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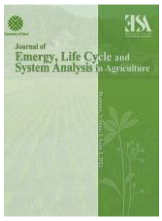

Investigation of organic and chemical fertilizer on the essential oil and seed yield of Moldavian balm (*Dracocephalum moldavica* L.)

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ABSTRACT

In order to examine the impact of biofertilizer on the essential oil and seed yield of Moldavian balm, a factorial experiment with a randomized complete block design and three replications were designed at the Khoy Agricultural Research Center in the West Azerbaijan Province. Five fertilization levels were applied: 100% urea (70 kg N ha−1), 75% urea (52.5 kg N ha−1) + 25% azocompost (3.85 ton ha^{-1}), 50% urea (35 kg N ha−1) + 50% azocompost (7.77 ton ha^{-1}), 25% urea (17.5 kg N ha−1) + 75% azocompost (11.55 ton ha^{-1}), and 100% azocompost (1555 ton ha^{-1}) on two Moldavian balm landrace (Urmia and the modern cultivar SZK-1). The results of this study indicated that a 100% urea fertilizer regime produced the highest dry herbage yield. The highest seed yield was achieved with a 100% urea fertilizer application of approximately 1,122 kg ha^{-1}. Inoculating seeds with Azotobacter and Azospirillum bacteria resulted in the highest harvest index, with a value of 22.4 percent. The Urmia landrace genotype had the highest yield of essential oil (20.5 kg ha^{-1}). Based on the findings, the 50% urea + 50% azocompost fertilizer treatment had the greatest effect on the majority of the investigated characteristics.

Highlights

- Five fertilization levels were examined on essential oil and seed yield to two Moldavian balm landraces.
- 100% urea fertilizer at 1,122 kg ha^{-1} yielded the most seeds.
- Azotobacter and Azospirillum bacteria inoculated seeds yielded the highest harvest index (22.4 percent).
- The 50% urea + 50% azocompost fertilizer treatment had the greatest impact on most of the characteristics.

1. Introduction

Since May 1978, the World Health Organization (WHO) has been studying medicinal plants. This study prompted the initial identification of 20,000 species of medicinal plants and a more detailed investigation of a short list of 200. Many of these plants have their origins in the world's tropical forests, and their present use is largely rooted in traditional medicines, which play a major role in maintaining the health and welfare of both rural and city dwellers in developing countries (Mahajan *et al.*, 2005). These days, around one third of human -needed medicines have herbal origins, and scientists, physicians, and pharmacologists all try to persuade people toward changing the other two thirds to medicinal plants too. Moldavian balm (*Dracocephalum moldavica* L., syn. Moldavian balm) is a perennial herb belonging to the Lamiaceae (Labiatae) family (El-Baky & El-Baroty, 2007). This plant is native to central Asia and has been naturalized in eastern and central Europe (Griffiths, 1994). In Iran, it is predominantly found in the north of the country, especially in the Alborz Mountains, where it is known as "Badarshoo." It is frequently consumed as a food additive (e.g., in yogurt) or as an infusion for its organoleptic properties. As a herbal drug, it is used in stomach and liver disorders, headache and congestion (Rechinger, 1986). Extracts of the plant are also used for their antitumor properties (Chachoyan & Oganesyan, 1996). Essential oil extracts from Moldavian balm have been reported to possess antibacterial,

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antimicrobial, and antioxidant activities (Kalinkina et al., 1991; Dastmalchi et al., 2007).

The indiscriminate use of nitrogen fertilizer in intensive agriculture has increased crop performance but also harmed ecosystems. Integrated nutrient management approaches advocate the controlled use of nitrogen fertilizer. Integrated nutrient supply involves the application of mineral fertilizers, bio-fertilizers such as those derived from *Azolla* spp., green manure crops, and bacterial inoculations (Richard & Woodbury, 1992; Epstein, 1997). The compost must be added to conventional NPK fertilizer to improve soil structure, making the soil easier to cultivate, encouraging root development, providing plant nutrients, and enabling their increased uptake by plants. Moreover, compost aids water absorption and retention by the soil, reducing erosion and run-off and thereby protecting surface waters from sedimentation; it also helps bind agricultural chemicals, keeping them out of waterways and protecting ground water from contamination (Leamaster et al., 1998). Members of the floating fern genus *Azolla* belong to the family Azollaceae. They host a symbiotic blue -green algae (*Anabaena azollae*), which can fix and assimilate atmospheric nitrogen. In Asia, *Azolla* spp. are used primarily to provide nitrogen nutrition to crops, such as rice. However, *Azolla* spp. can also accumulate other mineral nutrients, such as phosphorus and potassium, which become available to other plants when *Azolla* decomposes. Moreover, *Azolla* spp. are used as green manure, a water purifier, a biological herbicide, and as animal feed (Arora & Singh, 2003). Compost has already been established as a recommended fertilizer for improving the productivity of several medicinal and aromatic plants, such as mint, palmarosa, Moldavian balm, marjoram, *Acorus calamus* L., amaryllis, peppermint, *Tagetes erecta* L., chamomile, and basil (Rao, 2001; Kandeel et al., 2002; Naguib, 2003; Hussein et al., 2006; Gharib et al., 2008); However, little information is available about the effects of the combined application of azocompost and nitrogen fertilizer on the quality and quantity of essential oils in Moldavian balm plants. Thus, the objective of the study described herein was to determine the effects of different sources of nitrogen on the content and composition of essential oils in two genotypes of Moldavian balm in Iran.

2. Materials and methods

Field experiments were carried out during 2010 at the field research station of the Khoy Agricultural Research Center in West Azerbaijan Province (38º 35' N, 44º 52' E, and 1,040 m above sea level). Climate data for the growing seasons is provided in Table 1. The climate in this region is semi-arid, with warm and dry summers, a mean annual rainfall of 286.6 mm, and a mean annual temperature of 12 ºC. The soil is classified as a clay loam, with 38% clay, 22% silt, and 40% sand. The soil was air-dried and crushed before its pH, electrical conductivity (EC), and saturation percentage were evaluated. Next, we determined total organic carbon (using the Walkley and Black method, which involves sulfuric acid), total nitrogen (using the Kjeldahl method), available phosphorus (using the Olsen procedure), available potassium after extraction with ammonium acetate, and levels of the micronutrients iron, zinc, copper, and manganese after extraction with diethylene triamine pentaacetic acid (DTPA) (Tandon, 1995). Details of the properties of field soil are shown in Table 2. The experiment was laid out as a factorial in a randomized complete block design with three replications. They comprised a factorial combination of two genotypes (G1, the landrace genotype Urmia, and G2, the modern cultivar SZK-1), inoculation with bacteria (B1: Seed inoculation with Azotobacter and *Azospirillum* bacteria, and B2: No inoculation), and five fertilization regimes: F1, 100% urea (70 kg N ha^{-1}); F2, 75% urea (52.5 kg N ha^{-1}) + 25% azocompost (3.85 ton ha^{-1}); F3, 50% urea (35 kg N ha^{-1}) + 50% azocompost (7.77 ton ha^{-1}); F4, 25% urea (17.5 kg N ha^{-1}) + 75% azocompost (11.55 ton ha^{-1}), and F5, 100% azocompost (15.55 ton ha^{-1}). The physicochemical properties of the organic compost are shown in Table 3.

Table 1. Monthly temperature and precipitation during the growing season

Month	Average temperature (°C)			Total precipitation (mm)
	Minimum	Maximum	Mean	-
April	3.58	15.25	9.41	34.63
May	8.32	23.16	15.74	9.55
June	12.93	27.35	20.14	52.93
July	17.12	33.58	25.35	11.01
August	16.52	32.31	24.41	4.03

Table 2. Physicochemical characteristics of field soil

Soil Characteristics	Values
EC (dS m^{-1})	1.24
pH	7.7
Organic carbon (%)	0.81
Total N (%)	0.075
Available P (mg kg^{-1})	4
Available K (mg kg^{-1})	150
Fe (mg kg^{-1})	8.4
Zn (mg kg^{-1})	1.14
Cu (mg kg^{-1})	2.6
Mn (mg kg^{-1})	7.4

The composted manure and half of the total urea applied were broadcast by hand and incorporated immediately into the soil using a rototiller three days before planting. The remaining half of the urea was applied as a top dressing when the Moldavian balm seedlings were at the six-leaf stage. The plots were 3 m long and consisted of six rows, which were spaced 0.375 m apart. A 2-m alley was maintained between all plots to eliminate any influence of lateral water movement. Seeds were planted by hand on April 9, 2010, at a rate of 1 g of seed m^{-1} of row, and then were thinned at the four-leaf stage to achieve a density of approximately 133,333 seeds ha^{-1} of field. Weeds were controlled by hand weeding using a hoe and/or a rototiller whenever necessary. Throughout the growing season, sufficient water to support optimal plant growth was supplied. The plants were harvested once flowering was complete. We used an all-glass Clevenger-type apparatus to conduct 2.5 h of hydro-distillation on dried aerial parts (50 g) of *Dracocephalum moldavica* L., which were collected after flowering was complete. This method for the extraction of oils is recommended by the European Pharmacopoeia (European Pharmacopoeia, 1983). The analysis of variance (ANOVA) of the data from each attribute was computed using SAS version 9.1 (SAS Institute, 1988). Microsoft office Excel (2007) was used for figures drawing

3. Results and Discussion

The analysis of variance for dry herbage yield, essential oil yield, seed yield, and harvest index (HI) is summarized in Table 4. The results showed differences among genotypes were significant for essential oil yield and seed yield, and bacteria had a significant effect on seed yield and harvest index (P<0.05) (Table 4). Analysis of variance also showed that fertilizers had a significant effect on dry herbage yield, essential oil yield, and seed yield, and the interaction between bacteria treatments and fertilizers was just significant for essential oil yield (Table 4). The F1 (39003 kg ha^{-1}) and F5 (3083 kg ha^{-1}) regimes produced The highest and lowest dry herbage yields for each genotype was found on the F1 (3903 Kg ha^{-1}) and F5 (3083 Kg ha^{-1}) regime, respectively (Figure 1). Nitrogen is one of the most important nutrients for crop production and is essential for ensuring optimal dry matter production, leaf surface area, and rates of photosynthesis (Rao, 2001). Also, Heidarzadeh et al. (2021) showed that the use of chemical fertilizer had the highest biomass in *Dracocephalum kotschyi* Boiss. It can be due to the easy absorption and availability of chemical fertilizers (Heidarzadeh et al., 2021). Nitrogen deficiency decreases both vegetative and reproductive phonological development, yield components, and total yield in most plants. Available nitrogen in organic manure is released gradually into the soil, which results in a reduction in dry matter production relative to nitrogen fertilizers such as urea.

Table 4. Mean square values from analysis of variance (ANOVA) of dry herbage yield, essential oil yield, seed yield and harvest index under effect of genotype, fertilizer and bacteria.

S.O.V	df	Dry herbage yield	Essential oil yield	Seed yield	Harvest index
Rep.	2	334157.44ns	16.354ns	5718.156ns	8.63ns
Genotype	1	85.94ns	112.882*	57817.29**	23.69ns
Bacteria	1	313237.87ns	0.018ns	60872.01*	54.74*
Fertilizer	4	1316638.79*	104.54**	230131.82**	9.71ns
Genotype × bacteria	1	603237.68ns	28.649ns	711.143ns	6.94ns
Genotype × fertilizer	4	184820.07ns	32.735ns	4839.184ns	5.65ns
Bacteria × fertilizer	4	260457.47ns	64.004*	6344.001ns	5.78ns
Genotype × bacteria × fertilizer	4	281506.99ns	14.325ns	3400.1ns	3.07ns
Error	38	420913.39	20.602	10668.033	13.65
CV		18.33	23.68	10.9	17.26

* = p < 0.05, ** = p < 0.01, ns = non-significant

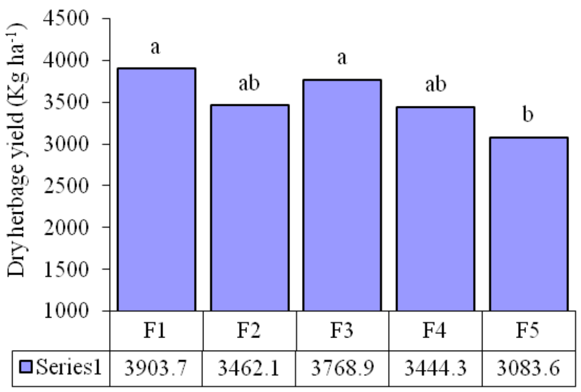

Figure 1. Effect of fertilizer treatments on dry herbage yield of Moldavian balm

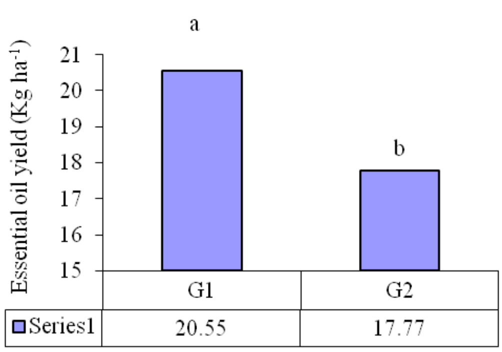

Figure 2. Effect of genotype on essential oil yield of Moldavian balm

The comparison between genotypes (G1, the landrace genotype Urmia, and G2, the modern cultivar SZK-1), showed that G1 (20.5 Kg ha^{-1}) had the highest amounts of essential oil yield and had a significant difference with G2 (17.7 Kg ha^{-1}) (Figure 2). The result of this study showed that there is no significant difference between the F1 and F3 fertilizer regimes for essential oil yield, but the highest essential oil yield was obtained with the F3 fertilizer regime (Figure 3). The lowest amount of essential oil yield was observed in the F5 fertilizer regime (Figure 3). Increases in the percentage oil content following the application of nitrogen and/or compost were observed in basil (Sifola & Barbieri, 2006), *Nigella sativa* L. (Ashraf et al., 2006), *Oenothera biennis* L. (Sekeroglu & Ozguven, 2006), Moldavian balm (Hussein et al., 2006), marjoram (Gharib et al., 2008), and chamomile (Naguib, 2003). Based on the comparison of interactions between fertilizer regimes and bacterial treatment, there is no significant difference among F1B1, F3B1, F4B1, F1B2, F2B2, and F3B2 treatments, but the highest amount of essential oil yield was measured in the F3B2 treatment (23.33 Kg ha^{-1}). But Delfieh et al. (2022) showed that there was no positive effect of biofertilizers (Azotobacter and Azospirillum) on fennel. The F5B1 treatment had the minimum amount of essential oil yield (Figure 4). There is a significant difference between G1 and G2 for seed yield, and the highest seed yield was obtained for the G2 genotype (980.9 kg ha^{-1}) (Figure 5).

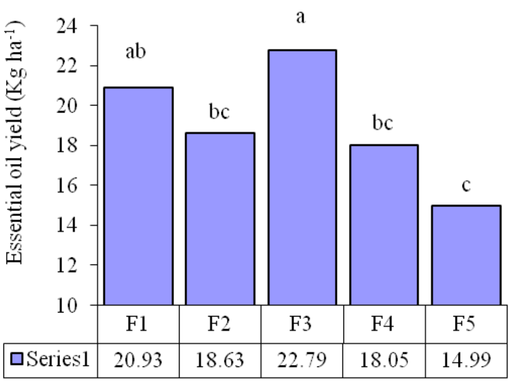

Figure 3. Effect of fertilizer treatments on essential oil yield of Moldavian balm

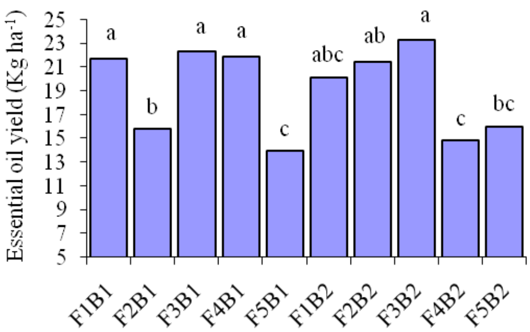

Figure 4. Effect of interaction of fertilizer×Bacteria treatments on essential oil yield of Moldavian balm

The results of Figure 6 showed that the application of the B1 treatment caused an increase in seed yield. The result of the comparison of fertilizer treatments for seed yield of both genotypes showed that the F1 fertilizer had a significant difference from other fertilizer treatments (Figure 7), and the highest seed yield was obtained in this treatment with an amount of about 1122 kg ha^{-1}. We observed that the harvest index (HV) of Dracocephalum moldavica L. can be affected by bacterial treatments. Our results showed that the highest HV was obtained in the B1 treatment, with a value of 22.4 percent.

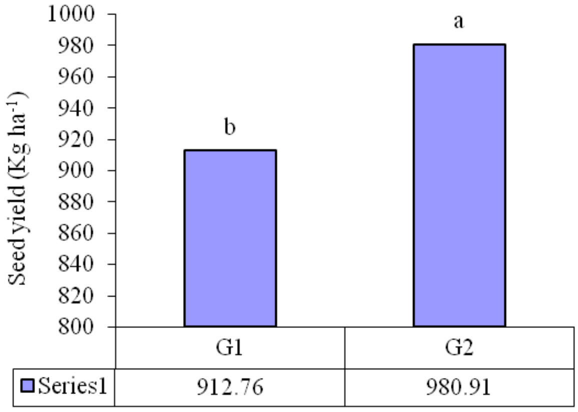

Figure 5. Effect of genotype on seed yield of Moldavian balm

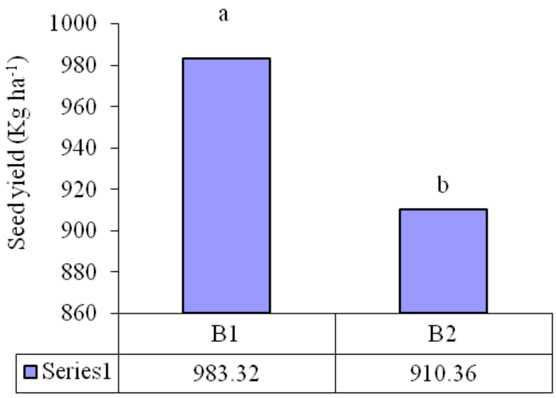

Figure 6. Effect of Bacteria treatment on seed yield of Moldavian balm

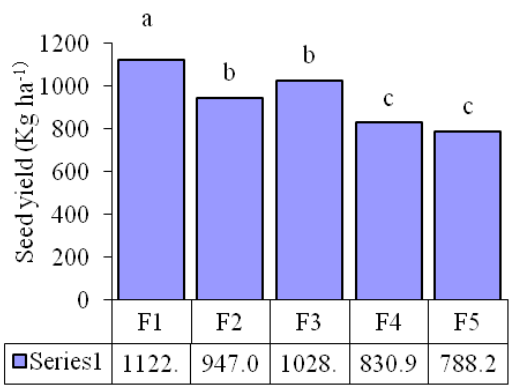

Figure 7. Effect of fertilizer treatments on seed yield of Moldavian balm

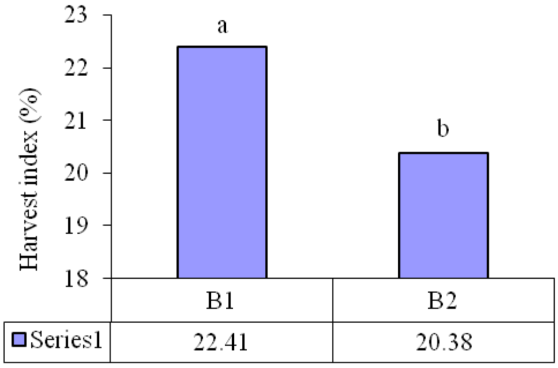

Figure 8. Effect of bacteria treatment on harvest index of Moldavian balm

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