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The effect of biofertilizer and potassium in reducing drought stress and improving black seed yield

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Abstract

A split factorial experiment was performed in the form of a randomized complete block design in three replications in Marvdasht city to investigate the application of mycorrhiza and potassium fertilizer in different irrigation methods of Nigella sativa. Four irrigation levels with 100, 75, 50, and 25% of the field capacity were considered the primary plot, and the application and non-application of mycorrhiza and Potassium fertilizer were considered secondary plots. As the drought stress increased, the number of follicles per plant, the number of seeds per follicle, 1000-seed weight, seed yield, oil percentage, and oil yield decreased. The percentage of Nigella sativa essential oil in irrigation treatment of 75% of the field capacity in the first year of the experiment had a maximum value of 1.89%. As the drought stress increases, the proline content, soluble sugars, and ion leakage increase under moisture stress conditions (25% of field capacity), and the phosphorus, potassium, and relative content of leaf water decreased. The application of mycorrhiza (simple effects) increased Nigella sativa traits, so its application showed a significant difference compared to its non-application. Also, the application of potassium fertilizer (simple effects) compared to the non-application of potassium fertilizer increased seed yield and some traits of Nigella sativa. Interaction effects showed that the application of mycorrhiza under the irrigation treatment of 75% of the field capacity increased Nigella sativa traits and no significant difference was found in some traits with irrigation treatment of 100% of the field capacity. Increasing the drought stress and the irrigation treatment of 25% of the field capacity reduced the efficiency of mycorrhiza and the application of potassium fertilizer. There was no significant difference between the application and non-application of mycorrhiza and the application and non-application of potassium fertilizer for most of the Nigella sativa traits.

Keywords: Proline, Essential oil percentage, Seed yield, Oil yield, Relative leaf water content.

Introduction

The correct consumption of chemical fertilizers, animal fertilizers, vegetable composts or green manures, and biological fertilizers is the most significant way to maintain and improve soil fertility conditions and increase the yield of agricultural products (Saleh Rastin, 2001). However, chemical fertilizers cannot be removed from the agricultural ecosystem at once. In this regard, the use of renewable and natural materials with the organic origin, and chemical fertilizers is crucial in maintaining the fertility, structure, and biological activity of the soil. The World Food and Agriculture Organization (FAO) has proposed the development of integrated systems of organic and chemical fertilizers for developing countries in recent years. According to the studies, the combination of chemical fertilizers and organic and biological resources has provided favorable results in increasing the yield of agricultural products, which can be a good way toward sustainable agriculture .A reduction in potassium causes a lack of plant growth or yellowing and burning of the ends of the leaves. Increasing the potassium concentration in the soil solution increases the plant's tolerance to salinity stress. When the amount of water accessible to the plant is low, increasing potassium even in high salinities (15decisiemens/m) increases the tolerance.

The use of potassium sulfate in saline conditions reduces the unfavorable effects of sodium and chlorine accumulation in plant leaves and ultimately increases the yield (Malakooti and Homae, 2004). Biological fertilizers are considered the most natural and desirable solution to keep the vital system of the soil alive and active. The supply of organic materials to the soil is the biggest advantage of such fertilizers thanks to meeting their significant requirements (Saleh Rastin, 2001). Supplying nutrients under the natural nutrition of plants and through creating biodiversity, intensifying vital activities, improving the quality and maintaining the health of the environment, and generally preserving and protecting national capitals (soil, water, and non-renewable energy sources) is considered one of the most significant advantages of biological fertilizers (Saleh Rastin, 2001). One of the biological fertilizers in today's agriculture is the use of mycorrhiza, which has different strains.

Identifying native fungi are necessary for every region to increase the yield. Sadat (2008) examined the effect of mycorrhiza species on the growth of wheat in saline conditions. He observed that wheat plants inoculated with Glomus intraradices absorbed higher contents of potassium, magnesium, and copper and lower contents of sodium and Chlorine compared to plants inoculated with Glomus etunicatum, and non-inoculated plants. Also, the parameters of spike length and weight, 1000-seed weight, and seed yield were higher in plants inoculated with this fungus.

The application of biological fertilizers, especially plant growth stimulating bacteria, and mycorrhiza (Azotobacter paspali), Azospirillum brasilense, and mycorrhiza symbiotic fungus (Glomus intraradaices) on the growth of Nigella sativa medicinal plant (Nigella sativa L.) showed that inoculation of Nigella sativa with biological fertilizers significantly increased plant height, leaf area index, maximum dry matter accumulation, and crop growth rate compared to the control. (Khorramdel et al., 2008).

Currently, the increase in their consumption of various crops has caused very serious problems such as water and air pollution and the resurgence of pests, etc. Thus, it is necessary to implement the management strategy of integrated consumption of different fertilizers in agriculture. The correct combination of applying chemical fertilizers and biofertilizers will be valuable to prevent the risk of irreversible destruction of energy production sources and to remove the disadvantages of chemical fertilizers. The application of micronutrient elements (control), zinc and iron, respectively, with concentrations of 2, 3, and 4 per thousand and a mixture of three elements in stress conditions (irrigation cycles with intervals of 7, 14, and 21 days) showed that the effect irrigation cycle significantly affected the plant height, number of branches, number of capsules per plant, number of seeds per plant and capsule, 1000-sed weight, seed and biological yield, and harvest index. By increasing irrigation intervals, the studied traits decreased. The highest seed yield was obtained in the 7-day irrigation cycle. Plant height, number of capsules per plant, number of seeds per plant and capsule, seed and biological yield, and harvest index were significant under the effect of micronutrient element foliar application. The differences between the treatments of foliar application of micronutrients and no foliar application (control) were significant in all the mentioned traits. The highest seed yield belonged to the treatment of the foliar application of a mixture of micronutrients (Shabanzadeh and Galui, 2011).

Also, climatic changes and drought conditions, and improper distribution of rainfall during the growing season of plants are other challenges in the agricultural sector. Since Iran has been located in an arid and semi-arid region and the rainfall level is less than the average level, the cultivation pattern and selection of plants can be fruitful.

Additionally, the highest 1000-seed weight was obtained in full irrigation treatment and Ardestan and Khomeinishahr ecotypes (Sardari et al., 2020). The results of the effects of drought stress caused by polyethylene glycol on shoot dry weight, ion leakage, relative leaf water content, photosynthetic pigments (chlorophyll a, b, total, and carotenoids), malondialdehyde and other aldehydes, anthocyanins, polyphenolic compounds, flavonoids, phenylalanine enzyme activity Ammonialiase, the content of soluble sugars and protein in Nigella sativa medicinal plant showed that drought stress significantly reduced the dry weight of aerial parts, the relative content of leaf water, photosynthetic pigments, anthocyanins, polyphenolic compounds, flavonoids, and protein and increased ion leakage, the amount of malondialdehyde and other aldehydes, the activity of the phenylalanine ammonialyase, and the content of soluble sugars (Kabiri et al., 2014).

The advantage of cultivating medicinal plants is clear to everyone. The significance of these plants can be a suitable cultivation model for fielders considering Iran's special weather conditions. Accordingly, the present study investigates the effects of drought stress on the yield and yield components of Nigella sativa by applying biofertilizers and potassium.

Methods and Materials

This experiment was conducted as a split factorial in the form of a randomized complete block design in three replications. The primary plot included four levels of irrigation (100, 75, 50, and 25% of the field capacity) and the secondary plot included the application and non-application of mycorrhiza and the application and non-application of potassium. Generally, 16 plots were considered in each block. The study was conducted in one year from 2017 to 2018 in Marvdasht city in Fatahabad agricultural lands. Table 1 presents the soil test results.

Table 1. Soil characteristics of the test site

Studied factor	Characteristics
Soil texture	loam - clay
pH	8.06
Electrical conductivity of soil (Disi Siemens)	1.17
Soil organic carbon percentage	0.076
Soil nitrogen percentage	0.05
absorbable phosphorus (ppm)	15.5
absorbable potassium (ppm)	176
Depth of sampled soil (cm)	0-30 cm

The plot length was also considered at 4 m. The interval between different irrigation levels was 1 m in the primary plot and 50 cm in the secondary plots. Each plot consisted of 8 crop lines at an interval of 25 cm. The sources of irrigation water were well water and canal water from Dorodzan Dam. Glomus mosseae mycorrhiza was used and Nigella sativa was inoculated with it before cultivation. The foliar application of potassium was used and Soluptas was used in this regard. It was used in the form of foliar application in two stages of 6-8 leaves and the reproductive stage of flowering. The maximum consumption of Soluptas in each stage was considered at 3 kg per hectare.

Quantitative and biochemical traits of Nigella sativa

To examine the relative water content of the leaves, a certain amount of Nigella sativa plant leaves were selected in the flowering stage and weighed with a precise scale (Abadi et al., 2018). The variance analysis of the data and mean comparisons were performed based on the minimum significant difference test at the 5% level in SAS software (version 9.4). Excel program was used to draw the graph.

Results

The results of the composite analysis indicated that the effect of year was not significant for traits related to yield and yield components of Nigella sativa for the traits of the number of side branches, 1000-seed weight, and biological yield. However, it was significant for other traits related to yield and yield components of Nigella sativa (Table 2).

Table 2. Composite variance analysis of different treatments on yield and yield components of Nigella sativa

Source of variations	df	Plant height	The number of lateral branches	The number of follicles per plant	The number of seeds in the follicle	1000-seed weight	Seed yield	Biologic yield	Oil content	Oil yield	Harvest index
Year	1	**513.23	ns1.83	**666.47	**2628.28	ns0.01	**578861.92	ns136786.48	**444.56	**90996.10	**1627.15
Error A	4	9.34	13.32	1.19	56.79	0.017	10868.72	202596.23	17.96	750.420	110.24
Irrigation	3	**3415.76	**1893.91	**488.73	**1546.94	**0.30	**1868543.04	**13140152.57	**1259.48	**266289.15	**990.65
irrigation*Year	3	ns10.30	**36.02	**15.67	*35.51	ns0.012	**44410.18	ns28481.03	ns15.67	**10279.10	ns18.15
Error B	12	13.51	7.80	2.16	41.84	0.036	*7015.30	79271.42	33.75	**2539.64	*35.27
Mycorrhiza	1	**829.32	**455.86	**87.54	**508.64	**0.006	**182048.17	**2922984.49	**114.19	**28097.04	ns0.84
potassium	1	**173.88	**44.19	**16.28	**165.97	ns0.13	**77935.55	**701689.02	**250.61	**19853.61	ns4.92
Irrigation* mycorrhiza	3	**52.92	**43.36	**6.63	ns17.06	ns000.002	**21600.32	**236601.69	ns4.73	**4079.43	ns5.71

International Journal of Multiphysics
Volume 18, No. 4, 2024
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Irrigation * potassium	3	ns13.18	ns4.37	ns2.61	ns1.83	ns0.021	ns5343.15	ns56225.78	ns3.04	*1779.33	ns12.54
Mycorrhiza* potassium	1	ns27.41	ns0.02	ns4.00	ns19.72	ns0.0003	ns2166.09	ns13.70	ns6.47	ns1186.05	ns1.21
Mycorrhiza* year	1	*30.91	ns5.94	ns1.31	ns1.36	ns00.012	ns45.39	ns173.24	ns0.05	ns10.07	ns29.54
Year*potassium	1	ns5.28	ns1.06	ns0.25	ns3.81	ns000.005	ns3657.25	ns13631.75	**49.52	ns1466.73	ns5.85
Irrigation*mycorrhiza*potassium	3	ns13.41	ns1.46	**9.17	ns3.70	ns000.006	ns2838.65	ns19752.43	ns4.66	ns755.61	ns9.32
Year* irrigation* Mycorrhiza	3	ns1.41	*17 .62	ns3.26	ns5.35	ns000.006	ns3283.52	ns69.6093	ns3.34	ns430.48	ns11.80
Year* irrigation* potassium	3	ns4.18	ns6.68	ns1.08	ns4.58	ns0000.006	ns3139.99	ns13538.27	ns5.16	ns642.38	ns2.03
year*Mycorrhiza*Potassium	1	ns0.01	ns12.27	ns0.20	*47.92	ns0.011	ns526.21	ns15413.19	ns7.08	ns420.62	ns11.51
year* irrigation* mycorrhiza* potassium	3	ns4.57	ns2.19	ns1.52	ns2.69	ns0.018	ns245.62	ns12001.71	ns0.33	ns88.86	2.58
error	48	7.99	4.90	1.62	10.86	0.017	3144.08	38570.86	7.68	548.55	17.56
Coefficient of variation		9.41	11.59	12.02	9.44	7.19	12.98	11.98	10.38	17.64	17.11

Ns, *, and **, respectively, represent no significance level, significance level at five and one percent, respectively.

The results of composite variance analysis on the biochemical and qualitative traits of Nigella sativa also showed that the effect of year on all traits was significant. Also, the effect of irrigation on all traits was significant (Table 3). The effect of mycorrhiza on all traits was significant. The effect of potassium was not significant only on the leaf nitrogen trait and significant on other traits (Table 3).

Table 3. Composite variance analysis of different treatments on biochemical and quantitative traits of Nigella sativa

Source of variations	df	Leaf nitrogen	leaf phosphorus	Leaf potassium	proline	Chlorophyll a	Chlorophyll b	Total chlorophyll	Soluble sugars	Ion leakage	Relative leaf water content	Essential oil percentage
Year	1	**6.10	**0.12	**179.8	**163.30	**1.26	**0.41	**3.12	**246.55	**382.26	**416.61	**3.42
Error A	4	6.04	0.001	2.15	1.29	0.008	0.007	0.013	69.12	34.02	31.06	0.05
Irrigation	3	**4.21	**0.11	**60.36	**150.07	**0.74	**0.11	**1.44	**9040.03	**5263.55	**4862.23	**2.06
irrigation*Year	3	ns0.20	**0.004	ns1.09	ns0.002	**00.088	**0.006	**0.14	ns7.19	ns20.40	ns10.85	**0.05
Error B	12	0.16	0.0017	1.54	0.81	**0.025	0.002	0.038	32.57	25.88	92.13	0.027
Mycorrhiza	1	**2.92	**0.041	**32.21	**30.40	**0.23	**0.054	**0.51	**1224.35	**943.00	**573.89	**0.37
potassium	1	ns00.07	**0.018	**15.92	**28.72	**0.13	**0.0087	**0.20	**948.92	**192.80	**89.73	**0.26

Irrigation* mycorrhiza	3	ns0.19	**0.003	ns1.50	**3.67	**0.054	**0.005	*0.051	**420.55	**70.10	**92.84	*0.042
Irrigation * potassium	3	ns00.08	**0.0031	**4.43	**3.79	ns0.026	ns00.0022	ns0.017	**108.01	**55.14	*41.04	ns0.008
Mycorrhiza* potassium	1	ns0.03	**0.005	ns0.24	**4.96	ns0.0008	ns0.0006	ns0.00001	**486.53	*52.98	ns1.66	ns0.004
Mycorrhiza* year	1	ns00.07	ns0.0002	ns1.13	ns00.06	ns0.10	ns0.002	ns0.023	ns8.22	ns1.39	ns18.38	ns0.000016
Year*potassium	1	ns0.007	ns0.00002	ns0.77	ns0.064	ns0.00002	ns0.0001	ns0.0002	ns3.04	ns34.20	ns5.07	ns0.0006
Irrigation*mycorrhiza*potassium	3	ns0.003	ns0.006	ns1.05	**2.16	ns0.005	**0.0054	ns0.018	ns61.38	*40.57	ns30.49	ns0.018
Year* irrigation* Mycorrhiza	3	ns0.038	ns0.0002	**2.55	ns0.04	ns0.0013	ns0.0004	ns0.0003	ns2.25	ns13.06	ns0.15	ns0.004
Year* irrigation* potassium	3	ns0.006	ns0.0002	ns0.09	ns0.046	ns0.006	ns0.0003	ns0.004	ns2.99	ns14.83	ns4.58	ns0.002
year*Mycorrhiza*Potassium	1	ns0.0002	ns0.0008	ns0.60	ns0.13	ns0.00003	ns0.00004	ns0.00000003	ns8.75	ns14.49	ns4.19	ns0.003
year* irrigation* mycorrhiza* potassium	3	ns0.012	ns0.0006	ns0.22	ns0.037	ns0.00007	ns0.0001	ns0.0003	ns13.04	ns11.16	ns15.01	ns0.0028
error	48	0.010	0.0005	0.62	0.59	0.011	0.0013	0.014	29.36	12.75	11.78	0.012

International Journal of Multiphysics
Volume 18, No. 4, 2024
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Coefficient of variation	16.41	9.44	9.18	8.49	15.18	10.42	11.26	8.89	7.12	5.13	8.00

Ns, *, and **, respectively, represent no significance level, significance level at five and one percent, respectively.

Analysis of the variance of different treatments on yield and yield components of Nigella sativa (the first year of the experiment):

The results of the variance analysis of different treatments on the yield and yield components of Nigella sativa in the first year of the experiment revealed that the effect of replication was significant for the traits of the number of seeds in the follicle, seed yield, biological yield, oil content, and harvest index. However, it was not significant for other traits (Table 4). The effect of different irrigation treatments on all the yield traits and yield components of Nigella sativa was significant. It was significant only for the 1000-seed weight trait at the 5% level and for the other traits; it was significant at the 1% level. The effect of fungus, as another source of variation, was not significant on all traits, except for two traits of 1000-seed weight and harvest index. It was significant for other traits at the level of 1%. Potassium, as another source of variation, was not significant for two traits of 1000-seed weight and harvest index. It was significant for other traits. The interaction effects of irrigation and mycorrhiza were not significant for the number of seeds per follicle, 1000-seed weight, oil percentage, and harvest index. However, they were significant for other traits. The interaction effects of irrigation and potassium were not significant for all traits. The interaction effect of fungus and potassium was not significant for all measured traits. Also, the three interaction effects were not significant for all traits (Table 2).

Table 4. Analysis of the variance of different treatments on the measured traits of Nigella sativa (the first year of the experiment)

Source of variations	df	Plant height	The number of lateral branches	The number of follicles per plant	The number of seeds in the follicle	1000-seed weight	Seed yield	Biologic yield	Oil content	Oil yield	Harvest index
replication	2	ns2.21	ns17.29	ns1.83	**95.68	ns0.019	**21665.60	*118441.05	*14.72	ns990.09	**180.81
Irrigation	3	**1576.60	**711.75	**333.85	**1024.65	**0.13	**1242153.30	**6964463.76	**529.21	**190571.59	**595.128
primary plot error	6	21.68	8.36	2.65	63.00	0.045	11579.59	75340.00	48.00	4091.64	53.92

Mycorrhiza	1	**269.99	**178.87	**55.14	**228.68	ns0.018	**82135.99	**1439080.82	**54.72	**13521.66	ns120.16
potassium	1	**119.89	*29.51	*10.29	*59.69	ns0.043	*23.913.65	**259859.53	**38.64	**5263.68	ns0.018
Irrigation* Mycorrhiza	3	*30.49	**35.51	**8.84	ns14.19	ns0.002	*14786.62	*90243.28	ns3.42	*2005.55	ns16.10
Irrigation * potassium	3	ns11.81	ns7.86	ns0.97	ns3.54	ns0.015	ns208.36	ns110.33.66	ns2.40	ns217.09	ns6.00
Potassium* Mycorrhiza	1	ns13.10	ns6.66	ns1.18	ns3.07	ns0.005	ns278.50	ns8173.22	ns0.006	ns97.04	ns2.61
Irrigation * Mycorrhiza * potassium	3	ns11.04	ns0.96	ns2.11	ns3.34	ns0.009	ns944.40	ns3149.38	ns1.99	ns183.52	ns6.29
error	24	10.61	5.95	1.97	11.94	0.018	3912.12	29049.17	3.29	548.52	17.01
Coefficient of variation		10.07	12.86	10.59	8.61	7.29	12.27	10.17	6.289	1432	23.67

Ns, *, and **, respectively, represent no significance level, significance level at five and one percent, respectively.

The effects of different levels of irrigation on yield and yield components of Nigella sativa (the first year of the experiment).

By increasing the drought stress, the plant height decreased. In the irrigation treatment of 25% of the field capacity, the lowest height of the Nigella sativa plant was observed at 19.73 cm (Table 5). The highest height was obtained in the irrigation treatment of 100% of the field capacity at 45.52 cm. In irrigation treatments of 75% and 50% of the field capacity, the height of the Nigella sativa plant was 37.55 and 26.50 cm, respectively. The results showed that the plant height decreased by 43% at the drought stress level of 50% of the field capacity. The percentage of reduction in the height of the Nigella sativa plant in the irrigation treatment of 75% of the field capacity was 18%. By reducing the available water content in the plant, the enzymatic activities of the plant decrease. Also, by reducing the enzymatic activities and plant growth, the growth and height of the plant decrease. Zareie et al. (2014) stated that plant height decreases under drought stress conditions since cell division and cell enlargement decrease due to a reduction in intracellular osmotic pressure.

The number of lateral branches was affected by different levels of irrigation and the lowest number of lateral branches (10.45) was obtained with the increase of drought stress and in the irrigation treatment of 25% of the field capacity. In irrigation treatment of 100% and 75% of field capacity, the number of lateral branches was 28.15 and 21.86 per plant, respectively. In irrigation treatment of 50 and 25 percent of the field capacity, the number of side branches did not decrease significantly as in 100 and 75 percent irrigation (Table 3). The percentage of a decrease in the number of lateral branches in two treatments of 50 and 25% of the field capacity compared to irrigation of 100% of the field capacity was 46 and 65%, respectively (Table 5). The results of the Ismail Pour et al.'s (2013) study showed that the number of lateral branches of the Savory plant was affected by different irrigation treatments. In the irrigation treatment of 100% of the field capacity, the number of secondary branches was 18. The number of sub-branches was 16 in the irrigation treatment of 60% of the field capacity and 12 in the irrigation treatment of 30% of the field capacity.

Comparing the mean number of seeds per follicle showed that the highest number of seeds was 49.45 in irrigation treatment of 100% field capacity (Table 3). By increasing drought stress and under the stress of 25% (irrigation treatment of 75% field capacity), the number of seeds in the follicle will reach 45.84. The number of seeds in the follicle was 36 and 29, respectively, in two treatments of 50 and 25% of the field capacity. The percentage of reduction in the number of seeds per follicle was 8, 26, and 41% in irrigation treatments with 75, 50, and 25% of the field capacity, respectively (Table 5). The presence of water in the reproductive phase causes an increase in photosynthetic activities and the growth of the plant. By increasing these activities, the number of seeds in the follicle increases. The number of follicles in a plant is more affected by moisture stress than the number of seeds in a follicle (Table 3). The 1000-seed weight of Nigella sativa was less affected by the irrigation treatments than other traits. The results showed that there was no statistically significant difference between the three irrigation treatments of 100, 75, and 50% of the field capacity and they were all in the same group.

The results showed that the weight of 1000 Nigella sativa decreased compared to other treatments in the irrigation treatment of 25% of the field capacity. This decrease led to a significant difference with the above three treatments (Table 5).

Comparing the mean percentage of Nigella sativa oil showed that in the irrigation condition of 100% of the field capacity, the highest percentage of oil was 35.79%, which was not significantly different from the irrigation treatment of 75% of the field capacity, which was 32.69% (Table 3). The results revealed that in the irrigation treatment of 50% of the field capacity, the percentage of oil decreased sharply and reached 25%. It reached 21% under severe stress and irrigation treatment of 25% of the field capacity (Table 5). No significant difference was observed between the irrigation treatment of 50% and 25% of the field capacity and they were placed in the same group. The results of Hassan et al.'s (2019) study on the thyme plant showed that the oil and plant yield decreased under severe stress.

Comparing the mean oil yield showed that in the irrigation treatment of 100% of the field capacity, the highest oil yield was 299 kg/ha, which was not significantly different from the irrigation treatment of 75% of the field capacity, which was 239 kg/ha (Table 5). The oil yield decreased sharply in the irrigation treatment of 50 and 25% of the field capacity, so it was 77 and 37 kg/ha in 50 and 25% of the field capacity, respectively (Table 3).

Analysis of variance of different treatments on quantitative and biochemical traits of Nigella sativa (in the first year of the experiment)

Table 5. Comparing the mean effects of different irrigation treatments on the measured traits of Nigella sativa (the first year of the experiment)

Irrigation level	Plant height	Number of lateral branches	Number of follicles per plant	The number of seeds in the follicle	1000-seed weight	Seed yield	Biologic yield	Oil percentage	Oil yield	Harvest index
Field capacity	cm				g	Kg/ha	Kg/ha	percentage	Kg/ha	Percentage
100	45.52 a	28.15 a	19.51 a	49.45 a	1.91a	833.30 a	25.28.25 a	35.79a	299.59 a	33.52a
75	37.55 b	21.87 b	15.71 b	45.86 a	1.89ab	731.79 b	2105.5b	32.69ab	236.67 a	35.41a
50	26.50 c	15.41 c	9.21c	36.13 b	1.89ab	297.20 b	1195.90 c	25.6bc	73.50b	24.95ab
25	19.73 d	10.45 d	8.57c	29.15 b	1.87b	176.01 c	887.06d	21.07c	37.24b	20.53b

Different letters in each column represent a significant difference at the 5% level.

The results of the analysis of variance on the measured traits showed that the effect of replication was not significant for the phosphorus traits, the relative content of leaf water, and the percentage of essential oil. However, it was not significant for other traits. The effect of different levels of irrigation on all measured traits of Nigella sativa was significant. The effect of mycorrhiza was also significant on all traits. The effect of potassium on the traits of nitrogen content, chlorophyll, ion leakage, and relative leaf water content was not significant. However, it was significant for other traits. The interaction effects of different levels of irrigation and mycorrhiza were not significant for phosphorus, potassium, proline, soluble sugars, relative leaf water content, and other traits. Additionally, the interaction effects of different levels of irrigation and potassium were not significant for the traits of phosphorus, leaf potassium, and the relative content of leaf water. However, it was not significant for other traits. The interaction effects of mycorrhiza and potassium were not significant for phosphorus, proline, soluble sugar content, and other traits. The mutual effects of the three investigated factors were significant only for the ion leakage traits and not significant for other traits (Table 6).

Effects of different levels of irrigation on quantitative and biochemical traits of Nigella sativa (the first year of the experiment)

Table 6. Analysis of variance of different treatments on the measured quantitative and biochemical traits (the first year of experiment)

Source of variation	df	Leaf nitrogen	leaf phosphorus	Potassium leaf	proline	Chlorophyll a	Chlorophyll b	Total chlorophyll	The content of soluble sugars	Ion leakage	Relative leaf water content	The percentage of essential oil
replication	2	ns0.031	**0.002	ns1.66	ns1.13	ns0.004	ns00.003	ns0.006	ns72.90	ns30.61	**52.64	**0.077

irrigation systems	3	**1.55	**0.04	**26.56	**74.92	**0.17	**0.034	**0.36	**4273.58	**2582.29	**2647.10	**1.36
primary plot error	6	0.15	0.0005	1.47	0.63	0.023	0.001	0.03	32.42	14.65	101.45	0.039
Mycorrhiza	1	**1.97	**0.0188	**10.640	**13.82	**0.074	**0.016	**0.16	**716.72	**435.60	**193.48	**0.18
potassium	1	ns0.06	*0.00888	*4.83	**12.97	**0.065	ns0.003	**0.096	**529.73	ns32.01	ns 26.06	**0.14
Irrigation* mycorrhiza	3	ns 0.15	*0.0016	*2.05	*2.01	ns0.019	ns0.002	ns0.022	**200.36	ns16.04	**43.94	ns0.010
Irrigation * potassium	3	ns0.037	ns0.0011	*2.29	*2.20	ns0.006	ns0.0005	ns0.005	ns69.53	ns 25.54	*32.29	ns00.006
Potassium* mycorrhiza	1	ns0.023	**0.0058	ns0.039	*3.36	ns0.0006	ns0.0005	ns0000.0000 07	**182.36	ns6.02	ns0.28	ns0.008
Irrigation * mycorrhiza * potassium	3	ns0.002	ns0.0023	ns1.09	ns1.07	ns0.0024	ns0.002	ns0.007	ns53.93	**41.56	ns 13.35	ns0.013
error	24	0.08	0.0004	0.63	0.60	0.0008	0.0009	0.0091	26.23	9.95	10.13	0.013
Coefficient of variation		16.85	9.41	10.93	9.92	15.43	10.70	10.79	8.63	6.63	4.61	7.24

ns, * , and ** represent non-significance, significance level at 5 and 1 percent, respectively.

Comparing the mean leaf nitrogen showed that the lowest leaf nitrogen was 1.29% in irrigation treatment of 100% of the field capacity, and it had a significant difference with other treatments. By increasing drought stress and in the irrigation treatment of 25% of the field capacity, the most nitrogen was 2.06%. Also, it was 1.99% in the irrigation treatment of 50% of the field capacity (Table 7). The amount of leaf phosphorus, compared to the amount of leaf nitrogen, showed a different trend. By increasing the drought stress and irrigation treatment of 25% of the field capacity, the amount of phosphorus decreased sharply and reached 0.16%. However, the amount of leaf phosphorus was 0.29% under normal irrigation treatment of 100% of the field capacity. The results revealed a significant difference between the four levels of irrigation regarding the amount of phosphorus. In irrigation treatments with 75 and 25%, the field capacity of leaf phosphorus was 0.24 and 0.19%, respectively (Table 8). In Mohammadpour et al.'s (2018) study, the amount of phosphorus in rosemary plants decreased by increasing drought stress.

Table 7. Comparing the mean effect of different irrigation treatments on quantitative and biochemical traits of Nigella sativa (the first year of the experiment)

Irrigation levels	Leaf nitrogen	leaf phosphorus	Leaf potassium	proline	Chlorophyll a	Chlorophyll b	Total chlorophyll	The content of soluble sugars	Ion leakage	Relative leaf water content	Essential oil
Filed capacity	percentage	percentage	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	percentage	percentage	percentage	percentage
100	1.29b	0.29a	8.89a	5.20d	0.73a	0.35a	1.08a	39.77d	30.89d	85.42a	1.80a
75	1.58b	0.24b	8.13ab	6.32c	0.68a	0.31a	0.99a	47.40c	42.30c	76.85a	1.89a
50	1.99a	0.19c	6.22bc	9.03b	0.50b	0.26b	0.76b	70.26.c	54.47b	60.83b	1.53b
25	2.06a	0.16c	5.80c	1067a	0.49b	0.23b	0.72b	79.93c	64.69a	52.76b	1.14c

Different letters in each column represent a significant difference at the 5% level

Table 8. Comparing the mean effects of mycorrhiza treatments on quantitative and biochemical traits of Nigella sativa (The first year of the experiment)

Mycorrhiza	Leaf nitrogen	leaf phosphorus	Leaf potassium	proline	Chlorophyll a	Chlorophyll b	Total chlorophyll	The content of soluble sugars	Ion leakage	Relative leaf water content	Essential oil
	percentage	percentage	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	percentage	percentage	percentage	percentage

Mycorrhiza application	1.93a	0.24a	7.73a	7.5b	0.64a	0.31a	0.94a	55.47b	45.07b	70.97a	1.65a
Mycorrhiza non-application	1.53b	0.20b	6.79b	8.43a	0.56b	0.27b	0.83b	63.20a	51.10a	66.96b	1.53b

Different letters in each column represent a significant difference at the 5% level

The amount of leaf potassium was also affected by different levels of drought stress. By increasing drought stress, the amount of leaf potassium decreased, so the lowest amount of leaf potassium was related to the irrigation treatment of 25% of the field capacity. In this treatment, the amount of potassium was 5.80 mg/g of dry matter. Also, the results revealed that in irrigation treatment of 100% field capacity, the highest amount of potassium was 8.89 mg/g of dry matter, which was not significantly different from other treatments, and were placed in the same group (Table 5). Heydari and Jahan Tighi (2013) concluded that the amount of potassium in Nigella sativa decreased and the amount of sodium and calcium elements increased by increasing drought stress.

Comparing the mean leaf proline also showed that the amount of proline increased linearly by increasing drought stress. In the irrigation treatment of 25% of the field capacity, the amount of proline reached 10.67 mg/g of leaf weight. However, in the irrigation of 100% of the field capacity, the amount of proline in leaves reached 20.5 mg/g of leaf weight (Table 5). Comparing the mean of the measured traits and the chlorophylls (a, b, and total) showed that the amount of chlorophyll decreased as drought stress increased. In irrigation treatment of 25% of the field capacity, the lowest amounts of chlorophyll a, b, and total was observed (Table 7). The results of Aslani et al.'s study (2011) revealed that the effect of irrigation cycles on the amount of chlorophyll (a, b, and total) of basil showed that the highest amount of chlorophyll was observed in the four-day irrigation cycle and the lowest was observed in the 12-day irrigation cycle.

The effect of mycorrhiza on quantitative and biochemical traits of Nigella sativa (the first year of the experiment).

Comparing the means of the application and non-application of mycorrhiza on quantitative and biochemical traits showed a significant difference between the application and non-application of mycorrhiza (Table 8). The results showed that mycorrhiza can affect the amount of these activities in the plant under drought-stress conditions. Mycorrhiza through mycelia and filamentous branches can enter the soil and pores that are not accessible to the plant's roots and filaments.

Comparing the means of application and non-application of potassium showed no significant difference regarding the leaf nitrogen, chlorophyll b, ion leakage, and relative leaf water content, and they were placed in the same group (Table 9). Potassium, as one of the essential macro-elements, plays a significant role in osmotic potential in plants. Potassium plays a key role in stomatal guidance, enzyme activation, protein synthesis, gas exchange, plant sap transfer, and resistance to environmental stress.

Table 9. Comparing the mean effects of different potassium treatments on quantitative and biochemical traits of Nigella sativa (the first year of the experiment)

Potassium fertilizer	Leaf nitrogen	leaf phosphorus	Leaf potassium	proline	Chlorophyll a	Chlorophyll b	Total chlorophyll	The content of soluble sugars	Ion leakage	Relative leaf water content	Essential oil
Field capacity	percentage	percentage	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	percentage	percentage	percentage	percentage
Potassium application	1.77a	0.23a	7.58b	7.29b	0.64a	0.30a	0.93a	56.02b	47.27a	69.70a	1.64a
Potassium non-application	1.69a	0.21b	6.94b	8.33a	0.55b	0.28a	0.84b	62.66a	48.91a	68.23a	1.52b

Different letters in each column represent a significant difference at the 5% level

Conclusion

The results revealed that the medicinal plant of Nigella sativa showed different reactions to different levels of drought stress. Irrigation treatment of 75% of the field capacity and the application of mycorrhiza can compensate for the damage caused by drought stress and make the conditions the normal irrigation of 100% of the field capacity. The results also showed that the yield and components of Nigella sativa decreased significantly with irrigation at 50% of the field capacity. However, in the irrigation of 50% of the field capacity, the application of mycorrhiza compared to the non-application of mycorrhiza led to an increase in traits related to yield and yield components of Nigella sativa. Thus, the efficiency of mycorrhiza was evident at this stress level. It is recommended to test the application of potassium sulfate fertilizer in the form of soil application under drought-stress conditions. Also, it is recommended to evaluate the drought stress conditions in the range of 50 to 80% of the field capacity along with other fertilizers such as potassium and zinc. Moreover, it is recommended to evaluate the effect of drought stress in different developmental and reproductive stages under the application of biological fertilizers such as nitroxin, azotobacter, and other growth stimulants.

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