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Research article

Promotion of Brahmi (*Bacopa monniera*) Growth by Cyanobacterium *Nostoc* sp. Extract

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saponin

Abstract

Bacopa monniera (Brahmi), a saponin-containing medicinal and culinary herb, can be used to enhance memory and rejuvenate the brain. Moreover, it has other human health benefits. The cyanobacterium *Nostoc* sp. has been shown to produce bioactive compounds and plant growth regulators like indol acetic acid (IAA), which regulates various aspects of plant growth and development. This research aimed to study the effects of *Nostoc* sp. extract (NTE) supplement concentration in liquid medium on Brahmi survival rate, growth parameters, and saponin content. The experiments were designed using randomized complete block design with five treatments and three replications, including Bristol's medium (BT), pH 6.0 (control solution), and BT with four concentrations of NTE: 0.001, 0.01, 0.1, and 1.0%(w/v). The results indicated that IAA was found to increase with increase in NTE concentration. The IAA concentration in 1.0%(w/v) NTE was 43.56±3.21 pmol.mg^{-1} Ch-a. NTE supplementation at 0.001%, and 0.01%(w/v) had no negative effect on Brahmi survival rate, while the Brahmi survival rate decreased with increase in NTE concentration up to 0.1% (w/v). The optimal NTE concentration for Brahmi growth was 0.01%(w/v), which produced the highest shoot number, shoot height, root number, root length, leaf number, and chlorophyll content. Brahmi grown under all treatments was extracted and determined for the saponin content. The results showed that the levels of saponin from Brahmi grown in 0.01%(w/v) NTE had increased by two-fold over the control. This study showed that NTE supplementation enhanced the growth and saponin levels of Brahmi and NTE could be used as bio-promoting agent for plant growth and development.

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1. Introduction

Bacopa monniera (Brahmi), a nootropic ayurvedic herb, has been utilized in both traditional medicines and food supplements. Brahmi has a wide range of pharmacological activities including neuroprotective, antiparkinson, anti-Alzheimer, antidepressant, antioxidant, anti-inflammatory, anticancer, antianxiety, antidiabetic, antihypertensive, antimicrobial, antinociceptive, and antidiarrhea activities [1-4]. The herbal medicine contains numerous active constituents including saponins, flavonoids, alkaloids, glycosides, sapogenin, phytochemicals, and others [5]. The major bioactive compounds in Brahmi responsible for memory and cognition enhancement are saponin glycosides, which are mainly found in the shoots and aerial parts of the plant [6]. Previous studies reported that saponin glycosides in Brahmi extract include bacoside A3, bacopaside X, bacopasides I, II, and bacopasaponin C [4, 6, 7]. Brahmi can grow and thrive in the moist soil and marshy areas of Asia, including Thailand. Recently, the use of herbal medicinal products and supplements has been increasing worldwide. However, conventional Brahmi cultivation requires three to six months before harvesting. The yield and quality of Brahmi depend on environmental variables such as temperature, altitude, soil type, and seasonal changes [8, 9].

Cyanobacteria are oxygenic photosynthetic prokaryotes that are one of the most morphologically diverse groups: unicellular, multicellular, or filamentous, and examples are *Synechococcus* sp., *Merismopedia* sp. and *Nostoc* sp. [10-13]. Cyanobacteria, which produce a wide variety of secondary bioactive metabolites, have recently emerged as potential candidates for a wide range of applications in the fields of bioenergy, biotechnology, natural products, medicine, agriculture, and environment [14, 15]. *Nostoc* is a genus of filamentous nitrogen-fixing cyanobacteria that live in symbiotic relationships with plants [10-13]. Previous studies reported that the members of *Nostoc* sp. are a potential source of phytochemicals, polysaccharides, and lipids that are associated with their biological activities. In addition, *Nostoc* sp. can produce and release the phytohormone indole-3-acetic acid (IAA) synthesized by the indole-3-pyruvic acid pathway [14]. IAA is one of the most abundant and important plant auxins that regulate various aspects of plant growth and development [15-19]. IAA has commercial use in the agricultural sector for plant growth and yield enhancement.

Diverse techniques such as tissue culture, cell culture, and hydroponic systems have been used to enhance economic plant growth and secondary metabolite production [20-24]. Currently, hydroponic cultivation is popular because soilless culture offers advantages in terms of yield and avoidance of general limiting factors [25]. Moreover, hydroponic is a context for conducting precise research studies regarding mineral uptake, root activity, and metabolism [26, 27]. The positive effects of integration and application of beneficial microorganisms or their extracts or bioactive compounds in cropping systems have been presented [28-30]. The aim of this research was to determine the survival rate, growth parameters, and saponin content of Brahmi grown in hydroponic medium supplemented with various *Nostoc* sp. extract concentrations. Chlorophyll content is one of the plant growth parameters that indicate plant growth and plant health because chlorophyll is an important leaf photosynthetic pigment, and because changes in leaf chlorophyll content are a key parameter of plant stress and nutrient status.

2. Materials and Methods

2.1 Preparation of cyanobacterium *Nostoc* sp. extract (NTE)

Nostoc sp. cells were grown using BG11 medium at pH 7.6 and at 28°C under continuous white light for 14 days. Cells were harvested by centrifugation at 5,000 rpm at 4°C for 10 min. Then, cell

pellets were washed twice with Bristol's medium and kept at 4°C until further use. Preparation of *Nostoc* sp. extract was reported by Kachensuwan *et al.* [31]. 1.0 g Fresh cells of *Nostoc* sp. in 100 ml of Bristol's medium was homogenized by sonicator at 30% amplitude using cycles of 20 s on and 10 s off for 10 min. Next, the homogenate was centrifuged at 5,000 rpm at 4°C for 10 min. Then, the cell-free supernatant, namely 1.0%(w/v) NTE, was collected. Serial dilutions of 1.0% (w/v) NTE were performed to obtain a concentration series ranging from 0.1, 0.01, and 0.001%(w/v) NTE using Bristol's medium.

2.2 Indol-3-acetic acid (IAA) determination

IAA determination of NTE was done using a modified method from Kamnev *et al.* [32]. NTE (500 µL) was added with 1000 µL of Salkowski reagent. Each sample was incubated in dark conditions for 30 min. The absorbance of each sample was measured by a UV-Vis spectrophotometer (Jenway6405 UV/VIS) at 530 nm. The quantity of IAA was determined by comparing the absorbance of each sample with a standard curve of pure IAA (MERCK) and expressed as $pmol.mg^{-1}$ Ch-a.

2.3 Effects of *Nostoc* sp. extract on growth of Brahmi

The experiments were designed using randomized complete block design (RCBD) with five treatments and three replications including Bristol's medium, pH 6.0 (control solution), and Bristol's medium at pH 6.0 with 0.001%, 0.01%(w/v), 0.1%(w/v), and 1.0%(w/v) NTE, respectively. The Brahmi was collected from Chatuchak Park, Bangkok. Before the experimental treatment, 1 cm long Brahmi stems with nodes and leaves were prepared and soaked with control solution for 1 h. Nine Brahmi cuttings were placed onto a sponge sheet sized 5x5x0.5 cm and soaked in 100 mL of treatment solutions in a hydroponic system plastic container (8 cm in diameter and 5 cm long) (Figure 1) using half-strength Hoagland's solution [33]. The Brahmi cuttings were grown in a closed system to keep optimum moisture and allowed to grow for four weeks. The growth parameters investigated were survival rate, shoot bud number, shoot height, root number, root length, leaf number, and chlorophyll content.

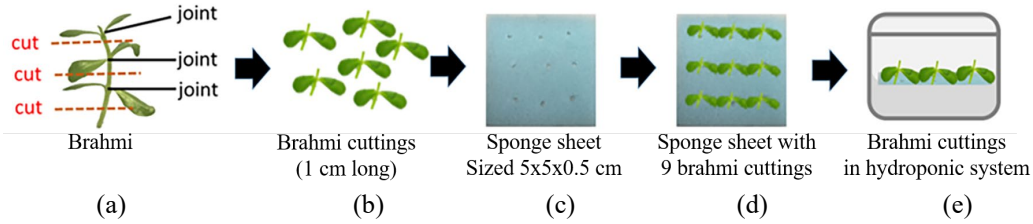

Figure 1. Preparation of Brahmi plant cuttings
(a) the position of taking cuttings, (b) Brahmi cuttings, (c) the positions for cuttings on the sponge sheet, (d) sponge sheet with 9 brahmi cuttings, and (e) Brahmi cuttings in the hydroponic system

The Brahmi survival rate was calculated by counting the number of Brahmi plant cuttings that showed shoot heights of more than 0.2-cm in length, root lengths of more than 0.2-cm in length, and green color, and by then dividing that number by the number of starting plants and multiplying that fraction by 100 to express it as a percentage of survival. The shoot height (cm) was measured from the ground level to the tip of the terminal shoot using a scale ruler. The root length (cm) was measured from the ground level to the tip of the terminal root using a scale ruler. The growth

parameters including number of shoots, shoot height, number of roots, root height, leaf number, and chlorophyll content were measured every week for 4 weeks.

2.4 Chlorophyll determination

Chlorophyll content was determined according to the method described by De Marsac and Houmard [34]. For Brahmi, fresh Brahmi plant cutting (0.1 g) was ground and dissolved in 90% methanol in darkness at room temperature for 2 h. For *Nostoc* sp., 0.1 g Fresh cells of *Nostoc* sp. were extracted by 90% methanol in darkness at room temperature for 2 h. The extract was centrifuged at 5,000 rpm for 10 min. The absorbance was measured at 665 nm using a UV–Visible spectrophotometer (Jenway6405 UV/VIS). The chlorophyll content was calculated using the following equation: OD_{665}x13.9.

2.5 Saponin determination

Saponin determination was modified following Uematsu *et al.* [35] and Sharma *et. al.* [36]. Each Brahmi crude extract (1 g in 100 mL methanol) was dissolved in 2 mL ethyl acetate. Then, 1 mL of reagent A (p-anisaldehyde in ethyl acetate (0.5:99.5)) and 1 mL of reagent B (H_2SO_4: ethyl acetate in equal parts) were added. Each mixture was shaken and incubated at 60°C for 10 min in a water bath. The solutions were cooled at room temperature for 10 min and analyzed by spectrophotometric technique at wavelength 430 nm. Ethyl acetate was used as a control blank and saponin solutions containing 0-200 µg in 2 mL of ethyl acetate were used to obtain a calibration curve. The total saponins in the extracts were calculated from the calibration curve of standard saponin and expressed as pmol.mg^{-1} Ch-a.

2.6 Data analysis

The data were expressed as means±standard deviation (SD) of three replicates (n=3). Differences between the means of individual groups were assessed using analysis of variance (ANOVA) with Duncan's New Multiple Range Test (DMRT) at a 5% level of significance.

3. Results and Discussion

3.1 Indol-3-acetic acid concentration of *Nostoc* sp. extract

Fresh extracts of 0.001%, 0.01%, 0.1%, 1.0%(w/v) NTE, and control solution (Bristol's medium) were analyzed for IAA concentration as shown in Table 1. The results showed that the IAA concentration of the 1.0%(w/v) NTE was 43.56 pmol.mg^{-1} Ch-a. Ten-fold serial dilutions of NTE reduced the concentration of IAA by a factor of ten.

3.2 Effects of *Nostoc* sp. extract on Brahmi survival rate

Brahmi cuttings were grown in Bristol's medium, pH 6.0 (control solution), and Bristol's medium at pH 6.0 with various concentrations of NTE. A 100% survival rate of Brahmi was observed under control, 0.001%, and 0.01%(w/v) NTE conditions, while the survival rate of Brahmi decreased with increase in concentration of *Nostoc* sp. extract up to 0.1% (w/v). The Brahmi survival rate at 0.1%(w/v) NTE decreased to the percentage of 85.00±4.13 in week 1, to 80.00±3.75 in week 2, and

to 75.00±3.65 in week 3. Moreover, the Brahmi survival rate decreased to the percentage of 50.00±2.48 in week 1 and 40.00±1.65 in week 2 at 1.0%(w/v) NTE (Table 2).

Table 1. The IAA concentration of *Nostoc* sp. extract

Treatment	IAA concentration (pmol.mg^{-1} Ch-a)
Control	0.00±0.00^{e}
0.001%(w/v) NTE	0.04±0.00^{d}
0.01%(w/v) NTE	0.42±0.04^{c}
0.1%(w/v) NTE	4.24±0.36^{b}
1.0%(w/v) NTE	43.56±3.21^{a}

Means with different letter(s) in columns are significantly different at $P \leq 0.05$.

Table 2. The survival rate of Brahmi cuttings grown in hydroponic growing medium supplemented with various *Nostoc* sp. extract concentrations

Treatments	Survival rate (%)				
	Week-0	Week-1	Week-2	Week-3	Week-4
Control	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
0.001%(w/v) NTE	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
0.01%(w/v) NTE	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
0.1%(w/v) NTE	100.00±0.00^{a}	85.00±4.13^{b}	80.00±3.75^{b}	75.00±3.65^{b}	75.00±3.45^{b}
1.0%(w/v) NTE	100.00±0.00^{a}	50.00±2.48^{c}	40.00±1.65^{c}	40.00±2.05^{c}	40.00±1.85^{c}

Means with different letter(s) in columns are significantly different at $P \leq 0.05$.

3.3 Effects of *Nostoc* sp. extract on shoot numbers and shoot heights of Brahmi

The shoot numbers and shoot height of Brahmi cuttings grown in hydroponic growing medium supplemented with various concentrations of *Nostoc* sp. extract are shown in Table 3. The highest shoot numbers and shoot heights were observed in Brahmi treated with 0.01%(w/v) NTE (2.35 shoots/plant and 3.65 cm of shoot length).

3.4 Effects of *Nostoc* sp. extract on root numbers and root heights of Brahmi

Table 4 shows the root numbers and root lengths of Brahmi cuttings grown in hydroponic growing medium supplemented with various NTE concentrations. The results showed that the root numbers of Brahmi treated with all concentrations of NTE were significantly different compared with the control but were not significantly different compared to each other. The highest root numbers and root length was observed in Brahmi treated with 0.01%(w/v) NTE (3.29±0.27 roots/plant and 6.98±0.59 cm of root length).

Table 3. The shoot numbers and shoot heights of Brahmi cuttings grown in hydroponic growing medium supplemented with various *Nostoc* sp. extract concentrations

Treatments	Shoot numbers (shoots/plant)				
	Week-0	Week-1	Week-2	Week-3	Week-4
Control	0.00±0.00[a]	1.00±0.00[c]	1.00±0.00[c]	1.00±0.00[c]	1.00±0.10[c]
0.001%(w/v) NTE	0.00±0.00[a]	1.15±0.05[b]	1.23±0.07[b]	1.44±0.07[b]	1.57±0.05[b]
0.01%(w/v) NTE	0.00±0.00[a]	1.36±0.07[a]	1.52±0.09[a]	1.86±0.11[a]	2.35±0.13[a]
0.1%(w/v) NTE	0.00±0.00[a]	1.38±0.05[a]	1.54±0.07[a]	1.83±0.09[a]	2.29±0.11[a]
1.0%(w/v) NTE	0.00±0.00[a]	1.19±0.07[b]	1.36±0.09[b]	1.53±0.11[b]	1.61±0.09[b]
Treatments	**Shoot height (cm)**				
	Week-0	**Week-1**	**Week-2**	**Week-3**	**Week-4**
Control	0.00±0.00[a]	0.25±0.05[b]	0.52± 0.07[b]	1.45±0.09[b]	1.66±0.11[b]
0.001%(w/v) NTE	0.00±0.00[a]	0.32±0.05[b]	0.68±0.07[b]	1.58±0.09[b]	1.78±0.13[b]
0.01%(w/v) NTE	0.00±0.00[a]	0.68±0.07[a]	1.15±0.09[a]	2.86±0.14[a]	3.65±0.19 [a]
0.1%(w/v) NTE	0.00±0.00[a]	0.73±0.09[a]	1.26±0.11[a]	2.62±0.15[a]	3.45±0.17[a]
1.0%(w/v) NTE	0.00±0.00[a]	0.75±0.11[a]	1.32±0.07[a]	2.54±0.13[a]	3.47±0.18[a]

Means with different letter(s) in columns are significantly different at $P \leq 0.05$.

Table 4. The root numbers and root lengths of Brahmi cuttings grown in hydroponic growing medium supplemented with various *Nostoc* sp. extract concentrations

Treatments	Root numbers (roots/plant)				
	Week-0	Week-1	Week-2	Week-3	Week-4
Control	0.00±0.00[a]	1.13±0.13[b]	1.25±0.13[b]	1.46±0.07[b]	1.54±0.07[b]
0.001%(w/v) NTE	0.00±0.00[a]	1.42±0.07[a]	1.67±0.07[a]	2.42±0.09[a]	3.13±0.13[a]
0.01%(w/v) NTE	0.00±0.00[a]	1.46±0.07[a]	1.79±0.09[a]	2.54±0.17[a]	3.29±0.27[a]
0.1%(w/v) NTE	0.00±0.00[a]	1.38±0.13[a]	1.75±0.15[a]	2.46±0.17[a]	3.25±0.13[a]
1.0%(w/v) NTE	0.00±0.00[a]	1.33±0.07[a]	1.67±0.07[a]	2.42±0.17[a]	3.21±0.21[a]
Treatments	**Root length (cm)**				
	Week-0	**Week-1**	**Week-2**	**Week-3**	**Week-4**
Control	0.00±0.00[a]	0.52±0.07[c]	1.06±0.11[c]	2.54±0.27[c]	2.84±0.24[c]
0.001%(w/v) NTE	0.00±0.00[a]	0.81±0.09[b]	1.68±0.15[b]	3.49±0.31[b]	4.69±0.44[b]
0.01%(w/v) NTE	0.00±0.00[a]	1.25±0.11[a]	2.48±0.23[a]	4.82±0.51[a]	6.98±0.59[a]
0.1%(w/v) NTE	0.00±0.00[a]	1.21±0.09[a]	2.34±0.21[a]	4.62±0.38[a]	6.79±0.67[a]
1.0%(w/v) NTE	0.00±0.00[a]	1.19±0.07[a]	2.21±0.19[a]	4.57±0.29[a]	6.68±0.58[a]

Means with different letter(s) in columns are significantly different at $P \leq 0.05$.

3.5 Effects of *Nostoc* sp. extract on leaf number and chlorophyll content of Brahmi

The maximum leaf number (about 12 leaves) was obtained with the 0.01%(w/v) NTE, 0.1%(w/v) NTE, and 1.0%(w/v) NTE treatments (Table 5). Treatment of Brahmi with NTE extract could also increase the chlorophyll concentration in leaves as shown in Table 6. The highest chlorophyll content (2.48±0.29 and 2.41±0.27 mg/g fresh weight) was observed in the 0.01, and 0.1%(w/v) NTE treatments, respectively.

Table 5. The leaf number of Brahmi cuttings grown in hydroponic growing medium supplemented with various *Nostoc* sp. extract concentrations

Treatment	Leaf number (leaves/plant)				
	Week-0	Week-1	Week-2	Week-3	Week-4
Control	2.00±0.00^{a}	2.08±0.07^{c}	4.04±0.13^{c}	4.33±0.07^{c}	6.08±0.15^{c}
0.001%(w/v) NTE	2.00±0.00^{a}	5.63±0.13^{b}	7.42±0.17^{b}	9.67±0.11^{b}	10.75±0.43^{b}
0.01%(w/v) NTE	2.00±0.00^{a}	7.25±0.21^{a}	9.17±0.33^{a}	10.96±0.23^{a}	12.25±0.23^{a}
0.1%(w/v) NTE	2.00±0.00^{a}	7.18±0.13^{a}	8.88±0.13^{a}	10.63±0.13^{a}	12.08±0.19^{a}
1.0%(w/v) NTE	2.00±0.00^{a}	7.06±0.25^{a}	8.71±0.09^{a}	10.46±0.17^{a}	11.96±0.37^{a}

Means with different letter(s) in columns are significantly different at $P \leq 0.05$.

Table 6. The chlorophyll content of Brahmi cuttings grown in hydroponic growing medium supplemented with various *Nostoc* sp. extract concentrations

Treatments	Chlorophyll content (mg/g fresh weight)				
	Week-0	Week-1	Week-2	Week-4	Week-5
Control	0.25±0.03^{a}	1.37±0.05^{c}	1.42±0.07^{c}	1.75±0.11^{c}	1.84±0.09^{c}
0.001%(w/v) NTE	0.25±0.02^{a}	1.52±0.11^{b}	1.73±0.09^{b}	1.86±0.17^{b}	2.14±0.23^{b}
0.01%(w/v) NTE	0.25±0.04^{a}	1.71±0.09^{a}	1.89±0.09^{a}	2.34±0.21^{a}	2.48±0.29^{a}
0.1%(w/v) NTE	0.25±0.05^{a}	1.69±0.13^{a}	1.86±0.05^{a}	2.28±0.23^{a}	2.41±0.27^{a}
1.0%(w/v) NTE	0.25±0.02^{a}	1.49±0.05^{b}	1.68±0.09^{b}	1.82±0.12^{b}	2.07±0.19^{b}

Means with different letter(s) in columns are significantly different at $P \leq 0.05$.

3.6 Effects of *Nostoc* sp. extract on saponin content of Brahmi

The levels of saponin of Brahmi grown in NTE treatments and control condition at week 4 are shown in Table 7. The highest saponin concentration was observed at 0.01%(w/v) NTE (47.48 pmol.mg^{-1} Ch-a), followed by 0.1%(w/v) NTE, 1.0%(w/v) NTE, 0.001%(w/v) NTE, and control.

Table 7. The saponin content of Brahmi cuttings grown in hydroponic growing medium supplemented with various *Nostoc* sp. extract concentrations at week 4

Treatments	Saponin concentration (pmol.mg^{-1} Ch-a)
Control	21.00±1.36^{c}
0.001%(w/v) NTE	29.56±1.48^{b}
0.01%(w/v) NTE	47.48±2.41^{a}
0.1%(w/v) NTE	45.39±2.46^{a}
1.0%(w/v) NTE	35.64±1.93^{b}

Means with different letter(s) in columns are significantly different at $P \leq 0.05$.

The levels of IAA in *Nostoc* sp. extracts were quantified. IAA concentration (Table 1) increased with increasing concentration of NTE. Previous studies showed that IAA was produced by cyanobacteria [14, 17, 37], microalga [38], and bacteria [39]. Sergeeva *et al*. [14] reported that the intracellular concentrations of IAA of symbiotic and free-living *Nostoc* strains grown in the absence of tryptophan were in the ranges of 7.2-24.2 and 6.0-15.3 pmol.mg^{-1} Ch-a, respectively. Our results indicated that NTE could be used as plant bio-stimulant. The effects of *Nostoc* sp. extract (NTE) supplement concentration in hydroponic growing medium on Brahmi survival rate, Brahmi growth, and saponin content were determined. The results showed that NTE enhanced Brahmi growth significantly compared to the control, with a progressive increase in biomass during the growth period. The optimal NTE concentration for Brahmi growth was at 0.01% (w/v). The highest number of shoot buds (2.35±0.13 shoots/plant), roots (3.29±0.27 roots/plant), and leaves (12.25±0.23 leaves/plant) were observed, along with approximately 3.65±0.19 cm shoot length and 6.98±0.59 cm root length, respectively. This was because NTE contains IAA, and various organic and inorganic substances such as sugars, amino acids, plant hormones, nitrate ions (NO_3^-), ammonium ions (NH_4^+), sodium ions (Na^+), and potassium ions (K^+), and the beneficial effects of these substances on plant growth have been well demonstrated [13, 15, 16, 40-42]. Jaiswal *et al.* [43] reported that NTE contains IAA which promotes rice seed germination. Furthermore, previous researchers reported that cyanobacteria extracts contain secondary metabolites and phytohormones that could induce strong physiological responses in plants and increase shoot and/or root weight [17, 44-46]. Mahender *et al.* [47] and Chauhan and Shirkot [48] reported that IAA increases the shoot bud numbers of Brahmi. Under hydroponic cultivation, the nutrients are absorbed directly into plant cells without a root system. So, the different osmotic pressure on either side of the cell membrane affects membrane structure, enzyme activity, and protein synthesis, and eventually affects the photosynthesis and survival rate of the plant [15, 17, 49-52]. Previous studies reported that Brahmi growth decreased when the plants were cultured in medium-high nutrient concentrations and that the yields of Brahmi plant decreased under NaCl concentrations greater than 15 mM [9, 53]. The application of IAA concentrations that were too-high decreased plant growth.

In addition, the positive effect of IAA on the chlorophyll content of Brahmi was also found (Table 6). This was probably because IAA regulates cell division, cell elongation, and development of adventitious root, embryogenesis, and increases pigment content, photosynthetic rate, stomatal conductance, and accumulation of sugars in plants [54, 55]. Previous reports revealed that extracts obtained from seaweed, microalgae, and cyanobacteria enhanced chlorophyll content in plants treated with the extracts [56, 57]. Moreover, the saponin contents in Brahmi significantly increased with increasing NTE concentration compared to control (Table 7). Especially in the 0.01%(w/v) NTE solution, the saponin content was a 2-fold increase in the amount of Brahmi likely because saponins were distributed in every part of Brahmi. Phrompittayarat *et al*. [57] reported that saponin

content in Brahmi shoots and leaves were significantly higher than those in the lower parts of the plant and in the roots in every season.

4. Conclusions

Hydroponics and elicitation with cyanobacterium *Nostoc* sp. extracts promoted Brahmi growth and saponin content. The optimal *Nostoc* sp. extract concentration at 0.01% (w/v) gave the highest shoot numbers, shoot heights, root numbers, root lengths, leaf numbers, and chlorophyll content, which were all significantly higher than control. Saponin accumulation in Brahmi in all tested NTE treatments were significantly greater than control. This is probably because *Nostoc* sp. extract contains a diversity of biologically active molecules such as phytohormones, including IAA, amino acids, and others. These results suggest that cyanobacterium *Nostoc* sp. extract could be used as exogenous elicitor in hydroponic systems to enhance the growth and development of plants and bioactive compound accumulation. In addition, *Nostoc* sp. extract could be used as alternative low-cost sustainable IAA source to replace chemical IAA in agriculture.

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