativa, Trifolium repens,T. pratense,* and *Melilotus officinalis*, of different concentrations: 0, 0.050, 0.075, and 0.100 g/mL(w/v), on the seed germination and seedling growth of Echinochloa crusgalli (barnyard grass), has been evaluated. Results indicated that the allelopathic effects of different legume species on barnyard grass varied. Seed germination of barnyard grass was significantly inhibited by the water extracts of hairy vetch and yellow sweet clover, and the inhibition rate exceeded 60%. Seedling growth, especially that of the root, was greatly retarded by the water extract of alfalfa. The results also show that the allelopathic inhibition rate increased with intense concentration of the water extracts (Xiao-xia *et al.*, 2008).

Melilotus alba Desr., a leguminous weed, grows as a competitor in wheat fields, has been evaluated for allelopathic effects of its roots and shoots. The different concentrations of root and shoot extracts of *M. alba* were tested on the seed germination and seedling establishment of wheat and was observed that the seed germination was more affected by the root extracts than shoot extracts and the seedling establishment was also inhibited by root extracts. Hence, a decrease in the percentage of seed germination and seedling establishment was recorded with the increasing concentration of aqueous extracts (Shweta & Sharma, 2008).

The allelopathic potential of *Datura stramonium* on *Paspalum scrobiculatum* (kodo) and its common weeds *Chenopodium album*, *Echinochloa colonum*, *Cynodon dactylon, Melilotus alba, Eclipta alba* and *Ageratum conyzoides* was investigated. The different extracts of *D. stramonium* did not show any detrimental effects on the targeted weeds (Oudhia & Tripathi, 1998). In a recent comparative allelopathic evaluation of two weeds, *Chenopodium murale* and *Malva parviflora* on growth and photosynthesis of barley (*Hordeum vulgare* L.), *C. murale* was found highly aggressive, as it affected almost all growth and physiological traits compared with *M. parviflora* (Al-johani *et al.,* 2012).

Mirabilis jalapa L., native to Tropical America, had become a major invasive plant in China. To evaluate the relationship between allelopathy and invasiveness in *M.jala*pa, the micronucleus test of *Vicia faba* toot tip cells was conducted to detect the genetic toxicity induced by the aqueous extracts of *M. jalapa*. The results showed that the aqueous extracts of *M. jalapa* disturbed mitosis of *Vicia faba* root tip cells. The mitotic index decreased and the micronucleus frequency increased, and aberrant chromosomes such as micronucleus, chromosome bridge,chromosome break and chromosome lag occurred. These effects were concentration dependent and time dependent. The allelopathic effect of overground organ was stronger than that of underground organ. The water-solubility allelopathic substance in *M. jalapa* into environment through eluviations and secretion inhibited growth of other plants, and allelopathy may be one of the mechanisms of *M. jalapa* rapid spread. The micronucleus test of Vicia fabra root tip cells has great value testing allelopathy of invasive plants (Xiao-kui *et al.*, 2008).

In another study the allelopathic effect of *Mirabilis jalapa* L. on *Oenothera biennis* L.. *Oenothera biennis* L. seeds has been investigated. The results showed that germination potential, germination rates, activity index and germination index all reached the highest compared to the other groups when the concentration of water extract from *Mirabilis jalapa* fruit was 0.025 g/mL. It has been concluded that the water extract from *Mirabilis jalapa* fruit could promote seed germination and raise fresh weight, seedling length of *Oenothera biennis*, but its root growth was inhibited (Zhang, 2011).

There is a strong need to study the allelopathic effects of weeds on crops in order to assess interactions of particular weeds with certain crops in agricultural fields. Understanding how weeds interact with crops and within their natural environments is important for developing weed management tools.

Conclusion

Allelo-chemical screening of invasive plants and weeds has great significance to Pakistan and Japan. In Japan database of most of the indigenous and naturalized plants has already been developed. However Pakistan with a diverse flora has no database available regarding allelopathic activity of indigenous and invasive plants although some current efforts are going on in this respect. Allelopathy evaluation can play an important role in the biological control of species that can negatively impact agriculture and forestry projects in both the countries. In Pakistan, cash crops like wheat, maize, rice and cotton already under stress from weed interference. Therefore, biological control of weeds is of prime importance as the phyto-chemical properties of most plants are not yet explored.

Acknowledgement

The authors are very much thankful to Higher Education Commission of Pakistan for financial support of this research endeavor as part of Post Doctoral Research work carried out by the authors.

References

Ali, S.I. 1978. *The Flora of Pakistan*: Some general and analytical remarks *Notes Roy. Bot. Gard. Edinburgh,* 36: 427-439.

Al-johani, N., A. A. Aytah and T. Boutraa. 2012. Allelopathic impact of two weeds, *Chenopodium murale* and *Malva parviflora* on growth and photosynthesis of barley (*Hordeum vulgare* L.). *Pak. J. Bot.*, 44(6): 1865-1872.

Asai, Y. 1993. *Green invaders*. Asahi-shinbunsha Press: Tokyo.

Bais, H.P., R. Vepached, S. Gilroy, R.M. Callaway and M.V. Jorge. 2003. Allelopathy and exotic plant invasion: From molecules and genes to species interactions *Science,* 301 (5638): 1377-1380.

Chaudhri, M.N. 1977. The Pakistan Herbarium. *Pak Syst,* 1: 100-105.

Dalton, H.J. 1875. *Flora of British India* London .

Enomoto, T. 1999. Naturalized weeds from foreign countries into Japan. In: *Biological Invasions of Ecosystem Pests and Beneficial Organisms*. (Eds.): E. Yano, K. Matsuo, M. Shiyomi and D.A. Andow,. Yokendo, Tokyo.

Frodin, D.G. 1984. *Guide to Standard Floras of the World*. Cambridge Univ. Press, Cambridge.

Fujii, Y., M. Furukawa, Y. Hayakawa, K. Sugawara and T. Shibuya. 1991. Survey of Japanese medicinal plants for the detection of allelopathic properties *Weed Res Japan.,* 36: 36-42.

Fujii, Y., M. Matsuyama, S. Hiradate and H. Shimozawa. 2005. Dish pack method: A new bioassay for volatile allelopathy. In: (Eds.): J.D.I. Harper, M. An, H. Wu and J.H. Kent, *Proceedings of the 4th World Congress on Allelopathy, "Establishing the Scientific Base"*, Fourth World Congress on Allelopathy, Wagga Wagga, New South Wales, Australia. pp. 493-497.

Fujii, Y., D. Pariasca, T. Shibuya, T. Yasuda, B. Kahn and G.R. Waller. 2007. Plant-Box Method: A specific bioassay to evaluate allelopathy through root exudates. In: *Allelopathy: New Concepts and Methodology*. (Eds.): Y. Fujii and S. Hiradate, Science Publishers, Inc. Enfield, NH, USA.

Fujii, Y., S. Parvez, M.M. Parvez, Y. Ohmae and O. IIDA. 2003. Screening of 239 Medicinal plant species for allelopathic activity using the sandwich method. *Weed Biol. Manag.,* 3: 233-241.

Fujii, Y., T. Shibuya and T. Yasuda. 1990. Survey of Japanese weed and crops for the detection of water-extractable allelopathic chemicals using Richard's function fitted to lettuce germination test. *Weed Res Japan,* 35: 362-370.

Gilani, S.A., Y. Fujii, Z.K. Shinwari, M. Adnan, A. Kikuchi, and K.N. Watanabe. 2010. Phytotoxic studies of medicinal plant species of Pakistan. *Pak. J. Bot*., 42(2): 987-996.

Henderson, L. 2001. Alien weeds and invasive plants: a complete guide to declared weeds and invaders in South Africa. In: *Plant Protection Research Institute, Handbook No. 12*. Agricultural Research Council. LNR: South Africa.

Hussain, A. and R.M. Zarif. 2003. Invasive alien tree species - A threat to biodiversity (Pakistan) *Pak. J. For.,* 53(2): 127-141.

Hussain, M., M.S.A. Ahmed, M. Hameed, M. Aqeel, S. Rasool and A. Iftikhar. 2012. Threats to rainfed and canal irrigated agro-ecosystems of the Punjab, Pakistan by weed infestation. *Pak. J. Bot.,* 44(171-178).

Ito, M. and A. Morita. 1999. *The underground invasion of clonal weeds*. Kyoto University Press: Kyoto Kasahara, Y. 1976. Genealogy of crops and weeds in Japan (2) *Weed Res Japan.,* 21: 49-55.

Khan, H., K. B. Marwat, G. Hassan and M. A. Khan. 2012. Chemical Control of Parthenium hysterophorus L. at different growth stages in non-cropped area. *Pak. J. Bot*., 44(5): 1721-1726.

Kasahara, Y. 1976. Genealogy of Crops and Weeds in Japan(2). *Weed Research Japan* 21: 49-55. *(in Japanese).*

Leon, W.B. 1976. *Phytotoxicitéinduite par les résidus de récolte de Sorghum vulgaredans les sols sableuxd'ouest Africain.* Université de Nancy, France.

Maekawa, F. 1943. Prehistoric-naturalised plants to Japan proper. *Acta Phytotaxonomica et Geobotanica,* 13: 274-279.

Michael, P.W. 2001. Plant links between eastern Asia and eastern Australia. *Weed Biol. Manag.,* 1: 157-163.

Nasir, Y.J. 1991. Threatened plants of Pakistan. In: *Plant Life of South Asia*. (Eds.): S.I. Ali and A. Ghaffar, Shamim Press, Karachi.

Oudhia, P. and R.S. Tripathi. 1998. Allelopathic potential of Datura stramonium L. *Crop Research (Hisar),* 16(1): 37-40.

Pallewatta, N., J.K. Reaser and A. Gutierrez. 2003. Prevention and Management of Invasive Alien Species. In: *Proceedings of a Workshop on Forging Cooperation throughout South and Southeast Asia. Global Invasive Species Programme*, Cape Town, South Africa. pp.

Pimentel, D., L. Loch, R. Zuniga and D. Morrison. 2000. Environmental and economic costs of non indigenous species in the United States *BioScience,* 50: 53-65.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O'Connell, E. Wong, L. Russel, J. Zern, T. Aquino and T. Tsomondo. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agri Ecosyst Env,* 84: 1-20.

Putnam, A.R., J. DeFrank and J.P. Barnes. 1983. Exploitation of allelopathy for weed control in annual and perennial cropping systems. *J. Chem. Ecol.,* 9: 101-111.

Saqib, Z., R. N. Malik., M. I. Shinwari and Z. K. Shinwari. 2011. Species Richness, Ethnobotanical Species Richness and Human Settlements along a Himalayan Altitudinal Gradient: Prioritizing Plant Conservation in Palas Valley, *Pakistan. Pak. J. Bot.*, 43 9 (SI): 129-133.

Shimizu, N., H. Morita and S. Hirota. 2001. Naturalised plant invaders of Japan-600 invaders: 199.

Shinwari, M.I., O. Iida, M.I. Shinwari and Y. Fujii. 2008a. Evaluation of allelopathic activity of 160 Japanese medicinal plants by sandwich method. *J. Weed Sci. Technol.,* 53: 73.

Shinwari, M.I., M.I. Shinwari and Y. Fujii. 2008b. Allelopathic invasive plants of Pakistan. *J. Weed Sci. Technol.,* 53: 72.

Shinwari, Z.K. & S. Gilani. 2003. Sustainable harvest of medicinal plants at Bulashbar Nullah, Astore (Northern Pakistan). *J. Ethnophormacology*., 84(2003): 289-298.

Shinwari, Z.K. & M. Qaisar 2011. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot*., 43(Special Issue): 5-10.

Shweta, P. and S.C. Sharma. 2008. Allelopathic Effects of Melilotus alba on Seed Germination and Seedling Establishment of Wheat var (RR 21) *Vegetos,* 21(2).

Weston, L.A. 1996. Utilization of allelopathy for weed management in agroecosystems *Agron. J.,* 88: 860-866.

Wu, C.X., X.X. Guo, Z.H. Li and Y.X. Shen. 2010. Feasibility of using the Allelopathic potential of yellow sweet clover for weed control *Allelopath. J.,* 25(1): 173-184.

Xiao-kui, Z., M. Dan-wei, Z. Quan-quan, H. Wei and C. Peng. 2008. The genetic toxicity of an invasive plant,Mirabilis jalapa L. *Southwest China Journal of Agricultural Sciences.,* 21(1): 152-155.

Zhang, Y.M. 2011. Effects of Water Extract from Mirabilis jalapa L. Fruit on Seed Germination and Seedling Growth of Oenothera biennis L. *Chinese Agricultural Science Bulletin,* 27: 188-19.

(Received for publication 1 September 2012)