The effect of nitrogen and phosphorous fertilizers on morphophysiological properties of *Althaea officinalis*

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Abstract

Althaea officinalis has a significant role in preventing and treating different diseases. The present study was aimed to assess the effect of N and P fertilizers on leaf, seed, and flower properties in a semi-arid area of Iran. N was applied as NH_2CONH at three stages (before cultivating stage, at 4-leaf stage, and before reproductive stage) as 50, 100, and 150 kg/ha. P was used as P_2O_5 before cultivating in three concentrations as 30, 60 and 90 kg/ha. Results showed that N fertilizer significantly increased plant height, leaf area, and leaf chlorophyll content, whereas P fertilizer only influenced the plant height. For seed properties, mucilage was not influenced by P fertilizer. Nor was 1000 seed weight affected by N fertilizer. P fertilizer significantly increased oil content. In contrast, a 0.5 % decrease in seed oil was observed in 150 kg/ha with respect to control. All flower properties (phenol, flavonoid, and antioxidant activity) of *A. officinalis* significantly increased by enhancing N and P concentrations. In most traits, 150 kg/ha N and 90 kg/ha P fertilizers were selected as most effective treatments.

Keywords: NH_2CONH; P_2O_5; *Althaea officinalis*; antioxidant activity; chlorophyll content

Abbreviations
N: Nitrogen; P: Phosphorous; *A. officinalis: Althaea officinalis*

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Introduction

Althaea officinalis belonging to family Malvaceae is native to Europe and parts of Asia and is cultivated throughout the world. *A. officinalis* is an eminent medicinal plant consumed in case of inflammation of nasal and oral cavities, lipemia, gastric ulcer, platelet aggregation, cystitis, and irritating coughs (Sutovska et al., 2009; Hage-Sleiman et al., 2011). Its flowers are terminal and axillary, with short peduncles, each bearing one, two, or three flowers. The petals are pale pink, reddish pink, and rarely white in color (Sadighara et al., 2012). The extract of *A. officinalis* has shown the strong antioxidant activity in different antioxidant tests (Elmastas et al., 2004). Antioxidant activity of *A. officinalis* is accounted for approximately 70% of the activity of the reference compound alpha-tocopherol (Kardosova and Machova, 2006).

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Table 1
The soil properties of the case study in Karaj

Texture	EC(ds/m)	pH	Organic C (%)	N (%)	P (mg/kg)	K (mg/kg)
Loam-Sandy	3.5	5.7	0.19	0.023	4.5	180

Nitrogen is an essential element required for optimum plant growth. Although inorganic nitrogen compounds (NH_4^+, NO_2^-, and NO_3^-) are considered less than 5% of total nitrogen in soil, they are the main form of the element absorbed by most plants (Commoner, 1970). Organic and inorganic fertilizers are used to maintain the nutritional condition of plant in different soils. For an organic agricultural system, continuous use of manure increases the essential elements such as nitrogen (N), phosphorus (P), potassium (K), calcium, and magnesium content in soil (Havlin et al., 2005). When organic fertilizers are applied to soils, mineralization begins, which results in releasing inorganic nitrogen absorbed by plants. On the other hand, mineralization rate is controlled by agricultural management systems, soil features, microorganism, temperature, water content, and the type of organic fertilizer (Stanford and Smith, 1972).

Phosphorus (P) is essential for plant growth. Its functions cannot be carried out by any other nutrient and an adequate supply of this element is required for optimum growth and reproduction. The total P in agricultural crops generally varies from 0.1 to 0.5 percent. It is vital for plant growth and can be found in every living plant cell (Pierzynski et al. 2005). P covers some key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next. It utilizes light energy in the presence of chlorophyll to combine carbon dioxide and water into simple sugars, with the energy being captured in ATP. Subsequently, the ATP is available as an energy source for many reactions occurring within the plant, and the sugars are applied as building blocks to produce other cell structural and storage component (Ruttenberg, 2003).

Regarding the importance of medicinal plants and their wide application instead of chemical drugs, it is necessary to use the growth stimulators to obtain the optimum efficiency of these plants. In agricultural soils, there is a significant annual reduction of N and P because of leaching (Adeniyan and Ojeniyi, 2005). So, the use of N and P fertilizers in agricultural soils are essential particularly for medicinal plants. Various studies have shown the significant effects of N and P fertilizers on quantitative and qualitative properties of different plants. For example, an increase of phenolic compounds and antioxidant properties was reported for basil (*Ocimum basilicum* L.) under nitrogen fertilization (Nguyen and Niemeyer, 2008). Moreover, El-Gendy et al. (2015) showed that N and P fertilizer have a significant effect on chlorophyll contents and essential oil of *Anthriscus cerefolium* L. However, Chrysargyris et al. (2016) indicated that P and N fertilizers have different impacts on morphological and biochemical properties of *Lavandula angustifolia* Mill so that N decreased the chlorophyll contents while P increased it. In addition, most morphological parameters such as plant height and fresh and dry weights increased by adding both N and P fertilizers. Hence, the resent study was conducted to assess the effect of N and P fertilizers on quantitative and qualitative properties of *A. officinalis* in Iran.

Materials and Methods

Site description

The study was carried out based on factorial as completely random design with three replications in Karaj, Iran (35˚48´ N, 51˚01´ E, and 1287 m a.s.l.). The fourteen-year records of the meteorological data (1996-2010) taken from the meteorological station nearest to the field, Karaj Meteorological Station (35˚ 45´ N, 51˚ 02´ E, and 1291 m a.s.l.), indicates that the mean annual precipitation is 257.6 mm (SE: ± 21.4 mm). The meteorological data shows that the wettest and driest months are March (42.4 mm; SE: ± 9.7 mm) and August (1.5 mm; SE: ± 0.6 mm), respectively.

The dry period begins in May and ends in October. The wet period, extends from November to April and historically accounts 85% of the total annual precipitation. The mean annual temperature is 17.2 ˚C (SE: ± 0.1 ˚C); August is the warmest month with average temperature of 28.4 ˚C (SE: ± 0.3 ˚C) and January is the coldest month (3.1 ˚C; SE: ± 0.8 ˚C). Soil parameters of the case study have been presented in Table 1.

Experimental treatments

N was applied as NH_2CONH at three stages (before cultivating stage, at 4-leaf stage, and before reproductive stage) as 50, 100, and 150 kg/ha. P was used as P_2O_5 before cultivating at three levels as 30, 60, and 90 kg/ha.

Measuring the traits

Pigment content assay

Chlorophyll a, chlorophyll b, and carotenoid content were extracted according to Arnon (1949). Samples consisting of 200 mg fresh leaves were homogenized in 8 ml 80% acetone with a homogenizer. Homogenates were then centrifuged at 4 °C for 15 min (3000 rpm). The supernatants were used for analyzing pigments. Absorbance was determined at 645, 652, 663, and 470 nm.

Total phenolic content

Folin-Ciocalteu method was used for the total phenolic content of the ethanolic extracts (Kahkonen et al., 1999). Briefly, each evaporated thick and viscous extract (0.8 to 0.9 g ±0.01 mg) was diluted by 5 ml methanol. Samples of plant extract solution (200l) was transferred into a tube and then mixed thoroughly with 1 ml of Folin-Ciocalteu reagent. Subsequently, sodium carbonate was added after mixing for 3 min, 0.8 ml of 7.5% (w/v). The mixtures were agitated with a vortex mixer, then allowed to stand for a further 30 min in the dark, and centrifuged at 3300 g for 5 min. The absorbance of plant extracts and a prepared blank were measured at 765 nm with a spectrophotometer (UV–vis model 1601, Shimadzu, Kyoto, Japan). The concentration of total phenolic compounds in all plant extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight of plant, using the linear Eq. (3) derived from Eq. (2), which was determined as common concentrations of garlic acid standard.

$$\text{Absorbance (at 765nm)} = \text{constant} \times (\text{galic acid coincentration}) \quad (1)$$

$$\text{Gallic acid equivalents} = \text{Absorbance (at 765nm)}/0.0508. \quad (2)$$

Total flavonoid content

The total flavonoid content was measured according to Dowd method (Meda et al., 2005). Briefly, 5 mL of 2% aluminum dichloride (AlCl3) in methanol was mixed with the same volume of the extract solution (0.4 mg/mL). Absorption was done with the spectrophotometer after 10 minutes against a blank sample consisting of 5 mL extract solution with 5 mL ethanol without $Alcl_3$. The total flavonoid content was measured using standard curve drown based on catching (0-100 mg/L) as a standard. It was finally expressed as mg of catching equivalents (CE) g of extract.

Antioxidant activity

Antioxidant activity was determined according to the method of Hatano et al. (1988). Briefly, the reaction mixture contained 0.1 ml of DPPH radical solution (5 mM) and different concentrations of tested compounds (from 0.0026 mM to 83 mM). Total volume of the reaction mixture was 3 ml. Absorption of DPPH radical at 515 nm was determined after 10 min against a blank solution that contained only methanol.

%DPPH radical scavenging activity = ((Ac-As)/Ac) ×100 where As = absorbance of sample and Ac = absorbance of control (DPPH radical solution in methanol without sample).

According to Ordoudi et al. (2006), Kinetic parameter EC_{50} (efficient concentration of the

Table 2a
Analysis of variance for N and P fertilizers on leaf properties of *A. officinalis*

Variable source	df	Plant height	Leaf area	Chll a	Chll b	Total chll
N	3	589.6**	1013.1**	$6^{*}10^{-2**}$	$1^{*}10^{-2**}$	$12^{*}10^{-2**}$
P	3	179.5**	146.9ns	$2^{*}10^{-3ns}$	$4^{*}10^{-4ns}$	$5^{*}10^{-4ns}$
N*P	9	2.87ns	1.44ns	$1^{*}10^{-4ns}$	$2^{*}10^{-5ns}$	$4^{*}10^{-5ns}$
Error	-	2.16	61.54	$1^{*}10^{-3}$	$2^{*}10^{-4}$	$3^{*}10^{-4}$
CV(%)	-	1.34	28.3	14.16	14.33	12.96

Table 2b
Analysis of variance for N and P fertilizers on seed and flower properties of *A. officinalis*

Variable source	df	Mucilage	Oil Content	1000 Seed Weight	Phenol	Flavonoid	Antioxidant Activity
N	3	9.96**	1.73**	0.03^{ns}	0.003**	0.009**	11.02**
P	3	3.09^{ns}	2.28**	0.4*	0.95**	0.16**	185.35**
N*P	9	0.33^{ns}	0.16^{ns}	0.0002^{ns}	0.53^{ns}	0.24^{ns}	40.9^{ns}
Error	-	1.008	0.23	0.11	0.0001	0.0001	2.68
CV(%)	-	4.36	4.47	2.48	3.52	4.93	3.12

antioxidant necessary to decrease the initial DPPH radical concentration by 50%) was calculated. Also, T_{EC50} (reaction time needed to reach the steady state at EC_{50}) was determined. Finally, the calculated EC_{50} and T_{EC50} values were applied to calculate the antiradical index AE (antiradical efficiency) as $AE = 1/(EC_{50} \times T_{EC50})$.

Statistical Analysis

All data were expressed as mean ± standard deviation. The data were submitted to SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Analysis of variance was performed by ANOVA. Duncan multiple range test was applied to mean comparison. A significant difference was considered at the level of $P \leq 0.05$.

Results

Analysis of variance

Analysis of variance showed the simple effect of N significantly changed all leaf properties, while P only influenced the plant height (Table 2a) ($P \leq 0.05$). All flower properties (phenol, flavonoid, and antioxidant activity) of *A. officinalis* significantly were affected by N and P concentrations (Table 2b) ($P \leq 0.05$). For seed properties mucilage was not influenced by P fertilizer. Also, 1000 seed weight was not affected by N fertilizer. In addition, there was no significant effect of N and P interaction on leaf, seed, and flower properties of *A. officinalis* (Table 2) ($P \leq 0.05$).

Mean comparison of leaf properties

Our findings showed N and P fertilizers influenced the leaf properties of *A. officinalis*. The highest and lowest plant height was observed in control (0 kg/ha) and 150 kg/ha concentration of N fertilizers as 102.3 cm and 115.7 cm, respectively. This was recorded from 105.4 cm in control to 115.8 cm for P fertilizer. The maximum leaf areas (2759 cm^2 and 2759 cm^2) were recorded in 150 kg/ha N and 90 kg/ha P fertilizers, respectively (Table 3).

Chlorophyll content was significantly influenced by N fertilizer so that the highest total chlorophyll content (0.15 mg/g FW) was observed in 150 kg/ha N. In contrast, there was no significant difference between the effects of different concentrations of P fertilizer on total chlorophyll (Table 3).

Mean comparison of seed properties

The mucilage increased by enhancing the concentration of N and P fertilizers. The maximum mucilage was found in 150 kg/ha N fertilizer (24.26%) and 90 kg/ha P fertilizer (23.56%). Oil content under N fertilizer decreased while it

Table 3
Effect of N and P fertilizers on plant height and leaf properties of *A. officinalis*

Fertilizer	Concentration (kg/ha)	Plant height (cm)	Leaf area (cm2)	Chll a (mg/g FW)	Chll b (mg/g FW)	Total Chll (mg/g FW)
N fertilizer	0	102.3±6.21d	2532.5 ±2.47c	0.067±0.005c	0.026±0.002c	0.1±0.006c
	50	107.5±5.5c	2654.25±2.53b	0.088±0.002b	0.034±0.00 06b	0.13±0.002b
	100	112.6±4.32b	2659.91±1.8b	0.1±0.001b	0.038±0.0005b	0.14±0.002b
	150	115.7±7.1a	2759.5±2.42a	0.12±0.006a	0.0048±0.002a	0.18±0.008a
P fertilizer	0	105.4±9.1d	2647.08±2.43b	0.089±0.007a	0.034±0.002a	0.136±0.099a
	30	108.1±7.9c	2649.5±2.42b	0.093±0.006a	0.036±0.002a	0.141±0.01a
	60	11.9±9.5b	2653.3±2.43b	0.096±0.007a	0.037±0.003a	0.147±0.01a
	90	115.8±9.9a	2655.1±2.45a	0.099±0.006a	0.038±0.003a	0.15±0.009a

Table 4
Effect of N and P fertilizers on seed properties of *A. officinalis*

Fertilizer	Concentration (kg/ha)	Mucilage (%)	Oil content (%)	100 seed weight (gr)
N fertilizer	0	22.25±0.32c	10.45±1.1b	4.41±0.5a
	50	23.15±0.43b	10.34±1.3a	4.35±0.4ab
	100	22.45±0.32bc	10.30±1.5a	4.22±0.05b
	150	24.26±0.25a	9.95±1.5a	4.39±0.02a
P fertilizer	0	22.5±0.43b	10.2±0.8c	4.25±0.06b
	30	22.7±0.39ab	10.5±1.2bc	4.35±0.05ab
	60	23.35±0.38ab	10.9±1.1b	4.39±0.07a
	90	23.56±0.36a	11.6±0.9a	4.42±0.08a

increased by P fertilizer application. 1000 seed weight had different behavior under P and N application. The maximum 1000 seed weight under N fertilizer was observed in control (4.41 g) whereas the maximum 1000 seed weight under P was obtained in 90 kg/ha P (4.42 g) (Table 4).

Mean comparison of flower properties

As shown in Fig. I, the phenol of *A. officinalis* flowers significantly increased by both N and P fertilizers. The highest phenolic compounds was observed in 150 kg/ha N fertilizer (4.7 mg GAE/g DW) and 90 kg/ha P fertilizer (4.4 mg GAE/g DW). In our study, a significant increase was found in flavonoid content under N fertilizers so that the maximum and minimum flavonoid contents were observed in 150 kg/ha and control, respectively. In contrast, P fertilizer had no effect on this trait.

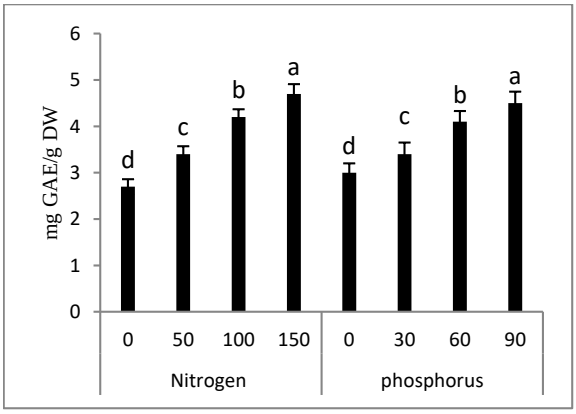

Fig. I. The effect of N and P fertilizers on the phenol of *A. officinalis flowers*

Discussion

Previous studies have shown an increase in plant height and leaf area under P and N fertilizers (Adeniyan et al., 2003; Adesemoye et al., 2009; Pinto et al., 2016). Due to its participation in protein structure, N increases internode length of plants which results in enhancing plant height and leaf area (Pinto et al., 2016). The uptake of different forms of N can be observed after two weeks in plants. In addition, the reaction of plants to N concentrations is higher with respect to other chemical fertilizers (Zhang et al., 2012). Hence, N can easily increase plant height and leaf area. The increment in plant height and leaf area shows the

importance of P for cell division activity, leading to the increase in plant height and branches and consequently increase in the plant dry weight (Tesfaye et al., 2007). Hokmalipour and Darbandi (2011) found an increase in chlorophyll content due to application of N fertilizer. N is a main component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from water and carbon dioxide (i.e., photosynthesis). It is also a major component of amino acids, the building blocks of proteins. Without proteins, plants wither and die. Some proteins act as structural units in plant cells while others act as enzymes, making possible many of the biochemical reactions on which life is based. Increasing the N concentration leads to enhancing the component of energy-transfer compounds, such as ATP and nucleic acids such as DNA, the genetic material that allows cells (and eventually whole plants) to grow and reproduce (Feller and Fischer, 1994).

The plant and soil analyses enable a more flexible calculation of fertilizer rate because net release of soil-N is predicted more accurately (Wiesler, 1998b). Moreover, physiological studies have indicated that there are critical growth phases in the N-nutrition of oilseed rape when a high N-supply is needed for yield formation (Behrens, 2002; Barlog and Grzebisz, 2004a). Strong relations were found between N uptake during the reproductive growth and yields. Additionally, it is obviously shown that under conditions of reduced N-supply, early N-application mainly increases vegetative biomass while late/delayed N-fertilization leads to reduced growth until beginning of the flowering but improves yield formation thereafter. Compared with high (240 kg N/ha) N-supply, split application of N fertilization only slightly affected seed yield on a field site near Goettingen (northern Germany) when timing was postponed to the beginning of shooting (Behrens, 2002). Nevertheless, in semi-arid areas early N-application may support yield formation due to higher soil water status in spring. Reduction in N-fertilization normally limits the N-balance surplus in oilseed rape production (Behrens et al., 2003b) as well as the amount of soil mineral N after harvest (Lickfett et al., 2001). The biology of phosphorus in the plant and the relationship

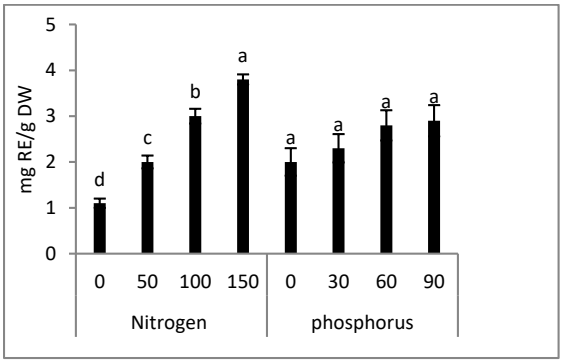

Fig. II. The effect of N and P fertilizers on the flavonoid of *A. officinalis flowers*

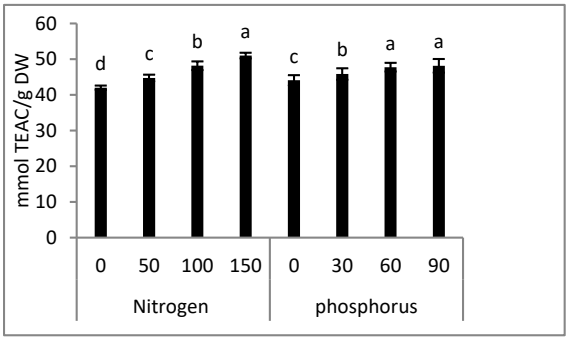

Fig. III. The effect of N and P fertilizers on the antioxidant activity of *A. officinalis flowers*

between phosphorus uptake and translocation are not well understood. Cassman et al. (1981) found that total seed phosphorus content increased as phosphorus fertility levels in the soil increased. Variations in total seed phosphorus concentrations under varying levels of fertilization are missing from most studies and need to be researched. Understanding this relationship is extremely important because many of the production areas in which these seeds are grown tend to have high levels of available phosphorus (Sims et al., 2000). Raghothama (1999) indicated that the prevalence of low phosphorus-containing soils in many production environments in the developing world also makes understanding seed deposition of soil phosphorus an important goal for improving international agriculture.

The importance of phenolic compounds as antioxidant in human health such as prevention of coronary heart disease and cancer is also of raising

5. Conclusion

A. officinalis as an eminent medicinal plant can be used because of its seed, flower, and leaves. How to use P and N fertilizers is important in increasing the yield of plants especially medicinal plants. In

the present study the N and P fertilizers were used as NH_2CONH (50, 100, and 150 kg/ha) and P_2O_5 (30, 60 and 90 kg/ha), respectively. Both N and P fertilizers had some significant impacts on seeds, flowers, and leaves of *A. officinalis.* However, N was relatively more efficient with respect to P fertilizer. N because of its participation in protein structure increases internode length of plants which results in enhanced plat height and leaf area. Increase in N and P fertilizer concentrations leads to enhanced ATP (the energy-transfer compound) and nucleic acids such as DNA and this allows cells and whole plants to grow. Antioxidant capacity as a main part of medicinal plants is significantly increased by increasing N and P fertilizers. Based on this study 150 kg/ha N fertilizer and 90 kg/ha P fertilizer are recommended as optimum concentrations that the producers of *A. officinalis* can use to achieve more medicinal characteristics of *A. officinalis.*

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