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N and K nutrition levels affect growth and essential oil content of costmary (*Tanacetum balsamita* L.)

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Abstract

Vegetative growth and essential oil content of costmary (*Tanacetum balsamita* L.) were evaluated in response to N and K. Experimental treatments included different concentrations of N (100, 200, 300 and 400 mg L^{-1}) and K (100 and 200 mg L^{-1}) based on the modified Hoagland's nutrient solution. Results showed that fresh and dry weight of plants increased by increasing N concentration up to 200 mg L^{-1} in the nutrient solution. The highest leaf number was observed in 200 mg L^{-1} N. Chlorophyll content of leaves increased significantly by adding nitrogen concentration in the nutrient solution up to 400 mg L^{-1}. However, K concentration had no effect on the chlorophyll content. N and K treatment combinations in the solution affected growth characteristics and essential oil content of costmary, so that the highest essential oil content (0.54%) and yield (11 ml m^{-2}) were obtained in $N_{200}K_{200}$ treatment. Essential oil content in $N_{200}K_{200}$ treatment was 8% higher than that of $N_{400}K_{100}$ treatment. Moreover, essential oil yield in $N_{200}K_{200}$ treatment was 35% higher than that of $N_{100}K_{100}$ treatment. N and K treatment combinations influenced fresh and dry weight of leaves (medicinal material), and the greatest amount was recorded in $N_{200}K_{200}$, which had the highest leaf area as well. The total net photosynthesis was affected by treatments, and the highest data was recorded in $N_{200}K_{100}$ treatment. Different concentrations of N and K influenced height of the plants, the greatest height being observed in $N_{200}K_{200}$ treatment. It can be concluded that different concentrations of N and K have remarkable effects on the growth and essential oil content of costmary.

Key words: Costmary, N, K, growth, essential oil content, essential oil yield.

Introduction

Costmary (*Tanacetum balsamita* L.) is a perennial rhizomaceous yellow flowered plant with Mediterranean origin that was cultivated in Eurasia till medieval times [2-6]. This plant and its essential oil have been used as hepatoprotective, antibacterial, antiallergic, sedative and cardiotonic [1-3, 5, 6].

In recent years interest in greenhouse and hydroponics production of medicinal and aromatic plants has been increased dramatically. Root medium of hydroponically grown plants is rich in water, air and nutrients, and absorptive and metabolic activities in roots of these plants increase and lead to the accelerated development of aerial parts and some fold increase in economical and biological products [7, 8]. Several factors affect growth, yield and active substance quality of medicinal and aromatic plants. Nutrition is one of the most important controllable factors that has a vital role in biosynthesis and yield of active principles in medicinal plants [9]. Macronutrients have a crucial role in primary metabolism of plants, and growth, development and active substance quality of medicinal plants completely depend on these nutrients. N and K, described as the most important macronutrients due to their effects on chlorophyll content, photosynthesis rate and leaf area development, have an essential role in increasing of biomass and active substance content and quality as well as promotion of secondary metabolism of medicinal plants [10]. Borlina Maia *et al.* [11] in a study of effects of different macronutrient concentrations on hydroponically grown Japanese mint reported that dry weight and aerial parts yield of plants had positive correlation with high N concentration in the nutrient solution [11]. Baranauskiene *et al.* [12] showed that N fertilization increased yield of thyme but differences in essential oil yield were not significant. Diatloff [13] reported that growth and essential oil yield of three species of *Leptospermum* did not respond to N fertilization. K as one of the most important macronutrients has an important role in photosynthesis, transpiration, cell permeability, hormonal and carbohydrate translocation and activity of some enzymes that have vital role in biosynthesis of active substances at cellular level [1]. However, there is very little literature about K effects on quantity and quality of active substances in medicinal and aromatic plants. Abdallah *et al.* [14] reported that K fertilization increased seed yield and essential oil content and yield of fennel. Borlina Maia *et al.* [11] reported that in Japanese mint, with increasing of K levels in the nutrient solution, essential oil yield per pot increased. Costmary is one of the most important traditional medicinal plants of Iran and by now, there has not been any scientific paper on the nutritional requirements of this plant. The aim of this experiment was to study the effects of different nutritional levels of N and K on growth characteristics and essential oil content of costmary in hydroponics condition.

Material and Methods

Plant material, growing conditions and experimental treatments: Rhizome cuttings (10 cm long) of native plants of established fields supplied by Khalat-Pushan research center were used as propagules. The rhizomes were planted in the pots (14 L) filled with perlite and vermiculite (9:2 v/v). Ambient temperature, relative humidity and photosynthetically active radiance in greenhouse were set at 20-35°C, 40% and 500 μmol m^{-2} s^{-1}, respectively. Experimental design was factorial based on the completely randomized block design with three replications and eight treatment combinations of N and K. Four concentrations of N (100, 200, 300 and 400 mg L^{-1}) and two concentrations of K (100 and 200 mg L^{-1}) based on the modified Hoagland's nutrient solution were applied to the plants. Irrigation of the plants with nutrient solutions was carried out manually. In order to avoid of salt accumulation and salinity stress, growing media were eluted with tap water weekly. It is noteworthy that concentrations of other nutrient elements in treatment combinations were the same (P 20; Ca 105; Mg 50; S 66; Fe 2.5; B 0.5; Mn 0.24; Zn 0.1; Cu 0.02 and Mo 0.01 mg L^{-1}).

Determination of essential oil content and yield: Essential oil content was determined by hydrodistillation. To do this, 25 g of air-dried leaf sample of plants, harvested in button stage of inflorescences was subjected to hydrodistillation for 120 minutes by means of the Clevenger type apparatus according to the European Pharmacopeia. Essential oil yield was calculated by the multiplication of essential oil content to the related leaf dry weight and reported in ml m^{-2}.

Leaf area determination: At the harvest time leaf number per plant and plant height were recorded. Then leaf area of plants was measured by means of leaf area meter (Li-cor Model Li-1300-USA). At the next stage, fresh weight of leaves and stems (aerial parts) and underground parts were recorded and these materials were dried in an air-forced oven at 70°C for 72 hours. Individual leaf area was calculated from ratio of leaf area to leaf number. Aerial to underground parts ratio was calculated based on dry weight.

Measurement of chlorophyll content and photosynthesis: Chlorophyll content of plants was measured by means of chlorophyll meter (Spad-502-Minolta-Japan) in mid-season. In each experimental unit 20 data were recorded from middle of blades of young fully expanded leaves. The mean of 20 data was subjected to statistical analysis.

Net photosynthesis of plants was measured by means of photosynthesis meter (Walz, Model HCM-1000-Germany). Time of measurement was mid-season from young fully expanded leaves within hours of 9 to 14. Measurement parameters were: flow rate 800 mL min^{-1}, PAR 1600 μmol $m^{-2}s^{-1}$, CO_2 absolute 450 μmol $m^{-2}s^{-1}$. Total net photosynthesis of plants was obtained by multiplication of recorded data from photosynthesis meter to the related leaf area as μmol CO_2 s^{-1} $plant^{-1}$.

Statistical analysis: Analysis of variance was carried out by SAS 8.2 software. The mean comparisons were performed by Duncan's test at 0.01 and 0.05 probability levels.

Results and Discussion

Different concentrations of N in the nutrient solution affect growth characteristics and yield of plants. In the present study, different N concentrations influenced fresh and dry weight of aerial parts (leaves plus stems) and underground parts (roots plus rhizomes). In both traits, the highest data belonged to 200 mg L^{-1} N (Table 1). In most of the standard nutrient solutions utilized in hydroponics systems, especially in the Hoagland nutrient solution (the most common and commercial nutrient solution), optimum N concentration is nearly 200 mg L^{-1}. Borlina Maia *et al.* [11] reported that in Japanese mint aerial parts fresh and dry yields are in direct relationship with N concentration in the nutrient solution. Balanced levels of N in the nutrient solution increase aerial parts biomass of plants. Different N concentrations influenced aerial to the underground parts ratio, and the highest ratio was observed in 400 mg L^{-1} N (Fig. 1). It seems that different N concentrations affect nutrient partitioning in plants, because in higher concentrations of N more carbohydrate had been translocated to shoots and aerial parts for growth and development of plants. Leaf number was significantly ($P<0.05$) affected by concentration

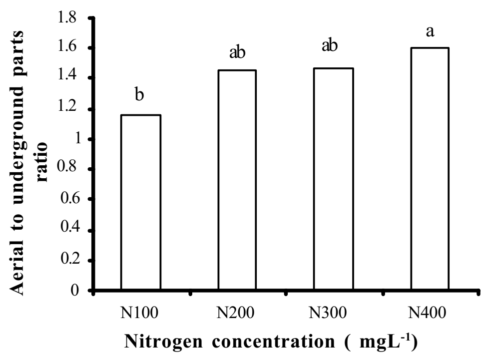

Figure 1. Effect of nitrogen concentrations on aerial to the underground parts ratio of costmary.

Table 1. Effects of N and K nutrition levels on aerial and underground parts fresh and dry weight, total net photosynthesis and height of costmary.

Treatment	Aerial parts†		Underground parts††		Total net photosynthesis (μMol CO_2 s^{-1} $plant^{-1}$)	Height (cm)
	Fwt (g)	Dwt (g)	Fwt (g)	Dwt (g)		
N (mg L^{-1})						
100	635.9 b	95.5 b	242.3 a	82.7 a	18.9	29.8
200	743.5 a	116.0 a	251.3 a	79.0 a	21.8	41.5
300	671.6 b	103.3 b	207.3 b	71.6 ab	20.0	29.8
400	621.6 b	98.6 b	190.4 b	61.0 b	18.1	27.4
K (mg L^{-1})						
100	652.2	100.5	221.2	71.8	20.2	29.7
200	684.1	106.2	224.5	75.6	19.2	34.1
ANOVA						
N	**	**	**	**	ns	**
K	ns	ns	ns	ns	ns	**
N&K	ns	ns	ns	ns	*	**

Mean separation within columns by Duncan's multiple range test. ns, *, ** Not significant or significant at P=0.05 or 0.01 respectively.
†Leaves plus stems, †† Root plus rhizome.

Table 2. Effects of N and K nutrition levels on essential oil content and yield, leaf fresh weight, leaf dry weight, leaf number and area as well as individual leaf area.

Treatment combination	Essential oil		Leaf				Individual leaf area
	Content (%)	Yield (ml m^{-2})	Fwt (g)	Dwt (g)	Number	Area (cm^2)	(cm^2)
N (mg L^{-1})							
100	0.4	7.08	551.1	83.5	223 b	13398	60.2 a
200	0.47	9.35	606.4	91.6	267 a	14381	55.7 a
300	0.41	7.15	537.3	81.6	233 ab	12546	54.1 a
400	0.48	7.62	501.1	78.1	264 a	12039	45.9 b
K (mg L^{-1})							
100	0.44	7.53	540.8	81.9	249	13101	53.1
200	0.45	8.07	557.1	85.5	245	13081	54.8
ANOVA							
N	*	**	**	*	*	**	**
K	ns	ns	ns	ns	ns	ns	ns
N&K	*	**	*	*	ns	*	ns

Mean separation within columns by Duncan's multiple range test. ns, *, ** Not significant or significant at P=0.05 or 0.01 respectively.

of N in the nutrient solution, and the greatest number was recorded in 200 mg L^{-1}N (Table 2). As mentioned above, optimum N concentration in the nutrient solution causes balanced growth and branching of plant and subsequently greater leaf number. Sifola and Barbieri [10] reported that N fertilization in basil increased leaf number per plant and this caused increase in LAI and aerial parts biomass. Furthermore, N concentrations affected individual leaf area, and the lowest amount was observed in the highest concentration of N, i.e., 400 mg L^{-1} (Table 2). Upon increasing leaf number any decrease in individual leaf area is completely rational. Sifola and Barbieri [10] reported that N fertilization did not affect individual leaf area in basil and increase in aerial parts biomass and leaf area was greatly due to greater leaf number. Chlorophyll content of leaves increased significantly (P<0.05) by increasing N concentration in the nutrient solution, and the greatest content was observed in 400 mg L^{-1} N (Fig. 2). Increase in chlorophyll content by applying higher N concentrations reflects the better efficiency of plants for N utilization in chlorophyll biosynthesis. This is in accordance with findings of Sifola and Barbieri [10] in basil. Gokang and van Iersel [15] reported that there is a direct relationship between N concentration in the nutrient solution and chlorophyll content in sage. In case of K concentrations, they did not have any significant effects upon the measured traits.

Treatment combinations of N and K affected growth characteristics and essential oil content and yield of costmary. The highest essential oil content and yield were observed in $N_{200}K_{200}$ (Fig. 3). This trend shows that for the improvement of essential oil production in costmary, accurate and balanced application of these two elements is crucial. Munsi [18] reported same nutrient balance for N and P in the Japanese mint production. Treatment combinations influenced fresh and dry weight of leaves (medicinal material) of costmary significantly (P<0.05), and the greatest amount for these traits was observed in $N_{200}K_{200}$ (Tables 2 and 3). Jeliazkova *et al.* [16] reported that N and K fertilization increased fresh and dry weight of peppermint plants. Abdallah *et al.* [14] reported that N and K fertilization increased seed yield and essential oil content and yield of fennel. Singh *et al.* [17] reported that NPK fertilization influenced growth, yield and essential oil content of French basil and increased plant height, shoot fresh weight, dry matter content and essential oil yield compared with unfertilized control plants. Because essential oil bearing glandular trichomes are prevalent among leaves, both leaf number and size are important factors for essential oil production in essential oil plants. Leaf area is dependent upon leaf number and size, and in the present experiment $N_{200}K_{200}$ treatment had the highest leaf number and size as well as leaf area (Table 2, Fig. 4). It seems that optimum concentrations of N and K, especially N concentration, affect essential oil content and yield of costmary by increasing

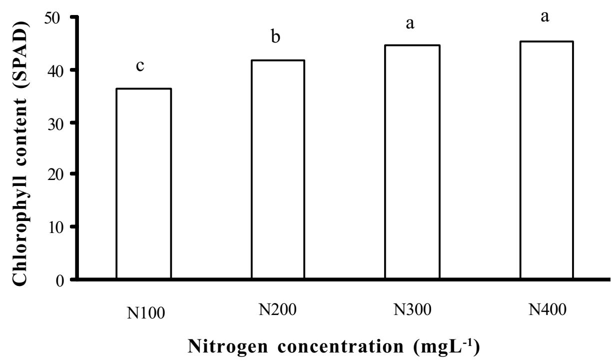

Figure 2. Effect of nitrogen concentrations on chlorophyll content of costmary.

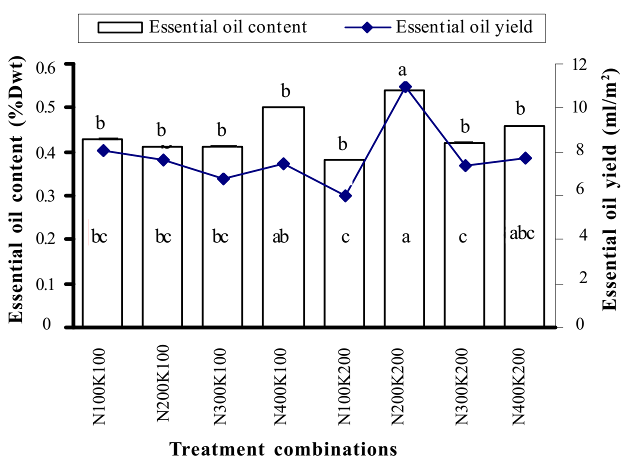

Figure 3. Effects of treatment combinations of N and K on essential oil content and yield of costmary.

Table 3. Mean comparison for N and K interactions within columns by Duncan's multiple range test.

Treatment combination	N (mg L^{-1})	K (mg L^{-1})	Leaf Fwt (g)	Leaf Dwt (g)	Height (cm)
A	100	100	598.5 ab	90.7 ab	31.3bc
B	200	100	577.8 abc	85.6 abc	34.6b
C	300	100	516.8 cd	79.3 bc	27.6c
D	400	100	470.3 d	72.0 c	25.3c
E	100	200	503.0 cd	76.3 c	28.3bc
F	200	200	635.1 a	97.6 a	48.3a
G	300	200	557.7 abc	84.0 bc	30.5bc
H	400	200	531.9 bcd	84.3 bc	29.5bc

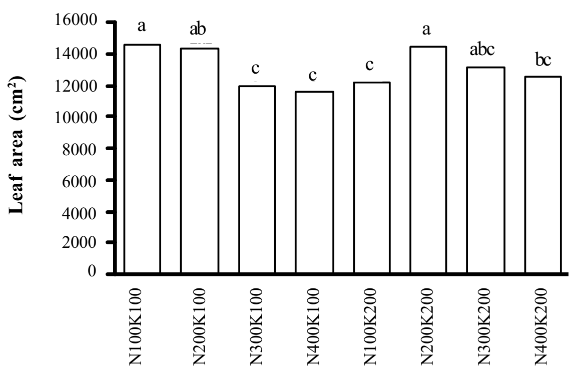

Figure 4. Effects of treatment combinations of N and K on leaf area of costmary.

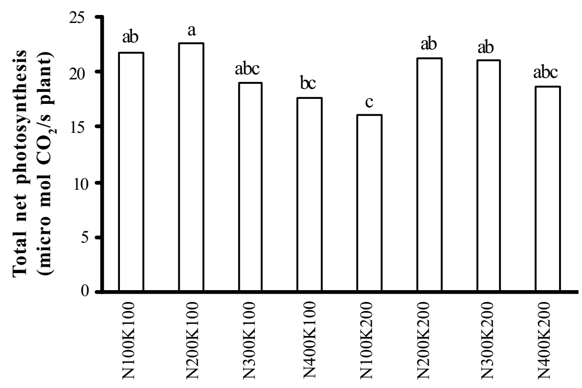

Figure 5. Effects of treatment combinations of N and K on total net photosynthesis of costmary.

leaf biomass in unit area, development of leaf area (leaves number and size) and promotion of plant efficiency for utilization of nutrient solutions in hydroponics systems. Sifola and Barbieri [10] reported that in these optimum growth conditions efficiency of plants for utilization of assimilates, especially glyceraldehyde-3-phosphate and pyruvate in terpenoid and essential oil biosynthesis pathway, increased and led to elevated essential oil content and yield. In our experiment high essential oil yield in $N_{200}K_{200}$ treatment is not only due to increase in leaf biomass, but also increase in essential oil content of leaves plays a crucial role in this case. These observations emphasize the positive and key role of optimum nutrient elements ratio on biosynthesis and accumulation of essential oil in leaves of costmary. Treatment combinations influenced total net photosynthesis of plants (Fig.5). In contrast with other traits the highest amount for total net photosynthesis was recorded in $N_{200}K_{100}$ treatment (Fig. 5). This finding is in consistency with long time demonstrations that often there is a weak correlation between net photosynthesis and plant yield. Several factors cause this paradox to be existing: 1) Leaf part or leaf itself that used for measurement is not representative of whole canopy of the plant. 2) There is a diurnal and seasonal change in photosynthesis rate. 3) Respirative CO_2 efflux of roots and shoots was not measured. Different treatment combinations influenced height of costmary significantly ($P<0.01$) and the highest data was observed in $N_{200}K_{200}$ treatment (Tables 1 and 3). Probably light interception is the most important factor that affects plant growth, and light interception itself is dependent upon leaf area. Taking into account that in $N_{200}K_{200}$ treatment plant height and leaf area were greater due to more branching, it seems that light efficiency of these plants for biomass production and essential oil accumulation was higher than that of the other treatments.

Conclusions

Appropriate application of nutrient solutions, especially N concentration, in the growing medium affects N metabolism, carboxylation activity and essential oil accumulation of medicinal plants by increasing of medicinal material fresh biomass and promotion of biochemical pathways involved in essential oil biosynthesis. Unbalanced N and K levels, especially high N rates, negatively affected essential oil content and yield of costmary. This result is supported by the following facts: 1) Maximum growth of plants in balanced levels of N and K indicates that crop can use available nutrients efficiently. 2) Effects of N rate on essential oil content was greater than that of K and this well agreed with research data from other findings. 3) The unbalanced levels of nutrients, especially increased rates of N in the nutrient solution, correlated by a decrease of essential oil content and yield. Finally it can be concluded that for high economic yields of costmary in hydroponics systems particularly for essential oil production which is a basic raw material for medicinal use, concentration of 200 mg L^{-1} is appropriate for both N and K.

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