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Comparative effects of organic and inorganic fertilizers on growth, antioxidant activity and bacoside content in *Bacopa monnieri* (L.) Wettst.

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Abstract Brahmi (*Bacopa monnieri* (L.) Wettst.) has been recently studied extensively for using in the Ayurvedic system of medicine. The effects of organic fertilizers and chemical fertilizers on the growth and bacoside contents during the growth phase of the Brahmi plant were investigated. The aged plants of two weeks after propagation were treated with fertilizers consisting of organic fertilizer-swine manure extract (SME) and bat guano extract (BGE) and inorganic fertilizers (N-P-K: 46-0-0, 16-16-16 or 24-7-7) for 8 weeks. The soil application of SME was significantly elevated the fresh and dry weights, relative growth rate and the promotion of root numbers in plants compared to the chemical fertilizers. Plants growth using the chemical fertilizer treatments had the highest total bacoside and phenolic compounds contents, and the highest scavenging activity compared to the SME and BGE treatments. These results indicated to benefit in combining SME with chemical fertilizer for Brahmi yield enhancement. The results indicated an alternative, inexpensive method to improve the yield and pharmaceutical components of Brahmi plants.

Keywords: Antioxidant activities, *Bacopa monnieri*, Bacoside, Fertilizers, Swine manure

Introduction

A creeping annual plant, *Bacopa monnieri* (L.) Wettst. belongs to Scrophulariaceae and known as ‘Brahmi’ which plays a crucial role in the Ayurvedic system of medicine. It is used as a brain tonic, memory enhancer, as a revitalizer of sensory organs, and anti-anxiety, cardio-tonic, diuretic, antidepressant and anticonvulsant agent (Russo and Borrelli, 2005). The presence of bioactive saponins called ‘bacosides’, which bacoside A is a major

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chemical entity, has been shown to be responsible for the memory-facilitating action of Brahmi (Singh and Dhawan, 1997). The mixture of triglycosidic saponins includes bacoside A_3, bacopaside II, jujubogenin and bacopasaponin C (Deepak *et al*., 2005).

These secondary metabolites play an important role in plants, providing a strong plant interaction with the environment that is important for their survival and fitness, making these essential as primary metabolites (Verma and Shukla, 2015). The biosynthesis of secondary metabolites in medicinal and aromatic plants is strongly influenced by environmental factors (Alizadeh *et al*., 2010). Most secondary metabolites such as terpenes, phenolics, and alkaloids, are classified according to their biosynthetic pathways, origins and biological activities, and are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, biopesticides and food additives (Murthy *et al*., 2014). There are a number of physical and chemical factors that may also be effective in increasing the production of secondary metabolisms in plant cells, such as light intensity, temperature, water, soil type and chemicals (minerals/fertilizers) (Murthy *et al*., 2014; Verma and Shukla, 2015). Proper understanding and rigorous analysis of these parameters will pave the way toward successful secondary metabolite production in plants. Furthermore, plants need appropriate amounts of these components for their development, growth and survival. Plant nutrients play an important role in the regulation of the secondary metabolite content. The total phenolic content and antioxidant activity of *Satureja hortensis* L. was increased by applying chemical fertilizer (Alizadeh *et al*., 2010). The total phenolics and flavonoid contents in *Labisia pumila* Benth were enhanced 12% and 22%, respectively, using organic fertilizer treatment compared to inorganic fertilization (Ibrahim *et al*., 2013).

As mentioned, soil fertility can enhance the contents of secondary plant metabolites. Chemical fertilizers consist of soluble inorganic nitrogen and other nutrients that are more directly available to plants than organic fertilizers. However, long-term chemical fertilizer application caused soil compaction and acidification (Nadarajan and Sukumaranorganic, 2021; Wang *et al*., 2019; Vašák *et al*., 2015), whereas organic fertilizer application increased soil organic matter which promoted plant yields. In particular, the availability of inorganic nitrogen has the potential to influence the synthesis of secondary plant metabolites, proteins, and soluble solids (Mitchell *et al*., 2007). The contribution of organic farming in agricultural production is continuously increasing. Bat guano is not only used traditionally as natural fertilizer that has high contents of macronutrients (N-P-K), as well as secondary minerals, such as calcium and magnesium that help to control soil pH but also contains high levels of living microorganisms which are beneficial to plants (Grantina-Ievina and Ievinsh, 2015; Misra *et al*., 2019; Shetty *et al*., 2013). Nitrogen in guano is known to enhance crop growth, while phosphorus in guano induces root development, shoot budding, and multiple branches and flowering (Sridhar *et al*., 2006). Swine manure extract (SME) is a liquid form of plant nutrient derived from steeping dry swine manure in water, contains a full profile of both the macronutrients and micronutrients required by plants (Kanto *et al*., 2012). Kanto *et al*. (2013) have shown that 5-aminolevulinic acid (ALA) is contained in concentrated SME. ALA is an aliphatic precursor in the biosynthesis of all porphyrin compounds such as chlorophyll, heme and phytochrome (Wang *et al*., 2004).

Some studies have focused on the effects of macro elements and the nitrogen source on plant growth and the bacoside content in Brahmi cells and organ culture (Srivastava *et al*., 2017; Almusawi *et al*., 2017; Kashyap *et al*., 2017). However, there is little information available on conventional field cultivation of Brahmi and the fertilizer applications to optimize the bacoside content in commercial Brahmi production are not well understood. Consequently, the purpose of the current study was to determine the effects of inorganic and organic fertilizer applications on the growth and active constituents of *Bacopa*, especially the bacoside A content in Brahmi under field conditions.

Materials and methods

Preparation and chemical properties of swine manure extract, bat guano extract and fertilizers

The concentrated swine manure extract (CSME) and concentrated bat guano extract (CBGE) were prepared by fermenting dried swine manure or dried bat manure in water at a ratio of manure and water of 3:10 (w/v) for 72 h and filtered out of solids (using the tea-bag method). The properties and plant nutrient compositions of CSME and CBGE were analyzed as shown in Table 1. CSME was slightly alkaline and high electrical conductivity, whereas CBGE had a more neutral pH and low electrical conductivity. Both CSME and CBGE were diluted with water at a ratio of 1:30 (w/v) to produce swine manure extract (SME), and bat guano extract (BGE) for soil application. Three chemical fertilizers containing nitrogen, phosphorus and potassium contents of 46-0-0 (Total N, 46%, P_2O_5 0 %, K_2O 0%), 24-7-7 (Total N, 24%, P_2O_5 7 %, K_2O 7%) and 16-16-16 (Total N, 16%, P_2O_5 16 %, K_2O 16%) were used as inorganic fertilizers.

Table 1. Chemical composition of concentrated swine manure extract (CSME) and bat guano extract (CBGE)

Organic fertilizer	CSME	CBGE
pH	7.58	7.05
ECe (dS/m)	28.7	5.82
OM (%)	0.49	0.03
N (%)	0.32	0.08
P (%)	0.1	0.02
K (%)	0.21	0.03
Cl (%)	0.11	0.05
S (%)	0.03	0.02
Ca (mg/kg)	19.39	10.36
Mg (mg/kg)	3.15	39.08
Na (mg/kg)	272.98	85.58
Cu (mg/kg)	4.38	1.24
Fe (mg/kg)	10.67	4.53
Mn (mg/kg)	0.23	0.7
Zn (mg/kg)	1.97	0.45
B (mg/kg)	0.76	0.63

Plant material

Brahmi (*Bacopa monnieri* (L.) Wettst.) was obtained from Chachoengsao province, Thailand. The plant was verified by The Forest Herbarium, Department of National Parks, The Ministry of Wildlife and Plant Conservation, Thailand (voucher specimen No. BKF-194458). The plant materials were propagated for 14 days to produce more homogenous shoot formations and then were subjected to fertilizer treatments at the Faculty of Science and Technology, Valaya Alongkorn Rajabhat University under the Royal Patronage. The experimental plants were grown in 30 plastic pots of 20.32 cm in diameter and contained 1,000 g of growing medium for 60 days. Each pot was planted with 6 pieces of Brahmi 15 cm long from the apical shoot. The field capacity of the soil medium was maintained by applying tap water at 500 ml per pot throughout the experiment to maintain 60% moisture content at field capacity. The pots were placed under ambient conditions where the average day/night temperature was 33.7/27.2 ℃, the relative humidity (RH) was 49.9/76.8% and the average daily photosynthetic photon flux density (PPFD) 629.5 μmol PPF m^{-2} s^{-1}. Properties of the medium both before and after the treatment were analyzed as shown in Table 2.

Table 2. Soil chemical properties at 60 days after planting Brahmi after treatment with organic or inorganic fertilizers

Soil sample	pH (1:1)	OM (%)	ECe (dS/m)	Plant available nutrients (mg/kg)			
				P	K	Ca	Mg
Control (Before)	6.45	22.81	7.09	1332.54	754.65	4053.24	725.26
Control (After)	7.29	12.06	4.11	729.32	1314.71	4364.34	552.40
SME	7.31	12.37	5.56	691.39	1686.40	5011.04	655.00
BGE	7.16	12.17	6.35	571.91	1465.03	5075.47	684.31
46-0-0	7.31	11.69	4.35	623.24	1096.21	4943.63	586.52
24-7-7	7.19	13.41	4.98	637.78	1128.91	4988.04	576.66
16-16-16	7.31	12.81	5.00	816.58	1279.80	4893.70	533.36

SME = swine manure extract; BGE = bat guano extract; 46-0-0 = total N, 46%, P_2O_5 0 %, K_2O 0%; 24-7-7 = total N, 24%, P_2O_5 7 %, K_2O 7%; 16-16-16 = total N, 16%, P_2O_5 16 %, K_2O 16%.

Sample collection

The experimental pots were randomly divided into 6 groups of 5 pots in each group. The pots in each group were randomly assigned to a fertilizer treatment as follows: no fertilizer (control), chemical fertilizer 46-0-0, 24-7-7 and 16-16-16, organic fertilizer as SME or BGE. Chemical fertilizers (0.5 g/pot) were applied only once at 1 week after planting. Applications of SME and BGE were carried out 5 times (each 360 ml/pot) throughout the experiment.

Table 3. Rates of macronutrients and micronutrients added to soil in 6 treatments

Nutrient	Control	SME	BGE	46-0-0	24-7-7	16-16-16
N (g/pot)	na	0.192	0.048	0.23	0.120	0.08
P (g/pot)	na	0.06	0.012	na	0.035	0.08
K (g/pot)	na	0.025	0.018	na	0.035	0.08
Cl (g/pot)	na	0.066	0.030	na	na	na
S (g/pot)	na	0.018	0.012	na	na	na
Ca (mg/pot)	na	1.163	0.622	na	na	na
Mg (mg/pot)	na	0.189	2.345	na	na	na
Cu (mg/pot)	na	0.263	0.074	na	na	na
Fe (mg/pot)	na	0.640	0.272	na	na	na
Mn (mg/pot)	na	0.014	0.042	na	na	na
Zn (mg/pot)	na	0.118	0.027	na	na	na
B (mg/pot)	na	0.046	0.038	na	na	na

na = not applicable; SME = swine manure extract; BGE = bat guano extract; 46-0-0 = total N, 46%, P_2O_5 0 %, K_2O 0%; 24-7-7 = total N, 24%, P_2O_5 7 %, K_2O 7%; 16-16-16 = total N, 16%, P_2O_5 16 %, K_2O 16%.

Determination of plant growth and SPAD values and mineral content

The stem lengths of the experimental plants were recorded at 30 and 60 days after planting. At 60 days after planting, all experimental plants were removed from the soil medium, washed with distilled water, and examined for fresh weight (FW), dry weight (DW), chlorophyll content, and nitrogen, magnesium and sodium contents. The relative growth rate was defined as (ln DW after treatment–ln DW before treatment)/treatment duration (Kingsbury *et al*., 1984). The leaf chlorophyll content was measured using a SPAD–502 chlorophyll meter (Minolta Camera Co., Osaka, Japan). The dried plants from each treatment were analyzed for nitrogen concentration using the micro-Kjeldahl method and for magnesium and sodium using atomic absorption and flame emission spectrophotometry (Shimadzu AA-6300, Shimadzu Corporation, Japan).

Quantification of bacoside A

Bacoside A was extracted from the samples and purified following the procedure based on Deepak *et al*. (2005) and further modified by Srivastava *et al*. (2012). Approximately 100 mg of the powdered plant material were weighed into a 50 ml vial with a screw cap to which was added about 10 ml of 70% (v/v) methanol and mixed for 30 seconds followed by 15 minutes of ultrasonication. The extracts were centrifuged at 11,000 rpm and 4 ℃ for 10 minutes and the supernatant was transferred to a 20 ml volumetric flask. The residue extraction was repeated twice (2×5 ml) with 70% (v/v) methanol, all alcohol fractions were combined and added to 20 ml with 70% methanol before passing through a 0.22 µm membrane filter. The final solution called Brahmi extract that was obtained for high performance liquid chromatography (HPLC) quantification. Quantifications were carried out using external standard curves plotted by taking the known quantities of standard compounds (individually bacoside A_3, bacopaside X, bacopaside II and bacopasaponin C) (ChromaDex, Inc., California, United States). Standard solutions were contained 10 mg/ml of the standards 1-7, in a medium of methanol (stock solution). Six additional calibration levels were prepared and the standard curves were obtained using the peak areas of six different concentrations in six replicated assays and were expressed using a linear least square regression equation.

HPLC instrumentation and analytical method

Solutions containing bacoside A were injected (20 µl, Waters 2998 photodiode array (PDA) detector, Waters 600 Controller, Waters 717 plus

Autosampler, Waters Corporation, Milford, U.S.A) onto the HPLC column (Spherisorb, 250 mm × 4.6mm, C18 ODS2 with 5 µm packing and equipped with a Spherisorb S5 guard column; 10 mm × 4.6 mm, C18 ODS2 with 5 µm packing, Waters Associates, Harrow, UK). The mobile phase was 0.2% phosphoric acid: acetonitrile at a ratio of 65:35 (v/v). The flow rate was 1 ml/min. The injection volume was 20 µl, and the running temperature was 30 °C. The total time required for the analysis was 40 min. Detection used the PDA at 205 nm. The peak corresponding to the bacoside derivative eluted at bacoside A_3, 19.84 min, bacopaside II, 21.55 min, bacopaside X, 25.073 min and bacopasaponin C, 28.471 min. The chromatograms obtained were analyzed and calibration plots were made.

Quantitative determination of total phenolic content and determination of DPPH scavenging activity

The Brahmi extracts from the preparation for quantification of the bacoside content were also determined for their phenolic content and DPPH activity determination. The total phenolic content of the methanolic extract was determined using a modified Folin-Ciocalteu method with gallic acid (Sigma-Aldrich, Gillingham, UK) as a standard, as described by Mohd-Esa *et al*. (2010). All measurements were made in 4 replicates and the results was expressed as milligrams of gallic acid equivalent (GAE) per 100 g of dry weight. DPPH scavenging activity was determined according to González-Mendoza *et al*. (2010). The methanol extracts from above were added to 1,200 µl of DPPH solution (0.025 g/l). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was measured at 517 nm using a spectrophotometer with methanol as a blank. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percentage of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging (RSA) was calculated using the equation: %DPPH RSA = [(Initial absorbance - Final absorbance)/Initial absorbance] × 100.

Statistical analysis

The data were subjected to analysis of variance and the differences among means were compared on Duncan's multiple range test. Pearson correlation coefficients between traits were analyzed. All analyses were conducted using the SPSS version 25 software (IBM Corporation, USA). Means differing at $p \leq 0.05$ were significantly considered.

Results

Plant growth and root number

The growth performance of Brahmi treated with inorganic and organic fertilizers at 60 days after planting is shown in Figure 1.

Figure 1. Pictorial growth at 60 days after planting of Brahmi treated with no fertilizer, organic fertilizer-swine manure extract (SME), bat guano extract (BGE) and N-P-K chemical fertilizer treatments of 46-0-0, 24-7-7 or 16-16-16

Plants growing with either organic or inorganic fertilizers had significantly higher fresh weights, dry weights and root numbers than the control plants. Among the fertilizer treatment groups, the plants receiving an SME application had significantly higher fresh weights and dry weights than the other treatments (Figure 2A−B). There were no differences in the fresh weights of Brahmi grown with BGE and the chemical fertilizers treatments (Figure 2A). The dry weight of the plants on the 46-0-0 treatment was significantly higher than for the BGE, 24-7-7 and 16-16-16 fertilizer treatments (Figure 2B). Plants on organic fertilizer treatments (SME and BGE) had significantly higher root numbers than those on chemical fertilizer treatments (Figure 2D). There were no significant differences in the RGR of the plants in the control, organic (SME or BGE) and 46-0-0 fertilizer treatments. However, the RGR values of the plants in these three treatments were significantly higher than those on the 24-7-7 and 16-16-16 fertilizer treatments. There were no significant differences in the RGR values of the plants on the 24-7-7 and 16-16-16 treatments (Figure 2C). Plants treated with organic fertilizers (both SME and

BGE) had significantly higher root numbers than those on the chemical fertilizer treatments (Figure 2D).

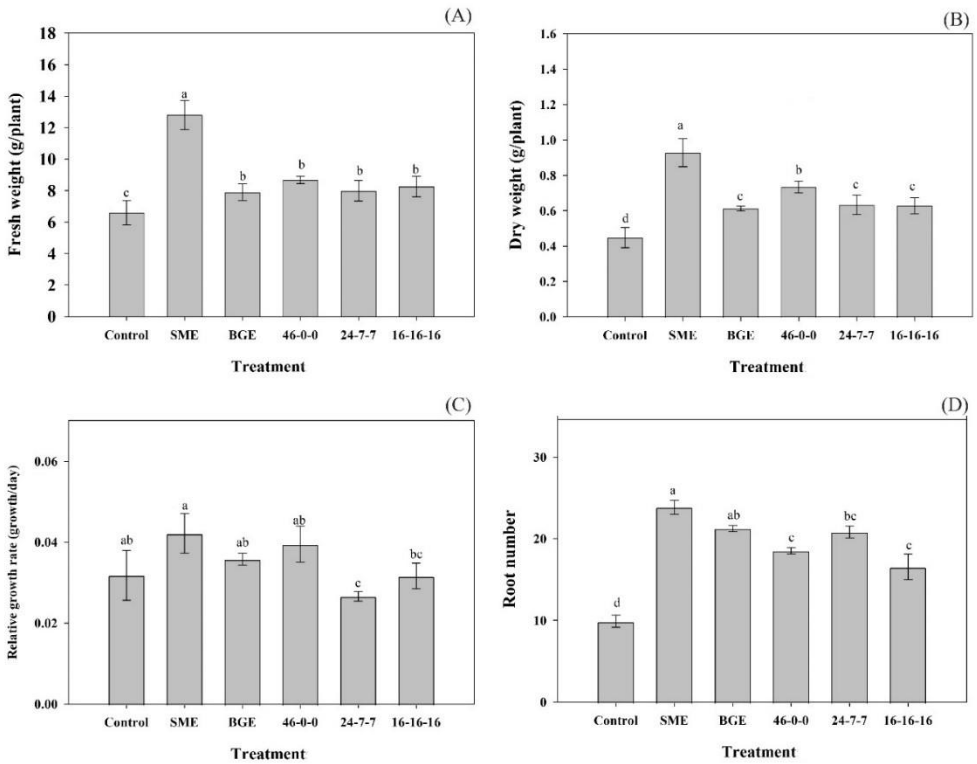

Figure 2. Effects of organic and inorganic fertilizers on fresh weight (A) dry weight (B) relative growth rate (C) and root number (D) at 60 days after planting of Brahmi treated with no fertilizer (control), swine manure extract (SME), bat guano extract (BGE), N-P-K chemical fertilizer at 46-0-0, 24-7-7 and 16-16-16, respectively. Values are means of 4 replicates. Mean ± standard deviation values with different lowercase letters in same graph are significantly different at $p < 0.05$, according to Duncan's multiple range test

Nitrogen, magnesium and sodium concentrations and SPAD values

The nitrogen, magnesium and sodium concentrations and the SPAD values at 60 days after planting of Brahmi treated with inorganic and organic fertilizer are shown in Figure 3. There were no significant differences in the total magnesium concentration in Brahmi among the fertilizer treatments (Figure 3B). However, there were significant differences in the nitrogen and sodium concentrations and SPAD values among the organic and inorganic fertilizer treatment groups. SME application was significantly increased the nitrogen concentration in Brahmi compared with the other treatments (Figure 3A). There were no significant differences in the nitrogen concentrations among the BGE, 24-7-7 and 16-16-16 treatment (Figure 3A). SME-treated plants had the lowest sodium concentrations (Figure 3C), but it produced the highest SPAD values compared to the other treatments (Figure 3D). There were no significant differences in SPAD values among the chemical fertilizer treatments (Figure 3D).

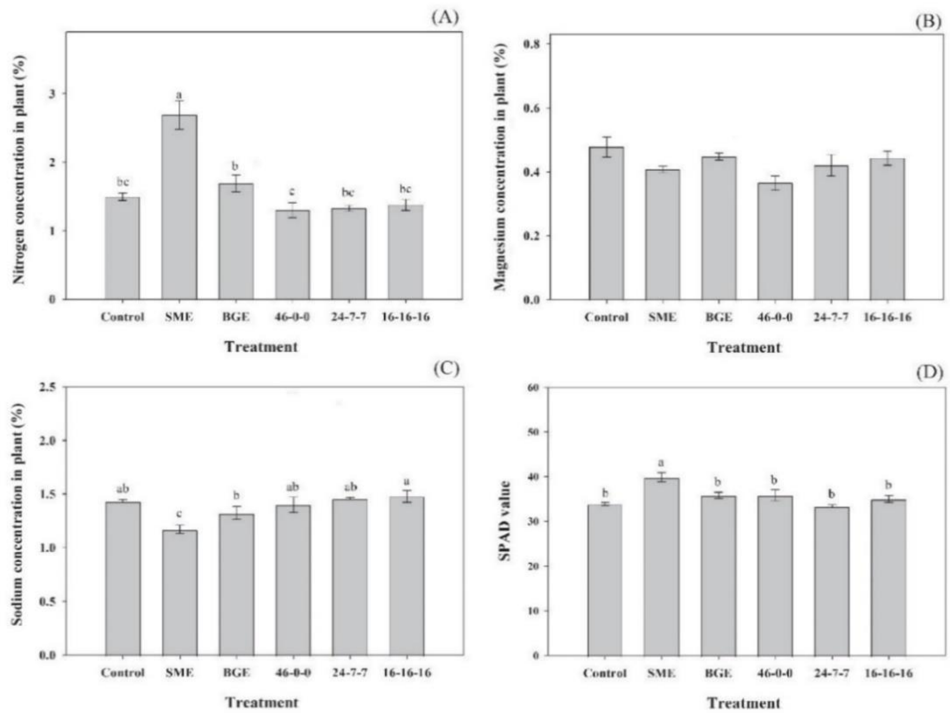

Figure 3. Effects of organic and inorganic fertilizers on: (A) N concentrations (B) Mg concentrations (C) Na concentrations in plant parts and (D) SPAD values at 60 days after planting of Brahmi treated with no fertilizer (Control); Swine manure extract (SME), bat guano extract (BGE), N-P-K chemical fertilizer at 46-0-0, 24-7-7 and 16-16-16, respectively. Values are means of 4 replicates. Mean ± standard deviation values followed by different lowercase letters in same graph are significantly different at $p < 0.05$, according to Duncan's multiple range test

Bacoside contents

The bacoside contents, consisting of a mixture of bacoside A_3, bacopaside II, bacopaside X and bacopasaponin C, in Brahmi of all treatments which are shown in Table 4. There were highly significant ($p < 0.01$) differences in the components of bacoside and the total bacoside contents among the organic fertilizer and inorganic fertilizer treatment groups (Table 4). Brahmi plants grown using chemical fertilizer had significantly higher bacoside A_3, bacopaside II and total bacoside contents than the plants treated with SME or BGE. The bacoside A_3 and total bacoside contents in the control treatments were significantly higher than those in the plants treated with organic fertilizer. The chromatograms of *B. monnieri* extracted from the 46-0-0 treatment and the standard total bacoside A are shown in Figure 4.

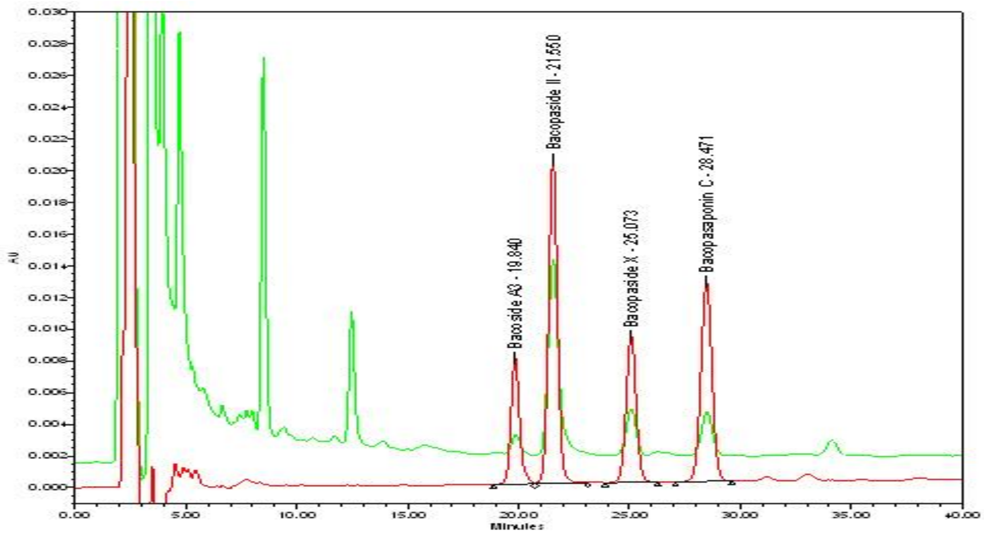

Figure 4. Example of HPLC-chromatogram of *B. monnieri* extract from 46-0-0 fertilizer application (green line) compared to standard total bacoside A (red line)

Total phenolic content and DPPH scavenging activity

The total phenolic content and DPPH scavenging activities in Brahmi under all fertilizer treatments are shown in Figure 5. Brahmi grown using chemical fertilizer and no fertilizer (control) had significantly higher total phenolic contents and % DPPH scavenging activities than those in the SME and BGE treatments (Figure 5A−B). The SME-treated plants had the lowest total phenolic contents and % DPPH scavenging activities compared to the chemical fertilizer groups and BGE treatments ($p < 0.05$). There were no significant differences in the total phenolic contents and % DPPH scavenging activities among the chemical fertilizer treatments (Figure 5A−B).

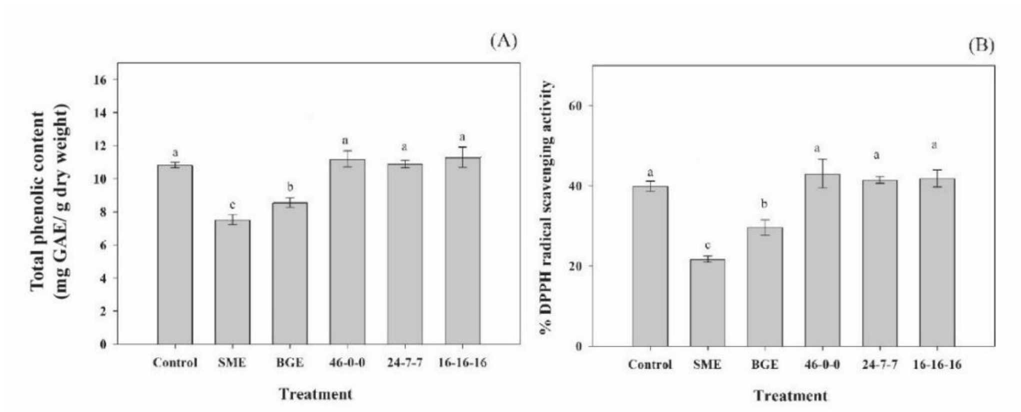

Figure 5. Effects of organic and inorganic fertilizers on: (A) total phenolic content and (B) % DPPH radical scavenging activity at 60 days after planting of Brahmi treated with no fertilizer (Control), swine manure extract (SME), bat guano extract (BGE), and N-P-K chemical fertilizer at 46-0-0, 24-7-7 or 16-16-16. Values are means of 4 replicates. Mean ± standard deviation values followed by different lowercase letters in same graph are significantly different at $p < 0.05$, according to Duncan's multiple range test

Table 4. Bacoside contents at 60 days after planting of Brahmi treated with no fertilizer (Control), swine manure extract (SME), bat guano extract (BGE) and N-P-K chemical fertilizer at 46-0-0, 24-7-7 or 16-16-16

Treatment	**Bacoside A_3**	**Bacopaside II**	**Bacopaside X**	**Bacopasaponin C**	**Total Bacoside**
			(mg/g)		
Control	3.81 ±1.90^{a}	8.46 ±2.32^{c}	74.55 ±0.94^{a}	5.21 ±1.48^{a}	92.02 ±4.86^{b}
SME	2.22 ±0.84^{c}	7.47 ±3.47^{c}	43.28 ±3.87^{c}	4.51 ±2.35^{b}	57.49 ±10.13^{c}
BGE	2.62 ±0.82^{b}	7.71 ±4.95^{c}	63.89 ±3.11^{b}	5.20 ±1.21^{a}	79.41 ±6.53^{c}
46-0-0	3.59 ±0.95^{a}	14.80 ±3.46^{a}	69.64 ±2.01^{ab}	4.62 ±1.60^{b}	92.66 ±2.35^{a}
24-7-7	3.66 ±1.65^{a}	13.80 ±4.55^{a}	64.95 ±4.48^{b}	4.43 ±2.46^{b}	86.84 ±12.07^{a}
16-16-16	3.59 ±1.70^{a}	10.55 ±14.00^{b}	64.86 ±3.84^{b}	4.36 ±1.95^{b}	83.37 ±11.65^{b}
p-value	<.0001	<.0001	<.0001	0.0026	<.0001

Values are means of 4 replicates. Mean ± standard deviation values followed by different lowercase letters in same graph are significantly different at $p < 0.05$, according to Duncan's multiple range test.

Relationships between plant growth variables

Relationships among the plant growth variables were investigated by determining the correlation between each pair of variables. A significant positive correlation was obtained for the relationship between leaf total nitrogen and fresh weight, root number and SPAD value. Total sodium levels in plant parts were also significantly positive and correlated to the bacoside A_3 contents and DPPH activities (*P*<0.01, R= 0.601, R=0.879). A strong negative correlation was found between total nitrogen and bacoside A_3, bacopaside X, total bacoside, phenolic content and DPPH activities in plant parts (*P*<0.01, R=-0.718, R=-0.774, R=-0.761, R=-0.804, R=-0.608). Furthermore, there was a substantially negative correlation between total magnesium and DPPH activities (*P*<0.01, R=-0.702).

Discussion

Brahmi in the SME treatment had significantly higher growth of biomass than with the chemical fertilizer treatments. Although there were low N, P and K concentrations in SME, it contained the full profile of plant nutrients, especially micronutrient such as Fe, Mn, Cu and Mn that promote higher photosynthetic activity and higher biomass growth of the plants. These results were in agreement with Kanto *et al*. (2012) who showed that a monthly foliar application of SME crucially enhanced the chlorophyll content in cassava leaves aged 4 months. Brahmi in the SME treatment had significantly higher SPAD values and significantly higher nitrogen concentrations in plant parts. In addition, there was a significant correlation between the SPAD value and the total nitrogen content in the plants. Furthermore, the SME-treated plants had significantly higher root numbers compared to plants treated with chemical fertilizers. SME contains 5-aminolevulinic acid (ALA) (Kanto *et al*., 2013) which could improve the chlorophyll status in Brahmi leaves. ALA also stimulated root elongation of rice and *Arabidopsis* (Hotta *et al.*, 1997; An *et al*., 2019). Our results indicated that micronutrients and 5-aminolevulinic acid in SME may play important roles in chlorophyll status as well as the SPAD value of Brahmi. The higher SPAD value may lead to increased photosynthesis and a higher yield of Brahmi. Verma and Shukla (2015) suggested that micronutrients enhanced the availability of primary metabolites that may be enhanced secondary metabolite production in *Cassia angustifolia*, where the chlorophyll, protein and phenol contents are also influenced by $FeSO_4$, $ZnSO_4$, and $CuSO_4$.

The N-P-K chemical fertilizer treatments (46-0-0, 24-7-7 or 16-16-16) failed to promote higher values for fresh weight, dry weight, RGR or root numbers of Brahmi in this study which probably due to the lack of microelements required by the plants in the treatments using chemical fertilizers. However, chemical fertilizer treatment could improve the bacoside contents in Brahmi parts. The results of the current study indicated that plants in the high nitrogen fertilizer treatments (46-0-0 or 24-7-7) had significantly higher total bacoside A contents than those in the 16-16-16, control group, SME and BGE treatments. Długosz *et al*. (2018) reported that nitrogen is the limiting source of saponin synthesis in *Calendula officinalis*. Therefore, nitrogen fertilizer application is still essential for production of bacoside A in Brahmi. Our finding was consistent with Naik *et al*. (2011) who reported that nitrogen in the form of NH_4NO_3 is recommended for the most efficient bacoside A production. Therefore, elemental nitrogen played an important role in bacoside A synthesis. Furthermore, our results revealed strong positive correlations between the Na concentration in Brahmi and the total bacoside contents in Brahmi. The growth of plants could be inhibited by the Na content in the plants. In this study, no fertilizer produced proved to be a high bacoside concentration in the plant parts. Importantly, chemical fertilizer application elevated the Na concentration in Brahmi and this might be elevated the antioxidant activities in terms of the phenolic content and %DPPH scavenging activities. The Na concentration in the control plants and the chemical fertilizer treatments might come from Na in the saline soil. Similarly, Ahire *et al*. (2013) reported that the bacoside A content of *B. monnieri* could be increased by moderate stress (100 mM NaCl), since the synthesis of higher triterpene saponin might prevent the membrane damage induced by NaCl. However, a higher NaCl concentration resulted in a substantial decline in the bacoside A content.

In the current study, SME application increased the yield of Brahmi but failed to promote the bacoside content. This might be due to the lower stress conditions compared to the chemical fertilizers and control groups. Brahmi in the SME treatment had significantly higher nitrogen but significantly lower Na concentrations in the plant parts than those in the chemical fertilizer treatments. This phenomenon might be due to the application of SME resulting in the elevation of the soil Ca concentration which ameliorated Na uptake by plants. Awada *et al*. (1995) demonstrated the mechanisms of Ca to ameliorate the effect of Na stress in snapbeans. This was in agreement with Rains and Floyd (1969) who showed that the presence of Ca in the aging medium did not promote the absorption of Na into aged bean stem slice tissue.

Phenolic compounds are a class of antioxidant agents that act as free radical scavengers and are responsible for antioxidant activities in medicinal plants. The DPPH (2, 2- diphenyl-1-picryl hydrazyl) free radical scavenging method is used for the determination of the antioxidant activity of plant extracts (Alizadeh *et al*., 2010). The total phenolic content and DPPH of Brahmi were enhanced in the inorganic fertilizer and without fertilizer treatments (control) compared to the organic fertilizer treatments. This phenomenon differed from other studies with *Satureja hortensis* (Alizadeh *et al*., 2010), *Labisia pumila* (Ibrahim *et al*., 2013) and *Manihot esculenta* (Omar *et al*., 2012), which demonstrated that plants in organic fertilizer treatments had a higher level of total phenolic content and DPPH activity than in inorganic fertilizer treatments. The results of the present study revealed the relationship between a high Na content in Brahmi grown using inorganic fertilizers and in the control treatment and the high levels of total phenolic content and DPPH activities. It is possible that the antioxidant capacity was stimulated by salt treatment since the degree of oxidative cellular damage in plants exposed to abiotic stress which is controlled by the capacity for protection against oxidative agents (Oueslati *et al*., 2010).

In conclusion, the current results are clearly revealed that SME as an organic fertilizer is an appropriate solution for Brahmi yield improvement. In contrast, the chemical fertilizer applications in the study had the highest total bacoside, phenolic compound and DPPH contents in the plants. Notably, SME was superior to BGE in improving the yield of Brahmi. Finally, studying on the effects of SME applications in combination with chemical fertilizers was useful to achieve optimal efficiency in commercial Brahmi production.

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