J. Med. Plants 2023; 22(87): 77-88

Institute of Medicinal Plants

Journal of Medicinal Plants

Journal homepage: www.jmp.ir

Research Article

Variation of antioxidant activity and phenolic compositions of *Marrubium vulgare* L. as influenced by organic acids

Maryam Zahedifar[1,*], Sharareh Najafian[2]

[1] *Department of Range and Watershed Management (Nature Engineering), Faculty of Agriculture, Fasa University, Fasa, Iran*
[2] *Department of Agriculture, Payame Noor University, Tehran, Iran*

ARTICLE INFO

Keywords:
Humic acid
Fulvic acid
Marrubium vulgare L.
Medicinal plants
Organic fertilizer
DPPH activity

ABSTRACT

Background: In sustainable agriculture, reduction of chemical fertilizers- induced environmental pollutions is mainly considered. Therefore, recently application of organic fertilizers particularly their foliar applications received increased attention. Besides, herbal medicine with higher antioxidant activity will be able to limit the formation of free radical species. **Objective:** Therefore, we aimed to determine the effect of foliar application of organic fertilizers on antioxidant activity and polyphenolic compounds of *Marrubium vulgare* L. in greenhouse experiment. **Methods:** In a completely randomized design experiment with three replications that was carried out in 2019, the applied treatments consisted of four levels (0, 250, 500, and 1000 mg L^{-1}) of foliar application of humic and fulvic acids (0, 250, 500, and 1000 mg L^{-1}). Some phenolic compounds including gallic acid, chloregenic acid, coumarin, hesperidin, and eugenol were detected and quantified in the *Marrubium vulgare* L. extracts. **Results:** Application of 250 mg humic acid L^{-1} resulted in the highest extraction of phenolic compounds, coumarin, reducing this content at a higher applied level and by fulvic acid addition. As such, the foliar application of low concentrations of humic acid before the flowering stage was a useful and effective method to increase the synthesis of phenolic compounds. Among the studied organic acids, the application of 250 mg humic acid L^{-1} showed the highest antioxidant activities. These compounds can be effective in controlling diseases with free radicals. **Conclusion:** Based on the findings of the present research, a more appropriate management of the growth and propagation of medicinal plants and their quality can be applied. In addition, humic substances application reduces the chemical fertilizers used, thereby maintaining the environment.

1. Introduction

The traditional system of medicine has tended to plant origin products. Herbal medicines such as the Lamiaceae family, are going to be more consumed in many countries of the world and attention to them is increasing

Abbreviations: EC, electrical conductivity; CCE, calcium carbonate equivalent; CEC, cation exchange capacity; OM, organic matter; N, total nitrogen; P, phosphorus; Mn, manganese; Fe, iron; Zn, zinc; Cu, copper; DPPH, 2,2-diphenyl-1-picrylhydrazyl; AOA, antioxidant activity; IC50, Half maximal inhibitory concentration; HA, humic acid; FA, fulvic acid.

*Corresponding author: zahedifar@fasau.ac.ir
doi: 10.61186/jmp.22.87.77
Received 20 August 2023; Received in revised form 10 December 2023; Accepted 13 December 2023

day by day. *Marrubium vulgare* L. (White horehound) as a perennial herb, is responsible for different pharmacological activities including antihypertensive, antioxidant, antidiabetic, digestive stimulant, antibacterial, and antifungal effects. The phenolic and antioxidant compounds are main secondary metabolites in White horehound [1, 2]. Phenolic compounds, as secondary metabolites, are divided into phenolic acids and polyphenols. These compounds have several biological activities and functions in plants, especially in confronting environmental factors, and stress conditions [3]. They are used as drugs for ulcer formation, heart ailments, neural disorders, mutagenesis, and bacterial infection [4]. The phenolic compounds improve food quality and value due to retardation in oxidative degradation of lipids [5]. According to Valko [6], antioxidant composites have protective mechanisms by avoiding the formation of free radical types, interrupting radical chained reactions, turning free radicals into less harmful molecules, and fixing oxidative impairment. The main function of antioxidant is its effect to trap free radicals [7]. The application of herbicides, fungicides, and insecticides will result in the higher amount of antioxidant compounds in organic foods [8].

Humic substances such as dark-colored compounds with large molecular weights and complex structures result from the decomposition of plant or animal remains [9]. According to the other investigators, humic substances or organic based compounds such as biochar influence soil fertility**,** increase productivity, alter the soil physical properties, raise the chelation of elements, and increase their availability to plants, or alter chemical forms of the element and pollutants in soil and environment [10-18]. Pizzeghello *et al.* [19] reported that humate application improves photosynthesis and root respiration. Humic acid with carboxyl, alcoholic hydroxyl, ketone, quinoide, and phenolic hydroxyl [20], influences cell permeability and division [21]. Van – Hees *et al.* [22] stated that fulvic acid is s one of the major components of organic matter (OM) and dissolved-organic carbon (DOC) pool of soils. Overall, plant physiological characteristics are affected by humic substances. Besides, fulvic and humic acids, as the most significant components of OM in soils and municipal waste compost, influence the elements cycling in the environment and ecological roles of soil [23]. The application of humic acid increased the uptake of nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg), and iron (Fe) in garden thyme [24]. According to Nikbakht *et al.* [25], humic acid increased the number of flowers and encourages the root growth of gerbera.

Nowadays, fertilization as foliar application which affects growth of plant at different growth stages, also the yield quality received more attention [26]. Under water stress, the humic substances foliar application increased antioxidant metabolism and leaf water retention [27, 28]. The objective of this study was to estimate the effects of foliar application of different levels (0, 250, 500, and 1000 mg L^{-1}) of humic (HA) and fulvic (FA) acids on antioxidant activity and polyphenolic content of *Marrubium vulgare* L. under greenhouse conditions.

2. Materials and methods

2.1. Soil sampling and analysis

A topsoil (0-20 cm) loamy calcareous soil was collected, air-dried, and sieved through a less than 2 mm sieve. Some physicochemical properties were determined based on standard methods used by others [29-34]: clay, silt, and sand fractions according to the hydrometer method; pH in saturated paste by pH meter; electrical conductivity (EC) by EC meter; calcium carbonate equivalent (CCE) by neutralization method with normal hydrochloride acid and titration with sodium hydroxide [35]; cation exchange capacity (CEC) by displacement cations with ammonium acetate [36]; OM by wet oxidation method [37]; total nitrogen (N) according to Bremner [38]; sodium bicarbonate extractable phosphorus (P) [39]; diethylenetriaminepentaacetic acid (DTPA)-extractable manganese (Mn), iron (Fe), zinc (Zn) and copper (Cu) were extracted by DTPA [40] and their concentration were determined by atomic absorption spectrophotometer (Table 1).

2.2. Experimental design

The research was done (in 2019) as completely randomized plan with 3 replications, in Eram Garden greenhouse which is located in Shiraz, IR Iran (29° 38′ 09″ N and 52° 31′ 31″ E). Treatments involved of four levels of humic acid (0, 250, 500 and 1000 mg L^{-1}) and fulvic acid (0, 250, 500, 1000 mg L^{-1}) (Table 2).

Table 1. Some physicochemical properties of the studied soil

Soil property	Sand (%)	Silt (%)	Clay (%)	pH	EC*	OM	CCE	CEC	P	N	Fe	Mn	Zn	Cu
Value	32	34	24	7.87	0.39	1.55	42	15	4.45	0.07	2.32	3.66	0.97	1.02

*EC, electrical conductivity (dS/m); OM, organic matter (%); CCE, calcium carbonate equivalent (%); CEC, cation exchange capacity, ($cmol_+$/kg); P, phosphorus (mg/kg); N, nitrogen (%); Fe, Mn, Zn, Cu, iron, manganese, zinc and copper, respectively (mg/kg).

Table 2. Summary of the applied treatments in the study

Organic acid	Applied concentrations for foliar application (mg L^{-1})			
Humic acid	0	250	500	1000
Fulvic acid	0	250	500	1000

2.3. Greenhouse conditions and planting

A greenhouse experiment was designed to evaluate the effect of organic acid foliar spray on phenolic compounds and the antioxidant activity of horehounds. Each plastic pot consisted of 2 kg of studied soil. Before planting to avoid lack of nutrients, all of the pots received 20 mg P kg^{-1} soil as $Ca(H_2PO_4)_2$. H_2O, 10, 10 and 5 mg Mn, Zn, and Cu kg^{-1} soil as sulfate, respectively and 200 mg N kg^{-1} soil as urea (it was added at the time of planting and 4 weeks after germination). The seedlings of *Marrubium vulgare* L. were planted in pots and were kept in the greenhouse conditions (the temperature and relative humidity of nearly 12 to 40 °C and 60 to 75 %, respectively). Pots watered daily near the field capacity, FC conditions. At the 4th and 8th weeks after planting, humic and fulvic acids were foliar applied at the rate of 250, 500 and 1000 mg L^{-1}. The deionized water was sprayed as the control sample. Plants were harvested 90 days after planting and plant materials were prepared for analysis.

2.4. Using DPPH micro plate method to determine antioxidant activity

The standard antioxidant and extracts of antioxidant activity of the plant were assessed according to the radical eliminating impact of fixed DPPH free radicals. For preparing a standard solution, vitamin E was used. In an altered evaluation 18, 200 μL of a 100 mmol L^{-1} solution of DPPH radical in methanol was mixed with 20 μL of 12.5-3200 μg/ml extracts of methanol and vitamin E, respectively.

[DOR: 20.1001.1.2717204.2023.22.87.5.0]

[DOI: 10.61186/jmp.22.87.77]

Dilutions were stored at ambient temperature for 30 minutes. The prohibition of DPPH radical was evaluated utilizing a Biotek ELx808 micro plate reader (at 515 nanometers). Each specimen IC50 (concentration needed for inhibiting 143 formation of DPPH radical by 50 %) was evaluated by MATLAB software. The methanol solution extract (without DPPH) was utilized as a blank to be reduced from all measurements. The antioxidant activity (AOA) is determined using the equation (1):

AOA =100- [(A) sample-(A) blank) × 100/ (A) control] (1)

where A indicates absorbance.

The value of IC50 of each specimen is the test sample concentrations which lead to a 50 % decrease in the primary concentration of DPPH. It was measured from the nonlinear regression Log curve of test extract concentration (μg/ml) versus the mean percentage of the activity of radical removing [41, 42].

2.5. Determination of polyphenolic compounds and HPLC analysis

The samples were ground and powdered and plant methanolic extracts were prepared in the following way: one g of dried plant was waterlogged in 10 mL methanol for a full day. The extract and the below solution were cleared by membrane filters and stored in a freezer, in the dark and at the temperature of -18 °C until analysis by HPLC [43, 44]. The liquid chromatographic device involved an on-line degasser pump and controller joint with a photodiode array sensor set with an automatic injector interfaced with chromatography manager software. Separations were completed on a 150 × 4.6 μm id, 5 μm particle sizes, inverse phase C 18 analytical column working at 30 °C at the flow rate 1mL min^{-1}. Finding was done at 280 and 320 nm. Elution was revealed with a ternary non-linear gradient of the different ratios methanol to formic acid 1 % including 10 to 90, 25 to 75, 60 to 40, and 70 to 30. In order to make of standards and range of calibration curves, components were recognized by relating retention times and UV-VIS spectra with those of pure standards. The values are known as mean as values, ± standard deviation.

2.6. Statistical analysis

Data were analyzed statistically using ANOVA in SPSS software (v. 25.0) and the mean values were compared using the Duncan’s Multiple Range Test at the probability level of 0.05.

3. Results

The variance analysis in Table 3 indicated that foliar application of organic acid influenced significantly phenolic compounds.

Table 3. ANOVA for the effect of organic acids and their applied levels on some phenolic compounds.

		Mean squares of the studied variables (%)				
	DF	**Gallic acid**	**Chloregenic acid**	**Coumarin**	**Hesperidin**	**Eugenol**
Organic acid (fertilizer)	1	24.25*	0.00ns	0.12**	0.03**	0.04**
Applied levels	3	9.97ns	0.00ns	0.17*	0.02**	0.02**
Organic acid * Applied Level	3	9.97 ns	0.00ns	0.17*	0.01	0.01**
Error	16	5.37	0.00	0.29	0.01	0.00

* and ** means statistically significant at the probability levels of 0.05 and 0.01, respectively and ns means not significant at the probability level of 0.05.

3.1. Effect of organic acids on phenolic compounds

Results showed that some phenolic compounds including gallic acid, coumarin, chloregenic acid, hesperidin, and eugenol were detected and quantified in the *Marrubium vulgare* L. extracts and the highest value (Fig. 1) was obtained for eugenol compound in control (0.229 mg g^{-1}). The data obtained show changes in the phenolic contents in response to organic acids application.

With regard to the findings, the maximum concentration of coumarin was observed with 250 mg humic acid L^{-1} (0.519 mg g^{-1}). The lowest concentration of coumarin was obtained with control (0.088 mg g^{-1}) (Fig. 1).

According to Fig. 1, the highest concentration of eugenol (0.470 mg g^{-1}) was obtained with the lowest dosage of humic acid (250 mg L^{-1}). Results showed that the highest concentration of phenolic compounds (0.264 mg g^{-1}) was observed for eugenol after application of 250 mg fulvic acid L^{-1} (Fig. 2). However, this content was lower in the samples for which fulvic acid was used at higher amounts. The coumarin concentration was not significantly affected by fulvic acid as compared to that of control. The maximum concentration of hesperidin was obtained with 250 mg fulvic acid L^{-1} addition (0.196 mg g^{-1}).

The comparison of mean value showed that the application of 250 mg humic acid L^{-1} increased significantly the coumarin concentration by 24.35 % as compared to that of the control. In interpreting results from Fig. 3, humic acid application as compared to that of fulvic acid, showed a significant increase in coumarin concentration. A significant increase in hesperidin concentration was found that can be attributed to the 500 and 1000 mg L^{-1} application of humic acid relative to the control.

According to the mean values, the application of 250 mg organic acids L^{-1} increased significantly the hesperidin by 85.66 % as compared to that of the control. Data cited in Fig. 3 showed that humic acid compared with fulvic acid, increased this compound significantly. Foliar organic acid application resulted in the highest Eugenol concentration by 59.86 % relative to the control.

3.2. Effect of organic acids on antioxidant activity

According to the results, there were significant differences among treatments with regard to IC50 (mg L^{-1}). As shown in Fig. 4, the addition of 250 mg humic acid L^{-1} decreased significantly the IC50 value by 25.14 % than control.

On the other words, foliar 250 mg L^{-1} humic acid application resulted in the lowest IC50 (1051mg L^{-1}).

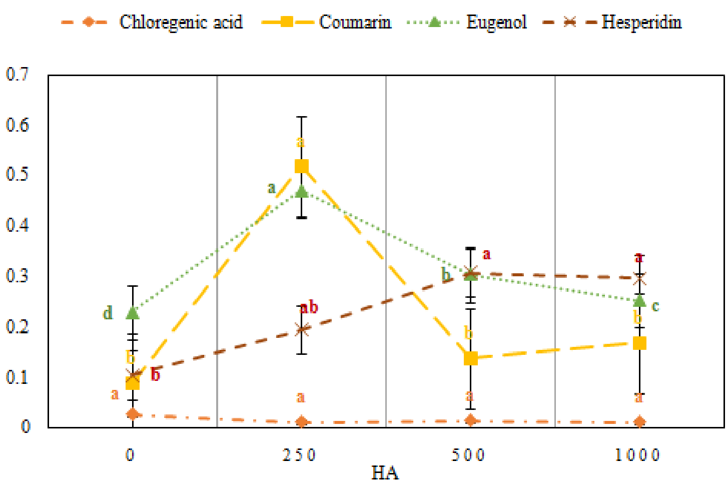


Fig. 1. Effect of different levels of humic acid, HA (mg L^{-1}) on phenolic compounds of *Marrubium vulgare*. Different lowercase statistical letters show significant differences between the applied treatments based on the Duncan's Multiple Range Test at the 0.05 probability level.

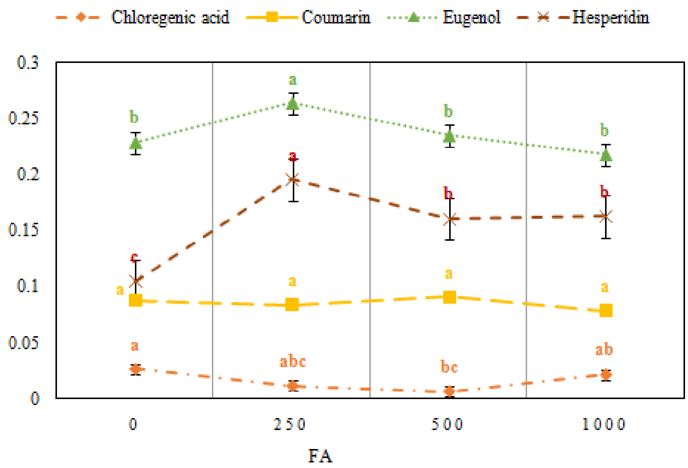


Fig. 2. Effect of different amounts of fulvic acid, FA (mg L^{-1}) on phenolic compounds of *Marrubium vulgare*. Different lowercase statistical letters indicate significant differences between the applied treatments based on the Duncan's Multiple Range Test at the at the 0.05 probability level.

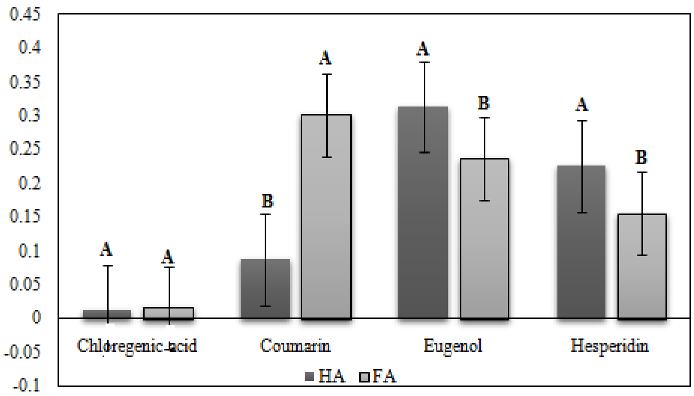


Fig. 3. The mean content of some phenolic compositions as influenced by humic acid (HA) and fulvic acids (FA) applications. Different capital statistical letters indicate significant differences between the applied treatments based on the Duncan's Multiple Range Test at the 0.05 probability level.

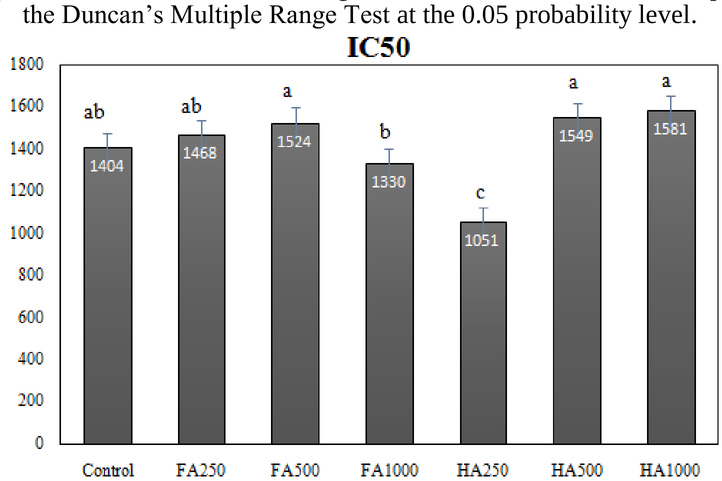


Fig. 4. Effect of different levels (0, 250, 500, and 1000 mg L^{-1}) of organic acids (humic, HA and fulvic, FL acids) on IC_{50} of *Marrubium vulgare*. Different lowercase statistical letters indicate significant differences between the applied treatments based on the Duncan's Multiple Range Test at the 0.05 probability level.

4. Discussion

Nowadays, studies on phenolic compounds are very much considered because of the role of these compounds as antioxidants, by chelating metal ions, consequently preventing radical formation. In sustainable agriculture, the application of humic substances instead of chemical amendments is very important to preserve the environment. Findings showed that foliar application of low concentration of humic acid before the flowering stage is a useful and effective method to increase the synthesis of phenolic compounds. Results showed that humic acid application increased total polyphenols in Echinacea [43] and in marigold [44]. According to Nguyen et al. [45], plant carbohydrates, as a building block, are essential to phenol synthesis, so, an increase in their quantity can increase in the substrate for phenolic compounds. Reda et al. [46] reported that foliar application of bioregulators increased biosynthesis and enzyme systems activities of Thyme, resulting the increase in certain main phenolic compounds.

Asami *et al.* [47] reported significantly higher total phenolic in Marion berries grown with organic agricultural methods as compared with the conventional methods. Olsson et al. [48] also showed that levels of all antioxidants including total the phenolic, flavanols, and ellagic acid in strawberries grown organically was higher than conventionally grown plants. Besides, the phenolic content of cabbages with organic management was higher than conventional management [49]. Karakurt et al. [50] indicated that humic acid addition significantly influenced the total yield of pepper and total carbohydrate content. According to Rivas-San Vicente and Plasencia [51], the phenolic compounds concentration will differ based on the salicylic acid applied, the method used, besides the tested plant species. The significant differences between treatments were obtained in association with IC50. As known, the lower the IC50 value, the higher the antioxidant activity. Regarding the IC50 quantified, humic acid application at the minimum content is the best treatment to increase antioxidant activity. According to Rimmer [52], the greater soil humic substances, the stronger antioxidant activity. Winter and Davis [8] stated that the lower use of insecticides, fungicides, and herbicides, the higher antioxidant compounds in organic foods. Karakurt *et al.* [50] showed that organic fertilizer increased the plant sugar content. Wang and Shin [53] illustrated that organic acids increased significantly the carbohydrate content and total soluble solids. Aminifard et al. [54] indicated that the application of 250 mg fulvic acid kg^{-1} soil increased the pepper capsaicin content more than the control. They showed that the antioxidant activity of pepper decreased with increasing fulvic acid levels and the maximum antioxidant activity was observed with 100 mg fulvic acid kg^{-1} soil. According to Davarpanah et al. [55], foliar application of humic acid had no effects on the total phenols and juice's antioxidant activity. There are some factors including light intensity, temperature, cultivar, kind of soil and the soil humic compounds values can influence the total antioxidant capacity of plants. Among them, the humic and fulvic acids are more effective on plant growth and antioxidant concentrations [56, 57]. Zahedifar and Najafian [58] indicated that the application of 500 and 1000 mg L^{-1} FA and 1000 mg L^{-1} HA increased significantly the antioxidant activity of yarrow. Erkan et al. and Yang et al. [59, 60] stated that plants are the main sources of polyphenols and antioxidants including various compounds of flavonoids and non-flavonoids such as phenolic acids, which found variations in their anti-oxidative and biological potential. The increases of antioxidant compounds in organic agriculture restrict the use of insecticides, fungicides, and herbicides and plants apply greater resources to fight pathogen attacks [8].

5. Conclusion

Recent progress in using organic substances has received much attention due to improved crop production, quality, and environmental safety. Our findings showed that some polyphenol compounds of *Marrubium vulgare* L. increased significantly as a result of 250 mg humic acid L^{-1} addition. As such, the highest polyphenol compound content was obtained for coumarin. However, it was decreased at a higher applied level. Besides, the maximum antioxidant activity was observed after the application of 250 mg humic acid L^{-1}. Considering that antioxidants prevent free radical abilities, using organic acid as humic acid in foliar applications can propose as a good candidate to increase antioxidant activity in medicinal plants.

Author contributions

M.Z. and Sh. N. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors acknowledge financial support from the Fasa University, Fasa, IR Iran and Payame Noor University.

[DOR: 20.1001.1.2717204.2023.22.87.5.0]

[DOI: 10.61186/jmp.22.87.77]

References

1. Meyre-Silva C and Cechinel-Filho V. A review of the chemical and pharmacological aspects of the genus *marrubium. Curr. Pharm. De*. 2010; 16(31): 3503-3518. doi: 10.2174/138161210793563392.

2. Lodhi S, Vadnere GP, Sharma VK, Usman MDR. *Marrubium vulgare* L.: A review on phytochemical and pharmacological aspects. *J. Intercult Ethnopharmacol*. 2017; 6: 429-452. doi: 10.5455/jice.20170713060840.

3. Sakihama Y, Cohen MF, Grace SC and Yamasaki H. Plant phenolic antioxidant and prooxidant activities: phenolisc-induced oxidative damage mediated by metals in plants. *Toxicol*. 2002; 177(1): 67-80. doi: 10.1016/s0300-483x(02)00196-8.

4. Kondo T, Oyama KI and Yoshida K. Chiral molecular recognition on a supramolecular metal complex pigment from blue flowers of *Salvia* patents. *Angew Chem. Int. Ed. Engl.* 2001; 40(5): 894-897. doi: 10.1002/1521-3773(20010302).

5. Wojdyło A, Oszmiański J and Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chem*. 2007; 105(3): 940-949. doi: 10.1016/j.foodchem.2007.04.038.

6. Valko M, Rhodes CJ, Moncol J, Izakovic M and Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Mini-review. *Chem. Biol. Interact*. 2006; 160(1): 1-40. doi: 10.1016/j.cbi.2005.12.009.

7. Wu YY, Li W, Xu Y, Jin EH and Tu YY. Evaluation of the antioxidant effects of four main theaflavin derivatives through chemiluminescence and DNA damage analyses. *J. Zheijang Univ. Sci. B.* 2011; 12: 744-751. doi: 10.1631/jzus. B1100041.

8. Winter CK and Davis SF. Organic foods. *J. Food Sci*. 2006; 71(9): 117-124. doi: 10.1111/j.1750-3841.2006.00196.x.

9. Lee CH, Shin HS and Kang KH. Chemical and spectroscopic characterization of peat moss and its different humic fractions (Humin, Humic acid and fulvic acid). *J. Soil Ground. Water Environ*. 2004; 9(4): 42-51.

10. Gulser F, Sonmez F and Boysan S. Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition. *J. Environ. Biol*. 2010; 31(5): 873-876.

11. Gavili E, Moosavi AA and Kamkar Haghighi AA. Does biochar mitigate the adverse effects of drought on the agronomic traits and yield components of soybean? *Indus. Crops Prod.* 2019; 128: 445-454. doi: 10.1016/j.indcrop.2018.11.047.

12. Gavili E, Moosavi AA and Moradi Choghamarani F. Cattle manure biochar potential for ameliorating soil physical characteristics and spinach response under drought. *Arch. Agron. Soil Sci.* 2018; 64(12): 1714-1727. doi: 10.1080/03650340.2018.1453925.

13. Gavili E, Moosavi AA, Zahedifar M. Integrated effects of cattle manure-derived biochar and soil moisture conditions on soil chemical characteristics and soybean yield. *Arch. Agron. Soil Sci*. 2019; 65: 1758-1774. doi: 10.1080/03650340.2019.1576864.

14. Zahedifar M and Moosavi AA. Assessing cadmium availability of contaminated saline-sodic soils subjected to biochar using the adsorption isotherm models. *Arch. Agron. Soil Sci*. 2020; 66(12): 1735- 1752. doi: 10.1080/03650340.2019.1694145.

15. Zahedifar M and Moosavi AA. Modeling desorption kinetics of the native and applied zinc in biochar-amended calcareous soils of different land uses. *Environ. Earth Sci.* 2017; 76: 567. doi: 10.1007/s12665-017-6895-z.

16. Zahedifar M. Sequential extraction of zinc in the soils of different land use types as influenced by wheat straw derived biochar. *J. Geochem. Explor*. 2017; 182: 22-31. doi: 10.1016/j.gexplo.2017.08.007.

17. Zahedifar M. Effect of biochar on cadmium fractions in some polluted saline and sodic soils. *Environ. Manage.* 2020; 66: 1133-1141. doi: 10.1007/s00267-020-01371-9.

18. Zahedifar M and Najafian Sh. *Ocimum basilicum* L. growth and nutrient status as influenced by biochar and potassium-nano-chelate fertilizers. *Arch. Agron. Soil Sci.* 2017; 63(5): 638-650. doi: 10.1080/03650340. 2016.1233323.

19. Pizzeghello D, Francioso O, Ertani A, Muscolo A and Nardi S. Isopentenyladenosine and cytokinin-like activity of different humic substances. *J. Geochem. Ex*. 2013; 129: 70-75.

20. Canellas LP, Olivares FL, Aguiar NO, Jones DL, Nebbioso A, Mazzei P and Piccolo A. Humic and fulvic acids as biostimulants in horticulture. *Sci. Hort*. 2015; 196: 15-27. doi: 10.1016/ j.scienta. 2015.09.013.

21. Nardi S, Pizzeghello D, Muscolo A and Vianello A. Physiological effects of humic substances on higher plants. *Soil Biol. Biochem*. 2002; 34(11): 1527-1536. doi: 10.1016/S0038-0717(02)00174-8.

22. Van-Hees PAW, Jones DL, Finlay R, Godbold DL and Lundstrom US. The carbon we do not see the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. *Soil Biol. Biochem*. 2005; 37(1): 1-13. doi: 10.1016/ j.soilbio.2004.06.010.

23. Vaughan D and Linehan DJ. The growth of wheat plants in humic acid solutions under axenic conditions. *Plant Soil*. 2004; 44: 445-449. doi: 10.1007/BF00015895.

24. Noroozisharaf AR and Kaviani M. Effect of soil application of humic acid on nutrients uptake, essential oil and chemical compositions of garden thyme (*Thymus vulgaris* L.) under greenhouse conditions. *Physiol. Mol. Biol. Plants* 2018; 24: 423-431. doi: 10.1007/s12298-018-0510-y.

25. Nikbakht A, Kafi M, Babalar M, Xia PY, Luo A and Etemadi NA. Effect of humic acid on plant growth, nutrient uptake, and postharvest life of gerbera. *J. Plant Nutr*. 2008; 31(12): 2155-2167. doi: 10.1080 /01904160802462819.

26. Bekhit RS, Hassan HR, Ramadan HM and El-Anani AM. Effect of different levels and sources of nitrogen on growth, yield and quality of potatoes grown under sandy soil conditions. *Ann. Agr. Sci*. 2005; 43: 391-394.

27. Vioratti Telles de Moura O, Luiz Louro Berbara R, França de Oliveira Torchia D, Fernanda Oliveira Da Silva H, Augusto van Tol de Castro T, Carlos Huertas Tavares O, Fernandes Rodrigues N, Zonta E, Azevedo Santos L and Calderín García A. Humic foliar application as sustainable technology for improving the growth, yield, and abiotic stress protection of agricultural crops. A review, *J. Saudi Soc. Agr. Sci*. 2023; 22(8): In press. doi: 10.1016/j.jssas.2023.05.001.

28. Preciado-Rangel P, Gaucin-Delgado JM, Salas-Perez L, Sanchez-Chavez E, Mendoza-Vllarreal R and Rodriguez Ortiz JC. The effect of citric acid on the phenolic compounds, flavonoids and antioxidant capacity of wheat sprouts. *Revista de la Facultad de Ciencias Agrarias UNCuyo*. 2018; 50(2): 119-127.

29. Najafian Sh, Zahedifar M and Ghasemi AR. Effect of organic and inorganic zinc foliar application on the natural product composition and antioxidant activity of lemon balm (*Melissa officinalis*). *Iran Agr. Res.* 2022; 40(2): 85-92. doi: 10.22099/ IAR.2022.42106.1466.

30. Zahedifar M. Iron fractionation in the calcareous soils of different land uses as influenced by biochar. *Waste Biomass Valor*. 2020; 11: 2321-2330. doi: 10.1007/s12649-018-0481-9.

31. Moosavi AA and Ronaghi AM. Influence of foliar and soil applications of iron and manganese on soybean dry matter yield and iron-manganese relationship in a calcareous soil. *Aust. J. Crop Sci.* 2011; 5(12): 1550-1556.

32. Moosavi AA and Ronaghi M. Growth and iron-manganese relationships in dry bean as affected by foliar and soil application of iron and manganese. *J. Plant Nutr*. 2010; 33(9): 1353-1365. doi: 10.1080/ 01904167. 2010.484095.

33. Najafian Sh and Zahedifar M. Productivity, essential oil components, and herbage yield, of sweet basil as a function of biochar and potassium-nano chelate. *J. Essent. Oil Bear. Plant*. 2018; 21(4): 886-894. doi: 10.1080/0972060X.2018.1510793.

34. Zahedifar M and Najafian Sh. Combined effect of soil applied iron and sulfur fertilisers on monoterpene content and antioxidant activity of *Satureja hortensis* L. extract. *Pertanika J. Trop. Agric. Sci*. 2015; 38(3): 361-374.

35. Loeppert RH and Suarez DL. Carbonate and Gypsum, In: Sparks DL, Page AL, Helmke PA, Loeppert RH. (Eds.): Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, ASA, Madison, WI, USA, 1996, pp: 437-474.

36. Sumner ME and Miller WP. Cation Exchange Capacity and Exchange Coefficients, In: Sparks DL, Page AL, Helmke PA, Loeppert RH. (Eds.): Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, ASA, Madison, WI, USA, 1996; 1201-1229. doi: 10.2136/ sssabookser5.3.c40.

37. Nelson DW and Sommers LE. Total Carbon, Organic Carbon, and Organic Matter, In: Sparks DL, Page AL, Helmke PA, Loeppert RH. (Eds.): Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, ASA, Madison, WI, USA, 1996; 961-1010. doi: 10.2136/ sssabookser5.3.c34.

38. Bremner JM. Nitrogen total. In: Sparks DL, Page AL, Helmke PA, Loeppert RH. (Eds.): Methods of Soil Analysis, Part 3. Chemical Methods. SSSA, ASA, Madison, WI, USA, 1996; 1085-1121. doi: 10.2136/ sssabookser5.3.c37.

39. Olsen SRC, Cole CV, Watanable FS, Dean LA. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. USDA Circular 939. Washington (DC): US Government Printing Office. 1954.

40. Lindsay WI and Norvell WA. Development of a DTPA soil test for Zinc, Iron, Manganese, and Copper. *Soil Sci. Soc. Am. J.* 1978; 42(3): 421-448. doi: 10.2136/ sssaj1978. 03615995004200030009x.

41. British Parmacopoeia B. Herbal drugs and herbal drug preparation kelp, V. III. The Stationary Office, London. 2009.

42. Zhou Z, Deng Z, Liang S, Zou X, Teng Y, Wang W and Fu L. Quantitative analysis of flavonoids in fruiting bodies of Sanghuangporus using ultra-high-performance liquid chromatography coupled with triple quadrupole mass spectrometry. *Molecules*. 2023; 28(13): 5166. doi: 10.3390/ molecules28135166.

43. Alizadeh Ahmadabadi A, Khorasani Nejad S and Hemati Kh. The effect of low irrigation and humic acid on morphological characteristics and phytochemical irrigation and Echinacea (*Echinacea purpurea* L.) root. *J. Crop.* Improvement. 2017; 19(1): 1-14. doi: 10.22059/jci.2017.60403.

44. Abedini T, Moradi P and Hani A. Effect of organic fertilizer and foliar application of humic acid on some quantitative and qualitative yield of Pot marigold. *J. Novel. Appl. Sci*. 2015; 4(10): 1100-1103.

45. Nguyen PM, Kwee EM and Niemeyer ED. Potassium rate alters the antioxidant capacity and phenolic concentration of basil (*Ocimum basilicum* L.) leaves. *Food Chem*. 2010; 123(4): 1235-1241. doi: 10.1016/ j.foodchem. 2010.05.092.

46. Reda F, Abdel-Rahim EA, El-Baroty GSA and Ayad HS. Response of essential oils, phenolic components and polyphenol oxidase activity of thyme (*Thymus vulgaris* L.) to some bioregulators and vitamins. *Int. J. Agri. Biol*. 2005; 7(5): 735-739.

47. Asami DK, Hong YJ, Barrett DM and Mitchell AE. Comparison of the total phenolic and ascorbic acid content of freeze-dried and airdried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *J. Agr. Food Chem*. 2003; 51(5): 1237-1241. doi: 10.1021/jf020635c.

48. Olsson ME, Andersson CS, Oredsson S, Berglund RH and Gustavsson K. Antioxidant levels and inhibition of cancer cell proliferation in vitro by extracts from organically and conventionally cultivated strawberries. *J. Agric. Food Chem*. 2006; 54(4): 1248-1255. doi: 10.1021/jf0524776.

49. Sousa C, Valentao P, Range J, Lopes G, Pereira JA, Ferreres F, Seabra RM and Andrade PB. Influence of two fertilization regimens on the amounts of organic acids and phenolic compounds of tronchuda cabbage (*Brassica oleracea* L. Var. costata DC). *J. Agric. Food*

Chem. 2005; 53(23): 91280-9132. doi: 10.1021/jf051445f.

50. Karakurt Y, Unlu H, Halime U and Padem H. The influence of foliar and soil fertilization of humic acid on yield and quality of pepper. *Acta Agric. Scand. B Soil Plant Sci.* 2009; 59(3): 233-237. doi: 10.1080/ 09064710802022952.

51. Rivas-San Vicente M and Plasencia J. Salicylic acid beyond defense: its role in plant growth and development. *J. Experim. Bot.* 2011; 62(10): 3321-3338. doi: 10.1093/jxb/err031.

52. Rimmer DL. Free radicals, antioxidants, and soil organic matter recalcitrance. *Eur. J. Soil Sci.* 2006; 57(2): 91-94. doi: 10.1111/j.1365-2389.2005.00735.x.

53. Wang SY and Lin SS. Compost as soil supplement enhanced plant growth and fruit quality of strawberry. *J. Plant Nutr.* 2002; 25(10): 2243-2259. doi: 10.1081/PLN-120014073.

54. Aminifard M, Aroiee H, Nemati H, Azizi M and Jaafar HZE. Fulvic acid affects pepper antioxidant activity and fruit quality. *Afr. J. Biotechnol*. 2012; 11(68): 13179-13185. doi: 10.5897/AJB12.1507.

55. Davarpanah S, Tehranifar A, Davarynejad GH, Abadía J and Khorasani R. Effect of humic acid on some physical and chemical characteristics of pomegranate (*Punica granatum* cv. Ardestani). *Plant Prod. Technol.* 2018; 10(1): 69-81. doi: 10.22084/ ppt.2018.9285.1525.

56. Ahn T, Oke M, Schofield A and Paliyath G. Effects of phosphorus fertilizer supplementation on antioxidant enzyme activities in Tomato fruits. *J. Agr. Food Chem*. 2005; 53(5): 1539-1545. doi: 10.1021/jf040248y.

57. Moukette BM, Pieme CA, Njimou JR, Nya Biapa CP, Marco B and Ngogang JY. In vitro antioxidant properties, free radicals scavenging activities of extracts and polyphenol composition of a non-timber forest product used as spice: *Monodora myristica*. *Biol. Res*. 2015; 48*:*15. doi: 10.1186/s40659-015-0003-1.

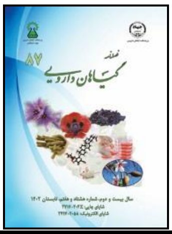
58. Zahedifar M and Najafian Sh. Evaluation of essential oil composition and antioxidant activity of yarrow as influenced by foliar application of humic substance-based products: Using multivariate exploratory method. *Sci. Hort*. 2023; 308: 111557. doi: 10.1016/j.scienta.2022.111557.

59. Erkan N, Akgonen S, Ovat S, Goksel G and Ayranci E. Phenolic compounds profile and antioxidant activity of *Dorystoechas hastata* L. Boiss et Heldr. *Food Res. Int*. 2011; 44(9): 3013-3020. doi: 10.1016/j.foodres.2011.07.015.

60. Yang Y, Hayden MR, Sowers S, Bagree SV and Sowers JR. Retinal redox stress and remodeling in cardiometabolic syndrome and diabetes. *Oxidative Medicine and Cellular Longevity* 2010; 3(6)*:* 392-403. doi: 10.4161/oxim.3.6.14786.

How to cite tis article: Zahedifar M, Najafian Sh. Variation of antioxidant activity and phenolic compositions of *Marrubium vulgare* L. as influenced by organic acids. ***Journal of Medicinal Plants*** 2023; 22(87): 77-88.
doi: 10.61186/jmp.22.87.77

فصلنامه گیاهان دارویی ۱۴۰۲؛ ۲۲(۸۷): ۷۷-۸۸



فصلنامه گیاهان دارویی

Journal homepage: www.jmp.ir

پژوهشکده گیاهان دارویی
جهاد دانشگاهی

مقاله تحقیقاتی

تغییرات فعالیت آنتی‌اکسیدانی و ترکیبات فنلی *Marrubium vulgare* L. تحت تأثیر اسیدهای آلی

مریم زاهدی‌فر[۱،*]، شراره نجفیان[۲]

[۱] گروه مرتع و آبخیزداری (مهندسی طبیعت)، دانشکده کشاورزی، دانشگاه فسا، فسا، ایران
[۲] گروه کشاورزی، دانشگاه پیام‌نور، تهران، ایران

اطلاعات مقاله

گل‌واژگان:
اسید هیومیک
اسید فولویک
Marrabium vulgare L.
گیاهان دارویی
فعالیت DPPH

چکیده

مقدمه: در کشاورزی پایدار، عمدتاً کاهش آلودگی محیط زیست توسط کودهای شیمیایی مورد توجه است. بر این اساس محلول پاشی کودهای آلی مورد تقاضا است. علاوه بر این، داروهای گیاهی با فعالیت آنتی‌اکسیدانی بالاتر قادر خواهند بود تشکیل گونه‌های رادیکال آزاد را محدود کنند. **هدف:** بنابراین آزمایش گلخانه‌ای به منظور تعیین اثر محلول‌پاشی کودهای آلی بر فعالیت آنتی‌اکسیدانی و ترکیبات پلی فنلی *Marrubium vulgare* L. انجام شد. **روش بررسی:** تیمارها شامل چهار سطح کاربرد اسید هیومیک و فولویک اسید (۰، ۲۵۰، ۵۰۰ و ۱۰۰۰ میلی‌گرم در لیتر) بود. برخی از ترکیبات فنلی از جمله اسید گالیک، اسید کلرژنیک، کومارین، هسپریدین و اوژنول در عصاره *Marrubium vulgare* L. شناسایی و اندازه‌گیری شد. **نتایج:** مصرف ۲۵۰ میلی‌گرم در لیتر هیومیک اسید منجر به بالاترین عصاره‌گیری از ترکیبات فنلی کومارین شد که این مقدار را در سطح کاربرد بالاتر و با افزودن اسید فولویک کاهش داد. به این ترتیب محلول‌پاشی اسید هیومیک با غلظت کم قبل از مرحله گلدهی روشی مفید و موثر برای افزایش سنتز ترکیبات فنلی است. در میان اسیدهای آلی مورد بررسی، کاربرد ۲۵۰ میلی‌گرم در لیتر هیومیک اسید بالاترین فعالیت آنتی‌اکسیدانی را نشان داد و ممکن است به عنوان منبع بالقوه عوامل آنتی‌اکسیدانی بیماری‌های مرتبط با رادیکال‌های آزاد مورد استفاده قرار گیرد. **نتیجه‌گیری:** یافته‌های مطالعه حاضر می‌تواند برای مدیریت تولید گیاهان دارویی و کیفیت فرآورده‌های آنها مورد استفاده قرار گیرد. علاوه بر این، استفاده از مواد هیومیک باعث کاهش مصرف کودهای شیمیایی و در نتیجه حفظ محیط زیست می‌شود.

مخففها: EC، قابلیت هدایت الکتریکی. CCE، کربنات کلسیم معادل ؛ CEC، ظرفیت تبادل کاتیونی؛ OM، ماده آلی؛ N، نیتروژن کل؛ P، فسفر؛ Mn، منگنز؛ Fe، آهن؛ Zn، روی؛ Cu، مس؛ DPPH، ۲،۲-دی فنیل-۱-پیکریل هیدرازیل؛ AOA، فعالیت آنتی اکسیدانی؛ IC50، نیمه حداکثر غلظت مهاری. HA، اسید هیومیک؛ FA، اسید فولویک

* نویسنده مسؤول: zahedifar@fasau.ac.ir

تاریخ دریافت: ۲۹ مرداد ۱۴۰۲؛ **تاریخ دریافت اصلاحات:** ۱۹ آذر ۱۴۰۲؛ **تاریخ پذیرش:** ۲۲ آذر ۱۴۰۲

doi: [10.61186/jmp.22.87.77](https://doi.org/10.61186/jmp.22.87.77)