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EFFECTS OF MACRONUTRIENT SUPPLEMENTATION ON PHYSIOLOGICAL, QUALITATIVE AND QUANTITATIVE TRAITS OF *SALVIA LERIIFOLIA* BENTH.

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Abstract

In order to study the effects of macronutrient fertilizers on physiological, qualitative and quantitative attributes of *Salvia leriifolia* Benth. (Salvia), an experiment was conducted, using a randomized complete block design with three replications at a research farm owned by the Islamic Azad University, Gonabad Branch during 2013–2015. Experimental factors included nitrogen (N) fertilizer in three levels (zero, 100 and 200 kg.ha^{-1} based on pure N), phosphorus (P) in three levels (zero, 50 and 100 kg.ha^{-1} based on P_2O_5) and potassium (K) fertilizer in two levels (zero and 50 kg.ha^{-1} based on K_2O). Results showed that three-way interactions of N, P and K application were significant on all variables examined. A marked decline in chlorophyll a, b, total chlorophyll, stomatal diameter, biological yield, and seed characteristics was observed under low doses of fertilizers. However, the highest stomatal number (167.88 mm^{-2} of leaf blade) and essential oil content was observed under no application of NPK fertilizer. The highest and the lowest seed oil content (38.68 and 17.61%, respectively) was recorded in the treatment of 100 kg.ha^{-1} N, 100 kg.ha^{-1} P, and 50 kg.ha^{-1} K, and control treatment (no fertilizer), respectively. Based on the results recorded, although salvia was a wild plant, its response to fertilizer application was similar to any other domesticated plant. The best fertilizer application levels were found to be 200 kg.ha^{-1} of N, 100 kg.ha^{-1} P, and 50 kg.ha^{-1} K fertilizer, at which all growth and physiological attributes expressed maximally. Of course, more investigation is needed regarding nutritional demands of salvia in different soils and climates.

Key words: K, Stomata, N, P, Yield, Chlorophyll.

Introduction

Supply of optimal amount of nutrients to crops is among the most important factors for increasing quantitative and qualitative yield (Malakooti & Belali, 2004). It is believed that optimum fertilizer application is the most effective, easiest, and most economical way to achieve higher yield per land area. Nitrogen is one of the major nutritional elements, which plays a key role in growth and seed production of crops (Shah *et al*., 2003). In addition, it is involved in all biochemical processes, energizing components and energy transfer mechanisms (Malakooti & Belali, 2004). Potassium supply is essential to ensure plant resistance against disease. It is an essential element in nature, which constitutes about 2.3 percent of the Earth's crust (Jamialahmadi *et al*., 2006).

Salvia leriifolia Benth. (salvia) is an important medicinal plant of Lamiaceae family, and this valuable plant is endemic to Iran and Afghanistan. This species is reported to grow in Khorasan and Semnan provinces of Iran (Rechinger, 1982). Frequent collection of seeds of this plant from the natural habitats by local residents as refreshment and nut indicates its nutritional value (Filekesh, 2005). Antioxidant, antifungal, antibacterial and detoxification properties of this herb have been reflected in modern medicine (Hosseinzadeh *et al*., 2009). Since *S. leriifolia* is under deep grazing pressure as well as harvesting, its domestication as a medicinal plant can be considered to remove the pressure from natural habitats and at the same time fulfil the market demand. Meanwhile, given special importance of nutritional elements in cultivated plants, including salvia, it is necessary to investigate how far chemical fertilizers can improve its growth and quality. Therefore, in this experiment we studied the physiological responses and qualitative and quantitative yield of salvia under different levels of N, P and K fertilizers.

Materials and Methods

This experiment was conducted during 2013–2015 at the research farm of Gonabad Islamic Azad University. A randomized complete block design with three replications was used. Experimental factors included N fertilizer in three levels (zero, 100 and 200 kg.ha^{-1} based on pure N), P in three levels (zero, 50 and 100 kg.ha^{-1} based on P_2O_5) and K fertilizer in two levels (zero and 50 kg.ha^{-1} based on K_2O). In order to determine soil characteristics, particularly the concentrations of soil N, P, and K, soil samples were chemically analyzed and the results were presented in Table 1. Since there was no any previous report, regarding salvia fertilizes acceptability, we used the recommendation of N, P and K fertilizer application for cereals in similar soils.

Table 1. Physical and chemical characteristics of soil in the farm under research.

Soil trait	Desirable level	Value	Units
pH	6.5 – 7.5	7.97	-
EC	< 4	3.85	dS.m^{-1}
Saturation percent	35-45	24.50	(%)
N	0.15 <	0.01	(%)
P	10-20	3.81	(mg.kg^{-1})
K	350 <	250.00	(mg.kg^{-1})
Fe	10	1.14	(mg.kg^{-1})
Zn	1.5-2	0.18	(mg.kg^{-1})
Cu	0.5 – 1	0.40	(mg.kg^{-1})
Mn	10-15	7.84	(mg.kg^{-1})
Organic matter content	1.5 <	0.04	(%)
Calcium carbonate	20 >	19.50	(%)
Gypsum	1.4 – 1.7	1.33	(%)
Sand	35-45	60.60	(%)
Silt	35-45	18.40	(%)
Clay	2-35	21.00	(%)

Seeds of *S. leriifolia* were collected from the Bajestan region, in Khorasan Razavi province and their immature and broken seeds were eliminated. Salvia has a very hard seed coat that prevents its easy germination, therefore, to accelerate germination, seed coats were broken and then sown inside the small pots filled with sand. Following germination and early seedling growth inside the greenhouse, seedlings of 4-5 leaves were transferred to the field on 5th of March 2013 in plots of size 2.5 × 3 m. P and K fertilizers were added to the soil after preparation of plots and N fertilizers were applied in three stages as emergence, early flowering and seed filling stage. *S. leriifolia* is a perennial plant. Therefore, N treatments were applied in the second year of the experiment, when the plant was well established in the field.

Sampling for chlorophyll measurement and stomatal number and diameter, leaf oil content was done at the flowering stage in the first part of each plot, while the second part was kept for yield measurement. Essence extraction was conducted using the water distillation method (Mirza *et al*., 1996). Number and diameter of leaf stomata were measured using an imaging microscope and finally the size of stomata was measured.

Data were statistically analyzed using the SAS software (SAS version 9.2). When the ANOVA indicated significant treatment effects (at 5 or 1%) based on the F-test, the LSD test (p=0.05) was calculated to determine which treatment is statistically different from the others. Tables and graphs were plotted using the Excel software.

Results and Discussion

Leaf chlorophyll content: Results showed that simple and interaction effects of N, P, and K fertilizers were significant on chlorophyll a, b and total chlorophyll in salvia leaves (Table 2). The lowest chlorophyll a content was recorded at the control treatment without fertilizer (0-0-0), and the highest chlorophyll a content (2.8 mg/g DW) at the highest level of N-P-K fertilizers (200-100-50) (Fig. 1). Nitrogen is the highly consumed nutrient element, which plays a role in the molecular structure of various proteins, enzymes, co-enzymes, nucleic acids and cytochromes (Hassegawa *et al*., 2008). It is also a crucial part of chlorophyll molecule and adequate supply of N is associated with higher growth and darker leaf green color. The increasing amount of P fertilizer causes increased leaf N concentration (Peng *et al*., 1999). K element plays a significant role in chlorophyll preservation and increased photosynthesis in plants through increase in leaf area and chlorophyll content (Evans, 1983) and decreased production of reactive oxygen species (Shen *et al*., 2000).

Based on the results, the effect of N and P application on chlorophyll b content of leaves was higher than that of K (Fig. 2). Therefore, when high levels of N and P (200-100) were used, K fertilizer application (50 $kg.ha^{-1}$) had no significant effect ($p < 0.01$) (Table 2) on chlorophyll b content of this plant. Nerson *et al*., (1999) also stated that when P was supplied in the soil, the soil N content and positive effects of N, were found to be beneficial in muskmelon growth performance. In general, P fertilizer enhances the effectiveness of N (Noormohammadi *et al*., 2001). Interaction effects of the levels of N and phosphate led to a proportional increase in chlorophyll content, leaf photosynthesis and N concentration of *Allium altissimum* (Arefi *et al*., 2016. On the other hand, application of K fertilizers, led to an increase in greenness and the chlorophyll contents in the wheat leaves, especially under stressful conditions (Alavi Matin *et al*., 2015).

Table 2. The statistical significance of different chemical fertilizers (N in three levels (0-100-200 kg ha^{-1} based on pure N) and three levels of P (0-50-100 kg ha^{-1} based on P_2O_5) and K fertilizer in two levels (0 -50 kg ha^{-1}, based on K_2O) on the qualitative and quantitative traits of salvia.

Source of variation	DF	Chlorophyll a	Chlorophyll b	Total chlorophyll	Biological yield	Stomatal numbers	Seed yield	Leaf essential oil	Seed oil content
Year	1	**	*	**	ns	ns	ns	ns	ns
Block (replication)	2	**	**	ns	**	**	ns	ns	**
N	2	**	**	**	**	**	**	ns	**
K	1	**	**	**	**	**	**	ns	ns
P	2	**	**	**	**	**	**	ns	**
N ×Year	2	ns	ns	ns	ns	ns	ns	ns	ns
K ×Year	1	ns	ns	ns	ns	ns	ns	ns	ns
P ×Year	2	ns	ns	ns	ns	ns	ns	ns	ns
N × K	2	ns	*	ns	ns	ns	**	ns	**
N × P	4	**	**	**	**	ns	**	ns	**
P × K	2	ns	ns	ns	**	ns	ns	ns	ns
N ×P × K	4	**	**	**	**	ns	**	ns	**
N × K×Year	2	ns	ns	ns	ns	ns	ns	ns	ns
N × P×Year	4	ns	ns	ns	ns	ns	ns	ns	ns
P × K×Year	2	ns	ns	ns	ns	ns	ns	ns	ns
N×P× K×Year	4	ns	ns	ns	ns	ns	ns	ns	ns

×ns, Non-significant, * Significant at 5%, ** Significant at 1% probability

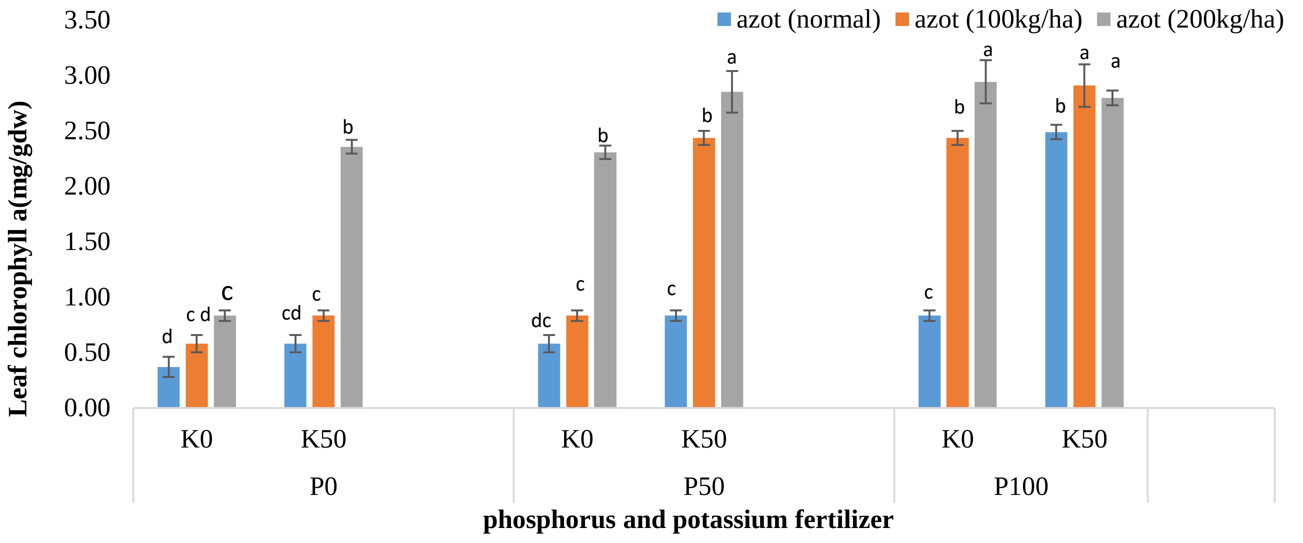

Fig 1. Mean leaf chlorophyll *a* content in salvia under the interaction of N (control, 100 and 200 kg.ha-1), K (control, 50 kg.ha-1) and P fertilizer (control, 50 and 100 kg.ha-1) application in the second year.

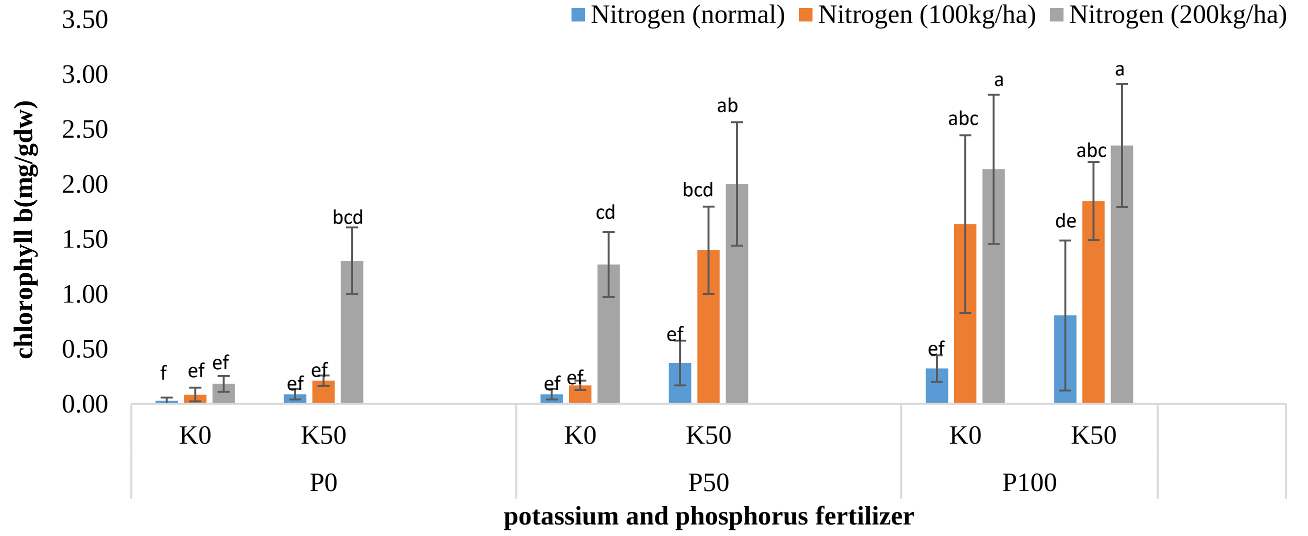

Fig 2. Mean leaf chlorophyll *b* content in *Salvia leriifolia* under the interaction of N (control, 100 and 200 kg.ha^{-1}), K (control, 50 kg.ha^{-1}) and P fertilizer (control, 50 and 100 kg.ha^{-1}) application during the second year.

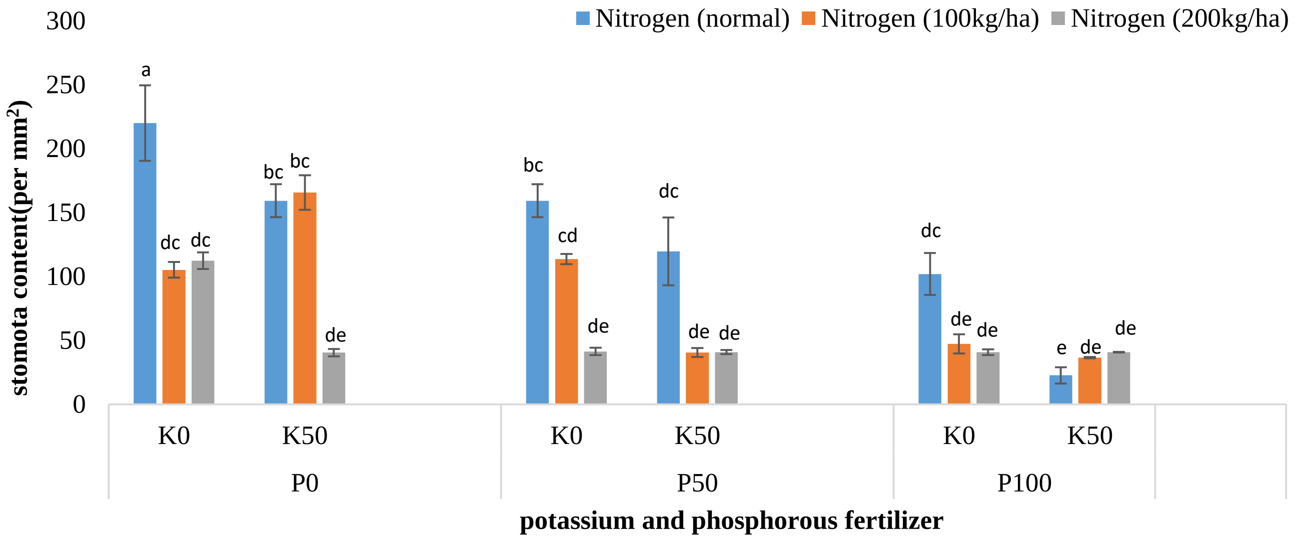

Fig 3. Stomatal frequency in *Salvia leriifolia* under the interaction of N (control, 100 and 200 kg.ha^{-1}), K (control, 50 kg.ha^{-1}) and P fertilizer (control, 50 and 100 kg.ha^{-1}) application during the second year.

Stomatal numbers: According to the results, the simple and interactive effects of N, P, and K fertilizers on the stomatal frequency were significant ($p < 0.01$) (Table 2). The highest number of leaf stomata per mm^2 was obtained in no fertilizer (0-0-0), and the lowest frequency of stomata was obtained in the treatment of maximum N-P-K fertilizer application (200-100-50) (Fig. 3). Nitrogen fertilizer might have a significant effect on the development of leaves mainly through increasing leaf width (Wheeler, 1963). Since frequency of stomata was measured based on leaf area, it was possible that the number of stomata might have reduced with increasing N, P, and K fertilizers, because of the effective role of these fertilizers in promoting leaf growth and increasing the leaf area, thereby reducing number of stomata per mm^{-2} (Sarmadnia & Koocheki, 1999).

Biological and seed yield: Results showed that application of N and P fertilizers imposed a significant impact on biological yield of salvia (Table 2). The lowest biological yield was recorded in the control treatment without fertilizer (0-0-0) application, and the highest biological yield was found at the highest level of N-P-K fertilizers (200-100-50) (Fig. 4). It is believed that the production of dry matter in many plants is directly dependent on the supply of N and P, and in the case of absence of these elements, dry matter production is reduced (Dordas, 2009). The results for *Kochia scoparia* showed that N and P application led to an increase in relative chlorophyll content, green area index, chlorophyll fluorescence and dry matter accumulation (Khaninejad *et al*., 2013).

N, P, and K fertilizers and their interactive effects caused a significant increase in seed yield of salvia (Table 2). The lowest seed yield (201.4 $kg.ha^{-1}$) was recorded when neither of the fertilizers was applied, but the highest seed yield was obtained (1383.0 $kg.ha^{-1}$) under the highest dose of N-P-K fertilizers (Fig. 5). In no N fertilizer application, the increase in P and K fertilizer levels could not impose a significant impact on increasing seed yield of salvia. Therefore, the highest effect for P and K fertilizers was obtained, when N fertilizer applied at 200 $kg.ha^{-1}$. On the other hand, in case of P fertilizer absence, N fertilizer application could not lead to the increase in seed yield in *S. leriifolia* Benth (Table 2). Akbarinia *et al*., (2003) also reported that seed yield increased in *Trachyspermum copticum* by increasing the application of chemical fertilizers and they reported that the highest seed yield was obtained with the application of 120 kg N and 80 kg P per ha. Takahashi & Anwar (2007) reported that the application of P fertilizer is vital to the growth of wheat and the application of N fertilizer alone will yield little positive effect. It is believed that K plays its role in the synthesis of osmolytes to cope with environmental stresses and maintain turgor pressure, which leads to increased seed yield.

Seed and leaf essential oil content: N, P, and K and their interactive effects were significant on leaf essential oil content in salvia (Table 2). The increased levels of N fertilizer led to a reduction in essence content of salvia (Fig. 6). Results indicated that the highest percent essence was under no fertilizer (0-0-0) and the lowest essence percentage was under the highest N, P and K fertilizer application (200-100-50) (Fig. 6). Findings obtained by Yousefi *et al*., (2014) also indicated that salvia had the highest essence percentage in lower fertile soils. Krishnamurthy & Madalager (1999) found that use of chemical fertilizers had no impact on essence content of ajowan. Hussain (2012) also tested the effects of different fertilizers on the essence content of marigold and observed that different fertilizers had no significant effect on marigold's essence that is not in agreement with our results. The best way to obtain the maximum yield for medicinal plants (maximum essence and maximum biomass) is the manipulation of plant nutrient formula to achieve a balance between biomass accumulation and essential oil content (Omid Beygi, 2005). Application of N in black cumin increased photosynthesis, chlorophyll content, enzyme activity, dry matter and leaf development and essential oil (Ashraf *et al*., 2006).

Effect of nitrogen and interactive effect of P×N, and K×N× P were significant on seed oil content of *Salvia leriifolia* (Fig. 7). The lowest oil content was recorded at no fertilizer treatment (0-0-0) and the highest oil content at the N-P-K fertilizer treatment (50-100-100). Therefore, an increase in the N fertilizer level up to 100 $kg.ha^{-1}$ led to an increase in oil content. These findings were consistent with those of Baybordi (2006) who reported that increase in N fertilizer up to 100 $kg.ha^{-1}$ led to increased oil content in safflower, and use of 100 $kg.ha^{-1}$ N fertilizer led to the reduction in oil content of this plant.

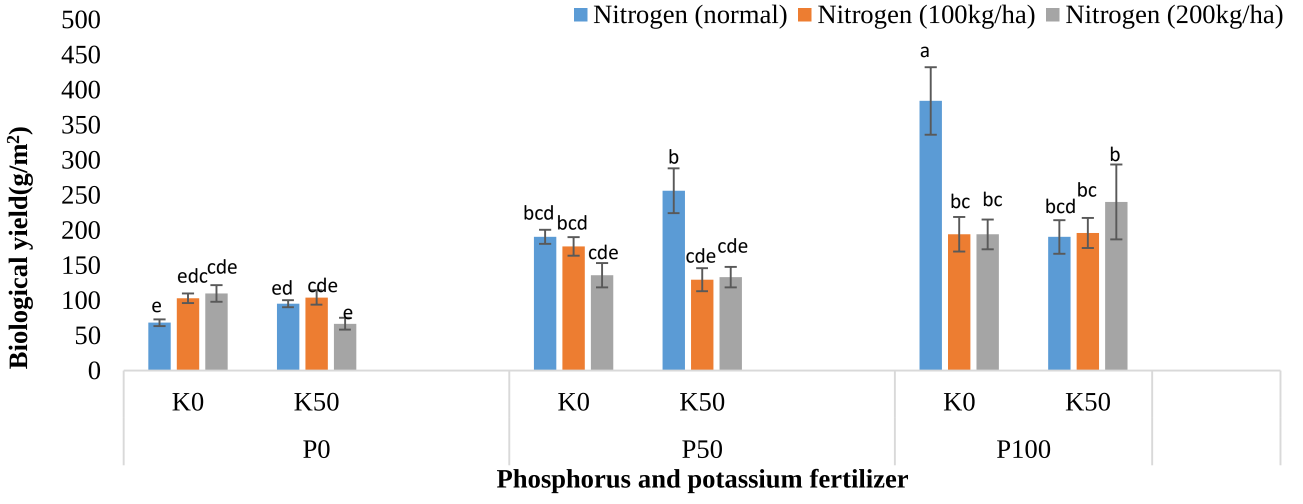

Fig 4. The biological yield of salvia under the interaction of N (control, 100 and 200 $kg.ha^{-1}$), K (control, 50 $kg.ha^{-1}$) and P fertilizer (control, 50 and 100 $kg.ha^{-1}$) application during the second year.

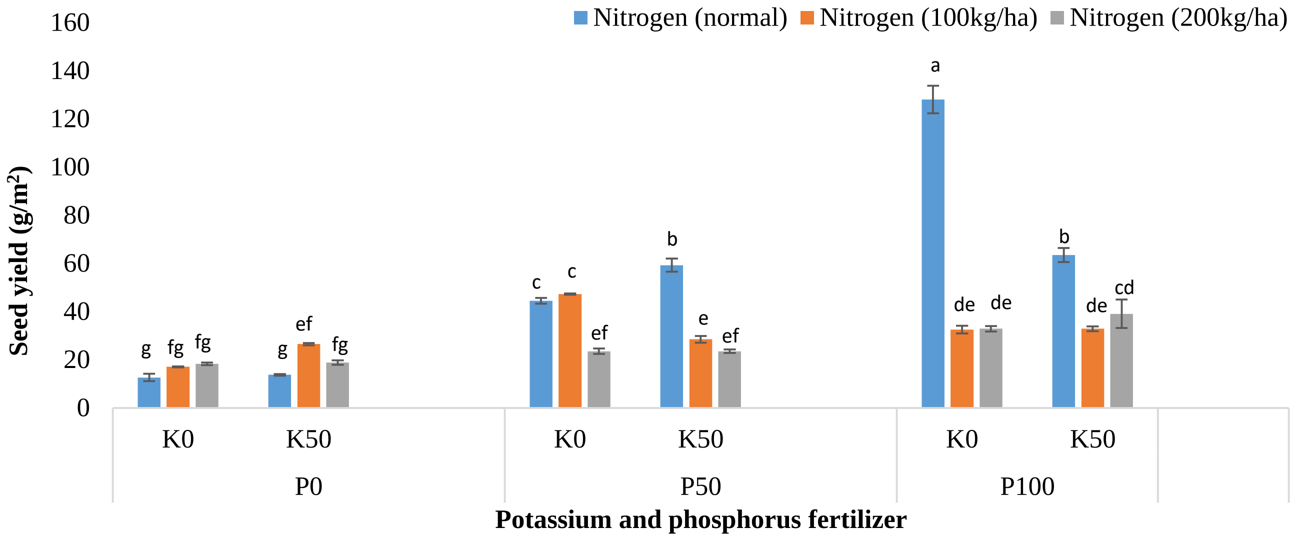

Fig 5. The average seed yield in salvia under the interaction of N (control, 100 and 200 kg.ha^{-1}), K (control, 50 kg.ha^{-1}) and P fertilizer (control, 50 and 100 kg.ha^{-1}) application during the second year.

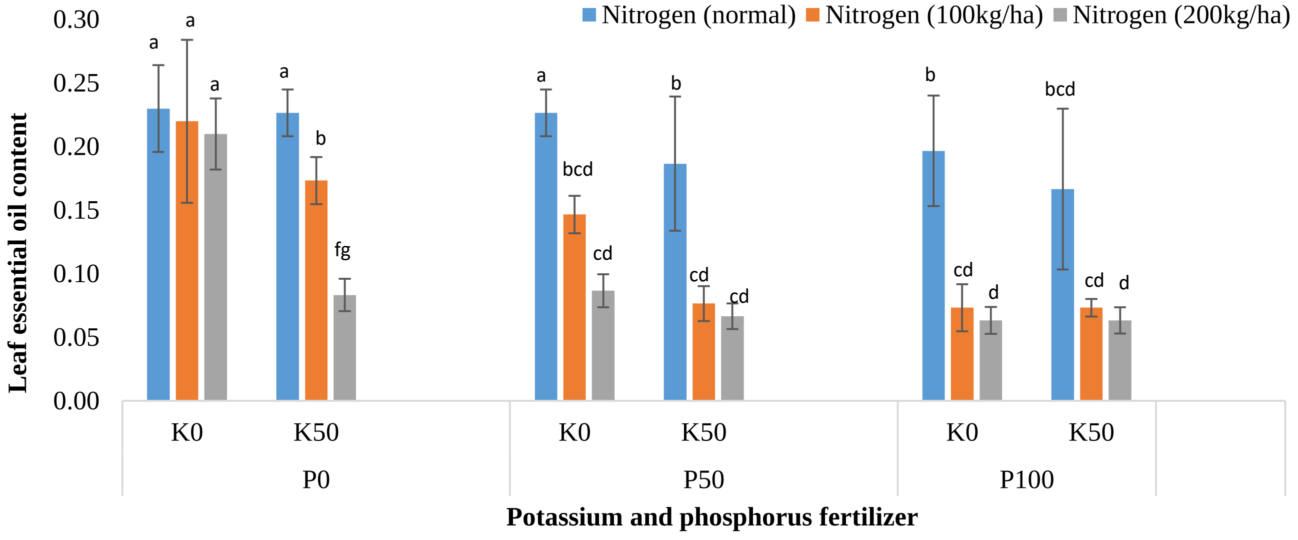

Fig 6. Leaf essential oil content (%) in salvia under the interaction of N (control, 100 and 200 kg.ha^{-1}), K (control, 50 kg.ha^{-1}) and P fertilizer (control, 50 and 100 kg.ha^{-1}) application during the second year.

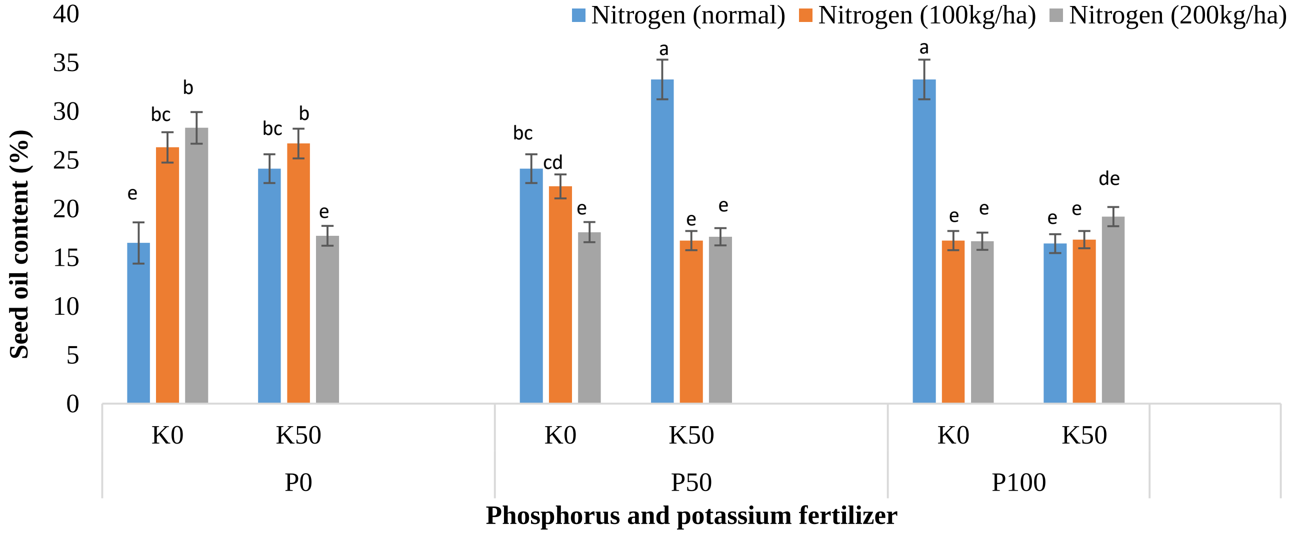

Fig 7. Mean seed oil content in salvia under the interaction of N (control, 100 and 200 kg.ha^{-1}), K (control, 50 kg.ha^{-1}) and P fertilizer (control, 50 and 100 kg.ha^{-1}) application during the second year.

Conclusion

Considering the findings of the current research, the best performance in terms of chlorophyll, stomatal diameter, the biological and seed yield were obtained in the treatment of 200 kg N, 100 kg P, and 50 kg K per ha. However, the highest stomatal numbers (167.88 mm^2) and essence percentage were recorded in no NPK treatment. The highest and the lowest seed oil content (38.68 a

nd 17.61%, respectively) was obtained in 100 kg N+100 kg P+50 kg K, and control treatment (no fertilizer), respectively. Although wild non-domesticated crops usually show a marginal response to increased fertilizer application rate, but salvia showed a performance like any other domesticated crops. The best fertilizer application level was 200 $kg.ha^{-1}$ of N, 100 $kg.ha^{-1}$ P, and 50 $kg.ha^{-1}$ K fertilizer, which improved the physiological and agronomical characteristics measured in *Salvia leriifolia* in Gonabad conditions. The interactive effects of fertilizers showed that use of P and K fertilizers could be useful to achieve better efficiency of N fertilizers. Of course, more investigation is needed regarding nutritional demands of *Salvia leriifolia* in different soils and climates.

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