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Influence of Chemical Fertilizers and Animal Manure on Morphological Traits of Medicinal Plants in Northern Iran

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ABSTRACT

In 2022, we conducted a comprehensive investigation into the effects of chemical fertilizers and animal manure on three distinct medicinal plants. Employing a factorial experiment design with three repetitions, we examined the first factor - chemical fertilizers - at three distinct levels, comprising the recommended 100% NPK dosage as a control, 50% of the recommended NPK (NPK50), and 75% of the recommended NPK (NPK75). The second factor was animal fertilizers, which we studied at four levels: cow manure, sheep manure, chicken manure, and a control group. Notably, the highest dry weight of the entire peppermint plant, an impressive 22.98 g, was achieved through a treatment utilizing 75% of the recommended NPK in conjunction with chicken manure. Conversely, the lowest dry weight for the entire peppermint plant was recorded in the recommended 50% NPK treatment without the use of animal manure, yielding a mere 13.9 g. Lemon balm and zufa plants, under the recommended 100% NPK treatment without animal manure, displayed maximum dry weights of 18.18 and 5.07 g, respectively. In contrast, the lowest dry weight for these plants was observed in the recommended 50% NPK treatment without animal manure, with rates of 5.87 and 18.1 g, respectively. Our results demonstrated the positive impact of employing 100% of the recommended NPK fertilizer without animal manure on the growth of the studied plant organs. Conversely, by reducing the application of both chemical fertilizers and animal manure, the growth conditions of these plants were markedly improved, resulting in significant enhancements in various measured parameters. It is worth noting that an excessive influx of inputs did not lead to increased growth.

***Keywords**:* Soil Enrichment, Agricultural Practices, Animal Manure and Fertilizer Impact

INTRODUCTION

Peppermint (*Mentha piperita* L.), zufa (*Hyssopus officinalis*), and lemon balm (*Melissa officinalis* L.) are all members of the Lamiaceae family. These three plants hold considerable value in the realm of medicinal herbs, owing to the extensive use of their essential oils in the production of various medical products, including antibacterial, antispasmodic, and cold remedies (Mehdiniya Afra *et al*., 2023).

Maximizing agricultural yield often entails the extensive utilization of various inputs, prominently among them being chemical fertilizers. However, an inherent dilemma arises as scientific research underscores the detrimental consequences of excessive chemical fertilizer usage. This includes the depletion of soil fertility, the compaction and hardening of agricultural land, and the escalation of environmental pollution (Zamani *et al*., 2023). This challenge was effectively addressed, and sustainable agricultural practices were ensured by prioritizing the preservation of soil health. In doing so, not only were the adverse environmental impacts mitigated, but the efficacy of development programs aimed at meeting the nutritional requirements of plants was also enhanced. In essence, striking a balance between agricultural productivity and environmental stewardship was hinged upon the promotion of soil health as a fundamental pillar of modern farming practices (Eyni *et al*., 2023).

In the contemporary agricultural landscape, the primary objective of fertilizer application is the optimization of production per unit area. However, this laudable goal is marred by a troubling imbalance in the consumption of chemical fertilizers. This imbalance often results in a mismatch between the nutrients supplied and the actual needs of the plants, leading to suboptimal outcomes. The persistent adherence to this prevailing trend of excessive and imbalanced chemical fertilizer use poses a significant and, in many cases, irreversible threat to a wide range of natural ecosystems (Zeidali *et al*., 2022; Fathi, 2022). Previous research has demonstrated that the provision of nutrients via fertilizers to medicinal plants had the potential to enhance their physiological attributes and elevate the production of secondary metabolites (Farnia *et al*., 2022). Sustainable agriculture practices primarily rely on organic inputs, such as animal manure, to supply the essential nutrients needed by both the soil and crop plants (Kamkar and Mahdavi Damghani, 2008). Animal manures gradually release nutrients into the soil, reducing the risk of nutrient loss through leaching. Furthermore, in addition to serving as a rich source of essential nutrients, these fertilizers also contribute to the enhancement of the soil's physical, chemical, and biological properties (Zamil *et al*, 2004). According to a report, the incorporation of organic matter into the soil has the potential to augment the quantity of available nitrogen for plants, enhance soil structure, and influence the diversity and composition of soil organisms (Shamim and Ahmed, 2010). Research conducted on a range of agricultural and medicinal plants has revealed that the enhancement in crop yield resulting from an escalation in the application of NPK chemical fertilizers is primarily attributed to the heightened accessibility of nutrients to the plants. Additionally, it has been established that relying solely on the mineralization of soil organic matter is insufficient to fulfill the nutritional requirements of these plants (Fathi *et al*., 2020). Furthermore, additional research studies have reported notable improvements in both the quantitative and qualitative attributes of ginger when subjected to the influence of chemical fertilizers, specifically NPK fertilizers (Akbari *et al*., 2022). In response to the challenges posed by the excessive use of chemical fertilizers and the consequent decline in soil fertility, the utilization of organic fertilizers has emerged as a promising solution. Organic fertilizers have demonstrated the capacity to enhance soil structure, stimulate microbial activity, and promote plant growth. The present study was undertaken to explore and analyze the impacts of both organic and chemical fertilizers on the attributes and performance of plant roots and shoots.

MATERIALS AND METHODS

This research project was conducted within the confines of the greenhouse facilities at the Technical and Vocational University, which is situated in Amol city. The precise geographical coordinates of the location are a latitude of 31 degrees and 34 minutes north and a longitude of 48 degrees and 53 minutes east. Additionally, the greenhouse is positioned at an elevation of 20 meters above sea level. The experimental framework adopted for this study entailed the application of a factorial design, specifically a randomized complete block design, and this was replicated three times for robustness. Within this design, two key factors were under scrutiny:

Chemical Fertilizer

This factor featured three distinct levels, which encompassed the Utilization of 100% of the recommended NPK as a control; Deployment of 50% of the recommended quantity of NPK50 and Application of 75% of the recommended quantity of NPK75.

Animal Manure

The second factor was comprised of four diverse levels, encompassing: Usage of cow manure as an input, Utilization of sheep manure, Incorporation of chicken manure and a control group where no animal manure was added.

Prior to the commencement of the experiments, soil sampling was undertaken from the pots utilized within the greenhouse. The outcomes of this initial soil sampling process are tabulated in Table 1, providing essential baseline information regarding soil characteristics. In addition, the research incorporated the examination of organic fertilizers. The results of the tests conducted on these organic fertilizers are presented in Table 2. These findings likely pertain to the composition, quality, or other relevant attributes of the organic fertilizers utilized in the study.

Table 1. Results from analysis of soil samples at a depth of 0-30 cm

soil texture	Ph	salinity)dS.m^{-1}(	organic matter (%)	K)ppm(	P(ppm)	N (ppm)
Clay loam	7.4	2.1	0.68	169	5.1	0.08

Table 2. Test results for used organic fertilizers

Fertilizer	nitrogen) %(	Phosphate)%(	potassium)%(
sheep manure	3.68	0.68	2.98
cow manure	2.27	0.64	2.4
chicken manure	3.62	1.99	1.67

The spacing between plants in each row was fixed at 4 centimeters. Before sowing, organic fertilizers were manually blended with the soil in the pots. Specifically, for nitrogen, the full recommended amount, which amounted to 2.5g per pot, was sourced from urea. Half of this nitrogen was applied during planting, while the remainder was administered when the plants reached the four-leaf growth stage. Additionally, phosphorus and potassium fertilizers were calculated and utilized at a rate of 2.5g per pot, in adherence to the recommended 100% dosage, drawing from sources such as triple superphosphate and potassium sulfate.

In treatments following the NPK50 regimen, fertilizer consumption was halved, while in the NPK75 treatments, all nitrogen, phosphorus, and potassium fertilizers were administered at a reduced rate of 1.87 g per pot. Plant seeds were manually sown at a depth of 1-2 centimeters. The initial irrigation was performed immediately after planting, and subsequent watering sessions were conducted at seven-day intervals, adjusted according to prevailing environmental conditions.

For the statistical analysis of the data acquired from soil sampling, SAS 9.1 statistical software was employed. To compare the means of the desired traits, the protected LSD (Least Significant Difference) test was applied.

Table 3. Analysis of the variance of the investigated traits

		Root volume			root fresh weight			Dry weight of the whole plant			Fresh weight of the whole plant			The length of the main stem		
	DF	zufa	lemon balm	peppermint	zufa	lemon balm	peppermint	zufa	lemon balm	peppermint	zufa	lemon balm	peppermint	zufa	lemon balm	peppermint
R	2	0.51**	0.18n.s	0.2**	1.08n.s	0.001n.s	0.64n.s	1.08n.s	0.66n.s	0.64n.s	23.4*	3.52n.s	11.9*	5.6n.s	4.1n.s	1.5n.s
Chemical fertilizers	2	0.1n.s	0.1n.s	0.2*	66.45**	0.003n.s	39.31**	66.45**	0.39n.s	39.3**	119.64**	2.07n.s	61.04**	63.9**	4.01n.s	17.7**
Manure	3	0.01n.s	2.08**	0.02	3.80**	0.15**	2.25**	3.8**	7.53**	2.25**	6.79n.s	39.87**	3.46n.s	3.3 n.s	149.1**	0.9n.s
Chemical fertilizer x manure	6	0.04n.s	0.76**	0.02	36.32**	0.02**	21.49**	36.32**	2.77**	21.49**	90.79**	14.6**	46.32**	23.5**	60.1**	6.5**
Residual	22	0.07	0.05	0.03	0.64	0.003	0.37	0.64	0.21	0.37	6.63	1.13	3.385	1.82	4.9	0.5
C.V. (%)		4.14	6.25	4.83	7.95	4.80	7.26	3.67	6.25	3.52	5.31	6.56	5.87	3.67	8.91	3.72

** ,* and ns, respectively, showed significant differences in the level of five percent, one percent and no differences are significant.

Table 4. Comparison of the average traits measured in different sources of chemical fertilizers and manure

NPK	manure	Root volume			root fresh weight			Dry weight of the whole plant		
		peppermint	lemon balm	zufa	pepper mint	lemon balm	zufa	pepper mint	lemon balm	zufa
50% recommended NPK	control	0.43a	0.97h	0.47a	4.94j	3.09g	6.43f	13.94j	5.87g	18.1f
	cow manure	0.41a	1.08fg	0.46a	5.95i	3.40fg	7.74ef	14.95i	6.47fg	19.4ef
	sheep manure	0.41a	1.11efg	0.46a	8.95e	3.86cde	11.63c	17.95e	7.3cde	23.3c
	chicken manure	0.42a	1.05gh	0.46a	5.97i	3.37fg	7.77ef	14.97i	6.40fg	19.5ef
75% recommended NPK	control	0.42a	1.20cde	0.46a	8.46f	3.75def	11c	17.46f	7.1def	22.7c
	cow manure	0.75a	1.25bcd	0.82a	9.27d	4.14vcd	12.05c	18.27d	7.8bcd	23.8c
	sheep manure	0.44a	1.15def	0.48a	7.32g	3.98bcde	9.51d	16.32g	7.5bcde	21.2d
	chicken manure	0.43a	1.42a	0.47a	13.98a	4.25bc	14.45b	22.98a	8.08bc	26.2b
100 % NPK recommended as control	control	0.68a	1.33ab	0.75a	11.12b	5.07a	18.18a	20.12b	9.64a	29.9a
	cow manure	0.51a	1.26bc	0.56a	10.47c	4.30b	13.62b	19.47c	8.17b	25.3b
	sheep manure	0.56a	1.24bc	0.62a	8.52f	3.97bcde	11.07c	17.52f	7.5bcde	22.8c
	chicken manure	0.62a	1.16cdef	0.68a	6.79h	3.59ef	8.82de	15.79	6.81ef	20.5de

In each column, the mean of each treatment, which has the same letters, does not differ significantly from the LSD test.

Table 5. Comparison of the average traits measured in different sources of chemical fertilizers and manure

		Fresh weight of the whole plant			The length of the main stem			The number of sub-stems		
NPK	manure	peppermint	lemon balm	zufa	peppermint	lemon balm	zufa	peppermint	lemon balm	zufa
50% recommended NPK	control	26.45f	13.5g	37f	17 fg	16.06h	32.3g	6.67e	6.4h	8.7e
	cow manure	27.04f	14.8fg	37.9f	17.92fg	21.48fg	34fg	7.33e	8.5fg	9.5e
	sheep manure	32.50bc	16.8cde	45.5bc	19.26	22.85efg	36.6cde	7e	9.14efg	9.1e
	chicken manure	29.18def	14.7fg	40.9def	17.81fg	23.98def	33.8fg	7e	9.5def	9.1e
75% recommended NPK	control	30.37cde	16.4def	42.5cde	18.94def	27.44abcd	36def	8.33d	10.9abcd	10.8d
	cow manure	32.56bc	18bcd	45.6bc	20.05bcd	26.99bcd	38.1bcd	9.67ab	10.8bcd	12.6ab
	sheep manure	30.3cde	17.3bcde	42.5cde	19.59bcde	26.04cde	37.2bcde	8.33d	10.4cde	10.8d
	chicken manure	34.33b	18.5bc	48.1b	20.38bc	29.83ab	38.7bc	8.67cd	11.9ab	11.3cd
100 % NPK recommended as control	control	38.75a	22.1a	54.2a	22.78a	30.93a	43.3a	9.33bc	12.3a	12.1bc
	cow manure	34.20b	18.8b	47.9b	20.53b	28.81abc	39b	10.33a	11.5abc	13.4a
	sheep manure	31.9bcd	17.3bcde	44.7bcd	19.58bcde	27.95abc	37.2bcde	9.67ab	11.1abc	12.6ab
	chicken manure	27.79ef	15.67ef	38.9ef	18.45ef	19.24gh	35.1ef	9bcd	7.6gh	11.7bcd

In each column, the mean of each treatment, which has the same letters, does not differ significantly from the LSD test.

Table 6. Analysis of the variance of the examined traits

		The number of sub-stems			Number of leaves per plant			Fresh weight of leaves			Dry weight of leaves		
	DF	zufa	lemon balm	peppermint	zufa	lemon balm	peppermint	zufa	lemon balm	peppermint	zufa	lemon balm	peppermint
R	2	3.1**	0.65n.s	1.86**	2.63n.s	5.93n.s	5.93n.s	2.65n.s	0.88n.s	1.56n.s	0.009n.s	0.091n.s	0.006n.s
Chemical fertilizers	2	35.2**	0.64n.s	20.86**	95.42**	5.77 n.s	214.7**	29.95**	0.51n.s	17.72**	0.81**	0.054n.s	0.566**
Manure	3	3.1**	23.8**	1.85**	2.56 n.s	214.7**	5.77n.s	1.56n.s	9.96**	0.92n.s	0.02n.s	1.037**	0.014n.s
Chemical fertilizer x manure	6	0.45 n.s	9.62**	0.26n.s	38.5 **	86.66**	86.66**	11.01**	3.6**	6.51**	0.15**	0.38**	0.106**
Residual	22	0.32	0.79	0.19	3.18	7.15	7.15	0.85	0.28	0.5	0.01	0.029	0.011
C.V. (%)		5.22	8.90	5.24	8.87	9.46	8.98	6.38	6.43	6.25	4.98	6.46	4.80

** ,* and ns, respectively, showed significant differences in the level of five percent, one percent and no differences are significant.

Table 7. Comparison of the average traits measured in different sources of chemical fertilizers and manure

NPK	manure	Dry weight of leaves			Fresh weight of leaves			Number of leaves per plant		
		peppermint	lemon balm	zufa	peppermint	lemon balm	zufa	peppermint	lemon balm	zufa
50% recommended NPK	control	1.85i	2.18c	2.22h	9g	6.75g	11.70g	19.27h	17.37h	12.85h
	cow manure	2.05g	2.40bc	2.46fg	9.92fg	7.44fg	12.89fg	25.77fg	23.87fg	17.18fg
	sheep manure	2.12f	2.72bc	2.54efg	11.26cde	8.44cde	14.64cde	27.42efg	25.52efg	18.28efg
	chicken manure	1.99h	2.37bc	2.39gh	9.81fg	7.36fg	12.75fg	28.78def	26.88def	19.19def
75% recommended NPK	control	2.28d	2.65bc	2.74cde	10.94def	8.20def	14.22def	32.92abcd	31.02abcd	21.95abcd
	cow manure	2.37c	2.92abc	2.85bcd	12.05bcd	9.04bcd	15.67bcd	32.39bcd	30.49bcd	21.59bcd
	sheep manure	2.19e	2.80abc	2.63def	11.59bcde	8.69bcde	15.07bcde	31.24cde	29.34cde	20.83cde
	chicken manure	2.69a	3.00abc	3.23a	12.38bc	9.29bc	16.10bc	35.80ab	33.90ab	23.87ab
100 % NPK recommended as control	control	2.52b	3.58a	3.02ab	14.78a	11.09a	19.22a	37.12a	35.22a	24.75a
	cow manure	2.40c	3.03ab	2.87bc	12.53b	9.40b	16.29b	34.57abc	32.67abc	23.05abc
	sheep manure	2.36c	2.80abc	2.83bcd	11.58bcde	8.68bcde	15.05bcde	33.54abc	31.64abc	22.36abc
	chicken manure	2.21e	2.53bc	2.65cdef	10.45ef	7.84ef	13.58ef	23.08gh	21.18gh	15.39gh

In each column, the mean of each treatment, which has the same letters, does not differ significantly from the LSD test.

RESULTS AND DISCUSSION

Root volume

Based on the results derived from this experiment, it was observed that the influence of chemical fertilizer, livestock treatment, and their interaction had a statistically significant impact on the root volume of lemon balm at a significance level of 1%. Conversely, this significance was not observed in the case of zufa and peppermint, as indicated in Table 3. Among the treatments applied to lemon balm, the treatment involving 75% of the recommended NPK and the utilization of chicken manure yielded the highest root volume, measuring at 1.42 cubic centimeters. In contrast, the lowest root volume in lemon balm was recorded in the treatment that employed 50% of the recommended NPK and did not incorporate any animal manure, resulting in a volume of 0.43 cubic centimeters, as outlined in Table 4. The research suggests that the accumulation of organic matter by soil bacteria played a pivotal role in augmenting root development and facilitating greater nutrient accessibility. This enhanced condition, driven by the organic matter, is believed to have contributed to increased plant performance, a phenomenon supported by prior research (Khatami *et al*., 2019).

root fresh weight

The results obtained from this experiment revealed significant effects of chemical fertilizer, livestock treatment, and their interaction on the root fresh weight of all three plant types: lemon balm, zufa, and peppermint, with statistical significance observed at the 1% level (Table 3). Peppermint exhibited its highest root fresh weight when subjected to the recommended 75% NPK treatment, in combination with chicken manure, resulting in a remarkable 13.98 g of fresh root weight. Conversely, the lowest root fresh weight for peppermint was recorded when the recommended 50% NPK treatment was applied, and no animal manure was utilized, yielding a meager 4.94 g (Table 4).

Lemon balm and zufa plants, on the other hand, displayed their highest root fresh weights under the recommended 100% NPK treatment, coupled with the absence of animal manure, at 18.18 g and 5.07 g, respectively. The lowest root fresh weight for both lemon balm and zufa was found under the recommended 50% NPK treatment and the omission of animal manure, resulting in root fresh weights of 3.09 g and 6.43 g, respectively (Table 4).

Similar research studies have consistently reported the advantageous impacts of organic fertilizers such as vermicompost and cow manure on the growth of lemon balm. This beneficial effect is often associated with the enhancement of morphological traits, including plant height, the number of stems per plant, the number of leaves per plant, dry shoot weight, root length per plant, root fresh weight per plant, dry root weight per plant, and the ratio of dry root weight to aerial part weight. These improvements have been found to significantly increase the percentage of essential oil in the plant, underscoring the profound biological implications of these fertilization practices (Badakhshan *et al*., 2019).

Dry weight of the whole plant

The results of this experiment demonstrated significant effects of chemical fertilizer, livestock treatment, and their interaction on the dry weight of the entire plant across all three plant varieties: lemon balm, zufa, and peppermint, with statistical significance observed at the 1% level (Table 3). Peppermint exhibited its highest dry weight when subjected to the recommended 75% NPK treatment, combined with 22.98 g of chicken manure, resulting in a substantial dry weight of 13.9 g for the entire plant. In contrast, the lowest dry weight for the entire peppermint plant was observed under the recommended 50% NPK treatment, with no animal manure used, yielding a reduced weight of 13.9 g (Table 4). In previous studies, it has been established that nutrient fertilizer has a significant impact on plant growth and dry weight. These essential nutrients are crucial for various aspects of plant development (Saleem *et al*. 2023).

Lemon balm and zufa plants displayed their maximum dry weights for the whole plant when the recommended 100% NPK treatment was applied in conjunction with the absence of animal manure, resulting in dry weights of 18.18 g and 5.07 g, respectively. Conversely, the lowest dry weights for the entire plants of both lemon balm and zufa were found under the recommended 50% NPK treatment, with no animal manure employed, yielding dry weights of 5.87 g and 18.1 g, respectively (Table 4).

Increased organic matter in the soil was found to augment nutrient availability and improve their absorption capacity, leading to a more balanced nitrogen content and enhanced phosphorus absorption efficiency. Additionally, the incorporation of animal manure into the soil not only supplied essential nutrients but also improved soil structure, enhanced moisture retention, facilitated better aeration, and created an environment conducive to root system growth and overall plant development. This, in turn, resulted in heightened vegetative growth, improved quality, and increased crop yields.

Previous studies, such as the work by Badakhshan *et al*. (2019), have underscored the positive influence of combining animal and chemical fertilizers. This combination has been associated with increased plant height, enhanced vegetative growth, augmented leaf count and leaf area, greater yields in terms of fresh and dry stem weight, fresh and dry leaf weight, and fresh and dry total plant weight, ultimately leading to improved economic performance in the case of lemon balm. Furthermore, the significance of treatments involving chicken manure and chemical fertilizers in influencing the trait of shoot dry weight can be attributed to the notable differences observed in the levels of photosynthetic pigments, as highlighted in research by Purbajanti *et al*. (2016).

Fresh weight of the whole plant

The results of this experiment revealed significant effects of both chemical fertilizer, livestock treatment, and their interaction on the fresh weight of the entire plant in all three plant varieties: lemon balm, zufa, and peppermint, with statistical significance at the 1% level (Table 3). The highest fresh weight for the entire plant was consistently observed in all three plant types when subjected to the recommended 100% NPK treatment and in the absence of animal manure. Specifically, for peppermint, lemon balm, and zufa, these treatments resulted in fresh plant weights of 38.75 g, 22.1 g, and 54.2 g, respectively. Conversely, the lowest fresh weight for the entire plant in all three plant types was observed under the recommended 50% NPK treatment, without the use of animal manure, yielding fresh weights of 26.45 g, 13.5 g, and 37 g for peppermint, lemon balm, and zufa, respectively (Table 5).

The researchers also reported that in peppermint, the highest quantities of fresh and dry weight of aerial organs were achieved during the budding stage, particularly when using nitrogen fertilizer in the range of 200-300 kg per hectare (Zeinali *et al*., 2014). These findings highlight the critical role of appropriate fertilizer treatments in maximizing plant growth and yield for specific plant species.

The length of the main stem

The results of this experiment highlight the significant effects of both chemical and animal fertilizer treatments, as well as their interaction, on the length of the main stem in all three plant varieties: lemon balm, zufa, and peppermint, with statistical significance at the 1% level (Table 3). The maximum length of the main stem was consistently achieved in all three plant types when the recommended 100% NPK treatment was employed, and no animal manure was utilized. Specifically, for peppermint, lemon balm, and zufa, these treatments resulted in main stem lengths of 27.78 cm, 30.93 cm, and 43.3 cm, respectively. Conversely, the minimum main stem lengths in all three plant types were observed under the recommended 50% NPK treatment, with no animal manure applied, yielding lengths of 17 cm, 16.06 cm, and 32.3 cm for peppermint, lemon balm, and zufa, respectively (Table 5).

These results underscore the pivotal role of chemical fertilizers in promoting crop growth and improving environmental conditions. By supplying the necessary nutrients required by plants, they enhance the relative growth rate and overall plant yield (Fathi and Mehdinia Afra, 2023).

Nitrogen, in particular, plays a crucial role in plant growth. The absorption of nitrogen during various growth stages is highly variable and depends on the consistent supply of nitrogen throughout the plant's growing season. Nitrogen availability significantly influences plant growth and contributes to biomass accumulation, as nitrogen is the second most abundant element in plant biomass after carbon. Furthermore, nitrogen's involvement in the formation of chlorophyll and proteins, along with its direct impact on plant growth, demonstrates its integral relationship with the accumulation of dry matter (Fathi, 2022; Fallah *et al*., 2018). These findings emphasize the essential nature of providing adequate nitrogen for plant growth and development, as well as its crucial role in protein function and chloroplast structure.

The number of sub-stems

The results of this experiment indicate that the effect of both chemical fertilizer and livestock treatment, as well as their interaction, had a significant impact on the number of sub-stems in all three plant varieties: lemon balm, zufa, and peppermint, with statistical significance at the 1% level (Table 3).

Peppermint demonstrated the highest number of sub-stems when subjected to the recommended 100% NPK treatment, combined with cow manure, resulting in a remarkable 10.33 sub-stems. Conversely, the lowest number of sub-stems for peppermint was observed under the recommended 50% NPK treatment, without the use of animal manure, yielding a reduced number of 6.67 sub-stems (Table 5). Lemon balm also displayed the highest number of sub-stems under the recommended 100% NPK treatment, in conjunction with a control group, with a count of 12.3 sub-stems. Conversely, the lowest number of sub-stems in lemon balm was found under the recommended 50% NPK treatment, without the application of animal manure, resulting in a count of 6.4 sub-stems (Table 5).

In the case of zufa, the highest number of sub-stems was obtained when the recommended 100% NPK treatment was combined with cow manure, yielding a count of 13.4 sub-stems. Conversely, the lowest number of sub-stems for zufa was observed under the recommended 50% NPK treatment, without the use of animal manure, with a count of 8.7 sub-stems (Table 5).

The study also found that the application of cow manure led to an increase in various morphological traits, including plant height, the number of branches, and essential oil content, when applied at a rate of 20 tons per hectare. Additionally, nitroxin inoculation was associated with a significant improvement in these traits (Razipour *et al*., 2016). These findings emphasize the positive impact of specific fertilization practices on plant growth and development, as well as the importance of considering both chemical and organic sources in optimizing plant characteristics.

Dry weight of leaves

The results of this experiment demonstrate the significant effects of both chemical fertilizer, livestock treatment, and their interaction on the dry weight of leaves in all three plant varieties: lemon balm, zufa, and peppermint, with statistical significance at the 1% level (Table 6).

Peppermint exhibited the highest dry weight of leaves when the recommended 75% NPK treatment was applied, along with chicken manure, resulting in a substantial 2.69 g of dry leaf weight. Conversely, the lowest dry weight of leaves for peppermint was recorded when subjected to the recommended 50% NPK treatment, without the use of animal manure, yielding a reduced weight of 1.85 g (Table 7).

Lemon balm also displayed its highest dry weight of leaves when treated with the recommended 100% NPK regimen, coupled with a control group, achieving a weight of 3.58 g. Conversely, the lowest dry weight of leaves for lemon balm was found under the recommended 50% NPK treatment, without the application of animal manure, resulting in a dry leaf weight of 2.18 g (Table 7). In the case of zufa, the highest dry weight of leaves was obtained when the recommended 100% NPK treatment was combined with cow manure, yielding a dry leaf weight of 3.23 g. Conversely, the lowest dry weight of leaves for zufa was observed under the recommended 50% NPK treatment, without the use of animal manure, with a weight of 2.22 g (Table 7).

The researchers' conclusion emphasized the positive impact of using sheep manure, which resulted in a higher number of leaves, an increased number of flowers, greater dry weight of leaves and stems, and a higher yield of dry matter per unit area. To mitigate environmental pollution, they recommended the use of sheep manure as an organic fertilizer (Sheykholeslami, 2015). These findings underscore the importance of selecting appropriate organic fertilizers for enhancing plant characteristics and overall yield while also considering environmental sustainability.

Fresh weight of leaves

The results of this experiment reveal that both chemical fertilizer, livestock treatment, and their interaction had a significant impact on the fresh weight of leaves in all three plant varieties: lemon balm, zufa, and peppermint, with statistical significance at the 1% level (Table 6). Peppermint displayed the highest fresh weight of leaves when treated with the recommended 100% NPK regimen, reaching an impressive weight of 14.78 g. In contrast, the lowest fresh weight of leaves for peppermint was observed under the recommended 50% NPK treatment, without the use of animal manure, yielding a weight of 9 g (Table 7).

Lemon balm also exhibited the highest fresh weight of leaves under the recommended 100% NPK treatment, with a control group, resulting in a weight of 11.09 g. The lowest fresh weight of leaves for lemon balm was observed under the recommended 50% NPK treatment, without the application of animal manure, with a weight of 6.75 g (Table 7). In the case of zufa, the highest fresh weight of leaves was obtained when the recommended 100% NPK treatment was applied, with a weight of 19.22 g. Conversely, the lowest fresh weight of leaves for zufa was recorded under the recommended 50% NPK treatment, without the use of animal manure, yielding a weight of 11.7 g (Table 7).

The research results of Rafieiolhossaini *et al*. (2019) emphasized that the application of animal manure significantly influenced various indicators, including fresh leaf yield, dry leaf yield, the number of leaves per stem, biological yield, essential oil percentage, and essential oil yield. These findings underscore the importance of considering the impact of animal manure and other organic fertilizers on plant growth and productivity, particularly when aiming to optimize essential oil content in aromatic plants like lemon balm, zufa, and peppermint.

Number of leaves per plant

The results of this experiment illustrate that the effect of both chemical fertilizer, livestock treatment, and their interaction significantly influenced the number of leaves in each plant of lemon balm, zufa, and peppermint, with statistical significance at the 1% level (Table 3).

Peppermint, lemon balm, and zufa plants consistently demonstrated the maximum number of leaves per plant when subjected to the recommended 100% NPK treatment, without the use of animal manure, achieving counts of 37.12, 35.22, and 24.75 leaves, respectively. Conversely, the lowest number of leaves per plant in all three plant types was observed under the recommended 50% NPK treatment, without the application of animal manure, resulting in leaf counts of 19.27, 17.37, and 12.85 leaves, respectively (Table 5). These findings emphasize the pivotal role of chemical fertilizer treatments in influencing the leaf count of aromatic plants such as lemon balm, zufa, and peppermint. Appropriate fertilization practices play a crucial role in optimizing plant characteristics, including leaf production, which is often indicative of overall plant health and potential yield.

CONCLUSION

The results of the study indicate that employing 100% of the recommended NPK fertilizer, without the addition of animal manure, has a positive impact on the growth of the plant organs under investigation. However, it's worth noting that reducing the consumption of chemical fertilizers and animal manure resulted in improved growth conditions for the studied plants. In fact, in certain traits, this reduction in chemical fertilizers led to significant increases in measured parameters. This suggests that the excessive application of inputs to the plants did not yield further growth benefits. These findings highlight the importance of optimizing the balance between chemical fertilizers and organic inputs like animal manure to achieve optimal growth conditions for plants, underscoring the principle that more is not always better when it comes to plant nutrition.

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