Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2024.132.145

Research Article
Effects of Fertilizer Rate on Growth Performance and Phytochemical Compounds of *Gynura procumbens*

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Abstract

Background and Objective: *Gynura procumbens* (Lour.) Merr., is a herbaceous plant of the Asteraceae family and the phytochemical contents of this plant play a significant role in health benefits. Optimizing fertilizer application is among the key strategies in enhancing the growth and phytochemical content of *Gynura procumbens*. Thus, the experiment was conducted to evaluate the effects of chicken manure rates on growth, physiological characteristics and phytochemical contents of leaves of *Gynura procumbens*. **Materials and Methods:** The research was structured using a Randomized Complete Block Design (RCBD) with the inclusion of four replicates. The experiment consisted of five fertilizer rates of processed chicken manure, ranging from 0, 100, 200, 300 and 400 kg ha^{-1} and applied in two split applications. The study was conducted from January until March, 2017. **Results:** The highest plant growth and total dry matter were recorded at application of 300 to 400 kg ha^{-1} fertilizer. As the fertilizer rate increased, there was a noticeable decline in total phenolic content, total flavonoid content, antioxidant activity and chemical markers. A significant interaction between the fertilizer rate and leaf maturity on kaempferol-3-glucoside and kaempferol-3-rutinoside content was also observed. Young leaf was found to contain higher chemical markers. **Conclusion:** Considering the plant growth and phytochemical contents, the application of 300 kg ha^{-1} was recommended as the optimum fertilizer rate for *G. procumbens*.

Key words: *Gynura procumbens*, chicken manure, DPPH activity, leaf maturity, organic fertilizer, phytochemical contents

Citation: Majid, W.H.D.W., M.M. Yusoff, A. Misran and F.M. Arisah, 2024. Effects of fertilizer rate on growth performance and phytochemical compounds of *Gynura procumbens*. Asian J. Plant Sci., 23: 132-145.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Crop yield of plants is the output of various physiological processes that occurred due to the influence of plant character development which usually can be modified through management of agricultural practices. The management strategy for plant nutrition is significantly important to improve plant growth and yield production[1]. Fertilization is one of the factors that has been reported to affect the quantity and quality of crop yield. In crop cultivation, fertilizer application provides sufficient nutrients to the plants, ensuring their overall health and well-being, as well as the maintenance of their optimal growth[2].

Management of the optimal amount of nitrogen fertilizer is one of the crucial aspects of crop fertilization for sustainability production. The excessive application of nitrogenous fertilizers beyond the plant's utilization capacity can result in adverse consequences. These include increase in soil acidity, inhibited crop growth, disrupted maturation processes and an increase in unwanted weed populations[3,4]. This situation may lead to low-quality crop production and pose potential risks to human health. According to Chakwizira *et al*.[5], the requirement for nitrogen supply is contingent upon the soil's fertility status and crops' response to nitrogen availability. Balancing the two to meet the crop's demand, is crucial to achieving the most efficient and effective utilization of nitrogen resources. When the supply of nitrogen falls short of the required demand, plants may struggle to produce an adequate number of leaves and achieve the desired leaf area, which can lead to a reduced leaf area index and a decline in the amount of light interception.

In addition, a suitable amount of nitrogen influences the growing stage of a plant and markedly affects the amount of photosynthesis activity[6]. Plants metabolize nitrogen in two ionic forms NH^{4+} and NO^{3-} which affect plant growth development as well as nutritional quality[6,7]. Nitrogen is also important as it influences plant primary and secondary metabolic pathways, thus aiding in the accumulation of plant secondary metabolites[8,9]. Increasing the accumulation of the secondary metabolites will result in decreasing chlorophyll content in leaves which subsequently lead to reduce plant biomass[10]. However, in a study by Ibrahim *et al*.[11] reported a contrasting pattern in which the accumulation of plant secondary metabolites was higher under optimal nitrogen application levels. Considering the diverse pattern observed, it is crucial to assess for the optimum fertilizer rate to promote the plant growth, thus enhancing its downstream application.

Chicken manure has been regarded as one of the excellent nitrogen sources. Its application has been demonstrated to have a significant impact on increasing the water-soluble salt concentration in soil[12]. This resulted in increment of nitrogen level up to 40-60 and 17-38% in sandy and loam soil, respectively. A study conducted by Affendy *et al*.[2] found that the growth of *Orthosiphon stamineus* was most favorable when treated with chicken manure, specifically at a rate of 135 kg ha^{-1}, as opposed to the use of oil palm empty fruit bunch (EFB). The application of chicken manure can have a notable impact on soil properties, primarily attributed to the humus content of the chicken manure. This leads to several positive effects on soil fertility such as increase of pH soil, enhancement of cation exchange capacity of the soil, promoting formation of soil aggregates and stimulation of beneficial soil microbes[12]. In addition, it also helps in decreasing the bioavailability of heavy metals such as Cu, Pb and Zn in soil[13].

The previous studies on *G. procumbens* had only focused on its content of bioactive compounds[14-17]. However, none of the research has been conducted on the suitable agronomic practices to ensure the high production of bioactive compounds. It was reported that the cultivation aspect requires a specific standard of practices such as nutrient sources to produce a high quantity of bioactive compounds. Therefore, gaining a comprehensive understanding of how *G. procumbens* responds to various agronomic practices is crucial for the successful commercial cultivation of this herbal plant with high phytochemical contents. Thus, this study was conducted to examine the effect of different rates of chicken manure on growth performance, physiological characteristics and total dry matter production of *G. procumbens.* It was also aimed to measure the phytochemical compounds of *G. procumbens* as affected by different rates of chicken manure and leaf maturity.

MATERIALS AND METHODS

Experimental site and duration of study: The location of the experiment was at Universiti Agriculture Park, Universiti Putra Malaysia, Serdang, Selangor (2°59'09.5'N, 101°42'24.7'E). The experimental site had been previously free from any planting for 2 years. The study was conducted from January until March, 2017.

Experimental design and treatment: The study was laid out in a Randomized Complete Block Design (RCBD) in four replicates. The experiment consisted of five fertilizer rates of processed chicken manure, ranging from 0, 100, 200, 300 and 400 kg ha^{-1}. The fertilizer was applied in two split applications. One-half of the total fertilizer of each rate was applied one week before transplanting and the balance was applied 3 weeks after transplanting to the field. The nitrogen, phosphorus and potassium content of the chicken manure was 2.09, 0.86 and 1.71%, respectively.

Harvesting, drying and grinding: *Gynura procumben* was harvested at week 12 after being transplanted in the polybags. The plants were harvested starting from the first node above the soil surface using sharp secateurs, washed and placed in plastic containers for post-harvest handling. Plant samples were air-dried to remove the excessive water. Leaves were separated from the stems and divided into two parts: Young and mature parts. The classification of young leaves was based on the number of nodes. Specifically, the leaves on the 7th node and above were classified as "young" parts, while those below the 7th node were categorized as "mature" parts. The total fresh weight of plants and leaf area was recorded before being oven-dried. Then, the plants were cut into small pieces of length (4-5 cm) and placed in labelled brown paper bags. Plant samples were oven-dried (Memmert ULM 500, Schwabach, Germany) at 50°C for about three days. During the drying period, the paper bags were flipped upward and downward to make sure the plant samples were dried. Dried plants were then ground into superfine powder using a plant tissue grinder (Thomas No. 123C63, USA) and stored in plastic vials for further analysis.

Statistical analysis: Data were statistically analyzed using SAS software version 9.3 (SAS Institute). Data on plant growth, leaf area index and total dry matter production were analyzed using one factor of effect on different rates of chicken manure as a One-way Analysis of Variance (ANOVA) while data on phytochemical content and plant nutrient uptake were analyzed using two factors of effects of chicken manure rates and leaf maturity as two-way ANOVA and means comparison was carried out using least significant difference (LSD). Mean differences at $p \leq 0.05$ were considered to be statistically significant.

Fresh weight and total dry matter production: Leaves were separated from the stem and weighed using a digital balance (Jadever, China), placed in labelled brown paper bags and dried in the oven at 50°C until completely dried at about 3 days. The total dry matter of plants was immediately recorded after taking them out of the oven (Memmert ULM 500, Schwabach, Germany).

Crop growth rate: Analysis of plant crop growth rate was calculated using the following formula[18]:

$$\text{Crop growth rate } (g\ m^{-2}\ w^{-1}) = \frac{W_2 - W_1}{(GA)\ T_2 - T_1} \quad (1)$$

Where:

GA = Ground area

W_1, W_2 = Dry weight of plant m^{-2} recorded at time T_1 and T_2

Plant height: The plant height (cm) was measured using a ruler and a measuring tape starting from ground level until the shoot tip.

Number of branches: The number of plant branches was counted starting from the first branch at the base of the stem up to the top of the stem. Only stems and branches longer than 10 cm were counted manually.

Leaf area index (LAI): The total leaf area of the plants was measured using the leaf area meter (LI-3100, LI-COR, United Stated). For every treatment, plants for each replicate were measured and recorded. Each of the plant's leaf area index was calculated using the following formula[18]:

$$\text{Leaf area index} = \frac{\text{Total leaf area } (m^2)}{\text{Ground area } (A = \pi r^2 m^2)} \quad (2)$$

The amount for the community leaf area index is then divided by the total plant used for sampling.

Preparation of leaf extract: Samples of dried leaves were used in the extraction process following the method described by Kaewseejan and Siriamornpun[14]. Dried ground leaves of 20 g was extracted using 200 mL of 95% ethanol for 24 hrs at room temperature. A conical flask containing the leaves sample was covered with aluminum foil and stirred using an orbital shaker (WS-100D, Wiggens, Beijing). After 24 hrs, the mixture was filtered using a vacuum pump (NVC-2200, Eyela, Japan) through Whatman No. 1 filter paper. The plant extract was then concentrated under a vacuum at 40°C using a rotary evaporator (N-1210, Eyela, Japan).

Total phenolic content: Total phenolic content (TPC) was analyzed using the Folin-Ciocalteu reagent method[14]. A volume of 200 µL of each extract was pipetted into the test tubes. Folin-Ciocalteu reagent of 1 mL (10% v/v) was added to the extract and incubated for five minutes. Then, 800 µL of 7.5% (w/v) of sodium carbonate solution was mixed and incubated for another 30 min at room temperature. A blank sample was prepared as a control by replacing the 200 µL of the extract with 95% ethanol. The absorbance of the solution was measured using a UV-visible spectrometer (Fisher Thermo Scientific, Multiskan Go, United Kingdom) at 765 nm. A series of gallic acid standard solutions (0-200 µg mL^{-1}) was prepared. The total phenolic content was calculated as mg gallic acid equivalent per gram of dry-weight plant material.

Total flavonoid content: The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method[14]. A volume of 250 µL of leaf extract solution was mixed with 1.25 mL of distilled water and 75 µL of Sodium Nitrite ($NaNO_2$) solution. The mixture was incubated for 6 min. After the incubation, 150 µL of 10% Aluminum Chloride ($AlCl_3$) solution was added. The mixture was allowed to settle for 5 min and an additional 500 µL of 1 M Sodium Hydroxide (NaOH) was added. Distilled water was added to the mixed solution to make the total volume 2.5 mL. A blank was prepared by substituting 95% ethanol for the 250 µL extract. The absorbance was measured at 510 nm using a UV-visible spectrophotometer (Fisher Thermo Scientific, Multiskan Go, United Kingdom). A calibration curve was constructed using quercetin with concentration range of 0 to 500 µg mL^{-1} which was diluted in ethanol for quantification of flavonoid content. Total flavonoid content was expressed as mg quercetin equivalent per gram of dried sample.

DPPH radical scavenging activity assay: Antioxidant activity was determined using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical based on the electron transfer reaction between the DPPH reagent and the plant extracts[19]. A volume of 40 µL of plant extract was added with 195 µL of 0.1 mM ethanolic DPPH solution. The mixture was then incubated at room temperature for 30 min in the dark condition before being measured at 515 nm using a spectrophotometer (Fisher Thermo Scientific, Multiskan Go, United Kingdom). The percentage of antioxidant inhibition was calculated using the following formula[20]:

$$\text{Inhibition (\%)} = \frac{A_{515} \text{ of control} - A_{515} \text{ of sample}}{A_{515} \text{ of control}} \quad (3)$$

Where:

A_{515} of control = Absorbance of control at 515 nm
A_{515} of sample = Absorbance of sample at 515 nm

Ferric reducing antioxidant power assay: The ferric reducing antioxidant power assay (FRAP) reagent was prepared by mixing 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-Tri[2-pyridyl]-s-triazine (TPTZ) and 20 mM $FeCl.6H_2O$ in the prepared ratio of 10:1:1[19]. Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$) was used as a standard while the TPTZ working reagent was used as the blank. A volume of 200 µL (1 mg mL^{-1}) of plant extract was mixed with 3 mL FRAP reagents. The mixture was then incubated at 37°C for 30 min. The FRAP absorbance was measured at 593 nm using a spectrophotometer. The antioxidant capacity, based on the ability to reduce ferric ions of the extract, was calculated as a percentage of the antioxidant. The percentage of antioxidants was calculated using the following formula[20]:

$$\text{Inhibition (\%)} = \frac{A_{593} \text{ of sample} - A_{593} \text{ of control}}{A_{593} \text{ of sample}} \quad (4)$$

Where:

A_{593} of sample = Absorbance of the sample at 593 nm
A_{593} of control = Absorbance of control at 593 nm

High-Performance Liquid Chromatography (HPLC) analysis: High-Performance Liquid Chromatography (HPLC) analysis was used for the detection of chemical markers in *G. procumbens.* This analysis was performed using Agilent 1100/1200 quaternary pump, photodiode array detection[15]. The extract was filtered using a 0.45 µL membrane filter and injected into the chromatographic separation column of Discovery PS5-1852D, C18 with a specification of 5 µm at 25 cm long and 4.6 mm wide (Supelco). The mobile phase consisted of water and 0.1% acetic acid (solvent A) and acetonitrile (solvent B) at a flow rate of 1.0 mL min^{-1}. The gradient elution was separated for 27 min. The solvent B was run for 5 min at 18 to 23% and increased to 90% in the next 1 min. The gradient was decreased to 80% for 16 min before further decreased to 18% for another 5 min. The bioactive components of flavonoids were separated using a column at a temperature of 38°C with an injection volume of 10 µL. The eluted bioactive compounds were detected using a 370 nm UV-photo diode array. A calibration curve was made with the standard marker compounds: Myricetin, kaempferol-3-glucoside and kaempferol-3-rutinoside for qualification and quantification purposes.

Plant nutrient analysis: The samples of leaf powder were subjected to H_2SO_4-H_2O_2 digestion for quantitative nutrient analysis. Ground leaves of 0.25 g were placed in 250 mL digestion tubes with 5 mL of sulfuric acid (H_2SO_4). The mixture was heated at 285°C for 45 min. Hydrogen peroxide (H_2O_2) of 10 mL was added to each tube and heated continuosly until the solution became clear. The digestion tube was removed from the digestion block and placed at room temperature for the cooling process. Then deionized water was added to the mixture to reach a total volume of 100 mL. The mixture was vortexed and filtered through the Whatman No. 41 filter paper. Auto analyzer (System 4, Chemlab) was used to measure total N and P while K, Ca and Mg were analyzed using an atomic absorption spectrophotometer (310, Perkin Elmer, United State).

RESULTS

Effect of chicken manure rate on growth performance of *Gynura procumbens*: The plant growth measurements were significantly affected by the chicken manure rates (Table 1). The increment in yield due to the chicken manure application can be observed in total dry matter production. It was observed that production of total dry matter increased significantly with increasing chicken manure rate, except at 300 and 400 kg ha^{-1} rates. Total dry matter yield recorded an increase of 57.45 and 61.55% on plants applied with 300 and 400 kg ha^{-1}, respectively, as compared to the control. The crop growth rate increased with the increasing rate of chicken manure. The highest crop growth rates were achieved on application 300 and 400 kg ha^{-1} followed by 200 and 100 kg ha^{-1}. Application of chicken manure at 300 and 400 kg ha^{-1} increased plant height by 35.3 and 33.39%, respectively, as compared to the control which exhibited the lowest plant height.

The number of branches as presented in Table 1 indicated significant differences among the chicken manure rates. The number of branches increased with increasing chicken manure rates. The highest number of branches was produced by plants applied with 300 and 400 kg ha^{-1} of chicken manure rates with 24 and 27 branches, respectively. However, no significant difference was detected between these two chicken manure rates. In addition, the increase in chicken manure rate had significantly increase the plant leaf area index of *G. procumbens*. The application of chicken manure at 300 and 400 kg ha^{-1} displayed the highest leaf area index of 6.02 and 6.57, respectively, with no significant statistical difference was recorded ($p \geq 0.05$). Meanwhile, plants in the control treatment exhibited the lowest number of branch and leaf area index.

Effect of chicken manure rate on total phenolic content and total flavonoid content of *Gynura procumbens*: Table 2 indicates a significant interaction between the rate of chicken manure and leaf maturity on total phenolic content. The total phenolic content on mature leaves decreased with increasing chicken manure rates. An increment of up to 8.33 mg GAE g^{-1} DW was recorded in the young leaf with the application of 200 kg ha^{-1} of chicken manure (Fig. 1a). However, the phenolic content was decreased with the application of the higher rate of chicken manure. The significant interaction between the rate of chicken manure and leaf maturity implied the effect of chicken manure rates on *G. procumbens* on different leaf maturity. The reduction of total phenolic content was more prominent in the young leaves as compared to the mature leaves.

Table 1: Effects of different rates of chicken manure (0 to 400 kg ha^{-1}) on *Gynura procumbens* harvested at 12 weeks after cultivation

Rate of chicken manure (kg ha^{-1})	Total dry matter (kg ha^{-1})	Crop growth rate (g m^{-2} w^{-1})	Plant height (cm)	No. of branches (per plant)	Leaf area index
0	2869.20^{d}	28.00^{d}	46.80^{d}	7^{d}	2.23^{d}
100	3885.80^{c}	38.00^{c}	63.47^{c}	12^{c}	3.34^{c}
200	5674.20^{b}	56.03^{b}	77.75^{b}	16^{b}	5.32^{b}
300	6744.40^{a}	66.55^{a}	88.96^{a}	24^{a}	6.02^{a}
400	7462.60^{a}	73.95^{a}	82.47^{ab}	27^{a}	6.57^{a}
Significance	**	**	**	**	**

Means within column followed by the same letter are not significantly different by LSD, * = $p \leq 0.05$, $p \leq 0.01$, ns= not significant

Table 2: Effect of different rates of chicken manure (0 to 400 kg ha^{-1}) and stages of leaf maturity on total phenolic content (TPC) and total flavonoid content (TFC)

Treatment	TPC (mg GAE g^{-1} DW)	TFC (mg QUE g^{-1} DW)
Rate of chicken manure (R) (kg ha^{-1})		
0	7.31^{bc}	17.91^{ab}
100	8.13^{ab}	18.88^{a}
200	8.47^{a}	18.63^{a}
300	7.02^{c}	17.51^{bc}
400	5.17^{d}	16.66^{c}
Leaf maturity (L)		
Mature	7.72^{a}	17.79^{a}
Young	6.72^{b}	18.05^{a}
Significance level		
Rate of chicken manure	**	**
Leaf maturity	**	ns
R×L	*	**

Means within column followed by the same letter are not significantly different by LSD, *= $p \leq 0.05$, $p \leq 0.01$, ns= not significant

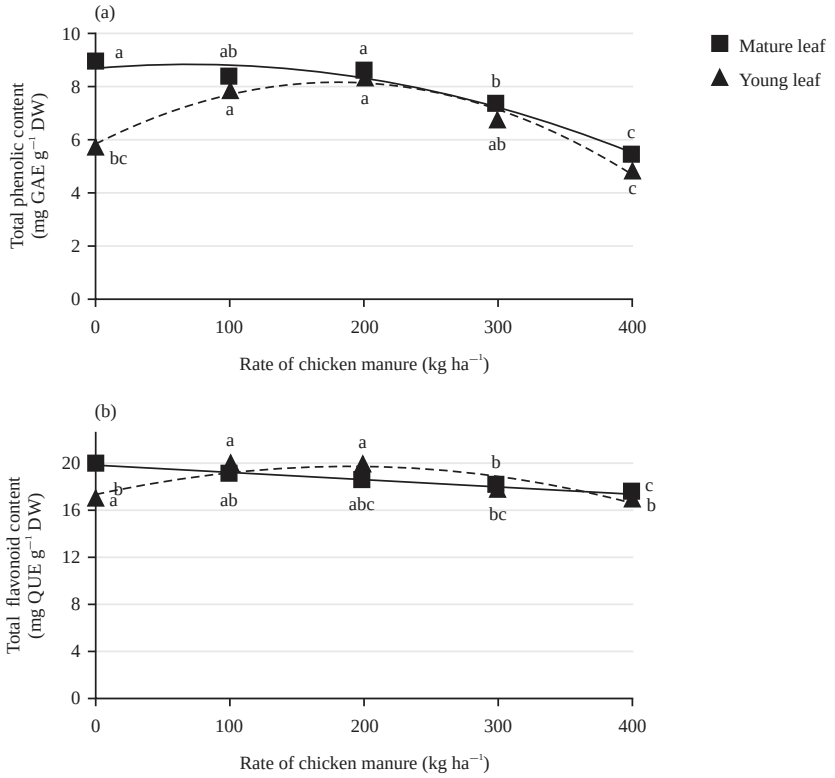

Fig. 1(a-b): Relationship between rate of chicken manure and leaf maturity on (a) TPC and (b) TFC of *Gynura procumbens*
Different letters denoted the statistical significance of the data according to the LSD test ($p \leq 0.05$), TPC: Total phenolic content, TFC: Total flavonoid content, Mature leaf: Dark line with square marker and Young leaf: Dotted line with triangle marker

Table 3: Effects of different rates of chicken manure (0 to 400 kg ha^{-1}) and stages of leaf maturity on DPPH free radical scavenging activity and ferric reducing antioxidant power (FRAP) assay of *Gynura procumbens*

Treatment	DPPH free radical scavenging activity (inhibition (%))	FRAP (inhibition (%))
Rate of chicken manure (R) (kg ha^{-1})		
0	51.71^{c}	57.86^{b}
100	57.58^{abc}	65.22^{a}
200	62.39^{a}	67.15^{a}
300	57.52^{ab}	67.26^{a}
400	53.99^{bc}	64.03^{ab}
Leaf maturity (L)		
Young	56.99^{a}	68.78^{a}
Mature	56.69^{a}	60.23^{b}
Significance level		
Rate of chicken manure	*	*
Leaf maturity	ns	**
R×L	*	ns

Means within column followed by the same letter are not significantly different by LSD, *= $p \leq 0.05$, $p \leq 0.01$, ns= not significant

The interaction effect between chicken manure rates and leaf maturity showed that it significantly affected the total flavonoid content of *G. procumbens* (Table 2). The mature leaves of *G. procumbens* exhibited a decreasing trend in total flavonoid content as the application rates of chicken manure increased (Fig. 1b). Meanwhile, a curvilinear pattern of total flavonoid content was observed in the young leaves with the highest concentration exhibited by plants treated with 100 and 200 kg ha^{-1}.

Effect of chicken manure rates on antioxidant activity: The percentage of antioxidant activity as determined by DPPH free radical scavenging was affected by different chicken manure rates and leaf maturity (Table 3). The main effect of chicken manure rates and leaf maturity were recorded with significant differences in FRAP of *G. procumbens*. In general, the percentage of inhibition in FRAP ranged from 57.86 to 67.26%. The increase in inhibition percentage was prominent in the young leaves as compared to the mature leaves.

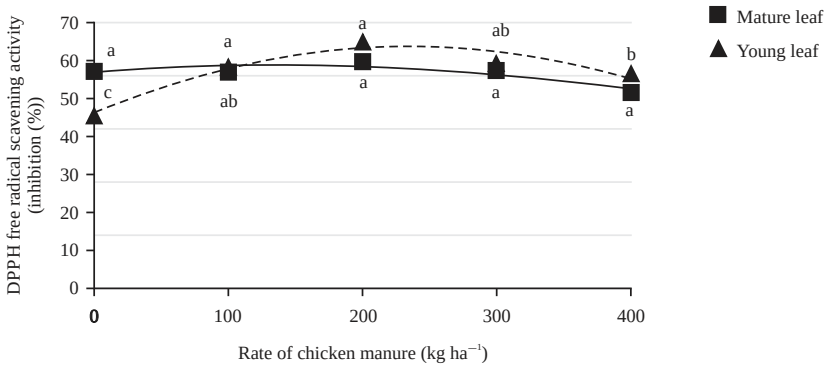

Fig. 2: Relationship between rate of chicken manure and leaf maturity on DPPH free radical scavenging activity of *Gynura procumbens*

Different letters denoted the statistical significance of the data according to the LSD test ($p<0.05$), Mature leaf: Dark line with square marker and Young leaf: Dotted line with triangle marker

Table 4: Effects of different rates of chicken manure (0 to 400 kg ha^{-1}) and stages of leaf maturity on kaempferol-3-rutinoside, kaempferol-3-glucoside and myricetin contents of *Gynura procumbens*

Treatment	Kaempferol-3-rutinoside (mg g^{-1} DW)	Kaempferol-3-glucoside (mg g^{-1} DW)	Myricetin (mg g^{-1} DW)
Rate of chicken manure (R) (kg ha^{-1})			
0	0.16^{ab}	0.28^{a}	0.04^{a}
100	0.17^{a}	0.30^{a}	0.04^{a}
200	0.20^{a}	0.32^{a}	0.04^{a}
300	0.09^{c}	0.20^{b}	0.03^{a}
400	0.12^{bc}	0.20^{b}	0.04^{a}
Leaf maturity (L)			
Young	0.19^{a}	0.26^{a}	0.03^{a}
Mature	0.11^{b}	0.26^{a}	0.04^{a}
Significance level			
Rate of chicken manure	**	**	ns
Leaf maturity	**	ns	ns
R×L	**	**	ns

Means within column followed by the same letter are not significantly different by LSD, *= $p \leq 0.05$, $p \leq 0.01$, ns= not significant

The response of DPPH inhibition in young leaves mirrored the pattern observed in total flavonoid content, with a notable decrease in the percentage of inhibition following the application of 200 kg ha^{-1} of chicken manure (Fig. 2). Meanwhile, the DPPH content in mature leaves remained unaffected by the varying rates of chicken manure application.

Effect of chicken manure rates on chemical marker: Three flavonoids, namely kaempferol-3-rutinoside, kaempferol-3-glucoside and myricetin, were identified using HPLC-PDA analysis. Table 4 demonstrates a significant interaction between the chicken manure application rate and leaf maturity on the levels of kaempferol-3-rutinoside and kaempferol-3-glucoside. However, it's important to note that Fig. 3a-b do not provide a visual representation of the mean comparisons across the combined treatments.

Figure 3a shows the interaction between chicken manure rates and leaf maturity significantly affected the concentration of kaempferol-3-rutinoside. The concentration of kaempferol-3-rutinoside exhibited a quadratic trend for mature leaves in response to different chicken manure application rates and leaf maturity. The kaempferol-3-rutinoside decreased after the application of more than 300 kg ha^{-1} of chicken manure. However, an inconsistent response to varying chicken manure rates was observed in the young leave. Despite that, the concentration of kaempferol-3-rutinoside was higher in young leaves than in mature leaves.

The interaction effect between the rate of chicken manure and leaf maturity on kaempferol-3-glucoside content was determined to be statistically significant solely in the young leaves (Fig. 3b). Lower kaempferol-3-glucoside content was exhibited by the young leaves in which it showed a quadratic response with varying chicken manure rates. Moreover, prominent reduction of kaempferol-3-glucoside content was recorded when the plants was applied with 300 and 400 kg ha^{-1}. Conversely, the myricetin content in *G. procumbens* leaves did not exhibit any significant differences across various rates of organic fertilizer application or between different leaf maturity stages (Table 4).

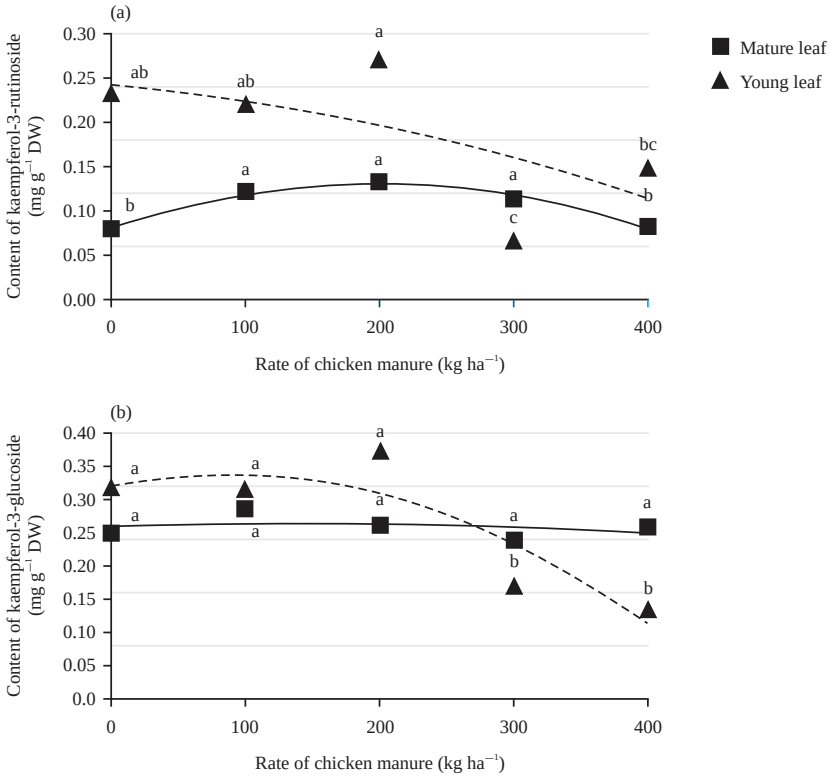

Fig. 3(a-b): Relationship between rate of chicken manure and leaf maturity on content of (a) Kaempferol-3-rutinoside and (b) Content of kaempferol-3-glucoside of *Gynura procumbens*

Different letters denoted the statistical significance of the data according to the LSD test ($p<0.05$), Mature leaf: Dark line with square marker and Young leaf: Dotted line with triangle marker

Table 5: Effects of different rates of chicken manure (0 to 400 kg ha^{-1}) and stages of leaf maturity on nutrient (N, P, K, Mg and Ca) content of *Gynura procumbens*

Treatment	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
Rate of chicken manure (R) (kg ha^{-1})					
0	0.77^{ab}	0.38^{c}	3.71^{bc}	0.55^{a}	2.82^{a}
100	0.70^{bc}	0.43^{c}	3.68^{bc}	0.53^{a}	2.41^{b}
200	0.64^{c}	0.63^{a}	3.27^{c}	0.53^{a}	2.54^{ab}
300	0.81^{a}	0.52^{b}	3.90^{ab}	0.51^{a}	2.66^{ab}
400	0.83^{a}	0.51^{b}	4.39^{a}	0.52^{a}	2.63^{ab}
Leaf maturity (L)					
Young	0.61^{b}	0.47^{b}	4.06^{a}	0.52^{a}	2.52^{a}
Mature	0.90^{a}	0.52^{a}	3.51^{b}	0.53^{a}	2.70^{a}
Significance level					
Rate of chicken manure	**	**	**	ns	ns
Leaf maturity	**	**	**	ns	ns
R×L	ns	*	ns	ns	ns

Means within column followed by the same letter are not significantly different by LSD, *= $p\leq0.05$, $p\leq0.01$, ns= not significant, N: Nitrogen, P: Phosphorus, K: Potassium, Mg: Magnesium and Ca: Calcium

Effect of chicken manure rates on plant nutrient uptake: No significant interaction effect between the rate of chicken manure and leaf maturity on the percentage of nitrogen (N) and potassium (K) uptake in *G. procumbens* (Table 5). Plants applied with 400 kg ha^{-1} of chicken manure exhibited the highest percentage of N with no significant difference with 0 and 300 kg ha^{-1} of chicken manure. As for the K content, the highest percentage was showed the highest by plants treated with 300 and 400 kg ha^{-1}. In terms of leaf maturity, the highest uptake of N was observed in mature leaves, while the highest uptake of K occurred in young leaves. However, there was no interaction effect detected between the rate of chicken manure and leaf maturity for the uptake of magnesium (Mg) and calcium (Ca) by *G. procumbens*.

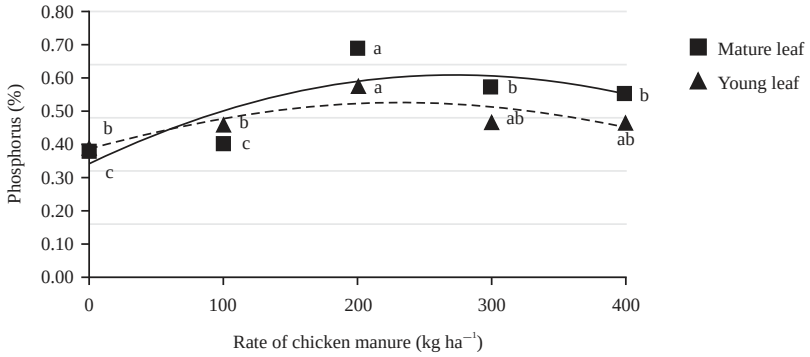

Fig. 4: Relationship between rate of chicken manure and leaf maturity on percentage of phosphorus content of *Gynura procumbens*

Different letters denoted the statistical significance of the data according to the LSD test ($p<0.05$), Mature leaf: Dark line with square marker and Young leaf: Dotted line with triangle marker

The percentage of phosphorus (P) in *G. procumbens* showed a significant interaction effect between the rate of chicken manure and leaf maturity (Table 5). The content of P in mature leaves was found to be highest in plants applied with 200 kg ha^{-1} of chicken manure. Phosphorus content increased by 45.49% in mature leaves compared to the control (Fig. 4). The P content in young leaves was observed to decrease after the plants were treated with more than 200 kg ha^{-1} of chicken manure.

DISCUSSION

Yield production is the manifestation of different physiological processes which affect the characteristics of plants. This is normally influenced by enforced management practices. A significant difference among the chicken manure rates was found in the total dry matter production of *G. procumbens*. Chicken manure application up to 300 kg ha^{-1} increased significantly the total dry matter. However, as the chicken manure rate increased above 400 kg ha^{-1}, there were no significant differences was recorded in the total dry matter between plants fertilized with 300 kg ha^{-1}. As reported by Masclaux-Daubresse *et al.*[4], high vegetative biomass production obtained was attributed to the role of nitrogen in forming the dry matter of plants and the presence of energy-rich compounds in the regulation of plant production and photosynthesis. In addition, nitrogen also play a crucial role in the creation of amino acids, which are the building blocks of proteins. This, in turn, significantly contributes to the growth and development of plants, leading to an increase in fresh weight, ultimately contributing to the production of total dry matter[21]. Nitrogen, as a macroelement and a major component of protein molecules, plays a pivotal role in energy synthesis and metabolic transfer. These processes have a direct and substantial impact on both the vegetative and reproductive phases of plant growth and development[22].

The effect of a high fertilizer rate in increasing the dry matter weight was also reported by Affendy *et al.*[2]. Such pattern was recorded due to the presence of organic matter, as well as the availability of essential major and minor nutrients in the soil. Furthermore, the combinations of nitrogen with plant matter produced during the photosynthesis process also led to an increase in the total leaf dry weight[23]. Increasing the nitrogen fertilizer rate has proven to significantly affect the increment of lettuce leaf dry weight. The nitrogen was found to stimulate the growth of plants and increase the leaf area, thus resulting in a higher rate of photosynthesis process and dry weight of lettuce.

In the present study, the growth rate of *G. procumbens* was significantly affected by the different rates of chicken manure. The crop growth rate was increased with the increase rate of chicken manure. The maximum crop growth rate was showed by plants applied with fertilizer rates of 300 and 400 kg ha^{-1}. Concerning the total dry matter, the increment of crop growth rate in the high rate of chicken manure was due to the high accumulation of total dry matter produced by the plants[24]. The observed increase in crop growth rate in response to the application of chicken manure is likely attributable to the presence of available nitrogen. This nitrogen is known to enhance the development of a larger leaf area and facilitate the production of higher levels of photosynthates, ultimately leading to the accumulation of dry matter in the plants[25]. Thus, as the nitrogen rate in chicken manure increases, a higher crop growth rate is obtained.

The highest plant height was exhibited by the plants treated with 300 and 400 kg N ha^{-1}. However, there was no significant difference between these two different rates. According to Farhad *et al.*[26], the increase in plant height at high levels of fertilizer was mainly due to more availability of nutrients supplied by manure throughout the growing season. Chicken manure was found to contribute to the supplementation of essential elements such as nitrogen, phosphorus and potassium for crop growth. Moreover, chicken manure also is useful in improving soil properties which ultimately enhances the vigorous growth of crop[27]. In addition, the improvement of plant height with increasing fertilizer rate could be attributed to chlorophyll synthesis due to an increase in nitrogen uptake[4]. Hence, the assimilation of carbon dioxide and the photosynthesis process will lead to the enhancement of plant growth. The reduced plant height in plants subjected to control treatment might be attributed to soil nitrogen deficiency that subsequently stunted the plants growth.

The effect of chicken manure was shown to significantly increase the production of branches which contribute to the absorption and translocation of assimilates and stimulation of apical and lateral meristems to grow[28]. Mustafizur Rahman *et al.*[16] stated that, the application of cow dung and poultry manure on *Mentha arvensis* had resulted in the highest number of branches compared to those plants with no fertilizer or mixed fertilizer applied. This is due to the high nitrogen content in cow dung and poultry manure which enhanced the protein synthesis and allowed the plants to grow faster. In addition, it also stimulated the apical growth as well as increases in branch and leaf numbers. Furthermore, a study on the effects of various boron rates on peanuts indicated that as the boron rate increased, there was a noticeable pattern of enhanced plant branching. This phenomenon contributed to increased side branching and promoted vegetative growth[29].

The leaf area index is essentially used for maintaining the rate of photosynthesis and plant yield performance. The significant response on leaf area index with chicken manure rate would indicate that nitrogen was successfully taken up by plants. Increasing the dose of chicken manure promotes an increase in the leaf area index of plants. In a study by Masclaux-Daubresse *et al.*[4], a highly significant response was observed on leaf area index on high rates of nitrogen fertilizer. Apart from that, the insufficient nitrogen accumulation under low fertilization will reduce the leaf area index which indirectly reduced the surface of the light interception process. According to Elli *et al.*[30], the highest leaf area index in high-dose fertilizer might have caused the increased incidence of radiation on the middle and lower third leaves of the plant canopy and promoted the greater degree of global radiation interception and efficiency of solar radiation.

Extraction in plants is a crucial first step in the quantification of bioactive compounds, especially phenolic compounds in herbal plants which have received increasing attention due to their health benefits and biological activity[14]. When passing from lower to higher fertilizer rates, TPC in mature leaves decreased after the application of more than 200 kg ha^{-1} of chicken manure. Total phenolic content in young leaves was increased up to the optimum level and decreased after the application of more than 300 kg ha^{-1} of chicken manure. The amount of phenolic content was higher at a certain level and decreased after the application of a high chicken manure rate. The increasing of secondary metabolites on plants under the low amount of nitrogen happens due to an increase of enzymatic activity and non-structural carbohydrate[31]. As phenylalanine is a precursor for the synthesis of phenolic and flavonoid compounds, increasing the chicken manure rate could influence the activity of phenylalanine where it was found to be the lowest when treated with high fertilizer and the highest phenylalanine was demonstrated when the was no application of chicken manure. According to Ibrahim *et al.*[32], increased phenylalanine activity under depletion of fertilizer suggests that enhancing phenylalanine activity would lead to the accumulation of polyphenolic compounds. This result indicated that plants with application of different fertilizer rates were composed of different individual phenolic compounds and antioxidant activity. Besides that, the content of phenolic compounds could be related to the antioxidant capacity. As shown in Fig. 1a, the concentration of phenolic compounds was significantly higher in mature leaves. Light plays an important role in synthesizing biochemical compounds. According to Quamruzzaman *et al.*[29], parts of plants that were exposed to less light could result in lower content of chemical compounds. The contradiction of this finding might have happened due to differences in the agricultural management system of the crops, harvesting time and regional climatic environment.

According to the present result, it was apparent that plants grown at 0, 300 and 400 kg ha^{-1} of chicken manure rate had the lowest quercetin equivalent of total flavonoid content in young leaves. An increase in chicken manure rates above 200 to 400 kg ha^{-1} did provoke the plants to reduce the total flavonoid content. This finding of decreasing trend in quercetin equivalent of total flavonoids content with increasing chicken manure rate was in line with the results reported by Ibrahim *et al.*[11] High flavonoids content was reported as *Labisia pumila* was applied with 180 and 270 kg ha^{-1} of fertilizer. This response was purported to

happen due to a decrease in carbon to nitrogen ratio under high fertilization[33]. When the availability of nitrogen in the soil is higher, plants tend to increase their sink size compared to producing secondary metabolites due to direct nitrogen supply. Increasing use of chicken manure has resulted in negative effects on soil which would disturb the nutritional equilibrium in plants. Besides, the increment of production of flavonoids is closely related to the enhancement of phenylalanine content in plant secondary metabolites. Phenylalanine and nitrogen can inhibit flavonoid synthesis by increasing the incorporation of phenylalanine into proteins[34]. This situation would have resulted in a decreasing level of phenol and flavonoid contents for a certain amount of phenylalanine. The inconsistent effect of nitrogen on total flavonoid content also can be seen in two previous reports by researchers[33-35]. It was reported that the total flavonoid content exhibited a linear decrease with increasing fertilizer rates, particularly evident in mature leaves. Based on these results, it appears that there are no distinct physical or environmental factors that singularly contribute to the expression of flavonoids. Instead, flavonoid expression seems to result from the combined influence of various factors, including light intensity and temperature. Under specific stress conditions, the induction of various enzymes plays a crucial role in the biosynthesis of flavonoids in plants. The decreasing trend in TFC in mature leaves closely resembles the effect of chicken manure on total phenolic content in mature leaves.

Principally, the DPPH method involves the use of molecules that contain stable free radicals, which have the ability to donate hydrogen atoms and in the process, neutralize other stable free radicals. In the process of electron pairing, DPPH radicals interact with appropriate reducing agents and the purple solution gradually loses its color in a stoichiometric manner, depending on the number of electrons taken up during the reaction[36]. The changes in DPPH absorbance after the addition of testing materials is the indicator antioxidant capacity of the materials. Young leaves of *G. procumbens* that were applied with optimum chicken manure rate had a high ability to neutralize the DPPH radicals by absorbing the hydrogen in antioxidants. The results provide evidence to suggest that a high supply of chicken manure to young leaves may have a detrimental impact on antioxidant activity. This present study is in agreement with Sheikh and Ishak[22] who observed the percentage of inhibition in DPPH was reduced after the application of a high or low rate of fertilizer. The accumulation of reactive oxygen species (ROS) was stimulated due to increasing nitrogen rate above the optimum level where the percentage of inhibition was reduced as the ROS production exceeded the production of antioxidants.

Apart from that, high antioxidant activity of the extract under the optimum chicken manure might also be achieved because of the high accumulation of TPC and TFC in young leaves. The content of antioxidants in plants is dependent on the mechanism and function of phenolic and flavonoids[33]. The contribution of phenolic compounds to the antioxidant activity of *G. procumbens* was found to be related to the mechanism and function of phenolic which contributes to the antioxidant activity. Kaewseejan and Siriamornpun[14] and Rosidah *et al.*[17] showed that, the antioxidant activity was greatly influenced by the phenolic group. The connection between antioxidants and the total phenolic and flavonoid compounds in *G. procumbens* leaf extract is influenced by the presence and position of hydroxyl groups within the structure of the phenolic compounds. The high percentage of antioxidant activity was parallel with the increase of hydroxyl group occurrence on the aromatic ring of the phenolic compounds[1].

In the reducing power assay, leaf extract from the young part shows an increment of percentage of inhibition as compared to leaves from the mature part which was in accordance with the result obtained by Izreen and Fadzelly[37]. The presence of antioxidants would result in the reduction of Ferric ions (Fe^{3+}) to Ferrous ions (Fe^{2+}) by donating an electron. Therefore, the present result of the study shows that young leaves have a high capacity to reduce Fe^{3+} to Fe^{2+}. A decreasing percentage of inhibition in mature leaves could happen due to the reduced ability of the defense system to neutralize the overproduction of reactive oxygen species[38].

Kaewseejan and Siriamornpun[14] found that the flavonoid compounds including kaempferol and myricetin were identified in different fractions of *G. procumbens* leaf extract. Flavonoids were widely distributed in health-related properties of plants due to antioxidant activity contents in the plants themselves. Based on the results of their study, it was determined that the kaempferol content was generally higher in most fractions of *G. procumbens* leaves when compared to myricetin. The same pattern was reported in several previous studies[39]. In the present study, a relationship between TPC, TFC and antioxidant activity was observed. An increase of chemical markers upon application of up to 200 kg ha^{-1} in young leaf extract contributes to the antioxidant properties of *G. procumbens*. Rosidah *et al.*[17] also reported a similar pattern of increasing kaempferol 3-O-rutinoside content with antioxidant properties. This pattern was observed due to the role of myricetin and kaempferol in antioxidant activity. Kaempferol is known to have benefits of anti-inflammatory properties and exert antioxidant activity while myricetin has various functions of biological activity including anti-oxidative actions and anti-viral[15].

The quantity of nutrient supply for *G. procumbens* is reflected in the amount of nutrient composition that is absorbed by the plants. The mineral content percentage of N, P and K was influenced by chicken manure rates and leaf maturity. Amount of N and K were found to be higher in plants applied with 300 and 400 kg ha^{-1} of chicken manure. This is probably due to the slow release of nutrients in the organic fertilizer and their beneficial effect in soil improvement. The deficiency of nitrogen in leaves was found to have increased the total flavonoid content. This observation becomes apparent when considering that the plant with the lowest nitrogen uptake exhibited an increase in the accumulation of TPC, TFC, as well as kaempferol-3-rutinoside.

CONCLUSION

The application of fertilizer at 300 kg ha^{-1} was regarded as the optimum rate to produce both high yield and better quality of phytochemical compounds from *G. procumbens*. Higher fertilizer rate application will result in lower concentration of phytochemical compounds in addition to insignificant effect of biomass production. Moreover, it was also confirmed that the phytochemical content and antioxidant activity were not affected by the leaf maturity.

SIGNIFICANCE STATEMENT

Gynura procumbens has demonstrated potential health benefits which are contributed by its phytochemical content. Despite that, the effect of agronomic practices on the phytochemical content of the plant was less explored. Therefore, gaining insight into how these compounds respond to agronomic practices is essential for the successful commercial cultivation of *G. procumbens*. Hence, this study was undertaken to assess the impact of varying fertilizer rates on the growth performance, physiological characteristics and total dry matter production of *G. procumbens*. Additionally, it also aimed to evaluate the phytochemical composition of the plant at different stages of leaf maturity. Result showed that the application of 300 kg ha^{-1} of fertilizer yielded plants with optimum growth and high phytochemical content.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Ministry of Agriculture and Food Security, Malaysia for funding the research under the NKEA Research Grant Scheme (NRGS) (Project number: NH1015A025).

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