Journal of Agriculture and Crops
ISSN(e): 2412-6381, ISSN(p): 2413-886X
Vol. 5, Issue. 11, pp: 218-225, 2019
URL: https://arpgweb.com/journal/journal/14
DOI: https://doi.org/10.32861/jac.511.218.225

Academic Research Publishing Group

Original Research Open Access

Leaf Gas Exchange Attributes and Quality Performance of Kacip Fatimah (*Labisia Pumila* Blume) Under Different Sources of Organic and Inorganic Fertilizers

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Abstract

An experiment was designed with Randomized Complete Block Designed to investigate the effects of organic and inorganic nitrogen fertilizer at 90 kg N/ha on growth and leaf gas exchange in two varieties of *Labisia pumila* at 15 weeks after transplanting (WAT). In this study total biomass and photosynthesis (A) were significantly ($P \leq 0.05$) different among all factor. The results indicated that chicken manure enhanced the net photosynthetic rate (11.10 μmol/m²/s), stomatal conductance (0.40 mmol/m/s) and transpiration rate (5.30 mmol/m²/s) of L.pumila significantly after 31 weeks of planting. Chicken manure and Gobi indicated higher accumulation on total phenolic and total flavonoid compared to NPK green and control. Organic fertilizers (chicken manure and Gobi) were positively influenced the leaf gas exchange and growth of L.pumila as compared to the NPK green and control. This study proved that organic fertilizer can produce a quality of L. pumila compared to NPK green. Based on the principle of organic fertilizer which is slowly release from organic fertilizer will limit the nutrient availability for plant growth and allocate more carbon to produce secondary metabolites.

Keywords: Labisia pumila; Leaf gas exchange; Nitrogen organic; Fertilizers; Secondary metabolites.

1. Introduction

Labisia pumila Benth is a medicinal herb came from Myrsinaceae family that is naturally grown in Malaysian forest and normally distributed the tropics and sub-tropics [1]. It is popularly known and named as Kacip Fatimah, Selusoh Fatimah, Rumput Siti Fatimah, Akar Fatimah, Tadah Matahari, Bunga Belangkas Hutan dan Pokok Pinggang [1, 2]. This plant is a shade loving plant and grows well under thinned forest with 70% to 90% shade [1]. Enhancing quality and growth rates of *L. pumila* has become one of the most important herb aims for herbal Malaysian herbal industry. *Labisia pumila* has drawn special interest because of its bioactivity that promotes health in women. Traditionally in Malaysia, the leaves of *L. pumila* have generally been used by Malay women for generations. The leaves of *L. pumila* are claimed to have health benefits for women, especially in the reproductive system. The leaf extracts contain secondary metabolites which include flavonoids, phenolics, and antioxidants that are valuable in promoting health in women.

Manipulation of nutrients in the soil can influence patterns of photosynthate especially in analyzing productivity for food, fuel and many other useful products for man [3]. The quality and quantity of incident light, temperature and availability and utilization of nutrient (fertilizers) in the soil are main factors that affect plant productivity. Plants have a variety of pigments that responsible for trapping the sun's energy for primary production and without which growth and biomass accumulation in plants could be adversely affected or cease completely. The productivity of *L.pumila* is a function of the production of assimilates by photosynthesis and translocation of assimilates to plant sinks [1]. Application of nitrogen fertilizer can play a major role in photosynthetic activity and yield capacity

The secondary metabolites production can be influenced by fertilizer management and environmental conditions. Cultivation of *L. pumila* varieties (*pumila* and *alata*) under favorable environmental conditions with suitable use of fertilizers can give good responses in *L. pumila* in terms of quality and growth. Since fertilization factor plays an important role in the plant's secondary metabolites production, it was hypothesized that complete organic or inorganic fertilization would increase the plant phenolic content. In this experiment, the response in the production of secondary metabolites and growth may be dependent on the type and source of nitrogen fertilizer. Currently, studies have shown that application of organic fertilizer (chicken manure) at 90 kg N/ha increased the production of total phenolics and total flavonoids [4], and the N rate excess of 90 kg N/ha reduced the level of secondary metabolites. Previous research also documented that lycopene tomatoes produced higher nitrogen

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fertilizer at 90 kg N/ha with Caralampides [5]. However, the effect of different sources of complete organic fertilizer in enhancing secondary metabolites production and plant growth of *L. pumila* has not been determined yet.

Organic fertilizers are known to be slow release fertilizers because the nutrients are released gradually. Organic fertilizer has also been the preferred fertilizer for herbs cultivation due to the high demand for organic medicinal plants has increased in the last decades. Chicken manure is the most commonly used form of organic matter used due to its high N content compared to other manure waste [6] and easily available. Besides the use of chicken manure has been practiced by farmers for centuries and in the recent years in Malaysia.

Recently, Gobi, which is a coffee-based commercially prepared organic fertilizer, has become popular among the farmers. Its nutrient composition is more stable than organic manures. The NPK composition of Gobi contains 8% of nitrogen, 8% of phosphorus and 8% of potassium, thus making it more reliable and ready to use. Whereas, nutrient composition of chicken manure is lesser than Gobi which is 1.7% of N, 4.1% of P and 5.3% of K and always variable. Gobi also comparing cheaper than chicken manure based on plant herbal requirement. However, whether Gobi is a better choice to use on *Labisia pumila* in order to enhance not only growth but also its pharmacological quality needs to be investigated. Previous research documented that *Cosmos caudatus* contained higher ascorbic acid when fertilized with Gobi compared to NPK green [7].

On the other hand, inorganic fertilizers are known as fast release fertilizers due to their nutrients that can be readily absorbed by plants as compared to the organic fertilizers. A compound NPK fertilizer (15:15:15) containing three main elements (nitrogen, phosphorus and potassium) essential in plant nutrition, is also commonly used by the growers. It is compound fertilizer with a high nutrient composition which easies to apply and able to enhance growth and biomass is stable, whether NPK green can also enhance the secondary metabolite in *L. pumila* need to be examined.

Examining these different types of commonly used complete organic and inorganic fertilizers as the source of nitrogen may give better alternative options to better not only herbal plant growth but also the quality. Therefore, this study was aimed to elucidate the responses of organic and inorganic fertilization at 90 kg N/ha toward the total biomass of *L. pumila,* lea*f* gas exchange and total plant secondary metabolites. This study was designed to determine the most suitable source of nitrogen either organic or inorganic fertilizers in enhancing *L. pumila* varieties growth and it pharmaceutical quality (secondary metabolite).

The hypothesis attempts in this study are based on carbon-nitrogen balance hypothesis. The limited nitrogen for plant uptake can result indirectly producing carbon-rich metabolites such as total phenolic and total flavonoid at the optimum time. Organic fertilizer is assumed can affect the secondary metabolites content compared to inorganic fertilizer due to N limitation inorganic fertilizer. While applied of inorganic fertilizer improves the synchrony between the supply and demand of N.

2. Material and Method

2.1. Experiment Treatment

A glasshouse experiment was conducted at Field 2, Faculty of Agriculture Glasshouse Complex II, Universiti Putra Malaysia. Seedlings of the two *Labisia pumila* varieties were collected from a place of origin at Hulu Langat Selangor and raised under acclimatized glasshouse conditions for 1 month before used in the research. A four-month-old of two varieties of *L. pumila* seedlings were treated with four nitrogen sources (chicken manure, Gobi, NPK green and control) at two harvesting times (5 and 15 weeks after transplanting). The polybags sized 36 cm x 36 cm was filled with 5: 5: 1 mixture soilless media of rice husk, cocopeat and chicken manure (1.7% of N). 5 liters of soilless media were filled in each polybag. The soilless media and chicken manure were analyzed a month before transplanting of the seedlings.

2.2. Experimental Layout

This three factorial glasshouse experiment consisted of two varieties of *L. pumila* (*alata* and *pumila*), four nitrogen sources (Gobi, chicken manure, NPK green and control) and two harvesting times (5, and 15 weeks after transplanting) with combined treatment of 16. *Labisia pumila* varieties were arranged in a split plot with the main plot, nitrogen sources as the sub-plots and harvest time as the subplot, combined treatment were blocked three times with two sampling unit per treatment at each harvest. Each combined treatment consisted of 6 sample plants totaling 96 plants (6 x 16 combined treatments) used in the experiment.

2.3. Total Biomass (G/plant)

Harvested plants were divided into stems, leaves, and roots and the oven-dried biomass was determined. The soilless media around the plant was dug to extract the roots and whole plant. The plant roots were cleaned first and dried on newspaper. The plant parts were placed in paper bags and plant dry biomass was determined from oven dried at 60ºC until constant weight was reached for three days. Total plant biomass was recorded using an electronic weighing scale (Model BP303-S/ Mettler Toledo/0.001gm -310 gm).

2.4. Leaf Gas Exchange

The plant samples were tested using LICOR 6400 was 12 plants per treatment. The gas exchange measurements were carried out using a closed system with an infra-red gas analyzer LICOR 6400 Portable Photosynthesis system (IRGA: LICOR Inc. Nebraska, USA). The instrument was warmed and calibrated for 30 minutes with ZERO IRGA mode before use. Two steps are required in the calibration procedures which are the initial zeroing process for the built-in flow meter and zeroing process for the infrared gas analyzer. The measurements used optimal cuvette conditions at 800 µmol/m^2/s photosynthetically photon flux density (PPFD), 400 µmol/mol CO_2 30 ºC cuvette temperature, and 60% relative humidity with air flow rate set at 500 cm^3/min (Ibrahim, 2012). A single fully expanded leaf was inserted in the leaf cuvette to record photosynthesis rate (A), stomatal conductance (g_s) and transpiration rate (E). The operation was automatic and the data were saved in the LI-6400 console and analyzed by "Photon Assistant" software (Version 3, Lincoln Inc, (USA). There were some precautions taken during measurement. The leaf surfaces were cleaned and dried using dry tissue before enclosing in the leaf cuvette to avoid errors. The leaf gas exchange data was recorded between 8.00 am to 11.00 am.

2.5. Maceration

Leaf extraction was carried out using the maceration method (ICS-UNIDO, 2008). Dried and ground leaves were weighed (0.5 g) into a conical flask and extracted at a leaf to solvent ratio of 1:100 w/v with aqueous ethanol 50%, respectively. The leaf samples were mixed and sealed well with parafilm to avoid contamination and to minimize solvent evaporation. The samples were soaked for 5 days. After 5 days, the extract solution was filtered (Whatman^{TM} No. 1) and the filtrate was used for the quantification of total phenolics and total flavonoids.

2.6. Total Phenolics and Total Flavonoids

The leaves part of *L. pumila* were determined for secondary metabolites, the stems and roots part were not determined due to the low content of secondary metabolites [8]. The total phenolics and flavonoids were determined using the procedures described by Ibrahim and Jaafar (2012). A 200 µL of sample leaf extract was mixed with 1.5 mL of Follin-Ciocalteu (diluted 10-fold) and allowed to stand at room temperature (25ºC) for 5 minutes. Then 1.5 ml $NaNO_3$ solution was added to the solution and allowed to stand at 25ºC for 2 hours before absorbance was measured at 725 nm. The results are presented as mg/g gallic acid equivalent (mg GAE/g dry sample). Whilst, for total flavonoid quantification, 1 mL of sample extract was mixed with 0.3 mL $NaNO_3$ in a test tube covered with aluminum foil and left for 5 minutes. Later 0.3 mL of 10% $AlCl_3$ and 2 mL of 1M NaOH were added to the solution. The absorbance was measured at 510 nm using a spectrophotometer with rutin as a standard and the results are expressed as mg/g rutin dry sample.

2.7. Statistical Analysis

Data were analyzed using analysis of variance by SAS version 9.2. Mean separation test between three treatments (N sources, N levels and harvest time) were performed using LSD test and standard error of differences between means was calculated with the data were normally distributed and equally replicated. Pearson correlation also was tested to measure the strength and direction of the linear relationship between pairs of continuous variables in this experiment.

3. Result

3.1. Total Biomass

Total biomass was significantly affected by three factors interaction. Total biomass was higher when Gobi was fertilized on *pumila* plants at 15 weeks at 6.24 g and followed by NPK when harvested at week 15. However, *alata* plants at all fertilizer treatment and harvest time showed no significant difference on total biomass production (Figure 1). The single factor of variety and harvest time also affected plant total dry biomass acutely (Table 1). Variety *pumila* indicated higher total biomass accumulation with 4.79 gram per plant compared to alata (0.93 gram/plant). The higher total biomas accumulation of variety *pumila* due to plant itself that is greater than *alata*. At last harvest showed more accumulation in total biomass compared to week 5.

3.2. Leaf Gas Exchange

3.2.1. Net Photosynthesis (Pn)

Net photosynthesis was collected using a LICOR 6400 Portable Photosynthesis system on weeks 5 and 15 WAT. There was an interaction effect among all factors on the photosynthesis activity of *L. pumila* plants (Figure 2a-b). Chicken manure fertilized on variety *alata* and *pumila* indicated higher photosynthesis activity compared to other combination treatments. The *pumila* and *alata* plants fertilized with chicken manure showed high photosynthesis activity at 15 week after transplanting (WAT) with values of 14.67 and 13.64 µmolCO_2/m/s respectively, followed by application of Gobi on *alata* and *pumila* at 15 WAT (10.62 and 10.60 µmolCO_2/m/s) which were not different to result of *alata* under Gobi at 5 WAT and *pumila* under chicken manure at 5 WAT. Fertilizer sources and harvest stage factor were also had a very strong effect on photosynthesis except on variety of *L. pumila* (Table 1). Chicken manure has promoted highest rate of photosynthesis rate (11.10 µmolCO2/m/s), followed by Gobi (9.24 µmolCO_2/m/s), NPK green (6.89 µmolCO2/m/s) and control (5.22 µmolCO_2/m/s). Differences in the variety of *L. pumila* indicated no difference in response of photosynthesis rate.

3.2.2. Stomatal Conductance (g_s)

Both the interaction between fertilizers and harvest time (WAT) and a single factor of variety or fertilizer imposed significant difference on the stomatal conductance of *L. pumila*. Chicken manure that fertilized on *L. pumila* plants showed higher stomatal conductivity at week 15 with 0.48 mmol/m/s than that of Gobi (0.35

mmol/m/s); p≤0.05 (Figure 3). Lower activity of stomatal conductivity at week 15 than week 5 was demonstrated by NPK green and followed by the control. Significantly stomatal conductivity was also contributed by fertilization with chicken manure followed by Gobi, NPK green, and control treatment (Table 1) with a respective value of 0.396, 0.311, 0.254 and 0.224 mmol/m/s. Variety of *alata* showed more response on stomatal conductance compared to *pumila* variety.

3.2.3. Transpiration Rate (E)

Transpiration rate (E) was affected by the interaction between fertilizer sources and harvest time of *L.pumila*. The interaction between fertilizers sources at the first harvest was higher as compared to the last harvest of transpiration rate was higher (Figure 4). This interaction offer on transpiration rate was observed differently from photosynthesis and stomatal conductance activity where the reduced impact was observed at week 15 after transplanting compared to 15 WAT. Thus the transpiration rate was significantly decreased as time progressed as referred to Table 1. *Labisia pumila* transpired more when fertilized with chicken manure (5.30mol/m^2/s) and Gobi (4.99 mol/m^2/s) as compared to NPK green and control with the respective value of 4.46 and 4.07 mol/m^2/s (Table 1). The variety *alata* (4.90 mol/m^2/s) also documented a higher transpiration rate than variety *pumila* (4.56 mol/m^2/s).

3.2.4. Secondary Metabolites

3.2.4.1. Total Phenolics (mg Gallic Acid E/g Dry Leaves Sample)

Fertilizer sources also had a significant impact on total phenolics of *L. pumila*. Chicken manure and Gobi indicated more TP accumulation 0.198 mg and 0.195gallic acid E/g dry sample and the control 0.191 mg gallic acid E/g dry sample. Application of NPK green, on the other hand, had significantly lowered the total phenolic value 0.185 mg gallic acid E/g dry leaves sample compared to all other treatments (Table 1).

3.2.4.1. Total Flavonoid (Mg Rutin E/g Dry Leaves Sample)

Total flavonoid (TF) of *L. pumila* was significantly affected by fertilizer sources. Single factor fertilizer sources imposed an effect on total flavonoid of *L. pumila* plant (Table 1). Where total flavonoid recorded higher range when fertilized using chicken manure with 0.085 mg rutin E/g dry leaves sample compared the NPK green (0.072 mg rutin E/g dry leaves sample).

Table-1. Single factor effect on parameter measurement

Factor	Total biomass	A	gs	E	Total phenolic	Total phenolic
	g/plant	µmolCO2/m/s	mmol/m/s	mmol/m/s	mg gallic acid E/g dry	mg rutin E/g dry
	ns	*	**	**	**	**
Chicken manure	2.65^a	11.10^a	0.40^a	5.30^a	0.196^a	0.082^a
Gobi	2.70^a	9.24^b	0.33^b	4.99^a	0.199^a	0.080^a
NPK green	3.00^a	6.89^c	0.25^c	4.46^b	0.178^b	0.067^b
Control	3.10^a	5.22^d	0.22^d	4.07^b	0.191^a	0.076^{ab}
Variety (V)	**	ns	**	**	ns	ns
alata	0.93^b	8.02^a	0.31^a	4.92^a	0.191^a	0.077^a
pumila	4.79^a	8.20^a	0.29^b	4.49^b	0.191^a	0.075^a
Harvest time (H)	**	**	ns	**	ns	ns
5 WAT	2.51^b	7.02^b	0.31^a	7.52^a	0.188^a	0.075^a
15 WAT	3.21^a	9.21^a	0.29^a	1.89^b	0.194^a	0.078^a
Interaction						
F x V	ns	**	ns	ns	ns	ns
F x H	*	ns	ns	ns	ns	ns
V x H	*	**	**	**	ns	ns
F x V x H	*	*	ns	ns	ns	ns
Mean	2.86	8.11	0.30	4.7	0.190	0.076

Means followed with the same letter in the same column and source are not significantly different at P≤0.05 by Least Significant Different (LSD) test. ns, not significant at P≤0.05. Significant different at **P≤0.01 or *P≤0.05

3.2.5. Correlation

This analysis is to indicate how well data measured in this study were related. This correlation can show high (0.5 -1.0), medium (0.3-0.5) and low (0.1-0.3) correlation of that data measured. Table 2 showed the correlation between all parameter measured in this study. Photosynthesis rate indicated a positive correlation with stomatal conductance (r= 0.708). However, stomatal conductance recorded a low positive correlation with transpiration rate (r= 0.315). Total flavonoid was observed to have a low positive correlation with total phenolic (r= 0.282). Total phenolic has a linear positive correlation with total flavonoid. Majority of flavonoids are phenolic that means they contain at least single phenolic group which influences the total phenolic content of plants.

Table-2. Pearson's correlations for all parameters in the experiment

	1	2	3	4	5	6
Total biomass	1.000					
Photosynthesis	0.072	1.000				
Stomatal conductance	-0.106	0.708**	1.000			
Transpiration	-0.220	-0.166	0.315*	1.000		
Total phenolic	0.048	0.298*	0.251	-0.092	1.000	
Total flavonoid	-0.039	0.066	0.028	-0.077	0.282*	1.000

4. Discussion

The main objective of this study was to determine the total biomass, leaf gas exchange and pharmaceutical quality in terms of secondary metabolite content and production of different varieties of *Labisia pumila* when applied with different sources of complete fertilizers (organic and inorganic fertilizers) and harvested at different time after transplanting. Generally, *pumila* plants fertilized with Gobi and chicken manure at 15 WAT showed maximum total biomass production and photosynthesis rate. These effects were qualified by the significance of interaction among fertilizer sources, *L. pumila* variety and harvesting time. Organic fertilizer is known as slow-release fertilizer. It was suggested that the slow-release fertilizer could be the main source of N [9, 10] compared to NPK green and the control.

It was found that total biomass of this plant was not statistically affected by fertilizer treatment alone. However, the leaf gas exchange attributes and secondary metabolites (total phenolic and flavonoid) contents varied significantly with change in fertilizer sources used. The significant effect of this study was also influenced by the different variety of *Labisia pumila* used. *Pumila* variety was observed to be significantly more responsive in growth parameters due to the size of the plant itself. Variety also depends on its vegetative and reproductive characters.

The interaction effect among all factor had a significant change in total biomass accumulation. Maximum total biomass accumulation was observed on *pumila* variety at 15 WAT when fertilizes with Gobi. The morphology of *pumila* variety is a factor that contributed to the interaction of total biomass. In this study, the variety *pumila* had better growth performance than *alata*. With variety *pumila* having higher plant height, thus consequently, had affected positively leaf area and total biomass. Different variety had different phenotypic plant characteristic that can cause varietal differences effect [11]. Hence, variety *pumila* harvested at 15 WAT could be the best choice for enhanced biomass as indicated by the significant result in the study.

Overall results of leaf gas exchange attributes showed that there were interaction effects by fertilizer sources and harvesting time on stomatal conductance and transpiration rate. Generally, chicken manure was observed to result in high photosynthesis, stomatal conductance and transpiration rate than the other fertilizer source followed closely by Gobi. This was due to probably more nutrients can be sustained in the soilless media especially at week 15 after transplanting because of the slow release fertilizer function in organic fertilizer [12]. At 5 WAT, all fertilizers showed almost similar transpiration values and were significantly higher than recorded on 15 WAT when using chicken manure followed by Gobi, NPK green and control. High transpiration rate at all fertilizer treatment at 5 WAT may be especially important in the acquisition of mobile nutrients to uptake by the plant [13] compared at 15 WAT and also when Pn was high at 15 WAT, E was low due to much H_2O used for Pn before the water vapor was released in E process [14].

It has been reported that the availability of nitrogen in the soilless media affects leaf gas exchange in *Labisia pumila* plants [1]. In the current work, photosynthesis rate of both varieties was significantly higher when fertilized with chicken manure followed by Gobi fertilizer, particularly when harvest on week 15 compared to weeks 5. This situation happened when N content in the leaves accumulated more as time progressed thus, in turn, increase the photosynthesis rate [15]. Higher rates of leaf photosynthesis were generally obtained with the mature leaves [15, 16]. The organic fertilizer is known as slow-release fertilizer that slowly releases the N nutrient until week 15 after transplanting. The increase in N availability might improve photosynthetic capacity [17].

There were statistically significant differences in leaf gas exchange attributes (photosynthesis rate, stomatal conductance and transpiration rate) as affected by fertilizer sources. The net photosynthesis was influenced by different fertilizer sources, but no varietal differences were observed. As expected, the photosynthetic characteristic was strongly affected by the nitrogen fertilization from different sources of complete fertilizers in this experiment, which is in agreement with many previous studies [17, 18]. The significant difference in photosynthesis rate, stomatal conductance and transpiration rate were probably due to variations in nutrient availability in the complete organic and inorganic fertilizers, and different mineralization process occurred under environmental and horticultural conditions [19, 20]. This happened because most of the N is used for the synthesis of components in the photosynthesis apparatus and carbon dioxide assimilation [21-23], whether in organic or inorganic form. The highest values of photosynthesis, stomatal conductance rate and transpiration rate were obtained in *L. pumila* fertilized with chicken manure followed by Gobi. Lower photosynthetic performance of *L. pumila* plants was control treatment and it may associate with lower nitrogen content in soilless media. Available nitrogen contents can increases cell wall rigidity and photosynthetic capacity in plants [24, 25]. There is a compensation mechanism in any limiting condition like organic fertilizer, where growth get restricted but the photosynthesis continues and diverts the surplus carbon to secondary metabolites [26].

The stomatal conductance had a positive correlation with photosynthesis rate (R^2= 0.708) and transpiration rate (R^2=0.315). In general, the photosynthesis process is known to have been associated with leaf gas exchange especially stomatal density [27, 28]. This correlation between photosynthesis rate and stomatal conductance was also found in previous studies [29, 30]. Stomatal conductance is correlated linearly with transpiration process which transpiration will be higher when the stomata pressure lead to increase [31]. This correlation probably implied a humidity factor in glasshouse situation. In protected space like glasshouse is observed that increasing photosynthesis rate has relation with stomatal conductance and transpiration rate which implied relative humidity [31, 32]. The stomatal conductivity of water was positively correlated with transpiration process and cause water in leaf to evaporate. Mechanism of opening-closure stomata played a very vital role in carbon assimilation for the photosynthesis process and water elimination of transpiration rate [31]. Hence, enhanced the stomata conductance is known to improve intercellular CO_2 concentration followed to enhance photosynthesis rate. These results suggest nitrogen supply improved leaf gas exchange attribute in a glasshouse experiment. Nitrogen could improve the plants stomatal regulation and also well conserve the physiological function of photosynthetic apparatus [21, 33], thus affected the correlation between leaf gas exchange attributes.

L. pumila variety *alata* showed significantly higher on stomatal conductance values and transpiration rate activity compared to variety *pumila*. The increase in net photosynthesis of variety *alata* might also probably recognized to higher Rubisco per unit chlorophyll surface area that can enhance net photosynthesis [34]. Variety *pumila* has lower net photosynthesis values could result in stomata sensitivity of the plants varieties [35] and a greater leaf production often compensates for a low rate of photosynthesis per unit of leaf area or dry weight [36] that correlates the effect of stomatal conductance and transpiration rate.

Total phenolic and flavonoid contents recorded for the experiment did not differ significantly between varieties of *L. pumila*. However, fertilizer sources played a critical ($p \leq 0.05$) role in determining the total phenolic and total flavonoid. Application of both chicken manure and Gobi resulted in almost maximum total phenolic value and total flavonoid compared to other fertilizer treatments. Organic fertilizers are known as slow-release fertilizer that can release the nutrients slowly to the plant. *Labisia pumila* plants growing in slow-release fertilizer possibly lead to high levels of secondary metabolites content as also found in other studies [37, 38]. This is in similar with results in other studies [1, 39] which state that the accumulation of polyphenolic components in plant tissues is often enhanced under conditions of limited nitrogen supply [38]. Thus, subsequently induces plant defense mechanisms by increased poly-phenolic accumulation [38, 40] as a defense mechanism against nutritional stress. This may be supported by carbon-nutrient balance (CNB) hypothesis which anticipates that the accumulation of excess carbon in response to nutrient stress leads to the increased production of carbon-based secondary metabolites (CBSM) and their precursors [41, 42]. Further explanation within the growth-differentiation balance (GDB) theory, the carbon/nutrient balance (CNB) hypothesis was more focused on the effects of fertilization on plant resource allocation. The CNB theory states that under limited nutrient conditions, plants increase their production of secondary metabolites. Low nitrogen availability leads to increased concentrations of carbon metabolites such as polyphenolic compounds. However, in this study all the fertilizer rates were similar, but the types of fertilizer may influence the N availability to the plant. It has been documented that higher tomato yields were produced by organic-mineral compounds, which was greater than those produced by mineral fertilizers were applied at the same rate [43]. Organic nutrient sources can play a role in determining the quality of *L. pumila* leaves.

This study was aimed to observe the differences in effect on growth and quality between complete organic and inorganic fertilizer given at 90 kg N/ha on *Labisia pumila* varieties and harvested at three different times. Generally, fertilizer treatment did not affect plant growth performance, which was more influenced by variety and harvest time in this study. Generally, chicken manure had shown to correspond with improved of leaf gas exchange attribute, whilst both chicken manure and Gobi affected the quality as in secondary metabolites contents in *L. pumila*. However, *L. pumila* varieties that fertilized under Gobi fertilization at 31 weeks of study indicates a positive effect on total biomass, total phenolic and total flavonoid contents compared to chicken manure when harvested at week 15 after transplanting. In practice, the use of slow release fertilizer and stabilized fertilizer is primarily concern and increased in the glasshouse, golf courses, and professional lawn management, as well as by consumers (home and garden) and landscape gardeners [44]. The need to decline costs of fertilizing herbal plants with renewable forms of energy has revitalized the application of organic fertilizers around the world. Thus, Gobi fertilizer cost indicates more cheaply available on herbal plants production compared to chicken manure.

The manipulation of complete fertilizer sources in the soilless media can influence patterns of photosynthate allocation between growth and the production of secondary metabolites. When nitrogen availability is improved, by applying suitable complete fertilizer sources it may affect the photosynthesis of the plant by altering the synthesis of secondary compounds [45]. This pattern of study has been associated with Growth-Differentiation Balance (GDB) as stated in the above paragraph.

5. Conclusion

This study showed that complete organic fertilizer sources showed a significantly higher effect especially on leaf gas exchange attributes and secondary metabolites contents (total phenolics and flavonoid). Fertilization practice, in particular nitrogen from different sources of complete fertilizer availability, has been shown to affect secondary metabolites concentration in *L. pumila* leaf tissues. Nitrogen limitation in organic fertilizer enhances leaf phenolics content. Organic fertilizers responded approximately at the last harvest (15 WAT). Thus organic fertilizers proved to be efficacious as a good source of fertilizer that promoted especially plant secondary metabolites.

Acknowledgments

The authors would like to thank the Ministry of Higher Education (MoHE), Universiti Sultan Zainal Abidin (UniSZA), Terengganu, for financial support of Nurrul Akmar Rosni and Universiti Putra Malaysia (UPM), Selangor. Special thanks also to the laboratory and field staffs of the Department of Crop Science and Soil Science UPM, who has abundantly helpful and offered invaluable assistance.

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