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Research Article

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Application of Nano-Biofertilizer under Abiotic Stress on the Vegetative Growth, Greenhouse Gas Emission, and Essential Oil Production of Rosemary (*Rosmarinus Officinalis* L.)

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Abstract

This study examines the effects of year, drought stress, and different fertilizer treatments on rosemary's growth, nutrient assimilation, and essential oil yield. Optimal growth was observed in the second year, while drought stress negatively impacted growth, which could be ameliorated by the application of nano-bio-fertilizers and bio-fertilizers. During the two-year study on rosemary, the second year displayed superior vegetative growth, but drought stress in the first year reduced essential oil percentage to 0.325% without fertilizers. Applying bio-fertilizers and nano bio-fertilizers, especially the nano-biofertilizer, increased the essential oil percentage to 1.57% in the second year despite a 30% accessible moisture condition. Under drought, rosemary's potassium levels in leaves increased, while nitrogen and phosphorus decreased, signifying shifts in nutrient uptake. Our research suggests that strategic fertilizer application can mitigate the adverse impacts of drought stress, optimizing growth and essential oil production in rosemary. However, more research is needed to understand these observations and create more effective cultivation strategies for medicinal plants.

Keywords: Biomik, Drought Stress, Phosphorus, Nutrient Assimilation, Intercellular Oxidation.

1. Introduction

Rosemary, a perennial medicinal plant native to the Mediterranean, has numerous phenolic compounds, contributing to its natural antioxidant properties, making it popular in pharmaceutical and food industries [1-3]. Irrigation and fertilization can optimize the medicinal content of plants, with environmental stresses influencing their secondary metabolites production [4,5]. Although different cropping practices have great impact on soil properties and health, water stress significantly affects growth, yield, gene expression, and secondary metabolite levels in plants [6]. Water shortage leads to soil salinity, reducing photosynthesis and, thus, plant growth and yield, in this situation different crop and soil practices can change soil health indicators to help plant survive under stressors [7]. Water stress has shown to affect growth parameters and nutrient concentrations in rosemary, impacting essential oil yield and composition [8,9]. A study found watering cycle frequency affected leaf nutrient concentrations and essential oil content.

Nanotechnology can enhance nutrient use efficiency and reduce environmental protection costs[10]. Nano-fertilizers, gradually releasing nutrients into the environment, can improve nutrient absorption, minimize soil pollution, and promote sustainable agriculture [11]. Given the challenge of managing agricultural inputs for optimal yield in harsh conditions, this study aims to determine the best fertilizer and water amount for maximum yield in rosemary.

2. Material and Methods

2.1 Soil Preparation

An experiment was conducted from 2019 to 2020 at Zabol University (35.6004°N 53.4367°E), Iran, in an area characterized by cold, dry winters and hot, dry summers. The study employed a randomized block design with three replications, under extremely hot and dry conditions as per Köppen's classification. The physical and chemical characteristics of the soil and meteorological statistics are provided in the study's tables 1 and 2.

Year	Rainfall (mm)	Maximum temperature (°C)	Minimum temperature (°C)	Average Temperature (°C)	Daily evaporation (mm)	Relative humidity (%)
2017	35.5	49.0	-2.2	23.1	13.7	19.1
2018	110.9	47.4	-4.0	23.7	12.45	19.6
2019	22..2	48.4	-4.0	22.5	12.3	20.1
average	53.0	49.0	-7.0	21.7	13.6	39.2

Table 1: The average meteorological data for the study area (Zahak Synoptic Station) during the years 2019 to 2020 were as follows

Soil texture	pH	EC[1] (dS.m^{-1})	Nitrogen (%)	Phosphorus (ppm)	Potassium (ppm)	Manganese (ppm)	Copper (ppm)	Zinc (ppm)	Iron (ppm)
Sandy-loam	8.4	1.45	0.02	4.60	100.0	5.60	1.15	0.46	10.40

[1] Electro Conductivity

Table 2: Physiochemical properties of soil before experiment in the depth of 0-30 cm.

Soil moisture levels were measured daily to determine optimal irrigation intervals. When the soil reached 30%, 50%, 70%, and 90% of plant-available water capacity, drip irrigation was applied. Manual weed management was performed five times during each growth period. The study evaluated drought stress at four levels (30%, 50%, 70%, and 90% plant-available moisture) as a primary factor, and the use of nano-bio fertilizer (Biomic), bio-fertilizer (Nitroxin and Mycorrhiza), and no-fertilizer as a secondary factor.

2.2 Nutrient Preparation

The nutrient requirement of rosemary is 125 kg per hectare of nitrogen, 75 kg per hectare of P2O5, and 50 kg per hectare of K2O [12]. The nano-bio fertilizer, Biomic, consists of Azotobacter, Bacillus, Pseudomonas, Azospirillum, 32% humic acid, 2% fulvic acid, 0.1% molybdenum, 12% potassium, 0.36% magnesium, 3.4% manganese, 0.36% calcium, 10% zinc, 9.5% iron, and various amino acids. The bio-fertilizer Nitroxin contains nitrogen-fixing bacteria from the genus Azotobacter chorococum, Azospirillum lipoferoum, and phosphate solubilizing from the genus Pseudomonas sp., with 10^8 live cells per milliliter. The bio-fertilizer Mycorrhiza includes two species, Glumus intraradices and Glumus etunicatum. The bio-fertilizers Biomic and Nitroxin were used at rates of three and five kilograms per hectare, respectively, by powder spraying.

2.3 Mycorrhiza Inoculation and Plant Preparation

The study utilized mycorrhiza inoculation by powder spraying two grams of active fungal organ from each species. The powder was prepared by mixing wheat bran with a gum arabic solution and then immersing it with Biomic and Nitroxin fertilizers, as well as G. intraradices and G. etunicatum fungi. This mixture was spread in the plots and watered. The Biomic fertilizer was produced by Biozer company, and the bio-fertilizers by Mehr Asia Biotechnology Company (MABCO). During the study, no chemical fertilizers or pesticides were used. Fertilizer treatments were repeated annually, coinciding with plant growth resumption in February.Harvesting was done during peak flowering in late June from a 4.5 square meter area. The harvested samples were shade-dried to maintain oil quality. Multiple factors like plant height, branch count, leaf nutrient content, and essential oil content were measured on ten plants per plot. Essential oil yield was calculated based on oil content and dry weight. The oil was extracted using a Clevenger apparatus, dehydrated, weighed, and stored for compound analysis. The constituents of the oil were identified using gas chromatography.

2.4 Statistical Analysis

The study's characteristics, based on 10 samples, were averaged over two years and subjected to analysis of variance and mean comparison. To confirm significant differences, Tukey's multiple range test was used at a 5% significance level. The data was statistically analyzed using SAS software version 9.2.

3. Results and Discussion

3.1 Vegetative Growth

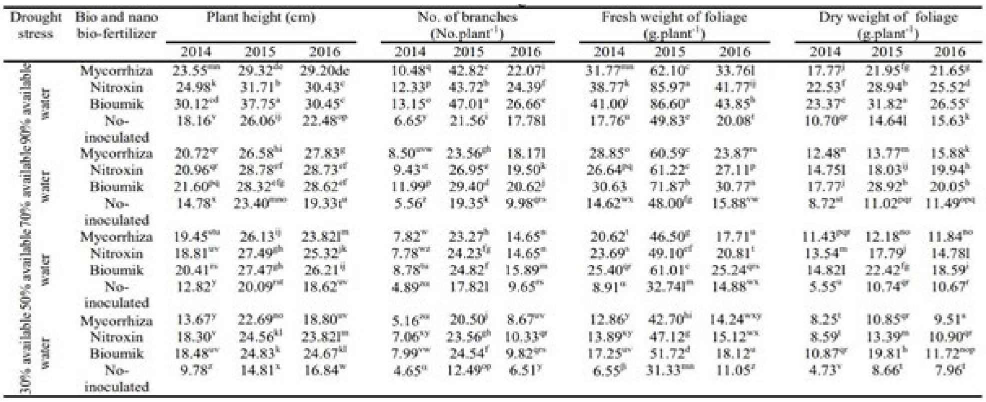
The primary effects of the experimental year, drought stress, fertilizer application, and their interactions had a significant impact on plant height, branch number per plant, and the fresh and dry weight of branches and leaves at a 1% probability level (Table 3).

S.O.V.	df	Plant height	No. branch	Foliage yield	Leaf Nitrogen	Leaf phosphorus	Essential oil	Essential oil yield
Year	2	660.83**	4089.03**	896480.36**	0.0004^{ns}	0.16838**	0.0031**	280419**
Drought stress	3	465.51**	998.38**	2481370.53**	139.188*	0.41437**	0.0029**	28153.4**
Drought stress × Year	6	3.83**	144.15**	10691.30**	0.0192^{ns}	0.00102**	0.554**	54326.2**
Bio-fertilizer	3	506.46**	515.99**	2226653.71**	137.280**	0.233175**	0.0005**	41680.3**
Drought stress × Bio-fertilizer	9	11.83**	24.82**	67784.29**	17.55**	0.02569**	1.539**	36002.1**
Bio-fertilizer × Year	6	1.03**	52.50**	114334.26**	0.1521**	0.0128**	0.0288**	55447.8**
Drought stress× Biofertilizer ×Year	18	5.30**	15.57**	11926.32**	0.0572**	0.00097**	0.1134**	13244.1**
Error	72	0.09	0.05	165.17	1.25	1.64	2.51	

*, ** and ns: significant at 0.05 and 0.01 probability levels and not significant, respectively.

Table 3: Combined analysis of variance of the effects of drought stress and bio and nano bio-fertilizers on rosemary vegetative characteristics, elements concentration and foliage and essential oil yield during 2019-2020

The optimal plant height (37.75 cm), branch number per plant (47.01 branches), and fresh and dry weight of branches and leaves (respectively 86.60 g and 31.82 g per plant) were observed under the irrigation treatment with 90% plant-available moisture and the application of nanobiomic bio-fertilizer in the second year. Conversely, the least robust growth parameters were found in the first year under the irrigation treatment with 30% plant-available moisture and without the application of fertilizer (Figure 1).



Drought stress	Bio and nano bio-fertilizer	Plant height (cm)			No. of branches (No.plant^{-1})			Fresh weight of foliage (g.plant^{-1})			Dry weight of foliage (g.plant^{-1})		
		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016
90% available water	Mycorrhiza	23.55^{mn}	29.32^{de}	29.20de	10.48^{q}	42.82^{c}	22.07^{i}	31.77^{mn}	62.10^{c}	33.76l	17.77^{j}	21.95^{fg}	21.65^{g}
	Nitroxin	24.98^{k}	31.71^{b}	30.43^{c}	12.33^{p}	43.72^{b}	24.39^{f}	38.77^{k}	85.97^{a}	41.77^{ij}	22.53^{f}	28.94^{b}	25.52^{d}
	Bioumik	30.12^{cd}	37.75^{a}	30.45^{c}	13.15^{o}	47.01^{a}	26.66^{e}	41.00^{j}	86.60^{a}	43.85^{h}	23.37^{e}	31.82^{a}	26.55^{c}
	No-inoculated	18.16^{v}	26.06^{ij}	22.48^{op}	6.65^{y}	21.56^{i}	17.78l	17.76^{u}	49.83^{e}	20.08^{t}	10.70^{qr}	14.64l	15.63^{k}
70% available water	Mycorrhiza	20.72^{qr}	26.58^{hi}	27.83^{g}	8.50^{uvw}	23.56^{gh}	18.17l	28.85^{o}	60.59^{c}	23.87^{n}	12.48^{n}	13.77^{m}	15.88^{k}
	Nitroxin	20.96^{qr}	28.78^{ef}	28.73^{ef}	9.43st	26.95^{e}	19.50^{k}	26.64^{pq}	61.22^{c}	27.11^{p}	14.75l	18.03^{ij}	19.94^{h}
	Bioumik	21.60^{pq}	28.32^{efg}	28.62^{ef}	11.99^{p}	29.40^{d}	20.62^{j}	30.63	71.87^{b}	30.77^{n}	17.77^{j}	28.92^{b}	20.05^{h}
	No-inoculated	14.78^{x}	23.40^{mno}	19.33t^{u}	5.56^{z}	19.35^{k}	9.98^{qrs}	14.62^{wx}	48.00^{fg}	15.88^{vw}	8.72st	11.02^{pqr}	11.49^{opq}
50% available water	Mycorrhiza	19.45^{stu}	26.13^{ij}	23.82l^{m}	7.82^{w}	23.27^{h}	14.65^{n}	20.62^{t}	46.50^{g}	17.71^{u}	11.43^{pqr}	12.18^{no}	11.84^{no}
	Nitroxin	18.81^{uv}	27.49^{gh}	25.32^{jk}	7.78^{wz}	24.23^{fg}	14.65^{n}	23.69^{s}	49.10^{ef}	20.81^{t}	13.54^{m}	17.79^{j}	14.78l
	Bioumik	20.41^{rs}	27.47^{gh}	26.21^{ij}	8.78^{tu}	24.82^{f}	15.89^{m}	25.40^{qr}	61.01^{c}	25.24^{qrs}	14.82l	22.42^{fg}	18.59^{i}
	No-inoculated	12.82^{y}	20.09^{rst}	18.62^{uv}	4.89^{za}	17.82l	9.65^{rs}	8.91^{a}	32.74l^{m}	14.88^{wx}	5.55^{u}	10.74^{qr}	10.67^{r}
30% available water	Mycorrhiza	13.67^{y}	22.69^{no}	18.80^{uv}	5.16^{za}	20.50^{j}	8.67^{uv}	12.86^{y}	42.70^{hi}	14.24^{wxy}	8.25^{t}	10.85^{qr}	9.51^{s}
	Nitroxin	18.30^{v}	24.56^{kl}	23.82l^{m}	7.06^{xy}	23.56^{gh}	10.33^{qr}	13.89^{xy}	47.12^{g}	15.12^{wx}	8.59^{t}	13.39^{m}	10.90^{qr}
	Bioumik	18.48^{uv}	24.83^{k}	24.67^{kl}	7.99^{vw}	24.54^{f}	9.82^{qrs}	17.25^{uv}	51.72^{d}	18.12^{u}	10.87^{qr}	19.81^{h}	11.72^{nop}
	No-inoculated	9.78^{z}	14.81^{x}	16.84^{w}	4.65^{a}	12.49^{op}	6.51^{y}	6.55^{b}	31.33^{mn}	11.05^{z}	4.73^{v}	8.66^{t}	7.96^{t}

Figure 1: Mean comparison of the effects of drought stress and bio and nano bio-fertilizers interactions on rosemary vegetative characteristics during 2019-2020 by SAS outcomes.

Vegetative growth characteristics exhibited variance across the experimental years, with the most vigorous vegetative growth recorded in the second year and the least in the first year. As rosemary is a perennial plant, it does not fully establish its root system in the first year, resulting in decreased nutrient uptake and, consequently, minimal growth characteristics. Drought stress and salinity was observed to diminish vegetative growth characteristics in long-term treatments, more similar researches recorded the same results resulting plants biomass reduction or gene expression alternations under long-term exposure, even for saline-resistance cultivars [13-16]. Plant height, branch number per plant, and fresh and dry weight of branches and leaves, akin to any other vegetative or reproductive organ, are profoundly influenced by nutrients and water [17]. Sreevalli suggest that the decline in performance with escalating levels of drought stress could be attributed to a shift in the allocation of photosynthetic materials from the aerial part of the plant towards the roots. Drought stress results in a decrease in water content, turgor pressure, total water potential, induces wilting, prompts stomatal closure, and curtails cell expansion and vegetative growth. The quality and quantity of a plant's vegetative growth are contingent upon cell division, cell enlargement, and differentiation, all processes impacted by drought stress [18]. According to drought stress triggered a decrease in the vegetative growth attributes of Thyme (Thymus vulgaris L.) [19]. Bio-fertilizers and Nano bio-fertilizers have demonstrated their efficacy in promoting vegetative growth attributes. Notwithstanding the diminution in water intake and consequential instigation of drought stress that reduced the dry matter yield in the plant, the application of Nano bio-fertilizers and bio-fertilizers, notably at elevated stress strata, could abate the adverse aftermath of drought stress on plant growth. This could be ascribed to the positive influence of bio-fertilizers in enhancing the plant's nutritional milieu under stress circumstances. Numerous sources have underscored the consequential role and impact of microorganisms in ameliorating the growth and performance of medicinal plants. As reported by bio-fertilizers contributed to an enhancement of the vegetative growth traits of thyme. The research conducted by 9. suggested that the utilization of Azospirillum and Azotobacter bio-fertilizers and mycorrhizal fungi resulted in an upsurge in parameters such as plant height, leaf surface area, dry matter accumulation, and growth velocity of black cumin (Nigella sativa L.) relative to the control [20,21]. A combination of mycorrhiza and Azospirillum was found to exert the most substantial influence on magnifying the examined traits. Furthermore, reported that inoculation of basil (Ocimum basilicum L.) with various strains of Azotobacter bacteria and Glomus fungi led to an increment in biomass, growth rate, and essential oil content. The application of Azospirillum and Azotobacter bio-fertilizers culminated in increased plant height and fresh and dry weight of the aerial parts of Sage (Salvia officinalis L.) across the first and second cuttings during two successive seasons [22]. Considering the comprehensive nature of nanobiomic bio-fertilizer relative to the other fertilizers employed, an improvement in plant growth subsequent to its application is anticipated. The incorporation of nanobiomic bio-fertilizer into the soil not only augments the provision of required nutrients for the plant but also ameliorates the physical conditions and biological processes of the soil.

3.2 The Percentage of Nitrogen, Phosphorus, and Potassium in the Leaves

The study conducted at Zabol University, Iran, during 2019-2020, investigated the influences of drought stress and fertilizer treatments on rosemary's growth, nutrient assimilation, and essential oil yield. The second year yielded optimal growth, attributed to mature root systems. Drought stress affected growth negatively, but the use of nano-bio-fertilizers and bio-fertilizers mitigated these effects. Drought stress led to a decrease in leaf nitrogen and phosphorus, while potassium levels increased. Cover cropping practices such as (Trifolium resupinatum L.) engaged with application of bio-fertilizer or appropriate nano-fertilizer promoting root growth, nutrient uptake, and improving soil conditions [23,24]. Under drought stress, production of secondary metabolites, including essential oil, increased. Both nano-systemic and biological fertilizers further augmented essential oil yield, demonstrating their effectiveness in high stress conditions. The research found a negative correlation between plant growth and essential oil percentage. Strategic fertilizer application could counteract the adverse effects of drought stress, optimizing rosemary's growth and essential oil yield [25,26]. However, further research is required to understand these mechanisms and develop more effective cultivation strategies for medicinal plants.

3.3 Percentage and Performance of Essence

The study found that the year, drought stress, and fertilizer, as well as their combined effects, significantly influenced the percentage and yield of rosemary essential oil (Table 3). The highest percentage of ess ential oil (1.57%) was achieved with 30% accessible moisture and Biomik nano-bio-fertilizer in the second year. The greatest essential oil yield was also obtained from this treatment (Table 4).

Drought Stress	fertilizer Nano + Bio	Essential oil (%)			Essential oil yield (g. plant^{-1})			Essential oil yield (Kg.h^{-1})		
		2014	2015	2016	2014	2015	2016	2014	2015	2016
90% available water	Mycorrhiza	0.515	0.680	0.615	10.93	14.93	11.15	601.25	821.07	613.30
	Nitroxin	0.595	0.805	0.645	14.53	23.29	15.18	799.33	1281.40	835.35
	Bioumik	0.605	0.895	0.735	17.18	28.49	16.06	944.98	1566.87	833.70
	No-inoculated	0.325	0.635	0.370	3.96	5.34	5.08	217.85	293.84	279.52
70% available water	Mycorrhiza	0.525	0.690	0.675	8.42	9.51	8.34	463.53	523.12	458.70
	Nitroxin	0.610	0.885	0.650	9.581	15.95	12.16	527.28	877.6	669.15
	Bioumik	0.615	1.115	0.845	6.97	12.08	6.13	383.44	665.07	337.30
	No-inoculated	0.365	0.675	1.480	0.895	7.69	19.82	7.35	422.91	1090.38

50% available water	Mycorrhiza	0.605	1.575	0.995	10.81	31.22	8.85	594.94	1716.93	487.01
	Nitroxin	0.610	0.635	0.370	3.96	5.34	5.08	217.85	293.84	279.52
	Bioumik	0.680	0.610	0.465	2.19	5.28	3.46	120.94	290.54	190.57
	No-inoculated	0.415	0.605	0.405	2.25	6.49	4.42	123.80	357.33	243.56
40% available water	Mycorrhiza	0.645	1.115	0.845	6.97	12.08	6.13	383.44	665.07	337.30
	Nitroxin	0.675	0.675	1.480	0.895	7.69	19.82	7.35	422.91	1090.38
	Bioumik	0.755	1.575	0.995	10.81	31.22	8.85	594.94	1716.93	487.01
	No-inoculated	0.435	0.610	0.465	2.19	5.28	3.46	120.94	290.54	190.57

Table 4: Mean comparison of the rosemary essential oil percentage and yield under the interactions of drought stress and bio and nano bio-fertilizers during 2019-2020.

In contrast, the lowest percentage of essential oil (0.325%) was found with 90% accessible moisture without any biological fertilizer in the first year (Table 6). The smallest yield was obtained with 30% accessible moisture and no fertilizer in the first year (Table 6). Drought stress was found to increase the percentage and efficacy of the plant's essential oil, confirming previous research findings in various plant species [27]. However, how environmental conditions impact secondary metabolites in medicinal plants remains unclear. The Carbon-Nutrient Balance (CNB) hypothesis suggests a balance between photosynthesis and growth affects the production of secondary metabolites [28,29]. Under drought conditions, the production of active substances in plants increases due to their role in preventing intracellular oxidation. Some critical enzymes involved in the biosynthesis pathway of secondary metabolites showed higher levels under drought stress, leading to an increase in essential oil percentage in several medicinal plants such as mint oregano sage, chamomile, and wormwood [30,31].

4. Conclusion

In recent years, interdisciplinary collaboration has become increasingly prevalent as various fields endeavor to develop innovative solutions to mitigate greenhouse gas emissions and address the ramifications of global warming. This trend is evident across a multitude of disciplines, ranging from nanotechnology to chemistry and from transportation to geology [32,33]. For instance, the pioneering application of nanotechnology in areas such as fertilization and the advent of Cooperative Automated Vehicle (CAV)-based traffic control systems underscore the concerted efforts of the scientific community in addressing these pressing environmental challenges. Our results indicated that drought stress led to a decrease in nitrogen and phosphorus concentration but increased the potassium concentration in the leaves. Nevertheless, the appropriate application of biological and nano-biological fertilizers with deep consideration on the amount of their application enhanced nutrient availability and improved soil conditions, promoting root growth and increasing access to essential mineral elements [34]. Drought conditions increased the plant's essential oil percentage, peaking at 1.57% in the second year with the aid of Biomik nano-bio-fertilizer. The use of bio-fertilizers and nano bio-fertilizers demonstrated significant potential in counteracting drought's adverse effects, improving growth and essential oil yields. The application of nano-systemic and biological fertilizers further enhanced the percentage and efficacy of the essence [35-61]. Given our findings, future research could delve deeper into optimizing the synergy between bio-fertilizers and plant growth phases, offering promising avenues to maximize rosemary's yield and essential oil concentration, especially in environments prone to drought and greenhouse gas emission.

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