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Acclimatization of *In Vitro* Propagated Turmeric (*Curcuma longa* Linn.) in a Hydroponic System

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ABSTRACT

The increasing demand for disease-free, high-quality planting materials of *Curcuma longa* Linn in the Philippines has led to evaluating tissue culture-based micropropagation as an alternative to traditional, time-consuming propagation methods. This study investigates the feasibility of using a Simple Nutrient Addition Program (SNAP) hydroponics system for acclimatizing *in vitro* propagated turmeric plantlets, comparing its efficacy with traditional soil media. The hydroponic system positively influenced the growth performance of turmeric plantlets, exhibiting improved characteristics in terms of stem length, stem girth, number of leaves, leaf area, shoot fresh weight, root length, and root fresh weight at both 30 and 60 days after planting (DAP). While the survival rate did not differ significantly between the hydroponics system and soil media, both achieved a 100% survival rate. The SNAP hydroponics system positively influenced the growth performance of turmeric plantlets. Despite the similar survival rates with soil media, the hydroponics system significantly improved various growth parameters. This suggests the potential for hydroponics to be a valuable and efficient method for optimizing turmeric plantlet development. Further investigation and implementation of hydroponic systems for turmeric cultivation is recommended, focusing on optimizing hydroponic conditions, nutrient formulations, and system designs tailored to turmeric requirements. Cost-benefit analyses and comparisons of resource efficiency with traditional methods may also be conducted to provide insights for practical implementation.

KEYWORDS: Growth performance, Soil-based media, Simple Nutrient Addition Program (SNAP)

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1. INTRODUCTION

The advantages of micropropagation can be appreciated when plants grow successfully in a greenhouse[1]. The last and most crucial stage of the micropropagation process is the acclimatization of *in vitro* propagated meriplants in the nursery. *In vitro* propagated plants must be acclimatized to their ex vitro environment to increase their survival rate and functionality[1].

Micropropagated plants typically have a significant mortality rate during acclimatization, mainly due to abiotic stresses. Abiotic stresses include reduced relative humidity, increased light intensity, decreased nutrition availability, and non-sterile conditions that cause few to survive acclimatization[1,2]. Therefore, in a successful tissue culture system, it is crucial to increase the potential of micropropagated plants to adapt to greenhouse or field conditions during the acclimatization stage.

Many efforts have been made recently to improve the conditions that allow *in vitro* plants to harden during acclimatization. These efforts include but are not limited to employing plant growth retardants[1], creating an *in vitro* photoautotrophic system[3], and optimizing mineral nutrition in media[4]. Plants that have been micropropagated are often acclimated by gradually introducing them to *ex vitro* settings by transplanting them into a soil mixture in a controlled environment[5]. However, this method is expensive, time-consuming, and labor-intensive for large-scale micropropagation.

In a hydroponic system, plants are grown in nutrient solutions or nutrient-enriched water using the soilless hydroponics technique, either in controlled or uncontrolled growth conditions. The plant roots are suspended in water and nutrient solutions, either in mist or static, continuously aerated flow[6]. Plant growth and development are significantly impacted by the formula, concentration, electric conductivity (EC), pH, and temperature of the nutrient solution[6].

It has been acknowledged that hydroponics is a resource-efficient approach and has been widely adopted for large-scale commercial applications[7,6]. In addition, the feasibility of acclimatizing micro-propagated plants in hydroponic systems has been investigated. These plants include cassava[8] and wasabi[9]. The results indicated that a hydroponic system might be an ideal alternative for the acclimatization of tissue-cultured plants more efficiently and effectively than regular soil acclimatization.

Except for the work of Zapata et al. (2003), little is known about acclimatizing tissue-cultured plants before field planting in turmeric. The hydroponic system enables plants to adapt to decreasing relative humidity. The plants had a high survival rate and continued to grow for six weeks, by which time they had fully developed[10]. Literature is scarce on hydroponics in the acclimatization of turmeric meriplants, particularly on using the Simple Nutrient Addition Program (SNAP) hydroponics system, a non-circulating, passive aeration hydroponics system developed by Santos and Ocampo (2002). This technique has gained popularity because of its ease of use and as an alternative agricultural system for production in areas with limited space or soil unavailable. Hence, this study will explore using the SNAP hydroponic system for the acclimatization of turmeric meriplants before field planting.

2. METHODOLOGY

The study employed an experimental research design to examine the effect of a hydroponics system in the acclimatization of the *in vitro* propagated turmeric (*Curcuma longa* Linn.) under nursery conditions. Descriptive statistics was used to identify the influence of soil media and hydroponics system on the growth performance and survival rate of turmeric meriplants. Inferential statistics was employed to compare the two groups for each parameter.

The Simple Nutrient Addition Program (SNAP) hydroponics system, a non-circulating, passive aeration system, was employed. The system involved preparing growing pots with styrofoam cups, culture boxes with styrofoam boxes, and a nutrient solution based on SNAP Hydroponics Nutrient Solution. The nutrient solution, consisting of SNAP A and SNAP B, was replenished every 30 days during the acclimatization period. Polyethylene bags were filled with a sun-sterilized mixture of 1 part loam soil and 1 part vermicompost for the soil-based media.

In vitro rooted plants or meriplants were selected from the Plant Tissue Culture Laboratory of Isabela State University. These meriplants were carefully prepared, assigned to either Group A (soil media) or Group B (hydroponic system), and planted accordingly. One meriplant was placed in each growing pot in the hydroponic system, with ten established hydroponic systems. The control group comprised 80 rooted plantlets cultivated in soil media under greenhouse conditions.

The unpaired student T-Test was employed to compare the two groups (Group A – soil media, Group B – hydroponic set-up using SNAP solution). Data analysis focused on assessing the growth parameters and survival rates of turmeric meriplants, evaluating the effectiveness of the hydroponics system compared to traditional soil media.

3.0 RESULTS

Growth Performance and Survival Rate of the Plantlets

Table 1 presents the growth performance and survival rates of acclimatized turmeric plantlets in hydroponic and soil media 30 and 60 days after planting (DAP). The turmeric plantlets exhibited increased growth performance in both media, from 30 DAP to 60 DAP. Furthermore, a 100% survival rate was observed in plantlets cultivated in the hydroponic system and soil media.

Table 1 - Growth performance and survival rates of acclimatized turmeric

Treatments	**Stem Length (mm)**		**Stem Girth (mm)**		**Number of Leaves**		**Leaf Area (mm^2)**		**Shoot Fresh Weight (g)**		**Root Length (mm)**		**Root Fresh Weight (g)**		**Survival Rate (%)**	
	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP	30 DAP	60 DAP
Soil Media	53.78	90.62	3.8	5.38	4.90	5.15	508.2	897.06	1.1	1.55	50.7	81.53	0.46	2.47	100.	100
Hydroponics System	63.25	124.58	4.82	7.22	5.85	5.95	591.27	3331.41	1.62	5.3	82.32	163.07	1.47	6.23	100	100
T-test	**	**	**	**	**	**	ns	*	**	**	**	**	**	**	ns	ns

* - significant at a 5% level of significance
** - significant at 1% level of significance
ns – non-significant

Comparing the two media, it is evident that the plantlets grown in the hydroponic system displayed improved growth performance than those in the soil media. Significant differences were noted between the two media in various growth parameters, except for leaf area at 60 DAP and survival rates at 30 DAP and 60 DAP, where no significant differences were observed.

At both 30 DAP and 60 DAP, the turmeric plantlets in the hydroponics system exhibit higher growth performance in terms of stem length, stem girth, number of leaves, leaf area, shoot fresh weight, root length, and root fresh weight. Significant differences between the hydroponics and soil media were evident in all measured parameters, except for leaf area at 30 DAP and survival rates at 30 DAP and 60 DAP.

4. DISCUSSION

The growth performance parameters indicated an improved growth and development of the plantlets in the hydroponics system than in soil. The findings suggest that the hydroponics system positively influences the growth performance of turmeric plantlets, leading to the development of better above-ground (stem, leaves, shoot) and below-ground (roots) components.

Plantlets grown through tissue culture are typically fragile due to their cultivation under low-intensity artificial light and high humidity conditions. In addition, the roots of the *in vitro* propagated plants are fragile and susceptible to mechanical damage. For this reason, the plants can die a few days after they are transplanted into the pots[11, 12]. Consequently, they lack sufficient "hardening off" and may easily experience water loss upon exposure to the surrounding environment. When tissue culture plants are transferred from the laboratory to the soil, they are exposed to abiotic stresses, like altered temperature, light intensity, and humidity conditions, hence the need for acclimatization of the plants under nursery conditions before field planting.

The methodology employed in this study involves a gradual reduction in relative humidity, which may likely trigger changes in leaf structure and function in response to altered environmental conditions. Furthermore, the hydroponic system supplies essential nutrients for plant growth. These conditions support the revitalization of *in vitro* roots and the absorption of minerals for the autotrophic growth of turmeric plants[10].

The proliferation of the above-ground (stem, leaves, shoot) and below-ground (roots) components of the turmeric may be attributed to the gradual changes in relative humidity. Changes in relative humidity may significantly affect some of the events that occur during the acclimatization of plants in the greenhouse, such as the regulation of the stomatal opening and closing, the biosynthesis of the epicuticular wax, and the development of the internal structure of the leaf[10].

In addition, the favorable response of the plants to the hydroponic system may also be attributed to the availability of necessary nutrients for plant growth. Plant growth highly depends on the availability of all required nutrients, ideally in well-balanced ratios. These conditions favor the invigoration of the *in vitro* roots and the absorption of minerals for the growth of *C. longa* plants[10].

The nutrient solution formula highly influences crop growth, yield, and quality. Hydroponic nutrient solutions require well-balanced quantities of critical macro- and microelements[13]. In addition, a far greater survival rate (100%) was reached when the *in vitro*-grown plants were supplied with nutritional solutions[14]. These plants outperformed the others, showing remarkable growth and significantly higher biomass. Because a nutrient solution of the hydroponic system can provide enough nutrients and compensate for water loss for plant growth compared to soil conditions, it can harden micro-propagated plants effectively and conveniently.

The SNAP Hydroponic solution used in this study contained essential elements such as nitrogen (6.10% wt./vol), calcium (4.245% wt/vol), potassium (3.09% wt/vol), phosphorus (0.376% wt/vol), magnesium (0.494% wt/vol), and iron (0.151% wt/vol). It also contains boron, manganese, and molybdenum in trace amounts. The turmeric plantlets responded well to the SNAP solution, as shown by the increased growth of the above-ground and below-ground parts.

The importance of various nutrients, such as Potassium (K), Nitrogen (N), Phosphorus (P), Magnesium (Mg), Iron, Boron (B), and Manganese (Mn), for the growth and development of *in-vitro*-grown turmeric plants, was demonstrated in the study. Potassium (K) significantly enhances plant height, tiller number, and rhizome production. Phosphorus (P) is a fundamental building block for biomolecules crucial in energy production1[5, 16, 17, 18]. Nitrogen (N) affects photosynthesis[19]. Nitrate transporters, such as NRT3.1 and NRT2.5, are essential for increased $NO3^-$ and $NH4^+$ uptake, promoting seedling growth[20].

Moreover, Potassium (K^+) is an enzyme activator, regulating water utilization and participating in ATP and protein synthesis[21]. Magnesium (Mg) is essential for photosynthetic CO2 assimilation, playing a key role in chlorophyll pigments[22, 23, 24]. Iron is vital for metabolic activities, maintaining chloroplast structure, and participating in chlorophyll synthesis[25]. Boron (B) contributes to cell wall formation, plasma membrane integrity, and various physiological processes[26]. Manganese (Mn) is a cofactor for the photosynthetic machinery[27, 28].

These findings underscore the importance of nutrients in plant growth, such as Potassium (K), phosphorus (P), nitrogen (N), magnesium (Mg), iron (Fe), boron (B), and manganese (Mn) for their roles in various physiological processes, such as photosynthesis, enzyme activation, metabolism, chlorophyll synthesis, and overall plant development. The study emphasizes the significance of nutrient balance in hydroponics, providing precise control over nutrient levels for optimal plant growth.

The results demonstrate the potential of hydroponics as a cultivation method for optimizing the growth of turmeric plantlets, offering advantages over traditional soil-based cultivation. The findings of the study are aligned with several studies demonstrating the successful use of hydroponics for the acclimatization of *in vitro*-generated plantlets. These researches encompass a variety of plant species, including *Solanum tuberosum* L.[29, 30, 31], *Stemona curtisii* Hook.[32, 33],*Grammatophyllum speciosum* Blume[33, 31], *Colocasia esculenta* spp.[32]. More recently, researchers have explored the feasibility of acclimatizing micro-propagated plants through hydroponic systems in crops such as cassava[9], wasabi[8], and caladium[34]. The findings from these studies collectively demonstrate that hydroponics serves as a viable alternative for acclimatizing in vitro plantlets in a clean and water-saving manner.

The studies on hydroponic systems across various plant species reveal notable physiological responses, emphasizing the impact of nutrient availability, cultivation techniques, and environmental conditions on plant development. These studies focus on the physiological responses of different crops, such as tomatoes, lettuce, cucumbers, and *Stevia rebaudiana*, to various aspects of hydroponic systems. Hydroponics allows precise control over nutrient levels in tomatoes, influencing processes like photosynthesis and enzyme activity[35]. The importance of optimal nutrient balance is emphasized in lettuce hydroponics, affecting essential element uptake and determining growth[36]. The impact of different hydroponic systems was also studiedin cucumber physiology, considering factors like root oxygenation and nutrient availability[37]. Hydroponic systems also improve the nutrient uptake and water-use efficiency in lettuce and spinach [38]. In addition, the physiological responses, including changes in leaf morphology, photosynthetic rates, and metabolite production of *Stevia rebaudiana* on hydroponic cultivation, are associated with the nutrient concentrations[39].

The research then highlights the potential of hydroponics for plant acclimatization since results demonstrate that there is improved growth and development of turmeric plantlets in a hydroponic system compared to soil due to the nutrient-rich hydroponic solution that plays a crucial role in promoting robust growth and achieving a 100% survival rate. The balance of nutrition and availability in the system is also essential in plant physiology and the advantages of hydroponics in providing a controlled and nutrient-rich environment for plant cultivation.

5. CONCLUSION

Based on the result of the preceding study, it can be concluded that the SNAP hydroponics systems had positively influenced the growth performance of turmeric plantlets in terms of stem length, stem girth, number of leaves, leaf area, shoot fresh weight, root length, and root fresh weight. However, in terms of survival rate, both the hydroponics system and soil media influenced the high survival rate.

REFERENCES

1. Hazarika, B.N. (2003) Acclimatization of tissue-cultured plants. Current Science, 85, 1704-1712.
2. Osório, M.L., Gonçalves, S., Coelho, N., Osório, J., Romano, A. (2013). Morphological, physiological, and oxidative stress markers during acclimatization and field transfer of micropropagated Tuberaria major plants. Plant Cell Tissue Organ Culture, 115, 85–97. https://doi.org/10.1007/s11240-013-0343-x
3. Tisarum, R., Samphumphung, T., Theerawitaya, C., Prommee, W., Cha-um, S. (2018). In vitro photoautotrophic acclimatization, direct transplantation and ex vitro adaptation of rubber tree (Hevea brasiliensis). Plant Cell Tissue and Organ Culture, 133, 215–223. https://doi.org/10.1007/s11240-017-1374-5
4. El-Hawaz, R., Park, D., Bridges, W.C., Adelberg, J. (2016). Optimizing in vitro mineral nutrition and plant density increases greenhouse growth of Curcuma longa L. during acclimatization. Plant Cell Tissue Organ and Culture, 126, 33–42. https://doi.org/10.1007/s11240-016-0974-9
5. Chen, L., Yang, Y., Jiang, Y., Zhao, J., Zang, H., Wang, X., Hu, Y., Xue, X. (2019). RNA-Seq analysis reveals differential responses of potato (Solanum tuberosum L.) plantlets cultured in vitro to red, blue, green, and white light-emitting diodes (LEDs). Journal of Plant Growth Regulation, 38, 1412–1427. https://doi.org/10.1007/s00344-019-09944-7
6. Hosseinzadeh, S., Verheust, Y., Bonarrigo, G., Van Hulle, S. (2017). Closed hydroponic systems : operational parameters, root exudates occurrence, and related water treatment reviews. In: Environmental Science And Bio-Technology, 16(1), 59–79. https://doi.org/10.1007/s11157-016-9418-6
7. Bradley, P. and Marulanda, C. (2001). SIMPLIFIED HYDROPONICS TO REDUCE GLOBAL HUNGER. Acta Hortic. 554, 289-296. https://doi.org/10.17660/ActaHortic.2001.554.31
8. Castañeda-Méndez, O., Ogawa, S., Medina, A., Chavarriaga, P., & Selvaraj, M. G. (2017). A simple hydroponic hardening system and the effect of nitrogen source on the acclimation of in

vitro cassava (Manihot esculenta Crantz). In Vitro Cellular & Developmental Biology-Plant, 53, 75-85.

9. Hoang, N., Kitaya, Y., Shibuya, T., Endo, R. (2019). Development of an in vitro hydroponic culture system for wasabi nursery plant production—effects of nutrient concentration and supporting material on plantlet growth. Scientia Horticulturae. 245, 237–243. https://doi.org/10.1016/j.scienta.2018.10.025
10. Zapata, E.V., Morales, G.S., Lauzardo, A.H., Bonfil, B.M., Tapia, G.T., Sánchez, A. DJ., Del Valle, M.V., Aparicio, A.J. (2003). In vitro regeneration and acclimatization of plants of Turmeric (Curcuma longa L.) in a hydroponic system. Biotecnología Aplicada. 20, 25-31
11. Zobayed FA, Zobayed SM, Kubota C, Kozai T. (1999). Supporting material influences the root growth and morphology of sweet potato plantlets cultured photoautotrophically. In Vitro Cell Dev Biol Plant; 35: 470-4.
12. Pospísilová J, Synková H, Haisel D, Catský J, Wilhelmová N, y Srámek F.(1999). Effect of elevated CO2 concentration on acclimation of tobacco plantlets to ex vitro conditions. J Exp Bot; 50(330):119-26.
13. Trejo-Téllez, L. & Gómez-Merino, F.C. 2012. Nutrient solutions for hydroponic systems. In Hydroponics-A Standard Methodology for Plant Biological Researches, edited by Asao, T. London: InTech. pp. 1-22
14. Duan, J.-X.; Duan, Q.-X.; Zhang, S.-F.; Cao, Y.-M.; Yang, C.-D.; Cai, X.-D. Morphological, Physiological, Anatomical and Histochemical Responses of Micropropagated Plants of Trichosanthes Kirilowii to Hydroponic and Soil Conditions during Acclimatization. Plant Cell Tissue Organ Cult. 2020, 142, 177–186.
15. Stigter KA, Plaxton WC. Molecular Mechanisms of Phosphorus Metabolism and Transport during Leaf Senescence. Plants (Basel). 2015 Dec 16;4(4):773-98. doi: 10.3390/plants4040773. PMID: 27135351; PMCID: PMC4844268.
16. Park, C.J., Caddell, D.F., Ronald, P.C. 2012. Protein phosphorylation in plant immunity: insights into the regulation of pattern recognition receptor-mediated signaling. Front Plant Sci. 3:177. doi: 10.3389/fpls.2012.00177. PMID: 22876255; PMCID: PMC3411088.
17. Morris P. C. 2001. MAP kinase signal transduction pathways in plants. The New Phytologist, 151(1), 67–89. https://doi.org/10.1046/j.1469-8137.2001.00167.x

18. Stone JM, Walker JC. 1995. Plant protein kinase families and signal transduction. Plant Physiol. 108(2):451-7. doi: 10.1104/pp.108.2.451. PMID: 7610156; PMCID: PMC157363.
19. Hachiya T., Sakakibara H. 2017. Interactions between Nitrate and Ammonium in Their Uptake, Allocation, Assimilation, and Signaling in Plants. J. Exp. Bot. 2017;68:2501–2512. doi: 10.1093/jxb/erw449.
20. Ma H., Zhao J., Feng S., Qiao K., Gong S., Wang J., Zhou A. (2020). Heterologous Expression of Nitrate Assimilation Related-Protein DsNAR2.1/NRT3.1 Affects Uptake of Nitrate and Ammonium in Nitrogen-Starved Arabidopsis. Int. J. Mol. Sci. 21:4027. doi: 10.3390/ijms21114027.
21. Silva, J.A., Uchida, R. (2000). Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa
22. Cakmak, I., & Kirkby, E. A. (2008). Role of magnesium in carbon partitioning and alleviating photooxidative damage. Physiologia plantarum, 133(4), 692-704.
23. Karley, A. J., & White, P. J. (2009). Moving cationic minerals to edible tissues: potassium, magnesium, calcium. Current opinion in plant biology, 12(3), 291–298. https://doi.org/10.1016/j.pbi.2009.04.013
24. Peng, Y. Y., Liao, L. L., Liu, S., Nie, M. M., Li, J., Zhang, Ma, J.F., L. D., Chen, Z. C. (2019). Magnesium deficiency triggers SGR–mediated chlorophyll degradation for magnesium remobilization. Plant Physiology, 181(1), 262-275.
25. Rout, G.R., Sahoo, S. (2015). Role of iron in plant growth and metabolism. Reviews in Agricultural Science, 2015, Volume 3, Pages 1-24, https://doi.org/10.7831/ras.3.1
26. Brown, P. H., Bellaloui, N., Wimmer, M. A., & Bassil, E. S. (2002). Boron in plant biology. Plant Biology, 4(2), 205-223.
27. Alejandro, S., Höller, S., Meier, B., Peiter, E. 2020. Manganese deficiency in plants: the impact on photosystem II. Front. Plant Sci., 26. 11-2020. https://doi.org/10.3389/fpls.2020.00300
28. Schmidt, J. J., Gagnon, G. A., & Jamieson, R. C. (2016). Microalgae growth and phosphorus uptake in wastewater under simulated cold region conditions. Ecological Engineering, 95, 588-593.

29. Banijamali, S. M., Feizian, M., Bayat, H., &Mirzaei, S. (2018). Effects of nitrogen forms and calcium amounts on growth and elemental concentration in Rosa hybrida cv.'Vendentta .'Journal of Plant Nutrition, 41(9), 1205-1213.
30. Phantong, P., Machikowa, T., Saensouk, P. & Muangsan, N. (2018). Comparing growth and physiological responses of *Globba schomburgkii* Hook. f. and *Globba marantina* L. under hydroponic and soil conditions. Emirates Journal of Food and Agriculture 30(2): 157-164
31. Sutthinon, P., Pan-aon, K., Meesawat, U. & Jantasilp, A. (2015). Acclimatization of in vitro germinated seedlings of tiger orchid (*Grammatophyllum speciosum* Blume.) in hydroponic culture using dynamic root floating technique (DRFT) with chitosan spraying. Thai Journal of Agricultural Science 48(2): 47-53
32. Nhut, D.T., Nguyen T.M.H., Pham, Q. T., Le, T. M. N., Nguyen, T. H. T.,Ngo, C. C.,Nguyen H. N., Do, N. V. (2006). Liquid culture as a positive condition to induce and enhance quality and quantity of somatic embryogenesis of Lilium longiflorum. Scientia Horticulturae 110(1):93-97. DOI: 10.1016/j.scienta.2006.05.015
33. Palee, J., Dheeranupattana, S., Jatisatienr, A., Mungkornasawakul, P. Pyne, S., Ung, A., Sastraruji,T. (2010). Influence of Plantlet Age and Different Soilless Culture on Acclimatization of Stemona curtisii Hook.f. Asian Journal of Plant Sciences 11(6):294-299. DOI: 10.3923/ajps.2012.294.299
34. Phonpho, S. & Saetiew, K. 2017. Selection of appropriate species of plants for indoor vertical garden. International Journal of Agricultural Technology 13(1): 119-129.
35. Nguyen, N. T., McInturf, S. A., & Mendoza-Cózatl, D. G. (2016). Hydroponics: A Versatile System to Study Nutrient Allocation and Plant Responses to Nutrient Availability and Exposure to Toxic Elements. Journal of visualized experiments : JoVE, (113), 54317. https://doi.org/10.3791/54317
36. Ahmed Z.F.R., Alnuaimi, A.K.H., Askri, A, Tzortzakis, N. (2021). Evaluation of lettuce (Lactuca sativa L.) production under hydroponic system: nutrient solution derived from fish waste vs. inorganic nutrient solution. Horticulturae. 7(9):292. https://doi.org/10.3390/horticulturae70902923.
37. Giban, M.G., Salas, R.A. (2023). Growth, yield and quality of cucumber (Cucumis sativus L.) grown in hydrophonic system as influenced by different aggregate compositions and pruning techniques. Science and Humanities Journal 17: 55-73. DOI: 10.47773/shj.1998.171.4

38. Kumar, K., Kumari, K., Acharya, S., Tsewang, T., Mishra, A., Verma, A., Chaurasia, O. (2023). Evaluation of spinach and lettuce production (growth, morphological characteristics, biomass yield, and nutritional quality) in NFT hydroponic system in greenhouse, room, and open environment at Leh, India. Section A-Research paper Eur. European Chemical Bulletin. 12. 5421-5435. 10.31838/ecb/2023.12.5.419.
39. Janakiramudu, S., Raja Kumar, K.N. , Ganesh Babu, R., Someswara Rao, C.H.. (2022). Optimization of nutrient concentration and grow media for stevia crop under hydroponic farming. The Pharma Innovation Journal 2022; 11(12): 5603-5608. www.thepharmajournal.com.