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Effect of humic acid and some bio-fertilizers on growth, yield and chemical constituents of marjoram plants

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Abstract

During 2022 and 2023 summer seasons, a field experiment was done at a private farm in Gerga district, Sohage Government, Egypt. This study was assessed to evaluate the effect of humic acid rate (0, 2, 4 and 6 kg/ feddan), bio-fertilizer type [without inoculation, Mycorrhizae (My), Azolla (Az) and mixture of My + Az] and their combination treatments on growth, yield and some marjoram chemical constituents. Using any humic acid rate significantly increased plant height, branches number per plant, herb fresh and dry weights per plant and per feddan and volatile oil yield per feddan as well as N, P and K percentages compared to control (untreated plants) in the 1^{st}, 2^{nd} and 3^{rd} cuts. The highest values in these traits were achieved when marjoram plants fertilized with 6 kg/ feddan. Inoculated marjoram plants by the mixture of Mycorrhizae and Azolla gave the best values in plant growth, herb yield (fresh and dry weights of herb per plant and per feddan as well as volatile oil yield per feddan) and herb chemical contents (nitrogen, phosphorus and potassium percentages) compared to control or individual inoculation of Mycorrhizae or Azolla. The obtained results came to conclusion that, utilizing the treatment of 6 kg/ feddan in combination with mixture between Mycorrhizae + Azolla recorded significant increase in marjoram growth, productivity and some chemical constituents as compared to the other combination treatments under study in most cases.

Keywords: Marjoram, humic, bio-fertilizer, growth, yield and NPK percentages

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1. Introduction

Because of its stimulating and antispasmodic qualities, Egyptian marjoram (*Majorana hortensis*, Moench) is utilized as a spice and medicinal source around the world in the form of essential oil in aromatherapy. It is a perennial herbaceous plant in the Lamiaceae (Labiatae) family. Variations across plant species were identified by analyzing the chemical makeup of marjoram oils. According to [1], it is regarded as a significant commercial agricultural export crop in Egypt. According to several reports [2 & 3], marjoram's essential oil components include thymol, linalool, thujanol, gamma-terpinene, trans-sabinene hydrate, and terpinen-4-ol. Because marjoram can generate and preserve essential oil, which is utilized in the perfume and cosmetics industries, marjoram farming has an economic influence.

According to [4], the total marjoram cultivated area in 2020 in Egypt was 4143 feddan (3450 feddan in new land and 693 feddan in old land) which produced 13369 tons (11385 tons from new land and 1984 tons from old land) with average 3.227 ton/feddan (3.300 tons / feddan in new land and 2.863 tons/ feddan in old land), through summer season. Whenever, through winter season, the total marjoram cultivated area in 2020 in Egypt was 2889 feddan (2429 feddan in new land and 460 feddan in old land) which produced 5390 tons (4412 tons from new land and 978 tons from old land) with average 1.886 ton/feddan (1.816 tons / feddan in new land and 2.126 tons/ feddan in old land).

Because humic acid (HA) affects physiological and metabolic processes, it is typically used to plant nutrition and increase root, growth and yield characteristics. Humic acid has been shown to be effective and to play a beneficial function in plant physiological and biochemical processes. It also has an indirect effect on improving the physical, chemical, and biological aspects of soil [5]. Humic acid, which are naturally occurring organic compounds utilized to plants to improve crop quality attributes and nutrition efficiency, are one of the various types of plant bio-stimulants [6]. HA improve respiration, nutrition absorption, and nucleic acid production, all of which are processes that the cell membrane regulates [7]. Also, [8] suggested that the utilization of bio-stimulants, such as humic acid, can optimize the production potential of Egyptian crops and enhance the physical, chemical, and biological characteristics of soil. Moreover, [9] found that humic acid treatments significantly boosted the vegetative growth traits of *Lepidium sativum* plants, such as plant height (cm), branches number per plant, and fresh and dried weights per plot (kg). *Origanum vulgare* yielded the most fresh herbs, dried herbs, and dry leaves at HA 50 in both years; nevertheless, the essential oil concentration rose in the first season at both HA 50 and HA 30, but was highest at HA 50 in the second season [10].

For plants to perform better growth and productivity, root-associated microorganisms-particularly bacteria and fungi-are crucial [11& 12]. More than 80% of terrestrial plant species, including aromatic and medicinal plants [13 & 14], are known to have symbiotic relationships with arbuscular mycorrhizal fungus (AMF) of the phylum Glomeromycota [15]. This type of symbiosis aids plants in obtaining additional water and mineral nutrients, such as oligo-elements, nitrogen, and phosphorus, from the soil. As payment, the host plant provides the AMF with photo-assimilates, or carbohydrates [15, 16 &17].

Azolla is an effective nitrogen fixer that floats on fresh water. By developing a symbiotic relationship with the cyanobacterium *Anabaena azollae*, which is found in the cavity of the dorsal lobe of its leaf, azolla plants fix atmospheric nitrogen [18]. *Azolla* is a great source of protein and vital amino acids. It also has beta-carotene, vitamin A, and vitamin B12. Minerals including calcium (Ca), phosphorous (P), nitrogen (N), potassium (K), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) are also abundant in it [19]. *Azolla*'s protein content ranges from 25 to 35 percent dry weight. Since azolla can fix between 30 and 60 percent of the nitrogen in the atmosphere per hectare, it is seen to be one of the most promising bio-fertilizers and has the potential to replace 25 percent of nitrogen mineral fertilization. *Azolla* breaks down over the course of eight to ten days, releasing its nitrogen content into the soil solution so that plants can absorb it [20].

Thus, this study set out to assess the effects of azolla, mycorrhizae and humic acid rate on herb yield, plant growth, and certain chemical components of marjoram (*Majorana hortensis*, Moench) plants.

2. Materials and Methods

At a private farm in Gerga district, Sohage Government, Egypt, a field experiment was conducted in the summers of 2022 and 2023.aiming to investigate the effects of four biofertilizer types (mycorrhizae, Azolla, and a combination of them) and four humic acid rates (0, 2, 4, and 6 kg/feddan) as well as their combination treatments on the herb yield, volatile oil, and various chemical constituents of marjoram plants. Prior to planting, a randomized soil sample was taken in order to perform a conventional method of physical and chemical examination, as shown in Table 1 by [21].

2.1. Plant Material and cultivation:

The marjoram seeds were obtained from Research Centre of Medicinal and Aromatic Plants, Dokky, Giza. It was sow in nursery beds on 8^{th} and 10^{th} December then seedlings were transplanted at 8^{th} and 10^{th} March at space of 25 cm between hills, on two sides of the row just after irrigation during the 1^{st} and 2^{nd} seasons, respectively. Experimental plot area (3.00 × 3.60 m) 10.80 m^2 included 6 rows; each row was 60 cm apart and three meters in length.

2.2. Humic acid source and application

The Abo Zaabal Company to Fertilizers' vegetarian humic acid fertilizer has 96% humic acid by weight. Three times a month following the transplant date and following the first and second cuts, humic acid rates of 2, 4, and 6 kg/ feddan were applied to the plant root region. Prior to each addition, the humic acid was dissolved in a set volume of irrigation water.

2.3. Mycorrhizae and Azola source and application

The Microbio. Dept. of the Soils, Water and Environment Res. Instit. (ARC), Giza provided the mycorrhizae and azola. Soil inoculation was carried out using vesicular arbuscular mycorrhizae (VAM) fungi, which were found to contain three efficient strains of *Glomus etunicatum, Glomus intraradices* and *Glomus fasciculatum*. Five milliliters per hill was the inoculation rate for the VAM on sowing hills. About 200 VAM spores per hill were present in the amount. Mycorrhizae and azola were added three times: The first dose was before transplanting but the two ones were applied alone at the soil subsurface near the plant roots at 8^{th} and 10^{th} May and July.

2.4. Fertilization

The following NPK levels were used: 100 kg/feddan for potassium sulfate (48% K_2O), 200 kg/feddan for ammonium sulfate (20.5% N), and 150 kg/feddan for calcium superphosphate (15.5% P_2O_5). Soil preparation involved the application of phosphorus fertilizer. Fertilizers containing potassium and nitrogen were added to the soil at 30, 90, and 110 days following transplanting, respectively, in three equal amounts.

2.5. Experimental Design

This experiment was set up in a split-plot design with three replicates. The main plots were occupied by four humic acid rates. The sub plots were entitled to four bio-fertilizer types. The combination treatments between HA rates and bio-fertilizer types were 16 treatments.

2.6. Data Recorded

2.6.1. Plant growth

In both seasons, marjoram plants were harvested thrice annually by cutting the aerial parts of each plant (10 cm) above the soil surface. The three cuts were taken on 8^{th} and 10^{th} May, July and September in 1^{st} and 2^{nd} seasons, respectively. In addition, plant height (cm) and number of branches/plant were listed.

2.6.2. Herb yield components and volatile oil yield

Herb fresh and dried weights (g)/plant (it is dried in oven at 45°C) after every cut were determined. Also, fresh and dried weights of herb per feddan (ton) were calculated after every cut then total yields were recorded in both seasons. Following the three cuts, the volatile oil was extracted using hydro distillation for three hours from the dried herb of marjoram plants that had been collected. [22] was followed in order to extract the volatile oil, and after calculating the volatile oil yield per plant (ml), the volatile oil yield per feddan (l) was given.

Table 1: Physical and chemical properties of experimental soil (average of both seasons)

<table>
<tr><td colspan="9">Physical analysis</td><td colspan="3">Soil texture</td></tr>
<tr><td colspan="3">Clay (%)</td><td colspan="3">Silt (%)</td><td colspan="3">Sand (%)</td><td colspan="3" rowspan="2">Clayey</td></tr>
<tr><td colspan="3">57.64</td><td colspan="3">29.72</td><td colspan="3">12.64</td></tr>
<tr><td colspan="12">Chemical analysis</td></tr>
<tr><td rowspan="2">pH</td><td rowspan="2">E.C. dSm^{-1}</td><td rowspan="2">Organic matter (%)</td><td rowspan="2">$CaCO_3$ (%)</td><td colspan="4">Soluble cations (meq./ l)</td><td colspan="4">Soluble anions (meq. /l)</td></tr>
<tr><td>Ca^{++}</td><td>Mg^{++}</td><td>Na^{+}</td><td>K^{+}</td><td>CO_3^{--}</td><td>HCO_3^{-}</td><td>Cl^{-}</td><td>SO_4^{--}</td></tr>
<tr><td>7.82</td><td>3.65</td><td>1.88</td><td>0.76</td><td>9.13</td><td>12.71</td><td>3.97</td><td>3.17</td><td>0.00</td><td>8.85</td><td>5.90</td><td>16.23</td></tr>
<tr><td colspan="12">Available nutrient (mg kg^{-1}soil)</td></tr>
<tr><td>N</td><td>P</td><td>K</td><td>Fe</td><td>Zn</td><td>Cu</td><td>Mn</td></tr>
<tr><td>56.22</td><td>17.53</td><td>276</td><td>2.47</td><td>0.86</td><td>0.71</td><td>0.54</td></tr>
</table>

2.6.3. Chemical constituents

In dry leaf samples taken from the third cut during both seasons, N, P and K were determined according to the methods [23].

2.7. Statistical Analysis

The statistical layout of this experiment was split-plot experiment in completely randomized block design. Data were analyzed according to [24]. The means were compared using computer program of Statistix version 9 [25].

3. Results and Discussion

3.1. Plant growth

Results presented in Tables 2 and 3 reveal that there was a gradual increase regard increasing humic acid rates in 1st, 2nd and 3rd cuts during both seasons. The highest values in marjoram height (52.84 and 54.00, 44.69 and 46.63 as well as 39.97 and 40.75 cm) and branches number per plant (16.33 and 18.75, 49.33 and 52.92 as well as 42.58 and 45.84 branch) were noticed under 6 kg humic acid / feddan in the first, second and third cut during 1st and 2nd seasons, respectively. In general, all humic acid rates significantly improved marjoram growth parameters compared to control, in most cases, during all cuts in the two consecutive seasons. According to [26], humic acids are a crucial component of soil that can influence several significant chemicals, biological, and physical aspects of soils as well as increase nutrient availability. Also, [27] noticed that applying bio-fertilizers increased the plant height and number of branches per plant of rosemary (*Rosmarinus officinalis* L.) plants.

Concerning the bio-fertilizer types effect, inoculating marjoram root zone with and type (mycorrhizae or/and azola) significantly increased marjoram growth parameters compared to control (without inoculation) in both seasons (Tables 2 and 3). The highest increase in plant height were achieved with mycorrhizae + azola treatment, while, the highest values in number of branches per plant were noticed when marjoram was inoculating with azola alone or mycorrhizae + azola without significant differences between them, in mostly. When bio-fertilizers were used in combination, the outcomes were superior to those obtained by using nitrogen fixers (*Azos. brasiliense*, *Azot. chroococcum* and *B. polymyxa*) or *B. circulans* alone in terms of plant height, stem diameter, and number of branches per marjoram plant [28]. According to [29], caraway (*Carum carvi* L.) plants grow taller and have more branches when Mycorrhiza and Azolla were applied.

Data listed in Tables 2 and 3 indicate that increasing humic acid rates under any bio-fertilizer type gradually increased marjoram growth parameters (plant height and branches number/ plant) in 1st, 2nd and 3rd cuts during both seasons. The highest values in marjoram height (54.40 and 55.60, 46.00 and 48.00 as well as 41.20 and 42.00 cm) and branches number per plant (17.00 and 19.67, 53.67 and 57.67 as well as 45.67 and 49.00 branch) were obtained when marjoram plant applied with humic acid at 6 kg/ feddan and inoculated with My +Az in the first, second and third cut during 1st and 2nd seasons, respectively. Likewise, marjoram plants fertilized with organic-biofertilizers recorded the highest significant values for plant height and number of branches compared to control treatment [30].

3.2. Herb and volatile oil yield

Data of both seasons presented in Tables 4 and 5 demonstrate that using different humic acid rates (2, 4 and 6 kg/ feddan) significantly increased herb fresh and dry weights per plant compared to control in 1st, 2nd and 3rd cuts during both seasons. Comparing to control, there were a significant enhance regard herb fresh and dry weights per feddan in three cuts during 2022 and 2023 seasons (Tables 6 and 7). The highest values in yield of herb per plant (g) and per feddan (ton) were observed when marjoram plants applied with humic acid at 6 kg/ feddan in both seasons. In most cases, all humic acid rates significantly raised volatile oil yield per feddan compared to control (Table 8) during the two tested seasons.

The concentration of humic acid has a significant impact on the biomass of the leaves and aboveground parts of the *Hyssopus officinalis* plant as well as the production of essential oils [31]. *Plantago psyllium* plants' seed output per plant, per feddan, and mucilage content per feddan were all significantly impacted by humic acid. The highest results were achieved when 4 kg of humic acid was added per feddan [32]. Additionally, humic acid treatments greatly enhanced the fruit yield per plot and per feddan (kg), as well as the volatile oil percentages and yields/feddan in *Lepidium sativum* seeds [9].

Application of mycorrhizae and azola alone or in combination increased the yield components of marjoram in terms of herb fresh and dry yields per plant and per feddan as well as volatile oil yield per feddan during three cutting (Tables 4, 5, 6, 7 and 8). The highest values in fresh herb yield per feddan of marjoram (4.96 and 5.78, 17.23 and 18.50 as well as 14.09 and 15.17 ton) and volatile oil yield per feddan (21.08 and 24.10, 55.89 and 60.07 as well as 71.82 and 77.19

tons) were noticed under the combination between mycorrhizae and azola in the first, second and third cut during 1st and 2nd seasons, respectively. Additionally, throughout the growth period of the crop, soluble minerals were bound to the plants via soil mobilization brought about by microorganisms. This enhanced crop nutrition led to a higher yield [33]. Furthermore, the inoculation of *Septoglomus viscosum* increased the output of essential oil in oregano, according to [34].

The best combination treatment concern herb and volatile oil yields of marjoram between humic acid rates and bio-fertilizer types was humic acid at 6 kg/feddan and azola alone or mycorrhizae + azola compared to the other combination treatments under study in both seasons (Tables 4, 5, 6, 7 and 8). In general, increasing humic acid rates from 2, 4 to 6 kg/ feddan gradually improved fresh or dry herb yields per plant and per feddan as well as volatile oil yield per feddan under any bio-fertilizer types in three cuts during the two seasons. In addition, yield components and volatile oil production were all greatly enhanced by inoculating caraway plants with azola and mixed mycorrhizae fungi at a high concentration of 300 ml/l of humic acid [29].

3.3. Nitrogen, phosphorus and potassium percentages

Data presented in Table 9 show that using humic acid rates significantly increased total nitrogen, total phosphorus and potassium percentages in marjoram herb compared to control in the three cuts during 1st and 2nd seasons. The highest values in N, P and K percentages were obtained with 4 or 6 kg /feddan of humic acid without significant differences between them, in most cases. Furthermore, it was shown that 4 liters per feddan of humic acid produced the highest percentages of N, P, and K in *Hibiscus sabdariffa*, followed by 2 liters per feddan, with a significant difference between them and the control plants [35].

Inoculated marjoram plant with azola alone or with mycorrhizae combination gave the highest values in the nitrogen, phosphorus and potassium percentages compared to control and mycorrhizae alone in both seasons (Table 9). Utilizing any bio-fertilizer type significantly increased NPK percentages in marjoram herb compared to control (un-inoculation plants) during 1st and 2nd seasons. According to [12], *Trifolium alexandrinum* plants with arbuscular mycorrhizal symbiosis had higher total biomass, N content, and N fixation. When compared to the untreated plants in the two cuts of both experimental seasons, the application of various N2-fixing bacteria resulted in a significant improvement in the chemical composition of the plant, including NPK percentages in dried rosemary herb [36].

Under any bio-fertilizer type (mycorrhizae or/ and azola) increasing humic acid rates gradually increased nitrogen, phosphorus and potassium percentages in the two seasons, in most cases (Table 9). The best combination treatment between humic acid and bio-fertilizer types was 4 or 3 kg/ feddan of humic acid interacted with azola alone or with mycorrhizae combination in both seasons. In addition, the high rate of humic acid (2.0 g/l) was the most effective application for enhancing nitrogen, phosphorus and potassium percentages in marjoram shoot [37]. Moreover, [38] indicated that phosphorein + Ceraline treatmentgave the highest values of P and K % of rosemary (*Rosmarinus officinalis* L.). Also, [39] reported that biofertilizer, *Pantoea agglomerans* and *Pseudomonas putida* significantly increased phosphorus and potassium contents of *Nigella sativa* compared to control.

Table 2. Effect of humic acid rate (A), bio-fertilizer type (B) and their interactions (A×B) on plant height (cm) of *Majorana hortensis* plant during 2022 and 2023 seasons

Humic acid rate (kg/feddan) (A)	Bio-fertilizers type (B)									
	Control	MIC	AZO	MIC +AZO	Mean (A)	Control	MIC	AZO	MIC +AZO	Mean (A)
	First season					Second season				
	First cut									
Control	45.33	47.63	48.40	48.97	48.58	46.33	48.67	49.50	50.03	48.63
2	48.57	49.33	49.87	50.77	49.64	49.63	50.43	50.97	51.87	50.73
4	49.47	50.37	50.87	51.27	50.50	50.53	51.43	51.97	52.37	51.58
6	51.70	52.20	53.07	54.40	52.84	52.83	53.33	54.23	55.60	54.00
Mean (B)	48.77	49.88	50.55	51.35		49.83	50.97	51.67	52.47	
LSD at 5%	A= 0.39		B= 0.13	A×B= 0.25		A= 0.04		B= 0.05	A×B= 0.9	
	Second cut									
Control	38.33	40.00	40.97	41.37	40.17	40.00	41.87	42.77	43.17	41.95
1	41.00	41.77	42.17	42.87	41.95	42.83	43.57	44.00	44.70	43.78
2	41.87	42.57	42.97	43.30	42.68	43.67	44.40	44.83	45.20	44.53
3	43.77	44.10	44.87	46.00	44.69	45.67	46.03	46.80	48.00	46.63
Mean (B)	41.24	42.11	42.75	43.39		43.04	43.97	44.60	45.27	
LSD at 5%	A= 0.45		B= 0.23	A×B= 0.64		A= 0.17		B= 0.09	A×B= 0.22	
	Third cut									
Control	34.33	36.00	36.77	37.07	36.04	35.00	36.70	37.47	37.77	36.74
2	36.77	37.30	37.77	38.43	37.57	37.47	38.08	38.50	39.17	38.31
4	37.27	38.07	38.43	38.77	38.14	38.03	38.80	39.17	39.53	38.88
6	39.13	39.43	40.13	41.20	39.97	39.87	40.02	40.93	42.00	40.75
Mean (B)	36.88	37.70	38.28	38.87		37.59	38.02	39.02	39.62	
LSD at 5%	A= 0.25		B= 0.13	A×B= 0.57		A= 0.10		B= 0.05	A×B= 0.18	

Table 3. Effect of humic acid rate (A), bio-fertilizer type (B) and their interactions (A×B) on number of branches / plant of *Majorana hortensis* plant during 2022 and 2023 seasons

Humic acid rate (kg/feddan) (A)	Bio-fertilizers type (B)									
	Control	MIC	AZO	MIC +AZO	Mean (A)	Control	MIC	AZO	MIC +AZO	Mean (A)
	First season					Second season				
	First cut									
Control	12.67	14.67	15.67	15.00	14.50	14.67	16.67	18.00	17.33	16.67
2	13.67	14.67	15.67	16.00	15.00	15.67	17.00	18.00	18.33	17.25
4	14.00	15.67	16.67	17.33	15.92	16.33	18.00	19.00	19.67	18.25
6	14.67	16.33	17.33	17.00	16.33	17.00	18.67	19.67	19.67	18.75
Mean (B)	13.75	15.43	16.34	16.33		15.92	17.59	18.67	18.75	
LSD at 5%	A= 0.17	B= 0.13	A×B= 0.25			A= 0.56	B= 0.37	A×B= 0.73		
	Second cut									
Control	34.67	44.00	50.33	46.00	43.75	37.33	47.33	54.00	49.33	47.00
2	38.67	45.33	46.33	45.33	43.92	41.67	49.00	50.00	49.00	47.42
4	39.67	46.33	47.33	46.33	44.92	42.67	50.00	51.33	50.33	48.58
6	41.00	50.33	52.33	53.67	49.33	44.00	54.00	56.00	57.67	52.92
Mean (B)	38.50	46.50	49.08	47.83		41.42	50.08	52.83	51.58	
LSD at 5%	A= 1.54	B= 1.26	A×B= 2.52			A= 0.62	B= 0.55	A×B= 1.11		
	Third cut									
Control	31.67	38.00	39.00	41.00	37.42	34.00	40.67	42.00	44.00	40.17
2	36.00	39.33	41.33	42.33	39.75	38.67	42.33	44.33	45.67	42.75
4	37.00	41.33	42.33	44.33	41.25	39.67	44.33	45.67	47.67	44.34
6	38.00	42.33	44.33	45.67	42.58	41.00	45.67	47.67	49.00	45.84
Mean (B)	35.67	40.25	41.75	43.33		38.34	43.25	44.92	46.59	
LSD at 5%	A= 1.52	B= 0.99	A×B= 1.99			A= 0.70	B= 0.40	A×B= 0.79		

Table 4. Effect of humic acid rate (A), bio-fertilizer type (B) and their interactions (A×B) on herb fresh weight / plant (g) of *Majorana hortensis* plant during 2022 and 2023 seasons

Humic acid rate (l/feddan) (A)	Bio-fertilizers type (B)									
	Control	MIC	AZO	MIC +AZO	Mean (A)	Control	MIC	AZO	MIC +AZO	Mean (A)
	First season					Second season				
	First cut									
Control	101.33	111.23	116.56	116.45	111.39	117.33	129.13	137.67	135.77	129.98
2	114.10	117.64	126.89	120.55	119.80	133.20	137.57	147.00	142.90	140.17
4	119.68	126.87	131.44	132.33	127.58	138.77	146.90	152.67	153.00	147.84
6	123.67	132.24	136.69	137.80	132.60	143.33	153.13	158.43	159.43	153.58
Mean (B)	114.70	122.00	127.90	126.78		133.16	141.68	148.94	147.78	
LSD at 5%	A= 8.21	B= 5.40	A×B= 10.81			A= 3.36	B= 1.97	A×B= 3.94		
	Second cut									
Control	278.13	354.97	380.43	393.37	351.73	299.33	380.97	408.23	422.03	377.64
1	293.77	372.33	418.37	430.87	378.84	315.43	399.90	448.87	462.57	406.69
2	304.57	406.03	431.80	456.33	399.68	326.67	435.70	463.23	489.77	428.84
3	302.20	443.77	456.67	481.87	421.13	331.43	476.33	489.90	517.10	453.69
Mean (B)	294.67	394.28	421.82	440.61		318.22	423.23	452.56	472.87	
LSD at 5%	A= 18.99	B= 11.62	A×B= 23.24			A= 9.01	B= 5.23	A×B= 10.47		
	Third cut									
Control	253.33	309.33	328.47	330.37	305.38	272.00	333.10	353.90	356.10	328.78
2	320.53	332.47	340.70	350.53	336.06	344.90	357.33	367.20	377.10	361.63
4	333.80	349.23	361.67	363.43	352.03	359.67	376.13	389.10	391.23	379.03
6	343.97	379.10	392.63	396.30	378.00	370.90	403.43	421.13	427.23	405.67
Mean (B)	312.91	342.53	355.87	360.16		336.87	367.50	382.83	387.92	
LSD at 5%	A= 25.04	B= 12.52	A×B= 34.17			A= 9.09	B= 4.54	A×B= 12.53		

Table 5. Effect of humic acid rate (A), bio-fertilizer type (B) and their interactions (A×B) on herb dry weight / plant (g) of *Majorana hortensis* plant during 2022 and 2023 seasons

Humic acid rate (kg/feddan) (A)	Bio-fertilizers type (B)									
	Control	MIC	AZO	MIC +AZO	Mean (A)	Control	MIC	AZO	MIC +AZO	Mean (A)
	First season					Second season				
	First cut									
Control	25.78	28.23	29.11	29.44	28.14	29.78	32.33	33.89	33.89	32.47
2	28.78	31.33	31.89	32.22	31.06	33.22	35.78	36.78	37.33	35.78
4	29.66	32.33	33.44	34.11	32.39	34.22	37.11	38.78	39.11	37.31
6	30.78	36.00	37.67	38.22	35.67	35.00	41.44	43.11	43.89	40.86
Mean (B)	28.75	31.97	33.03	33.50		33.06	36.67	38.14	38.56	
LSD at 5%	A= 3.33	B= 1.66	A×B= 5.02			A= 3.36	B= 1.97	A×B= 5.06		
	Second cut									
Control	56.00	61.89	63.67	64.78	61.59	60.22	66.89	67.89	69.33	66.08
2	64.67	70.11	73.11	73.89	70.45	69.22	75.44	78.44	79.33	75.61
4	66.00	78.44	82.22	83.66	77.58	70.78	84.22	88.44	90.00	83.36
6	69.11	89.67	93.89	96.66	87.33	73.78	96.44	100.33	102.78	93.33
Mean (B)	63.95	75.03	78.22	79.75		68.50	80.75	83.78	85.36	
LSD at 5%	A= 3.95	B= 1.97	A×B= 5.23			A= 1.62	B= 0.81	A×B= 2.03		
	Third cut									
Control	63.89	82.89	86.11	86.66	79.89	68.33	88.78	91.89	92.66	85.42
2	74.34	76.56	89.44	92.11	83.11	79.34	91.78	95.44	98.89	91.36
4	76.67	102.66	105.56	107.00	97.97	82.00	109.44	113.00	114.00	104.61
6	79.67	112.00	115.11	121.22	107.00	85.00	119.66	123.22	129.78	114.42
Mean (B)	73.64	93.53	99.06	101.75		78.67	102.42	105.89	108.83	
LSD at 5%	A= 7.01	B= 3.91	A×B= 10.81			A= 2.54	B= 1.32	A×B= 3.74		

Table 6. Effect of humic acid rate (A), bio-fertilizer type (B) and their interactions (A×B) on herb fresh weight /feddan (ton) of *Majorana hortensis* plant during 2022 and 2023 seasons

Humic acid rate (kg/feddan) (A)	Bio-fertilizers type (B)									
	Control	MIC	AZO	MIC +AZO	Mean (A)	Control	MIC	AZO	MIC +AZO	Mean (A)
	First season					Second season				
	First cut									
Control	3.96	4.35	4.56	4.55	4.36	4.59	5.05	5.38	5.31	5.08
2	4.46	4.60	4.96	4.72	4.69	5.21	5.38	5.75	5.59	5.48
4	4.46	4.96	5.14	5.18	4.99	5.43	5.75	5.97	5.98	5.78
6	4.84	5.17	5.35	5.39	5.19	5.61	5.99	6.20	6.24	6.01
Mean (B)	4.49	4.77	5.00	4.96		5.21	5.54	5.83	5.78	
LSD at 5%	A= 0.32	B= 0.21	A×B= 0.42			A= 0.08	B= 0.08	A×B= 0.15		
	Second cut									
Control	10.88	13.88	14.88	15.38	13.76	11.71	14.90	15.97	16.50	14.77
2	11.49	14.56	16.36	16.85	14.82	12.34	15.64	17.56	18.09	15.91
4	11.91	15.88	16.89	17.85	15.63	12.77	17.04	18.12	19.16	16.77
6	11.82	17.36	17.86	18.85	16.47	12.96	18.63	19.16	20.22	17.74
Mean (B)	11.53	15.42	16.50	17.23		12.45	16.55	17.70	18.50	
LSD at 5%	A= 0.74	B= 0.46	A×B= 0.91			A= 0.35	B= 0.20	A×B= 0.41		
	Third cut									
Control	9.91	12.01	12.85	12.92	11.95	10.64	13.03	13.84	13.93	12.86
2	12.54	13.00	13.32	13.71	13.14	13.49	13.98	14.36	14.75	14.15
4	13.05	13.66	14.14	14.22	13.77	14.07	14.71	15.22	15.30	14.83
6	13.45	14.83	15.36	15.50	14.79	14.51	15.78	16.47	16.71	15.87
Mean (B)	12.24	13.40	13.92	14.09		13.18	14.38	14.97	15.17	
LSD at 5%	A= 0.98	B= 0.49	A×B= 1.33			A= 0.35	B= 0.18	A×B= 0.49		

Table 7. Effect of humic acid rate (A), bio-fertilizer type (B) and their interactions (A×B) on herb dry weight / feddan (ton) of *Majorana hortensis* plant during 2022 and 2023 seasons

Humic acid rate (l/feddan) (A)	Bio-fertilizers type (B)									
	Control	MIC	AZO	MIC +AZO	Mean (A)	Control	MIC	AZO	MIC +AZO	Mean (A)
	First season					Second season				
	First cut									
Control	1.01	1.10	1.14	1.15	1.10	1.16	1.26	1.33	1.33	1.27
2	1.13	1.23	1.25	1.26	1.22	1.30	1.40	1.44	1.46	1.40
4	1.16	1.26	1.31	1.33	1.27	1.34	1.45	1.52	1.53	1.46
6	1.20	1.41	1.47	1.50	1.40	1.37	1.62	1.69	1.72	1.60
Mean (B)	1.13	1.25	1.29	13.31		1.29	1.43	1.50	1.51	
LSD at 5%	A= 0.11	B= 0.07	A×B= 0.16			A= 0.04	B= 0.02	A×B= 0.05		
	Second cut									
Control	2.19	2.42	2.49	2.53	2.41	2.36	2.62	2.66	2.71	2.59
2	2.53	2.74	2.86	2.89	2.76	2.71	2.95	3.07	3.10	2.96
4	2.58	3.07	3.22	3.27	3.04	2.77	3.29	3.46	3.52	3.26
6	2.70	3.51	3.67	3.78	3.42	2.89	3.77	3.92	4.02	3.65
Mean (B)	2.50	2.94	3.06	3.12		2.68	3.16	3.28	3.34	
LSD at 5%	A= 0.15	B= 0.08	A×B= 0.20			A= 0.06	B= 0.03	A×B= 0.08		
	Third cut									
Control	2.50	3.24	3.37	3.39	3.13	2.67	3.47	3.59	3.62	3.34
2	2.91	2.99	3.50	3.60	3.25	3.10	3.59	3.73	3.87	3.57
4	3.00	4.02	4.13	4.18	3.83	3.21	4.28	4.42	4.46	4.09
6	3.12	4.38	4.50	4.74	4.19	3.32	4.68	4.82	5.08	4.48
Mean (B)	2.88	3.66	3.88	3.98		3.08	4.01	4.14	4.26	
LSD at 5%	A= 0.27	B= 0.15	A×B= 0.31			A= 1.00	B= 0.05	A×B= 1.04		

Table 8. Effect of humic acid rate (A), bio-fertilizer type (B) and their interactions (A×B) on volatile oil yield/ feddan (l) of *Majorana hortensis* plant during 2022 and 2023 seasons

Humic acid rate (kg/feddan) (A)	Bio-fertilizers type (B)									
	Control	MIC	AZO	MIC +AZO	Mean (A)	Control	MIC	AZO	MIC +AZO	Mean (A)
	First season					Second season				
	First cut									
Control	15.11	17.17	18.07	18.09	17.11	17.34	19.57	20.83	20.69	19.61
2	17.43	19.10	19.78	20.04	19.09	19.97	21.72	22.62	23.01	21.83
4	18.07	49.97	21.03	21.35	20.11	20.72	22.75	24.15	24.39	23.00
6	18.86	22.84	24.21	24.84	22.69	21.37	26.11	27.56	28.32	25.84
Mean (B)	17.37	19.77	20.77	21.08		19.85	22.54	23.79	24.10	
LSD at 5%	A= 2.73	B= 1.36	A×B= 3.10			A= 1.06	B= 0.53	A×B= 1.15		
	Second cut									
Control	34.90	41.43	43.33	44.00	40.92	37.68	44.96	46.44	47.26	44.09
2	42.69	47.02	49.87	51.18	47.69	45.89	50.81	53.71	55.19	51.40
4	44.23	53.66	57.15	58.94	53.50	47.62	57.86	61.73	63.66	57.72
6	46.89	63.34	67.23	69.42	61.72	50.28	68.39	72.16	74.17	66.25
Mean (B)	42.18	51.36	54.40	55.89		45.37	55.51	58.51	60.07	
LSD at 5%	A= 3.61	B= 1.80	A×B= 5.18			A= 1.51	B= 0.75	A×B= 2.02		
	Third cut									
Control	42.46	56.90	59.57	60.19	54.78	45.63	61.22	63.87	64.57	58.82
2	51.03	53.20	62.51	64.53	57.82	54.71	63.95	66.96	69.57	63.80
4	53.03	71.60	74.48	75.74	68.71	56.99	76.68	80.12	81.13	73.73
6	55.55	79.03	82.18	86.80	75.89	59.57	84.85	88.39	93.47	81.57
Mean (B)	50.52	65.18	69.69	71.82		54.23	71.68	74.84	77.19	
LSD at 5%	A= 6.17	B= 3.15	A×B= 9.31			A= 2.28	B= 1.14	A×B= 3.41		

Table 9. Effect of humic acid rate (A), bio-fertilizer type (B) and their interactions (A×B) on N, P and K percentages of *Majorana hortensis* plant during 2022 and 2023 seasons in the third cut

Humic acid rate (kg/feddan) (A)	Bio-fertilizers type (B)									
	Control	MIC	AZO	MIC +AZO	Mean (A)	Control	MIC	AZO	MIC +AZO	Mean (A)
	First season					Second season				
	Total nitrogen (%)									
Control	2.35	2.36	2.48	2.36	2.39	2.31	2.42	2.49	2.47	2.42
2	2.45	2.43	2.53	2.54	2.49	2.44	2.49	2.51	2.54	2.50
4	2.49	2.47	2.58	2.59	2.53	2.48	2.54	2.56	2.58	2.54
6	2.53	2.43	2.56	2.56	2.52	2.54	2.56	2.58	2.61	2.57
Mean (B)	2.46	2.42	2.54	2.51		2.44	2.50	2.54	2.55	
LSD at 5%	A= 0.08	B= 0.07	A×B= 0.15			A= 0.02	B= 0.01	A×B= 0.04		
	Total phosphorus (%)									
Control	0.050	0.050	0.053	0.050	0.051	0.046	0.049	0.049	0.050	0.049
2	0.052	0.053	0.054	0.052	0.053	0.048	0.049	0.050	0.050	0.049
4	0.053	0.054	0.056	0.052	0.054	0.050	0.051	0.051	0.052	0.051
6	0.053	0.053	0.056	0.052	0.054	0.050	0.052	0.053	0.053	0.052
Mean (B)	0.052	0.053	0.055	0.052		0.049	0.050	0.051	0.051	
LSD at 5%	A= 0.028	B= 0.024	A×B= 0.048			A= 0.002	B= 0.001	A×B= 0.003		
	Potassium (%)									
Control	2.25	2.64	2.44	2.83	2.54	2.07	2.42	2.52	2.61	2.41
2	2.45	2.72	2.62	2.92	2.68	2.26	2.59	2.69	2.80	2.59
4	2.61	2.81	2.71	3.09	2.81	2.85	2.70	2.80	2.90	2.81
6	2.51	2.83	2.71	3.09	2.79	2.40	2.80	2.90	3.00	2.78
Mean (B)	2.46	2.75	2.62	2.98		2.40	2.63	2.73	2.83	
LSD at 5%	A= 0.20	B= 0.20	A×B= 0.41			A= 0.22	B= 0.019	A×B= 0.37		

4. Conclusions

Because humic acid and certain types of bio-fertilizer (mycorrhizae or/ and azola) naturally stimulate plant development and improve the nutritional content of marjoram, they can be given to the soil as a soil drench. Furthermore, it was thought to be a potential method of farming for growing aromatic plants.

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