J. Hortic. Sci.
Vol. 20(1), 2025

https://doi.org/10.24154/jhs.v20i1.3062

Original Research Paper

Optimization of bacoside A3 content in hydroponically cultivated *Bacopa monnieri*

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ABSTRACT

Bacopa monnieri L. is a popular ayurvedic plant known for its cognitive properties. The active principle in *B. monnieri* responsible for its brain-stimulating character is a group of saponins, specifically, bacoside A (bacoside A3, bacopaside II, and bacopasaponin C). In this study, the impact of different nutrient formulations-Hoagland & Arnon (1938) (control), GL-L, Higro-Super, and Hoagland & Arnon (1950), and different light conditions *viz*., white LED lights (WL) & multi-spectral LED lights (ML) on the growth and bacoside A3 content of *B. monnieri* in a nutrient film technique (NFT) hydroponic system were investigated. The data was analyzed using the ANOVA ($p<0.05$) and DMRT test. It was found that GLL+ML as a treatment showed the highest number of shoots/plant (51.2±3.6), number of leaves/plant (518±21.2), leaf area (1.28±0.04), fresh weight (28.82±5.7 g/plant), and dry weight (2.2±0.26 g/plant). Conversely, the Higro-Super+ML treatment illustrated the highest bacoside A3 content (3528 mg/kg) along with suitable growth, followed by Hoagland & Arnon (1938) +ML (3204 mg/kg). These research findings shed light on the importance of considering both light spectrum and nutrient availability in optimizing growth conditions for *B. monnieri* cultivation.

Keywords: GL-L, higro-super, HPLC, medicinal plant, nutrient film technique

INTRODUCTION

Bacopa monnieri (L.), commonly known as '*Brahmi*', is a highly valued herb in ayurvedic medicine for improving intellect and memory. The active compound in *B. monnieri*, known as bacoside A (bacoside A3, bacopaside II, and bacopasaponin C), is a type of triterpenoid saponin that plays a crucial role in producing its nootropic effects (Bansal et al., 2016). Among all the components of bacoside A, bacoside A3 is the most significant molecule (Goyal et al., 2018), and its detection serves as a bioindicator or marker, indicating and providing an idea of the quantification of bacosides in the extract. This compound has been extensively studied for its memory-enhancing effects (Maneeply et al., 2018).

B. monnieri is listed significantly under the National Medicinal Plants Board (NMPB) scheme and National Ayush Mission (NAM) due to its over-exploitation from its natural habitats and provided with a 30% subsidy to prioritize its conservation and cultivation (Gowthami et al., 2021). The estimated annual trade of *B. monnieri* is 1000 tonnes (Sanyal et al., 2022). Additionally, the time for *B. monnieri* harvest in traditional soil cultivation is typically three to four months (Maneeply et al., 2018), with seasonal variations in plant quality and active bacoside content.

To meet the growing demand, traditional farming cannot be viable; hence, there is a necessity to investigate and develop optimal cultivation techniques that can effectively maximize the yield and concentration of bioactive constituents in *B. monnieri*. A study by Aggarwal & Mathur (2020) proposes hydroponic culture as a sustainable alternative for the scalable cultivation of *B. monnieri*. Kaur et al. (2022) reported that hydroponically produced *B. monnieri* had a 7.5% higher bacoside-A concentration than soil-cultured. Similarly, Maneeply et al. (2018) reported that hydroponically grown *B. monnieri* had much higher accumulations of all active components of bacoside A per plant than soil. In consideration of market requirements, several studies have identified

hydroponics as a better and more efficient technique of farming than conventional cultivation, as it can ensure higher biomass production per unit area with uniform yield and a high percentage of bioactive molecules (Pomoni et al., 2023). Thus, the cultivation of *B. monnieri* through hydroponics is a promising approach in achieving high-yielding and good-quality plants. This research set out to contribute to the advancement of hydroponic cultivation techniques for *B. monnieri* by identifying the most suitable nutrient treatment and light conditions for optimal growth and yield.

MATERIALS AND METHODS

The present investigation was conducted at an indoor hydroponics facility developed in 2016 by the Higronics Division, HiMedia Laboratories Private Limited, Thane. The location of the study was at an altitude of 31 meters above mean sea level, situated at approximately 19° 05' 36" N latitude and 72° 54' 42" E longitude. If not stated otherwise, all necessary materials and instruments required for the experiment were obtained from Higronics, HiMedia. The bacoside A3 (≥ 95% purity) HPLC grade was sourced from Sigma-Aldrich (USA).

Plant materials and growing conditions

B. monnieri cuttings (6 cm long, having 6-8 leaves and 3-4 nodes) were taken from the two-month-old mother plants grown under soil culture. These cuttings were inserted into cocopeat plugs (40 mm). The cuttings were divided into four groups, and for the first three weeks, they were supplied with half-strength nutrient solutions of four different nutrient formulations (pH: 5.8±0.2 and EC: 0.5-0.8) to facilitate proper root development and anchorage. Optimal conditions for the saplings were maintained, including a 16-hour photoperiod and a temperature of 25±2°C. After three weeks, the developed cuttings were transferred to net pots and placed in an NFT hydroponic system for an additional four weeks.

Experimental design and nutrient formulations

The experimental rack setup consisted of two distinct racks: rack 1 and rack 2, equipped with multi-spectrum LED lights (ML) and white LED lights (WL), respectively (Fig. 1). Each rack consisted of two layers, which were further divided into four zones each. The NFT channels (UPVC food-grade plastic) in the racks were designed with dimensions of 1000 mm x 118 mm x 56 mm. Each channel had 5 pre-punched cavities for net pots and a planting space of 15 cm between them. Each of these zones containing 25 plants were supplied with four different nutrient formulations *viz*., Hoagland & Arnon (1938) (control) (Wu et al., 2015), GL-L™ (HiMedia-Higronics commercial nutrient mixture), Higro-Super™ (HiMedia-Higronics commercial nutrient mixture), and Hoagland & Arnon (1950) (Purohit, 2020), under two different light conditions: neutral WL (380-780 nm) and ML- red (600 to 700 nm) and blue (400 to 500 nm) lights.

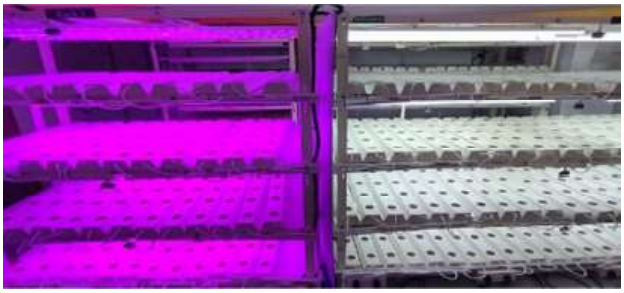


Fig. 1 : Racks 1 (left side rack) and 2 (right side rack) equipped with ML and WL, respectively

The experiment in the NFT system was conducted for four weeks with a total of 8 different treatments *viz*., Hoagland & Arnon (1938) + ML, GLL+ ML, Higro-Super + ML, Hoagland & Arnon (1950)+ML, Hoagland & Arnon (1938)+WL, GLL+WL, Higro-Super + WL and Hoagland & Arnon (1950) +WL. The pH of the nutrient solution was continuously monitored and adjusted within a target range of 6.0±0.10 while the EC of the nutrient solution was systematically increased from 1.0 mS/cm to 1.5 mS/cm (Maneeply et al., 2018).

After harvest, the plant growth traits, such as shoot length (cm) and number of shoots per plant, chlorophyll content using a Nunes handheld chlorophyll meter (VT-B), and fresh weight and dry weight per plant, were measured for 5 plants per treatment using 2 biological and 5 technical replicates. Furthermore, the plant samples were oven-dried at 50°C for 15 hours (Silpa et al., 2019) and powdered. For bacoside A3 content estimation, 0.1 g of dried plant powder was dissolved in 3 mL of methanol for 1 h at room temperature. The methanolic extracts were sonicated for 15 min, and the supernatant was kept at 4°C in the dark. This extraction step was repeated thrice, and the final volume of the extracted solution was adjusted to 10 mL and filtered using a nylon

syringe filter (0.45 µm). The extracted solution was used to analyze bacoside A3 contents by HPLC. Measurement of bacoside A3 was performed using an HPLC system (Agilent, 1200 series) comprising a solvent delivery module (LC-10ADVP) and a column oven (CTO-10AVP). The HPLC column used was a Zorbax Extend C18, 4.6x250x5. The mobile phase was a mixture of 0.71% anhydrous sodium sulphate (pH 2.3 with H_2SO_4): acetonitrile (685:315) at a flow rate of 1 mL/min. The injection volume was 20 µl using a UV-Vis detector (SPD-10AVP) with a wavelength set at 205 nm (Maneeply et al., 2018). The content of bacoside A3 in each treatment was determined using the following formula.

% Bacoside A3 content = (A_T/A_S) X (C_S/ C_T) X 100

Where, A_T is the peak area of bacoside A3 from the sample solution, A_S is the peak area from the standard solution, C_S is the concentration of bacoside from the standard solution, and C_T is the concentration of bacoside A3 from the sample solution

Statistical analysis of the data was performed using IBM SPSS software, version 29.0.1.0 (171). One-way analysis of variance (ANOVA) was conducted in a complete randomized design at 0.05 ($p<0.05$), followed by Duncan's new multiple range test (DMRT) at a significance level of 0.05 ($p<0.05$) to compare the means of the different treatments.

RESULTS AND DISCUSSION

B. monnieri saplings developed by cuttings were grown in NFT hydroponics systems for four weeks using four different nutrient formulations under two types of light conditions (Fig. 2, 3, & 4). The plants were analyzed based on the growth traits and according to bacoside A3 content, and the best treatment was identified.

Fig. 2 : 4-week-old *B. monnieri* plants in NFT systems in rack 1 & 2, respectively

Fig. 3 : 4-week-old *B. monnieri* plants under ML subjected to different nutrient treatments
a. Hoagland & Arnon (1938), b. GLL, c. Higro-Super, d. Hoagland & Arnon (1950)

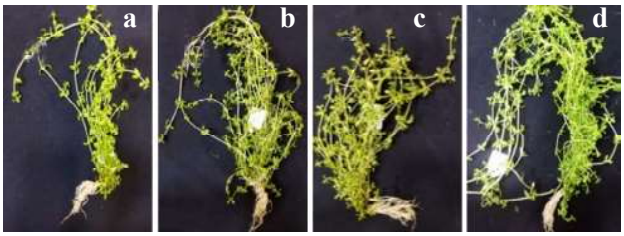


Fig. 4 : 4-week-old *B. monnieri* plants under WL subjected to different nutrient treatments
a. Hoagland & Arnon (1938), b. GLL, c. Higro-Super, d. Hoagland & Arnon (1950)

Table 1 : DMRT test output for the shoot length, shoot number, leaf number, chlorophyll content, fresh weight, and dry weight of *B. monnieri* plants grown under ML and WL subjected to different nutrient treatments

Treatment	Shoot length (cm)	No. of shoots/plant	No. of leaves/plant	Leaf area (cm^2)	Chlorophyll (SPAD)	Fresh weight (g/plant)	Dry weight (g/plant)
Multi-spectrum LED light (ML)							
Hoagland & Arnon (1938)	35.6 ± 5.3^{a}	24.0 ± 2.0^{a}	347.8 ± 26.6^{ab}	0.7 ± 0.01^{a}	44.4 ± 2.5^{b}	12.1 ± 3.9^{a}	1.1 ± 0.13^{a}
GLL	46.5 ± 0.7^{bc}	51.2 ± 3.6^{c}	518.0 ± 21.2^{d}	1.28 ± 0.04^{d}	45.9 ± 1.3^{b}	28.8 ± 5.7^{ab}	2.2 ± 0. 26^{b}
Higro-Super	46.2 ± 2.4^{bc}	38.6 ± 9.0^{abc}	484.4 ± 58.0^{cd}	1.1 ± 0.01^{c}	42.8 ± 1.1^{b}	16.6 ± 0.8^{a}	1.6 ± 0.29^{ab}
Hoagland & Arnon (1950)	39.1 ± 2.9^{ab}	30.3 ± 1.3^{ab}	352.4 ± 3.2^{ab}	0.1 ± 0.01 ^{b}	44.6 ± 2.3^{b}	13.4 ± 1.3^{a}	1.1 ± 0. 09^{a}
White LED light (WL)							
Hoagland & Arnon (1938)	52.1 ± 1.8^{c}	45.5 ± 6.9^{bc}	411.6 ± 52.4^{abcd}	1.27 ± 0.01^{d}	40.8 ± 1.3^{ab}	18.9± 1.8^{ab}	1.8^{a} ± 0.15^{b}
GLL	41.1 ± 3.5^{ab}	29.0 ± 4.0^{ab}	416.6 ± 46.6^{bcd}	0.9 ± 0.04^{b}	46.8 ± 2.0^{b}	13.7± 4.7^{a}	1.3 ± 0.24^{a}
Higro-Super	40.9 ± 2.4^{ab}	29.2 ± 0.4^{ab}	282.1 ± 26.3^{a}	0.8 ± 0.03^{a}	43.2 ± 2.7^{b}	12.0± 1.7^{a}	1.4 ± 0.01^{a}
Hoagland & Arnon (1950)	37.0 ± 1.4^{ab}	27.4 ± 6.4^{a}	380.4 ± 31.6^{abc}	1.1 ± 0.02^{c}	35.3 ± 1.2^{a}	9.4± 1.6^{a}	1.1 ± 0.18^{a}

Values are represented as means of 2 biological and 5 technical replicates ± standard error. Different letters in the same column indicate significant differences ($p<0.05$)

Plant growth traits

The phenotypic measurements of *B. monnieri* plants exhibited significant variations among the different treatments. Analysis of variance (ANOVA) revealed significant differences ($p<0.05$) in all growth traits. Duncan's new multiple range test (DMRT) was employed to compare significant differences ($p<0.05$) among treatment means, and the highest and lowest performing plants within treatments were determined (Table 1).

The highest mean shoot length was observed in *B. monnieri* plants grown with Hoagland & Arnon (1938) +WL (52.1±1.8 cm), followed by GLL+ML (46.5±0.7 cm) and Higro-Super+ML (46.2±2.4 cm). Conversely, the lowest mean shoot length was observed in *B. monnieri* plants subjected to Hoagland & Arnon (1950) +ML treatment (35.6 ± 5.3 cm). These findings are consistent with Karataş et al. (2016) who reported that R: B LEDs were more conducive to longer shoot growth compared to white LEDs. In terms of shoot number, the highest mean value was observed in GLL+ML (51.2±3.6), followed by Hoagland & Arnon (1938) +WL (45.5±6.9) and Higro-Super+ML (38.6±9), while, lowest was displayed in Hoagland & Arnon (1938) +ML (24±2).

Plants grown in GLL+ML showcased highest mean leaf value (518±21.2) followed by Higro-Super+ML (484.4±58) and GLL+WL (416.6±46.6), whereas, Higro-Super+WL displayed the lowest mean leaf number (282.1±26.3), followed by Hoagland & Arnon (1938)+ML (347.8±26.6) and Hoagland & Arnon (1950)+ML (352.4±3.2). The analysis of leaf area in *B. monnieri* plants revealed that the GLL+ML displayed the highest mean leaf area (1.28±0.04 cm^2), followed by Hoagland & Arnon (1938) +WL (1.27±0.01 cm^2) and Hoagland & Arnon (1950) +WL (1.1±0.02 cm^2), however, Hoagland & Arnon (1938) +ML exhibited the lowest mean leaf area (0.7±0.01 cm^2). The chlorophyll content of *B. monnieri* plants revealed that GLL+WL treatment exhibited the highest mean chlorophyll content (46.8 ± 2.0), which was followed by GLL+ML (45.9±1.3) and Hoagland & Arnon (1950) +ML (44.6±2.3), while, Hoagland group under WL displayed the lowest mean chlorophyll content (35.3±1.2).

The GLL+ML treatment exhibited the highest mean biomass, measuring 28.8±5.7 g, followed by Hoagland & Arnon (1938) + WL (18.9±1.85 g) and Higro-Super+ML (16.6±0.82 g), however, Hoagland group under WL displayed the lowest mean fresh weight (9.4±1.6 g). Comparing similar nutrient treatments under two different light conditions, we find that the ML is performing better than WL. The GLL nutrient mixture is low in N, P, and K (180 ppm, 30 ppm, 180 ppm, respectively) as compared to the other three nutrient formulations used. Nevertheless, it contained balanced minor nutrients such as Fe (2.0 ppm), Mn (1.5 ppm), Cu (0.05 ppm), and Zn (0.07 ppm), which contribute to higher photosynthesis, leading to more carbon conversion and contributing to fresh weight. Several studies have shown that a mixture of wavelengths of two ranges of lights *viz*., red (600-700 nm) and blue (400-500 nm) light enhances the plants' biomass and dry mass content of various vegetables (Su et al., 2025). The inconsistent performance of Hoagland & Arnon (1938) treatments under different light conditions highlights the complex interaction between nutrient availability and light spectrum. Significantly, the Hoagland & Arnon (1938) treatments showed contradictory results, with better plant growth traits performance observed under WL compared to ML. Hoagland & Arnon (1938) showed inferior growth trait values under ML as compared to the other three treatments. A study on the phytoremediation capability of *Bacopa* demonstrated that red and blue light are more effective than white LED light, resulting in a higher bio-concentration factor (BCF) for arsenic (As) and mercury (Hg) heavy metals (Dogan & Ugur, 2024).

The highest dry weight was observed in GLL+ML treatment at 2.16±0.3 g, which was followed by Hoagland & Arnon (1938) +WL (1.8±0.15 g) and Higro-Super+ML (1.6±0.3 g), while, lowest was observed in Hoagland & Arnon (1950) + WL (1.09 ± 0.18 g). The GLL+ML showed highest number of shoots (51.2±3.6), number of leaves (518±21.2), leaf area (1.28±0.04 cm^2/plant), fresh (28.82±5.74 g/plant) and dry weight (2.16 ±0.25 g/plant). Overall, ML outperformed WL across all growth traits.

Bacoside A3 content

Bacoside A3 content was measured using HPLC (Table 2). The Higro-Super+ML treatment showed the highest bacoside A3 content at 3528 mg/kg with legitimate growth, followed by Hoagland & Arnon (1938) +ML at 3204 mg/kg (Fig. 5).

Table 2 : Bacoside A content in four-week-old *B. monnieri* grown in NFT hydroponic systems under different grow lights

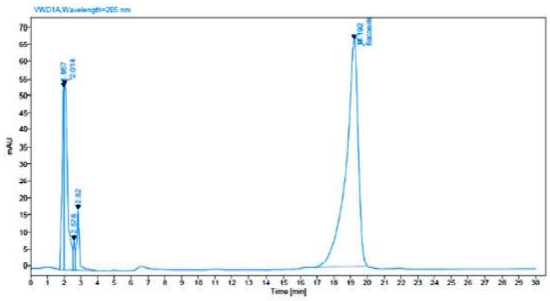
Treatment	Bacoside A3 content (mg/kg)* (% w/w dry weight)
Hoagland & Arnon (1938) + ML	3204 (0.3204)
GLL+ ML	351 (0.0351)
Higro-Super + ML	3528 (0.3528)
Hoagland & Arnon (1950) +ML	384 (0.0384)
Hoagland & Arnon (1938) + WL	740 (0.0740)
GLL+ WL	523 (0.0523)
Higro-Super+ WL	733 (0.0733)
Hoagland & Arnon (1950) +WL	590 (0.059)

*values are represented as means of 2 biological and 5 technical replicates

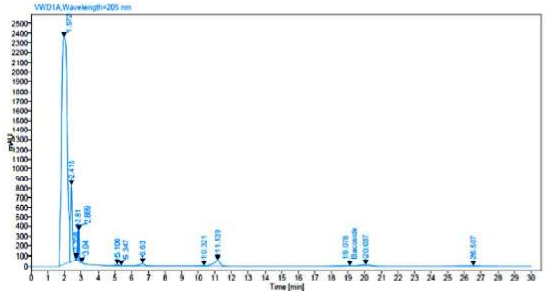
The Higro-super contains chelated forms of essential minor-nutrients (Fe, Mn, Zn, & Cu) and high concentrations of P (60 ppm), K (250 ppm), Cl (50 ppm) & B (1 ppm), which can be envisaged for the increased bacoside A3 content in Higro-Super+ML treatment. This finding agrees with a study that reported *Cyclocarya paliurus* plants in high-phosphorus and nitrogen treatments have significantly higher total triterpenoid contents (Xiaofang et al., 2024). Potassium and phosphorus are involved in several physiological and biochemical processes, where they play a crucial role in activating various enzymes that are important for critical metabolic processes (Naciri et al., 2022). Another study suggested that minor nutrients enhanced the availability of primary metabolites that impacted secondary metabolite production in *Cassia angustifolia* (Verma & Shukla, 2015). Some studies indicate that in the hydroponic system, NaCl at 50 mM and 100 mM concentrations stimulates the synthesis and accumulation of total flavonoid and polyphenol compounds (Eoh et al., 2024).

Apart from nutrients, environmental factors also significantly influence plant growth in hydroponic systems (Kumar & Saini, 2020). Factors such as temperature, drought, salinity and light intensity can impact both the quantitative and qualitative composition of saponins (Kundu et al., 2025). The bacosides are triterpenoid saponins that are synthesized by two independent and interlinked biosynthetic pathways: the mevalonic acid (MVA) pathway in the cytosol and mitochondria and the methylerythritol phosphate (MEP) pathway in plastids. The MEP pathway is stimulated by light (Noushahi et al., 2022). Red (600-700 nm) and blue light (400-500 nm) are found to be effective regulators of terpenoid biosynthesis (Zhang et al., 2021). A recent study revealed that green light enhanced the yield of bacosides by 1.5 to 2.8 times compared to white LED light. Additionally, it emphasized the influence of green LED light on bacoside content and gene expression in both diploid and tetraploid plants, underscoring the intricate interplay between genetic factors and light quality (Inthima & Supaibulwatana, 2025).

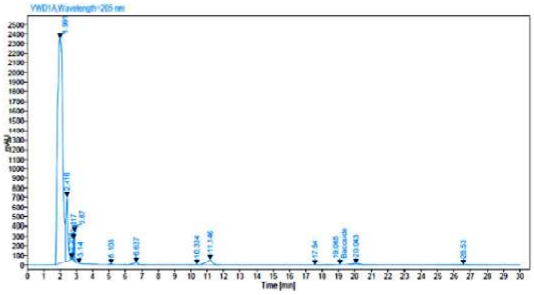
The ML, in combination with Higro-Super and Hoagland Arnon (1938), surpasses WL, in combination with all other treatments based on bacoside A3 content. These findings support the studies conducted on bacoside accumulation in *in vitro* culture of *B. monnieri*. They observed that blue and red light significantly increased the levels of triterpenoid saponin glycosides compared to white light, with a 1.7-fold and 1.5-fold increase, respectively. Among the different LED lights tested, blue light was found to be the most effective in promoting the accumulation of bioactive compounds (Watcharatanon et al., 2019). These findings showed that LED light exposure can boost the production of bioactive substances in *B. monnieri in vitro* cultures. The GLL+ML and Hoagland+Arnon (1950) +ML illustrated the lowest bacoside A3 content at 351 mg/kg and 384 mg/kg, respectively. This output can be envisaged to lower nutrient levels and external stress conditions compared to Higro-Super and Hoagland & Arnon (1938), which contain high levels of nutrient concentrations. These findings provide useful information on the advancement of increasing the bacoside A3 content in *B. monnieri*.



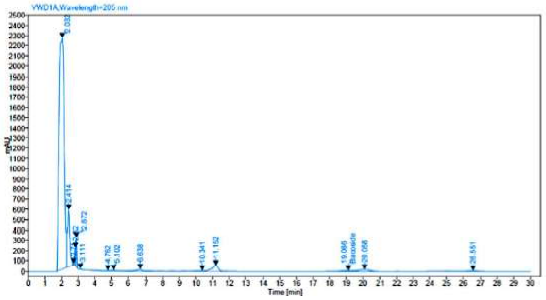
Standard bacoside A3



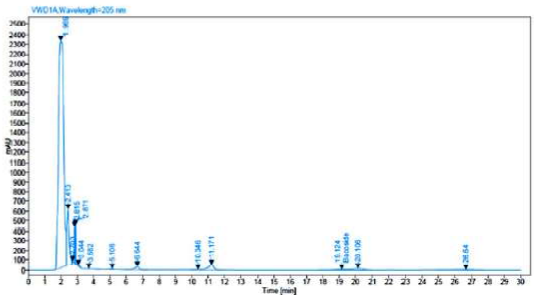
Hoagland & Arnon (1950) +ML



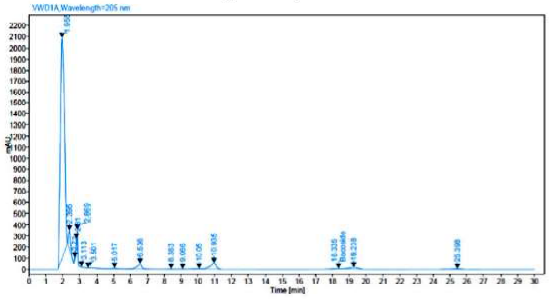
GLL+ML



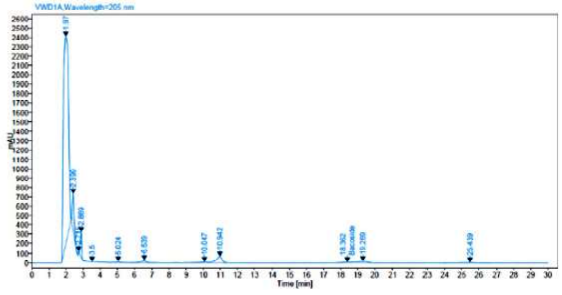
Higro-Super +ML



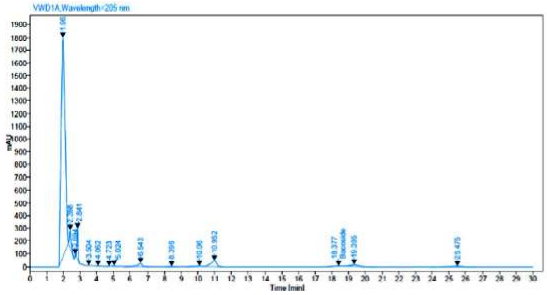
Hoagland & Arnon (1938) +ML



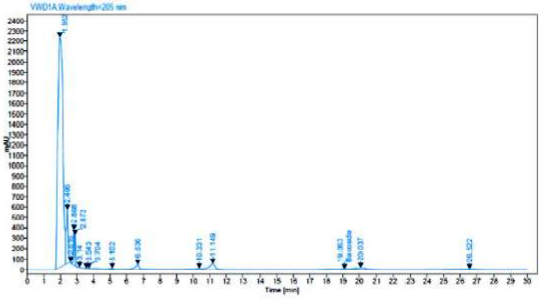
Hoagland & Arnon (1950) +WL



GLL+WL



Higro-Super +WL



Hoagland & Arnon (1938) +WL

Fig. 5 : HPLC chromatograms of the authentic bacoside A3 standard and *B. monnieri* extracts from 4-week-old plants under all 8 different treatments.

CONCLUSION

The growth and development of *B. monnieri* plants were investigated through the evaluation of various growth traits parameters and bacoside A3 content to understand their response to different nutrient treatments under ML and WL. In terms of plant growth traits across multiple parameters, the GLL and Hoagland & Arnon (1938) treatments under ML exhibited the highest values across four key parameters, namely shoot number, leaf number, fresh weight, and dry weight. However, the HPLC results showed the highest bacoside A3 content in Higro-Super+ML, which can be attributed to their high nutrient content. Since bacoside A3 content is a potential active metabolite in *B. monnieri*, and Higro-Super+ML showed satisfactory results in terms of plant growth parameters and high bacoside content. These findings indicate that the Higro-Super nutrient treatment, when combined with ML, resulted in favourable growth and development of *B. monnieri* plants, as evidenced by the significant improvements in these important growth indicators. Furthermore, when considering the overall growth across all parameters, ML emerged as the more favorable light condition compared to WL. Future studies could delve deeper into unraveling the intricate interplay between light intensity, spectrum, and nutrient treatments to gain a comprehensive understanding of their combined effects on *B. monnieri* growth and productivity.

ACKNOWLEDGEMENT

This work was supported by the Indo-German Science and Technology Centre for the 2+2 project -AutoNutri under grant number IGSTC/Call 2019/AutoNutri.

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(Received : 16.8.2024; Revised : 17.6.2025; Accepted : 21.6.2025)