



## Enhancing Growth and Bioactive Compound Contents in Brahmi (*Bacopa monnieri* (L.) Wettst.) Irrigated with Canteen Wastewater

Jirarat Thipnang<sup>1</sup>, Nipaporn Taweejun<sup>2</sup> and Narumol Piwpuan<sup>1,\*</sup>

<sup>1</sup> Department of Environmental Science and Natural Resources, Faculty of Interdisciplinary Studies, Khon Kaen University. Nong Khai Campus, 112 Nong Kom Ko, Muang Nong Khai, Nong Khai 43000, Thailand.

<sup>2</sup> Department of Science Technology and Business Enterprise, Faculty of Interdisciplinary Studies, Khon Kaen University. Nong Khai Campus, 112 Nong Kom Ko, Muang Nong Khai, Nong Khai 43000, Thailand

\*Corresponding author: narupi@kku.ac.th

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### Abstract

A medicinal plant, *Bacopa monnieri*, was grown under different canteen wastewater concentrations for five weeks. Their growth and certain secondary metabolite contents were measured, together with several water and soil parameters. Plants irrigated with 50%, 75% and 100% wastewater had higher biomass than those irrigated with tap water and plant growth appearing to be optimal under 50% wastewater treatment according to shoot fresh weight and root dry weight. The highest secondary metabolites measured as total phenolic contents ( $1.18 \pm 0.11$  mg GAE/g dry plant) and total flavonoid content ( $8.79 \pm 1.13$  mg QE/g dry plant) were observed in plants irrigated with 100% wastewater. This may be due to the plants irrigated with 100% wastewater were experienced to higher stress factors, such as increased potassium (K) and electrical conductivity (EC), than the plants in other treatments. These results suggest that canteen wastewater could be a suitable option for irrigation, potentially integrating wastewater management with medicinal plant cultivation.

**Keywords:** Antioxidants, Total flavonoids, Total phenolics, Water reclaim

### 1. Introduction

*Bacopa monnieri* (L.) Wettst., a member of the family Scrophulariaceae, is commonly known as water hyssop and locally called in Thai as “phak mi” or “phrommi”. It is a native plant in Asia and has been used for either ornamental or medicinal purposes. This medicinal herb has been used in India for centuries. As the active substances produced by the species have special properties for cognitive enhancement and relieve some neurological problems, the species is widely available in herbal markets throughout India and Southeast Asia [1]. In the Ayurvedic medicine system of India, *B. monnieri* has been used as a memory enhancer and sharpener and for a broad range of ailments such as diabetes, jaundice, throat infection, skin and blood disorders, etc. [2]. Among its many bioactive constituents, phenolics, terpenoids, flavonoids, and especially bacoside A are the main active phytochemicals responsible for its antioxidant activity and protection of DNA damage [3]. The secondary metabolite contents in *B. monnieri* can vary depending on several factors, including both biotic and abiotic factors within a plant's growth environment [4-8]. However, the different doses of specific substances usually result in variations in plant growth performance as well.

As *B. monnieri* can grow in terrestrial areas-preferably in wet places- as well as in aquatic conditions with both fresh and brackish water, and thrives exceptionally well under waterlogged conditions [1], its potential for wastewater treatment falls in the area of interest. Brahmi is recognized as a facultative halophyte and its ability to treat saline water having electrical conductivity (EC) as high as  $4,000 \mu\text{S/cm}$  has been proved [9]. The species has also been shown to grow well in horizontal subsurface flow constructed wetlands treating grey water in gravel and biochar bases over a period of three months [10]. Additionally, it has been tested also for the removal of coliform bacteria from sewage by 70% after 24 hours of incubation without any stress symptom based on chlorophyll contents [11]. Moreover, as a plant used on floating wetland, *B. monnieri* effectively removed

67-81% of biochemical oxygen demand (BOD) from artificial sugar mill effluent [12]. In addition, its ability to grow on and remediate metal contaminated soil is well documented [6,13,14].

Treated household wastewater such as municipal wastewater, black or grey water, has become an interesting source of water supply for irrigation particularly in arid regions of the world where water demand is increasing [15]. However, there are a few studies on using solely kitchen wastewater reported and the effects on soil properties appear to vary. For instance, Parwin and Paul [16] suggested that more attention should be given to the high mineral contents rather than toxic substances, while this concern was not the issue found in the study by Abegunrin et al. [17] which investigated on using kitchen wastewater for irrigation.

As *B. monnieri* has the ability to tolerate well with various types and concentration of substances, we expect that it may have potential to be cultivated in soil irrigated with kitchen wastewater. In addition, different growth conditions may influence its secondary metabolites production. Our study aims to explore the feasibility of using raw canteen effluent for the production of *B. monnieri* plants. By studying the effect of irrigating with canteen effluent at different concentrations on plant growth and the content of some secondary metabolites in *B. monnieri*, the findings from our study may provide the practical recommendation to reclaim water for specific plant production at either the household or community level.

## 2. Materials and Methods

### 2.1 Soil and soil analysis

Natural soil (300 g) and commercial soil for general planting (300 g) were mixed and filled into each pot. This soil mixed was used for all treatments in our present study, mainly aimed as a controlled factor for the experiment. For soil parameters analysis, soil samples were collected from each pot in all treatments. The soil samples from the same treatment were combined together and then divided into three replicates. The soil samples were air-dried, ground and sieved through 2 mm sieve, and kept for further analyses. Available phosphorus was extracted from the soil using Olsen solution and analyzed using Molybdenum blue method [18]. Available potassium (K) was extracted using  $\text{NH}_4\text{OAc}$  for 30 minutes and the extract was analyzed using atomic absorption spectrophotometry. Soil pH and EC were measured in water extract solution at a ratio of 1:5 (soil:water) using a pH meter and an EC meter, respectively [19].

### 2.2 Water collection and analysis

Wastewater was collected weekly and directly from effluent tube of three restaurants at the cafeteria in Building No. 2, Khon Kaen University, Nong Khai Campus, Nong Khai Province, Thailand. Solid waste was separated from wastewater by sieving before mixing all portions of wastewater together. The wastewater was then diluted with tap water to achieve the determined concentrations used in the treatments (50% wastewater, 75% wastewater and 100% wastewater, respectively). Each batch of water was prepared for using within one week. Water parameters were analyzed on days one and seven. Chemical oxygen demand (COD) was analyzed using a closed reflux method. Total Kjeldahl nitrogen (TKN) was analyzed using the Macro Kjeldahl Method [19]. Total phosphorus (TP) was analyzed using a digestion procedure and spectrophotometric analysis according to ascorbic acid method. Potassium (K) content in the water was analyzed using an Atomic Absorption Spectrophotometer [20]. The values of pH and electrical conductivity (EC) were measured using a pH meter and an EC meter, respectively.

### 2.3 Plant preparation and Experimental set up

Plant shoots were cut to a length of 7 cm from shoot tip and planted into the prepared pots (one shoot per pot). Tap water (100 mL) was applied twice daily for 50 days before starting the experiment. The treatments were assigned into four groups: 0% wastewater (control), 50% wastewater, 75% wastewater and 100% wastewater. Each treatment consisted of five replicates.

Before starting the experiment, the number of shoots and shoot length of each plant were measured and the average values were recorded. During the experimental period, each plant was applied with 100 mL of wastewater at the designated concentration twice daily. The experimental period was 5 weeks.

At the end of the experiment, shoots length was recorded and the plants were harvested by cutting the shoots at the base before cleaning and measuring their fresh weights. Roots of the plants were removed from the soils, rinsed with tap water to remove any debris before weighing. Then plant shoot and root samples were dried under 60 °C in a hot air oven for 48 hours and their dry weights were measured thereafter. Dried plant samples were subsequently used for estimation of bioactive compounds. Soil from each treatment was collected for analysis as mentioned above.

## 2.4 Estimation of bioactive compound contents

The bioactive compounds estimated in this experiment were total flavonoid and total phenolic compounds. Plant extracts were prepared based on the method described by Navarro del Hierro et al. [21], with some modification. Briefly, 0.5 g of dried plant sample was mixed with 15 ml of 95% ethanol and extraction was completed by sonication for 15 minutes at an output amplitude of 60% and temperature was maintained under 75 °C. After that, the sample was filtered to collect the extract for further analysis.

Total phenolic content was analyzed using the Folin-Ciocalteu method. An aliquot of the extract (0.2 mL) was mixed with 5 mL of deionized (DI) water before adding Folin reagent and sodium carbonate. The reaction was allowed to complete under darkness for 1.5 hours. Absorbance of the solution was measured at 725 nm to quantify total phenolic content using Gallic acid as the standard. Results were expressed as mg GAE/g dry plant.

Total flavonoid content was analyzed using aluminum nitrite colorimetric technique. An aliquot of the extract (0.3 mL) was mixed with DI water, then all reagents were added and the reaction was allowed to complete for five minutes. Absorbance was measured at 420 nm to quantify total flavonoid content using Quercetin as the standard. Results of total flavonoid contents were expressed as mg QE/g dry plant.

From the extraction methods used, the final volume of extract was in the range of 9.4–10.8 mL (average = 10.22±0.38 mL). Plant dry mass was in the range of 0.5000-0.5029 g (average = 0.5014±0.0009 g). The exact final volume and dry mass of plant used in extraction were incorporated in calculation of each content as following formulas;

$$\text{total phenolic content (mg GAE/g dry plant)} = [C \times V] / W$$

$$\text{total flavonoid content (mg QE/g dry plant)} = [C \times V] / W$$

As  $C$  = concentration of total flavonoid or total phenolic (mg/mL),  $V$  = final volume of extract (mL) and  $W$  = plant dry mass (g).

## 2.5 Statistical analysis

Means of water and soil parameters, plant length and biomass, and bioactive compound contents were compared using one-way analysis of variance (ANOVA) to determine significant differences among treatment groups. Post hoc comparisons were performed using Duncan's multiple range test to identify the significantly different groups at a significance level of 0.05 ( $p < 0.05$ ). The statistical program used to analyze the data was IBM SPSS Statistics Version 29.0.2.0 (20).

## 3. Results

### 3.1 Water quality parameters

In our study, wastewater was collected once a week and water quality were measured on days one and seven for each batch of water. Water quality parameters are presented as the average values of all measurements to enable comparison between treatments. The water parameters measured in this study were total phosphorus (TP), total Kjeldahl nitrogen (TKN), available potassium (K), chemical oxygen demand (COD), pH and electrical conductivity (EC).

The results showed that TP, COD, K and EC levels in the three treatment groups were significantly higher than those in the control group, and the concentration of each parameter reflected the treatment conditions (Table 1,  $p < 0.05$ ). In raw canteen wastewater (100% wastewater treatment), the concentrations of TP, K and COD were  $0.44 \pm 0.07$ ,  $29.06 \pm 13.15$  and  $56.03 \pm 6.14$  mg/L, respectively, and the EC was approximately  $454.17 \pm 60.80$  µs/cm. For TKN and pH values, these both parameters tended to be lower in the control group but the differences were not significant. Total Kjeldahl nitrogen was  $6.34 \pm 1.09$  mg/L and pH was  $6.42 \pm 0.28$  in raw wastewater.

**Table 1** Chemical oxygen demand (COD), Total Kjeldahl nitrogen (TKN), total phosphorus (TP), potassium (K), pH and electrical conductivity (EC) of water applied to each treatment (mean±S.E.).

| Treatment (%wastewater) | COD (mg/L)                 | TKN (mg/L)  | TP (mg/L)                 | K (mg/L)                   | pH                        | EC (µs/cm)                   |
|-------------------------|----------------------------|-------------|---------------------------|----------------------------|---------------------------|------------------------------|
| 0% (Control)            | 22.55 ± 11.27 <sup>c</sup> | 2.74 ± 0.33 | 0.04 ± 0.02 <sup>c</sup>  | 1.34 ± 0.007 <sup>b</sup>  | 7.84 ± 0.001 <sup>a</sup> | 241.27 ± 0.00 <sup>b</sup>   |
| 50%                     | 28.84 ± 5.31 <sup>b</sup>  | 5.56 ± 1.12 | 0.22 ± 0.05 <sup>bc</sup> | 7.58 ± 1.33 <sup>ab</sup>  | 6.46 ± 0.23 <sup>b</sup>  | 329.20 ± 31.46 <sup>ab</sup> |
| 75%                     | 29.29 ± 3.47 <sup>bc</sup> | 5.86 ± 0.69 | 0.34 ± 0.07 <sup>ab</sup> | 14.88 ± 4.34 <sup>ab</sup> | 6.43 ± 0.22 <sup>b</sup>  | 325.40 ± 22.40 <sup>ab</sup> |
| 100%                    | 56.03 ± 6.14 <sup>a</sup>  | 6.34 ± 1.09 | 0.44 ± 0.07 <sup>a</sup>  | 29.06 ± 13.15 <sup>a</sup> | 6.42 ± 0.28 <sup>b</sup>  | 454.17 ± 60.80 <sup>a</sup>  |

Different letters above each column indicates significant differences between treatments ( $p < 0.05$ ).

### 3.2 Soil characteristics

After harvesting, soil samples were collected for available P, available K, pH and EC measurements and the results presented in Table 2. Available P concentrations in the soils were in accordance with concentrations in wastewater applied to the treatments, although the differences were not significant. However, available K and EC increased with the concentrations applied, and the clear differences were observed between the control and the three treatments. Soil pH was not different among any of the treatment conditions.

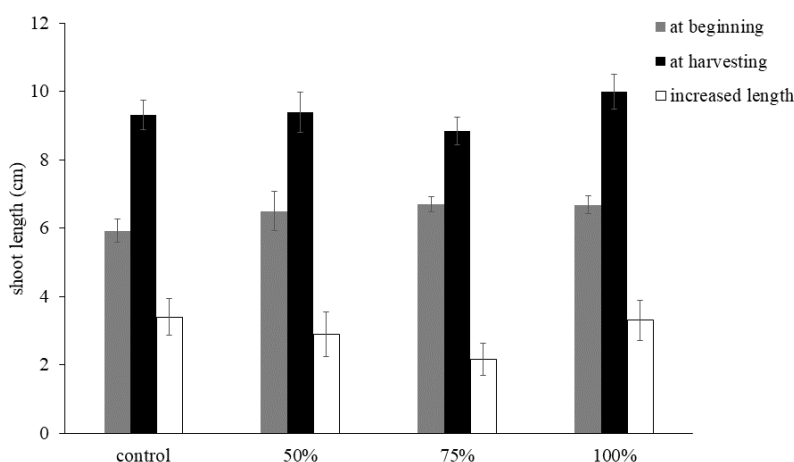
**Table 2** Available phosphorus (available P), available potassium (available K), pH and electrical conductivity (EC) of soils after harvesting (mean±S.E.).

| Treatment (%wastewater) | Available P (µg-P/g soil) | Available K (µg-K/g soil)     | pH          | EC (µs/cm)                 |
|-------------------------|---------------------------|-------------------------------|-------------|----------------------------|
| 0% (Control)            | 36.97 ± 3.69              | 1172.43 ± 28.01 <sup>c</sup>  | 5.58 ± 0.17 | 157.90 ± 3.50 <sup>c</sup> |
| 50%                     | 125.04 ± 75.52            | 4025.40 ± 34.01 <sup>b</sup>  | 5.41 ± 0.03 | 216.43 ± 5.14 <sup>b</sup> |
| 75%                     | 65.30 ± 4.84              | 4339.74 ± 240.63 <sup>b</sup> | 5.45 ± 0.02 | 218.17 ± 5.24 <sup>b</sup> |
| 100%                    | 68.48 ± 13.55             | 4912.62 ± 147.68 <sup>a</sup> | 5.52 ± 0.02 | 246.00 ± 0.29 <sup>a</sup> |

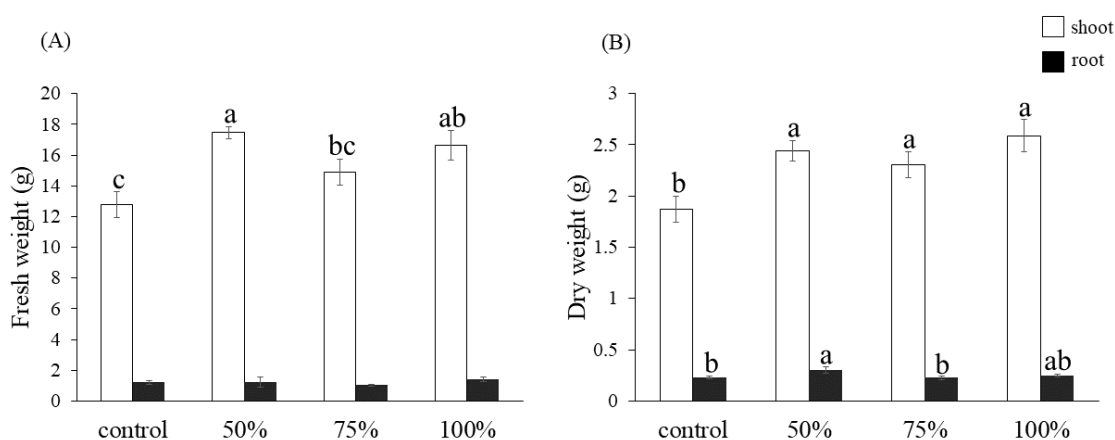
Different letters above each column indicates significant differences between treatments ( $p < 0.05$ ).

### 3.3 Effects on plant growth and bioactive compound contents

After five weeks of receiving specified concentrations of canteen wastewater, plant growth was determined by measuring their shoot length as well as shoot and root biomass. The average shoot length and elongation of plants did not differ among the treatments (Figure 1). However, both the fresh and dry weights of the shoots were significantly higher in the three treatment groups compared to the control group (Figure 2A,  $p < 0.05$ ). The results also showed that root growth, as measured by fresh and dry weights, was not affected by the treatment conditions (Figure 2B).

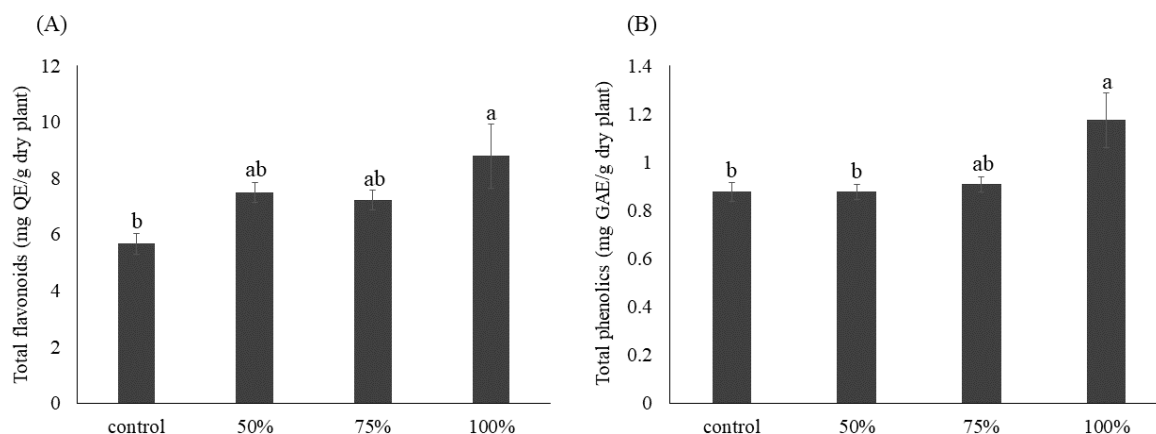


**Figure 1** Length of shoots of Brahmi grown in each treatment.



**Figure 2** Fresh weight (A) and dry weight (B) of shoots and roots of Brahmi in each treatment. Different letters above each column indicates significant differences between treatments ( $p < 0.05$ ).

In this study, we basically evaluated the effects of the treatments on bioactive compound contents by measuring total flavonoid and total phenolic contents in shoots and calculated based on plant dry biomass. Total flavonoid contents were significantly higher in the three treatments compared to the control group ( $p < 0.05$ ), and the highest value observed in the treatment of 100% wastewater ( $8.79 \pm 1.13$  mg QE/g dry plant) (Figure 3A). Total phenolic contents were not different between the control and the treatment of 50% wastewater, however, the highest total phenolic content was found in the treatment of 100% wastewater ( $1.18 \pm 0.11$  mg GAE/g dry plant) (Figure 3B), similar to the pattern observed for total flavonoid contents.



**Figure 3** Total flavonoid contents (A) and total phenolic contents (B) in above ground part of Brahmi in each treatment. Different letters above each column indicates significant differences between treatments ( $p < 0.05$ ).

#### 4. Discussion

In this study, wastewater was collected directly from effluent pipes of three restaurants in the university canteen. The characteristics of the wastewater were measured before application, and subsequently, the consequences of the treatments on soil properties, plant growth and their secondary metabolite contents were evaluated.

The results of water quality analysis showed that the canteen wastewater used in the present study contained higher amounts of elements compared to tap water. However, the concentrations of COD, TP and TKN found in our study were much lower compared to all reports cited in the paper published by Li et al. [22]. In addition, salinity hazards, as expressed by EC values, were lower, while the concentration of K was higher than that found in kitchen wastewater examined in the study by Parwin and Paul [16]. However, the EC values found in our study still fall within the range considered suitable for irrigation water according to the US Salinity Laboratory (USSL) classification system, and do not exceed the limits established by the FAO standard, as referenced by Parwin and Paul [16].

Some soil properties changed after irrigation with canteen wastewater, and most of these changes reflected the amounts of mineral contents- such as P, K and EC- in the applied wastewater. The soil pH was slightly lower, though not significantly, in the three treatments with canteen wastewater compared to the control group. This finding is consistent with results found in the study on the use of kitchen wastewater for cucumber irrigation [17]. As the authors mentioned, the significantly lower pH may result from the decomposition of organic matter. Electrical conductivity of the soils in the treatments irrigated with wastewater were higher than that of the control group. The only parameter that might pose detrimental effects on irrigated soil and plant, especially in the long term, is potassium accumulation, as this was much higher in the treated soils compared to the control group, reflecting the high concentration of K found in the wastewater.

Regarding plant growth, all treatments irrigated with canteen wastewater had higher shoot biomass compared to the control group, and no adverse effects on *B. monnieri* growth were observed. This might be due to the increased nutrient contents supplied to the soils by the wastewater that allow the treated plants to uptake and assimilate them into their biomass which in consequence helps improve plant growth. Compared between the groups of 50%, 75% and 100% wastewater treatments, growth of Brahmi appeared to be better under the 50% wastewater treatment, as the plants in this treatment achieved higher shoot fresh weight and root dry weight. Most study on Brahmi growth revealed that optimum nutrient concentrations are not always at the highest level, and supplied form could be either organic or inorganic. A report by Pierce et al. [23] showed that *B. monnieri* immobilized lower amounts of N and P compared to another wetland plants namely *Leersia oryzoides*. Poornananda et al. [24] found that *B. monnieri* grown on full-strength MS medium had the highest biomass, while plants grown on 0.25, 0.5 and 0.75 MS media had moderate levels of biomass and plants grown on the two highest

concentration of nutrients (1.5 MS and 2 MS media) had the lowest biomass. When comparing hydroponic and soil cultures, *B. monnieri* grew better in hydroponic conditions due to not only a better supply of essential nutrients [25-26] but also to greater light availability [26]. Studies on soil culture have mostly found that higher nutrient availability from farmyard manure (FYM) and vermicomposts can improve *B. monnieri* growth over NPK-fertilizers [27], and it can be grown organically using FYM fertilizer at 100% N equivalent [28]. Su et al. [29] found that increased salinity posed detrimental effects on phosphorus uptake in alfalfa plants, but the application of phosphorus fertilizer improved salt tolerance and growth of the plant. In addition, considering that *B. monnieri* is a salt tolerant species itself [1,9], this trait supports its survival under salt stress. In terms of biomass production, our results show that the slightly higher K contents in the treated soils did not pose adverse effects on Brahmi growth, however, this may pose negative impacts in the long-term application.

Increased antioxidant activity is one of the mechanisms that plants use to cope with various types of stress [30] and it is normally correlated with enhancement of secondary metabolites such as phenolics and flavonoids in many plant species [31]. Brahmi samples with high bacoside and phytochemical contents (such as total phenolics, total flavonoids and total triterpenoids) also exhibit high antioxidant activity [3]. Compared with tap water, the results from recent study shows that nutrients supplied from canteen wastewater positively affect total phenolic and total flavonoid contents together with promoting plant growth. Plant nutrients affect differently on production and activity of secondary metabolites in this plant species. Poornananda et al. [24] found that *B. monnieri* grown on full-strength MS medium had higher bacoside A contents compared to plants grown on higher (1.5 MS and 2 MS) and lower (0.25, 0.5 and 0.75 MS) concentrations of nutrients in growth media. Most studies in *B. monnieri* found that effects of differences in macronutrient supply on secondary metabolites or antioxidant activity were less noticeable than effects of stress factors. Factors such as metals and metal contamination [4-6], plant hormones [7,32], organic acids such as salicylic acid [8], changes of seasons [33], water stress [34] and salt stress [35-37] have been proved to alleviate secondary metabolite production in *B. monnieri*. From our results, we therefore suggest that enhanced phenolic and flavonoid contents might be caused by response to other factors rather than amount of nutrient supply.

Maikami et al. [35] found that sodium (Na) stress, which might occur by nutrient imbalance under inorganic fertilizer condition, was the most important factor that increased bacoside content, total phenolic content and antioxidant activity in *B. monnieri* grown with organic and inorganic fertilizers. Also, Anupama et al. [36] reported that *B. monnieri* under the highest salt concentration (400 mM NaCl) and moderate urea application (500 mg) produced the highest phenolic and flavonoid contents, respectively. Although the composition of  $\text{Na}^+$ ,  $\text{K}^+$  and other ions is defined as soil salinity, studies on salt stress are mainly focused on effects of high  $\text{Na}^+$  concentrations. However, K-induced salt stress has been reported to pose more detrimental effects on sensitive plants than  $\text{Na}^+$  at the same concentration. Nevertheless, salt-tolerant species can cope with this problem through various mechanisms such as limited  $\text{K}^+$  accumulation, maintain the ability to uptake nutrients and increase antioxidants and osmoprotectants [38]. In our study, the accumulation of  $\text{K}^+$  and other ions-implied from elevated EC- in the soil, especially in the treatment of 100% wastewater, may have caused some stress in the treated plants, resulting in higher total phenolic and total flavonoid contents than other treatments. As commonly found in other plants, increased antioxidant activity has been proved to correlate with enhancement of secondary metabolites such as phenolics and flavonoids in *B. monnieri* [3,39], we extrapolate that the plant irrigated with canteen wastewater would exhibit higher antioxidant activity than the plant irrigated with tap water.

Biological factors such as pathogens and specific types of microbes are among various factors that influence secondary metabolite production in the plant. The study by Gupta et al. [40] strongly suggests that chitinolytic microbes (*Chitiniphilus* sp. MTN22 and *Streptomyces* sp. MTN14) enhanced activities of enzymes involved in the biosynthetic pathway of bacosides and also phenolic compounds in *B. monnieri*. On the contrary, pathogen infection reduced those enzyme activities to even lower than the controlled plant. Hence, results of wastewater usage on microbial communities in the treated soils should be considered for further studies, in order to evaluate the consequences of wastewater reuses for specific plant production.

Therefore, our study demonstrates that using canteen wastewater for irrigation could help crop production without growth retardant and enhance secondary metabolites production. The underlined mechanism in this case might be due to the species ability to cope with  $\text{K}^+$  induced salt stress or ions imbalance. Nevertheless, in order to acquire knowledge on the relevant mechanisms and long-term effects, scopes of further study should be expanded to other possible stress factors such as nutrient imbalance or biotic stress, and bioactive substances and antioxidant activity responding to those factors.

## 5. Conclusions

Our results reveal that *B. monnieri* grew better when irrigated with canteen wastewater, especially at the concentration of 50% wastewater, compared to tap water. This practice could be a valuable option to support water for irrigation, either at the household or community levels, especially during the summer when fresh water

is scarce. In addition, using canteen wastewater may promote secondary metabolite production in plants, which makes incorporating wastewater management with medicinal plant cultivation even more interesting.

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## 7. Author Contributions

Thipnang, J.: Conceptualization, Formal analysis, Investigation, Visualization; Taweejun, N: Methodology, Resources; Piwpuan, N: Conceptualization, Formal analysis, Methodology, Resources, Supervision, Visualization, Writing – original draft

## 8. References

- [1] Rojas-Sandoval J. *Bacopa monnieri* (water hyssop). CABI Compendium [Internet]. 2018 [cited 2025 July 20]. Available from <https://doi.org/10.1079/cabicompendium.112638>.
- [2] Pandey AK. *Bacopa monnieri* (Linn.) Pennell - A possible plant for impossible diseases (A review). Adv Pharmacol Pharm. 2022;10(1):35-53.
- [3] Nopparat J, Sujipuli K, Ratanasut K, Weerawatanakorn M, Prasarnpun S, Thongbai B, et al. Exploring the excellence of commercial Brahmi products from Thai online markets: Unraveling phytochemical contents, antioxidant properties and DNA damage protection. Heliyon. 2024;10(2):e24509.
- [4] Krishnaraj C, Jagan EG, Ramachandran R, Abirami SM, Mohan N, Kalaichelvan PT. Effect of biologically synthesized silver nanoparticles on *Bacopa monnieri* (Linn.) Wettst. plant growth metabolism. Process Biochem. 2012;47:651–658.
- [5] Lala S. Enhancement of secondary metabolites in *Bacopa monnieri* (L.) Pennell plants treated with copper-based nanoparticles in vivo. IET Nanobiotechnol. 2020;14(1):78-85.
- [6] Patel A, Jaiswal N, Srivastava PK, Patra DD. Enhancing secondary metabolite production and antioxidants in *Bacopa monnieri* grown on tannery sludge contaminated soil. Ind Crop Prod. 2022;187:115365.
- [7] Laloknam S, Tengnoy S, Kaewkham W, Bualuang A, Boonburapong B. Promotion of Brahmi (*Bacopa monniera*) growth by Cyanobacterium *Nostoc* sp. extract. Curr Appl Sci Technol. 2023;23 (5):1-12.
- [8] Vinay CM, Shetty DN, Sanjay KU, Venkatasai NN, Rai PS. Impact of salicylic acid elicitation on the growth, photosynthesis, and metabolites of *Bacopa monnieri* (L.) Pennell growing in a hydroponic control system. Sci Hortic. 2025;342:114056.
- [9] Lastiri-Hernández MA, Álvarez-Bernal D, Cruz-Cárdenas G, Silva-García JT, Conde-Barajas E, Oregel-Zamudio E. Potential of *Epipremnum aureum* and *Bacopa monnieri* (L.) Wettst for saline phytoremediation in artificial wetlands. Water. 2023;15:194.
- [10] Aylan RA, Al-Abbawy DAH, Yaseen DA. Development of the horizontal flow wetland using palm waste biochar for greywater reclamation. J Ecol Eng. 2023;24(8):236–249.
- [11] Nagarajan P, Sruthy KS, Lal VP, Devan VP, Krishna A, Lakshman A, et al. Biological treatment of domestic wastewater by selected aquatic plants. In: 2017 International Conference on Technological Advancements in Power and Energy (TAP Energy); 2017 Dec 21-23; Kollam, India.: IEEE; 2017. p. 1-4.
- [12] Kumar J, Pandey VK, Singh R, Singh S, Malyan SK. Wastewater treatment of artificial sugar mill effluent through medicinal plant sweet flag and water hyssop on floating wetland systems. Int J Curr Microbiol App Sci. 2020;9(12):3266-3275.
- [13] Dineshkumar M, Sivalingam A, Thirumarimurugan M. Removal of lead from battery industry effluent using *bacopa monnieri* (waterhyssop). Adv in Nat Appl Sci. 2016;10(2):177-182.
- [14] Dineshkumar M, Sivalingam A, Thirumarimurugan M. Phytoremediation potential of *Bacopa monnieri* in the removal of heavy metals. J Environ Biol. 2019;40:753-757.
- [15] Khan MM, Siddiqi SA, Farooque AA, Iqbal Q, Shahid SA, Akram MT, et al. Towards sustainable application of wastewater in agriculture: A review on reusability and risk assessment. Agronomy. 2022;12:1397.
- [16] Parwin R, Paul KK. Assessment of kitchen wastewater quality for irrigation. Appl Water Sci. 2020;10:240.

- [17] Abegunrin TP, Awe GO, Idowu DO, Onigbogi OO, Onofua OE. Effect of kitchen wastewater irrigation on soil properties and growth of cucumber (*Cucumis sativus*). J Soil Sci Environ Manage. 2013;4(7):139-145.
- [18] Olsen SR, Cole V, Watanabe FS, Dean LA. Estimation of available phosphorus in soil by extraction with sodium bicarbonate. United States Department of Agriculture Circ. 93;1954.
- [19] Land development department. Guide for analysis of soil samples, water, fertilizers, crops, soil improvement materials and analysis to verify product standards. Office science for land development. Bangkok; 2003.
- [20] AOAC Peer Verified Methods Program. Manual on Policies and Procedures. AOAC International. Gathersburg; 1998.
- [21] Navarro del Hierro J, Herrera T, García-Risco MR, Fornari T, Reglero G, Martin D. Ultrasound-assisted extraction and bioaccessibility of saponins from edible seeds: quinoa, lentil, fenugreek, soybean and lupin. Food Res Int. 2018;109:440–447.
- [22] Li LW, Liu H, Deng L, Zhang Y, Ma Y, Chen Y. Recent advances in treatment refinement of kitchen digested wastewater: Feasibility, prospects, and technicalities. Water Cycle. 2024;5:20–30.
- [23] Pierce SC, Pezeshki SR, Larsen D, Moore MT. Hydrology and species-specific effects of *Bacopa monnieri* and *Leersia oryzoides* on soil and water chemistry. Ecohydrol. 2009.
- [24] Poornananda MN, Godbole M, Nagella P, Murthy HN. Influence of different media, medium strength and carbon sources on adventitious shoot cultures and production of bacoside A in *Bacopa monnieri* (L.). Ceylon J Sci. 2017;46(4):97-104.
- [25] Maneeply C, Sujipuli K, Kunpratun N. Growth of Brahmi (*Bacopa monnieri* (L.) Wettst.) by NFT and DFT hydroponic systems and their accumulation of saponin bacosides. NU Int J Sci. 2018;15(2):114-124.
- [26] Kaur P, Chhina GS, Kaur A. Response of brahmi (*Bacopa monnieri* L.) cultivars to different growing media in hydroponic system. Eco Env & Cons. 2022;28(Suppl. Issue):S215-S219.
- [27] Vikram N, Sarkar NC, Sridhar R, Ghosh B, Doon MK, Bochalya KS. Growth attributes and economics of Brahmi (*Bacopa monnieri* L.) cultivation influenced by organic sources of nutrients. Int J Econ Plants. 2023;10(4):302-307.
- [28] Smitha GR, Kalaivanan D, Sujatha S. Influence of shade and organic nutrition on growth, yield and quality of memory enhancing herb, Brahmi (*Bacopa monnieri* L.). Med Plants. 2021;13(1):72-90.
- [29] Su R, Zhang Z, Chang C, Peng Q, Cheng X, Pang J, et al. Interactive effects of phosphorus fertilization and salinity on plant growth, phosphorus and sodium status, and tartrate exudation by roots of two alfalfa cultivars. Ann Bot. 2022;129(1):53-65.
- [30] Jan R, Asaf S, Numan M, Lubna, Kim K-M. Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. Agronomy. 2021;11:968.
- [31] Esmaeili AK, Taha RM, Mohajer S, Banisalam B. Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *In Vivo* and *In Vitro* grown *Trifolium pratense* L. (Red Clover). BioMed Res Int. 2015.
- [32] Masoumian M, Zandi M. Secondary metabolite of *Bacopa monnieri* under plant growth regulators treatment on fibroblast and cancer cells. Gene Cell Tissue. 2025;12(1):e151058.
- [33] Phrompittayarat W, Jetiyanon K, Wittaya-areekul S, Putalun W, Tanaka H, Khan I, et al. Influence of seasons, different plant parts, and plant growth stages on saponin quantity and distribution in *Bacopa monnieri*. Songklanakarin J Sci Technol. 2011;33(2):193-199.
- [34] Singh V, Jain N, Shri R. Effect of abiotic factors on bacoside A content, acetylcholinesterase inhibitory and antioxidant activities of *Bacopa monnieri* (L.) Wettst. J Appl Pharm Sci. 2021;11(Suppl1):063-070.
- [35] Maikami M, Kanto U, Sonjaroon W, Promdang S. Comparative effects of organic and inorganic fertilizers on growth, antioxidant activity and bacoside content in *Bacopa monnieri* (L.) Wettst. Int J Agric Technol. 2022;18(4):1683-1700.
- [36] Anupama VC, Sreenand TM, Revathi P. Impact of chemical stress on secondary metabolites of BACOPA MONNIERI (L.) WETTST. K R J. 2024;11(1):15-24.
- [37] Ahire ML, Walunj PR, Kishor PBK, Nikam TD. Effect of sodium chloride-induced stress on growth, proline, glycine betaine accumulation, antioxidative defence and bacoside A content in in vitro regenerated shoots of *Bacopa monnieri* (L.) Pennell. Acta Physiol Plant. 2013;35:1943–1953.
- [38] Pantha P, Oh D-H, Longstreth D, Dassanayake M. Living with high potassium: Balance between nutrient acquisition and K-induced salt stress signaling. Plant Physiol. 2023;191:1102–1121.
- [39] Jauhari N, Bharadwaj R, Sharma N, Bharadvaja N. Assessment of bacoside production, total phenol content and antioxidant potential of elicited and non-elicited shoot cultures of *Bacopa monnieri* (L.). Environ Sustain. 2019.

- [40] Gupta R, Singh A, Srivastava M, Singh V, Gupta MM, Pandey R. Microbial modulation of bacoside A biosynthetic pathway and systemic defense mechanism in *Bacopa monnieri* under *Meloidogyne incognita* stress. Sci Rep. 2017;7:41867.