# Growth of Brahmi (*Bacopa monnieri* (L.) Wettst.) by NFT and DFT hydroponic systems and their accumulation of saponin bacosides

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## ABSTRACT

Brahmi or *Bacopa monnieri* (L.) Wettst. has been used as a nootropic herb in Ayurvedic medicine for thousands of years. This plant can boost memory retention and protect against depression and Alzheimer's disease. The influence of NFT (Nutrient Film Technique) and DFT (Deep Flow Technique) hydroponic systems on growth of Brahmi and production of bacosides was investigated. Brahmi shoots were cultured in NFT and DFT hydroponic soilless systems for six weeks. NFT hydroponics grows plants using a very shallow recirculating nutrient solution, while DFT hydroponics employs a deep recirculating nutrient solution. Results showed that Brahmi biomass increased under hydroponic culture. The DFT technique stimulated vegetative growth of Brahmi more than the NFT technique, with the former generating higher dry weight, shoot number, leaf number and total leaf area. Highest dry weight of Brahmi plants grown under DFT hydroponics was 8.48±0.44 g/plant, 10.1 times higher compared to soil culture (control). Bioactive compounds in bacoside A consisting of bacoside A3, bacopaside II, bacoside X and bacopasaponin C were measured by high performance liquid chromatography (HPLC). High levels of bacoside A3, bacopaside II and bacoside X were observed in Brahmi grown in soil culture rather than by hydroponics, and Bacopasaponin C content (1.45±0.09% w/w dry wt) was significantly higher than control (0.81±0.05% w/w dry wt). Interestingly, accumulations of all active compounds of bacoside A per plant for Brahmi grown under hydroponics were significantly higher ($p$<0.01) than by soil culture. Findings indicated hydroponic cultures as an optimal alternative for Brahmi biomass production. Hydroponics gave a high-quality yield of Brahmi, high accumulation of bioactive compounds with fast crop production rate.

*Keywords: Brahmi, hydroponics, saponins, HPLC*

## INTRODUCTION

Brahmi (*Bacopa monnieri* (L.) Wettst.) is a succulent perennial medicinal plant belonging to the family Scrophulariaceae and widely found in wet soil and muddy shores of India, Australia, the United States and East Asia (Rai et al., 2017).

Brahmi is an important herb in Ayurvedic medicine, used for improving intellect and memory, with reported benefits against inflammatory diseases including asthma, bronchitis and rheumatism (Channa et al., 2006; Rao et al., 2012). Biologically active compounds in Brahmi include alkaloids, bacosides, flavonoids, glycosides, triterpenoids and saponins (Sivaramakrishna et al., 2005). Bacoside A, a primarily component of Brahmi extract, consists of four types of saponin compounds as bacoside A3, bacopaside II, bacoside X and bacopasaponin C which have been shown to improve memory, act as an antidepressant and protect neurons (Ramawat and Merillon, 2008). They have also been reported to ameliorate the effects of inflammatory diseases including asthma, bronchitis and rheumatism (Rao et al., 2012; Channa et al., 2006). Pharmacological effects of the Brahmi plant have generated high material demand as a herbal medicine and food supplement. Thus, collection of Brahmi plants in large numbers from wild habitats might result in overexploitation to the detriment of wild sources. Generally, conventional culture of Brahmi takes three to four months before harvesting and plant quality varies between different seasons. Moreover, Brahmi plant materials may be contaminated by chemical agents from fields or agricultural wastes washed down to wetlands. Thus, conventional culture involves many problems which can result in low quantity of raw Brahmi plant materials. To increase quantity and improve the quality of Brahmi production, diverse techniques such as tissue culture to enhance Brahmi biomass (Kachonpadungkitti and Jala, 2014; Kumari et al., 2015) and cell culture to stimulate bacoside accumulation (Rahman et al., 2002) have been investigated; however, no reports are available regarding the application of hydroponics for Brahmi cultivation. Hydroponics is a method for growing plant material without soil using mineral nutrients dissolved in water. Roots are submerged in nutrient solution and directly uptake the essential minerals. Hydroponic culture is widely used to increase the production of a variety of plants, especially economic plants such as lettuce (*Lettuce sativa*) (Park and Kurata, 2009), strawberry (Caruso et al., 2011), tomato (Roosta et al., 2011) and melon (Asao et al., 2013). Nowadays, hydroponic culture has become popular because the yield is greater than soil culture with high quality and less insect and pathogen infection. Here, the advantages of hydroponics were utilized to increase production of Brahmi and compare growth and bioactive compound accumulation under nutrient film technique (NFT) and deep flow technique (DFT) hydroponic systems.

## METHODOLOGY

### 1) Plant materials

Shoots of Brahmi (*Bacopa monnieri* (L.) Wettst.) (8 cm long, bearing 6 leaves) from one-month-old Brahmi plants grown under soil culture were cut and transferred into foam cubes in a net pot for acclimation and then cultured under DFT hydroponics for two weeks. Nutrient used was half-strength Hoagland's solution.

### 2) Comparison of growth and saponin contents of Brahmi cultured under nutrient film technique (NFT) and deep flow technique (DFT) hydroponic systems

Research was conducted from March to April 2017. Acclimated shoots were cultured in NFT and DFT hydroponic systems using half-strength Hoagland's solution (Hoagland and Arnon, 1950), with electrical conductivity (EC) 1.5 μS/cm, pH 5.8±0.2 and average air temperature 36±3 °C. The size of hydroponic system was 120 cm wide, 130 cm long and 130 cm high. The growing bed tray was made of uPVC (unplasticized polyvinyl chloride) in 10 cm wide, 120 cm long and 130 cm high. The length between individual Brahmi plant was 10 cm. For NFT hydroponics, a half-strength Hoagland's solution was pumped through the system at 1.0 L/min with solution depth at 1 to 3 mm. For DFT hydroponics, half-strength Hoagland's solution was pumped through the system at 1.5 L/min with solution depth at 4 cm. Conventional soil culture was used as control containing Brahmi shoots grown in wet soil with 6 cm water depth. The culture box of control was made of cement, about 40 cm high and 80 cm diameter. The experiment was conducted for six weeks, with the half-strength Hoagland's solution renewed in the hydroponic systems at the fourth week of culture. There were ten replicates for each experiment. Fresh weight, dry weight, plant height, number of shoots per plant and leaf area per plant were measured every two weeks. For dry weight, each Brahmi plant was dried in a hot air oven at 40 °C for 2 to 7 days or until constant weight. Total leaf area was measured using a leaf area meter LI 3100 (Licor, USA).

**3) Analysis of bacoside A contents by high performance liquid chromatography (HPLC)**

Shoots of six- week- old Brahmi plants grown under NFT and DFT hydroponics were cut and dried at 40 °C for 48 hours and ground into a fine powder. Then, 0.1 g of powder was dissolved by 3 mL of methanol for 1 hour at room temperature. Extracts were sonicated for 15 min and the supernatant was kept at 4 °C in the dark. The extraction step was repeated 3 times before the extracted solution was adjusted to 10 mL and filtrated using a nylon syringe filter (0.45 μm). The extracted solution was used for bacoside A measurement by HPLC (Phrompittayarat et al., 2011; Mishra et al., 2013). Measurement of bacoside A was performed using a Shimadzu HPLC system (Shimadzu, Japan) comprising a solvent delivery module (LC-10ADVP) and column oven (CTO-10AVP). The HPLC column used was a LiChroCART® 250-4.6 with 5 μm Purospher® Star RP-18 endcapped (Merck, Germany). The mobile phase was a mixture of 0.2% phosphoric acid: acetonitrile (65:35) at a flow rate of 1 mL/min. Injection volume was 20 μl using a UV-Vis detector (SPD-10AVP) with wavelength set at 205 nm. Bacoside A standard, as a mixture of bacoside A3, bacopaside X, bacopaside II and bacopasaponin C (≥95% purity) HPLC grade was obtained from Sigma-Aldrich (USA).

**4) Data analysis**

All data were analyzed by one-way analysis of variance (ANOVA) and treatment means were compared by Duncan's new multiple range test (DMRT) at the 0.05 and 0.01 probability level ($p<0.05$ and $p<0.01$).

## RESULTS AND DISCUSSION

### 1) Plant growth

Acclimated Brahmi shoots were cultured by NFT and DFT hydroponics for six weeks using half strength Hoagland's solution with growth measured as fresh weight and dry weight. Results showed that both NFT and DFT hydroponics triggered growth of Brahmi plants with a progressive increase in biomass during the growth period. NFT and DFT hydroponics produced dry weight of 6-week-old Brahmi plants significantly greater than the control culture at 8.5 and 10.1 folds respectively. DFT hydroponics showed greater potential for Brahmi production compared to NFT with significantly higher fresh weight (106.01±2.98 g/plant) and dry weight (8.48±0.44 g/plant) (**Table 1**).

Nutrients play a key role in the growth and development of plants. Hydroponics is a method of growing plants in soilless media by providing all essential nutrients the plant needs in a water solvent with optimal pH level for nutrient availability (Raviv and Lieth, 2008). The nutrient solution flows continuously throughout the crop. Thus, Brahmi plants grown in hydroponics can uptake nutrients more efficiently than plants grown in soil. Hydroponic cultures enhanced the growth of lettuce (*Lactuca sativa* L.) at twice the rate of traditional culture (Domingues et al., 2012). Awad et al. (2017) reported that a hydroponic system with rice biochar and perlite increased growth of leafy vegetables including cabbage, dill, mallow, red lettuce and tatsoi.

**Table 1.** Fresh and dry weights of Brahmi grown under NFT and DFT hydroponics.

| Sample | No. of weeks | Control | NFT | DFT |
|---|---|---|---|---|
| Fresh weight (g/plant) | 2 | $1.16\pm0.57^{b}$ | $4.58\pm0.24^{a}$ | $4.89\pm0.20^{a}$ |
| | 4 | $3.60\pm0.29^{c}$ | $31.79\pm1.50^{b}$ | $40.77\pm1.55^{a}$ |
| | 6 | $14.64\pm0.64^{c}$ | $69.18\pm3.38^{b}$ | $106.01\pm2.98^{a}$ |
| Dry weight (g/plant) | 2 | $0.07\pm0.01^{b}$ | $0.30\pm0.01^{a}$ | $0.28\pm0.01^{a}$ |
| | 4 | $0.21\pm0.01^{b}$ | $2.26\pm0.14^{a}$ | $2.42\pm0.10^{a}$ |
| | 6 | $0.84\pm0.03^{c}$ | $7.14\pm0.57^{b}$ | $8.48\pm0.44^{a}$ |

Note: Data are means±SE. Different letters in the same row indicate significant differences ($p<0.05$).

Here, DFT hydroponic provided a flow of nutrient solution at 4 cm depth with 1.5 L/min flow rate while the NFT system had 1 to 3 mm solution depth with 1.0 L/min flow rate. Thus, Brahmi plants were more efficient in essential nutrients uptake, resulting in increased development of the upper parts and root system. Root growth is a vital factor that controls mineral uptake of plants. As shown in **Figure 1**, quality of adventitious roots of Brahmi plants grown in DFT hydroponic was better than those in the NFT system. Plants grown in DFT hydroponic can encounter low levels of oxygen dissolved in the nutrient solution (Tongaram, 2007), hampering root cell respiration and leading to growth reduction. However, this problem was not observed in our experiment because Brahmi (*Bacopa monnieri* (L.) Wettst.) is an

aquatic plant which adapts to live in water by producing a honeycomb of aerenchyma with large cortical lacunae in the roots and stem (Anju et al., 2017). The aerenchyma supplies sufficient oxygen for cellular respiration in the roots and improves root growth.


**Figure 1.** Morphology of six-week-old Brahmi roots cultured in NFT (left) and DFT (right) hydroponic systems.

Reduction of growth in Brahmi plants cultured under NFT hydroponics might result from heat stress response because temperature of the nutrient solution was usually higher than DFT hydroponics (data not shown). Heat stress becomes a more serious problem in tropical regions (Tancho, 2005). Temperature is a key environmental factor that directly influences absorption of nutrients in solution near the roots (Raviv and Lieth, 2008) and this directly affects yield and quality (Costa et al., 2011). Masaru et al. (2016) revealed that high root-zone temperature reduced strawberry growth, whereas low root-zone temperature led to higher biomass production. High temperature also has a negative effect on membrane structure, enzyme activity and protein synthesis which eventually affects photosynthesis of the whole plant (Nxawe et al., 2010). Mohammed and Tarpley (2011) reported that high temperature reduced the productivity of rice (*Oryza sativa*) while Pramanik et al. (2000) indicated that high temperature retarded root growth and dry matter of cucumber in hydroponic culture.

Our results indicated that both DFT and NFT hydroponic cultures promoted growth of Brahmi by enhancing axillary shoot proliferation (**Figure 2**). Highest number of axillary shoots (63.20 ± 5.11 shoots/plant), leaf number (1,376.20±24.88 leaves/plant) and leaf area (1146.17±48.58 $cm^2$/plant) were observed on Brahmi

grown in DFT hydroponic (**Table 2**). At the end of the experimental period, leaf number and leaf area of Brahmi plants grown under the DFT system were 8.6 and

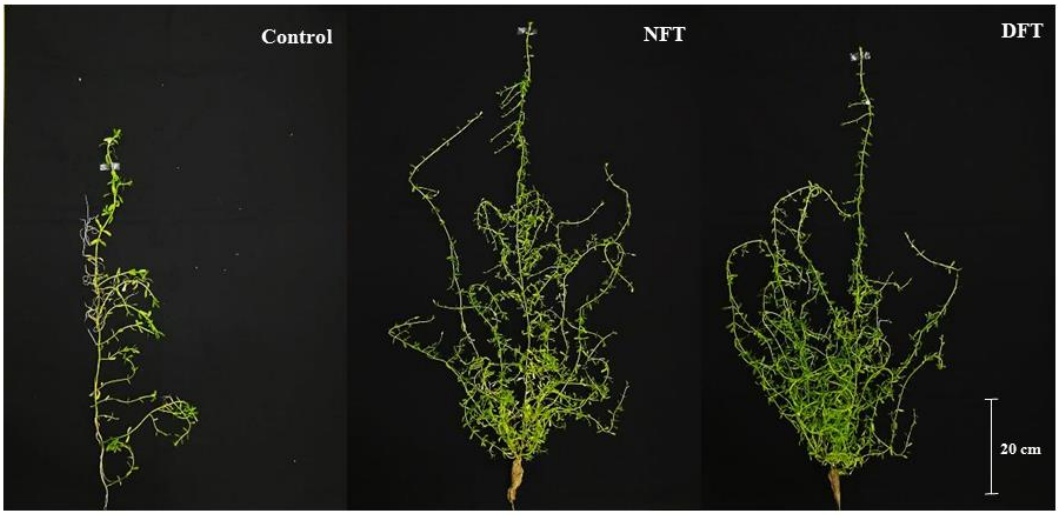


**Figure 2.** Six-week-old Brahmi plants grown in soil culture (control), NFT and DFT hydroponic systems.

**Table 2.** Plant height, shoot number, leaf number and leaf area of Brahmi plants grown under NFT and DFT hydroponic systems.

| Sample | No. of weeks | Control | NFT | DFT |
|---|---|---|---|---|
| Plant height (cm) | 2 | 14.73±0.54$^{b}$ | 24.46±1.03$^{a}$ | 24.00±0.76$^{a}$ |
| | 4 | 35.23±1.15$^{b}$ | 63.75±1.51$^{a}$ | 62.82±2.24$^{a}$ |
| | 6 | 87.00±2.04$^{b}$ | 99.60±1.93$^{a}$ | 95.60±2.81$^{a}$ |
| Axillary shoot number (shoots/plant) | 2 | 3.77±0.53$^{b}$ | 16.92±0.44$^{a}$ | 16.46±0.47$^{a}$ |
| | 4 | 10.31±0.71$^{c}$ | 34.08±1.00$^{b}$ | 40.00±0.92$^{a}$ |
| | 6 | 21.60±0.83$^{b}$ | 53.40±3.85$^{a}$ | 63.20±5.11$^{a}$ |
| Leaf number (leaves/plant) | 2 | 26.50±1.28$^{c}$ | 131.17±3.87$^{a}$ | 118.00±3.94$^{b}$ |
| | 4 | 86.60±5.26$^{b}$ | 482.00±6.15$^{a}$ | 509.00±15.16$^{a}$ |
| | 6 | 127.30±8.83$^{c}$ | 958.60±45.23$^{b}$ | 1,376.20±24.88$^{a}$ |
| Leaf area ($cm^2$/plant) | 2 | 20.88±2.95$^{b}$ | 71.28±6.38$^{a}$ | 66.35±5.32$^{a}$ |
| | 4 | 59.17±4.92$^{c}$ | 258.61±16.25$^{b}$ | 344.33±20.61$^{a}$ |
| | 6 | 132.66±14.47$^{c}$ | 670.72±57.13$^{b}$ | 1,146.17±48.58$^{a}$ |

Note: Data are means±SE. Different letters in the same row indicate significant differences ($p$<0.05).

10.8 times greater than control. Leaf sizes of Brahmi plants from DFT and NFT cultures were smaller than those of control (data not shown). Brahmi plants grown in both NFT and DFT hydroponics produced high numbers of auxiliary shoots. Phrompittayarat et al. (2011) reported that upper plant parts had high levels of total saponin. Thus, hydroponic culture enhanced the biomass of Brahmi by producing high numbers of axillary shoots.

**2) Bacoside A production in Brahmi cultured under hydroponic systems**

After six-week-hydroponic cultivations, quantities of bacoside A (bacoside A3, bacopaside II, bacopaside X and bacopasaponin C) in Brahmi were measured by HPLC and presented in **Table 3**. Highest levels of bacoside A3, bacopaside II and bacopaside X were detected in Brahmi grown in soil culture (control) at 0.75±0.04, 1.27±0.04 and 0.36±0.03% w/w dry weight, respectively, while bacopasaponin C was observed at high levels at 1.45±0.09 and 1.21±0.03 % w/w dry weight, in Brahmi plants grown by NFT and DFT hydroponics respectively. Bacosides, as secondary metabolites, are synthesized in greater quantities when plants are under stress conditions including abiotic and biotic stresses (Gupta et al., 2017). Brahmi plants grown in soil culture (control) were more exposed to stress environments such as osmotic stress, thermal stress and pathogen infection (Raviv and Lieth, 2008). Stresses are important factors that can alter physiological processes and increase secondary metabolites which are required for plant response and plant defense (Zhao et al., 2005). Recently, Gupta et al. (2017) reported that biotic stress induced by a microbe infection enhanced defense as enzyme activity, phenolic compounds, and bacoside A production in Brahmi, while Autaijamsripon et al. (2017) revealed that shoots of Brahmi grown in wild habitats had higher levels of total phenolic and flavonoid compounds than those grown in tissue culture. Interestingly, contents of all active compounds of bacoside A per plant grown in NFT and DFT hydroponics were significantly greater ($p$<0.01) than in soil culture. As shown in **Table 4**, highest amounts of bacoside $A_3$ and bacopaside II in Brahmi from DFT were 30.51±1.59 and 52.54±2.74 mg/plant at approximately 5 folds greater than control. Bacopasaponin C was observed at high levels in Brahmi plants grown by NFT and DFT hydroponics (**Table 3**). A very high accumulation in one Brahmi plant grown under NFT and DFT (**Table 4**) showed highest amount in DFT (102.55±5.35 mg/plant), significantly higher than control (6.83±0.27 mg/plant) by approximately 15 folds. This finding offers useful information for Brahmi application because bacopasaponin C is non-toxic and has been shown to improve memory retention while also possessing antileishmanial properties (Sinha et al., 2002). Therefore, bacopasaponin C might be considered for many aspects of medicinal application.

**Table 3.** Bacoside A contents in six-week-old Brahmi grown under NFT and DFT hydroponic systems.

| | Bacoside A contents (% w/w dry wt) | | | | |
|---|---|---|---|---|---|
| | Bacoside $A_3$ | Bacopaside II | Bacopaside X | Bacopasaponin C | Total Bacoside A |
| Control | 0.75 ± 0.04 [a] | 1.27 ± 0.04 [a] | 0.36 ± 0.03 [a] | 0.81 ± 0.05 [c] | 3.19 ± 0.05 [a] |
| NFT | 0.38 ± 0.01 [b] | 0.76 ± 0.02 [b] | 0.10 ± 0.01 [b] | 1.45 ± 0.09 [a] | 2.69 ± 0.08 [b] |
| DFT | 0.36 ±0.02 [b] | 0.62 ± 0.04 [c] | 0.11 ± 0.03 [b] | 1.21 ± 0.03 [b] | 2.30 ± 0.05 [c] |

Note: Data are means±SE. Different letters in the same column indicate significant differences ($p$<0.05).

**Table 4.** Bacoside A contents per plant accumulated in six-week-old Brahmi grown under NFT and DFT hydroponic systems.

| | Bacoside A contents (mg/plant) | | | | |
|---|---|---|---|---|---|
| | Bacoside $A_3$ | Bacopaside II | Bacopaside X | Bacopasaponin C | Total bacoside A |
| Control | 6.33±0.25 [b] | 10.71±0.42 [b] | 3.04±0.12 [c] | 6.83±0.27 [b] | 26.90±1.05 [b] |
| NFT | 27.13±2.19 [a] | 54.25±4.39 [a] | 7.14±0.58 [b] | 92.89±12.57 [a] | 181.41±17.54 [a] |
| DFT | 30.51±1.59 [a] | 52.54±2.74 [a] | 9.32±0.49 [a] | 102.55±5.35 [a] | 194.93±10.16 [a] |

Note: Data are means±SE. Different letters in the same column indicate significant differences ($p$<0.01).

## CONCLUSIONS AND SUGGESTIONS

Both NFT and DFT hydroponic systems strongly promoted growth of Brahmi giving high fresh weight, dry weight, plant height, number of shoots per plant and leaf area per plant. Growth of Brahmi plants under DFT hydroponic exceeded growth under NFT hydroponic, with increased potential for large scale biomass production. Three compounds of bacoside A (bacoside A3, bacopaside II and bacopaside X) were found at high levels in the control, while Bacopasaponin C was detected at a high level in Brahmi plants grown under NFT and DFT. In addition, accumulations of the four active compounds of bacoside A per plant cultured by DFT and NFT were greater than control. DFT hydroponic culture for six weeks was recommended as the most efficient method for large scale Brahmi biomass production with high yield of bacoside A contents, while hydroponic cultures supplemented with exogenous elicitors could be used to enhance accumulation of bacoside A.

## ACKNOWLEDGEMENT

The authors are grateful to National Research Council of Thailand and Naresuan University research fund (R2560B111) for financial support.

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