Evaluating the effects of Naphthalene acetic acid (NAA) on morpho-physiological traits of valerian (*Valeriana officinalis* L.) in aeroponic system

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Abstract

Aeroponic culture is an alternative method for optimizing growth of different plants in controlled conditions. Aeroponic systems are an efficient tool for the root studies and improving medicinal root production. In order to study the feasibility of valerian (*Valeriana officinalis* L.) production in aeroponic systems and assessing the effects of different NAA concentrations (0, 0.1, 0.3, and 0.5 mg/l) on some morpho-physiological traits of valerian, the current study was conducted based on a completely randomized design. The results indicated that the effects of the production system were significant for plant height, root length, number of leaves, volume of roots, and dry weight of the shoot and root. Application of NAA hormone had a significant effect on all of the studied traits and by increasing NAA concentration, leaf number, root length and volume, plant height, dry weight of root and shoot, root/shoot ratio, relative water content, and photosynthetic pigments increased significantly.

Key words: foliar application; growth hormones; medicinal plant; soilless culture

Abbreviations:
AP: Aeroponic system; EC: electrical conductivity; NAA: Naphthalene acetic acid; PS: production system; RWC: relative water content

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Introduction

Valerian (*Valeriana officinalis* L.) belongs to the family of (Valerianaceae). The plant is native to Europe and parts of Asia and has been introduced into different countries (Penzkofer et al., 2014). This is a perennial medicinal plant whose underground organs such as rhizomes and roots are one of the most commonly used herbal raw materials. The abovementioned vegetative organs are rich in essential oils. (Kwiatkowski, 2010). Dried root and rhizome of valerian used in various pharmaceutical formulations have antioxidative and neuroprotective properties. Pharmaceutical preparations made of valerian rhizome and roots have various roles in treating many disorders (Damnjanović et al., 2010). Although the sedative and useful effects of the plant's root have been known for a long time, the compounds which are responsible for its activities

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have not been identified and agreed upon. There is significant variation in the chemical components of plants with different growth conditions, storage and processing methods. The separation of valerian roots from the soil particles is labor and cost intensive, because of the necessity of hand hoeing by workers as well as the time and energy consuming for harvest and drying of the roots (Penzkofer et al, 2014).

Production of medicinal and aromatic plants in controlled conditions such as soilless systems could improve biomass production, yield quality, and bioactivity of raw materials and could produce high-quality herbs and raw materials which are free from weeds, pathogens, and soil (Dorais et al., 2001). These systems also allow homogeneity of the plant size. The promising soilless technique for the future is aeroponic culture, which is based on spraying the water and nutritional elements using an aerosol mist for bathing the plant roots (Otazú, 2010). In this system the underground organs are enclosed in a dark chamber and are supplied with a solution of mineral nutrients with a mist device.

Aeroponics optimizes aeration of roots, which is the main factor leading to increasing plant yield as compared to common hydroponic systems and also planting in soil (Asao, 2012). Other advantages include recirculating nutrient elements, increasing water use efficiency, and good controlling of nutrient levels and pH. Aeroponic technique has been successfully applied for the production of many different horticultural species (Biddinger et al., 1998; Molitor and Fischer, 1999).

It is documented previously that different plant hormones such as auxins have significant role in controlling metabolism, growth, and development of plants. Auxins are a group of tryptophan-derived signals which are involved in many aspects of plants growth and development (Woodward and Bartel, 2005). Auxins have a key role in formation of the main root, initiation of lateral root, shoot/root relation, and development of leaves (Petersson et al., 2009). In addition, amount of auxin in the plant may affect the abscission of leaves (Pop et al., 2011). Naphthalene acetic acid (NAA) is a type of plant hormone in the auxin family. Effects of this hormone on different plants often depend on the stage of the plant's development (Ding and Friml, 2010). This exogenous hormone normally acts by signaling the proteins to stimulate new cell resulting in the initiation and production of lateral roots (Durbak et al., 2012).

It seems that reducing the losses during root harvest and processing, as well as simplifying the cleaning of the roots and stimulation of root producing are the main aim for improving valerian production, so the first objective of this investigation was to compare an aeroponic system with conventional systems for valerian production. Although some reports have been published concerning the effects of the above mentioned plant growth regulators, they have investigated the plant production in the greenhouse or field conditions and the effects of the NAA have not been investigated on the valerian production in the aeroponic system. Therefore the second purpose is assessment of the effects of NAA on growth and some physiological traits of valerian in an aeroponic system.

Materials and Methods

For the first experiment the seeds of valerian were planted in small pots in greenhouse condition and after 30 days the similar plants (≈20 cm height) were transferred to aeroponic system in a controlled greenhouse. Two cultivation systems (conventional planting in soil and aeroponics) were utilized. The plants were grown in the experimental greenhouse of Malayer University, where day and night temperatures in the greenhouse maintained at 27 °C and 18 °C, respectively, and relative humidity around 70%. The diurnal light regime during the experiment was 16 hours light and 8 hours dark.

Aeroponic system

In the present study, we conducted a research on aeroponic system (phytorhizotron) which was designed previously for the production of potato minitubers. The phytorhizotron consisted of two compartments: the upper compartment was supplied with photoperiod control and the lower compartment was kept in darkness. The plants were planted on the board of the upper compartment with 13 × 13 spacing and about one-third of the length of the stems was placed inside the lower compartment. The shoots grew in the upper compartment and the roots developed in the lower compartment under dark conditions. The lower compartment was a closed container (120 × 100 × 100 cm; depth, width, and length, respectively), which had a removable front panel for root monitoring and harvesting. Plant roots were periodically sprayed (every 20 minutes for 20 seconds) with nutrient solution using 12 fog nozzles. Nutrient solution was renewed weekly. The residual nutrient solution was allowed to flow back into a collecting tank and was recirculated.

Nutrient solution and growing conditions

The component of nutrient solution used is shown in Table 1. Solution electrical conductivity (EC) was adjusted to 1.6 ± 0.2 dS/m. The initial pH was adjusted to 5.8 ± 0.2, whereas pH beyond that was not controlled. Plants grew in a greenhouse under a 16-h photoperiod.

Treatments

In the experiment the effects of foliar application of NAA (0, 0.1, 0.3, 0.5 mg/l) on some of morpho-physiological characteristics of valerian were assessed. Control plants were treated with distilled water. In all treatments, the Tween 20 (Polysorbat) at 0.05 ml/l concentration was used as wetting agent. Plants were foliar sprayed with NAA 30 and 50 days after transplanting in theaeroponic system. The experiment was carried out according to completely randomized design (CRD) layout with five replications.

Data recorded

Plants were harvested 6 months after transplanting. The total length of the plant, root length, number of leaves, and volume of roots, root fresh weight, root dry weight, shoot dry weight, and also amount of photosynthetic pigments were measured. Photosynthetic pigments of valerian leaves were determined according to Lichtenthaler (1987). The relative water content (RWC) of valerian leaf was determined as follows:

$$RWC = (FW - DW)/ (TW - DW) \times 100$$

Where, FW, DW, and TW are the fresh, dry, and turgid weight of leaf, respectively.

Data analysis

Primary statistical analyses such as normality test (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test) were conducted. After the analysis of variance (ANOVA), the means of treatment combinations were compared using Duncan's Multi Range Test (DMRT). All the above statistical analyses were carried out using SPSS version 21.

Table 1
Concentrations of nutrients used in aeroponic system (mg/l)

Elements	Concentration (mg/l)	Elements	Concentration (mg/l)
K	200	Fe	1
N	190	Mn	0.5
Ca	150	B	0.5
S	70	Zn	0.15
Mg	45	Cu	0.1
P	35	Mo	0.05

Table 2
Analysis of variance for measured traits of root production system in CRD layout

SOV	df	Leaf number	Total length	Root length	Root volume	Root dry weight	Shoot dry weight
PS	1	220.9**	2280.1**	1254.4**	67.6**	16.384**	13.064**
Error	8	4.9	8.250	9.750	3.250	0.762	0.16

PS:Production System; ns: non-significant; * and**: Significant at 0.05 and 0.01 probability level, respectively

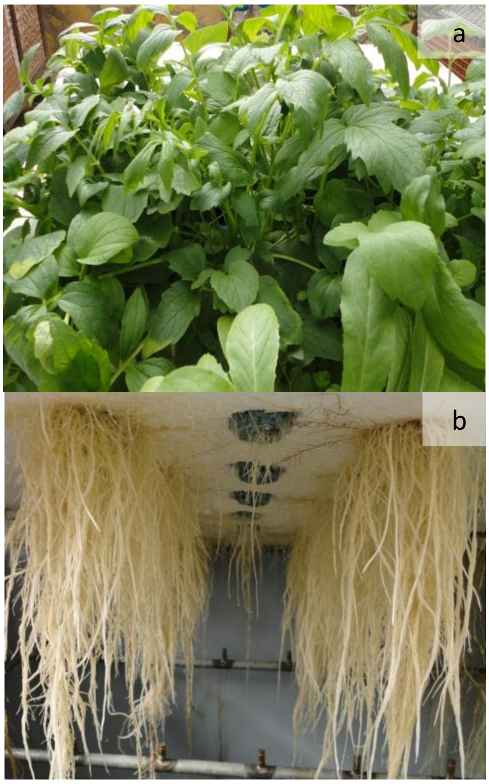

Fig. I. Root and shoot of Valerian 4 month after transplanting in aeroponic system

Fig. II. Root and shoot of Valerian 6 month after transplanting in aeroponic system

Results

The results of Kolmogorov-Smirnov normality test for all the measured traits in aeroponic and soil system indicated that all variables were normal. The results of Levene test proved the assumption of homogeneity of variances. Similar results were obtained for the effect of NAA as well. Therefore, conventional parametric statistics were used to analyze the traits under study.

According to the ANOVA (Table 2), the effects of production system (PS) were highly significant at 0.01 probability level for total length of the plant, root length, number of leaves, volume of the root, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight.

Mean comparison between aeroponic system and soil system indicated that valerian in aeroponic production (AP) system had the highest length of the plant (73.4 cm), root length (54.6 cm), number of leaves per plant (23.4), volume of the root per plant (11.4 cm^3/plant), root fresh weight (21.34 g/plant), root dry weight (5.84 g/plant), shoot fresh weight (18.48 g/plant), and

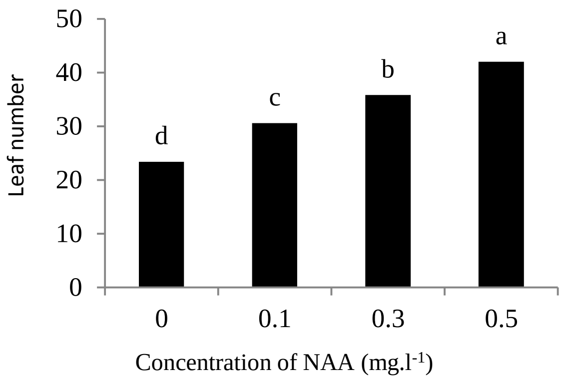

Fig. III. Effect of NAA application on leaf number

shoot dry weight (4.64 g/plant) while in the soil production system versus AP system showed total length of the plant (43.2 cm), root length (32.2 cm), number of leaves per plant (14), volume of the root (6.2 cm^3/plant), root fresh weight (10.52 g/plant), root dry weight (2.92 g/plant), shoot fresh weight (10 g/plant), and shoot dry weight (2.36 g/plant). These results could be verified through Figures Ib and IIb, which show that the AP system was better than the soil production system. It has been shown that stems' length

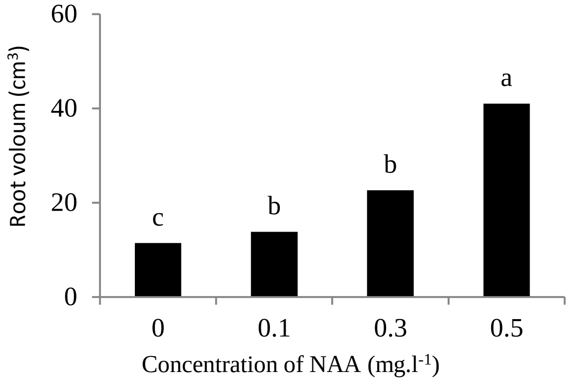

Fig. IV. Effect of NAA application on root volume

grown in aeroponics was longer than the ones grown in the other systems .

For the second experiment, according to the analysis of variance, the effects of different concentrations of NAA were highly significant at 0.01 probability level for total length of the plant, root length, number of leaves, volume of the root,

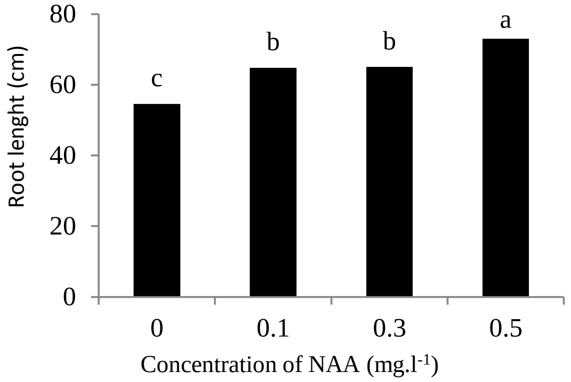

Fig. V. Effect of NAA application on root length

root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight. Application of NAA also significantly affected Chlorophyll a, Chlorophyll b, Chl a/Chl b ratio, carotenoids, and relative water content (Table 3). Mean comparison of the treatment indicated that concentration of 0.5 mg/l NAA in AP system had the highest number of leaves per plant (Fig. III), volume of the root (Fig. IV), root length (Fig. V), root dry weight (Fig. VI), plant height (Fig. VII) and shoot dry weight (Fig. VIII), while the control had the lowest plant height (73.4 cm), root length (54.6 cm), number of leaves per plant (23.4), volume of the root (11.4 cm^3/plant), root dry weight (5.84 g/plant), and shoot dry weight (4.64 g/plant). Increasing the NAA concentration up to 0.1 g/l resulted in increasing root dry weight/shoot dry weight ratio but in higher concentrations this ratio decreased slightly (Fig. IX).

In this experiment effect of NAA application on photosynthetic pigments was significant. All of the photosynthetic pigments (i.e., chlorophyll a, b, and carotenoids) of valerian leaves significantly increased by application of

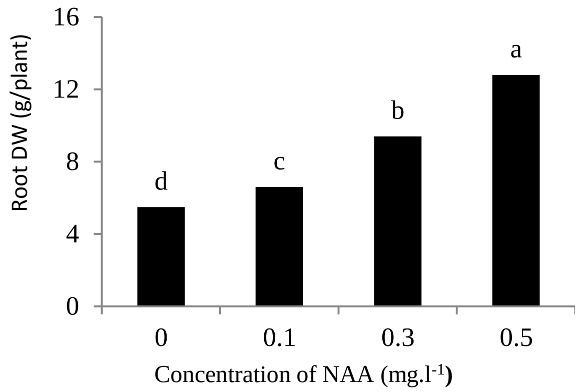

Fig. VI. Effect of NAA application on root dry weight

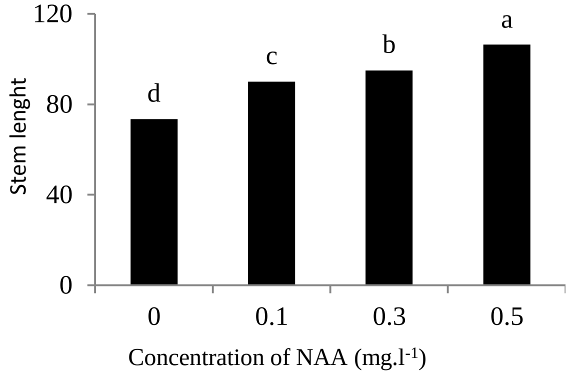

Fig.VII. Effect of NAA application plant height

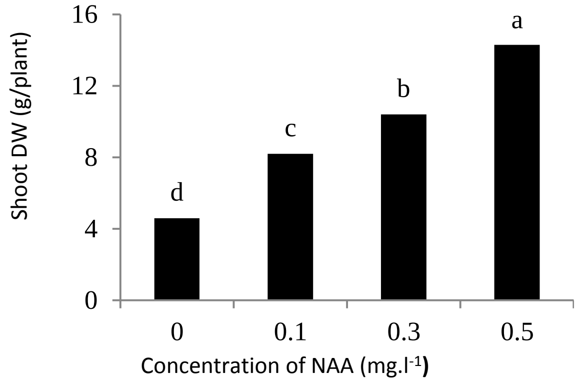

Fig. VIII. Effect of NAA application on shoot dry weight

NAA and the highest amount of chlorophyll a was observed at 0.1 mg/l NAA that was higher by 25.8% compared to the control.

Discussion

The principles of aeroponics are based on the possibility of cultivating vegetables whose roots are not inserted in a substratum (the case with hydroponics) or soil, but in containers filled with flowing plant nutrition. In these containers roots can find the best condition regarding oxygenation and moisture. These conditions allow for better plant nutrition assimilation in a more balanced way, with consequential faster development of the cultivated plants. Plant containers can be mounted on top of one another and because they are light and handy, they can be easily moved according to agricultural needs. Because the aeroponic system is a continuous-cycle in an enclosed space it reduces the agricultural labor to a series of mechanical routine operational tasks which are carried out daily and throughout the year. This enables workers to acquire considerable skill within a short period of time—a few months. The aeroponic equipment is sheltered within greenhouses or anti hail-storm coverings according to the latitude. Climate controls within the greenhouse ensure optimal growing conditions, assuring high yields. AP system optimizes root aeration, which is a major factor leading to increasing yield compared to classical production systems (Asao, 2012). In this study AP system has improved all measured traits of valerian in comparison to soil production systems. Our finding in significant and positive effect of AP system is in agreement with Hyden (2006), which indicated that aeroponic system is a useful option for producing medicinal herbs, rhizomes, and root crops. These results confirm the other findings which emphasize the good performance of aeroponic root in comparison to conventional production systems (Pagliarulo and Hayden, 2000). Results of similar a study showed that the aeroponic system led to increase in stem length, root length, stem diameter, yield, and quality of potato minitubers (Movahedi et al., 2012). The positive effects of aeroponic culture were found for many of plant species such as medicinal plants (Hyden, 2006). Spraying the nutrient solution could decrease the temperature of root environment.

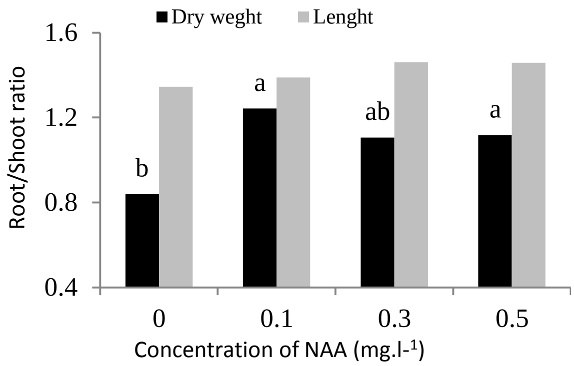

Fig. IX. Effect of NAA application on root/shoot ratio

High temperature of root environment involves nutrient deficiency symptoms on leaves, and usually has significant effect on enzymatic and phytohormone activities, roots morphology, nutrients uptake, and the relations between roots and shoots (Tan et al., 2002).

Also result showed the positive effect of NAA on valerian grown in an aeroponic system. It is commonly accepted that auxins have an important role in the plant rooting (Khan and Bi, 2012). Different hormones from the auxin group such as NAA could control growth and development in plants. Many studies have shown that exogenous application of auxins results in increased roots length and volumes and lateral

Table 3
Mean comparison of NAA effects on photosynthetic pigments and relative water content (RWC).

NAA	Chlorophyll a (mg/ml)	Chlorophyll b (mg/ml)	Chl a/ Chl b	Carotenoids (mg/ml)	RWC (%)
0.1	1.82b	0.96b	1.89ab	2.3b	83b
0.3	2.29a	1.17a	1.95a	2.6a	82b
0.5	2.14ab	1.3a	1.64b	2.77a	85ab
Control	2.23a	1.34a	1.66ab	2.8a	88a

ns: non-significant, * and**: Significant at 0.05 and 0.01 probability level, respectively root development is highly dependent on auxin and auxin transport (Chhun et al., 2003).

Based on the findings of the present study, by increasing the concentration of NAA, lengths, dry matter and volume of valerian roots increased significantly. Other researcher also reported that NAA can control many aspects of plant development including root initiation and elongation (Shan et al., 2012). Results of Raju and Prasad (2010) showed that the rooting percentage is changed significantly depending on the types and concentrations of the hormone used. Our results are in contrast with the results of other researchers who reported a rapid increase in root production at lower NAA concentration while the number of roots decreased at higher concentration of auxin (Sun and Hung, 2010). In this experiment, the effect of NAA application on photosynthetic pigments was significant. Although results of Tolentino and Cadiz (2005) showed that auxin compounds did not significantly change the chlorophyll and alkaloid content of bitter gourd, but they did not indicate the absence of their influence on these parameters. It has been shown that stems' length grown in aeroponics was longer than the ones grown in the other systems (Kang et al., 1996; Farran and Mingo-Castel, 2006). These findings are in agreement with the results of the present study (Fig. II A, which indicate that AP system had longer root length than the soil production system.

Chlorophyll is the main photoreceptor in photosynthesis, the light-driven process in which carbon dioxide is fixed to yield carbohydrates and oxygen (Quach et al., 2004). Biosynthesis of chlorophylls in valerian like other plants is susceptible to hormones and by reducing chlorophyll synthesis the photosynthesis will also be reduced. Photosynthesis is the most important physiological process in plants and plant metabolism is affected by photosynthesis and the rate of this process in higher plants could affect plant growth and development. Higher photosynthesis rate may increase the total dry matter of root and shoot.

It seems that NAA application could increase the valerian root dry matter through different ways such as increasing photosynthetic products (because of higher leaf number, photosynthetic pigments and RWC), and increasing root/shoot ratio by changing allocation pattern.

Conclusion

Soilless systems for production of different crops have several benefits compared with soil production system. The current results showed that an aeroponic system could be a proper system for valerian root production and study. The produced roots were healthy and free of infections. However, many aspects of aeroponic system have to be studied in order to optimize the root-producing method and it seems that some other important considerations must be addressed for a commercial system of root production. By increasing the concentration of NAA, dry matter production both in shoot and root improved; therefore, application of this plant hormone for increasing root yield and profit in aeroponic systems is justified.

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