Effects of Different Types of Organic Fertilizer on Biomass Yield, Bioactive Compounds and Heavy Metals Contents of *Phyllanthus Niruri*

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Abstract. This research aimed to study the effects of different types and rates of organic fertilizer on biomass yield, bioactive compounds, and heavy metals content of *Phyllanthus niruri*. Two types of organic fertilizers (animal-based and plant-based) with five different rates (0, 100, 200, 300, and 400 kg N ha⁻¹) were used in this study. There was an interaction between fertilizer types and rates on biomass yield, heavy metals content, and bioactive compounds. Plants are grown with 400 kg N ha⁻¹ of animal-based fertilizer, and plant-based fertilizer produced the highest fresh biomass. However, phyllanthin and hypophyllanthin concentrations of *P. niruri* were not affected by applying animal-based and plant-based fertilizers at all rates. In addition, Cd and Pb concentrations also decreased with the increasing rate of organic fertilizer and were below the Malaysia Permissible Levels (MPLs). Therefore, animal-based fertilizer at 400 kg N ha⁻¹ was selected as the optimum rate due to its reduced heavy metal concentration in plants and higher phyllanthin and hypophyllanthin yield.

Keywords: Organic fertilizer, Biomass yield, Bioactive compounds, Heavy metals.

1. Introduction

In the Third National Agricultural Policy (DPN3) and Economic Transformation Programme (NKEA), herbs have been identified as potential industrial crops in Malaysia. One of the potential herbs is *Phyllanthus niruri*. *Phyllanthus niruri* L. (Euphorbiaceae), locally also known as dukung anak, is a small annual herb found throughout the tropical and subtropical regions of both hemispheres. It is a worldwide distributed tropical plant acclaimed for its versatile medicinal properties. It has been traditionally used as a remedy against jaundice, syphilis, constipation, gonorrhea, kidney disorders [1,2], skin ulcer, and diabetes [3]. Several different phytochemicals present in this plant, such as phenol, flavonoid, terpenoid, and saponin, lead to antioxidant and antimicrobial activity [4]. Current researches show that Formononetin-7-O-Glucuronide from *Phyllanthus niruri* could serve as a potential drug candidate to control and fight against COVID-19 [5]. Catechin and Quercetin in *Phyllanthus niruri* also inhibit the expression of TNF-α, IL-1, IL-6, and iNOS, thus inhibiting the process of excessive inflammation and functioning as an immunomodulatory for Covid-19 [6].
On the other hand, the herbal business was seriously affected by a scarcity of raw materials and a lack of expertise in related herbs. Furthermore, Ultisols and Oxisols [7] account for 72 percent of Malaysian soils, highly weathered acidic soils known as acid sulfate soils that produce low agricultural production due to low pH, aluminum, and manganese toxicity. As a result, organic amendments must be incorporated.

Nowadays, wastes generated from the poultry farm industry and other agricultural activities are increasing. These wastes can be converted into valuable fertilizer to supply an adequate amount of nutrients for plant growth. People are becoming more interested in using organic resources as fertilizers for crop production to ensure long-term crop output [8,9]. Vegetable producers often use chicken dung as a fertilizer because it provides essential plant nutrients (N, P, and K) [10,11]. It provides a source of all necessary macro and micronutrients in accessible forms during mineralization, thus enhancing the physical and chemical qualities of soils [12]. Application of organic fertilizers, produced mainly by composting process, could increase soil pH and ameliorate slightly acidic tropical soils to improve crop production [13]. Organic addition has consistently generated good impacts on soil nutrients, physical soil conditions, and soil biological activity [14], thereby improving plant health.

Organically farmed herbs, particularly for medicinal uses, are in high demand right now. This study aimed to produce high-quality, safe *Phyllanthus niruri* for human consumption while also improving soil health and fertility. As a result, this research aims to see how different types and rates of organic fertilizers affect biomass yield and heavy metals production.

## 2. Materials and Method

A field experiment was conducted in the Organic Unit, Universiti Putra Malaysia. Two types of fertilizer, plant-based (P.L.) and animal-based (AN) were used in this study with five rates of organic fertilizer: 0, 100, 200, 300, and 400 kg N equivalent ha⁻¹. So, there were ten treatments, which were T1: PL0, T2: PL100, T3: PL200, T4: PL300, T5: PL400, T6: AN0, T7: AN100, T8: AN200, T9: AN300, and T10: AN400. The fertilizers were calculated based on N content where AN and P.L. fertilizers contained 2.57% and 2.52%, respectively. The characteristics of the soil, AN, and P.L. used in this study were given in Table 1. The experiment was laid out in a split-plot design where the main plot was types of fertilizers, and the subplot was the rates of fertilizers. There were four replications and 40 experimental units.

### 2.1 Chemical properties of the soil before planting, rice husk biochar (B.C.), chicken manure (CM), animal-based (AN), and plant-based (P.L.) used in this study.

| Chemical properties | Soil | BC | CM | AN | PL |
|---------------------|------|----|----|----|----|
| pH (H₂O)            | 4.77 | 9.66 | 9.20 | 7.25 | 7.92 |
| Total C (%)         | 2.31 | 8.09 | 14.47 | 29.04 | 42.09 |
| Total N (%)         | 0.201 | 0.107 | 1.086 | 2.57 | 2.52 |
| Available P (mg kg⁻¹) | 15.62 | -     | -    | -    | -    |
| Total P (%)         | -    | 2.10 | 1.68 | 1.07 |      |
| Total K (%)         | -    | 1.73 | 1.72 | 0.86 |      |
| Total Ca (%)        | -    | 4.78 | 3.22 | 0.64 |      |
| Total Mg (%)        | -    | 0.55 | 0.51 | 0.38 |      |
| CEC (cmol(+), kg⁻¹) | 6.19 | -    | -    | -    | -    |
| Exch. K (cmol(+), kg⁻¹) | 0.44 | -    | -    | -    | -    |
| Exch. Ca (cmol(+), kg⁻¹) | 2.58 | -    | -    | -    | -    |
| Exch. Mg (cmol(+), kg⁻¹) | 0.20 | -    | -    | -    | -    |
| Cd (µg kg⁻¹)        | 63.12 | 62.65 | 310.69 | 432.90 | 94.37 |
| Pb (mg kg⁻¹)        | 12.72 | 14.01 | 37.76 | 6.80 | 17.41 |
| As (µg kg⁻¹)        | N.D. | -    | -    | 71.6 | ND   |
2.1 Plot preparation and transplanting
An area (10 m x 23 m) was plowed and rotavated. Then, 40 beds of 1 m x 2 m each was prepared for one bed per experimental unit. A week before transplanting, the selected rate of soil amendment BC (10 t ha\(^{-1}\)) and CM (2.5 t ha\(^{-1}\)) from the previous experiment was incorporated into the soil. Then, the organic fertilizers were applied and incorporated into the soil according to the treatments on each plot a day before transplanting. After that, the silver shine was used for mulching the soil to control weeds. Finally, holes for planting were made on the silver shine following the planting distance of 30 cm x 30 cm. The plants were watered twice a day using a drip irrigation system.

2.2 Harvesting and soil and plant sampling
Plants were harvested by cutting the top shoot 5 cm above the soil surface on the 8\(^{th}\) week (56 days) after transplanting as suggested by Malaysia Agriculture Research and Development Institute (MARDI). The total fresh weight of the plants per plot was recorded, and five plants were selected for tissue analysis. The plants were oven-dried slowly for a week with a low temperature of 45 °C to prevent loss of biochemical. After a week, the dried plant samples were ground for tissue analysis. A composite soil sample from each plot was taken using an auger at 0-15 cm depth from five points. The soil was mixed and air-dried in the glasshouse for a week, ground, and sieved using a 2 mm mesh sieve for soil chemical analysis.

2.3 Chemical analysis
Soil chemical properties for pH was measured using a pH meter with the suspension of 1:2.5 (w/v) soil: distilled water, total carbon, and nitrogen were determined on dried samples using elemental analyzer LECO TruMac CNS Analyzer, available phosphorus was determined using Bray II Method, cation exchange capacity and exchangeable bases (K, Ca, Mg) were determined using leaching method (Baruah and Barthakur, 1997). Plant nutrients uptake (N, P, K, Ca, Mg) were determined using the wet digestion method (Miller and Miller, 1948), and concentrations of heavy metals (Cd, Pb, As, Hg) were determined using the dry ashing method (Jones, 1984). Extract *Phyllanthus niruri* was prepared to determine bioactive compounds (phyllanthin and hypophyllanthin) and determined using an Agilent 1200 Series HPLC system at an excitation wavelength of 280 nm.

2.4 Statistical analysis
Statistical analysis was performed using SAS version 9.4 (SAS Institute, Inc., Cary, N.C., USA.). A two-way ANOVA was conducted to determine the effects of the fertilizer rates and types and their interactions on biomass yield, nutrients uptake, heavy metals content and bioactive compounds measured as variables, and types and rates of organic fertilizer as factors. Whenever ANOVA revealed significant interaction effects among factors, data were analyzed using the least significant difference (LSD) at P<0.05 to determine the differences among treatment combinations. A regression analysis was also conducted when there were significant interactions between organic fertilizers' types and rates to predict *Phyllanthus niruri* growth characteristics (biomass yield, nutrients uptake, heavy metals content, and bioactive compounds) response towards different types and rates of organic fertilizer treatments.

3. Results

3.1 Biomass yield
Significant interaction effects between fertilizer types (F.T.) and fertilizer rates (F.R.) on biomass yield of *P. niruri* were observed. Figure 1 shows a significant linear response of dry weight shoot biomass of *P.*
niruri to rate of AN fertilizer but no significant response trend with P.L. fertilizer. Treatment with P.L. fertilizer produced 839.87 g plot$^{-1}$ while AN fertilizer produced 625.20 g plot$^{-1}$ with a 400 kg N ha$^{-1}$ which is about 25% higher than AN fertilizer and 42% than the control treatment. Figure 2 shows P. niruri that has been harvested after the 8th week of transplanting. It showed that the plant growth increased with the increasing rates of fertilizers for AN and P.L. fertilizer. Thus, P.L. fertilizer seems to have a better plant growth performance effect compared to AN fertilizer.

![Figure 1. Relationships between different types (AN, P.L.) and rates (0, 100, 200, 300, and 400 kg N ha$^{-1}$) on dry weight shoot biomass of P. niruri. The solid line indicates a significant regression trend at P<0.05 (n=4).](image)
Figure 2. Plants that had been harvested according to the different types and rates of organic fertilizer treatments, animal-based fertilizer (AN (a)) and plant-based fertilizer (P.L. (b)).

Plant nutrients uptake (N, P, K)
The uptake of macronutrients has significant interaction effects between fertilizer types and rates (F.T. x F.R.) (Figure 3). P.L. fertilizer showed a better response in plant nutrient uptake compared to AN fertilizer. Application of P.L. fertilizer uptake 43% N, 31% P, and 29% K higher than AN fertilizer at rate 400 kg N ha-1 and 58% N, 45% P, and 55% K compared to control treatment.
Figure 3. Relationships between different types (AN, P.L.) and rates (0, 100, 200, 300, and 400 kg N ha$^{-1}$) on N, P, and K uptake. The solid line indicates a significant regression trend at P<0.05 (n=4).

3.2 Heavy metal concentrations (Cd, Pb, As, Hg)  
Cadmium (Cd) and lead (Pb) concentrations were affected by interaction effects of F.T. x F.R. However, arsenic (As) and mercury (Hg) were not detected in the plant tissue of *P. niruri*. P.L. and AN fertilizers showed negative quadratic responses with the increasing fertilizer rates for Pb, while for Cd, P.L. fertilizer showed a negative quadratic response, and AN fertilizer showed a negative linear response with the increasing rates of organic fertilizers. Figure 4 shows that Pb concentration was below the Malaysian
Permissible Level (MPLs), 10.0 mg kg\(^{-1}\), while Cd concentration was slightly higher than MPLs level. Although Cd concentration was still above the MPLs, the concentrations decreased with the increasing rates of fertilizers for AN and P.L. This shows that applications of organic fertilizers can reduce the concentrations of Cd and Pb in a shoot of *P. niruri*.

![Graph showing relationships between different types (AN, P.L.) and rates (0, 100, 200, 300, and 400 kg N ha\(^{-1}\)) of organic fertilizer on Pb and Cd concentration of *P. niruri*. The solid line indicates a significant regression trend at P<0.05 (n=4). Maximum Permissible Limits (MPLs) by Malaysian Herbal Monograph, Pb: 10.0 mg/kg and Cd: 0.3 mg/kg.]

**Figure 4.** Relationships between different types (AN, P.L.) and rates (0, 100, 200, 300, and 400 kg N ha\(^{-1}\)) of organic fertilizer on Pb and Cd concentration of *P. niruri*. The solid line indicates a significant regression trend at P<0.05 (n=4). Maximum Permissible Limits (MPLs) by Malaysian Herbal Monograph, Pb: 10.0 mg/kg and Cd: 0.3 mg/kg.
3.3 Bioactive compounds (phyllanthin and hypophyllanthin)

Figure 5 phyllanthin and hypophyllanthin contents consistently followed linear trends due to different rates of fertilizers, particularly for plants applied with AN fertilizer. However, there was an effect of a significant interaction of F.T. x F.R., which indicated that application of organic fertilizer up to 400 kg N ha\(^{-1}\) tends to increase phyllanthin and hypophyllanthin contents. Phyllanthin and hypophyllanthin concentrations showed positive quadratic relationships with the rates of fertilizers (Figure 6). However, there was no significant quadratic response for AN fertilizer for both compounds. Application of AN and P.L. fertilizers showed a similar response for phyllanthin and hypophyllanthin concentrations and contents. However, when phyllanthin and hypophyllanthin were converted to yield, the value increased as the value was multiplied with the biomass yield of \textit{P. niruri}. This showed that the concentration of phyllanthin and hypophyllanthin were not affected by the increasing rates of fertilizers.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure5.png}
\caption{Relationships between different types (AN, P.L.) and rates (0, 100, 200, 300, and 400 kg N ha\(^{-1}\)) on phyllanthin yield (a) and hypophyllanthin yield (b) of \textit{P. niruri}. The solid line indicates a significant regression trend at P<0.05 (N=4).}
\end{figure}
Figure 6. Relationships between different types (AN, P.L.) and rates (0, 100, 200, 300, and 400 kg N ha⁻¹) on phyllanthin concentration (a) and hypophyllanthin concentration (b) of *P. niruri*. The solid line indicates a significant regression trend at *P*<0.05 (n=4).

4. Discussions

Application of AN and P.L. fertilizers at a 400 kg N ha⁻¹ improved the biomass yield of *P. niruri*. The increase in biomass yield of *P. niruri* might be due to the high concentration of N, P, K, Ca, and Mg in organic fertilizer (Figure 3). Organic fertilizers stimulate plant growth and supply nutrients needed by plants to increase biomass yield. Khaitov et al. [15] and Kugbe et al. [16] mentioned that plant growth performance and crop production increased with the incorporation of organic manure. Besides, Kyi et al. [17] observed an increment in growth, dry weight accumulation, and crop yield. Pegoraro et al. [18] reported an increase in dry matter weight of *M. piperita* and *M. arvensis* due to nutrient availability and uptake. Iqbal et al. [19] reported that incorporating organic manure in paddy cultivation improved the leaf
area causing higher photosynthesis and higher dry weight biomass yield. Manure can keep the nutrient for the plant, and during the growth periods, the nutrients decompose and are slowly released to be taken up by the plant [20]. This helps the plants to gain higher seed and biomass yields. The control treatment without N fertilizer has low biomass yield because low micronutrients are supplied for their growth. When N is insufficient, it will cause the plant to become smaller, and thus, translocation of carbohydrates to other plant parts will be affected [21]. Plant nutrients uptake of \textit{P. niruri} also increased with the application of both fertilizers. Figure 3 showed that plant N, P, and K response linear trend with AN and P.L. fertilizers. The plant's higher N, P, and K uptake could improve the biomass yield [22]. Nitrogen in organic fertilizer bound with organic materials and soil microorganisms help to convert the nutrient into available form for plant uptake [23]. Based on biomass yield and bioactive compounds, the plants treated with AN fertilizer have better yield because P, K, Ca, and Mg was higher in manure than plant-based fertilizer. Besides, nutrients in AN fertilizer decompose quickly and can be absorbed by plant roots compared to P.L. fertilizer that needs more time to decompose due to higher lignin content. Different types of organic fertilizers with different heavy metal concentrations affect the Cd and Pb in the shoot. From this study, P.L. and AN fertilizer decreased the Cd and Pb concentrations in a shoot of \textit{P. niruri} compared to the control treatment. AN fertilizer and P.L. fertilizer could reduce the concentration of Pb in a shoot of \textit{P. niruri}, and the concentration for Pb is more petite than MPLs level.

Although Cd concentration is still above the MPLs level, it is proven that organic fertilizer could reduce the heavy metal concentration in plants at increasing rates. The insoluble Cu-organic and Pb-organic complexes formed in the soil could decrease the metals in the shoot [24]. Also, the increased biomass with increasing fertilizer rates further reduces heavy metal concentrations in plant tissue. Soil chemical properties treated with different types and rates of organic fertilizer were improved after the 8th week of transplanting. Plant-based fertilizer affected the soil total N, available P, CEC, and exchangeable bases, while AN fertilizer affected the soil pH, total N, total C, available P, CEC, and exchangeable bases. The improvement in soil chemical properties might be due to microbial activities that help the root development and growth of shoots and transform the nutrient so that it becomes available for plants [25]. High biomass yield with manure fertilizer is because of an increase in root growth and absorption of nutrients that helps to increase the plant yield. Organic fertilizer can provide optimal conditions for plant growth by increasing the soil's chemical properties and continuously supply nutrients during the growing period. Furthermore, adding organic matter to the soil improves the soil structure, making it more favorable to healthy root formation [26] and microbial mineralization. As a result of organic fertilizers, plants can obtain nutrients for a better yield [27].

In this study, the soil pH was affected by the application of both fertilizers. This shows that organic fertilizers could alleviate the soil's acidity by increasing the soil organic matter, improving soil physical properties, and promoting the soil base saturation. Total N was highest with AN fertilizer because manure has lower ammonia losses and is easy to accumulate in the soil. Results for soil nutrient contents were significantly affected by different types of fertilizer. AN fertilizer showed a better response in available P and exchangeable bases with application rates of 300 kg N ha\(^{-1}\) and 400 kg N ha\(^{-1}\) than P.L. fertilizer. An increase in biomass yield with an adequate amount of N fertilizer also increases the phyllanthin and hypophyllanthin contents as the content obtained by multiplying with the dry weight shoot biomass of \textit{P. niruri}. This shows that N has an essential function in plant growth which is also involved in the production content of bioactive compounds [28]. Besides, the available micronutrients present in organic fertilizer might also contribute to the higher production of bioactive compounds content. Ibrahim et al. [29] reported that organically grown plants have micronutrient concentrations compared to plants planted using the conventional method. Nitrogen is an essential nutrient for plants because it helps in increasing the levels of photosynthesis that boost plant production. In this process, the growth hormone stimulator was transferred to the apex and laterals, increasing the production of higher nitrogen levels [30].
Phyllanthin and hypophyllanthin concentrations were not affected with the application of both fertilizers at all rates. However, some studies reported that the application of manure could increase the concentration of the bioactive compounds in herbs. Gerami et al. [31] stated that essential oil content and yield of *Origanum vulgare* plant as the amount of manure application increased.

5. Conclusion
In conclusion, the application of 300 kg N ha$^{-1}$ and 400 kg N ha$^{-1}$ produced the highest shoot biomass D.W. compared to control. For bioactive compound concentrations, the application of 400 kg N ha$^{-1}$ produced the highest phyllanthin and hypophyllanthin contents. Although the concentrations for phyllanthin and hypophyllanthin had no significant difference between treatments, total phyllanthin and hypophyllanthin contents were significantly higher in treatment 400 kg N ha$^{-1}$ application of organic fertilizer. The plant nutrient uptake of *P. niruri* also increased with the increasing fertilizers up to 400 kg N ha$^{-1}$ of organic fertilizer. As the rates of organic fertilizers increased, the heavy metal concentrations of *P. niruri* decreased. Based on all the results, AN fertilizer at 400 kg N ha$^{-1}$ was selected as the optimum rate for maximum improvement in biomass yield, bioactive compounds yield, and lower heavy metals uptake in plant tissues. However, Cd concentration was slightly higher than the MPL, which could be due to heavy metals in the soil. Thus, it is essential to check the soil for heavy metal contents before cultivating herbs for safe production.

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