5th EURASIA WASTE MANAGEMENT SYMPOSIUM
www.eurasiasymposium.com

# Hazelnut Husk Based Acclimatization Soil for in-vitro Propagated Ornamental Plants

***Saim Ozdemir[1], Merve Ozdemir[2]***

***Abstract***

*In-vitro propagated ornamental plantlets frequently stressed by newly exposed environmental conditions due to the weak root system, a poorly developed epicuticular structures, less vigor and hyperhydricity due to special conditions of in-vitro. Therefore, high mortality occurs after transplantation from in-vitro to ex-vitro acclimatization. Ornamental plantlets are quite sensitive to the same environmental conditions when propagated in-vitro and transferred to potting mix. In the present study, several ornamental tissue cultured plantlets were transplanted on a novel hazelnut husk substrate in order to test the ability to acclimatize ex-vitro conditions. Phytotoxicity test, acclimatization rate, and visual growth parameters were evaluated comparing to the standard potting substrate coco peat. Phytotoxicity test results were comparable to the standard coco peat in all tested hazelnut husk based substrates. Mortality rate of transplanted plantlets on either hazelnut husk based substrates or standard coco peat were higher for woody species Rosa hybridae and Loropetalum chinense, comparing to the succulent species Aloa vera and Hylocereus undatus. Growth parameters of all species correlating well with shoot development, chlorophyll fluorescence and root development.*

**Keywords:** in-vitro plantlets, acclimatization, potting mix, gyttja, hazelnut husk

## 1. INTRODUCTION

In-vitro propagation has been extensively used for propagating ornamental plant species due to the rapid multiplication of high quality, precious, rare commercial plants in a laboratory [1]. However, initial survival rate and growth of plantlets in ex-vitro conditions severely restrict the success of in-vitro technics, when in-vitro propagated plantlets transplanted to potting soil and exposed to ambient environmental conditions. A substantial number of plantlets do not survive when transferred from in-vitro to ex-vitro environmental conditions, due to the septic environment, lower relative humidity, temperature fluctuation and insufficient readily available nutrition that are stressful to micro-propagated seedling compared to in-vitro conditions [2]. In this context, the success of micro propagation not only depends on multiplication in culture media but also depends on high survival rate and healthy plantlets during acclimatization stage [3].

Most plant species require an acclimatization stage in order to ensure high number of plants survive when transferred to ambient conditions. It is widely known that substrate properties such as sterilization, biological stability and air-water balance are the most important structural properties for healthy plant life [4]. In

[1] *Corresponding author: Sakarya University Environmental Engineering Department, 54187, Serdivan/Sakarya*
*saimo@sakarya.edu.tr*

[2] *Sakarya University Environmental Engineering Department, 54187, Serdivan/Sakarya*
*merve.ozdemir17@ogr.sakarya.edu.tr*

addition, other ecological and growth promoting precautions can be complemented for the survival of plantlets, such as the use of humic substances and growth promoting microorganism or by products [5], [6].

The gradually changing environmental conditions during the acclimatization period also used to improve survival rate and plant hardening [7]. Growing media or substrate properties and their performance during acclimatization period are also important. Previous studies have demonstrated the addition of humic substances to substrates promotes the rooting [8], the growth and development of aerial structures, as well as visual quality of plantlets [9]. Essentially, growing media must provide a root environment that is initially free of pathogens and properties that guarantee an adequate gaseous exchange, water supply, and retain nutrients bioavailable [10]. To optimize the seedling survival and hardening the plantlets, bioresources such as crop residues, waste materials or by-products have great potential to be used as growing media constituents and stand-alone substrates [11]. Different waste organic materials or by-products have been investigated for fully or partly replacing non-renewable inorganic or peat-based substrates. Coco-peat, compost, and wood industry wastes are some organic materials that are already being tested for possible ornamental growing media [12]. In addition, some growth stimulating humic like materials, such as leonardite, biochar, clay granules, and vermicuite are used in mixture with peat and other combinations [11]. The main aim of those studies were primarily to decrease the mortality of newly transplanted in-vitro plantlets by either hasten the root and protective cuticular tissue formation to restrict the excess transpiration when plants are transferred from in vitro to ex vitro conditions [12]. Because, the decrease in water supply and the increase in transpiration frequently lead to drought stress in new environment.

A study about the effects of proposed growing media ingredient hazelnut husk for *ex-vitro* potting substrate has not been done yet, although hazelnut husk based growing media is widely used as a potting mixture for ornamental plants [13]. Hazelnut husk, when mixed into growing media provide a source of bio-stable fiber, as well as an important source of macro and micro plant nutrients. In addition, gyttja as a humic substances source might further assist the acclimatization by providing growth promoting and osmotic regulation against to wilting in early stage [14]. The expectation from the hazelnut and gyttja is a good rooting substrate, because hazelnut husk proved to be a good growing media and gyttja is a source of growth stimulating substances. Therefore, we investigated the effect of hazelnut husk as main substrate component and gyttja as a growth stimulating ingredient on in-vitro plantlet survival rate and growth of two woody and two succulent species during acclimatization period for their adaptation ability to ex-vitro conditions.

## 2. MATERIALS AND METHODS

### *2.1. Plant Materials*

The in-vitro micro propagated test materials; two woody ornamental species *Rosa hybridae* and *Loropetalum chinense*, and two succulent species *Aloa* vera and *Hylocereus undatus* obtained from the Tissue Culture Laboratory, Sakarya University, Turkey. The plantlets with sufficient roots were taken out of the culture vessels washed with flowing tap water to remove medium on planets surfaces and transplanted directly to the *ex vitro* potting mixtures.

### *2.2. Potting Soil Materials*

Hazelnut husk waste residue was collected from a nearby hazelnut producer after nut separation from the husk, milled with hammer mill to reduce the particle size to between 0 and 4 mm and composted before use. The scanning electron microscopic (SEM) and energy dispersive X-ray spectroscopic (EDS) analyses were also performed using the FEI Quanta model FEG 250 instrument (FEI Netherlands) at the Kastamonu University Research Laboratory, in order to evaluate the structural variability on acclimatization performance of plantlets.

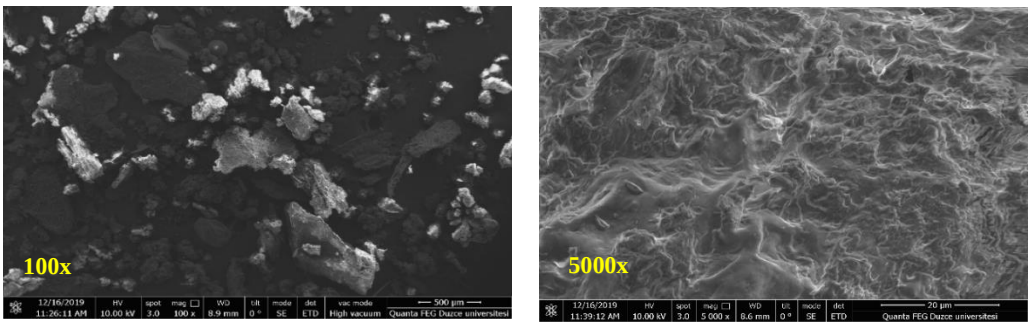
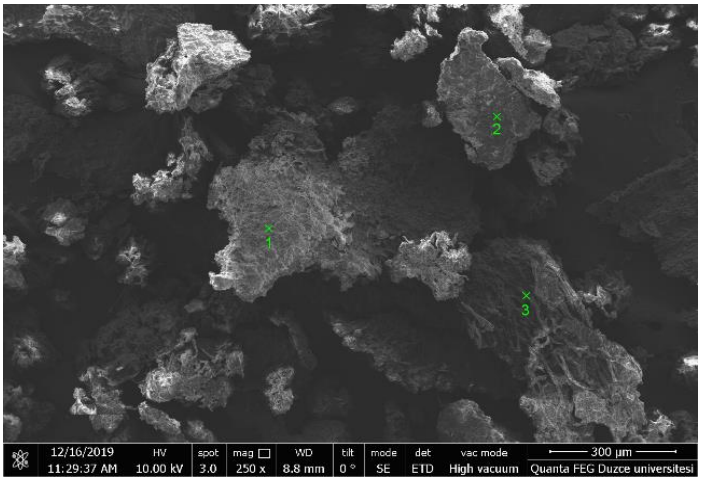



*Figure 1. SEM images of hazelnut husk*




Spectrum 1 Spectrum 2 Spectrum 3

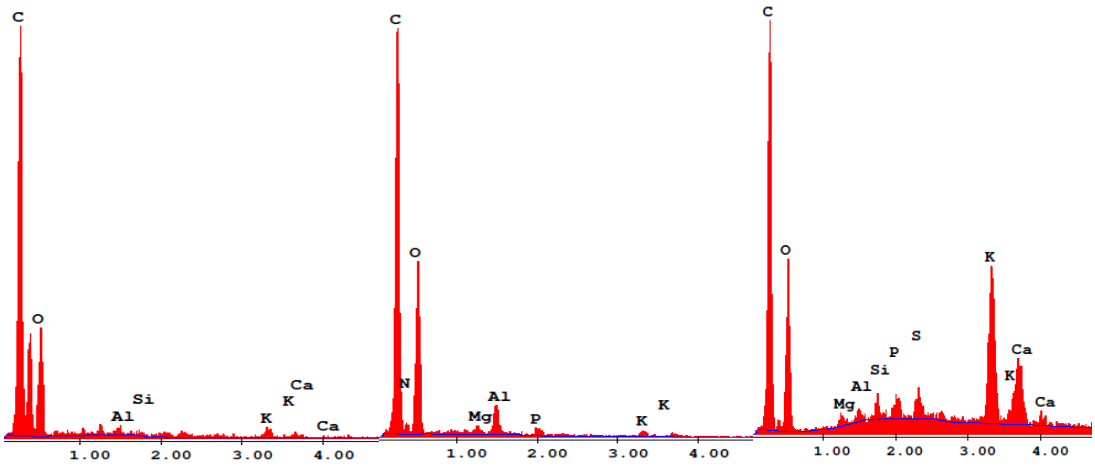



*Figure 2. EDS analysis of hazelnut husk and elemental compositions of selected spectrum*

The gyttja sample was purchased from the commercial market. The origin of the gyttja mine is Afşin-Elbistan lignite deposit in Kahramanmaras province, Turkey. The wasted gyttja during the mining of lignite at region processed for horticultural use, as it is containing mainly humic and fulvic substances that promote plant growth and development [14]. The main characteristics of gyttja presented in Table 1.

*Table 1. Physicochemical properties of hazelnut husk and gyttja.*

| Parameters | Hazelnut husk | Gyttja |
|---|---|---|
| Organic matter (%) | 86.15 ± 1.34 | 67.30 ± 1.83 |
| Ash (%) | 13.85 ± 0.66 | 32.82 ± 2.51 |
| pH | 6.87 ± 0.12 | 6.84 ± 0.11 |
| EC (mS/cm) | 2.02 ± 0.03 | 1.81 ± 0.06 |
| N (%) | 0.82 ± 0.03 | 1.01 ± 0.09 |
| P (%) | 1.12 ± 0.31 | 0.12 ± 0.01 |
| K (%) | 5.68 ± 0.74 | 0.07 ± 0.00 |
| Humic substances (%) | - | 58.43 ± 1.04 |

### *2.3. Phytotoxicity Tests*

Toxicity tests were performed germinating *Lepidium sativum* (garden cress) seeds in petri dishes using water eluates obtained from all hazelnut husk and gyttja combinations. One hundred grams of dry sample was put into a 1-L bottle and the 900 ml of ultra-pure water was added to prepare water eluate of the samples (1:10 w/v dilution). The suspension was shaken for 24 h at 20 rpm. After the solid particles had settled down, the supernatant of the samples was centrifuged for 30 min at 3000 rpm and filtered through a 0.45-mm Teflon nylon filter under vacuum application. 25 seeds of cress were put on filter paper that was placed into 9 cm Petri dishes and treated with 10mL of extracted solution. After 3-day incubation in germination cabinet at 25 ºC, the root length of healthy 20 seedlings were measured for the indicator calculations. Reference tests with ultra-pure water were performed as control treatment. The indicator indices such as the germination rate

(GR), the relative seed germination (RSG), the relative radicle growth (RRG) and the seed germination index (GI) calculated the test procedure described by [15] as follows:

GR = number of germinated seeds / number of total seeds × 100 (1)
RSG = (n of seeds germinated in sample / n of seeds germinated in control) × 100 (2)
RRG = (mean root length in sample / mean root length in control) × 100 (3)
GI = (RSG × RRG) / 100 (4)

### *2.4. Experimental Design*

The study was prepared in One-Way Anova Experimental Design for four plant species in order to obtain the suitability of hazelnut and gyttja combination as a potting substrate for in-vitro plantlets during acclimatization period. The experiment consists of one factor, growing media with combination of different compositions substrate, A0 = 100% hazelnut husk; A1 = 99% hazelnut husk + 1% gyttja; A2 = 98% hazelnut husk + 2% gyttja; A3 = 95% hazelnut husk + 5% gyttja, A4 = 90% hazelnut husk + 10% gyttja, and A6 = 100% coco-peat (control treatment). Every combination of treatment was 10 replications, so there were 60 treatments for each plant species.

### *2.5. Acclimatization Conditions*

The planted pots were sprayed with protective fungicides and placed in an acclimatization chamber (Figure 3). The environmental conditions in a chamber was operated with the following conditions: 14 h light/10 h darkness, quantum flux density 200 µmol $m^{-2}$ $s^{-1}$ temperature 20±1 °C. Relative humidity was gradually decreased from 90 to 70%. Transplanted plants were kept for four weeks in an acclimatization chamber. Following to the pre-acclimatization phase pots were placed in a tray and transferred to ambient environmental conditions under shade net for hardening. During the hardening period required humidity levels maintained with moistening for 2 consecutive months.


*Figure 3. Acclimatization conditions*

The plant growth parameters observed in this study were the survival rate of plantlets during acclimatization period (%), plant height (cm), base diameter (mm), and the Seedling Quality Index. Calculation of survival rates were performed at the end of the study using following formula:

$$Survival\ rate = \frac{number\ of\ live\ plant}{number\ of\ transplanted\ plant} \times 100$$

## 3. RESULTS AND DISCUSSION

### *3.1. Potting Soil Components*

Pysico-chemical structure of potting soil is essential for healthy root growth and root establishment especially for micro propagated plants during acclimatization period. Peat based and coco peat based substrates are standardized and commercialized for micro propagation. However, locally accessible organic waste materials such as harvest residue of hazelnut husk and mining residues gyttja, can be alternatively exploited as acclimatization substrate for *in vitro* plants. In the present study, SEM image (Figure 1) of the hazelnut husk proved to be an acceptable porous and relatively smooth surface structure as a main component of the potting soil. According to the previous studies [13], it is found that the macro pores were more prone to be formed in the total porosity of hazelnut husk media, and have characteristics of high air porosity and low water holding capacity. These properties could be beneficial for proving optimum gas exchange interface while providing necessarily moistened matrix for root proliferation. Similarly, organic matter, pH, EC and nutrient composition within the acceptable range (Table 1, Figure 2) for potting soil reported in previous literature [13]. Gyttja has similar pH, EC and nutritional characteristics to the hazelnut husk, but contain outstanding component humic substances (Table 1), which could be a stimulating effect on rooting and hardening of *in vitro* plantlets during acclimatization stage [5], [14].

### *3.2. Phytotoxicity*

In vitro plantlets are sensitive to *ex vitro* acclimatization and safe potting soil is essential for survival, plant growth and development during hardening stage. One of the potting soil indicator is seed germination test which evaluating toxicity of growing media [15]. Therefore, phytotoxicity tests that indicator of quality of growing media performed and germination test results of tested growing media comparing to standard substrate coco peat and deionized water are presented in Table 2. The results showed that number of germinated seed, root elongation and shoot elongation showed no statistical differences between the growing media that is prepared from hazelnut husk and gyttja combinations. Germination rate (GR) estimated from the germination percentage, relative shoot, and root growth data of respected growing media relative to the deionized water and coco peat control is said to be normal. The RSG*RRG (%) results showed that shoot and root length did not show significant differences between treatments and coco peat control substrates, small differences were within the insignificant range. It can also be observed from Table 2 that extracts obtained from 5% and 10% gyttja application showed small increase in GI values which further confirms stimulating effects of gyttja on seedling shoot and root growth.

Table 2. Germination test results and indicator indices

| | Toxicity indicator | | | |
|---|---|---|---|---|
| Treatments | GR (%) | RSG (%) | RRG (%) | RSG*RRG (%) |
| Deionized water | 96.0 | 100.0 | 100.0 | 100.0 |
| Coco peat (100%) | 96.0 | 100.0 | 102.5 | 102.5 |
| HH (%100) | 94.7 | 97.2 | 98.7 | 95.9 |
| HH + Gyttja (99:1%) | 97.3 | 101.2 | 102.2 | 103.4 |
| HH + Gyttja (98:2%) | 94.7 | 97.2 | 102.3 | 97.2 |
| HH + Gyttja (95:5%) | 96.0 | 100.0 | 105.1 | 105.3 |
| HH + Gyttja (90:10%) | 94.7 | 97.2 | 105.3 | 102.4 |
| *P* | NS | NS | NS | NS |

Previous studies report that a RSG*RRG value above 80% indicates the absence of phytotoxicity [13]. In the present study, RSG*RRG values varied between 95.9-105.3 which exhibited no phytotoxiciy and can be used safely as potting soil for ornamental plant seedlings.

### *3.3. Plant Survival Rate*

The first objective of the study was to test the survival rates of micro propagated plantlets when transplanted on potting soil during acclimatization period. Therefore, survival rate was used as an indicator of the success

of tested growing media comparing to control group that was transplanted in coco peat in the same conditions as the ex-vitro grown plantlets. In the first stage of the ex vitro acclimatization in the controlled environmental conditioned cabinet, most of the plantlets of all tested plant species were survived and growing media effects on survival rates were insignificant during the 4 initial weeks. However, by the end of the initial acclimatization period, no plantlets had developed any leaves or plant height increments observed.

The response of *in vitro* plantlets of four plant species to different growing media is presented in Figure 4. The survival rates of plantlets on average at the end of the experiment was 96 % for dragon fruit *Hylocereus undatus*, 74% for Aloa vera, 34% for miniature rose *Rosa hybridae* and 16% *Loropetalum chinense*. A survival rates up to 90% was obtained for the *Hylocereus undatus* for all tested potting soils.

The results obtained in this study indicated that, at three-month acclimatization, the survival rate of micro propagated plants of four plant species are significantly influenced by tested potting soils. It is observed that, after 3 months of acclimatization, the succulent plant species plantlets had a higher survival rate of over 80.8 %. Nonetheless, a higher survival was observed in plantlets that transplanted in coco peat control, T1, T2 and T3 treatments, respectively, for the both succulent species (Figure 4). These results demonstrated the role of the substrate components and their physico-chemical properties on the survival of micro propagated plantlets during the acclimatization stage.

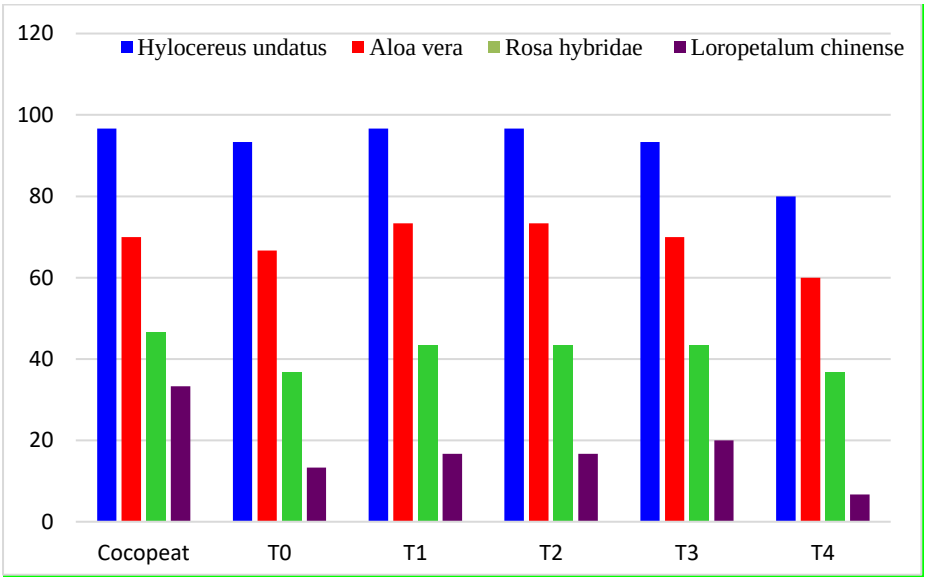



*Figure 4. Surviving percentage of different plant species in different potting soil after acclimatization*

Despite the succulent species, the plantlets of woody species exhibit significantly low survival rate probably due to the morphological and physiological differences, and rooting environments provided by different potting soil. For the woody species plantlets transplanted in coco peat control showed 40% of survival rate, followed by T3, T1 and T2 with above 30% survival (Figure 4). The lowest survival rate was found in the plantlets transplanted on hazelnut husk with high dose of gyttja.

The type of growing media is known to affect the survival rate of in vitro plantlets during acclimation either under controlled environmental conditions or semi sterile ex vitro environments [5]. The results of present study indicated that small dose of gyttja significantly improved the physico-chemical properties of potting soil and plant survival rates positively responded this conditions. Humic substances present in gytjaa might also be responsible for the improvements of survival rate. But, high rate of small particle size of gyttja (T4 10%) probably disturbed the macro and micro pore size distribution [16] and, this is in turn impaired the air-water balance in rooting medium, which is crucial factor for plant survival.

## 4. CONCLUSIONS

The successful acclimatization of in-vitro plants to soil conditions is a most crucial step for micropropagation technology, because of rapid desiccation of plantlets or their susceptibility to diseases under excess humidity that frequently used in order to prevent desiccation in acclimatization environment. In the present study, potting soil mixture prepared by locally waste resources were tested for the aiming to rapid rooting and improve survivor rate of plantlets of four different plant species. The survival rates were higher for the succulent species than that of woody species *Rosa hybridae* and *Loropetalum chinense*. In general, the survival rates were significantly improved in medium containing hazelnut husk with 2 to 5 % gyttja,

irrespective to the tested plant species. The plantlets transplanted into the gyttja received potting soil achieved 85, 75, 60 and 30 % survival for the plant dragon fruit, *Aloa vera*, rose and *Loropetalum chinense* species, respectively Moreover, the gyttja incorporation in to potting soil with the amount of 2 and 5% helped in the production of larger roots and shoot. The most important finding was that hazelnut husk derived from agricultural waste stream and gytjaa derived from mining waste did not adversely affect the survival rate of *in vitro* seedlings. However, more information about the physicochemical characteristics of growing media combination would help to establish a more suitable potting soil for possible use in *in-vitro* culture acclimatization.

## ACKNOWLEDGEMENT

The authors would like to thank Sakarya University, Tissue Culture Laboratory, and Prof.Dr Taki Demir for supplying the plantlets, advising us precious information for ex-vitro acclimatization and measurements. We wish to express our gratitude to the referees for reading the manuscript very carefully and for the very useful comments which helped us to improve the manuscript.

## REFERENCES

[1]. P. Kadlecek, I. Ticha, D. Haisel, V. Capkova, and C. Schäfer, "Importance of *in vitro* pretreatment for *ex vitro* acclimatization and growth", *Plant Science*, vol. 161, pp. 695-701, 2001.
[2]. B.N. Hazarika, "Acclimatization of tissue-cultured plants", *Current Science*, vol. 85, pp.1704-1712, 2003.
[3]. I. Siddique, and M. Anis, "An improved plant regeneration system and ex vitro acclimatization of Ocimum basilicum L.", *Acta physiologiae plantarum*, vol. 30, pp. 493-499, 2008.
[4]. Clapa, D., Fira, A., Simu, M. (2015). The role of rooting substrate in blackberry ex vitro rooting and acclimatization stage. *ProEnvironment/ProMediu*, *8*(22).
[4]. M.S. Barreto, and A. Nookaraju, "Effect of auxin types on in vitro and ex vitro rooting and acclimatization of grapevine as influenced by substrates", *Indian Journal of Horticulture*, vol. 64, pp. 5-11. 2007.
[5]. A. Mengesha, B. Ayenew, and T. Tadesse, "Acclimatization of in vitro propagated pineapple (Ananas comosuss (L.), var. smooth cayenne) plantlets to ex vitro condition in Ethiopia", *American Journal of Plant Sciences*, vol. 4, pp. 317-323, 2013.
[6]. A. H. Naing, S. H. Kim, M. Y. Chung, S. K Park, and C. K. "In vitro propagation method for production of morphologically and genetically stable plants of different strawberry cultivars", *Plant methods*, vol. 15, pp. 36, 2019.
[7]. J. Pospíšilová, I. Tichá, P. Kadlecek, D. Haisel, and S. Plzáková, "Acclimatization of micropropagated plants to *ex vitro* conditions", *Biologia Plantarum*, vol. 42, pp. 481-497, 1999.
[8]. Y. Wu, Y.P. Xia, J.P. Zhang, F. Du, L. Zhang, and H. Zhou, "Low humic acids promote in vitro lily bulblet enlargement by enhancing roots growth and carbohydrate metabolism", *Journal of Zhejiang University-SCIENCE B*, vol. 17, pp. 892-904, 2016.
[9]. M. S. Elmongy, X. Wang, H. Zhou, and Y. Xia, "Humic Acid and Auxins Induced Metabolic Changes and Differential Gene Expression during Adventitious Root Development in Azalea Microshoots". *HortScience*, vol. 55, pp. 926-935, 2020.
[10]. S. Petropoulos, A. Fernandes, D. Stojković, C. Pereira, O. Taofiq, F. Di Gioia, and I.C. Ferreira, "Cotton and cardoon byproducts as potential growing media components for Cichorium spinosum L. commercial cultivation", *Journal of Cleaner Production*, vol. *240*, pp. 118254, 2019.
[11]. N. S. Gruda, "Increasing sustainability of growing media constituents and stand-alone substrates in soilless culture systems", *Agronomy*, vol. 9, pp. 298, 2019.
[12]. S. Chandra, R. Bandopadhyay, V. Kumar, and R. Chandra, "Acclimatization of tissue cultured plantlets: from laboratory to land". *Biotechnology letters*, vol. 32, pp.1199-1205, 2010.
[13]. O. H. Dede, G. Dede, S. Ozdemir, and M. Abad, "Physicochemical characterization of hazelnut husk residues with different decomposition degrees for soilless growing media preparation. *Journal of Plant Nutrition*, vol.34, pp. 1973-1984, 2011.
[14]. F. Gülser, C. Yılmaz, and F. Sönmez, "Gidya ve kimyasal gübre uygulamalarının yetiştirme ortamı ile biber (Capsicum annuum l.) bitkisinde meyvelerin pomolojik ve biyokimyasal özelliklerine etkileri", *Toprak Bilimi ve Bitki Besleme Dergisi*, vol. 2, pp. 1-5, 2014.
[15]. Y. Luo, J. Liang, G. Zeng, M. Chen, D. Mo, G. Li, and D. Zhang, "Seed germination test for toxicity evaluation of compost: Its roles, problems and prospects", *Waste Management*, vol. 71, pp. 109-114, 2018.
[16]. S. Eskandari, A. Mohammadi, M. Sandberg, R.L. Eckstein, K. Hedberg, and K. Granström, "Hydrochar-Amended Substrates for Production of Containerized Pine Tree Seedlings under Different Fertilization Regimes", *Agronomy*, vol. 9, pp. 350, 2019.

## BIOGRAPHY

***Merve ÖZDEMİR*** *holds a master's degree at Sakarya University Environmental Engineering Department.*

*Özdemir received his BSc in Environmental Engineering in 2018 from Sakarya University, Sakarya, Turkey. She was in Hungary for 5 months for the Erasmus student exchange program while she was at the postgraduate degree.*

*She may be contacted at merve.ozdemir17@ogr.sakarya.edu.tr or* mrvzdmr123@gmail.com