Investigation of humic acid on the characteristics of Dracocephalum moldavica L. and its effect on E.Coli bacteria

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Abstract

organic fertilizers in the cultivation can accelerate their growth and better performance (Zarei 2019), D. moldavica L. has been able to have an inhibitory effect on various types of bacteria (Acimovic, Šovljanski et al. 2022)). This research was conducted in order to investigate the effect of different concentrations of humic acid on the vegetative and biochemical characteristics of the D. moldavica L. The experiment was conducted as a factorial in the form of a completely randomized block design in three replications.The results of analysis of variance showed that the effect of 1.5 g/ml humic acid on the measured traits was significant at 1% level. Average comparisons showed that the highest number of leaves was 33 and the lowest was 4 of the control, and the maximum root length, fresh weight, and dry weight at the concentration of 1.5 g/ml humic acid were significantly different from the control. The measurement of biochemical traits showed that the highest percentage of phenol, anthocyanin was related to humic acid with a concentration of 1.5 g/ml, but there was no significant difference compared to the control. The highest amount of DPPH with 46.3 percent is related to the treatment of 1.5 g/ml humic acid, which was increased compared to the control. Therefore, it can be concluded that humic acid can be used in order to reduce the consumption of chemical fertilizers and use organic fertilizers by supplying the plant's nutrient requirements and improving the quality of plant growth and inhibiting E. coli bacteria.

Key words: humic acid, antioxidant activity, DPPH
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Introduction

The lemon balm plant with the scientific name (Dracocephalum moldavica L.) from the Lamiaceae family is an annual herbaceous plant whose essential oil and extract are widely used in the pharmaceutical, food, cosmetic and health industries. (Yousefzadeh, Modarres-Sanavy et al. 2013, Nasiri 2021)) This plant is native to Central Asia and Eastern Europe and grows in the northern and northwestern regions of Iran. Secondary metabolites are controlled and synthesized by genetic factors, but their production is significantly influenced by environmental factors (Ameri, Nasiri-Mahallati et al. 2008). Due to the presence of effective substances such as citral (geranyl + neral) essential oil of lemongrass has antiseptic, antibacterial, antifungal, antiviral effects (Abd El-Baky and El-Baroty 2008) and has antioxidant properties (1386). (Saeed et al. phenolic compounds of D. moldavica, as a major group of secondary metabolites that have antibacterial properties

(Achakzai, Achakzai et al. 2009)). Creating better tillage conditions and using organic materials to provide Fertilizing the soil and providing the nutrients required by the plant in order to improve the performance and quality of the plant has been effective, and these plants have been used in the pharmaceutical industry (Farzanian and Yarnia 2014). The use of humic substances as organic fertilizers has improved the quality of the soil. (Ghaderimokri, Rezaei-Chiyaneh et al. 2022)). These materials are macromolecular natural biopolymers that are considered as organic and heterogeneous materials and are created from plant materials such as peat, brown coal, and deep sea sediments, and are mostly represented as humic acids (HAs). Schepetkin, Khlebnikov et al. 2002, Xia, Li et al. 2023)). Research indicates that the use of humic acids can work as an alternative to chemical fertilizers and prevent environmental pollution (Sharafabad, Abdipour et al. 2022)). Also, the use of organic fertilizers reduces the apparent specific mass of the soil and increases the water holding capacity in the soil and improves the qualitative and quantitative performance of the plant (Lee 2010, Mardi, Abbasifar et al. 2022.(

Test method

First, before planting, the physical and chemical analysis of the pot soil samples, their composition and the percentage of their main elements were determined (Table 1). Badrashboye seeds were purchased from Pakan Dasht Isfahan Company and at the end of September 1401, under the same conditions of light, temperature, Ph, tillage, in pots It was planted at a height of 35 cm with a hole diameter of 25 cm in the greenhouse and soil tests were done. (Table 1)

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Figure No. 1- Investigating the growth time of the lemon balm plant after a week

After 3 weeks and the emergence of buds, the plant was treated with concentrations of 0, 0.5, 1 and 1.5 grams/ml of humic acid solution. After four weeks of treatment, the product was collected and the morphological characteristics of the plant were evaluated. (Table 2)

Figure No. 2- Examining the morphological properties of the lemon balm plant using concentrations

AC: Absorption rate of blank
AS: sample absorption rate

The extract of dry samples of D. moldavica L. was prepared with a concentration (100 mg/ml). For this purpose, it was done by soaking in sterile distilled water at a temperature of 50 degrees Celsius in a bain-marie. For this purpose, 2 grams of dry plant leaf powder was soaked in 100 ml of sterile distilled water for 2 hours in a bain-marie, and then it was filtered and separated using filter paper. The obtained extract was stored in a sterile container impermeable to air and light in a refrigerator at a temperature of 4 degrees Celsius until the test was performed. To prepare the mother stock for the plant extract, a concentration equal to 200 mg/ml (2 grams of extract in 200 microliters of DMSO and 800 microliters of distilled water) was prepared and sterilized using a 0.2 micron microbial filter. 100 microliters of Mullerhinton Broth culture medium and 100 microliters of the extract prepared from the plant were poured into sterile flasks. The strain of Escherichia Coli, ATCC 25922, which is a gram-negative and rod-shaped bacterium, was obtained from Baharfshan Company. Bacterial suspension according to the standard turbidity of 0.5 McFarland (1.5 x 106-1 (CFU/mL) was prepared. 10 microliters of the bacterial suspension equivalent to half a McFarland was poured into the first tube and 10 microliters from the first tube was added to the second tube and then 10 microliters to the last tube. 10 microliters from the last tube was discarded and the dilution series was prepared and then the tubes together with the positive control tube which contained the culture medium and plant extract and with the negative control only bacterial culture were placed at 37 degrees Celsius for 24 hours and then From this time, the turbidity absorption of the tubes was read with a spectrophotometer at a wavelength of 63

Extraction was done according to the method of Hajimahdipour et al. 2009 with a slight modification. 0.1 g of the frozen leaf sample was powdered in a cold mortar with liquid nitrogen. Then, 20 ml of 80% methanol was added to the container containing the leaf powder and it was placed in an ultrasonic device at a temperature of 30 degrees for 30 minutes to completely disintegrate the leaf tissue. After this period, the samples were centrifuged for 15 minutes at 10000 rpm and 4 degrees Celsius and passed through filter paper. Finally, the filtered extract was used to measure phenol and antioxidant capacity. In order to measure the amount of total phenol in the leaf samples, a specific amount of methanolic leaf extract (200 microliters of the extract) was poured into clean glass tubes with 1200 microliters of 10% folin reagent and 960 microliters of 7% sodium carbonate and 180 microliters of distilled water were added to it. became Then the glass tubes were kept in the dark for 30-40 minutes at room temperature. The absorbance was read at a wavelength of 760 nm with a Dynamica (HALODB-20) spectrophotometer. The obtained absorbance (Y) was reported in the equation resulting from the placement calibration and the amount of total phenol (X) was reported in mg of gallic acid per gram of wet weight. (Slinkard and Singleton, 1977).
Y=0.0007X+0.0145
To measure the antioxidant capacity by the free method 1 diphenyl-2-picryl hydrazyl (DPPH), 12 ml of DPPH solution (0.004 g of DPPH powder in 100 cc of 80% methanol) to 400 microliters of the extract of the leaf samples of Badrashabiyeh was added. The resulting solution was shaken and kept at the laboratory temperature for 30 minutes. The absorbance of the samples was read at 517 nm using a spectrophotometer. To prepare the blank, proceed as above and only Instead of plant extract, 150 µL of 80% methanol was added (Chiou et al., 2007).

DPPH (%) = [(AC-AS)/AC]×100

Table-1 Physical and chemical properties of the tested soil

Soil texture	Clay%	Loam%	Sand%	Organic carbon	N%	P%	K%	EC dS/m	Ph

				(%)					
Sand-loam	25	38	28	2.7	0.15	13.8	223	3.7	8.6

Table-2 Vegetative characteristics of Badarshboyeh plant

Humic acid	number of leaf	leaf length	fresh weigh	Dry weight	stem height	root length
0.5 1 ml	6.33	2.4	0.3	0.07	6.46	4.93
1.5ml	10.6	3.36	0.5	0.05	12.8	5.83
	23.3	4.36	1.5	0.02	20.5	12.2
control	4.33	1.41	0.1	0.01	4.87	3.9

Chart No. 1- Morphological characteristics of Badrashboye plant

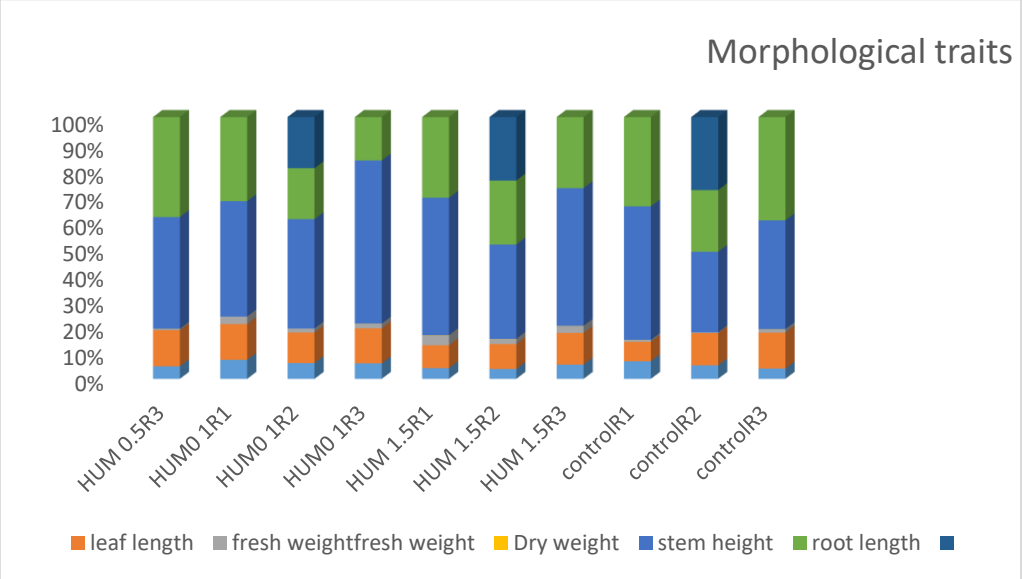
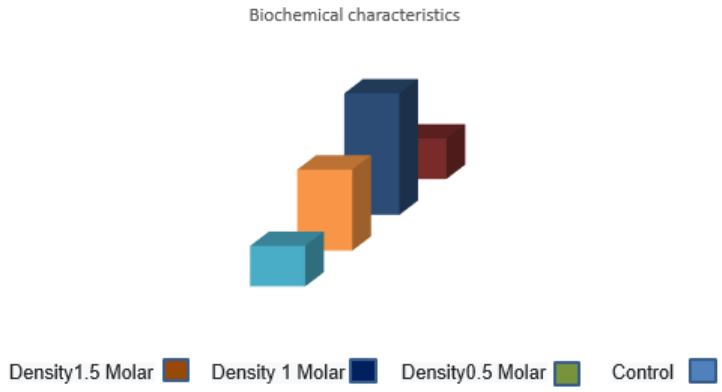


Table 3. Phytochemical characteristics of Badarshboyeh plant

Humic acid	Phenol	Anthocyanin	DPPH
HUM0.5ml	13.41	0.57	44
HUM1 ml	14.49	0.52	42.8
HUM1.5ml	15.14	0.98	46.3
Control	15.85	2.5	33.6

Diagram No. 2- Phytochemical characteristics of Badarsheboye plant



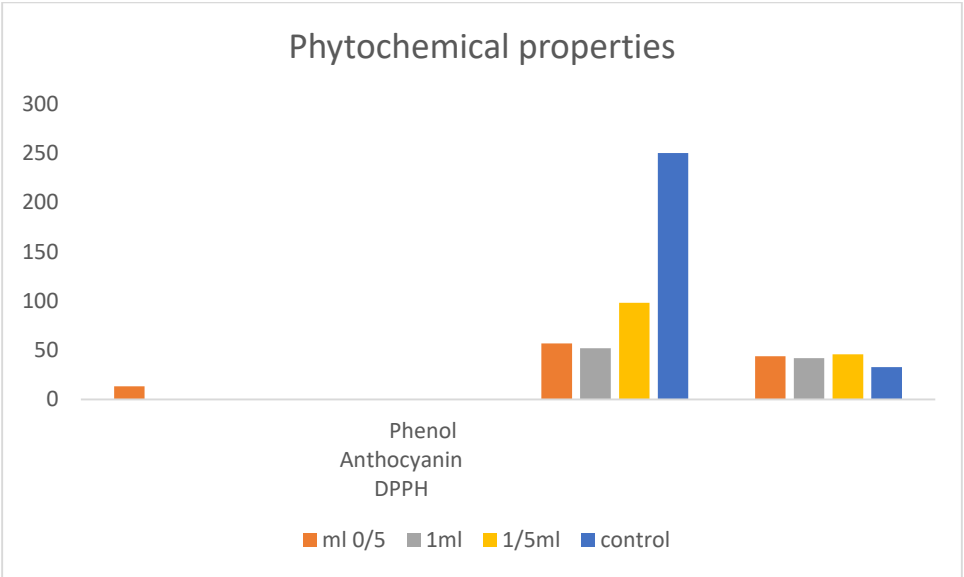
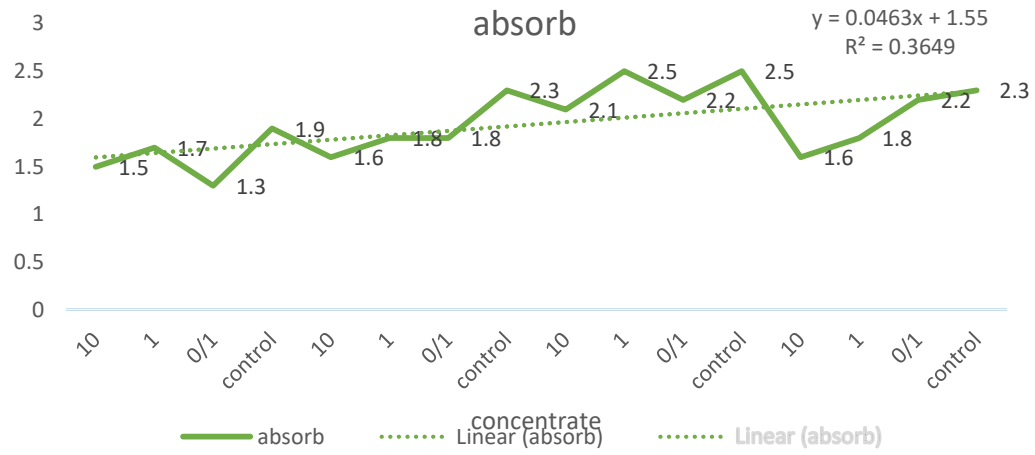


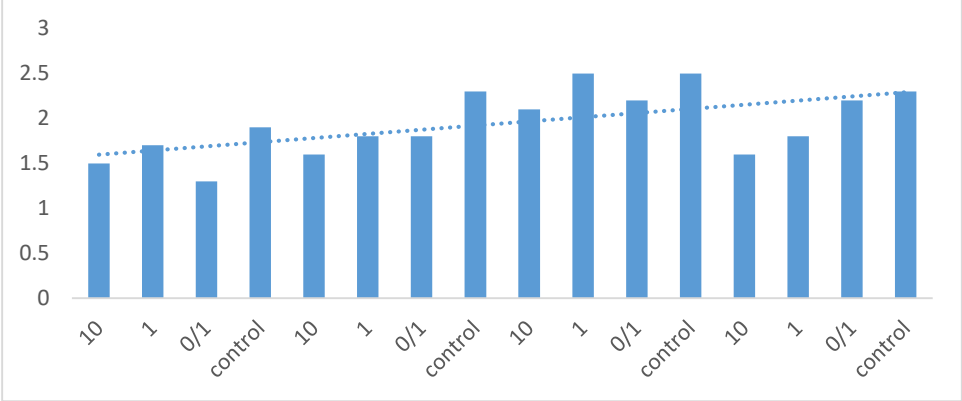
Table-4 - Antibacterial activity (MIC) concentrations of lemon balm extract with an average absorption of 630 (ml/ug)

microorganism1mili molarconcentration	Density 0/1mili molarDensity 0/01mili molar

Ecoliattraction 630 nm																
Control			2/3													2/2
			2/5													
Density ml 0.5HA			1/5						1/7						1/2	
Density ml 5 HA		1/6					1/8						1/8			
Density ml 1.52/1			2/0						2/3							

Chart No. 3- Drawing microbial activity in different concentrations of HA





Results and discussion

mM humic acid concentration had the highest effect (20.56 cm) and the control treatment had the lowest height. It was also found that in the treatment of 0.5, 1 and 1.5 mM humic acid concentrations, a significant difference was seen in the root length compared to the control, and the highest value belonged to the 1.5 (12.2 mM)

Morphological characteristics of Badarshaboye plant

The results of variance analysis of vegetative traits showed that the effects of humic acid concentrations on plant height had a significant difference, so comparing the average effect of 1.5

The results of the mean comparison (Table 3) showed that the humic acid concentration of 1.5 mM had the greatest effect on the phenolic content of 15.14 nm per plant, but there was no significant difference compared to the control. Also, the amount of anthocyanin (Table 3) in the concentration of 1.5 mM humic acid was the highest by 98% and the concentration of 1 mM humic acid was the lowest by 52%, which showed a significant difference compared to the control .with the amount of 25%

Also, the IC50 index (inhibition concentration with 50% extract) was used in order to inhibit free radicals from the DPPH method. (Yusuf, Lawal et al. 2018). Accordingly, the lower the 50IC value, the greater the inhibitory power of the extract (Sataei Mokhtari, Shahdadi et al. 2022). (Based on the results obtained from comparing the average of different treatments, the lowest DPPH value with 36.6 was related to the control group, which had a significant difference in different concentrations. The results of variance analysis of the data (Table 3) showed that the effect of thimaracidhumic on DPPH level was significant at the 1% probability level. In general, it can be concluded that the treatment with humic acid concentrations did not increase the phytochemical .characteristics compared to the control group

Antimicrobial of lemon balm plant

use of different concentrations of humic acid has had a significant effect on the qualitative and quantitative traits of Badrashboye plant and has .been able to inhibit microbial growth

Conclusion

In general, it can be concluded that the phenolic compounds and the antibacterial activity of plant extracts lead to the destruction of the cell wall and the death of bacterial cells, and these results once again proved the positive effects of the use of plant extracts on preventing the growth of pathogenic bacteria. As it is known to the general public that the use of chemical antibiotic compounds has harmful effects on human health and on the other hand, they have caused the emergence of antibiotic-resistant breeds, so the use of medicinal plant extracts can be a suitable, affordable and safe

concentration. Humic acid treatment with a concentration of 1.5 mM produced the highest fresh weight (1.25 grams) and the highest dry weight (0.029 grams), which was significantly different from the control (Table 2). The gradual use of nutrients increases the height of the plant. (Omrani et.al. 2016) comparison of the average plant height showed that the effect of humic acid treatment was the highest at 1.5 millimolar concentration (20.55) and the lowest at zero millimolar control concentration (4.87). The results of variance analysis of the data (Table 2) showed that the effect of humic acid treatment on the number of leaves was significant at the 1% probability level. Also, the highest number of leaves was related to humic acid concentration of 1.5 grams per millimolar with 23 leaves and the lowest number was related to the control effect with 4 leaves. The results showed that the effect of humic acid treatment on the phenolic content of Badrashboye plant was significant, but it was not significant in comparison with the control. From the examination of the morphological characteristics of the plant, it can be concluded that the concentrations of humic acid have improved the growth characteristics of the lemon .balm plant

Phytochemical properties of Badarshboyeh plant

effects

The antimicrobial effect of lemon balm plant in the interaction effect of Timaru and concentration in microbial inhibition was significant at the probability level of 1%. that the most inhibitory effect is at the humic acid concentration of 0.5 g/mM (Table No. 4) in the dilution series of 0.01 mM of the culture medium with an absorption length of 1.2 nm, which had a significant difference compared to the control. Previous research shows that the effect of extracts and essential oils can increase the permeability of ions, or the leakage of vital cell compounds or the failure of the bacterial enzyme system (Sandri, Zacaria et al. 2007)). Some researchers have shown that there is a direct relationship between the chemical structures of some of the dominant components in essential oils and their antibacterial activity (Friedman, Henika et al. 2002)). So that it can be concluded that the

and to be considered as a suitable solution for treatment.

approach for the environment. To control microbial activities in the field of food industry

REFRENCES

2. Achakzai, A. K. K., P. Achakzai, A. Masood, S. A. Kayani and R. B. Tareen (2009) . "Response of plant parts and age on the distribution of secondary

Cetkovic, J. ˇCanadanovic-Brunet and A. Miljkovic (2022). "Chemical Composition, Antioxidant, and

(Calendula afficinalis L.)." IranianJournal of Field Crops Research**5**(2): 315-325.

7. Farzanian, M. and M. Yarnia (2014). "Effects of microelement fertilizers and phosphate biofertilizer on some morphological, physiological traits, yield and essence of Purple coneflower in water stress conditions."
sativum L.)." Journal of Vegetables Sciences**5**(2): 1-17.

12. Nasiri, Y. (2021). "Crop productivity and chemical compositions of dragonhead (Dracocephalum moldavica L.) essential oil under different cropping patterns and fertilization." Industrial Crops and Products**171**: 113920.
13. Sandri, I., J. Zacaria, F. Fracaro, A. Delamare and S. Echeverrigaray (2007). "Antimicrobial activity of the essential oils of Brazilianspecies of the genus Cunila against foodborne pathogens and spoiling bacteria." Food chemistry**103**(3): 823-828.
14. Sataei Mokhtari, T., F. Shahdadi and A. Salehi Sardoei (2022). "The Effect of Different Solvents on the Percentage of Free Radical Scavenging DPPH and Antioxidant Activity of Native Medicinal Plants in Jiroft City." Karafan Quarterly Scientific Journal**18**(4): 145-158.
15. Schepetkin, I., A. Khlebnikov and B. S. Kwon (2002). "Medical drugs from humus matter: Focus on mumie." Drug development research**57**(3): 140-159.
16. Sharafabad, Z. H., M. Abdipour, M. Hosseinifarahi, A. Kelidari and L. Rashidi

1. Abd El-Baky, H. H. and G. S. El-Baroty (2008). "Chemical and biological evaluation of the essential oil of Egyptian Moldavian balm." Advances in food sciences**30**(3): 170-175.
3. metabolites on plants found in Quetta." Pak. J. Bot**41**(5): 2129-2135.
4. Acimovic, M., O. Šovljanski, V. Šeregelj, L. Pezo, V. Zheljazkov, J. Ljujic, A. Tomic, G.
5. Antimicrobial Activity of Dracocephalum moldavica L." Essential Oil and Hydrolate. Plants**11**: 941.
6. Ameri, A., M. Nasiri-Mahallati and P. Rezvani-Moghaddam (2008). "The effect of different amounts ofnitrogen and plant density on nitrogenuse efficiency, yield and activeingredients of marigold plant
8. Friedman, M., P. R. Henika and R. E. Mandrell (2002). "Bactericidal activities of plant essential oils and some of their isolated constituents against Campylobacter jejuni, Escherichia coli, Listeria monocytogenes, and Salmonella enterica." Journal of food protection**65**(10): 1545-1560.
9. Ghaderimokri, L., E. Rezaei-Chiyaneh, M. Ghiyasi, M. Gheshlaghi, M.L. Battaglia and K. H. Siddique (2022). "Application of humic acid and biofertilizers changes oil and phenolic compounds of fennel and fenugreek in intercropping systems." Scientific Reports**12**(1): 5946.
10. Lee, J. (2010). "Effect of application methods of organic fertilizer on growth, soil chemical properties and microbial densities in organic bulb onion production." Scientia Horticulturae**124**(3): 299-305.
11. Mardi, M., A. Abbasifar and B. ValizadehKaji (2022). "Comparison of the Effect of Biological and Non-Biological Fertilizers on Quantitative, Qualitative and Phytochemical Properties of Garlic (Allium

vermicomposting changes essential oil qua oils from two genotypes of dragonhead (Dracocephalum moldavica L.) in two regions of Iran." Food chemistry**138**(2-3): 1407-1413.

20. Yusuf, A. A., B. Lawal, M. A. Yusuf, A. O. Adejoke, F. H. Raji and D. L. Wenawo (2018). "Free radical scavenging, antimicrobial activities and effect of sub-acute exposure to Nigerian Xylopia Aethiopica seed extract on liver and kidney functional indices of albino rat." Iranian journal of toxicology**12**(3): 51-58.
21. Zarei, A. (2019). Sustainable Agriculture, Day System.

(2022). "Integrated humic acid and
17. ntity, and quality in field-grown Lavandula angustifolia L. intercropped with Brassica nigra L." Industrial Crops and Products**178**: 114635.
18. Xia, C., X. Li, Y. Wu, S. Suharti, Y. Unpaprom and A. Pugazhendhi (2023). "A review on pollutants remediation competence of nanocomposites on contaminated water." Environmental Research: 115318.
19. Yousefzadeh, S., S. A. M. Modarres-Sanavy, F. Sefidkon, A. Asgarzadeh, A. Ghalavand and K. Sadat-Asilan (2013). "Effects of Azocompost and urea on the herbage yield and contents and compositions of essential