Optimizing hydroponic culture media and NO$_3^-$/NH$_4^+$ ratio for improving essential oil compositions of purple coneflower (Echinacea purpurea L.)

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Medicinal plants represent a valuable commodity due to beneficial effects of their natural products on human health, prompting a need for finding a way to optimize/increase their production. In this study, a novel growing media with various perlite particle size and its mixture with peat moss was tested for hydroponic-based production of Echinacea purpurea medicinal plant under greenhouse conditions. The plant growth parameters such as plant height, total fresh leave weight, fresh root weight, total biomass, total chlorophyll, leaf area, and essential oil compositions were assessed. Perlite particle size in the growing media was varied from very coarse (more than 2 mm) to very fine (less than 0.5 mm), and the ratio between perlite and peat moss varied from 50:50 v/v to 30:70 v/v. In addition, two nitrate (NO$_3^-$) to ammonium (NH$_4^+$) ratios (90:10 and 70:30) were tested for each growing media. The medium containing very fine-grade perlite and 50:50 v/v perlite to peat moss ratio was found to be most optimal and beneficial for E. purpurea performance, resulting in maximal plant height, fresh and dry weight, leaf surface area, and chlorophyll content. It was also found that an increase in NO$_3^-$/NH$_4^+$ ratio caused a significant increase in plant growth parameters and increase the plant essential oil content. The major terpene hydrocarbons found in extract of E. purpurea with the best growth parameters were germacrene D (51%), myrcene (15%), α-pinene (12%), β-caryophyllene (11%), and 1-Pentadecene (4.4%), respectively. The percentages of these terpene hydrocarbons were increased by increasing of NO$_3^-$/NH$_4^+$ ratio. It can be concluded that decreasing the perlite particle size and increasing the NO$_3^-$/NH$_4^+$ ratio increased the plant growth parameters and essential oil compositions in E. purpurea.

Medicinal plants and their beneficial effects on human health are well known in various cultures for centuries.$^1$ Echinacea is a medicinal plant that belongs to the family of Asteracea/Compositae and is native to much of the United States.$^{2,3}$ The most popular species of the plant in medicine are E. purpurea, E. angustifolia, and E. pallida. The species has a black and pungent root and purple coneshape flowering head.$^4$ All parts of the E. purpurea species, especially root and coneflower, are rich in useful medicinal compounds, prompting significant attention of researchers to this species.$^5,6$ Using of E. purpurea essential oil in medicinal, cosmetic, and food industries is common in all over the world.$^7$ The effect of E. purpurea essential oils on antimicrobial properties has been proven in previous studies.$^8$ Also accepted is the role of some constituents of the essential oil of E. purpurea, including α-phellandrene, myrcene, limonene, α-pinene, β-pinene a, δ-cadinene, germacrene D, and β-caryophyllene, as antifungal, antiviral and antibacterial agents.$^{6,10}$ Extracts of essential oil obtained from E. purpurea are efficient in pest control and could regulate insect population at different life stages.$^{11}$ Numerous studies have been focused on prominent insecticidal influence of E. purpurea essential oil compositions and found the better influence of them in comparing with chemicals or a potential source of insecticides.$^{11}$ The antibacterial activity of E. purpurea essential oil is also reported against different food pathogens and bacteria in food industry.$^{12,13}$

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crop inconsistency, seed dormancy, water stress regimes, microbes, heavy metal ions and other pollutants can concentrate at the top portion of the container includes low aeration and high water-holding capacity.

Different hydroponic cultivation methods, such as artificial substrate media, water culture, and nutrient film techniques have been reported for *E. purpurea* cultivation. However, using artificial substrates in the hydroponic cultivation system reduces the cost of establishing advanced hydroponic cultivation systems and also enables the farmer to make a practical use of it by using commonly raw materials such as cocopeat, sand, and vermiculite as an initial plant growing media. Nevertheless, different inorganic products such as peat moss, perlite, mixed materials, etc. are fully or partially used instead of initial substrates due to their useful physical properties. The particle size of substrates is a critical factor in air and water-holding capacity, root distribution, and plant growth, which are different based on their origin and preparation conditions. A high volume of roots can concentrate at the top portion of the container includes low aeration and high water-holding capacity.

In addition to the importance of substrates properties in the hydroponic culture system, attention to the chemical composition of nutrient solution is important. In terms of chemical composition of nutrient solutions, two major inorganic forms of nitrogen (N), the NH4+ and the NO3−, can differentially impact the various plant properties, based on the plant species. Although the assimilation and metabolism of NH4+ form require less energy than that of NO3− in plants, the majority of plant species grow better on NO3− since NH4+ is toxic for plants and a few species grow well if NH4+ is the only source of N. The plant species and environmental conditions are two critical factors that affect the optimum NO3−/NH4+ ratio. It has been reported that different N application rates could affect the essential oil compositions of peppermint (*Mentha piperita* L.).

While the industrial application of *E. purpurea* essential oils is well established, several factors such as weather changes, plant growth stage, and method of cultivation may influence both the composition and production of *E. purpurea* essential oil. The open field cultivation of *E. purpurea* has some significant limitations such as crop inconsistency, seed dormancy, water stress regimes, microbes, heavy metal ions and other pollutants and loss of wild germplasm, that affect the different chemical composition of the plant extract. The above limitations have prompted a shift towards plant production under greenhouse conditions, especially in hydroponic (or soilless) culture systems. Growing in a greenhouse also offers additional advantages of more effective control of plant nutrition.

Table 1. Chemical properties of nutrient solution.

| Element        | Fertilizer type        | Amount             |
|----------------|------------------------|--------------------|
| Nitrogen (N)   | (NH4)2SO4, KNO3, Ca(NO3)2 | 15 mM              |
| Phosphorus (P) | H3PO4                  | 1 mM               |
| Potassium (K)  | KNO3                   | 6 mM               |
| Calcium (Ca)   | Ca(NO3)2               | 4 mM               |
| Magnesium (Mg) | MgSO4·7H2O             | 2 mM               |
| Sulfur (S)     | Sulfate fertilizers     | 2 mM               |
| Iron (Fe)      | Fe-EDTA                | 50 µM              |
| Manganese (Mn) | MnSO4·H2O              | 9 µM               |
| Copper (Ca)    | CaSO4·5H2O             | 0.3 µM             |
| Zinc (Zn)      | ZnSO4·7H2O             | 0.8 µM             |
| Boron (B)      | H3BO3                  | 0.05 µM            |
| Molybdenum (Mo)| H2MoO3(NH2)2·H4O        | 0.01 µM            |

Materials and methods

Growth conditions. The experiment was performed in a commercial greenhouse at Urmia University, West Azerbaijan, Iran. The air temperature was 22/18 °C (day/night) and the humidity ranged from 70 to 80%. The maximum photosynthetic photon flux density (PPFD) fluctuated from 550 to 750 µmol m−2 s−1 inside the greenhouse. The *E. purpurea* seeds were purchased from Iranian private joint-stock company, Pakan Bazr Esfahan (www. Pakanbazar.com). The seeds were sowed in plastic cups filled with a mixture of perlite and peat moss substrates as a medium to initiate germination. Irrigation was performed based on greenhouse conditions regularly. Seedlings (with four real leaves) were translocated to experimental plastic pots (2.5 L) containing a different ratio of perlite and peat moss as artificial substrates (100% perlite, 100% peat moss, 50% (v) perlite + 50% (v) peat moss, 70% (v) perlite + 30% (v) peat moss) with various perlite particle size containing less than 0.5 mm, 0.5–1 mm, 1–1.5 mm, 1.5–2 mm, and more than 2 mm. Chemical concentrations of nutrient solution are shown in Table 1. The pH and electrical conductivity (EC) of the nutrient solution were maintained between 5.7 to 6.2 and 1.0 to 1.5 dS m−1, respectively. According to the stage of the plant growth, 0.5 to 3.5 L day−1 was used in fertigation system.
Sample preparation. Plants were harvested at the end of the flowering stage (eight months). The plants were divided into roots, stems, flower heads, and lower and upper leaves after washing with tap water. Root, flower heads, and leaves samples were dried at 25 ± 1 °C, ground into a fine powder and collected for further analyses.

Plant growth parameters. The main growth parameters such as plant height (cm), fresh root weight (g plant⁻¹), total fresh leaf weight (g plant⁻¹), total biomass (g plant⁻¹), and leaf area (cm²) were determined for each plant at the matured stage. The leaf area was measured by using leaf area meter. Chlorophylls a and b were determined using 0.5 g of dry sample, which was homogenized with 10 mL acetone. Homogenized samples were centrifuged at 10,000×g for 15 min at 4 °C. The supernatant was separated, and the absorbance spectra were measured at 400–700 nm. The total chlorophyll was calculated at 645 nm and 663 nm respectively. So that:

\[ C = 20.2A_{645} - 2.350A_{663} \]  

where C is the total chlorophyll contents in mg/L of acetone extract, A₆₄₅, and A₆₆₃ are the absorption of the extract at 645 and 663 nm.

Extraction of essential oils. The E. purpurea plants which shown the best morphological properties (maximum height, dry and wet weight of leaves and roots, and leaf area) were selected for analysis of essential oil. Distilled water was added to 120 g powder samples (root, leaves, and flower head) at a 1:10 (g/mL−1) ratio. The essential oil was extract based on the distillation procedure using a commercial Clevenger apparatus.

Analysis of essential oil. The essential oil analysis was performed using gas chromatography (GC) with 30 m × 0.25 mm capillary column coated with 0.25 µm film; carrier gas, helium (He) with a flow rate of 32 cm s⁻¹; injection temperature of 260 °C and injection volume 0.2 µL. The programming was carried out from 90 °C for 2 min rising at 7 °C min⁻¹ to 180 °C, at 15 °C min⁻¹ to 220 °C. Identifications of different components were made by library search program on monoterpenoids and sesquiterpenoids mass spectral database and by comparing retention time with those of reference samples.

Gas chromatography–mass spectrometry. Gas Chromatography–Mass Spectrometry (GC–MS) spectra were recorded on a Varian-3400 model fitted with a fused silica capillary column (30 m × 0.25 mm i.d.) coated with 0.25 µm film. The GC was run from 60 to 250 °C at a programmed rate of 8 °C min⁻¹, hold at 100 °C for 2 min, using He as the carrier gas at a pressure of 1.6 kg cm⁻² and injector temperature of 250 °C. The GC column was coupled directly to the quadrupole mass spectrometer operated in the electron impact (EI) mode at 70 eV. Mass spectra were recorded at a scan speed of 9 at m/z 700–10.

Statistical analysis. The statistics was based on the factorial with completely randomize design with three replications. The factors contained different sizes of perlite, including very coarse perlite (more than 2 mm), coarse perlite (1.5–2 mm), medium perlite (1–1.5 mm), fine perlite (0.5–1 mm), and very fine perlite (less than 0.5 mm), two NO₃⁻/NH₄⁺ rations (90:10 and 70:30), and a mixture of peat moss with different size of perlite at 50% perlite + 50% peat moss medium with perlite particle size less than 0.5 mm growing medium at different NO₃⁻/NH₄⁺ ratios (90:10 and 70:30), and a mixture of peat moss with different size of perlite at 50% perlite + 50% peat moss medium with perlite particle size less than 0.5 mm growing medium at different NO₃⁻/NH₄⁺ ratios. Increasing NO₃⁻ proportion in the N nutrition of E. purpurea caused to increase in plant height and root weight considerably.

Essential oil analysis. The flower head, leaves, and root essential oil compositions of E. purpurea grown at the 50% perlite + 50% peat moss medium with perlite particle size less than 0.5 mm growing medium at different NO₃⁻/NH₄⁺ ratios are shown in Tables 3 and 4, respectively.
Table 2. Some morphological properties of *E. purpurea* growing on various culture media and NO$_3^−$/NH$_4^+$ ratio at the flowering stage. Pt: peat moss and Pe: perlite. The numbers in the parentheses show perlite particle size. Each value is expressed as mean ± SD (n = 3). Means bearing different letters in the same column are significantly different (P ≤ 0.01). The numbers show as mean ± standard deviation. The interaction effect of different treatments on total fresh leave weight, chlorophylls a and b, and leaf area was not significant.

| Culture media                  | NO$_3^−$/NH$_4^+$ ratio | Height (cm) | Total fresh leave weight (g plant$^−1$) | Fresh root weight (g plant$^−1$) | Total biomass (g plant$^−1$) | Total Chlorophyll (mg g$^−1$ FW) | Leaf area (cm$^2$) |
|--------------------------------|-------------------------|-------------|----------------------------------------|---------------------------------|-------------------------------|---------------------------------|------------------|
| 100% Pe (> 2 mm)              | 90:10                   | 5.3$±$0.27  | 1.3$±$0.13                             | 3.1$±$0.16                      | 4.4$±$0.25                   | 5.12$±$0.11                     | 5$±$0.41         |
|                               | 70:30                   | 3.2$±$0.21  | 1.5$±$0.11                             | 3.1$±$0.11                      | 4.1$±$0.14                   | 3.53$±$0.032                    | 4$±$0.35        |
| 100% Pt                       | 90:10                   | 55$±$2.9    | 10$±$2.1                               | 20$±$3.9                        | 30$±$4.1                     | 8.8$±$0.15                     | 20$±$0.25        |
|                               | 70:30                   | 47$±$2.1    | 8.2$±$1.1                              | 16$±$2.5                        | 24$±$3.2                     | 6.6$±$0.12                     | 15$±$0.14        |
| 50% Pt + 50% Pe (<0.5 mm)     | 90:10                   | 105$±$6.1   | 40$±$3.2                               | 75$±$4.6                        | 116$±$7.1                    | 18.5$±$0.11                    | 60$±$0.35        |
|                               | 70:30                   | 91$±$4.2    | 28$±$1.2                               | 52$±$3.2                        | 80$±$4.1                     | 15.1$±$0.11                    | 51$±$0.23        |
| 50% Pt + 50% Pe (0.5–1 mm)    | 90:10                   | 98$±$5.1    | 27$±$2.1                               | 53$±$4.1                        | 81$±$6.1                     | 16.2$±$0.13                    | 55$±$0.15        |
|                               | 70:30                   | 71$±$3.2    | 21$±$1.1                               | 48$±$2.5                        | 70$±$3.6                     | 13.2$±$0.11                    | 49$±$0.11        |
| 50% Pt + 50% Pe (1–1.5 mm)    | 90:10                   | 96$±$5.9    | 26$±$2.1                               | 50$±$3.2                        | 76$±$5.2                     | 14.6$±$0.16                    | 50$±$0.15        |
|                               | 70:30                   | 82$±$3.2    | 24$±$1.2                               | 43$±$2.4                        | 67$±$2.4                     | 12.8$±$0.11                    | 42$±$0.10        |
| 50% Pt + 50% Pe (1.5–2 mm)    | 90:10                   | 91$±$4.3    | 25$±$1.1                               | 45$±$2.5                        | 71$±$3.6                     | 13.8$±$0.14                    | 43$±$0.11        |
|                               | 70:30                   | 71$±$2.1    | 24$±$1.1                               | 40$±$1.9                        | 65$±$2.4                     | 12.2$±$0.12                    | 38$±$0.11        |
| 50% Pt + 50% Pe (>2 mm)       | 90:10                   | 85$±$3.3    | 23$±$2.1                               | 41$±$2.5                        | 64$±$4.1                     | 13.2$±$0.12                    | 40$±$0.14        |
|                               | 70:30                   | 66$±$2.5    | 21$±$1.1                               | 37$±$2.1                        | 58$±$3.5                     | 11.5$±$0.11                    | 32$±$0.10        |
| 30% Pt + 70% Pe (<0.5 mm)     | 90:10                   | 85.2$±$2.8  | 23$±$2.1                               | 50$±$3.1                        | 74$±$4.1                     | 16.1$±$0.13                    | 42$±$0.15        |
|                               | 70:30                   | 71.6$±$2.1  | 22$±$1.1                               | 43$±$2.4                        | 65$±$3.6                     | 12.3$±$0.11                    | 35$±$0.11        |
| 30% Pt + 70% Pe (0.5–1 mm)    | 90:10                   | 71.1$±$3.5  | 21.1.3                                 | 47$±$2.2                        | 68$±$4.1                     | 13.5$±$0.12                    | 38$±$0.15        |
|                               | 70:30                   | 63.9$±$1.9  | 20$±$1.1                               | 37$±$2.1                        | 58$±$3.1                     | 10.4$±$0.11                    | 30$±$0.11        |
| 30% Pt + 70% Pe (1–1.5 mm)    | 90:10                   | 66.1$±$2.5  | 18.8$±$2.1                             | 42$±$2.8                        | 61$±$4.2                     | 12.9$±$0.13                    | 32$±$0.13        |
|                               | 70:30                   | 52.7$±$2.1  | 15$±$1.3                               | 32$±$1.6                        | 47$±$2.6                     | 9.4$±$0.10                     | 25$±$0.11        |
| 30% Pt + 70% Pe (1.5–2 mm)    | 90:10                   | 55.5$±$3.2  | 18.6$±$1.2                             | 34$±$2.5                        | 53$±$3.1                     | 11.3$±$0.14                    | 28$±$0.13        |
|                               | 70:30                   | 43.1$±$2.1  | 14.1$±$1.1                             | 29$±$1.1                        | 43$±$2.1                     | 8.4$±$0.11                     | 23$±$0.11        |
| 30% Pt + 70% Pe (>2 mm)       | 90:10                   | 49.3$±$4.2  | 15.9$±$2.5                             | 32$±$2.1                        | 48$±$3.6                     | 10.4$±$0.13                    | 25$±$0.14        |
|                               | 70:30                   | 35.9$±$2.1  | 12.7$±$1.3                             | 28$±$1.5                        | 41$±$2.2                     | 8.3$±$0.11                     | 19$±$0.12        |

| Figure 1. *Echinacea purpurea* grown in (A) 100% peat moss, (B) 30% peat moss + 70% perlite (<0.5 mm), (C) 50% peat moss + 50% perlite (<0.5 mm), (D) 100% perlite (>2 mm) culture media, just at 90:10 NO$_3^−$/NH$_4^+$ ratio. (All photos were taken by F. Ahmadi). |
The essential oils were separated into 51 components, 38 of them were identified, comprising 92.8% of the total essential oil yield (Tables 3 and 4). The content and composition of the essential oil exhibited a variable pattern at different plant organs at different NO$_3^-$/NH$_4^+$ ratios (Tables 3 and 4).

The most abundant terpenes including, germacrene D, myrcene, α-Pinene, β-caryophyllene, and 1-pentadecene were found in chemical composition of *E. purpurea* extract by previous researchers. Comparing of the results in present study with other researches shows the noticeable increase in essential oil composition by using novel growing media and nutrition pattern (Table 5), which is related to improve physical properties of growing media (50% perlite + 50% peat moss medium with perlite particle size less than 0.5 mm and 90:10 NO$_3^-$/NH$_4^+$ ratio).

**Discussion**

Based on open hydroponic cultivation system in the present experiment, decreasing perlite particle size, increased the retention time of nutrient solution in the culture media. Increasing nutrient accessibility for plant roots by increasing retention time improves nutrient uptake and plant growth. However, the pure perlite culture system (100% perlite, < 0.5 mm) has a very low air-filled porosity (AFP) of 33% and water holding capacity (WHC) of 56% in comparison with other fine-perlite culture media (Table 6). Accordingly, the lowest growth parameters were obtained in pure perlite medium (Table 2), which can be attributed to the rapid withdrawal of nutrient solution from the culture medium and the inability of the medium to maintain the nutrient solution. Due to the high porosity of peat mass and nutrient solution retention capability, an increase of the plant morphological parameters is expected in the presence of peat moss in various cultural media (Table 2). The noticeable increase in chlorophyll content by reducing perlite particle size implies the significant effect of culture media on photosynthesizing pigments (Table 2). It has been reported that the application of N fertilizers in the fine perlite culture media increased N content of the plants, thereby increasing their chlorophyll content, subsequently, and their ability to absorb sunlight and produce photosynthates, which resulted in their higher leaf area, and growth and yield$^{18,28}$.

The variations in the concentrations of various essential oil compositions at different NO$_3^-$/NH$_4^+$ ratios (Tables 3 and 4) may be due to supply different amounts of NO$_3^-$ to the plant. The presence of N as a key factor can affect the production of essential oils in aromatic plants$^{29}$. Nitrogen is critical factor in biosynthesis pathway of essential oil in medicinal and aromatic plants$^{30}$. Nitrogen increases photosynthetic efficiency and plays an important role in increasing the amount of essential oil by increasing the number and area of leave and providing a suitable condition for receiving sunlight energy and also participating in the structure of chlorophyll and...
enzymes involved in photosynthetic carbon metabolism. Nitrogen is an essential nutrient in plants used to synthesize many organic compounds in plants such as nucleic acids, enzymes, proteins, and amino acids, which are necessary for essential oil biosynthesis. Besides, essential oils are terpenoids compounds whose constituent units (isomers) such as isopentenyl pyrophosphate and dimethyl allyl pyrophosphate are strongly formed into adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), and due to the effect of N in the production of these compounds, the amount of essential oil increased. Nitrogen increases the essential oil content of plants by increasing the dry weight (Nyalambisa et al. 2016). Comparing the results in Tables 3 and 4 indicated that increase of NO$_3^-$ concentration could increase the percentage of essential oil composition due to its effect on essential oil biosynthesis as demonstrated in previous researches.

Germacrene D, myrcene, α-Pinene, β-Caryophyllene, and 1-Pentadecene were the major compositions of essential oil of *E. purpurea* grown in very fine-grade (< 0.5 mm) perlite with 50:50 v/v perlite to peat moss ratio (Tables 3 and 4). The compositions have a valuable beneficial effects in medicine and agriculture industries.

| Components          | Class a | LRI b | KI     | Percentage | Flower heads | Leaves | Root |
|---------------------|---------|-------|--------|------------|--------------|--------|------|
| Heptane             | NT      | 776   | 732.68 | 0.018      | 0.013        | tr c   |
| Myrcene             | MH      | 921   | 773.24 | 15         | 11           | 1.1    |
| (Z)-3-Hexenol acetate | NT     | 930   | 794.80 | 0.32       | 0.25         | 0.11   |
| n-Tridecane         | NT      | 940   | 811.30 | 0.028      | 0.012        | tr     |
| δ-Elemene           | SH      | 965   | 832.24 | 0.12       | 0.097        | tr     |
| Cyclocassartene     | SH      | 968   | 837.15 | 0.32       | 0.25         | 0.11   |
| α-Ylangene          | SH      | 983   | 858.63 | 0.19       | 0.13         | 0.12   |
| α-Copaene           | SH      | 998   | 906.21 | 1.4        | 1.1          | 0.25   |
| α-Pinene            | SH      | 1003  | 919.32 | 12         | 8.1          | 1.15   |
| β-Bourbonene        | SH      | 1010  | 923.68 | 0.22       | 0.19         | 0.064  |
| β-Cabebeene         | SH      | 1018  | 969.90 | 0.19       | 0.17         | 0.013  |
| β-Elemene           | SH      | 1020  | 993.55 | 0.33       | 0.24         | 0.072  |
| n-Tetradecane       | NT      | 1022  | 1022.15| 0.96       | 0.52         | 0.15   |
| β-Caryophyllene     | SH      | 1031  | 1076.38| 11         | 7.6          | 1.1    |
| β-Copaene           | SH      | 1047  | 1093.50| 1.1        | 0.78         | 0.15   |
| γ-Elemene           | SH      | 1074  | 1110.86| 0.41       | 0.35         | tr     |
| trans-α-bergamotene | SH      | 1362  | 1143.80| 0.78       | 0.43         | 0.096  |
| Aromadendrene       | SH      | 1365  | 1159.81| 0.41       | 0.38         | tr     |
| α-Humulene          | SHS     | 1370  | 1205.62| 0.21       | 0.15         | tr     |
| cis-Murola-4(14), 5- diene | SH | 1390 | 1232.56| 0.063      | 0.022        | tr     |
| (Z)-8-dodecen-1-ol  | NT      | 1391  | 1240.25| 0.014      | 0.027        | tr     |
| Germacrene D        | SH      | 1412  | 1320.39| 51         | 43           | 1.6    |
| (E)-B-ionone        | AC      | 1422  | 1329.12| 0.28       | 0.21         | tr     |
| 1-Pentadecane       | NT      | 1430  | 1360.85| 4.4        | 2.1          | 0.91   |
| Bicyclogermacrene   | SH      | 1441  | 1372.13| 0.66       | 0.46         | tr     |
| α-Murolene          | SH      | 1468  | 1390.23| 1.2        | 0.89         | 0.031  |
| n-Pentadecane       | NT      | 1476  | 1396.14| 0.58       | 0.38         | tr     |
| (Z)-α-Bisabolene    | SH      | 1479  | 1409.09| 0.43       | 0.35         | tr     |
| trans-β-Guaiene     | SH      | 1488  | 1423.29| 0.11       | 0.082        | tr     |
| (E, E)-α-Farnesene  | SH      | 1415  | 1439.43| 0.062      | 0.024        | tr     |
| a-Bulnesene         | SH      | 1520  | 1452.14| 0.14       | 0.097        | tr     |
| δ-Amorphene         | SH      | 1548  | 1490.33| 0.19       | 0.85         | 0.053  |
| trans-γ-Cadinene    | SH      | 1561  | 1513.77| 0.057      | 0.032        | tr     |
| δ-Cadinene          | SH      | 1570  | 1521.36| 0.092      | 0.015        | tr     |
| Selina-3,7(11)-diene| SH      | 1575  | 1525.44| 0.083      | 0.021        | tr     |
| Germacrene B        | SH      | 1620  | 1537.14| 0.054      | 0.013        | tr     |
| Germacrene D-4-ol   | OS      | 1630  | 1540.32| 0.092      | 0.042        | tr     |
| Nerolidol acetate   | OS      | 1662  | 1554.56| 0.063      | 0.011        | tr     |

Table 3. Essential oil chemical composition of *E. purpurea* grown at the best growing media at 90:10 NO$_3^-$/NH$_4^+$ ratio. a-Class: AC apocarotenoids; MH monoterpenic hydrocarbons; SH sesquiterpenic hydrocarbons; NT non-terpenes; OS oxygenated sesquiterpenes. b*LRI* Linear Retention Index. cTrace (below 0.01%); *KI* Kovats Index.
is one of the important roles of germacrene compounds in humans. The antimicrobial properties of germacrene D were reported in previous researches. Anti-inflammatory, antimicrobial, and antioxidant effects of germacrene D are also well known. The anti-insect influence of germacrene D has been reported in previous studies. Myrcene is a terpene with anti-inflammatory and anti-depressant effects. Regulating the efficiency of other terpenes and cannabinoids by increasing of myrcene is recognized previously. Pinene has a several of potential benefits, including anti-inflammatory, antimicrobial, antitumor, antioxidant, and neuroprotective effects. It may also help counteract the short-term memory issues that many people experience. Beta-caryophyllene is also known for antioxidant and anti-inflammatory medicinal effects. It is especially useful to decrease pain and anxiety.

It was found that the mixture of peat moss into very fine-grade perlite (< 0.5 mm) at 50:50 v/v perlite to peat moss ratio had a significant increase in plant growth parameters, which increased by increasing of NO$_3^-$/NH$_4^+$ ratio. The essential oil content was significantly highest in the 50:50 v/v perlite to peat moss ratio (perlite particle size less than 0.5 mm) than others. The major terpene hydrocarbons found in extract of E. purpurea with the best growth parameters were germacrene D, myrcene, α-pinene, β-caryophyllene, and 1-Pentadecene, respectively.

| Components         | Classa | LRIb | KI   | Percentage | Flower heads | Leaves | Root |
|--------------------|--------|------|------|------------|--------------|--------|------|
| Heptane            | NT     | 776  | 732.68 | tr         | tr           | tr     | tr   |
| Myrcene            | MH     | 921  | 773.24 | 10         | 8.9          | 0.75   |
| (Z)-3-Hexenol acetate | NT   | 930  | 794.80 | 0.21       | 0.11         | 0.083  |
| n-Tridecane        | NT     | 940  | 811.30 | tr         | tr           | Tr     |
| β-Elemene          | SH     | 965  | 832.24 | 0.082      | 0.023        | Tr     |
| Cyclosativene      | SH     | 968  | 837.15 | 0.21       | 0.15         | Tr     |
| α-Ylangene         | SH     | 983  | 858.65 | 0.11       | 0.08         | 0.092  |
| α-Copaene          | SH     | 998  | 906.21 | 0.89       | 0.66         | 0.11   |
| α-Pinene           | SH     | 1003 | 919.32 | 8.5        | 6.1          | 0.75   |
| β-Bourbonene       | SH     | 1010 | 923.68 | 0.15       | 0.091        | Tr     |
| β-Cabene           | SH     | 1018 | 969.90 | 0.12       | 0.024        | Tr     |
| β-Elemene          | SH     | 1020 | 993.55 | 0.18       | 0.11         | 0.022  |
| n-Tetradecene      | NT     | 1022 | 1022.15| 0.59       | 0.39         | Tr     |
| β-Caryophyllene    | SH     | 1031 | 1076.38| 7.1        | 5.5          | 0.45   |
| β-Copaene          | SH     | 1047 | 1093.50| 0.84       | 0.62         | 0.083  |
| γ-Elemene          | SH     | 1074 | 1110.86| 0.22       | 0.11         | Tr     |
| trans-α-bergamotene | SH    | 1362 | 1143.80| 0.54       | 0.42         | Tr     |
| Aromadendrene      | SH     | 1365 | 1159.81| 0.21       | 0.15         | Tr     |
| α-Humulene         | SHS    | 1370 | 1205.62| 0.13       | 0.053        | Tr     |
| cis-Mutrola-4(14), 5-diene | SH | 1390 | 1232.56| tr         | tr           | Tr     |
| (Z)-8-dodecen-1-ol | NT     | 1391 | 1240.25| tr         | tr           | Tr     |
| Germacrene D       | SH     | 1412 | 1320.39| 47         | 33           | 0.95   |
| (E)-B-ionone       | AC     | 1422 | 1329.12| 0.15       | 0.042        | Tr     |
| 1-Pentadecene      | NT     | 1430 | 1360.85| 3.1        | 1.8          | 0.25   |
| Bicyclogermacrene  | SH     | 1441 | 1372.13| 0.25       | 0.16         | Tr     |
| α-Murolene         | SH     | 1468 | 1390.23| 0.87       | 0.64         | 0.034  |
| n-Pentadecane      | NT     | 1476 | 1396.14| 0.18       | 0.091        | Tr     |
| (Z)-a- Bisabolene  | SH     | 1479 | 1400.09| 0.23       | 0.15         | Tr     |
| trans-β-Guaiene    | SH     | 1488 | 1423.29| 0.052      | 0.014        | Tr     |
| (E, E)-α-Farnesene | SH     | 1415 | 1439.43| tr         | tr           | Tr     |
| α-Bulnesene        | SH     | 1520 | 1452.14| 0.064      | 0.013        | Tr     |
| δ-Amorphene        | SH     | 1548 | 1490.33| 0.092      | 0.032        | Tr     |
| trans-γ-Cadinene   | SH     | 1561 | 1513.77| tr         | tr           | Tr     |
| δ-Cadinene         | SH     | 1570 | 1521.36| 0.023      | 0.014        | Tr     |
| Selina-3,7(11)-diene | SH    | 1575 | 1525.44| tr         | tr           | Tr     |
| Germacrene B       | SH     | 1620 | 1557.14| tr         | tr           | Tr     |
| Germacrene D-4-ol  | OS     | 1630 | 1540.32| 0.017      | tr           | Tr     |
| Nerolidol acetate  | OS     | 1662 | 1544.56| 0.019      | tr           | Tr     |

Table 4. Essential oil chemical composition of E. purpurea grown at the best growing media at 70:30 NO$_3^-$/NH$_4^+$ ratio. a Class: AC apocarotenoids; MH monoterpenic hydrocarbons; SH sesquiterpenic hydrocarbons; NT non-terpenes; OS oxygenated sesquiterpenes. b LRI Linear Retention Index. c Trace (below 0.01%); KI Kovats Index.
The percentages of these terpene hydrocarbons were increased by increasing of $\text{NO}_3^-/\text{NH}_4^+$ ratio. Using of perlite and peat moss mixture for plant cultivation not only affects the plant growth parameters and essential oil compositions, but also reduces production costs in hydroponic systems.

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Table 5. Maximum percentage of major essential oil compositions of $E.$ purpurea reported in various previous studies.

| Culture media | Germacrene D | Myrcene | $\alpha$-Pinene | $\beta$-Caryophyllene | 1-Pentadecene |
|---------------|--------------|---------|----------------|----------------------|--------------|
| Present study | 51           | 15      | 12             | 11                   | 4.4          |
| Diraz et al. (2012) | 11        | -       | -              | 7.2                  | -            |
| Sitarik et al. (2017) | 19        | 0.12    | 1.1            | -                    | 0.73         |
| Thappa et al. (2003) | 33        | 10      | 6.6            | 9.3                  | 2.5          |
| Nyalambisa et al. (2016) | 20       | -       | 3.7            | 4.5                  | -            |
| Hudaib and Cavrini (2002) | 29        | 1.7     | 2.3            | 3.1                  | -            |
| Holla et al. (2005) | 4.8     | 2.1     | 5.1            | 3.6                  | 2.5          |
| Kyslychenko et al. (2008) | 25        | 11      | 7.5            | 5.2                  | 2.9          |
| Kan et al. (2008) | 32          | 9.4     | 4.2            | 4.3                  | 3.2          |

Table 6. Physical properties of media used in greenhouse $E.$ purpurea culture. The numbers in the parentheses show perlite particle size. Pt peat moss and Pe perlite.

| Culture media | Water holding capacity | Air-filled porosity | Bulk density | Total porosity |
|---------------|------------------------|---------------------|--------------|----------------|
| (% vol)       | (%)                    | (%)                 | (g cm$^{-3}$) | (%)            |
| 100% Pe (> 2 mm) | 56        | 33                  | 0.16         | 89             |
| 100% Pt       | 75          | 10                  | 0.21         | 85             |
| 50% Pt + 50% Pe (< 0.5 mm) | 68  | 24                  | 0.18         | 92             |
| 50% Pt + 50% Pe (0.5–1 mm) | 65  | 26                  | 0.18         | 91             |
| 50% Pt + 50% Pe (1–1.5 mm) | 64  | 28                  | 0.18         | 92             |
| 50% Pt + 50% Pe (1.5–2 mm) | 62  | 31                  | 0.17         | 93             |
| 50% Pt + 50% Pe (> 2 mm) | 60  | 33                  | 0.17         | 93             |
| 30% Pt + 70% Pe (< 0.5 mm) | 61  | 28                  | 0.14         | 89             |
| 30% Pt + 70% Pe (0.5–1 mm) | 58  | 30                  | 0.14         | 88             |
| 30% Pt + 70% Pe (1–1.5 mm) | 56  | 32                  | 0.15         | 88             |
| 30% Pt + 70% Pe (1.5–2 mm) | 53  | 33                  | 0.15         | 86             |
| 30% Pt + 70% Pe (> 2 mm) | 50  | 35                  | 0.16         | 85             |

The percentages of these terpene hydrocarbons were increased by increasing of $\text{NO}_3^-/\text{NH}_4^+$ ratio. Using of perlite and peat moss mixture for plant cultivation not only affects the plant growth parameters and essential oil compositions, but also reduces production costs in hydroponic systems.

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Table 5. Maximum percentage of major essential oil compositions of $E.$ purpurea reported in various previous studies.

| Culture media | Water holding capacity | Air-filled porosity | Bulk density | Total porosity |
|---------------|------------------------|---------------------|--------------|----------------|
| (% vol)       | (%)                    | (%)                 | (g cm$^{-3}$) | (%)            |
| 100% Pe (> 2 mm) | 56        | 33                  | 0.16         | 89             |
| 100% Pt       | 75          | 10                  | 0.21         | 85             |
| 50% Pt + 50% Pe (< 0.5 mm) | 68  | 24                  | 0.18         | 92             |
| 50% Pt + 50% Pe (0.5–1 mm) | 65  | 26                  | 0.18         | 91             |
| 50% Pt + 50% Pe (1–1.5 mm) | 64  | 28                  | 0.18         | 92             |
| 50% Pt + 50% Pe (1.5–2 mm) | 62  | 31                  | 0.17         | 93             |
| 50% Pt + 50% Pe (> 2 mm) | 60  | 33                  | 0.17         | 93             |
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| 30% Pt + 70% Pe (1.5–2 mm) | 53  | 33                  | 0.15         | 86             |
| 30% Pt + 70% Pe (> 2 mm) | 50  | 35                  | 0.16         | 85             |
Author contributions
F.A. performed the experiment and wrote the paper, A.S. conceived the idea, E.S. and A.R. reviewed the collected data, and S.S. and A.S. edited the paper. A.S. was responsible for editing, original data and text preparation. All authors took responsibility for the integrity of the data that is present in this study.

Competing interests
The authors declare no competing interests.

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