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GROWTH AND PHYSIOLOGICAL RESPONSES TO SHADE AND NITROGEN FERTILIZER LEVELS ON *GYNURA PROCUMBENS*

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Abstract

Gynura procumbens, is one of the most common medicinal plants belonging to the family of Asteraceae. Its non-toxic leaves have been documented as having phytochemicals with high potentials to be used in phytomedicine. It could be improved through varying agronomic practices such as light intensity and nitrogen fertilization that have been documented to be the main limiting factors in the production of primary and secondary metabolites. The present study evaluated 4 shade levels (0, 30, 50 and 70%) and 4 nitrogen fertilizer rates (0, 100, 200 and 300 kg N ha^{-1}) on growth and physiological responses of *Gynura procumbens.* Results showed that significant interaction between shade and nitrogen fertilizer were recorded on plants grown under 30% shade with 300 kg N ha^{-1} fertilizer rate resulting in high total leaf fresh weight (TLFW) (213.64 g), total fresh weight (TFW) (323.98 g), total leaf dry weight (TLDW) (21.26 g) and total dry weight (TDW) (43.13 g), together with increased number of branches, higher crop growth rate and relative growth rate. While, the control treatment of full sunlight (0% of shade) and no nitrogen application (0 kg N ha^{-1}) revealed the lowest fresh and dry biomass yield of TLFW (29.37 g), TFW (44.63 g), TLDW (2.70 g) and TDW (4.83 g) due to low net photosynthesis rate, total chlorophyll content, leaf area and number of branches under same treatment. The study concluded that for high biomass production, *Gynura procumbens* is to be grown under 30% shade level with 300 kg ha^{-1} nitrogen fertilizer.

Key words: *Gynura procumbens*, shade, nitrogen, growth, yield, biomass.

Introduction

Gynura procumbens is evergreen medicinal shrub belonging to Asteraceae family. It is widely distributed in Africa (Rahman and Al-Asad, 2013; Sukadeetad *et al*., 2018) and tropical regions of South-east Asia and China (Li *et al.,* 2016; Mou and Dash, 2016; Nasiri, 2016). In Peninsular Malaysia it is limited in its distribution to the western part (Kenget *et al.,* 2009).

Fresh leaves of *G. procumbens* are usually consumed as vegetables and green salad (Hew and Gam, 2011) without any toxic effect reported (Rohin *et al*., 2018). It has been traditionally used in the treatment of various ailments and health issues (Jiratchariyakul *et al*., 2000; Perry and Metzger, 1980) in countries such as Malaysia, Indonesia and Vietnam. The medicinal benefits of *G. procumbens*are said to be related to its bioactive compounds such as saponins, flavonoids and terpenoids (Mou and Dash, 2016; Tan *et al*., 2016).

The quality of medicinal plants is known by their superior genetic traits and high biomass production of consistently high secondary metabolite content (Kozai *et al.,* 2005). Concentrations of secondary metabolites of plants grown in the field are often influenced by environmental conditions such as temperature and light intensity (Mosaleeyanon *et al*., 2005). Light intensity strongly affects rate of photosynthesis as it enhances photosynthetic capacity, hence increased biomass production (Abrams and Mostoller, 1995; Nakano, Makino and Mae, 1997).

In addition, plant productivity is directly dependent on the photosynthetic capacity of the leaves which are the dominant photosynthetic organs in plants. Gregoriou, Pontikis and Vemmos, (2007) claimed that increase in shade levels reduces leaf thickness, leaf mass per area, stomatal conductance and net photosynthetic rate. In

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contrast, shoot length, inter-nodal length, chlorophyll content and leaf area were recorded to increase under the same shade levels. Consequently, the determination of optimum light intensity for chemical accumulation as well as plant growth and development is imperative in order to obtain a higher concentration of phytochemicals (Odabas *et al*., 2009).

Besides sunlight, nitrogen is one of the main nutrients required for plant growth in substantial amounts to ensure high yield. It strongly influences optimum use of environmental resources (water, light) because its metabolites (amino acids) and their derivatives (enzymes and co-factors) are of vital importance for plant growth and plant physiology (Ibrahim *et al*., 2011). Khaliq *et al*., (2008) and Khaliq *et al*., (2009) demonstrated that in sunflower, higher rates of nitrogen application led to rapid leaf area development, prolongedlife of foliage, increased leaf area duration and enhanced whole crop assimilation contributing to increases in yield. The properties of *G. procumbens* extracts have extensively investigated but studies covering responseson growth, physiology and yield as affected by shade and nitrogen fertilizer are still scarce. Therefore, the present study was conducted to investigate the influence of different shade levels and nitrogen fertilizer rate on growth and yield of *G. procumbens.*

Materials and Methods

Plant propagation

A total of 300 cuttings of *G. procumbens* were propagated in Field 2, Faculty of Agriculture, Universiti Putra Malaysia (UPM) to produce twice the number of cuttings required for the experiment. The cuttings were placed for three weeks in small paper cups and later transferred to poly bags filled with thoroughly sieved mixture of top soil, sand and peat moss in 3:1:2 (v/v) ratio.

Experimental design and treatments

The experiment was arranged in Nested design with four replicates. The treatments were consisted of four shade levels (0, 30, 50 and 70%) with four nitrogen fertilizer rates (0, 100, 200 and 300 kg N ha^{-1}). Treatments were distributed randomly by Randomized Complete Block Design (RCBD). Each treatment consisted of 15 plants, giving a total number of 960 plants. Plants were harvested at 12 weeks after transplanting.

Study Parameters

The study parameters measured included physiological parameters:net photosynthesis rate (P*N*), stomatal conductance (gs), transpiration rate (*E*), water use efficiency, photosynthetic pigments and plant growth were measured to determine the effects of shade levels and nitrogen fertilizer rates. Leaf chlorophyl 1 content was also measured using a modified method of Lichtentaler and Wellburn, (1983). Leaf weighing 0.2 g were collected from plant samples and stored in small plastic vials containing 20 mL of 80% (v/v) acetone. The vials were covered with aluminium foil and kept in the dark for 3-7 days until all chlorophyl 1 in the leaves was removed. The extracted chlorophyl1 was measured against a blank 80% acetone. The maximum absorbance of chlorophyl1-*a* was recorded at 662 nm, while absorbance in chlorophyll-*b* was recorded at 645 nm wavelength. Absorbance was measured using a Scanning Spectrophotometer Model UV 3101 PC and the total chlorophyll was calculated as the sum of chlorophyll-*a* and chlorophyll-*b* using the following formulae:

$$\text{Chlorophyll } a = 11.75 \text{ (Absorbance 662)} - 2.350 \text{ (Absorbance 645)}$$

$$\text{Chlorophyll b} = 18.61 \text{ (Absorbance 645)} - 3.960 \text{ (Absorbance 662)}$$

A portable photosynthesis system (LICOR-64001 LI-COR Inc., USA), was used to measure the plant physiological parameters: net photosynthesis rate, stomata conductance, transpiration rate and water use efficiency measured between 0900 to 1100 hr.

Plant height was measured from top of growing medium in each poly bag to terminal bud of each plant using a measuring tape. The number of branches was determined by counting the number of primary and secondary branches per plant. Total leaf area was measured using a leaf area meter (LI-3100 Area meter, USA). Specific leaf area (SLA, cm^2/g) was computed after measuring the total leaf area and dry weight of leaves using the formula: SLA = Total leaf area (cm^2) / Leaf dry weight (g) at 90 days after transplanting.

For measurement of growth rates, three plants per treatment were harvested. Fresh leaf, stem and root samples were weighed and subsequently dried in an oven at 45°C to reduce moisture content to a constant weight of dried samples. Both, the fresh and dry yield were determined using an electronic balance (BP 2100, Sartorius, Germany). The weights taken included total leaf fresh weight (TLFW), total fresh weight (TFW), total leaf dry weight (TLDW) and total dry weight (TDW).

Root-shoot ratio (RSR) was calculated after completing all harvest measurements using the formula RSR = TRDW / TShDW (TRDW= Total root dry weight; TShDW = Total shoot dry weight). Crop growth rate (CGR) was measured using the formula, CGR = (W2-W1) / (t2-t1). Relative growth rate (RGR) was calculated

Table 1: Effects of shade levels and nitrogen fertilizer rates on netphotosynthesis components of *Gynura procumbens.*

Shade level (%)	Net photosynthesis rate (µmol m^{-2} s^{-1})	Stomata conductance (mol.m^{-2}.s^{-1})	Transpiration rate (mmol.m^{-2}.s^{-1})	Water use efficiency (µmol mmol^{-1})
0	7.15 ± 0.41^{b}	0.034 ±0.0009^{a}	1.92 ±0.04^{a}	3.79± 0.27^{b}
30	10.50 ±0.47^{a}	0.031 ±0.0009^{b}	1.71 ±0.02^{b}	6.18± 0.32^{a}
50	9.11 ± 0.43^{a}	0.028 ±0.0008^{c}	1.56 ±0.03^{c}	5.94± 0.35^{a}
70	9.11 ± 0.46^{a}	0.029 ±0.0005^{c}	1.55 ± 0.03^{c}	5.91 ± 0.37^{a}
Nitrogen (N) (kg N ha^{-1})				
0	6.91 ±0.42^{c}	0.029 ±0.0007^{a}	1.87 ± 0.04^{a}	3.76 ± 0.26^{c}
100	8.86 ± 0.38^{b}	0.030± 0.001^{a}	1.67 ±0.05 ^{b}	5.42 ± 0.31^{b}
200	9.85 ±0.42^{a}	0.031± 0.001^{a}	1.62 ± 0.03^{bc}	6.17 ± 0.32^{a}
300	10.24 ±0.48^{a}	0.031± 0.001^{a}	1.61 ± 0.04^{c}	6.48 ± 0.36^{a}
S×N	ns	ns	ns	ns
Means with the same letters in columns are not significantly different at $p \leq 0.05$ (LSD)				

as RGR = (lnW2 – lnW1) / (t2 – t1), where (ln) natural logarithm, W1= total dry weight of plant at time 1 (t1) and W2 = total dry weight of plant at time 2 (t2).

Data were analysed using analysis of variance (ANOVA) and the means were separated using Least Significant Difference (LSD) at significant level of 0.05 using SAS 9.4 software. Pears on correlation analysis between parameters were calculated.

Results

There was no significant interaction between shade levels and nitrogen fertilizer rates in net photosynthesis rate (P*N*), stomatal conductance (gs), transpiration rate (*E*) and water use efficiency (WUE) ($p>0.05$) (Table 1).

Net photosynthesis (P*N*) of *Gynura procumbens* increased by 46.85% with increasing shade level from full sunlight (0% shade) to 30% shade but no significant difference was observed with 50 and 70% of shade. Similarly, nitrogen fertilizer rate at 300 and 200 kg N ha^{-1} produced significantly higher P*N* (10.24 and 9.85 µmol m^{-2} s^{-1}) than (6.91 µmol m^{-2} s^{-1}) at 0 kg N ha^{-1}. Stomata conductance significantly affected by shade level and recorded the highest value of 0.034 molm^{-2}s^{-1} at 0% shade than the lowest of 0.028 and 0.029 molm^{-2}s^{-1} at 50 and 70% shade, respectively. Meanwhile application of nitrogen fertilizer did not effect on gs of *G. procumbens*. In addition, the highest value of *E* of *G. procumbens* was 1.92 mmol m^{-2}s^{-1} at 0% shade and the lowest values were (1.56 and 1.55 mmol m^{-2}s^{-1}) at 50 and 70% shade levels, respectively. Transpiration rate~~s~~ declined by 16.14% with application of nitrogen fertilizer rates at 300 kg N ha^{-1} before application of nitrogen fertilizer (0 kg N ha^{-1}).

Accordingly shade levels and nitrogen fertilizer rates had significant effects ($p \leq 0.05$) on WUE of *G. procumbens* (Table 1). Water use efficiency was observed to increase by 63.06% with increases in shade levels from 0 to 30%. While, there were no significant differences among 30, 50 and 70% of shade level in WUE. The percentage increases were 64.09 and 72.34% at 200 and 300 kg N ha^{-1} compared with no nitrogen application, respectively.

Table 2: Effects of shade levels and nitrogen fertilizer rates on chlorophyll-*a* and chlorophyll-*b*.

Shade level (%)	Chlorophyll *a* (mgg^{-1})	Chlorophyll *b* (mgg^{-1})	Chlorophyll *a+b* (mgg^{-1})	Chlorophyll *a/b* (mgg^{-1})
0	7.81± 0.43^{c}	5.02 ±0.28^{b}	12.84 ±0.71^{c}	1.56 ±0.03^{b}
30	10.35± 0.70^{b}	6.04 ±0.29^{a}	16.39± 0.96^{b}	1.69 ±0.07^{a}
50	10.92± 0.59^{ab}	6.39 ±0.25^{a}	17.32 ±0.84^{ab}	1.69 ±0.03^{a}
70	11.98±0.66^{a}	6.80± 0.27^{a}	18.78± 0.91^{a}	1.75± 0.04^{a}
Nitrogen (N) (kg N ha^{-1})				
0	7.86 ±0.43^{c}	4.99 ±0.25^{d}	12.86 ±0.67^{d}	1.57± 0.04^{b}
100	9.69 ±0.57^{b}	5.69 ±0.23^{c}	15.38± 0.79^{c}	1.69 ±0.05^{ab}
200	10.93 ±0.58^{b}	6.42± 0.22^{b}	17.36 ±0.79^{b}	1.68 ±0.0 5^{ab}
300	12.59 ±0.66^{a}	7.15±0.27^{a}	19.74 ±0.92^{a}	1.75 ±0.05^{a}
S×N	ns	ns	ns	ns
Means with the same letters in same columns are not significantly different at $p<0.05$ (LSD)				

No significant interaction ($p>0.05$) was observed between shade levels and nitrogen fertilizer rates in chlorophyll (*a, b, a+b* and *a/b*) (Table 2). Based on table 2, chlorophyll-*a*, chlorophyll-*b*, total chlorophyll (a+b) and chlorophyll ratio (a/b) content in the leaf of *G. procumbens*were significantly affected by shade and nitrogen fertilizer ($p<0.05$). The content of chlorophylls increased with shade level from 0 to 70%. Comparable situation of increase occurred with increasing nitrogen fertilizer from 0 to 100, 200 and 300 kg N ha^{-1}.

However, the results showed significantly higher concentration of

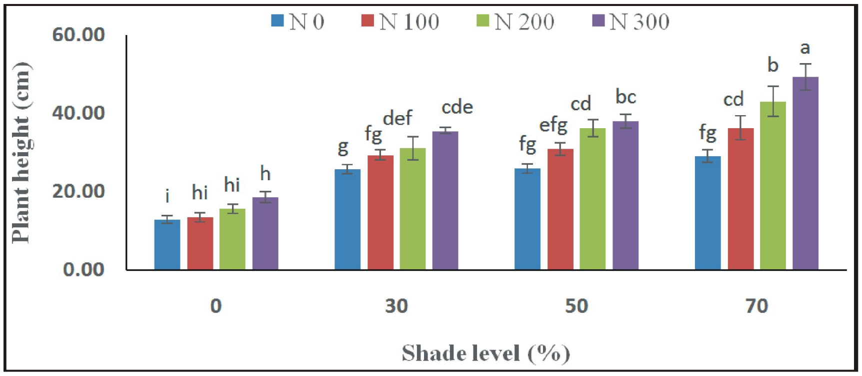

Fig. 1: Effects of shade levels and nitrogen rates on plant height of *Gynura procumbens*.

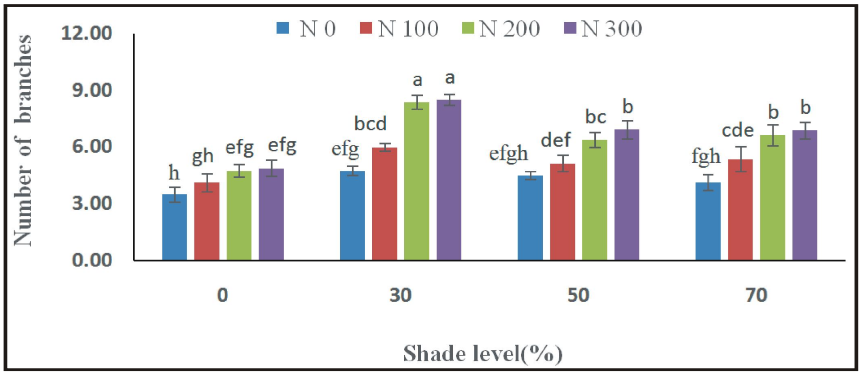

Fig. 2: Effects of shade levels and nitrogen rates on number of branches of *Gynura procumbens*.

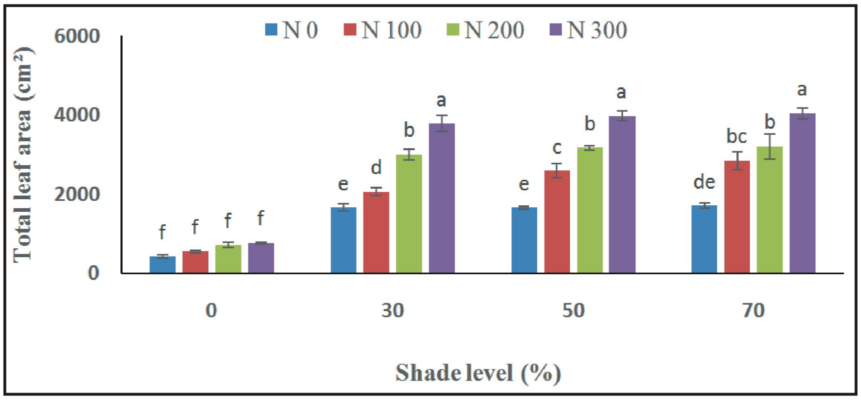

Fig. 3: Effects of shade levels and nitrogen rates on total leaf areaof *Gynura procumbens*.

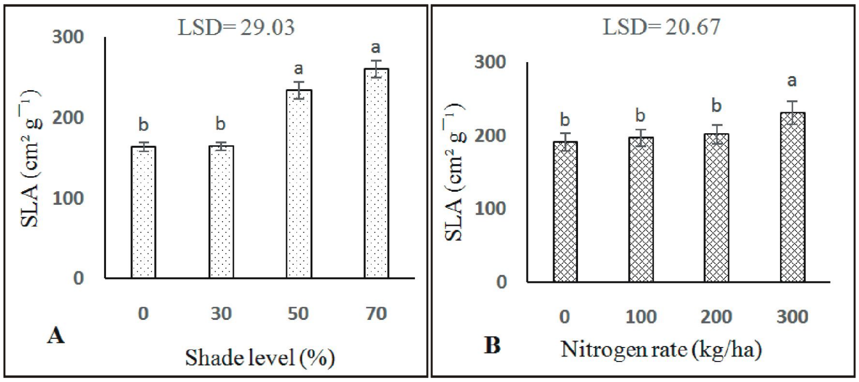

Fig. 4: Effects of shade levels (A) and nitrogen rates on specific leaf area (SLA) of*Gynura procumbens.*

chlorophyll-*a* (11.98 and 10.92mgg^{-1} fresh weight) at 70 and 50% shade levels as compared with 0% shade (7.81 mgg^{-1} fresh weight). While for chlorophyll-*b*, no significant difference among three shade levels of 30, 50 and 70% was observed with 6.04, 6.39 and 6.04 mgg^{-1} fresh weight, respectively compared (5.02 mgg^{-1} fresh weight with 0% shade). On the other hand, total chlorophyll (*a*+*b*) increased by 34.89 and 46.26% at 70 and 50% of shade levels respectively, as compared to 0% shade. Chlorophyll-*a*:*b* showed similar pattern with chlorophyll-*b* with 1.69 mgg^{-1} fresh weight under 30 and 50% shade levels and 1.75 under 70% shade level.

There were significant effects of nitrogen fertilizer rates on chlorophyll-*a*. Chlorophyll-*a* increased 23.28, 39.1 and 60% with increasing nitrogen rates from 0 to 100, 200 and 300 kg ha^{-1} N) in chlorophyll-*b* (7.15mgg^{-1} fresh weight) at 300 kgha^{-1} Nas compared to 0 kgha^{-1} N (4.99mgg^{-1} fresh weight); in chlorophyll *a*+*b* (19.74mgg^{-1} fresh weight) at 300 kg ha^{-1} N as compared to 0 kg ha^{-1} N (12.86mgg^{-1} fresh weight). Furthermore, chlorophyll-*a*:*b* (1.75, 1.68 and 1.69 mgg^{-1} fresh weight) at 300, 200 and 100 kg ha^{-1} N respectively as compared to 0 kg ha^{-1} N (1.57mgg^{-1} fresh weight).

There were significant interactions between shade levels and nitrogen fertilizer rates on plant height (fig. 1). Maximum plant height (49.38 cm) was recorded at 70% shade level and 300 kg N ha^{-1} of fertilizer application. The minimum plant height was recorded at 12.88 c mat 0% shade level and no application of N fertilizer however, no significant difference was observed with the plants applied with 100 and 200 kg N ha^{-1}.

Fig. 2, revealed that there was significant interaction between shade levels and nitrogen rates on number of branches. The maximum number of branches were 8.38 and 8.50 per plant at 30% shade under 200 and 300 kg N ha^{-1} fertilizer. While, the minimum number of branches was 3.50 at 0% shade and no nitrogen application (0 N kg ha^{-1}). However, application at 100 kg N ha^{-1}, did not show any significant effect with no application of nitrogen fertilizer.

Similarly, interaction between shade level and nitrogen application was observed on total leaf area (Fig. 3). The maximum leaf area was 3792.2, 3985.1 and 4045.28 cm^2 at 30, 50 and 70% shade under 300 kg ha^{-1} of nitrogen rate. On the other hand, the lowest value of leaf area was 425.66, 561.5, 714.2 and 771.94 cm^2 at 0% shade under 0, 100, 200 and 300 kg ha^{-1} of N, respectively.

No interaction effect occurred on maximum specific leaf area. The maximum specific leaf area was 260.05 and 233.46 cm^{2}g^{-1} observed under 70 and 50% shade. While, the lowest value was 164.43 and 163.56 cm^{2}g^{-1} under 30 and 0% shade (Fig. 4A.). The highest specific leaf area under nitrogen fertiliser was 231.33 cm^{2}g^{-1} observed under 300 kg N ha^{-1} and the lowest were 196.81 and 201.93 cm^{2}g^{-1} under 100 and 200 kg N ha^{-1}, respectively and 190.41cm^{2}g^{-1} with no application of nitrogen fertilizer (Fig. 4B).

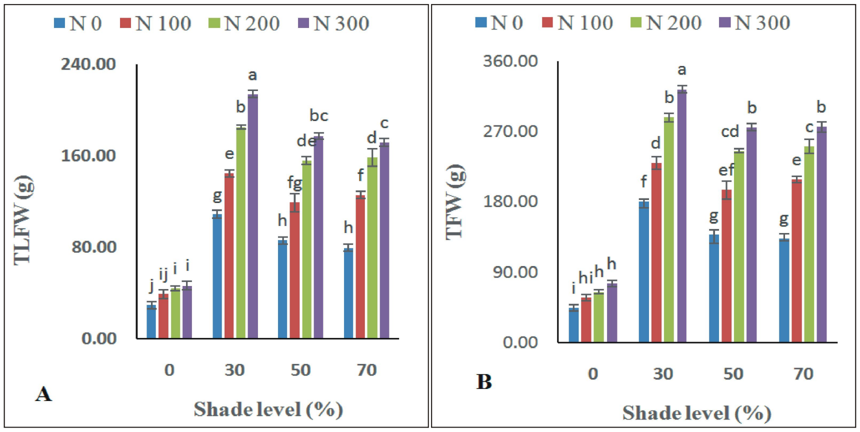

Fig. 5: Effects of shade levels and nitrogen rates on total leaf fresh weight (TLFW) (A) and total fresh weight (TFW) (B) of *Gynura procumbens.*

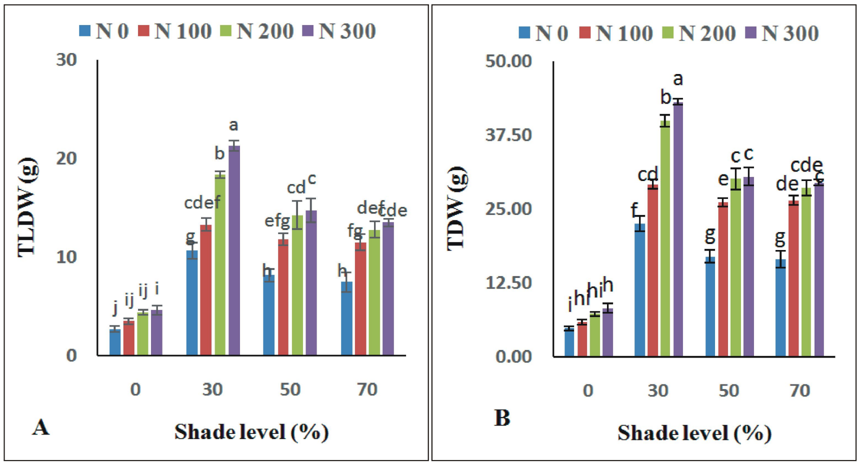

Fig. 6: Effects of shade levels and nitrogen fertilizer rates on (A) Total leaf dry weight (TLDW) and (B) Total dry weight (TDW) of *Gynura procumbens.*

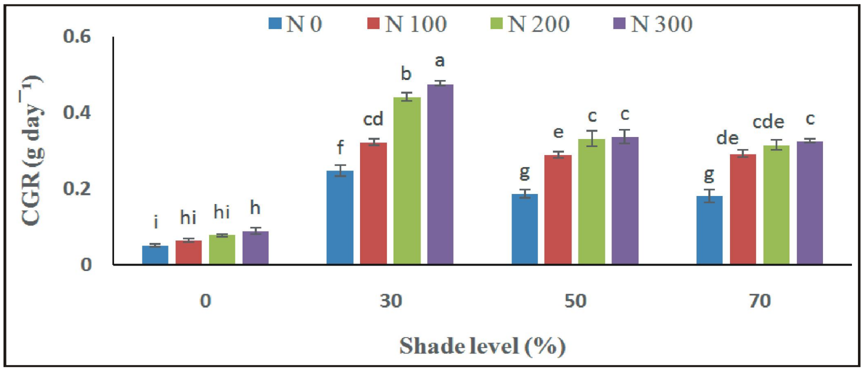

Fig. 7: Effects of shade levels and nitrogen rates on crop growth rate (CGR) of*Gynura procumbens.*

The results (Fig. 5A) showed that there were significant interaction effects of shade levels and nitrogen fertilizer rate ($p \leq 0.05$) on total leaf fresh weight per plant. The highest leaf fresh weight was 213.64 g at highest nitrogen rate (300 kg ha^{-1}) under 30% shade. The lowest total leaf fresh weight per plant was 29.37g at low nitrogen rate (0 kg ha^{-1}) under 0% shade however, no significant difference with application at 100 kg N ha^{-1}.

There was a significant interaction ($p<0.05$) recorded between shade and nitrogen in total fresh weight (TFW) of *G. procumbens* (Fig. 5B). The highest TFW was 323.98 g at 30% shade with 300 kg ha^{-1} of nitrogen rates. The minimum value of TFW indicated the similar pattern with TLFW.

Similar results were revealed in the total leaf dry weight (TLDW) and total dry weight (TDW) of *G. procumbens* under different shade levels and nitrogen rates (Fig. 6A). The maximum TLDW was 21.26 g at 30% shade with 300 kg N ha^{-1}. On the other hand, the lowest value of TDW was 2.70 g at 0% shade and no nitrogen application treatment (0 kg N ha^{-1}) but no significant difference if the plants was fertilized at 100 and 200 kg N ha^{-1}.

Fig. 6B, shows the TDW of *Gynura procumbens* grown under

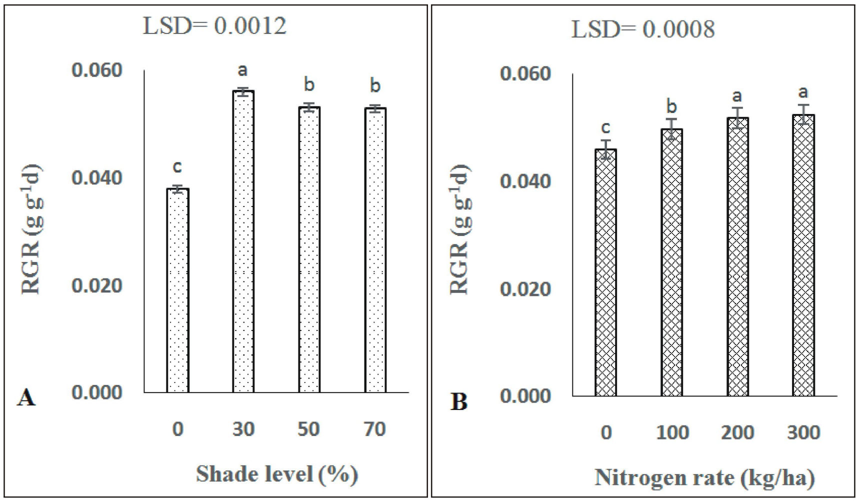

Fig. 8: Effects of (A) shade levels and (B) nitrogen fertilizerrates on relative growth rate of *Gynura procumbens.*

different shade levels and nitrogen fertilizer rates. The highest TDW of *Gynura procumbens* was 43.13 g at 30% shade with 300 kg N ha^{-1}. While, the minimum value of TDW was 4.83 g at 0% shade and no nitrogen application (0 kg N ha^{-1}).

There were an interaction effects between shade levels and nitrogen rates on crop growth rate (CGR) which produced with an average total of 0.48g day^{-1} under 30% shade and 300 kg N ha$^{-1,}$ whereas the lowest value of 0.05 g day^{-1} produced at no nitrogen application (0 kg N ha^{-1}) under 0% shade (Fig. 7).

The relative growth rate (RGR) of *Gynura procumbens* in fig. 8A, was higher at 0.056 g g^{-1}d under 30% shade compared to 0.038 g g^{-1}d grown without shade (0% shade). While, the higher RGR under nitrogen fertiliser were 0.052 and 0.051 g g^{-1}d observed at 300 and 200 kg N ha^{-1} rates respectively, compared with the lowest RGR of 0.045 g g^{-1}d under 0 kg N ha^{-1} (Fig. 8B).

The root- shoot ratio (RSR) of *G.procumbens* plants were not significantly different among all evaluated shade levels (Fig. 9A). However, the effect of nitrogen fertilizer rates on RSR was significant (Fig. 9B). The results revealed that RSR of *G.procumbens* decreased as nitrogen fertilizer rate applied either at 100, 200 or 300 kg N ha^{-1}. The corresponding percentages of decreased were 22.7, 30.8 and 28.6% for nitrogen rate of 100, 200 and 300 kg N ha^{-1} respectively, compared with no nitrogen application.

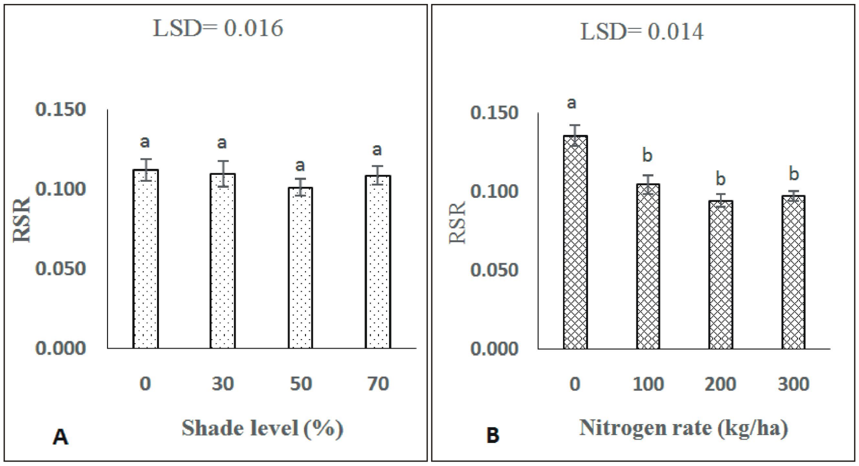

Fig. 9: Effects of (A) shade levels and (B) nitrogen rates on root-shoot ratio (RSR) of *Gynura procumbens.*

The correlation coefficient (r) between net photosynthesis rate (P*N*), total chlorophyll content a+b (*Chl*), plant height (PH), number of branches (Nb), leaf area (LA), specific leaf area (SLA), total leaf fresh weight (TLFW), total leaf dry weight (TLDW), total fresh weight (TFW) and total dry weight (TDW) of *G. procumbens* under different shade levels and nitrogen fertilizer rates are shown in table 3.

Discussion

The results showed that there was no interaction between light intensity levels and nitrogen fertilizer rates as observed in net photosynthesis rate, stomatal conductance, transpiration rate and water use efficiency. Mean while, net photosynthesis rate and water use efficiency was observed to be fluctuating under shade level and their peak point was at 30% shade level. While, the highest value of net photosynthesis rate and water use efficiency was at 200 and 300 kg N ha^{-1}. However, stomata conductance and transpiration rates were minimum under high shade level. Similar observations have been reported by a number of researchers which according to them, stomatal size decreases in response to low light intensity (Weidong, 2002; Wei *et al.*, 2005; Dai *et al.*, 2009). An increase in transpiration rates caused by high light intensity led to decrease photosynthesis in leaves (Albayrak and Camas, 2007). The results were in agreement with (Ghasemzadeh *et al.,* 2010; Gregoriou *et al.,* 2007; Ibrahim and Jaafar, 2012; Labrooy *et al.,* 2016; Zaliyatun Akhma *et al.,* 2014).

In the present study, results showed significant (P<0.05) decreases in chlorophyll contents under open field

Table 3: Correlation coefficient (r) between net photosynthesis rate (P*N*), chlorophyll content a+b (*Chl*), plant height (PH), number of branches (Nb), leaf area (LA), specific leaf area (SLA), total leaf fresh weight (TLFW), total leaf dry weight (TLDW), total fresh weight (TFW) and total dry weight (TDW) of *G. procumbens* under different shade levels and nitrogen fertilizer rates.

	P*N*	*Chl*	PH	Nb	LA	SLA	TLFW	TLDW	TFW	TDW	CGR	RGR	RSR
P*N*	-	0.57 ***	0.59 ***	0.78 ***	0.72 ***	0.26 *	0.76 ***	0.74 ***	0.75 ***	0.75 ***	0.75 ***	0.70 ***	-0.39 **
Chl		-	0.72 ***	0.58 ***	0.79 ***	0.49 ***	0.71 ***	0.64 ***	0.70 ***	0.64 ***	0.64 ***	0.65 ***	-0.50 ***
PH			-	0.57 ***	0.89 ***	0.64 ***	0.80 ***	0.71 ***	0.83 ***	0.75 ***	0.75 ***	0.80 ***	-0.30 *
Nb				-	0.75 ***	0.23ns ns	0.82 ***	0.80 ***	0.81 ***	0.81 ***	0.81 ***	0.72 ***	-0.46 ***
LA					-	0.62 ***	0.93 ***	0.86 ***	0.93 ***	0.88 ***	0.88 ***	0.88 ***	-0.42 ***
SLA						-	0.36**	0.15ns	0.39**	0.24ns	0.24ns	0.33**	0.02ns
LFW							-	0.95 ***	0.99 ***	0.97 ***	0.97 ***	0.94 ***	-0.38 **
LDW								-	0.94 ***	0.98 ***	0.98 ***	0.93 ***	-0.47 ***
TFW									-	0.97 ***	0.97 ***	0.95 ***	-0.36 **
TDW										-	0.99 ***	0.96 ***	-0.42 ***
CGR											-	0.96 ***	-0.42 ***
RGR												-	-0.37**
RSR													-

ns = not significant; *= significant at $p\leq0.05$; **=significant at $p\leq0.01$; ***= significant at $p\leq0.001$

(0% shade) and no nitrogen application. Under full sunlight, it was found that lowest chlorophyll (*a, b, a+b* and *a/b*) were 7.81,5.02, 12.84 and 1.56 respectively. Yellowing leaves of *G. procumbens* under 0% shade (full sunlight) suggesting that light intensity greater than 70% of sunlight (30% of shade) seriously impaired or inactivated the photosynthetic system. Decreases in Chlorophyll-*b* content have been suggested to be an indication of chlorophyll destruction by excess irradiance (Griffin *et al.,* 2004). Excess light can be harmful in open environments where plant metabolism is impaired by environmental stresses. Bertamini *et al*., (2006) documented that in high light intensity, photosynthetic apparatus absorbs excessive light energy, resulting in the inactivation or impairment of the chlorophyll-containing reaction centres of the chloroplasts. As a consequence, photosynthetic activity is depressed by photo-inhibition (Robinson and Osmond, 1994).

In contrast, under 70% of shade level, chlorophyll contents (*a, b, a+b* and *a/b*) recorded 11.98, 6.80, 18.79 and 1.75, respectively. Under high shade level (low light intensity) the *G. procumbens* leaf chlorophyll pigments increased as physiological response in order to absorbs high amount of light. The results were similar with other findings which reported increased chlorophyll contents due to plants grown under shade (Santo and Alfani, 1980). The increase in chlorophyll content of shade-grown plants is said to be due to more resources are channelled into chlorophyll synthesis in low light environment to maximise available light utilisation. Plants grown under shaded conditions are known to optimise their effectiveness of light absorption by increasing pigment density per unit leaf area (Wittmann *et al.,* 2001). Bailey *et al*., (2001) reported that plants balance light absorption for photosynthesis by regulating chlorophyll synthesis and in many plants, changes in light intensity elicit physiological responses at the level of leaf and chloroplast.

Studies on nitrogen supply in relation to chlorophyll content showed that an increase in chlorophyll content was associated to nitrogen supply where the highest chlorophyll content was achieved (Chlorophyll-*a*=12.59, Chlolophyll-*b*=7.15, Chlorophyll a+*b* =19.74 and Chlorophyll-*a/b*=1.75) at the highest nitrogen rate (300 kg Nha^{-1}). Tissue nitrogen contents increased with increasing nitrogen fertilisation and due to the fundamental role of nitrogen nutrition in the chlorophyll complex

(Barker, 1979). Pompelli *et al*., (2010) showed that decreased light captured via strong reductions in the concentrations of both Chlorophyll-*a* and Chlorophyll-*b* was evident in response to both high light and nitrogen deficiency. The results were in agreement with (Mao *et al*., 2007; Ghosh *et al.,* 2004).

In the present study, the high value of plant height, total leaf area and specific leaf area were observed at high shade level (lower light intensities) and high nitrogen fertiliser supplementation. However, the lower value of plant height, total leaf area, specific leaf area were observed to be stunted at 0% of shade (higher light intensity) and at lower rate of nitrogen fertiliser. It is clear that high shade level stimulated stem elongation. Stem elongation is typically considered as part of 'shade avoidance syndrome'. The differences observed in plant height as the effect of shade was stronger at higher N supplementation than at lower N. This is due to limitation of the resources at low nitrogen supply. The results are in agreement with the results reported in previous studies by (Morais *et al.,* 2006; Bote and Vos, 2017) who reported greater stem height of trees grown under shade compared to trees exposed to full sun. It increased number of branches as plants' response to increasing light absorbance under a 30 % light condition with high nitrogen supply. Higher nitrogen supplementation stimulated plant height, number of branches, leaf area and leaf dry weight (Bote and Vos, 2017) but disagreed with Campanha *et al*., (2004).

It was also noted that increasing shade levels and nitrogen supply generally resulted in an increase of leaf area and specific leaf area. The lower values of leaf area, plant height and specific leaf area in higher light intensity counteracted better performance observed. Branch length and leaf dry weight showed most positive plasticity in response to both radiation and N supply (Bote and Vos, 2017). The increase in specific leaf area under low light intensity which is accompanied by increase in the leaf area served to maximise the leaf surface area available for interception of the limited light incident. Partitioning of biomass is altered to provide maximum possible surface area for photosynthesis (Loach, 1970; Pons, 1977). Hughes *et al*., (1965) reported that increase in specific leaf area was attributed to greater expansion of existing cells rather than increased cell division. Typically leaves of plant grown under shade have fewer layers of palisade parenchyma cells than leaves plant grown under full sun (Pons, 1977). In the study conducted by Casey *et al*., (2002), 40% shade elicited a response of similar magnitude as that for 60% shade in terms of dry matter content, which manifested itself in greater leaf area expansion. Therefore, 40% of shade conferred double benefit of receiving enough light for greater biomass accumulation than 60 or 80% shade coupled with relatively large morphological response to shade. Urbas and Zobel, (2000) indicated that a decrease in leaves thickness and increase in leaf area under low light intensity led to reduce plant biomass due to decrease of some leaves per plant.

Results in the current study showed that nitrogen levels had significant effect on plant height and leaf area. Plants grow well if nutrient uptake in the shoot were high, but in contrasts, nitrogen deficiency could induce stunted cell division and consequently inhibited plant growth. (Khaliq *et al*., 2008 and 2009) demonstrated that high rate of nitrogen application leads to more rapid leaf area development, prolongs life of foliage, increases leaf area duration and enhances whole crop assimilation, accordingly contributing to increases in sunflower yield. While Bote and Vos, (2017) reported that higher nitrogen supplies stimulated plant height, number of branches, leaf area and leaf dry weight.

The smaller and thicker leaves (low specific leaf area) at high light intensity would help to maximise water-use efficiency (Parkhurst and Loucks, 1972) and also allow more light to pass to lower canopy layers, which is beneficial in high light intensity, where top leaves receive light in excess, saturating leaf photosynthesis (Bote and Vos, 2017). Moreover, under no nitrogen application (0 kg N ha^{-1}) plants adapt their leaf size to maintain high nitrogen content per unit leaf area, photosynthetic capacity and radiation use efficiency to the detriment of light interception per plant (Anten *et al*., 1995; Vos and Van der Putten, 1998). In order to survive under stress conditions (high light intensity and nitrogen deficiency) *G. procumbens* plant minimized its leaf size.

Moreover, shade treatments can reduce canopy temperature about 2-4°C as compared to exposure to full sunlight which reduces evapotranspiration *(DaMatta et al.,* 2007; *Geromel et al.,* 2008; Guyot *et al.,* 1996; Muschler, 2001; Vaast *et al.,* 2006). Increased root hydraulic conductivity and decreased evapotranspiration under nitrogen supply improve regulation ability of water balance in coffee plant trees (Yang *et al.,* 2011) and contribute to enhance yield.

In the present study, plant height and leaf area showed positive plasticity in response to shade levels. Leaf area declined from 2955.8 to 618.3 cm^2 and plant height declined from 39.5 to 15.1 cm for a decrease in shade from 70 to 0% shade while it was noted a decrease in leaf area from 3148.63 to 1368.1 cm^2 and decrease in plant height from 35.4 to 23.4 cm for a decrease in nitrogen supply from 300 to 0 kg ha^{-1}.

For root-shoot ratio (RSR), the result showed that only nitrogen fertilizer rates had significant effects. The highest value was under 0 kg N ha^{-1} than the lowest at 300 kg N ha^{-1}. The differences in biomass between above-ground parts and below ground parts is associated to nutrient limitation (Li *et al*., 2016; Zhang *et al.,* 2018). Bo *et al*., (2009) demonstrated that low nitrogen availability in the soil improved root biomass, whereas high levels of available nitrogen reduced root biomass. However, in nutrient-deficient conditions, growth was reduced in the above-ground parts of wheat plants than in the root system which indicated that stress had a relatively strong effect on growth of above-ground parts. The reduction in total root dry weight (data don't show) was lower than the reduction on total shoot dry weight when nitrogen fertilizer decreased from 300 to 0 kg N ha^{-1}. Accordingly, above ground part of *G. procumbens* is high sensitive to nitrogen deficiency than root. The results were in agreement with (*Wang et al.,* 2014; Yang *et al.,* 2018) but disagreed with (Bote and Vos, 2017).

Total fresh weight, total dry weight and crop growth rate were higher at 30% shade with 300 kg N ha^{-1} where as the lowest biomass was observed under 0% shade (full sunlight) with no nitrogen application (0 N kgha^{-1}). This was due to the observed increase in number of branches, net photosynthesis rate and crop growth rate under 30% shade with higher nitrogen supply which increased total biomass yield. Results in table 3 showed that there were strong and positive correlations between P*N*, *Chl* and Nb with (TLFW, TLDW, TFW and TDW) (r=0.76, 0.74, 0.75 and 0.75) for P*N*, (r= 0.71, 0.64, 0.70 and 0.64) for *Chl* and (r= 0.82, 0.80, 0.81 and 0.81) for Nb respectively, which positively reflected crop growth rate under 30% shade at high nitrogen supplementation. Contrary to the negative correlation between RSR and (TLFW, TLDW, TFW and TDW) which was (r= -0.38, -0.47, -0.36 and -0.42), there were strong and positive correlation between CGR and RGR with (TLFW, TLDW, TFW and TDW) the values of (r) were (r= 0.97, 0.98, 0.97 and 0.97) and (r= 0.94, 0.93, 0.95 and 0.96) respectively, which resulted in increased biomass yield of *G. procumbens* plant under 30% shade (70% of sunlight) at 300 kg N ha^{-1}. These observations confirmed the results reported by (Bote and Vos, 2017; Ibrahim *et al.,* 2011) however, in contrast with (Santo and Alfani, 1980) who found that there were no difference between full sunlight and 66% shade on dry matter production of mint (*Mentha piperita* L.).

Conclusion

Light intensity levels and nitrogen fertiliser rates significantly affected growth of *G. procumbens*. Leaf area, number of branches, leaf chlorophyll content changed according to growth conditions as plants response to survive or maximise biomass yield. The lowest leaf area, chlorophyll content and net photosynthesis rate were under full sunlight with no application of nitrogen fertiliser which resulted in low biomass yield. In contrast, the 30% shade with 300 kg N ha^{-1} was favourable for high biomass yield of *G. procumbens* due to high number of branches and net photosynthesis rate.

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