International Journal of Horticultural Science and Technology
Vol. 3, No. 2; December 2016, pp 243-253
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ ijhst.2016.62923
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Evaluation of allelopathic activity of 68 medicinal and wild plant species of Iran by Sandwich method

Somaye Amini[1*], Majid Azizi[2], Mohammad Reza Joharchi[3] and Farid Moradinezhad[4]

1. Department of Horticultural Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

2. Department of Horticultural Science, Ferdowsi University of Mashhad, Mashhad, Iran

3. Research Center of Plant Science, Ferdowsi University of Mashhad, Mashhad, Iran

4. Department of Horticultural Science, College of Agriculture, University of Birjand, Birjand, Iran

(Received: 11 May 2016, Accepted: 13 February 2017)

Abstract
This experiment was conducted in Ferdowsi University of Mashhad, in 2011 to investigate the allelopathic potential of 68 medicinal and wild plant species belong to 19 plant families grown in Iran. Results showed that among examined plants, stigma and style of *Crocus sativus*, leaves of *Artemisia kopetdaghensis*, *Mentha piperita*, *Zhumeria majdae*, *Frulago subvelutina*, flowers bud of *Eugenia caryophyllata*, flower of *Perovskia abrotanoides*, fruits of *Melia azedarach* and *Ruta graveolen* had the strongest inhibitory effects on lettuce seedling growth. Interestingly by using of very low amount of plant samples (10 mg)growth inhibitory effects of these plants were observed by more than 70%. Additionally, the leaf of *Atriplex canescens* and the flower of *Achillea millefolium* had the strongest inhibitory effect on radicle growth (more than 75%) compare to the growth of hypocotyl (less than 20%). Here we can suggest that plants with inhibitory effects on growth and development of other plants have the potential to be applied as biological herbicides; this finding can be highlighted as new sustainable herbicides for biological control of weeds..

Keywords: allelochemicals, biological herbicide, secondary metabolites, weeds.

Introduction

Allelopathy is a process by which chemical substances are released from plants by mechanisms including root exudation, leaching dew and rain from the plant surface, volatile compounds secretion or as consequence of decaying plant litters (Rice, 1984). For many years, it was thought that the allelochemicals are useless for practical weed managements but nowadays the protective potential of these compounds against biotic stresses such as pathogens, insects, and pests has been approved (Khanh *et al.*, 2005). Therefore, plants with capacity to produce allelochemicals can positively influence agricultural systems (Macias *et al*., 2007). Numerous growth inhibitors with allelopathic properties have been identified from allelopathic plants (Xuan *et al.*, 2005). These compounds vary in their chemical compositions and concentrations (Haddadchi and Massoodi Khorasani, 2006). The current research is aim to investigate allelopathic activity of some plant materials to solve ecological and agricultural problems with an emphasis on the use of allelochemicals as an alternative candidate for management of weeds. This will help to reduce the using of

* Correspondence Author, Email: S.amini@gau.ac.ir

synthetic herbicides and avoid their environmental hazards (Khanh *et al*., 2005).

In the agricultural system, application of chemical herbicides is one of the effective approaches in plants weed control (Batish *et al*., 2006). However, excessive uses of chemical herbicides in recent decades have caused serious concerns such as environmental and health problems due to long-term and large-scale herbicide application (Gao-Feng *et al.*, 2010). Moreover, induction of herbicide-resistant mechanisms in weeds (Quader *et al*., 2001) has raised needs for introduction of new herbicides (Xuan *et al*., 2005). Therefore, recent studies have mainly focussed on finding novel natural plant products to develop bio-herbicides (Khanh *et al*., 2005). In comparison with chemical herbicides, natural plant products exhibit important advantages including biodegradable property, structural diversity and complexity and low amount of halogenated atoms (Dayan *et al.*, 1999; Duke *et al*., 2000). Besides, they can act on unexploited target sites (Duke *et al.*, 1997).

In general allelopathic plants are used to introduce selective weed management strategies (Khanh *et al.*, 2005). In order to identify plants containing natural compounds with biological activity, allelopathic plants selection strategy is a general and an acceptable approach (Duke *et al.*, 2000). However, researches belong to allelopathic studies meet some difficulties. As an example, insufficient description of methodology may cause a serious concern due to the use of unnatural growth medium in laboratory condition which makes it rather difficult to explore the actual effects of allelopathy in artificial condition (Inderjit and Nilsen, 2003). This can be due the fact that in natural conditions plants generally are capable of adapting to the toxic compounds (Harper, 1977), and raise the question whether this capacity is lost in laboratory condition? (Weibhuhn and Parti, 2009). In addition, allelopathy bioassays are influenced by soil property as a complex biological system which causes different complexities during experiments (Inderjit, 2006).

To overcome these challenging problems, accurate laboratory experiments have been designed in the past few years. These experimental designs are used to distinguish the effect of individual elements such as light, water, and nutrients (Tang and Young, 1981). Sandwich method, is a new approach to specify bioassay test for assessing allelopathy by using of leaf litter plants in a nutrient free agar medium (Fujii *et al*., 2003); therefore this method exclude the mentioned difficulties. Furthermore, it is relatively fast and allows researchers to conduct numerous experiments in a short timeframe. In our experiment, to select the strongest allelopathic species, dried leaves of 239 plant species were assayed by the Sandwich method, using *Lactuca sativa* (lettuce) as the test plant (Fujii *et al*., 2003). Furthermore, allelopathic properties of 37 plant species were determined with this method (Ibrar Shinwari *et al*., 2013). Recently, 251 plant species collected from the Sino-Japanese Floristic Region were screened for allelopathic activity by sandwich method (Appiah *et al*., 2015)., Although many studies have been done by using this method, less information is available regarding the allelopathic activity of plant species from Iran, particularly this method is not regularly applied in former studies. Therefore such information is of considerable importance for any attempts to evaluate the allelopathic capacity of native plants ifrom Iran by using of newly advanced approaches like sandwich method.

Material and Methods

Plants material, preparation, and procedures

This study was conducted in Department of Horticultural Science, Ferdowsi University of Mashhad, Iran and Tokyo University of Agriculture and Technology. In our research, for evaluating allelopathic activity, the majority of test-plants were selected from medicinal and weed plants, due to the high

content of secondary metabolites in the medicinal plants and high aggressive power of the weeds which indicate proper identity to choose candidate plants for allopathic assessment (Table 1). Most of the plant materials collected from different regions of Khorasan Province, Northeast part of Iran. After authenticating samples in the Ferdowsi University of Mashhad Herbarium (FUMH) voucher samples were deposited in the herbarium for further studies. The samples were oven dried at 60 ˚C for less than 24 h and were approximately left for two days in a general drying chamber. Dried samples were placed in plastic bags and kept in an air-tight box until use.

Evaluation of allelopathic activity

To assess the allelopathic activity of the selected plants, multi-dishes plates with 3.5 cm diameter including 6 holes (wells) (Nunc Company) were used (Fujii *et al.*, 2003). Ten mg of dried samples were placed in all three wells in the upper row and 50 mg was placed in rest of the three lower wells.

For the preparation of the growth medium, commercially available agar was used (gelling temperature 30-31 °C, Nacalai Tesque, Kyoto, Japan). The medium was prepared as 0.5% (w/v) and autoclaved at 115 °C for 20 min. The autoclaved agar was cooled down to 45 °C in a water bath. Thereafter, five ml autoclaved agar was added to each well of the multi-dish plastic plate include plant samples (Fig. 1a). After gelatinizing the agar within 30-60 min at room temperature (25 °C), another five ml agar was added to all wells as the second layer and left at room temperature. This made a sandwich of dried leaves by two layers of agar (Fig. 1b). For the bioassay experiment, lettuce seeds (*Lactuca sativa* L. var. Great Lakes 366) were used as a test plant because lettuce is reliable plant to investigate the inhibitory and stimulatory allelochemicals at low concentrations (Fujii *et al.*, 1990). Five seeds of lettuce were planted on the agar surface of the wells and all treatments replicated four times. Each side of the prepared multi-dishes was then sealed with parafilm and wrapped by aluminum foil to prevent light penetration and was placed in incubator (25 °C) (Fig. 1c). After three days, germination rate and seedling growth (radicle and hypocotyl length) of the lettuce seeds were recorded and were compared with control samples (Fig. 1d). Agar medium without plant samples was used as control (Fig. 1e).

Table 1. Screening of 68 medicinal and wild plant species using the sandwich method

No.	Family	Scientific name	Part used	Plant samples amount in each well				Criteria
				10 mg		50 mg		
				Radicle	Hypocotyl	Radicle	Hypocotyl	
1	Berberidaceae	*Berberis vulgaris* L.	Leaf	32.3	2.6	79.3	67.3	
			Fruit	67.6	38.0	97.3	93.0	
2	Boraginaceae	*Borago officinalis* L.	Leaf	49.6	23.6	81.3	66.6	
		Caccinia macranthera (Barks & Soland.) Brand var. Crassifolia (Vent.) Brand	Leaf	46.6	-7.3	84.6	63.0	
		Echium italicum L.	Leaf	40.0	-19.0	73.6	19.3	
3	Chenopodiaceae	*Atriplex canescens* James	Leaf	81.3	21.0	95.0	89.0	R*
		Chenopodium botrys L.	Leaf	64.0	24.0	94.3	82.3	
		Halimocnemis mollissima Bge.	Leaf	58.0	25.0	95.3	91.0	
		Kraschenninnikovia ceratoides (L.) Gueldenst.	Leaf	51.6	-12.3	85.3	63.0	
		Kraschenninnikovia ceratoides (L.) Gueldenst.	Seed	54.6	9.3	74.0	39.3	
4	Cistaceae	*Helianthemum ledifolium* (L.) Miller	Leaf	33.6	-21.6	61.0	-6.0	

Table 1. Screening of 68 medicinal and wild plant species using the sandwich method

5	Compositae (Asteraceae)	*Achillea biebersteinii* Afan.	Leaf	56.3	2.0	87.3	71.3	
			Flower	71.6	30.6	93.3	80.6	
		Achillea millefolium L. subsp. Elbursensis Hub.Mor.	Flower	70.6	9.0	86.0	65.3	R*
		Achillea nobilis L. subsp. Neilreichii (Kerner) Formanek	Leaf	71.0	-18.3	90.3	70.3	R* H+
			Flower	48.3	-9.0	88.0	75.0	
		Achillea pachycephala Rech. F	Leaf	72.0	48.3	95.6	86.0	
		Artemisia kopetdaghensis Krasch., M. Pop. & Lincz. Ex poljak	Leaf	90.0	84.0	99.3	98.3	*
		Echinacea purpurea (L) Moench	Leaf	52.3	-22.3	68.6	20.0	
		Heteropappus altaicus (Willd.) Novopoker	Leaf	64.0	52.3	88.3	86.3	
		Lactuca serriola L.	Leaf	53.3	33.0	84.6	59.6	
		Pulicaria gnaphalodes (Vent.) Boiss.	Seed	61.0	25.3	77.6	63.3	
6	Cupressaceae	*Juniperus excelsa* M.B.	Leaf	-27.3	-16.0	9.3	7.3	R^+H^+
			Fruit	13.6	-10.0	57.0	25.0	
7	Euphorbiaceae	*Euphorbia aellenii* Rech. F.	Leaf	61.3	47.0	77.0	59.0	
8	Iridaceae	*Crocus sativus* L.	Style	79.3	68.6	96.3	90.0	*
			Leaf	67.6	51.3	77.0	63.0	
			Stigma	79.0	68.6	97.3	92.3	*
9	Labiatae (Lamiaceae)	*Hyssopus angustifolius* M.B.	Leaf	61.6	43.3	86.3	78.3	
			Flower	31.0	-2.3	58.6	28.6	
		Lavandula vera D.C.	Flower	76.0	48.6	99.6	99.3	
		Syn: *L. angustifolia* Miller	Leaf	38.6	14.0	98.0	94.6	
		Melissa officinalis L.	Leaf	50.0	21.0	86.0	75.6	
		Mentha piperita L.	Leaf	90.6	86.6	99.3	99.3	*
		Origanum vulgar L.	Leaf	41.0	27.6	75.3	64.0	
		Perovskia abrotanoides Karel.	Flower	72.3	66.3	97.3	93.0	*
		Phlomis cancellata Bunge.	Leaf	69.6	37.3	89.0	67.6	
		Rosmarinus officinalis L.	Leaf	22.6	3.6	61.0	54.6	
		Salvia nemorosa L.	Leaf	57.6	32.0	92.0	83.3	
		Satureja hortensis L.	Leaf	0.0	13.6	76.0	59.6	
		Stachys lavandulifolia Vahl.	Leaf	50.0	14.0	85.6	70.6	
		Stachys turcomanica Trautv.	Leaf	67.6	49.6	89.3	77.0	
		Teucrium polium L.	Leaf	50.6	25.6	86.6	71.6	
		Thymus vulgaris L.	Leaf	29.6	13.0	93.0	90.0	
		Zataria multiflora Boiss.	Leaf	-2.3	27.6	62.6	60.0	
		Zhumeria majdae Rech.	Leaf	86.3	73.0	100	100.0	*
		Ziziphora clinopodioides Lam.	Leaf	78.0	50.0	94.0	78.6	
10	Meliaceae	*Azadirachta indica* Adr. Ju	Fruit	39.3	14.0	68.3	41.6	
11	Myrtaceae	*Eugenia caryophyllata* Thunb.	Flower	73.6	60.6	96.3	91.3	*
12	Oleaceae	*Melia azedarach* L.	Fruit	76.3	72.6	95.3	91.3	*
		Melia azedarach L.	Flower	64.6	56.0	66.6	69.6	
13	Ranunculaceae	*Ranunculus cicutarius* Schlectend.	Leaf	61.3	24.6	80.6	57.3	
	Rosaceae	*Cotoneaster nummularia* Fisch. & C.A. Mey	Leaf	72.3	42.3	88.3	65.3	
14	Rutaceae	*Ruta graveolens* L.	Fruit	60.3	67.3	84.0	82.0	*
			Leaf	55.3	49.3	76.6	73.0	
15	Scrophulariaceae	*Verbascum speciosum*Schrad.	Leaf	41.0	3.0	83.3	56.6	
16	Solanaceae	*Hyoscyamus turcomanicus* Pojark.	Leaf	81.3	46.6	96.0	89.6	
		Lycium depressum Stocks	Leaf	55.6	38.6	93.0	85.6	
		Withania coagulans (Stocks) Dun.	Fruit	29.3	11.3	60.3	42.0	
		Withania somnifera (L.) Dun.	Leaf	31.0	8.0	81.0	45.6	
17	Umbellifera (Apiaceae)	*Bunium persicum* (Boiss.) B.	Straw	40.0	9.6	84.6	63.0	
			Flower	58.6	26.0	91.3	67.6	
		Dorema ammoniacum D. Don.	Leaf-2	65.0	37.6	94.3	79.6	
			leaf-1	44.6	21.0	80.6	49.6	
			Stem	43.6	4.0	43.6	25.6	
		Frulago subvelutina Rech.F	leaf	91.6	76.3	96.0	90.0	*
18	Verbinaceae	*Vitex pseudo-negundo* (Hausskn.)	leaf	37.0	5.6	54.0	38.0	
19	Zygophyllaceae	*Zygophyllum fabago* L.	leaf	39.3	21.6	69.0	53.0	

*(seventh cluster): highest growth inhibitory on radicle and hypocotyl.
R* (second cluster): inhibitory effects on radicle growth
R* H^+ (fourth cluster): inhibitory effects on radicle growth and stimulatory on hypocotyl.
R^+ H^+ (ninth cluster): stimulatory effects on radicle and hypocotyl growth.

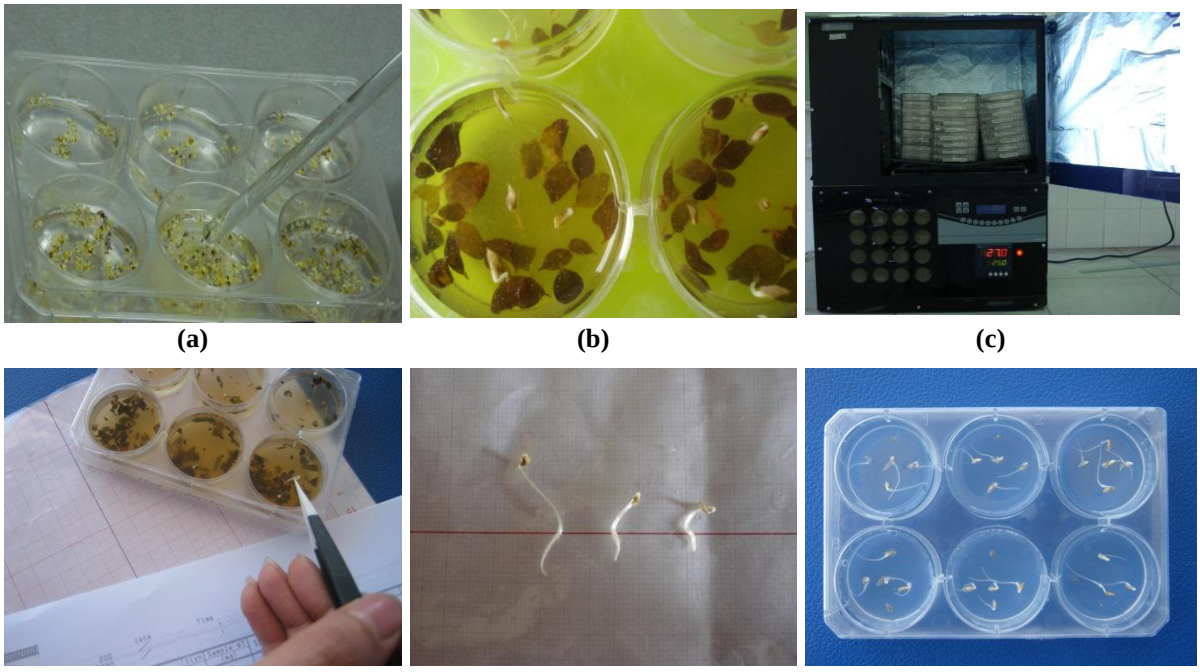


Fig. 1. Steps of the sandwich method; (a) five ml autoclaved agar was added to each well of the multi-dish plastic plate include plant samples; (b) five ml agar was added to all wells as the second layer and left at room temperature. Five seeds of lettuce were planted on the agar surface of the wells; (c) Each side of the prepared multi-dishes were sealed with parafilm and wrapped by aluminium foil to inhibit light penetration and placed in incubator (25 °C); (d) After 3 days, germination rate and seedling growth (radicle and hypocotyl length) of the lettuce seeds recorded and compared with control samples; and (e) Agar medium without plant samples was used as control

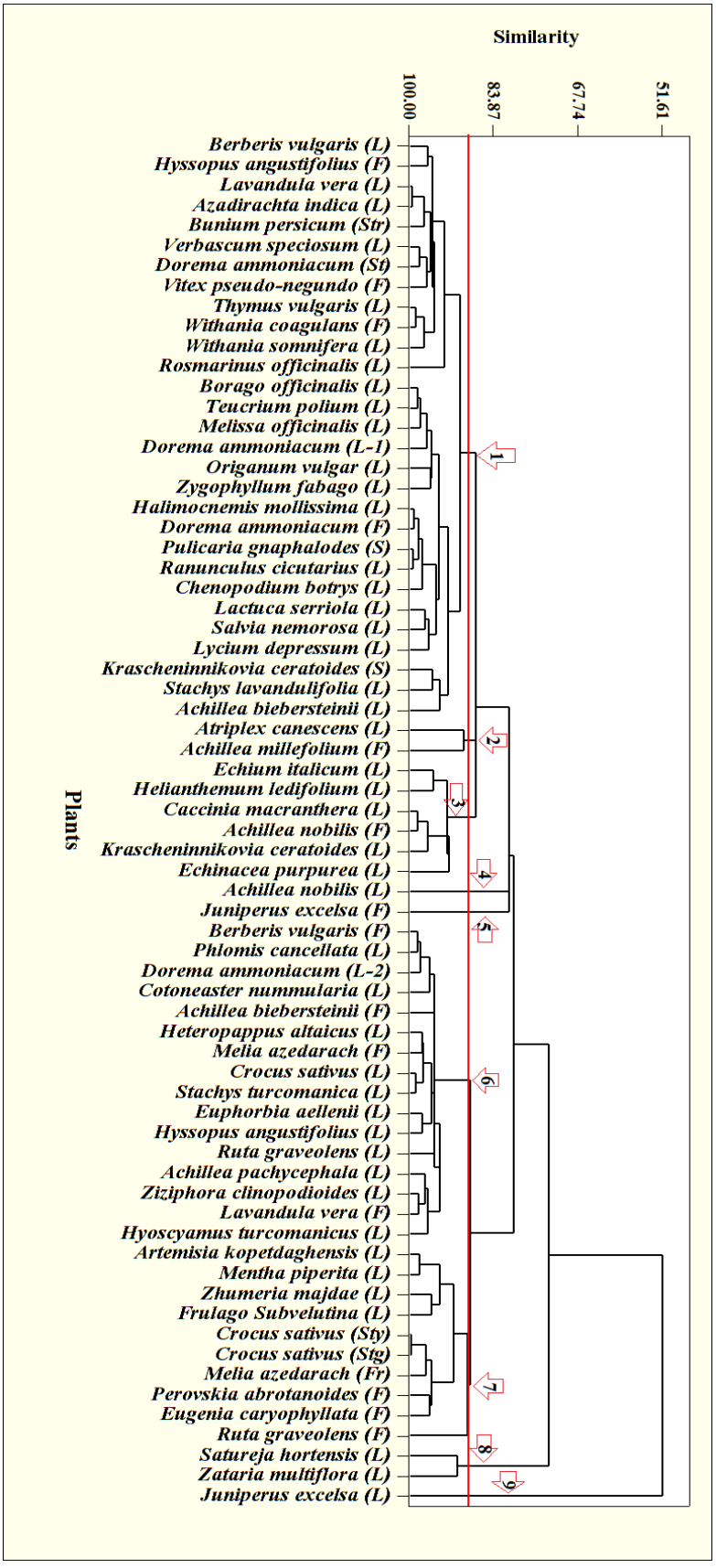
Statistical analysis

For initial statistical analysis, growth inhibition percentage was calculated. In this experiment, 68 plants including radicle and hypocotyl growth percentage in two concentrations of 10 and 50 mg plant samples were compared. Visual clustering was not conducted due to the large numbers of variables. For more convenient comparisons between plants Minitab software (version 16) was used. Clustering was performed by centroid method. Similarity level between plants, in each cluster, was 90%.

Results

Table 1 shows the growth inhibitory percentage of the radicle and hypocotyl of lettuce seedlings based on the dried samples of 68 plant specimens from 19 different families. Growth percentages of lettuce seedlings are presented according to either induction or inhibition effects. Negative and positive values represented induction and inhibition when compared to the corresponding controls, respectively. The initial results provided in Table 1 showed that among 68 plant samples, 57 plants had inhibitory responses while 11 plants induced seedling growth in lettuce.

A graphical analysis of clusters and associated plants was done with centroid method and presented in Figure 2. According to the results of clustering analysis, after adjustment, the data were strikingly clustered and all plants with their associated variables were categorized in nine clusters.



Wherever the horizontal line has cut the vertical lines that point is a cluster and the joined plants to each point are in the same cluster

Fig. 2. Graphical depiction of clusters and their associated plants by using of centroid method

Table 2. Clustering summary: similarity level between plants in each cluster is 90%

Cluster	Mean of inhibitory				Number of plants
	10 mg		50 mg		
	Radicle	Hypocotyl	Radicle	Hypocotyl	
7	79.97	72.43	96.13	92.77	10
2	76.00	15.00	90.50	77.17	2
4	71.00	-18.67	90.33	70.33	1
6	68.5	45.54	88.06	77.90	16
1	45.57	16.60	79.32	62.08	29
3	45.44	-15.28	76.89	39.06	6
5	13.67	-10.00	57.00	25.00	1
8	-1.17	20.67	69.33	59.83	2
9	-27.33	-16.00	9.33	7.33	1

Clustering summary has been indicated in Table 2. Properties of clusters including growth inhibitory percentage of radicle and hypocotyl in two different amounts of plant samples and also the number of plants in each cluster have been explained in this Table. Results showed that based on allelopathic effects four clusters are more important including inhibitory and stimulatory effects among nine clusters.

These four clusters are described as the following:

- The seventh cluster includes ten plant samples (from nine species) namely viz., stigma and style of *Crocus sativus,* leaves of *Artemisia kopetdaghensis*, *Mentha piperita*, *Zhumeria majdae*, *Frulago subvelutina*, flower buds of *Eugenia caryophyllata*, the flower of *Perovskia abrotanoides*, fruit of *Melia azedarach*, *Ruta graveolens.* Plants in this cluster had the highest growth inhibitory effects among all clusters. The growth inhibitory of these plants was more than 70% in 10 mg plant sample.
- The second cluster contains two plant samples including leaves of *Atriplex canescens* and flowers of *Achillea millefolium*. These plants had the strong inhibition growth effects on radicle growth. However, inhibitory effects on hypocotyl growth in this cluster were less than 20%. Similar to the seventh cluster, plants in this cluster can be considered for identification of allelopathic active compound or further compounds identification
- The fourth cluster includes leaves of *Achillea nobilis*. Allelopathic activity of this plant influenced the growth rate In both positive and negative manner, So that a significant allelopathic stimulation of hypocotyl and strong inhibition of radical growth was observed. Even though growth induction can be related to the allelopathic compounds, but their inhibitory effects are more noteworthy.
- The ninth cluster includes the leaf of *Juniperus excelsa* (Fig. 2). Stimulatory growth effects on radicle and hypocotyl was observer for this plant.

Clustering was done to facilitate the comparison among plants. This experiment was conducted according to 50 mg dried plant samples however, final selection and classification was performed based on 10 mg dried material. This was done due to the fact that most of the plants in 50 mg dried plant sample showed considerable inhibitory effects on lettuce seedlings. One of the possibilities for these strong inhibitory effects could be the high amount of elements or compounds other than allelochemicals which eventually results in seedlings death.

Discussion

The results presented in this paper indicated that among 68 specimens 12

plant samples had allelopathic effects as inhibitory growth on lettuce germination (second, fourth and seventh clusters). So far, there is little information on the allelopathic effects of most tested plants which have been used in this study. However, few reports have shown allelopathic properties of *Achillea millefolium*, *Crocus sativus*, *Melia azedarach* and *Ruta graveolens*.

Achillea millefolium is a medicinal plant that its therapeutic applications have been frequently reported in Europe and in Asia (Vitalini *et al*., 2011). Scientists have been indicated that high antioxidant capacity (Giorgi *et al*., 2009) and antispasmodic effects in *Achillea* genus (Saeidnia *et al*., 2011) are due to the high amount of phenolic and flavonoids contents. Phenolic and flavonoid compounds are classified in allelochemical groups (Lattanzio *et al*., 2006). Quercetin is a typical phenolic allelochemical (Lattanzio *et al*., 2006) that commonly found in *Achillea* genus (Saeidnia *et al*., 2011). Rutin is one of the main flavonoid compounds that have also been identified in flowers of *Achillea millefolium* (Benetis *et al*., 2008). Allelopathic properties of rutin in *Citrus unshiu* have been reported by Nishida (2005). Interestingly, fluorescent allelochemical such as austricine and azulenes have been identified in *Achillea millefolium*. These fluorescent compounds may be applied to the study of allelopathic functional mechanisms (Roshchina *et al*., 2012). Some flavonoids such as apigenin and luteolin are the major bioactive constituents of the *A. millefolium* bioactive compounds (Vitalini *et al*., 2011). Allelopathic effects of apigenin (Basile *et al*., 2000) and luteolin (Beninger and Christopher Hall, 2005) have been confirmed in former studies. These compounds possibly are effective in triggering of allelopathic responses in *A. millefolium*.

In our study, saffron showed also strong allelopathic effects (*Crocus sativus*) in stigma and style organs. Iran is considered as one of the main countries in saffron production. This autumn-flowering perennial plant grows mainly in the East and Southeast of Iran (Sariri *et al*., 2011). For a long time, Saffron is used for its taste, odor, and colour (Hori *et al*., 1988). The therapeutic effects of Saffron have been used in traditional and modern medicine (Hadizadeh *et al*., 2003; Hori *et al*., 1988; Magdalini *et al*., 2006). Antioxidant properties in stigma and remaining flowers of saffron are related to flavonoids and phenolic components (Magdalini *et al*., 2006; Sariri *et al*., 2011). Allelopathic effects of saffron stigma in this research could be related to the presence of phenolic compounds. These compounds have been previously introduced as allelochemical materials (Almeida Barbosa *et al*., 2007; Scognamiglio *et al*., 2012). Allelopathic effects have been also reported in the extract of saffron leaves and corm (Rashed *et al*., 2008). These allelopathic effects have been identified by dish pack method (Amini *et al*., 2014), and introduced with the name of safranal as a bioactive compound (Mardani *et al*., 2015) *Melia azedarach* L. is another plant species that in this experiment suppressed seed germination and inhibited seedling growth in lettuce. Consistent with our finding, phytotoxic effects of *M. azedarach* L. on germination and growth of *Lactuca sativa* L. was reported by Lungu *et al*. (2011). Analysing *M. Azedarach* allelochemicals on *Raphanus sativus* germination and growth showed that *Melia* allelochemicals caused an imbalance in oxidative status of cells and influenced peroxidation of membrane lipid and electrolytes leakage in radish seedlings (Akacha *et al*., 2013). Chemical analysis of the *Melia azedarach* fruit resulted in determination of 14 compounds with insecticidal effects. Although, three compounds including catechin and two kaempherols have been reported in previous studies (Italo Chiffelle

et al., 2009), the majority of these compounds are unknown. In addition, further studies showed that the secretion of catechin from roots of noxious weed is negatively influenced the plant-plant interactions (Bais and Kaushik, 2010). Catechin is also exists in *Melia azedarach* fruit and plays an important role in its allelopathic effect.

In the current experiment, *Ruta graveoloans* (Rutaceae) showed allelopathic effects on growth. This well-known medical plant is used in ancient civilizations for treating many diseases (Ahmadi Jalali Moghadam *et al*., 2012). The possible allelopathic activity of *R. graveolens* essential oil and some of its isolated constituents have been previously reported (De Feo *et al*., 2002).

Rutin isolated from leaves, stems, and fruits of *R. graveolens* (Mancuso *et al*., 2015) is introduced as an allelochemical (Cheng and Cheng, 2015).

The results obtained from current study revealed that the sandwich method is a very useful tool for screening different plants based on their allelopathic activities. In current research, an introductory screening was done on 68 plants to recognise their allelopathic activities. However, further studies are also needed on these plants to identify their effects on the weed plants.

Acknowledgment

The research was supported by JSPS (Japanese Society for the Promotion of Science), Ferdowsi University of Mashhad and Tokyo University of Agriculture and Technology.

References

1. Ahmadi Jalali Moghadam, M., H. Honarmand, S. Falah-Delavar, and A. Saeidinia. 2012. Study on Antibacterial Effect of *Ruta graveolens* Extracts on Pathogenic Bacteria. Ann. Biol. Res. 3(9): 4542-4545.
2. Akacha. M., N.G. Boughanmi, and R. Haouala. 2013. Effects of M*elia azedarach* Leaves Extracts on Radish Growth and Oxidative Status. IJBR. 3(2): 29-42.
3. Almeida Barbosa, L.C., A.J. Demuner, A. Dumont Clemente, V.F. De Paula, and M.D.I. Faiz. 2007. Seasonal Variation in the Composition of Volatile Oils From S*chinus terebinthifolius* Raddi. Quim. Nova. 30(8): 1959-1965.
4. Amini, S., M. Azizi, M.R. Joharchi, M.N. Shafei, F. Moradinezhad, and Y. Fujii. 2014. Determination of Allelopathic Potential in Some Medicinal and Wild Plant Species of Iran by Dish Pack Method. Theor. Exp. Plant Physiol. 26: 189-199.
5. Appiah, K.S., Z. Li, R.S. Zeng, Sh. Luo, Y. Oikawa, Y. Fujii. 2015. Determination of Allelopathic Potentials in Plant Species in Sino-Japanese Floristic Region by Sandwich Method and Dish Pack Method. IJBAS. 4: 381-394.
6. Bais, H.P., and Sh. Kaushik. 2010. Catechin Secretion and Phytotoxicity Fact, not Fiction. Commun. Integr. Biol. 3(5): 468-470.
7. Basile, A., S. Sorbo, S. Giordano, L. Ricciardi, S. Ferrara, D. Montesano, R. Castaldo Cobianchi, M.L. Vuotto, and L. Ferrara. 2000. Antibacterial and Allelopathic Activity of Extract From C*astanea Sativa* leaves. Fitoterapia. 71: 110-116.
8. Batish, D.R., H.P. Singh, R.K. Kohli, and G.P. Dawra. 2006. Potential of Allelopathy and Allelochemicals For Weed Management: 209-237. In Singh, H.P., Batish, D.R. and Kohli, R.K. Handbook of Sustainable Weed Management. Food Products Press, New York, London, Oxford.
9. Benetis, R., J. Radusiene, and V. Janulis. 2008. Variability of Phenolic Compounds in Flowers of *Achillea Millefolium* wild Populations in Lithuania. Medicina. 44: 775-781.
10. Beninger, C.W., and J. Christopher Hall. 2005. Allelopathic Activity of Luteolin 7-O-β-Glucuronide Isolated From C*hrysanthemum Morifolium* L. Biochem. Syst. Ecol. 33(2): 103-111.
11. Cheng, F., and Z. Cheng. 2015. Research Progress on the Use of Plant Allelopathy in Agriculture and the Physiological and Ecological Mechanisms of Allelopathy. Front. Plant Sci. 6: 1-16.
12. Dayan, F.E., A. Hernández, S.N. Allen, R.M. Moraes, J.A. Vroman, M.A. Avery, and S.O.

Duke. 1999. Comparative Phytotoxicity of Artemisinin and Several Sesquiterpene Analogues. Phytochemistry, 50: 607-614.

13. Duke, S.O., F.E. Dayan, J.G. Romagni, and A.M. Rimando. 2000. Natural Products as Sources of Herbicides: Current Status and Future trends. Weed Res. 40: 99-111.

14. Duke, S.O., F.E. Dayan, A. Hernandez, M.V. Duke, and H.K. Abbas. 1997. Natural Products as Leads For New Herbicides. In: Proceedings of Brighton Crop Protection Conference Weeds. Brighton, UK: 579-586.

15. Fujii Y., S.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.

16. Fujii, Y., T. Shibuya, and T. Yasuda. 1990. Survey of Japanese Weed and Crops, For the Detection of Water Extractable Allelopathic Chemicals Using Richards' Function Fitted to Lettuce Germination Test. Weed Res. 35: 362-370.

17. Gao-Feng, X., Z. Fu-dou, L. Tian-lin, and Z. Yu-hua. 2010. Induced Effects of Exogenous Phenolic acids on Allelopathy of a Wild rice Accession (*Oryza longistaminata*, S37). Rice Sci. 17: 135-140.

18. Giorgia, A., M. Mingozzia, M. Madeoa, G. Speranzab, and M. Cocuccia. 2009. Effect of Nitrogen Starvation on the Phenolic Metabolism and Antioxidant Properties of Yarrow (*Achillea Collina* Becker Ex Rchb.). Food Chem. 114(1): 204-211.

19. Haddadchi, Gh., and F. Massoodi Khorasani. 2006. Allelopathic Effects of Aqueous Extracts of S*inapis arvensis* on Growth and Related Physiological and Biochemical Responses of *Brassica napus*. J. Agr. Sci. Tech. 32: 23-28.

20. Hadizadeh, F., N. Khalili, H. Hosseinzadeh, and R. Khair-Aldine. 2003. Kaempferol from Saffron Petals. Iranian J. Plant Res. 2: 251-252.

21. Harper, J.L. 1977. The Population Biology of Plants. London. Academic Press.

22. Hori, H., K. Enomoto, and M. Nakaya. 1988. Induction of Callus from Pistils of C*rocus Sativus* L. and Production of Color Compounds in Callus. Plant Tissue Culture Letters. 5(2): 72-77.

23. Ibrar Shinwari M., M. Ibrar Shinwari, and Y. Fujii. 2013. Allelopathic Evaluation of Shared Invasive Plants and Weeds of Pakistan and Japan For Environmental Risk Assessment. Pak. J. Bot. 45: 467-474.

24. Inderjit. 2006. Experimental Complexities in Evaluating the Allelopathic Activities in Laboratory Bioassays: A Case Study. Soil Biol. Biochem. 38: 256-262.

25. Inderjit, and E.T. Nilsen. 2003. Bioassays and Field Studies for Allelopathy in Terrestrial Plants: Progress and Problems. Crit. Rev. Plant Sci. 22: 221-238.

26. Italo Chiffelle, G., F. Amanda Huerta, and R. Diego Lizana. 2009. Physical and Chemical Characterization of M*elia azedarach F*ruit and Leaf for Use as Botanical Insecticide. Chilean JAR. 69(1): 38-45.

27. Khanh T.D., N.H. Hong, T.D. Xuan, and I.M. Chung. 2005. Paddy Weed Control by Medicinal and Leguminous Plants From Southeast Asia. Crop Prot. 24: 421-431.

28. Lattanzio, V., V.M.T. Lattanzio, and A. Cardinali. 2006. Role of Phenolics in the Resistance Mechanisms of Plants Against Fungal Pathogens and Insects. Phytochem. Adv. Res. 2: 23-67.

29. Lungu, L., C.V. Popa, J. Morris, and M. Savoiu. 2011. Evaluation of Phytotoxic Activity of M*elia azedarach* L. Extracts on *Lactuca Sativa* L. Rom. Biotech. Lett. 16(2): 6089-6095.

30. Macias, F.A., J.M.G. Molinillo, R.M. Varela, and J.C.G. Galindo. 2007. Review Allelopathy-a Natural Alternative For Weed Control. Pest Manag. Sci. 63: 327-348.

31. Magdalini, A.P., D.K. Charalambos, G.P. Moschos, E. Spyros, C. Pau, M. Marigoula, and N.L. Fotini. 2006. Inhibitory Activity on Amyloid-â aggregation and Antioxidant Properties of C*rocus Sativus* Stigmas Extract and its Crocin Constituents. J. Agric. Food Chem. 54: 8762-8768.

32. Mancuso, G., G. Borgonovo, L. Scaglioni, and A. Bassoli. 2015. Phytochemicals from *Ruta graveolens* Activate TAS2R Bitter Taste Receptors and TRP Channels Involved in Gustation and Nociception. Molecules. 20: 18907-18922.

33. Mardania, H., T. Sekine, M. Azizi, M. Mishyna, and Y. Fujii. 2015. Identification of Safranal as the Main Allelochemical from Saffron (*Crocus Sativus*). Nat. Prod. Commun. 10: 1-3.

34. Nishida, R. 2005. Chemosensory basis of Host Recognition in Butterflies— multi-component System of Oviposition Stimulants and Deterrents. Chem. Senses, 30: 293-294.

35. Quader, M., G. Daggard, R. Barrow, S. Walker, and M.W. Sutherland. 2001. Allelopathy, DIMOBA Production and Genetic Variability in Accessions of *Triticum Speltoides*. J. Chem. Ecol. 27(4): 747-760.

36. Rashed, M.H., J. Gherekhloo, and M. Rastgoo. 2008. Allelopathic Effects of Saffron (*Crocus Sativus*) Leaves and Corms on Seedling Growth of Redroot Pigweed (*Amaranthus retroflexus*) and Lambsquarter (*Chenopodium album*). J. Iranian Field Crop Res. 7(1): 53-61. [In Persian].

37. Rice, E.L. 1984. Allelopathy. Academic Press. New York, 353 pp.

38. Roshchina, V.V., V.A. Yashin, A.V. Yashina, and M.V. Gol'tyaev. 2012. Microscopy for Modelling of Cell-cell allelopathic Interactions. Cheema, Z. A., Farooq, M., and Wahid, A. (Eds.). In: Allelopathy: Current trends and Future applications. Springer Science and Business Media. 407-427.

39. Saeidnia, S., A.R. Gohari, N. Mokhber-Dezfuli, F. Kiuchi. 2011. A Review on Phytochemistry and Medicinal Properties of the Genus *Achillea*. DARU J. Pharmaceut. Sci. 19: 173-186.

40. Sariri, R., R. Sabbaghzadeh, and F. Poumohamad. 2011. In-Vitro Antioxidant and Anti-tyrosinase Activity of Methanol Extracts From C*rocus Sativus* Flowers. Pharmacologyonline. 3: 1-11.

41. Scognamiglio, M., A. Esposito, B. D'Abrosca, S. Pacifico, V. Fiumano, N. Tsafantakis, P. Monaco, and A. Fiorentino. 2012. Isolation, Distribution and Allelopathic Effect of Caffeic acid Derivatives From *Bellis Perennis* L. Biochem. Sys. Ecol. 43: 108-113.

42. Tang, Ch.Sh., and Ch.Ch. Young. 1981. Collection and Identification of Allelopathic Compounds from the Undisturbed Root System of Bigalta Limpograss (*Hemarthria altissima*). Plant Physiol. 69: 155-160.

43. De Feo, V., F. De Simone, and F. Senatore. 2002. Potential Allelochemicals from the Essential Oil of *Ruta graveolens*. Phytochem. 61(5): 573-578.

44. Vitalini, S., G. Beretta, M. Iriti, S. Orsenigo, N. Basilico, S. Dall'Acqua, M. Iorizzi, and G. Fico. 2011. Phenolic Compounds From *Achillea millefolium* L. and their bioactivity. Acta Biochem. Polonica. 58(2): 203-209.

45. Weibhuhn, K., and D. Parti. 2009. Activated Carbon May have undesired Side Effects for Testing Allelopathy in Invasive Plants. Basic Appl. Ecol. 10: 500-507.

46. Xuan, T.D., T. Shinkichi, T.D. Khanh, and C.I. Min. 2005. Biological Control of Weeds and Plant Pathogens in Paddy Rice by Exploiting Plant Allelopathy: An Overview. Crop Prot. 24(3): 197-206.