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ORIGINAL ARTICLE

Hydroponic cultivation of *Withania somnifera* and comparative assessment with the soil grown plants

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ABSTRACT
Conventional method of agriculture is being challenged by various factors due to amplifying population and environmental stress. There is necessity for developing alternate methods for cultivation of crops as the globe is facing major planetary crisis leading to climate change and water scarcity. Soilless cultivation provides opportunity for better cultivation of crops with elite produce and less consumption. Hydroponics can be proficiently used for the cultivation of medicinal and aromatic plants which plays very integral role in the healthcare system. Ashwagandha is one of the important medicinal plants which has immense health benefits and vastly used in ayurveda. A study was undertaken to check the feasibility of Withania somnifera in Dutch bucket system and to compare its morphological and biochemical properties with that of soil grown plants. Three different ratios of growing media (cocopeat, perlite, lecaballs and vermiculite) were used in Dutch bucket system to compare the growth of plant with soil. The plant showed significant growth in soilless medium when compare to plants grown under soil. Pigments, protein, soluble sugar and amino acid observed higher in soilless cultivation, on the other hand total phenol content and starch obtained higher in the soil. Hence it is recommended to use hydroponics as a viable alternative for tackling the problems related to conventional method of agriculture in urban and semi urban areas.
Keywords: Hydroponics, Withania somnifera, protein, biochemical.

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INTRODUCTION

Traditional agriculture is facing major challenges due to various factors such as poor soil fertility, disease-causing organisms, pest incidence, poor drainage, soil reaction, unfavorable soil compaction, poor drainage in soil, degradation by soil erosion, unfavorable soil compaction and use of synthetic fertilizers, pesticides and insecticides. Other problem arising due to climatic change is water scarcity in developing countries specially India, and agriculture consumes surplus amount of water, and thus traditional agriculture is facing a serious threat due to water scarcity because of enormous demand of water in different sectors [1,2].

Soilless cultivation illustrates a credible opportunity in certain areas marked by severe soil erosion, degradation and restricted water supply. The massive potential offered by this cultivation approach is invincible and not only this approach is highly productive but also provides qualitative advantages being environmentally friendly because of its higher water and nutrients use efficiency [3]. The most important advantage of hydroponic system is water conservation because in particular water loss through evapotranspiration is low in a closed greenhouse [4]. Also, soilless cultivation has become important due to its promising approach for growing variety of crops. Through this approach many short-lived herbs and crops can be easily cultivated throughout the whole year by utilizing fewer land and labor [5].

Medicinal plants play an indispensable role in the human health care.Expansion of the use of herbal medicines and phytonutrients or nutraceuticals continues briskly across the world with a lot of population now resorting to herbal products for treatment of various health issues in different healthcare settings [6]. The cultivation of medicinal herbs and root crops in controlled environment bestows opportunities for enhancing and improving the bioactivity, quality, purity, biomass production and

consistency of the raw material [7]. Hydroponic systems can be efficiently used for the production of high-quality elite herbs along with root material free from any adulteration due to various external factors such as weeds, soil, environmental toxins including heavy metals in soils. Also, hydroponic cultivation can prove to be more conducive in controlling pests as beneficial organisms can be used in place of synthetic pesticides, thereby making it organic. This technique can be proficiently used for optimization of secondary metabolites and, for obtaining higher yields of target parts such as roots, leaves, or rhizomes [8]. Hydroponic technology can be efficiently applied for production of high-standard elite plant material all year round considering the possibility of controlling growing conditions along with stimulation of secondary metabolites by pertinent manipulation of mineral nutrients, pH and electrical conductivity [9].Keeping in consideration the above-mentioned points, the aim of the study is to analyze the suitability of the Dutch-bucket hydroponic system for the commercial cultivation of *Withania somnifera.*

MATERIAL AND METHODS

The hydroponic system was installed in the glasshouse of High-Altitude Plant physiology research centre, HNBGU, Srinagar Garhwal, during 2021-2022. The setup consisted of Dutch buckets, tank (200l), water pump and aerator. 30 Dutch buckets were used and attached with the help of PVC pipes having 140mm diameter and 2 m length to frame a hydroponic system. Both the ends of the pipes were closed with end caps. The Dutch buckets were covered with filter paper on the base and sides of the bucket for filling different ratios of growing substrate.The schematic diagram representing the hydroponic system is presented on the Fig 1. The experiment was done using RBD (randomized block design) with three treatments and ten replications.

Uniform-sized 30-day old seedlings of *W. somnifera* were planted in Dutch bucket system as well as soil. Hoagland's nutrient solution was used and supplied to the Dutch bucket using drip irrigation system with regulatory valves attached to the PVC pipes. The nutrients and water were supplied through the PVC pipes to the system at a uniform flow rate. Different parameters checked were pH, EC, TDC and salinity level of the nutrient solution. The pH was maintained between 5.5 and 6.5 at all stages of development. The requirement of nutrients and water was fulfilled by preparation and supplying the nutrient prepared in accordance to the different plant's developmental phase. Different ratios of the substrates were used for preparing 3 treatments in hydroponic system as depicted in the table 1.

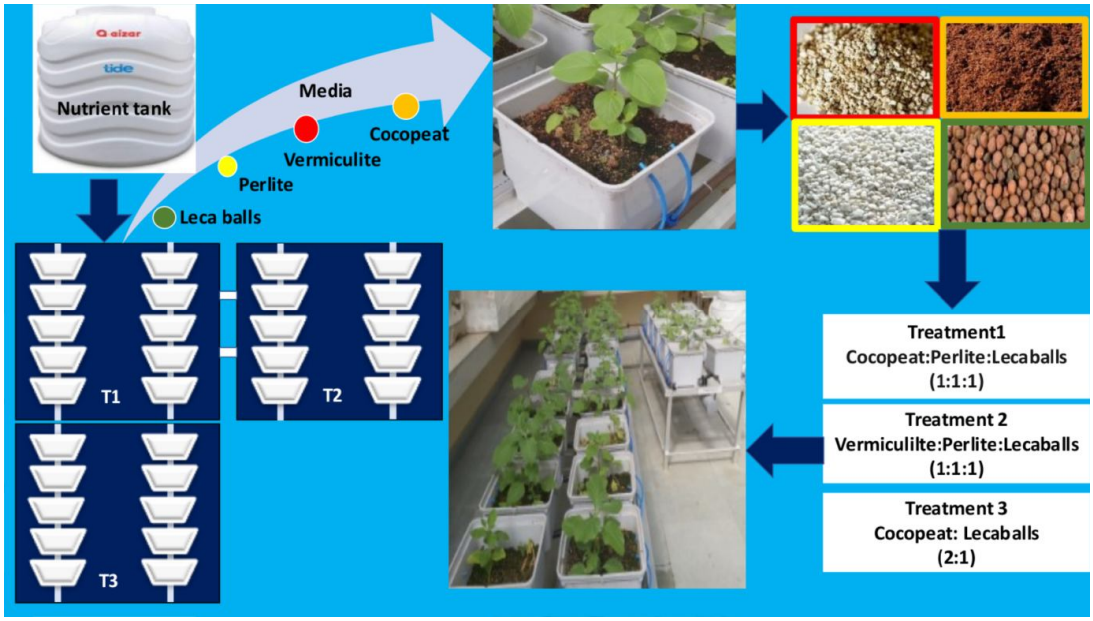

Fig.1 A schematic diagram of a hydroponic setup

Factors	Treatment
Growing media	Cocopeat:Perlite:Lecaballs (1:1:1)
	Vermiculite:Perlite:Lecaballs (1:1:1)
	Cocopeat:Lecaballs (2:1)
Control	Soil grown plants of *Withania*

Table 1. Treatments combinations used for hydroponics.

Plant and supporting media analysis

Crop biometric observations such as root length (cm), shoot length (cm), leaf number(cm), leaf area and specific leaf area (cm^2) were measured both at vegetative and reproductive phase in plants grown in hydroponics and compared with the plants grown in soil. The biochemical parameters of the third young leaf namely chlorophyll a, chlorophyll b, carotenoids[10], total protein content [11], amino acid[12],Total phenolic content [13]starch and total soluble sugars[14], were measured and compared with the soil grown plants. Regular and continuous monitoring of the media was done daily for seeing the changes in pH, EC and TDS at morning and evening.

Statistical analysis

The experimental data were compiled by using ANOVA (analysis of variance) with Turkey HSD. Data were analysed using SPSS 22.0. P values < 0.05 were considered as statistically significant.

RESULTS

1. Morphological changes-

Root length- The root length of the plant ranged between 2.03±0.98 to 1.46±0.67cm (Table 2) during the vegetative phase and 1.58±1.78 to 2.93±3.65 (Table 2) during the reproductive phase. The minimum root length observed in plants grown under geoponics however, maximum obtained in T3 which was statistically significant ($p<0.05$) in both of the seasons. On the other hand, no significant difference obtained between T1 and T2 during reproductive phase.

Shoot length- The shoot length of the plant ranged between 18.46±0.94 to 15±2.24 cm (Table 2) during the vegetative phase and 41.2±0.34 to 85.6±1.98 cm (Table 2) during the reproductive phase. The maximum shoot length procured by T3 which was statistically significant ($p<0.05$) and minimum observed in plants grown under geoponics in both of the seasons.

Leaf number- The number leaves ranged between 6±1.18 to 15±2.24 (Table 2) during the vegetative phase and 14±2.21 to 33±2.73 (Table 2)during the reproductive phase. The maximum number of leaves procured in T3 which was statistically significant ($p<0.05$) and minimum observed in plants grown under geoponics. No significant difference observed between T2 and plants grown under soil during vegetative phase. On the other hand, no significant difference procured between T1 and T2 under soilless condition.

Leaf area-The leaf area of the plant ranged between 8.93±0.34 to 15.56±0.34 cm^2 (Table 2) during the vegetative phase and 14±2.21 to 33±2.73 cm^2 (Table 3) during the reproductive phase. The maximum leaf area observed in T3 which was statistically significant ($p<0.05$) for vegetative phase while the minimum leaf area observed in plants grown under geoponic condition. On the other hand, no significant difference obtained between the treatments plant grown under soilless condition during the reproductive phase however, it was statistically significant ($p<0.05$) with plants grown under soil.

2. Biochemical analysis-

Chlorophyll a- The chla content ranged between 1.17±0.25 to 1.01±0.54 mg/g FW (Table 2) during the vegetative phase and 0.5±1.36 to 0.9±1.58 mg/g FW (Table 2) during the reproductive phase. Maximum chla analysed in T3 which was statistically significant ($p<0.05$) while minimum value observed in soil during vegetative phase. No significant difference observed between T1 and T3. On the other hand, maximum chla observed in T2 and minimum observed in T1 during reproductive phase.

Chlorophyll b- The chlb content ranged between 0.6±0.86 to 0.83±0.54 (Table 1) mg/g FW during the vegetative phase and 0.53±0.45 to 0.9±0.4 mg/g FW (Table 2) during the reproductive phase. The maximum chl b content observed in T1 and minimum obtained in T2 during the vegetative which was statistically significant ($p<0.05$). On the other hand, maximum chlb content observed in T1 while minimum observed in T3. The content was statistically significant ($p<0.05$).

Carotenoid content- The carotenoid content ranged between 0.07±0.45 to 0.09±0.25 mg/g FW (Table 2) during the vegetative phase and 00.14±1.56 to 0.16±0.45 mg/g FW (Table 2) during the reproductive phase. The carotenoid content observed maximum in T1 and minimum in soil during the vegetative phase which was statistically significant ($p<0.05$). No significant difference obtained between treatments of soilless cultivation. During reproductive phase, minimum carotenoid content observed in T3 and maximum in T1 which was statistically significant ($p<0.05$).

Protein content- The Protein content ranged between 54.04±0.98 to 60.70±0.46 mg BSAE/g (Table 2) of FW during the vegetative phase and 58.15±0.86 to 69.12±0.79 mg BSAE/g FW (Table 2) during the reproductive phase. The minimum protein content observed in T3 and maximum observed in T2 during vegetative phase which was statistically significant ($p<0.05$). However, no significant difference observed between T1 and soil. On the other hand, the protein content observed maximum in T3 and minimum in T1 during reproductive phase which was statistically significant ($p<0.05$). A rapid decrease in protein content of T1 has observed from vegetative to reproductive phase.

Total phenolic content- The total phenol content (TPC) ranged between 43.24±1.25 (Table 2) to 70.02±0.85 mg/g of FW during the vegetative phase and 73.90±0.78 to 81.25±0.76 mg/g FW (Table 2) during the reproductive phase. The maximum TPC observed in soil and minimum in T1 during vegetative phase which was statistically significant ($p<0.05$). On the other hand, maximum TPC observed in soil and minimum in T3 during the reproductive phase which was statistically significant ($p<0.05$). No significant difference observed between the treatments of hydroponic cultivation.

Amino acid- The amino acid ranged between 12.07±1.87 to 26.09±1.60 mg/g (Table 2) during the vegetative phase and 26.09±0.99 to 37.45±1.76 mg/g FW (Table 2) during the reproductive phase. The maximum amino acid observed in soil and minimum in T3 during vegetative phase. It was statistically significant ($p<0.05$). However, the maximum amino acid observed in T1 and minimum in T3 during the reproductive phase which was also significant ($p<0.05$). No significant difference observed between the treatments during both of the seasons.

Starch content- The starch content ranged between 101.25±2.56 to 202.25±0.54 mg/g of FW (Table 1) during the vegetative phase and 616.78±1.26 to 842.78±0.54 mg/g FW (Table 3) during the reproductive phase. The minimum starch content observed in soil while, maximum obtained in T2 during the vegetative phase which was statistically significant ($p<0.05$). No significant difference obtained in T1 and T3. On the other hand, minimum starch content observed in T1 and maximum in T3 during the reproductive phase which was statistically significant ($p<0.05$).

Total soluble sugar- The total soluble sugar (TSS) ranged between 485.25±0.46 to 661.65±1.26 mg/g FW (Table 1) during the vegetative phase and 306.5±2.36 to 564.6±1.25 mg/g FW (Table 3) during the reproductive phase. The maximum TSS observed in T2 and minimum in soil during vegetative phase which was statistically significant ($p<0.05$). No significant difference observed between T1 and T3. On the other hand, maximum TSS observed in T3 and minimum in T1 during the reproductive phase which was statistically significant ($p<0.05$).

Table 2: Morphological and biochemical variations in *W. somnifera* grown under hydroponics & geoponics condition during vegetative phase

Parameters	VT1	VT2	VT3	V Soil
Root length	1.95±0.87^{b}	1.67±2.34^{bc}	2.03±0.98^{a}	1.58±0.67^{c}
Shoot length	22.2±1.13^{bc}	28±1.50^{b}	30±1.14^{a}	18.46±0.94^{c}
Leaf number	10±2.81^{b}	7±1.16^{c}	15±2.24^{a}	6±1.18^{c}
Leaf area (cm)	13.98±0.87^{b}	9.67±0.65^{c}	15.56±0.34^{a}	8.93±0.34^{c}
Specific leaf area	49.75±1.33^{c}	99.69±1.34^{b}	51.69±1.22^{bc}	139±0.32^{a}
Chlorophyll a	1.14±0.15^{ab}	1.01±0.54^{b}	1.14±1.25^{ab}	1.17±0.25^{a}
Chlorophyll b	0.83±0.54^{a}	0.6±0.86^{c}	0.75±0.1^{b}	0.68±0.26^{bc}
Carotenoid	0.07±0.45^{a}	0.07±0.65^{b}	0.07±1.25^{b}	0.09±0.25^{a}
Protein content	59.24±1.25^{b}	60.70±0.46^{a}	54.04±0.98^{c}	58.01±1.56^{b}
TPC	43.24±1.25^{c}	52.25±0.25^{bc}	61.25±0.85^{b}	70.02±0.85^{a}
Amino acid	17.04±2.00^{b}	16.04±0.97^{b}	12.07±1.87^{b}	26.09±1.60^{a}
Starch	171.2±0.25^{b}	202.25±0.54^{a}	166.45±2.35^{b}	101.25±2.56^{c}
Total soluble sugar	570.3±1.25^{b}	661.65±1.26^{a}	571.54±0.64^{b}	485.25±0.46^{c}

TPC- Total phenolic content, FW- Fresh weight.

Alphabets (a, b, c) represent statistically significant values ($p < 0.05$) as determined by Tukey's HSD test.

Table 3. Morphological and biochemical variations in *W. somnifera* grown under hydroponics & geoponics condition during reproductive phase

Parameters	RT1	RT2	RT3	R Soil
Root length	2.91±0.93^{a}	2.76±1.98^{b}	2.71±3.65^{b}	1.46±1.79^{c}
Shoot length	71±1.5^{b}	59.4±1.76^{bc}	85.6±1.98^{a}	41.2±0.34^{c}
Leaf number	23±2.52^{b}	20±1.48^{b}	33±2.73^{a}	14±2.21^{c}
Leaf area (cm)	19.45±0.45^{a}	18.67±1.9^{a}	20.12±1.23^{a}	15.45±1.54^{b}
Specific leaf area	54.48±0.98^{c}	100.37±1.23^{b}	45.52±3.6^{c}	152.97±0.99^{a}
Chlorophyll a	0.5±1.36^{c}	0.9±1.58^{a}	0.82±0.65^{ab}	0.77±0.42^{b}
Chlorophyll b	0.9±0.4^{a}	0.59±1.58^{c}	0.53±0.45^{c}	0.76±0.94^{b}
Carotenoid	0.16±0.45^{a}	0.16±0.42^{a}	0.14±1.56^{ab}	0.15±0.48^{b}
Protein content	58.15±0.86^{b}	62.56±0.99^{ab}	69.12±0.79^{a}	68.24±0.98^{a}
TPC	74.92±1.25^{b}	74.31±0.98^{b}	73.90±0.78^{b}	81.25±0.76^{a}
Amino acid	37.45±1.76^{a}	29.76±1.87^{b}	26.09±0.99^{b}	34.33±2.3^{ab}
Starch	616.78±1.26^{c}	714.56±0.89^{b}	745.25±0.55^{ab}	842.78±0.54^{a}
Total soluble sugar	306.5±2.36^{c}	424.54±0.78^{bc}	564.6±1.25^{a}	494.36±2.65^{b}

TPC- Total phenolic content, FW- Fresh weight.

Alphabets (a, b, c) represent statistically significant values ($p < 0.05$) as determined by Tukey's HSD test.

DISCUSSION

The substrates in soilless cultivation have an impact on morphological, biochemical, physiological as well as anatomical traits of the plant. The growing medium facilitates plant growth, which may be ascribed towards the nutrients that are present in the medium. In this experiment, the, number of leaves, leaf and root length and leaf area were mostly highest in T3 (cocopeat and lecaballs). This may be because cocopeat has a high water-holding capacity, lecaball aeration, and both have a significant nutrient input with an acceptance rate for electric conductivity throughout the growing season. These findings are corroborated by earlier research [15].

The accumulation in primary and secondary biochemicals in leaves grown under soilless system may be due to the physical properties of inert media that stimulates the process of photosynthesis by enhancing chla and chlb. The amount of chla, chlb as well as carotenoids in plants varies relying on the growing factors [15]. In this study, the pigments obtained maximum in soilless culture. The development of leaf pigments depends on the nitrogen that accumulates in plants growing on inert media to promote aeration, water retention capacity, and biostability. Inert media specially lecaballs, perlite and vermiculite, which have better aeration as well as water retention capability in coco peat, and bio-stability of these substrates lead to absorption of nitrogen for the chlorophyll development. Previous studies found cocopeat as a potential substrate in soilless culture, which providing high concentration of pigments and affecting the plant growth and development [16].

In this study, plants grown in soil had a higher TPC than plants cultivated in soilless system. It might result from the plant's defense to physical injury, abiotic stress and biotic stress etc. in the soil [17] as phenolic chemicals are typically connected to plant defense mechanisms [18]. The amino acid also observed higher in soil during vegetative phase which may be due to amino acids entailed in coping up with stress [19].

This study showed that, there was a loss of proteins in T1 (Cocopeat, Perlite and Lecaballs) during reproductive phase. It has been shown in earlier study in *Hemerocallis* by Shahri who concluded that, plant start to transfer protein bi-products from sink to source as reproductive phase started [20]. The role of ethylene during reproductive stage has been already reported in previous report [21]. There has been evidence of a general drop in cell protein levels in leaves in both ethylene-sensitive as well as insensitive plants [22]. An apparent reduction in the concentration of amino acids during reproductive phase as observed in plants grown under soilless conditionsmaybe as a result of the accelerated transport of amino acids towards the growing pistil.

During the reproductive phase, the plants grown under T1 (Cocopeat, Perlite and Lecaballs) showed the minimum total soluble sugar in leaf of *W. somnifera* which may be due to early reproductive phase of the plant has been observed in soilless media. These results are supported by earlier studies which demonstrated that, in *Hemerocallis* [23] and *Helleborus orientalis*[20], maturation as well as reproductive phase are followed by a drop in total soluble sugar. Starch content observed higher in soil may be due to late reproductive phase as well as starch aids in plant defence by participating in pathogen-specific immune response [20, 24].

REFERENCES

1. Surendran U, Sushanth CM, Mammen George, Joseph EJ. (2014). Modeling the impacts of increase in temperature on irrigation water requirements in Palakkad district-a case study in humid tropical Kerala. J Water Clim Change 5(3); 471-487.
2. Surendran U, Sushanth CM, Mammen George, Joseph EJ. (2015). Modeling the crop water requirement using FAO-CROPWAT and assessment of water resources for sustainable water resource management: a case study in Palakkad district of humid tropical Kerala, India. Aquat Proc 4:1211-1219
3. Sambo P, Nicoletto C, Giro A, Pii Y, Valentinuzzi F, Mimmo T, Lugli P, Orzes G, Mazzetto F, Astolfi S, Terzano R, Cesco S. (2019). Hydroponic Solutions for Soilless Production Systems: Issues and Opportunities in a Smart Agriculture Perspective. Front Plant Sci. 2019 Jul 24; 10:923. doi: 10.3389/fpls.2019.00923. PMID: 31396245; PMCID: PMC6668597.
4. Zimmermann, M., & Fischer, M. (2020). Impact assessment of water and nutrient reuse in hydroponic systems using Bayesian Belief Networks. *Journal of Water Reuse and Desalination*. pp. 431-442.
5. Fussy A, Papenbrovck J. (2022). An overview of Soil and soilless Cultivation Techniques Chances, Challenges and the Neglected Question of Sustainability. Plants(Basel). (9): 1153
6. Chauhan, M., & Sati, P. (2009). Effect of covid-19 on trade of medicinal and aromatic plants in Uttarakhand, India. Medico-Biowealth of India Vol. III, 56.
7. Sati, P., Chauhan, M., Trivedi, V.L. (2022). Challenges and prospects for the in-vitro conservation of plants having anticarcinogenic potential in the Western Himalaya, India. Plant Cell Tiss Organ Cult 152, 237–252.
8. Hayden, A. L. (2006). Aeroponic and hydroponic systems for medicinal herb, rhizome, and root crops. HortScience, 41(3), 536-538.

9. Maggini R, Kiferle C, Guidi L, Pardossi A. and Raffaelli, A. 2012. Growing medicinal plants in hydroponic culture. Acta Hortic. 952, 697-704.
10. Holm-Hansen, O., Lorenzen, C. J., Holmes, R. W., and Strickland, J. D. H. 1965. Fluorometric Determination 496 of Chlorophyll. ICES J. Mar. Sci. 30 (1), 3–15. doi:10.1093/icesjms/30.1.3
11. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72(1-2), 248-254.
12. Moore, S., & Stein, W. H. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. biol. Chem, 211(2), 907-913.
13. Kruger, N.J. 2009. The Bradford Method For Protein Quantitation. In: Walker, J.M. (eds) The Protein Protocols Handbook. Springer Protocols Handbooks. Humana Press, Totowa, NJ.
14. Mc Cready RM, Guggolz J, Silviera V, Owens HS (1950) Determination of starch and amylose in vegetables. Anal Chem 22:1156–1158.
15. Rahman M.J., Quamruzzaman M., Uddain J., Sarkar M.D., Islam M.Z., Zakia M.Z., Subramaniam S.(2018). Photosynthetic Response and Antioxidant Content of Hydroponic Bitter Gourd as Influenced by Organic Substrates and Nutrient Solution. HortScience.53:1314–1318. doi: 10.21273/HORTSCI13226-18.
16. Kimura M., Rodriguez-Amaya D.B. (2008). Carotenoid Composition of Hydroponic Leafy Vegetables. J. Agric. Food Chem. 51:2603–2607. doi: 10.1021/jf020539b.
17. Pratyusha, S. (2022). Phenolic Compounds in the Plant Development and Defense: An Overview. Physiology. doi: 10.5772/intechopen.102873.
18. Edreva A., Velikova V., Tsonev T., Dagnon S., Gürel A.L., Aktas L. (2008). Stress-protective role of secondary metabolites: Diversity of functions and mechanisms. Gen. Appl. Plant. Physiol.34:67–78.
19. Trovato Maurizio, Funck Dietmar, Forlani Giuseppe, Okumoto Sakiko, Amir Rachel 2021.Amino Acids in Plants: Regulation and Functions in Development and Stress Defense . *Frontiers in Plant Science.* 12, 1664-462X.
20. Shahri, W., Tahir, I., Islam, S. T., & Bhat, M. A. (2011). Physiological and biochemical changes associated with flower development and senescence in so far unexplored Helleborus orientalis Lam. cv. Olympicus. Physiology and molecular biology of plants: an international journal of functional plant biology, 17(1), 33–39. https://doi.org/10.1007/s12298-010-0045-3.
21. Iqbal, N., Khan, N. A., Ferrante, A., Trivellini, A., Francini, A., & Khan, M. I. R. (2017). Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. Frontiers in plant science, 8, 475. https://doi.org/10.3389/fpls.2017.00475.
22. Van Doorn WG, Woltering EJ. (2008). Physiology and molecular biology of petal senescence. J Exp Bot 59:453–480. doi: 10.1093/jxb/erm356.
23. Gulzar S, Amin I, Tahir I, Farooq S, Sultan SM. (2005). Effect of cytokinins on the senescence and longevity of isolated flowers of daylily (Hemerocallis fulva) cv. Royal crown sprayed with cycloheximide. Acta Hort.669:395–403.
24. Tauzin, A. S., & Giardina, T. (2014). Sucrose and invertases, a part of the plant defense response to the biotic stresses. Frontiers in plant science, 5, 293. https://doi.org/10.3389/fpls.2014.00293.