



Original Article

Allelopathic Effects of Medicinal Plants of Lemon Balm, Lemon Verbena and Bitter Apple on Seed Germination and Early Seedling Growth Characteristics of Wild Mustard Weed

Reza Geimadil¹, Behzad Shokati^{2*} and Hassan Shahgholi³

¹Young Researchers and Elite Club, Aligoodarz Branch, Islamic Azad University, Aligoodarz, Iran

²Young Researchers and Elite Club, Maragheh Branch, Islamic Azad University, Maragheh, Iran

³Young Researchers and Elite Club, Aligoodarz Branch, Islamic Azad University, Aligoodarz, Iran

Article History: Received: 24 May 2015/Accepted in revised form: 12 October 2015

© 2013 Iranian Society of Medicinal Plants. All rights reserved.

Abstract

Allelopathy refers to the chemical inhibition or stimulation of one species by another which could influence on the growth or germination of other plant and used in control of weed in agricultural practices. In this sense in order to study the possibly allelopathic effects of lemon balm, bitter apple and lemon verbena medicinal plants on control of wild mustard weed, a laboratory experiment was arranged based on Completely Randomized Design (CRD) in three replications at laboratory of Mehrگان institute during 2014. In this study, effects of different aqueous extracts and essential oils of mentioned medicinal plants compared with control treatments. Evaluated values were germination rate, germination percentage, radicle length, shoot length, fresh and dry weight of radicle and shoot. Results showed that essential oil of Lemon verbena at 600 mg/L concentration had more meaningful effect on germination percentage and germination rate of wild mustard. Our results also indicated that aqueous extract of these plants had stimulating effect on weed seed development, while some essential oils treatments had an inhibitory effect on this weed seeds development.

Key words: *Aloysia citriodora*, *Citrullus colocynthis*, *Melissa officinalis*, *Sinapis arvensis*

Introduction

Weed is unwanted plant which enters to the farm unlike farmers and competes with main crop in field, garden or forest and decrease quantity and quality of crops yield. Recently regards to extra usage of herbicides, insecticides and other harmful chemical components in agricultural systems and their subsequently secondary impacts on agro-ecosystem health and contradiction of this issue with sustainable agriculture, however, less attention has been paid by farmers [1].

Allelopathy term is direct or indirect positive or negative effect of one species by another. Subsequently allelochemicals are subset of secondary metabolites which released by

allelopathic process to the natural growth environment of plant. Recently allelopathy is considered as a strategy for reducing environmental contaminations and issue for increasing agricultural products in sustainable agriculture and global importance of this issue is well addressed among the agricultural and environmental experts [2]. Allelochemicals could be classified as secondary metabolites in plants and generally do not play any role in primary metabolic processes essential for plant's survival [3]. Negative effects of secondary metabolites could be addressed to toxic gases which have no internal effect on the plant contrarily, some times in the case of pigment, it has a useful effect

* Corresponding author: Young Researchers and Elite Club, Maragheh Branch, Islamic Azad University, Maragheh, Iran. Post box: 5519747587

Email Address: Behzad.shokati66@gmail.com

on attracting pollinators [4]. Some allelochemicals are intermediates for lignification and can also activate plant defense after exposure to pathogens. Therefore, they have a structural or physiological role within plants [3]. Allelochemical affect different physiological and biochemical process such as water and nutrient absorption [4], photosynthesis [5], growth and germination of plant and activates of some enzymes and membrane [6]. In this sense, biometric is a method used to evaluate the allelopathic process by preparation of aqueous extract of leaves, roots or other parts of the plant to find the effect of this biochemical on seed germination, seedling growth and other physiological characteristics [7]. In this way, research by Batish *et al.* [8], showed that eucalyptus essential oil reduced the germination and growth of wheat, corn, foxtail and slept tumbleweed while the highest allelopathic effect had been observed on slept tumbleweed and the lowest of it was related to foxtail weed. It is reported that the aqueous extract of waste corn and sorghum showed an inhibitory effect on germination and growth of wheat [9]. Wild mustard (*Sinapisarvensis* L.) is an annual weed belongs to Brassicaceae and is common weed in 52 countries and 30 croplands such as sugar beet (*Beta vulgaris* L.), maize (*Zea mays* L.) and rapeseed (*Brassica napus* L.) [10]. Lemon balm (*Melissa officinalis* L.) is a perennial grass which is abundant in Iran [11, 12] and citronellal, polyphenols, tannins, flavonoid and rosmarinic acid are the most important constituent of this plant essential oil [13]. Bitter apple (*Citrulluscolocynthis*) belongs to Cucurbitaceae family which its fruit is like watermelon but smaller than it in about orange size in yellow or green color [14]. Lemon verbena (*Lippiacitriodora*) is a perennial shrub or subshrub growing to 2–3 m high and belongs to Lamiales order and Verbenaceae family [15]. In this sense, this study evaluated the possibly allelopathic effect of Lemon balm, Lemon verbena and bitter apple plants on germination and growth of wild mustard weed.

Material and Methods

A laboratory experiment was arranged based on completely randomized design in three replications at laboratory of Mehrgan institute during 2014. In this study, effects of different aqueous extracts and essential oils of lemon balm, bitter apple and lemon

verbena medicinal plants compared with control treatments according to the table 1. As essential oil is insoluble in the water so, gumarabic + water was used to reach the proposed concentrations thereby, there were two control treatments, where the results of essential oils treatments were compared with gumarabic+water treatments and results of aqueous extracts were compared with sole water treatment. Wild mustard seeds were gathered from spring wheat field of Larestan city which is abundant in canola and spring cereals fields. Aqueous extract was prepared by soaking of 100 g leaf and stems of each studied plants at 1000 mL water due 48 h and shake continuously by shaker device. Clean linen cloth and Whatman filter paper were used in order to filter the residuum. Finally the extracts were prepared based on 15, 30, 45, 55 g/L concentrations. Essential oil was achieved by Clevenger and they were solubilized in water and gum arabic in order to preparing 300 and 600 mL/L concentration. Then 50 wild mustard seeds disinfected by sodium hypochlorite (5% for two minutes) and treated by 5 mL of prepared solution. Samples maintained in incubator at 24 °C and 60 percentage humidity, until finishing of germination process. During this time samples germination and germinated seeds had been checked every day. Radicle length, shoot length, fresh and dry weight of radicle and shoot were measured at the end of this time. Germination percentage and germination rate were calculated by Azizi and Fuji [16], Asplund [17] and FallahHosseini, *et al.*, [18] equations given below respectively.

$$GP = \frac{G}{N} \times 100$$

GP: germination percentage
G: germinated seeds number
N: total seed number

$$GR = \sum_{i=1}^n \frac{S_i}{D_i}$$

GR: germination rate
S_i: germinated seeds per counting
D_i: The number of days to the n-th count
N: counting times

Data were analyzed by SAS 9.2 software and mean of comparison calculated by LSD test at P≤0.05.

Table 1 Studied treatments

T1= Water (control treatment)
T2= Arabic gum+water (control)
T3= Lemon verbena aqueous extract (15g\L)
T4= Lemon verbena aqueous extract (30g\L)
T5= Lemon verbena aqueous extract (45g\L)
T6= Lemon verbena aqueous extract (55g\L)
T7= Lemon balm aqueous extract (15g\L)
T8= Lemon balm aqueous extract (30g\L)
T9= Lemon balm aqueous extract (45g\L)
T10= Lemon balm aqueous extract (55g\L)
T11= Bitter apple aqueous extract (15g\L)
T12= Bitter apple aqueous extract (30g\L)
T13= Bitter apple aqueous extract (45g\L)
T14= Bitter apple aqueous extract (55g\L)
T15= Lemon verbena essential oil (300 mL/L)
T16= Lemon verbena essential oil (600 mL/L)
T17= Lemon balm essential oil (300 mL/L)
T18= Lemon balm essential oil (600 mL/L)
T19= Bitter apple essential oil (300 mL/L)
T20= Bitter apple essential oil (600 mL/L)

Results

Shoot length

According to Table 2, studied treatments had significant effect on shoot length of wild mustard ($P \leq 0.01$). Mean of comparison showed that maximum shoot length observed at 45 and 55 g/L lemon balm concentration treatments (52.3 and 51.5 mm respectively) while the inhibitory effects were related to 600 mg/L (11.1 mL), 300 mg/L lemon balm (11.4 mL) and 600 mg/L lemon verbena essential oil treatments (11.3 mL), compared to the control treatments (14.7 and 15.2 mL, respectively). All the lemon verbena, lemon balm and bitter apple extracts increased shoot length compare to the control treatment (Table 3).

Radicle Length

Studied treatments had meaningful effect on radicle length at $P \leq 0.01$ (Table 2). Mean of comparison indicated that the highest radicle length had achieved from both 45 g/L extracts of lemon balm and bitter apple treatments (43.5 and 43.3 mm respectively) and the lowest of it were related to 600 mg/L essential oils of lemon verbena, lemon balm and bitter apple (13.2, 13.0 and 22.2 mm, respectively). At lemon verbena extract treatments by increasing of extract concentration, radicle length reduced and they had meaningful differences with control treatment. By increasing essential oil concentration of lemon verbena, lemon balm and bitter apple radicle length decreased while there were no significant differences between 300 and 600 mg/L essential oil of lemon balm and 600 mg/L

essential oil of lemon verbena, however, they had meaningful differences with control treatment (Table 3).

Germination percentage

According to the Table 2, wild mustard had effected by studied treatment ($P \leq 0.05$). Mean of comparison revealed that although lemon balm (30 g/L) and Bitter apple aqueous extracts (45 and 55g/L) couldn't inhibit weed seeds germination percentage, however, minimum germination percentage was related to 600 mL/L essential oil of lemon verbena (89.3%). By increasing of lemon verbena essential oil germination percentage significantly decreased in compare to control treatment (Table 3).

Germination rate

Germination rate was strongly affected by application of treatments (Table 2). The highest germination rate obtained by 15g/L lemon verbena (24.8), 55 g/L bitter apple extracts (24.6) and water+gumarabic treatment respectively (24.3). The lowest germination rate related to 600 mL/L lemon verbena extract treatment (17.1). The results showed that by increasing of lemon verbena essential oil concentration germination rate decreased in compression to control treatments (Table 3).

Shoot Fresh Weight

All treatments had significant effect on shoot fresh weight of wild mustard (Table 2). Means of comparison showed that maximum shoot fresh weight was obtained by 45g/L lemon balm extract treatment (809 mg). The lowest fresh shoot weight were also seen at 600 mg/L lemon verbena essential oil (160 mg) which had no meaningful differences with lemon balm essential oil concentrations and control treatments. Table 3, revealed that by increasing of lemon verbena essential oil concentration shoot fresh weight decreased.

Radicle Fresh Weight

Results showed that treatments had significant effect on radicle fresh weight of wild mustard at $P \leq 0.01$ (Table 2). Maximum radicle weights were related to 15g/L lemon balm extract (182mm) and 55 g/L bitter apple (179mm) treatments and minimum of it obtained from 600 mg/L lemon verbena and lemon balm (10 ml) treatments. By increasing of lemon verbena and Lemon balm extract concentrations up to 30g/L and bitter apple

Table 2 Analysis of variances for different studied treatments on germination of wild mustard

S.V	df	Means of squares							
		shoot length	Radicle length	Germination percentage	Germination rate	shoot fresh weight	Radicle fresh weight	shoot dry weight	Radicle dry weight
Treatment	19	426.4 **	266.7 **	21.2 *	12.8 **	93660.5 **	7100.1 **	135.2 **	28.2 **
error	40	24.8	6.8	9	3	8820.9	44.8	14.6	0.5
C.V (%)		18.4	8.9	3.1	7.6	19.9	9.1	10.9	10.6

*, ** : significant at $P \leq 0.01$ and $P \leq 0.05$, respectively.

up to 45 g/L radicle weight increased, however, although by increasing of concentrations to more than that doses radicle fresh weight decreased but generally they had more values in compare to control treatments. Essential oil of lemon balm and lemon verbena by increasing of concentration decreased fresh radicle weight, as 600 mg/L essential oils have significant differences compare to control treatments (Table 3).

Shoot dry weight

Analysis of variances (Table 2), showed that shoot weight was affected by studied treatments ($P < 0.01$). Mean of comparison indicated that the highest shoot dry weight obtained by 55g/L bitter apple treatments (42.7 mL). Generally all extract treatments except 300 mg/L lemon verbena treatment, had the lowest shoot dry weight (Table

3).

Radicle dry weight

Allelopathic treatments had significant effects on radicle dry weight of wild mustard (Table 2). The highest radicle dry weight obtained by 15 g/L bitter apple extract treatment (15.7mg) and the lowest was related to 600 mg/L lemon verbena essential oil treatment (1mg). By increasing of lemon verbena essential oil concentration radicle weight significantly decreased and there was a statically difference between 600 mg/L essential oil of lemon verbena and control treatments. Essential oils of lemon balm in comparison of control treatment also significantly decreased radicle dry weight, however, there was no meaningful difference between lower concentration of bitter apple essential oil and control treatments (Table 3).

Table 3 Mean comparison for different studied treatments on germination of wild mustard

Treatments	shoot length(mm)	Radicle length(mm)	Germination percentage	Germination rate	shoot fresh weight(mg)	Radicle fresh weight(mg)	shoot dry weight(mg)	Radicle dry weight(mg)
T1	14.7f	24.7fgh	95.3ab	23.2ab	306fgh	38ij	33.7ab	4.3fgh
T2	15.2f	25.0fgh	98.0a	24.3a	306fgh	29j	32.0bcd	5.0fg
T3	23.2cdef	37.9abc	99.3a	24.8a	405defg	107cd	37.3ab	7.3cde
T4	31.4bcde	35.7bcd	98.0a	24.1ab	549bcde	128b	38.0ab	7.0cde
T5	29.3bcde	32.1cde	98.7a	24.1ab	546bcde	80ef	42.0a	7.7cd
T6	33.3bc	28.1efg	99.3a	24.2ab	558bcde	85gh	38.7ab	8.0bc
T7	33.3bc	31.5de	99.3a	23.9ab	595abcd	182a	36.0ab	8.0bc
T8	40.5bc	41.4ab	96.0ab	22.4ab	681abc	110c	40.0ab	8.3cd
T9	52.3a	43.5a	100.0a	22.8ab	809a	53hi	41.3ab	6.0def
T10	51.5a	29.4def	96.0ab	20.4abcd	763ab	52hi	40.7ab	5.7ef
T11	27.7cde	27.6efgh	97.3a	23.9ab	479cdef	70fg	40.7ab	15.7a
T12	28.3bcde	24.1h	97.3a	24.1ab	540bcdef	94de	40.7ab	9.7b
T13	32.8bc	43.3a	100.0a	24.1ab	612abcd	179a	40.7ab	8.7bc
T14	32.0bcd	35.7bcd	100.0a	24.6a	611abcd	70fg	42.7a	6.0def
T15	21.8cdef	24.5fgh	97.3a	19.7bcd	396defg	46hi	33.0abc	7.0cde
T16	11.3f	13.2i	89.3b	17.1d	160h	10k	25.7cd	1.0i
T17	11.4f	13.6i	93.3ab	19.5cd	206gh	26j	26.0cd	3.0h
T18	11.1f	13.0i	96.7ab	22.7abc	198gh	10k	25.3cd	3.0h
T19	20.2def	34.7cd	96.7ab	23.5abc	353efgh	46hi	24.3cd	4.0gh
T20	19.1ef	22.2gh	94.0ab	23.1abc	351efgh	78f	23.3d	6.0def

Discussion

According to the achieved data it could be resulted that high germination percentage in early stages of plant growth period is due to the hardness of seed coat of wild mustard which led to no significant allelochemical effect on seed germination rate and percentage. Hence, absorption of this allelochemicals increased by growth of plant and significantly affected all growth characters (Radicle length, shoot length, fresh and dry weight of radicle and shoot). Essential oil of lemon verbena at 600 mg/L had the best inhibitory effect on germination of wild mustard seeds.

Allelochemicals by inhabitation of nutrient absorption or direct interference in respiration or oxidative phosphorylation could reduce plant growth [19] by physiological changes such as damage to the cell membrane and dehydrogenase enzyme activate [20]. These organic herbicides could reduce chemical herbicide usage which is the key element in current agricultural approaches. Our results were agree with Azizi and Fuji [16] results who found that allelochemicals could effect on seed germination by inhabitation of Gibberellic acid, Amylases and Proteinases synthesis, which are necessary enzymes for seed germination [21, 22]. In about allelopathic effects of crops similarly Miri [11], showed that by increasing of *Carthamus tinctorius* extract concentration germination percentage of wild mustard significantly decreased. Ebrahimi-kia [23] indicated that essential oil of *Eucalyptus globulus* and *Eucalyptus citriodora* inhibit the germination and development of bean seeds and resulted that Cineole and Limonene which were the main components of the essential oil caused to this fact. Regards to allelopathic effect of lemon verbena in this study, Rezaei and Jaymand [24] and Yang *et al.*, [20] similarly reported that the most components of this plant essential oil were cineole, β -guanine limonene, spathulenol, ciphilin oxide which they had germination inhibitory effect on weed seeds.

Conclusion

Regards to inhibition or stimulation effects of allelochemicals, our results also indicated that extract of these plants had stimulating effect on weed seed development, while some essential oils treatments had an inhibitory effect on this weed

seeds development. According to the results it could be concluded that essential oil of lemon balm in both used concentrations and lemon verbena at 600 mg/L had strong allelopathic effect on seed germination and development of weed, however, most of aqueous extract had stimulating effects on wild mustard seeds. In general essential oil treatments of lemon balm and lemon verbena were more effective treatments in compare to others. Further long-term experiments will be necessary in order to demonstrate the application of such a technique to other medicinal and aromatic plant mixtures.

References

- Zargari A. Medicinal Plants. Vol. 3, 6th ed. Tehran. *University Publication*, Tehran, 1996. (In Persian).
- Ghahreman A. Flora of Iran / Flore de l' Iran en couleurs naturelles. vol. 9. Publie' par: institute des recherches des Forests etdes paturage Departement Botanique. Tehran, 1987; (In Persian).
- Einhellig FA. Allelopathy-current status and future goals. In: Allelopathy: Organisms, processes and applications. (Eds., K. M. Inderjit, M. Dakshini and F. A. Einhellig), Am Chem Soc Washington DC. 1995.
- Yong C, Lee C, Chou C. Effects of three allelopathic on chlorophyll accumulation of rice seedling: I. Inhibition of supply-orientation. *Botanic Bullein Academia Sci.* 2002;43:25-31.
- Tworokosi T. Herbicide effects of essential oils. *Weed Sci.* 2002;50:425-431.
- ChungIM, Ahn JK, Yun SJ. Assessment of allelopathic potential of barnyardgrass (*Echinochloa crus-galli*) on rice (*Oryza sativa* L.) cultivars. *Crop Prot.* 2001;20:921-928.
- Skaltsa H. Shamma G. Flavonoids from Lippia citriodora, *Planta Medica.* 1988;54:465.
- Batish DR, Setia N, Singh HP, Kohli RK. Phytotoxicity of lemon-scented eucalypt oil and its potential use as a bioherbicide. *Crop Prot.* 2004;23:1209-1214.
- Alam SM, Ala SA, Azmi AR, Khan MA, Ansari R. Allelopathy and its role in agriculture. *Online J Bio Sci.* 2001;5:308-315.
- Callihan B, Brennan J, Miller T, Brown J, Moore M. Mustard in mustards. Published bt Ag coomuncations, university of Idaho. 2000.
- Miri HR. Inhibitory Effect of safflower crop residues (*Cartamus tinctorius* L.) on germination of maize, wheat, wild mustard and wild safflower. *Journal of Plant Ecophysiology - Islamic Azad University Arsanjan.* 2009; 2:81-90. (In Persian).
- Makkizadeh Tafti M, Farhoudi R, Rastifar Tafti M. Effect of osmopriming on seed germination of Lemon balm (*Melissa officinalis* L.) under salinity

- stresses. Iranian J Med Arom Plants. 2011;4:573-586. (In Persian).
13. Scholey A, Gibbs Neale A, Perry C, Ossoukhova N, Bilog A, Kras V, Scholz M, Sass, CM, Buchwald-Werner S. Anti-Stress Effects of Lemon Balm-Containing Foods. *Nutrients*. 2014;6:4805-4821.
 14. Lee PL, Presbylla MP. The discovery and structural requirements of inhibitors of p-hydroxy pyroval dioxigenase. *Weed Sci*. 1997;45:601-609.
 15. Soltanipor M. Allelopathic Effects of essential oil *Zhumeria majdae* Rech on barnyard grass weeds and garden cress. *Iranian J. Med. Arom. Plants*. 2007; 65: 8-14. (In Persian).
 16. Azizi M, Fuji Y. Allelopathic effect of some medicinal plant substances on seed germination of *Amarantus refeelexus* and *Potulacea oleracea*. *Acta Horticultur*. 2006;699:61-66.
 17. Asplund RO. Monoterpenes, Relationship between structure and inhibition of germination. *Phytochem J*. 1968;7:1995-1997.
 18. Fallah Hosseini H, Heshmat R, Larijani B, Fakhr Zadeh H, Jafari Azar Z, Darvish Zadeh, F. *The clinical investigation of Citrullus Colocynthis (L.) Schrad, fruit in treatment of type II diabetic patients: A randomized, double-blind, placebo-controlled study*. *J Med Plants*. 2006;5:31-5.
 19. Torrent Marti MT. Some pharmacognostic and pharmacodynamics aspects of *Lippia citriodora*. *Rev R Acad, Farm. Barcelona*. 1976;14:39-55.
 20. Yang CM, Chang IF, Lin SJ. Effect of three allelopathic, phenolic on chlorophyll cumlation of rice seedling. *Boot Bull Acad*. 2004;45:112-125.
 21. Rice EL. *Allelopathy*. 2nd Edn. Academic Publishers, New York, 1984.
 22. Inderjit SO. Ecophysiological aspects of allelopathy. *Planta*. 2003;32:191-196.
- Beres I, Kazainczi G. allelopathy effects of shoot extracts and residues of weed on field crop. *Allelopathy J*. 2000;7:93-98.
23. Ebrahimikia F. Allelopathic effects of aqueous extracts and essential oils of eucalyptus leaves on two species of weeds and crops. MA thesis. Shiraz University, 2000. (In Persian).
 24. Rezaie MB, Jaymand K. Study of chemical constituent oil of *Lippia citriodora* H.B.K. Pajouhesh -va-Sazandegi in *Agron Horti*. 2002; 53:13-15.