African Journal of Biotechnology Vol. 8 (6), pp. 1024-1029, 20 March, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Assessment of allelopathic potential of selected medicinal plants of Pakistan

Abdul Latif Khan[1,2], Muhammad Hamayun[2], Javid Hussain[1], Hamayun Khan[5], Syed Abdullah Gilani[3], Akira Kikuchi[3], Kazou N. Watanabe[3], Eung Ho Jung[4], and In-Jung Lee[2]*

[1]Department of Chemistry, Kohat University of Science & Technology, Kohat-26000, Pakistan.
[2]School of Applied Biosciences, College of Agriculture & Life Science, Kyungpook National University, Republic of Korea.
[3]Gene Research Centre, Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan.
[4]Faculty of Environmental Science, Keimyung University, Daegu 704-701, Republic of Korea.
[5]Department of Chemistry, Malakand University Chakdara NWFP Pakistan.

Accepted 2 January, 2009

***Inula falconeri*, *Inula koelzii*, *Lactuca dissecta* and *Anthemis nobilis* were collected from Himalaya and Hindukush ranges of Pakistan and their allelopathic effect was studied through Sandwich and Homogenated Sandwich methods. The study also aimed at analyzing whether method and concentration can affect the overall results. The results showed that method has no significant value; however, concentration of leaf leachates has highly significant value for exploring the inhibition or stimulation pattern of the plant species.**

Key words: Allelopathy, Sandwich and Homogenated Sandwich methods, medicinal plants of Pakistan.

INTRODUCTION

After Prof. Hans Molisch coined the word 'allelopathy' in 1937, efforts have been made to explore the allelopathic potential of various plant species. To do so, high altitudinal medicinal plants, *Inula falconeri*, *Inula koelzii*, *Lactuca dissecta* and *Anthemis nobilis* were selected to know their allelopathic effect. *I. falconeri* is present in Pakistan and western Tibet (Dawar, 1998), while *I. koelzii* is only known from Laddakh but has also been found in Skardu region (Ali and Qaiser, 1992). The plants species of genus *Inula* were selected due to their diverse chemical composition, which are famous for cytotoxic, antioxidant, hepaprotective and bactericidal effects (Zhao et al., 2006; Khan et al., 2008). However, any exploratory study on the allelopathic effect of the plant species of the genus has not been found. *A. nobilis* has been used as herbal tea against indigestion, vomiting, and intestinal problems (Ahmad, 2007). *L. dissecta* is herb with milky latex. Leaves of *L. dissecta* are used as sedatives by the local people. Lactucol and lactupircin are the reported compounds (Shinwari et al., 2006).

*Corresponding author. E-mail: ijlee@knu.ac.kr, Tel: +82-53-950-5708 (Office), Fax: +82-53-958-6880.

Besides allelopathic potential of plants species, attempts have been made to illustrate it at laboratory level, however, little has been done at field level (Einhelling, 1987; Inderjit and Weiner, 2001; Macías et al., 2007). At field levels, allelochemicals play an important role in the agricultural and functional ecology (Vyvyan, 2002; Fujii et al., 2004). The importance of allelopathy in biological control of weeds and crop productivity has been highly recognized and various methods have been suggested to know the allelopathic effect (Rice, 1992; Wu et al., 1999; Fujii et al., 1991, 1992, 2003, 2004; Terzi, 2008; Taiwo and Makinde, 2005).

In one of the survey almost 96 different methods have been adopted so far to study the allelopathy phenomenon (Blair, 2007), which has led to many unsolved questions. It is normally difficult to demonstrate the actual environmental conditions at *in vitro* analysis, however, rout-specific bioassay methodologies have to be still developed and worked on (Fuji et al., 2004). Leaf litter leachates play an important role in natural conditions and in practical farming systems such as mulching. Mulching with straw is a common traditional farming system in many of the developing countries of Asia and Africa (Fuji et al., 2004; Rice, 1992). Sandwich method is thus replica-

Table 1. Geographical locations and collection areas of the plants species for allelopathic studies from Hindukush and Himalayan regions.

S/N	Plant names	Collection areas	Geographical locations
1	*I. falconeri*	Skardu	35º03' 23.26" N- 75º27'39.69" E
2	*I. koelzii*	Skardu	35º03' 23.26" N- 75º27'39.69" E
3	*L. dissecta*	Swat	35^0.02' N and 72^0, 33' E *
4	*A. nobilis*	Chitral	35-40 N and 71-40 E**

*Adnan, 2002; **Ahmad, 2007.

tion of natural condition at the laboratory levels (Fuji et al., 2003, 2004) to assess the allelopathic potential. Besides that various chemical compounds are solubilised in water while oozing out from fallen leaves or other parts of the plant. Looking into this natural phenomenon, a newly established Homogenated Sandwich method was used.

The objective of the study was to distinguish the allelopathic impact of the selected plant species and to compare that whether method and concentration can affect the inhibition rate of the growth of lettuce seed (*Lactuca sativa*). The present study is an addition to the list of plants species whose allelopathic effects have been reported earlier from Pakistan (Gilani et al., 2008; Khalid et al., 2002; Hussain, 1980; Shoukat et al., 2002).

MATERIALS AND METHODS

Plant materials

I. falconeri, *I. koelzii*, *A. nobilis* and *L. dissecta* were collected from different localities of Hindukush and Himalayan regions of almost same altitudes in August 2007 (Table 1). The aerial parts of the plant species were packed in polyethylene bags with silica gel; shade dried at room temperature for 10 days and was stored at 10ºC.

Preparation of medium

Agar powder (Nacalai Tesque Kyoto, Japan) with gelling temperature of 30-31 ºC was used as medium for both methods. For sandwich method, 0.75% (w/v) agar solution was prepared while for Homogenated Sandwich method, 0.5 and 1% (w/v) of agar solutions were prepared. The solutions were autoclaved at 115ºC for 15 min and cooled to *ca*. 45ºC.

Method description

1. **Sandwich method:** 10 and 50 mg of dried leaves were placed in each well of the multidish plastic plate (6 – wells micro-plate; 10 cm^2 area per each dish; Nalge Nunc Intl., Roskilde, Denmark) (Fujii et al., 2003, 2004). 5 ml of 0.75% of agar solution was added into each well of the plastic microplates on the leaves tissues. After solidification of agar, another 5 ml agar solution was added to each well of the microplate.
2. **Homogenated Sandwich method:** To have uniformity with sandwich method in concentration of leaf leachate, 60 and 300 mg of the dried leaves were ground using liquid nitrogen. The powdered forms of the dried leaves were mixed in 20 ml of distilled water (DW) in Falcon Tubes. The macerated solution was centrifuged (Kobota 5220, Japan) at 3000 rpm for 20 min to prepare supernatant solution. The supernatant was filtered. The 1 and 0.75% agar solution were added in the 6-well dish.

Lettuce seed and its measurement

Five Lettuce seeds (*Lactuca sativa* L. Great Lakes 366, Takii Seed Co. Ltd, Japan) were added to each well of the plate. The multidish plastic plates were sealed with plastic tape, labelled and kept in an incubator (BIOTEC 300-L, Shimadzu Rika Institute Co. Ltd, Kyoto, Japan) to grow the seedlings for 72 h at 25ºC under dark. Agar solution was used as control for the growth of lettuce seeds and experiment was conducted three times.

Statistical analysis

For each plant species, mean, SD variance (Fujii et al., 2003) and standard error were calculated to determine the inhibition pattern of (i) 10 and 50 mg and (ii) 60 and 300 mg of root and hypocotyl of the plants species. To explore that method and concentration (two factors) might have effects on the growth of root and hypocotyl of lettuce seeds, two ways ANOVA test in MSTATC Software with Randomized Complete Block Design (RCBD) was applied. The level of significance was 0.05.

RESULTS

Allelopathic effects of plant species

Bioassay results of the tested plant species belonging to Asteraceae family revealed that *I. falconeri* and *I. koelzii* have significant inhibitory effect towards the growth of lettuce seeds. *L. dissecta* has moderate effect while *A. nobilis* has stimulating effect. The allelopathic effects vary among species showing the potential allelopathic plant. A similar trend was also reported previously (Fujii et al., 2003; Gilani et al., 2008).

The results of sandwich method revealed that among four plant species, 50 mg dried leaf leachates showed higher inhibitory effect to the growth of root and hypocotyl compared to 10 mg. Using 50 mg of dried leaves, *I. falconeri*, *I. koelzii*, and *L. dissecta* have shown signifi-

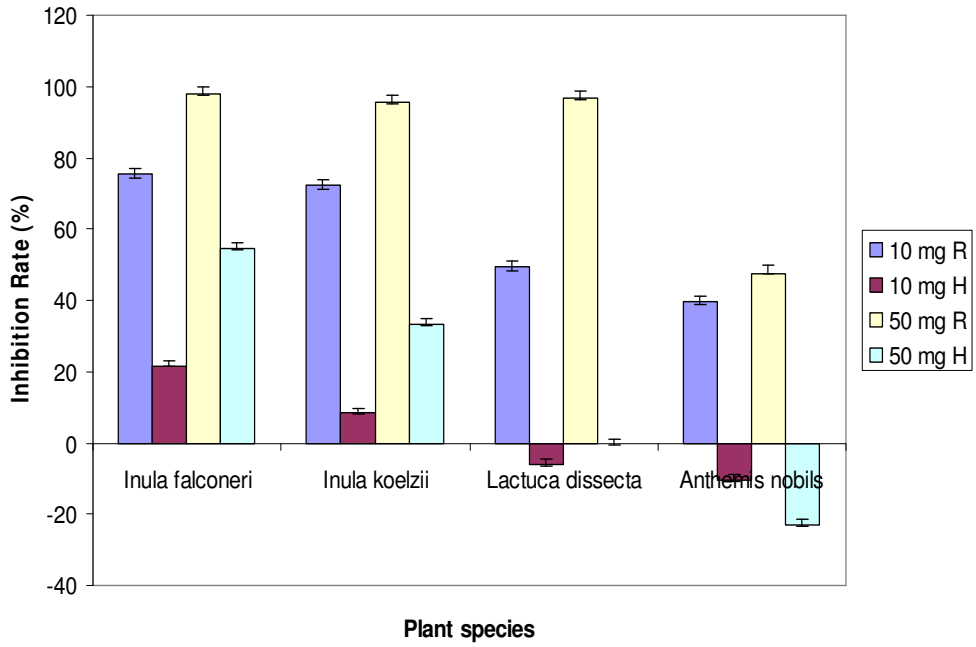

Figure 1. The effect of concentration on the growth inhibition/stimulation of root and hypocotyl of lettuce seedlings by Sandwich method (H: hypocotyl; R: root).

cant allelopathic distress with an inhibition rate of 98.0, 95.7, and 96.7% respectively (Table 2). In case of 10 mg concentration, *I. falconeri* and *I. koelzii* exhibited significant inhibitory rate of 75.3 and 72.3% respectively while *L. dissecta* has shown moderate effect of 49.3%. *A. nobilis* has shown stimulating effect both in 10 and 50 mg hypocotyl growth (Figure 1).

Following the Homogenated Sandwich methods, *I. falconeri,* and *I. koelzii* have shown considerable inhibitory effects (78.3, and 75.7% respectively) to the growth of roots on 300 mg concentration compared to other species (*A. nobilis* and *L. dissecta*). In case of hypocotyl, the inhibitory levels were not significant (Figure 2). The inhibitory effects for other species (*A. nobilis* and *L. dissecta*) were almost similar to the results of sandwich method with lower inhibition rate.

Comparison of the two methodsIn comparing the growth reticence of roots and hypocotyl, *I. falconeri* and *I. koelzii* showed the highest inhibition (Figure 3). *I. falconeri* has the highest inhibition rate of 75.3 and 61.1% when exposed to 10 and 60 mg of leaf leachates, respectively, while hypocotyl has 21.7 and 34.9%, respectively. For 50 and 300 mg, root inhibition was 98.0 and 78.3% while hypocotyl had 54.7 and 47.7% inhibition rate, respectively. *I. koelzii* inhibited the root of lettuce seed by 72.3 and 63.1% while hypocotyl by 8.3 and 41.9%, respectively, when exposed to 10 and 60 mg leaf leachates. For 50 and 300 mg, root growth was inhibited 95.7 and 75.7% while hypocotyl had 33.3 and 58.1% inhibition rate, respectively. The other two species showed moderate inhibitory effects towards the growth of lettuce seeds (Figure 3).

ANOVA

The ANOVA analysis shows that methods and concentration have no or less interdependency on the results (Table 2). For most of the plant species, method has no significant value in the evaluation of the allelopathic potential of plant (Table 2). However, it has been proved that the concentration of leaf leachates of plant species plays an important role in natural allelopathic phenomenon. Therefore, the present results are in compliance with the earlier reports and the same has also been proved here through statistical analysis (Table 2) (Gilani et al., 2008; Fujii et al., 2004).

DISCUSSION

I. falconeri and *I. koelzii* have shown significant inhibition rate to the growth of root and hypocotyl of lettuce seeds compared to other species (*A. nobilis* and *L. dissecta*). Genus *Inula* is famous for sesquiterpenes and monoterpenes (Zhao et al., 2006; Khan et al., 2008). These have been identified and reported to be allelochemicals in nature (Macías et al., 1999, 2002; Abdelgaleil and

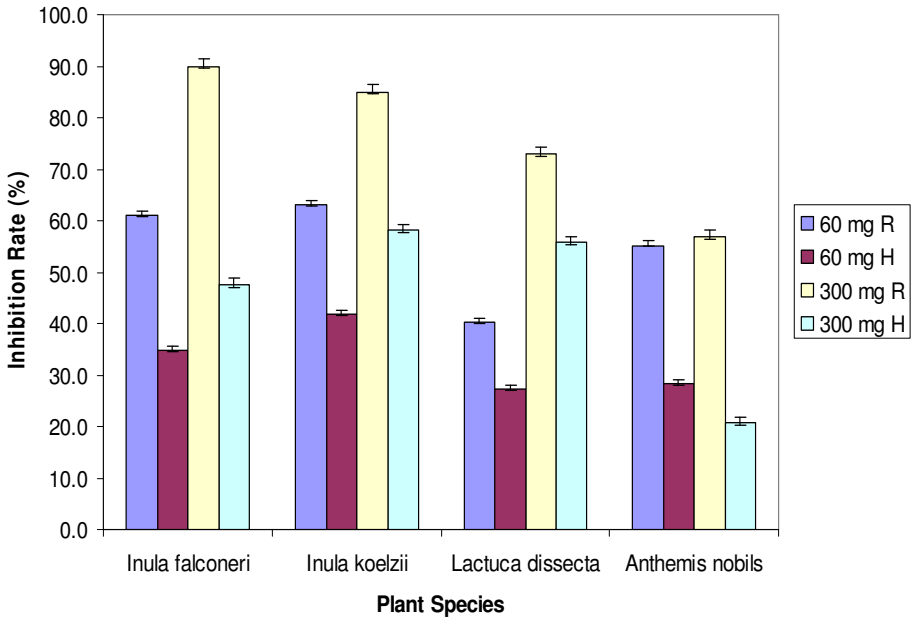

Figure 2. The effect of concentration on the growth inhibition of root and hypocotyl of lettuce seedlings using homogenated Sandwich method (H: hypocotyl; R: root).

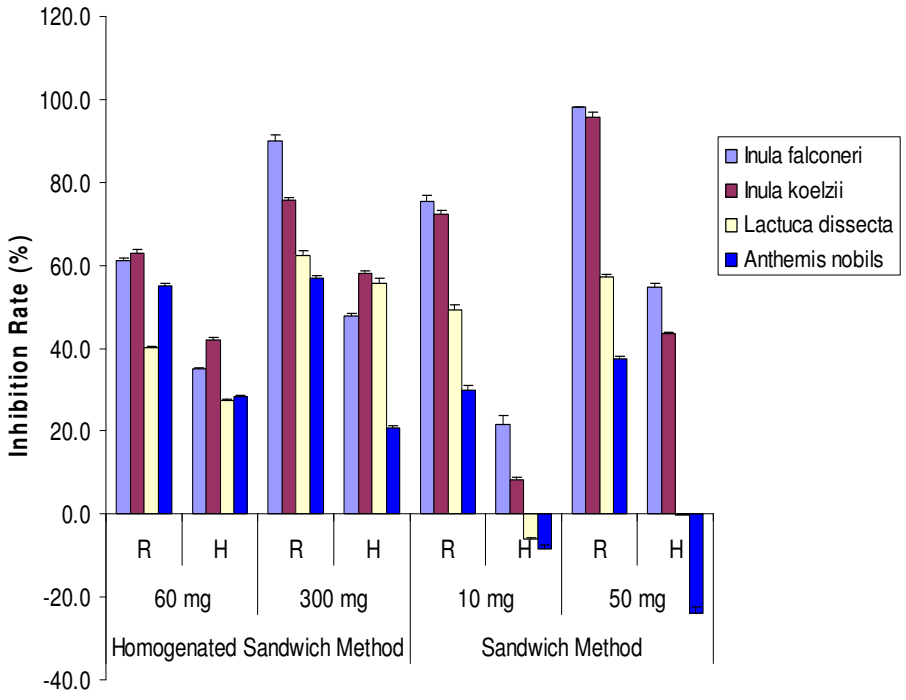

Figure 3. Comparison of the results of Sandwich and homogenated Sandwich methods (H: hypocotyl; R: root).

Hashinaga, 2007). Besides the presence of sesquiterpenes, various phenolic acids (caffiec acid and chlorogenic acid) and flavonoids (rutin, quercetin and kaempfrol) have been reported from *I. falconeri* and *I. koelzii* (Abid and Qaiser, 2003). Some of the phenolic compounds and flavonoids are experimentally proved to

Table 2. Comparative extension rates (%) of root and hypocotyls of lettuce seeds against different concentration of the selected four plants species used in both methods. The analysis of variance (two ways) showing the significance level of correlation of method and concentration.

Plant	HS method (%)				Sandwich method (%)				Sum of squares				Significance level				Relation
	60 mg		300 mg		10 mg		50 mg		M		C		M		C		
	R	H	R	H	R	H	R	H	R	H	R	H	R	H	R	H	M x C
L. dissecta	55.1	70.2	38.2	49.4	50.7	106.0	3.3	100.3	115.75	100.17	299.5	41.181	0.059b	0.07b	0.001a	0.022ab	0.026ab
I. falconeri	38.9	65.1	21.7	52.3	24.7	78.3	2.0	45.3	83.74	1.08	114.33	65.05	0.057b	-	0.0014a	0.034ab	0.367b
I. koelzii	36.9	58.1	24.3	41.9	27.7	91.7	4.3	66.7	63.48	42.67	94.64	62.42	0.011ab	0.057b	0.0082a	0.000a	0.057b
A. nobilis	44.8	71.7	43.2	79.3	60.3	110.3	52.3	123.0	33.1	73.26	9.63	0.175	0.14b	0.061b	0.025ab	-	0.068b

R = Root, H = hypocotyl, a = highly significant, ab = significant, b = non-significant, M = method, C = concentration, HS = Homogenated Sandwich.

carry allelopathic behaviour (Tsuzuki and Yamamoto 1987; Wu et al., 1998; Bias et al., 2003; Pervaiz et al., 2004; Golisz et al., 2007; Makoi and Ndakidemi, 2007). The presence of such class of compounds in the leaf leachates has inhibited the growth of lettuce seed.

As far as the methodology is concerned, through Sandwich method the lettuce seeds are directly exposed to the leaf leachates from the dried leaf. The more the volatile organic content in the leaf, the more significant will be inhibition rate. However, Sandwich method is an exercise of natural allelopathic phenomenon at laboratory level (Fujii et al., 2003). The allelochemicals thus released in natural system affect/regulate the growth of the other plant species (Rice, 1984). Because of the nature and effect of sesquiterpenes, we can conclude that the direct release of sesquiterpene (volatile component) from the leaves inhibited the growth of lettuce seeds in the Sandwich method.

The Homogenated Sandwich method is more about assessing the water soluble compounds to ooze out in the medium while rupturing the cell wall of the plant material and stopping the overall enzymatic reactions within the leave. Thus the lettuce seeds are exposed to water soluble, mix solution of chemical constituents, mostly phenolic acids and flavonoids. The leaf leachates in Homogenated Sandwich method had lower total activity compared to that of Sandwich method (Golisz et al., 2007; Hiradate et al., 2004).

The results also been proved that higher concentrations of allelochemicals can impose inhibitory or stimulatory effect to the growth of lettuce seeds. At higher concentration, *A. nobilis* showed stimulatory effect. Considering the ANOVA results, it is clear that method and concentration are not dependent on each other and the higher concentration of the leaf leachates furnish significant values in identification of allelopathic potential of plant species (Gilani et al., 2008; Fujii et al., 2003). Thus the present study has not only produced how nature oriented methodologies at laboratory level are important for assessing the allelopathy potential of a plant species and but also the difference in concentration of certain leaf leachates from allelopathic plant species can effect the neighboring plants as well. The identification of two potential allelopathic plant species, i.e., *I. falconeri* and *I. koelzii* can be further subject to isolation and characterization of allelochemicals, which can be helpful in development of environmental friendly herbicide.

ACKNOWLEDGEMENTS

The authors are thankful to Mr. Ismail Zafar, Divisional Forest Officer, Skardu for facilitation of the research work. The research study was supported by “On-Site Cooperative Agriculture Research Project (20070301080024)”, RDA, Republic of Korea.

REFERENCES

Abid R, Qaiser M (2003). Chemotaxonomic study of *Inula* L. (s.str.) and its allied genera (Inuleae - Compositae) from Pakistan and Kashmir. Pak. J. Bot. 35(2): 127-140

Abdelgaleil SAM, Hashinaga F (2007). Allelopathic potential of two sesquiterpene lactones from *Magnolia grandiflora* L. Bio. Syst. Ecol. 35: 737-742

Ali SI, Qaiser M (1992). *Asteraceae*. University of Karachi. Karachi Printing press.

Adnan SM (2002). Feasibility of community involvement in the sustainable use of medicinal plants in Roringar Valley, Swat. Ethnobotany Project WWF-P, Peshawar.

Ahmad E (2007). Indigenous plant use of Kelash Valley, Chitral. A report to NWFP Forest Department Chitral.

Bias HP, Walker TS, Kennan AJ, Stermitz FR, Vivanco JM (2003). Structure-Dependent Phytotoxicity of Catechins and Other Flavonoids: Flavonoid Conversions by Cell-free Protein Extracts of *Centaurea maculosa* (Spotted Knapweed) Roots. J. Agric. Food Chem. 51: 897-901.

Dawar R (1998). Biosystematic studies on Genus *Inula* of Pakistan and Kashmir. Ph.D dissertation, Department of Botany, University of Karachi, Pakistan, pp. 34-55.

Einhellig FA (1987). Interactions among allelochemicals and other stress factors of the plant environment. In Waller GR (ed.). Allelochemicals: Role In agriculture and forestry. Am. Chem. Soc., pp. 343-357.

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

Fujii Y (1992). The potential biological control of paddy weeds with allelopathy (allelopathic effects of some rice varieties). In: Proceedings of the International Symposium for Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia, National Agricultural Research Centre, Tsukuba, Japan. Food and Fertilizer Technology Centre for the Asian and Pacific Region, China, pp. 305-320.

Fujii Y, Parvez SS, Parvez MM, Ohmae Y and Iida O (2003). Screening of 239 medicinal plant species for allelopathic activity using sandwich method. Weed Biol. Manage. 3: 233-241.

Fujii Y, Shibuya T, Nakatani K, Itani T, Hiradate S, Parvez MM (2004). Assessment method for allelopathic effect from leaf litter leachates. Weed Biol. Manage. 4: 19-23.

Gilani SA, Fujii Y, Adnan SM, Kikuchi A, Shinwari ZK, Watanabe KN (2008). Allelopathic study of 81 medicinal plants of Pakistan. Weed Biol. Manage. (Accepted).

Golisz A, Lata B, Gawronski SW, Fujii Y (2007). Specific and total activities of the allelochemicals identified in buckwheat. Weed Biol. Manage. 7: 164-171.

Hiradate S, Morita S, Sugie H, Fujii Y, Harada J (2004). Phytotoxic cis-cinnamoyl glucosides from *Spiraea thunbergii.* Phytochemistry 65: 731-739.

Hussain F (1980). Allelopathic Effects of Pakistani Weeds: *Euphorbia granulata* Forssk. Oeco. (Berl.) 45: 267-269.

Inderjit, Weiner J (2001). Plant allelochemicals interference or soil chemical ecology? Persp. Plant Ecol. Eviron. Syst. 4: 3.

Khan AL, Gilani SA, Fujii Y, Watanabe KN (2008). Monograph on *Inula britannica* L. Mimatsu Corporation, Tokyo-Japan. ISBN 978-4-903242-24-8.

Khalid S, Ahmad T, Shad RA (2002). Control of seed allelopathy in agriculture. Austr. J. Plant Sci. 1: 292-297.

Macías FA, Oliva RM, Verala RM, Torres A, Molinillo JMG (1999). Allelochemicals from sunflower leaves cv. Peredovick. Phytochemistry 52: 613-621

Macías FA, Torres A, Galindo LG, Varela RM, Jose´ AA, Molinillo MG (2002). Bioactive terpenoids from sunflower leaves cv. Peredovick. Phytochemistry 61: 687-692.

Macías FA, Galindo LG Galindo CG (2007). Evolution and current status of ecological phytochemistry. Phytochemistry 68: 2917-2936.

Makoi JHJR, Ndakidemi PA (2007). Biological, ecological and agronomic significance of plant phenolic compounds in rhizosphere of the symbiotic legumes. Afr. J. Biotechnol. 6 (12): 1358-1368.

Pervaiz MM, Yokotani KT, Fujii Y, Konishi T, Iwashina T (2004). Effects of quercetin and its seven derivatives on the growth of *Arabidopsis thaliana* and *Neurospora crassa.* Biochem. Syst. Ecol. 32: 631-635.

Rice EL (1984). Allelopathy. 2nd Ed. Academic 17 Publishers, New York, p. 424.

Rice EL (1992). Allelopathic growth stimulation. In: Putnam, A.R. Chang (Eds.), The Science of Allelopathy. Wiley & Sons, New York, pp. 23-42.

Shoukat S, Siddiqui IA, Khan GH, Zaki MJ (2002). Nematicidal and allelopathic potential of *Argemone mexicana*, a tropical weed. Plant Soil 245(2): 239-247

Shinwari ZK, Watanabe T, Rehman M, Youshikawa T (2006). A pictorial guide to Medicinal Plants of Pakistan. Kohat University of Science & Technology, Pakistan. p. 247.

Taiwo LB, Makinde JO (2005). Influence of water extract of Mexican sunflower (*Tithonia diversifolia*) on growth of cowpea (*Vigna unguiculata*). Afr. J. Biotechnol. 4(4): 355-360.

Terzi I (2008). Allelopathic effects of Juglone and decomposed walnut leaf juice on muskmelon and cucumber seed germination and seedling growth. Afr. J. Biotechnol. 7 (12): 1870-1874.

Tsuzuki E, Yamamoto Y (1987). Studies on allelopathy among higher plants. Isolation and identification of phenolic substances from wild perennial buckwheat (*Fagopyrum cymosum* M.). Bull. Fac. Agric. Miyazaki Univ. 34, 289-295.

Wu L, Guo X, Harivandi MA (1998). Allelopathic effects of phenolic acids detected in buffalograss (*Buchloe dactyloides*) clippings on growth of annual bluegrass (*Poa annua*) and buffalograss seedlings. Environ. Exp. Bot. 39: 159-167.

Wu H, PratleyJ, Lemerle D and Haig T (1999). Crop cultivars with allelopathic capability. Weed Res. 39: 171-180.

Vyvyan JR (2002). Allelochemicals as lead for new herbicides and agrochemicals. Tetrahedron 58: 1631-1646.

Zhao YM, Zhang ML, Shi QW, Kiyota H (2006). Chemical constituents of Plants from the Genus *Inula*. Chem. Biodiver. 3: 371-384.