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GROWTH, PHYSIOLOGICAL RESPONSE, YIELD AND PHYTOCHEMICAL CONTENT OF VERNONIA AMYGDALINA AS AFFECTED BY DIFFERENT LIGHT INTENSITIES, GROWING MEDIA AND HARVEST TIMES

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Abstract: *Vernonia amygdalina* Del. is a botanical plant used for self-medication purposes. It is important to understand its acclimatization process, which is influenced by abiotic factors and agronomic practices on growth performance. The present study was conducted to determine the optimum light intensity, growing media, and harvest time required to maximize the growth performance of *V. amygdalina*. The treatments consisted of three light intensities (30, 50 and 100%), four growing media (soil, cocopeat, empty fruit bunch and burnt paddy husk) and six harvest times. The highest biomass yield of *V. amygdalina* was achieved with exposure to 50% light intensity. Highest plant height and specific leaf area were found on *V. amgdalina* grown under 30% light intensity, while 50% light intensity attained the highest photosynthetic rate on empty fruit bunch growing media. Both total phenolic and flavonoid contents of *V. amygdalina* increased when harvested at 9 and 18 weeks after transplanting. Therefore, the practice of using oil palm empty fruit bunch with 50% of light intensity exposure and harvest at 18 weeks after transplanting is recommended to optimize the growth and phytochemicals yield of *V. amygdalina*.

Keywords: *Vernonia amygdalina*, growing media, light intensity, harvest time, phytochemicals.

Introduction

Vernonia amygdalina is an herbal plant native to Africa from the Asteraceae family. It is also found growing wild in many countries in Asia (Wong *et al.,* 2013) where it is called "*Bitter leaf*" or "*Daun Bismillah*". The green leaves of *V. amygdalina* have a bitter taste and unique odor (Udochukwu *et al.,* 2015) and contain bioactive compounds used in pharmacological products (Ramachendrin, 2015). The highest amount of anti-nutritive constituents, such as alkaloids, tannins, glycosides and saponins, is found in the leaves (Adiukwa *et al.,* 2013). The major components found in the leaves of *V. amygdalina* with medicinal properties are sesquiterpene lactones, kaempferol flavonoid and saponin vernonin (Ademola & Eloff, 2010; Iwu, 2013). The potent antioxidant and free radical activities of this plant are probably due to the presence of flavonoids and phenolic compounds (Adaramoye, 2008; Udochukwu *et al.,* 2015). In depth studies of *V. amygdalina* found that its leaves exhibit antidiabetic, anticancer, antibacterial, antimalarial, antiparasitic and anthelmintic properties (Audu *et al.,* 2012). In Nigeria, the stems of *V. amygdalina* are applied as chew-sticks (Udochukwu *et al.,* 2015) while it roots are used to cure toothaches because of their antimicrobial properties (Imaga & Bamigbetan, 2013).

Light plays an important role in the plant's development and significantly affects plant growth, biomass and phytochemicals accumulation (Li *et al*., 2015). The plants can only fully self-regulate to absorb and transform light energy within their own light range for growth (Yao *et al.,* 2017). However, only few levels of light intensity could optimize the plant growth. For example, *Eryngium foetidum* performs well in producing biomass yield when planted under 25-50% of shading (Moniruzzaman *et al.,* 2009). Shading was found to decrease the moisture, temperature and light intensity requirement (Nur Faezah *et al.,* 2015).

Nonetheless, extreme irradiance can disrupt the pigment and inhibit photosynthesis (Ibrahim & Jaafar, 2012). When *Pothomorphe umbellate* was exposed to full sunlight, it demonstrated lower rate of photosynthesis, less plant yield and lower nutrient content (Mattana *et al.,* 2010). The chemical and physical characteristics of growing media ware important to supply water and nutrients to *V. amygdalina,* as this plant is rarely grown commercially. Nowadays, organic matter is a common amendment to natural soil to conserve fertility and efficiently use nutrients (Riaz *et al.,* 2015). Basically, organic matter improves physicochemical characteristics of nutrient availability (Suvitha & Babu, 2017) and enhances biological properties of soil (Demir & Gulser, 2015). These agricultural waste products: Empty fruit bunch (EFB), cocopeat and burnt paddy husk (BPH), can be used as organic growing media for *V. amygdalina* plant.

The phytochemicals content in plants varies according to harvest time (Chua *et al.,* 2015), and environmental factors of irradiance and CO_2 concentration, plant age, and leaf maturity, which all affect the synthesis of phenolics and flavonoids (Hemm *et al.,* 2004). Bagchi *et al.* (2003) found that harvesting Adhatoda *vasica* during the vegetative stage resulted in low vasicine concentration. The phytochemicals in medicinal plants *Melissa officinalis, Hypericum pruinatum* and *Artemisia annua* have been found to contain higher bioactive compounds when harvested during the flowering stage (Tajidin, 2017). However, exposure to environmental conditions can change the plant physiology and morphology by influencing the production of chemical compounds (Ghasemzadeh *et al.,* 2010). The synthesis of secondary metabolites is related to plant`s defence mechanism against predators, like insects, fungi and herbivorous mammals (Chua *et al.,* 2015).

Information on the underlying acclimatization process, agronomic practices and suitable harvest time is vital to develop technology to improve the commercial cultivation of this herb. In Malaysia, research into the medicinal properties of *V. amygdalina* has been carried out, but studies that focus on other aspects are lacking. By examining light intensity, growing media and harvest time, this study investigated their impact on plant growth, physiological response, yield, and phytochemical production of *V. amygdalina.*

Materials and Methods

Experimental Site, Plant Materials, Experimental Design and Treatments

The study was conducted at Field 15, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor. The stem cuttings of *V. amygdalina* were collected from the Malaysia Agricultural Research and Development Institute (MARDI), Serdang, Selangor. The plant was identified in the herbarium unit of the Institute of Bioscience at Universiti Putra Malaysia (UPM) with a voucher specimen of SK-3180/17. The 15 cm stem cuttings were propagated first under 70% light level using trays containing media of cocopeat:sand (3:1) before they were transferred to the experimental plot. The treatments consisted of four types of growing media. The soil was combined with three types of organic matter (cocopeat, empty fruit bunch (EFB) and burnt paddy husk (BPH)) and sand in a 3:2:1 ratio of soil: chicken manure: sand (T1), soil: cocopeat: sand (T2), soil: EFB: sand (T3) and soil: BPH: sand (T4). After one month, the rooted cuttings of *V. amygdalina* were moved into polybags (41 x 41 cm) and arranged in a nested design (3 replications) under three levels of light intensity: 30% (70% shading netting), 50% (50% shading netting) and 100% (without shade), classified as Experiment 1. Limitation of space to arrange the experimental units according to the treatments of different light intensity and growing media, led to the choice of the nested design.

The second batch of planting from the finding of optimum requirement of the light intensity and growing media on *V. amygdalina* from Experiment 1 (50% light intensity EFB) was applied in this study as Experiment 2 (Effects of different harvesting time on plant phytochemicals content). The treatment consisted of six different harvest times, which are 3,6,9,12,15 and 18 WAT and arranged

as Randomized Complete Block Design (RCBD). Leaf samples were taken and the phytochemical analyses were conducted at the laboratory of Physiology, Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor.

The data collection for plant growth parameters (Experiment 1) and phytochemicals (Experiment 2) were taken at three-weekly intervals after transplanting, starting from week 3 and extending to week 18.

Plant Growth Parameters

The parameters of plant growth were evaluated along the experimental duration to observe the impacts of light intensity and growing media on plant development. The parameters taken were plant height, dry matter and leaf area. The plant height was determined by using a tape, measured from the surface of the growing media until upper part of the plant. The data were taken on 36 samples plants. The leaf area was analyzed immediately after harvest using an automatic leaf area (MODEL LI-300, LI-COR), the leaves then were dried in the oven (50°C) for four days and the weight was taken using a digital scale to get the biomass yield. While for the specific leaf area, the following formula was used:

Specific leaf area ($cm^3\ g^{-1}$)
= Total leaf area/total leaf dry weight

where, biomass yield was calculated the following equation:

Biomass yield ($kg\ ha^{-1}$)
= (Dry weight of leaves/area per plant) x 10,000

Plant Physiology

Net Photosynthesis Rate

The net photosynthesis rate was analyzed using closed system, infrared gas analyzer by placing the cuvette head on fully young leaves supported by tripod stand (Ibrahim & Jaafar, 2012). The optimal conditions of 800 $\mu mol \cdot mol^{2} \cdot s$ photosynthetic photon flux density, 400 $\mu mol \cdot mol^{-1}\ CO_{2,}$ 60% relative humidity, 30°C cuvette temperature and 500 $cm^3 \cdot min^{-1}$ of air flow rate were set up on a portable photosynthesis system attached with a red LED light source (Licor LI-6400 Nebraska, USA). The measurements were carried out at 0900 to 1100. The operation was automatic, and the data was stored in the LI-6400 console and analyzed by "Photons Assistant" software (version 1.0; Dundee Scientific: Dundee, Scotland, UK, 2000).

Total Chlorophyll Content

The total chlorophyll content was evaluated by obtaining disks using a hole puncher on young fully expanded of *V. amygdalina* leaves. The leaf disks (3 mm diameter) were left in 80% acetone (20 mL) in glass bottles covered with aluminium foil for 36 hours or until all the leaves were decolorized. The spectrophotometer (UV-3101P, Labomed Inc, USA) was then used to measure the chlorophyll extraction at the wavelengths of 664 and 647 nm (Coombs *et al.*, 1986).

The total chlorophyll content was calculated as follows:

Chlorophyll a (mg cm^{-2} fresh leaf)
$= 13.19A_{664} - 2.57A_{647}$

Chlorophyll b (mg cm^{-2} fresh leaf)
$= 22.10A_{647} - 5.26A_{664}$

Total chlorophyll content (mg cm^{-2})

$$= \frac{3.4x\ (Chl\ a + Chl\ b)}{4}$$

where A_{647}, A_{664} are the absorbance of the solution at 647, 664 respectively while 13.19, 2.57, 22.1 and 5.26 are the absorption coefficients, 3.5 is the total volume used in analysis taken from the original solution (ml) and 4 is the amount of total disc area (cm^2).

Plant Phytochemical Contents

The leaf phenolic content was evaluated using the Follin-Ciocalteau reagent (gallic acid as a standard) (Kaewseejan & Siriamornpun, 2015). A 200 µL of leaf extract was mixed with 10% (v/v) Follin-Ciocalteau reagent (1.0 mL) and left to stand for five minutes, followed by the addition of 800 µL sodium carbonate (7.5%, w/v). The mixture was allowed to stand for a

further 30 minutes in the dark and the absorbance was measured at 765 nm using an ultraviolet spectrophotometer (WPA Biowave II, Biochrom Ltd, England). The TPC is expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW). The colorimetric method adopted to determine the flavonoid content followed the technique described by Kaewseejan and Siriamornpun (2015). In brief, 250 µL of extract was mixed with 1.25 mL of distilled water and then 75 µL of 5% $NaNO_2$ solution, 150 mL of 10% $AlCl_3$ was added to the mixture after leaving it to stand for 6 minutes. After five minutes, 500 µL of 1 M NaOH was added to the mixture, and the total volume was made up with distilled water to the volume of 2.5 mL. The absorbance of the mixture was measured at 510 nm with quercetin as a standard. Results are expressed as mg of quercetin equivalent per g of dry weight (mg QE/g DW). DPPH free-radical activity was determined by following the method described by Gulcin *et al.* (2015). One mL of extract was mixed first with 2 mL of DPPH (0.1 mM, dissolved in ethanol) radical solution. The mixture was left to stand in the dark for 30 minutes. Then, the absorbance was read at 517 nm with a blank containing methanol and DPPH solution in 96-well microplate. The percentage of inhibition was calculated as follows:

Percentage of inhibition:
(Blank sample - extract)/blank) x 100

Statistical Analysis

Analysis of Variance (ANOVA) for nested design (Experiment 1) and randomised complete block design (Experiment 2) data were obtained using the Statistical Analysis System (SAS 9.1, SAS Institute, Inc. Cary NC. USA). Least Significant Difference (LSD) test at $P<0.05$ was used for mean comparison.

Results and Discussion

Nutrient Contents of Growing Media

To investigate the influence of the different growing media on plant growth performance, the content of several main nutrients in the growing media were analyzed and the results are shown in Table 1.

Plant Growth Parameters

Light and media are the main aspects in supplying plants with energy sources and nutrients for growth. In general, differences in light intensity significantly affected all plant growth parameters measured at $P<0.05$. The findings from this study suggest that the response of *V. amygdalina* exposed to light intensity is like other shade tolerant plants.

Decreasing light intensity led to greater *V. amygdalina* plant height (Figure 1(a)). It was found that plants which were planted within 30% and 50% light intensity showed an increase in mean height of 33% and 22% respectively, compared to those under full sunlight. The height of *V. amygdalina,* followed a similar trend to the herbal plant of *Baccharis trimera*, where the higher shade levels increased the plant height (Fernandes *et al.,* 2013). The increase in plant height is probably due to high accumulation of auxin in the apex, resulting in apical dominance (Moniruzzaman *et al.,* 2009;

Table 1: Nutrient contents of growing media of control, cocopeat, EFB and BPH

Treatment	Nitrogen (N)	Phosphorus (P) (%)	Potassium (K)
T1 (Control)	0.23	0.005	0.008
T2 (Cocopeat)	0.31	0.001	0.019
T3 (EFB)	0.56	0.005	0.022
T4 (BPH)	0.52	0.002	0.019

Fernandes *et al.,* 2013). This acclimatization to light intensity favors a better light interception, leading to a higher biomass yield (Silva *et al.,* 2006). As there was limitation of light under shading, plants had phototropism responses which efficiently captured light for shoot growth (Kumar *et al.,* 2013). Therefore, it is not surprising that *V. amygdalina* grown within low light levels had increased height.

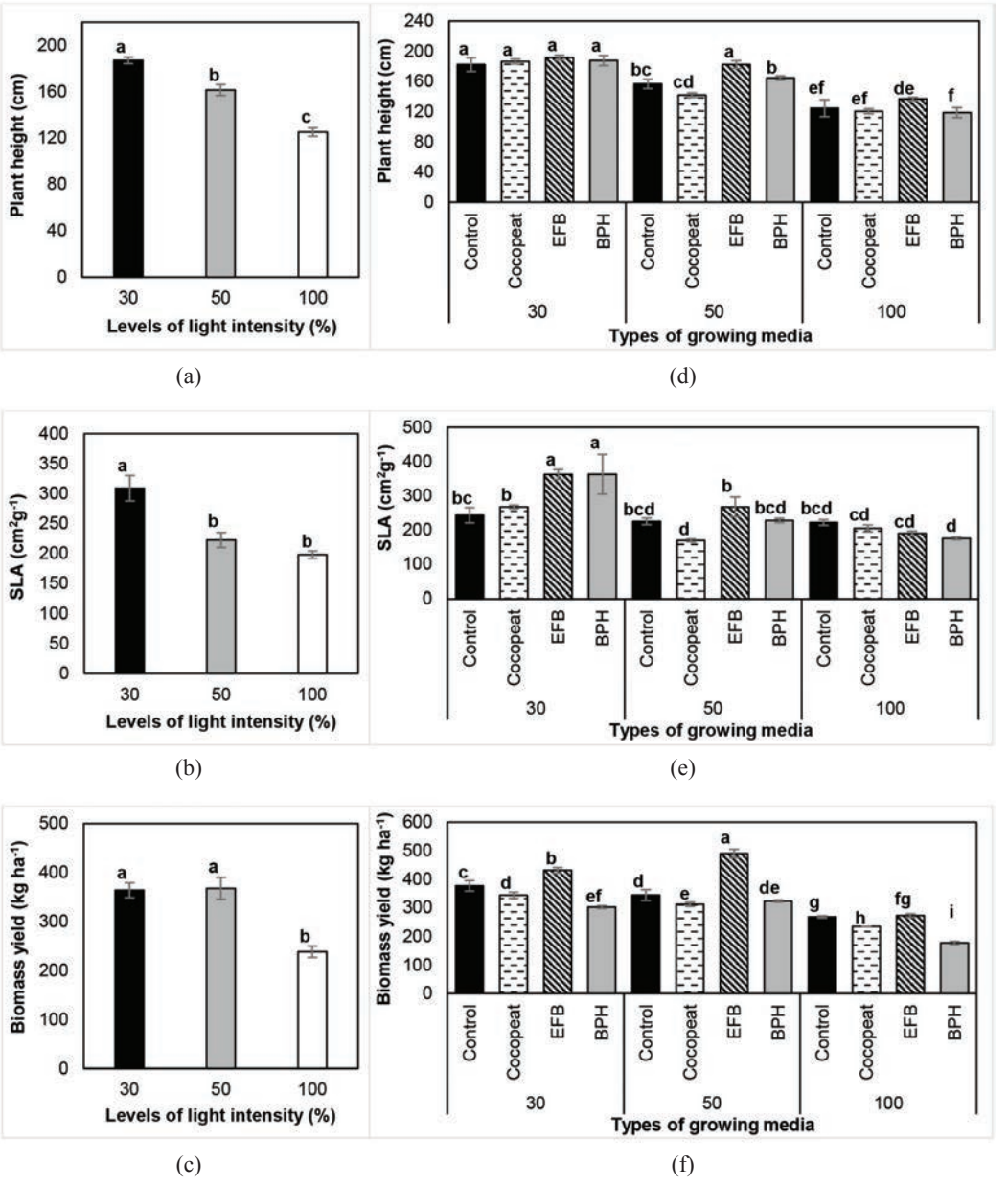

Figure 1: (a) Plant height, (b) specific leaf area (SLA) and (c) biomass yield of *V. amygdalina* under 30%, 50% and 100% light intensity, and (d) plant height, (e) specific leaf area (SLA) and (f) biomass yield of *V. amygdalina* using growing media of control, cocopeat, EFB and BPH. Different letter above each bar indicates significant differences at P≤0.05 using the Least Significant Difference (LSD) test

Plants respond to varying amounts of light physiologicaly and morphologicaly, as well as specific leaf area (SLA) to boost carbon fixation and light absorption in that particular environment for plant performance (Liu *et al.,* 2016). Decreased leaf thickness can lead to decreased leaf yield, therefore resulting in greater SLA (Karavin, 2013). In this study, SLA of *V. amygdalina* grown under 30% in comparison to 50% and 100% light levels had as much as 28% and 36% more leaf area, respectively (Figure 1(b)). A similar finding was made by Du *et al.* (2017) where *Solidago canadensis* (Family Asteraceae) also had thin and large leaves when grown under low light intensity. "Phenotypic plasticity" is the capability of the plant to perform various phenotypes to maintain better performance during acclimatization towards different environments (Feng & Van Kleunen, 2014). It was also found that *V. amygdalina* grown under 30% and 50% light intensities yielded significantly greater biomass compared to those grown in full sunlight, with the average difference around 34% and 35%, respectively (Figure 1(c)). This result is in agreement with Nur Faezah *et al.* (2015) who found that the medicinal plant of *Andrographis paniculata* produced higher yield when grown under 50% light level compared to those grown in the open field. In this situation, it is well-known as "balanced growth hypothesis", where the biomass is allocated more to shoots when the growth-limiting factor is above ground (e.g. light). Alternatively, when the limiting factor is below ground (e.g. nutrients), the biomass tends to distribute more to the roots (Poorter *et al.,* 2012).

The assessment of different types of growing media revealed that there was an impact on plant growth caused by different growing media. Most V. amygdalina plants showed a significant increase in growth when planted in the EFB medium. As shown in Figure 1(d), the different growing media of control, cocopeat, EFB and BPH (30% light intensity) and also EFB (50% light intensity) were observed to show high mean plant height. Similarly, EFB and BPH (30% light intensity) showed the highest SLA compared to other growing media (Figure 1(e)). EFB and BPH were indicated as the best media for *V. amygdalina* production and increased leaf area because of the higher rate of diffusion of oxygen to the roots (Zulkarimi *et al.*, 2010). Adapting the soil with BPH helps to decrease bulk density, and it increases organic matter and soil porosity, which both aid the roots' nutrient uptake (Demir & Gulser, 2015). Meanwhile, the biomass yield in different growing media varied significantly with EFB (50% light intensity) showing the highest biomass yield (Figure 1(f)). The EFB medium resulted in 64% more biomass yield compared to the lowest yield, which were plants grown in BPH under 100% light intensity. EFB is an essential source of nutrients and adding it to the soil improves soil biological activity and biomass yield, as found in *Orthosiphon stamineus* (Affendy *et al.*, 2010).

Plant Physiology

Net Photosynthesis Rate

Photosynthesis is the major physicochemical process for plant growth and involves plants using light energy to synthesis organic compounds. As shown in Figure 2(a), different light levels significantly changed the net photosynthesis rate (Pn rate). Of measured light intensities, 50% light intensity resulted in a 16% and 30% higher Pn rate of *V. amygdalina* than 100% and 30% light intensity. This result was similar to what was reported by Mattana *et al.* (2010) who identified that exposure of *Pothomorphe umbellate* to full light significantly decreases the rate of photosynthesis. This decrease as a result of photoinhibition that causes chromo-pigment degradation (Nur Faezah *et al.,* 2015). While the low photosynthetic rate under low levels of light intensity is by the reason of lower activity of RuBP carboxylase for carbon fixation through the photosynthetic pathway (Muthuchelian *et al*., 1989). Furthermore, the leaf morphology attributes of thicker and compact leaves, and tidier spongy and palisade tissue of chloroplasts contribute to the greater rate of photosynthesis (Xu-yang *et al.,* 2017). Nonetheless, light intensity levels only significantly affected

chlorophyll content of plants grown under 30% and 100% light intensity (Figure 2(b)).

Chlorophyll is a crucial component of the pigment-protein complex and plays a major role in photosynthesis. Any changes to chlorophyll content in leaves lead to a different photosynthesis rate (Gezahen, 2016). Chlorophyll content for plants exposed to 100% light intensity (open field) was lower by 24% compared to plants grown under 30% light intensity. However, no significant difference was detected with plants grown under 50% light intensity. Ma *et al.* (2015) also found that there was a lowering chlorophyll content of *Camptotheca acuminata* leaves when planted in an open field. This reduction may be the cause of the changes in leaf morphology (larger and thinner leaves) which exhibits high grana in chloroplast (Bote & Struik, 2011). Even though chlorophyll synthesizes typically in the presence of light, extreme light can disrupt and ultimately lower the amount of chlorophyll (deCarvalho Goncalves *et al.,* 2005). The different growing media had varying effects on the plant physiology of *V. amygdalina.* Figure 2(c) shows that plants that were grown in EFB (50% light intensity) had the highest Pn rate, while the plants grown in BPH, control and cocopeat (30% light intensity) had lower Pn rates by 1.3%, 1.4% and 5.1%.

Nitrogen is an element particularly found in chloroplast and is closely associated to photosynthesis process. Hence, sufficient N uptake from the EFB media that is rich in nitrogen (0.56%) (Table 1) can lead to a higher Pn rate and plant productivity, a result also found in sweet corn (Abdulrahman *et al.,* 2016). However, the chlorophyll content of plants grown in EFB of 30% light intensity was 24%

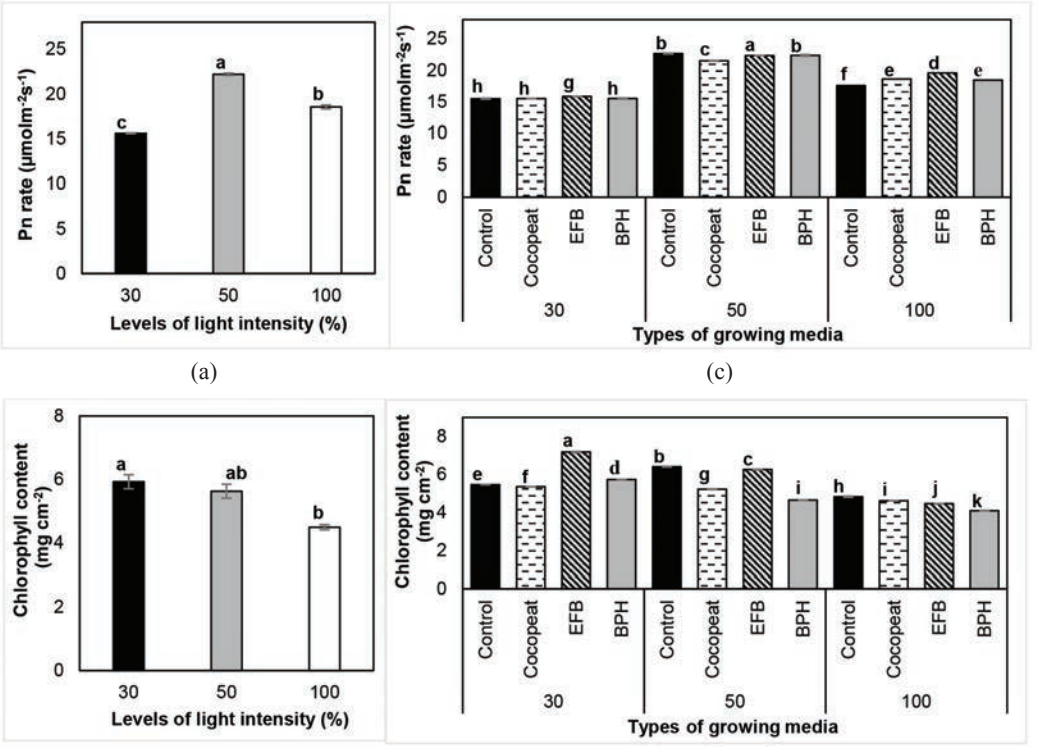

Figure 2: (a) Net photosynthesis rate and (b) chlorophyll content of *V. amygdalina* under 30%, 50% and 100% light intensity, and (c) net photosynthesis rate and (d) chlorophyll content of *V. amygdalina* using growing media of control, cocopeat, EFB and BPH. Different letters s above each bar indicates significant differences at P≤0.05 using the Least Significant Difference (LSD) test

higher compared to the lowest plant chlorophyll content of BPH (100% light intensity) (Figure 2(d)). Amending the soil with EFB improves the leaf chlorophyll in *V. amygdalina* which may be related to the high N content. Furthermore, N is a protein building nutrient that develops vegetative growth and plant reproduction, and it is derived entirely from the media (Lawlor, 2002). Addition of EFB was found to increase leaf chlorophyll content as there was increased the N uptake as an essential requirement of photosynthesis (Abdul Rahman *et al.,* 2016).

In the present study, *V. amygdalina* plants grown under 50% light intensity in EFB media showed optimum growth and physiology compared to 30% and 100% level of light intensity as well as other types of growing media (control, cocopeat and BPH). Therefore, it can be classified as a shade tolerant species due to the increased growth performance, biomass yield and rate of photosynthesis when grown under shade. Growing media of EFB was the best growing media compared to control, cocopeat and BPH because it improved plant growth and yield.

Plant Phytochemical Content

Regardless of plant performance, Elizabeth (2013) reported that *V. amygdalina* grown in open shade conditions (under the shade of a tree) leads to a higher production of flavonoids than those grown in an open field. The lower temperature of shade is a factor in increasing the phenolic and flavonoid components in plants (Nur Faezah *et al.,* 2016). On the contrary, another study on lettuce found increased content of phytochemicals when exposed to direct sun as compared to the greenhouse, as the higher UV radiation could enhance the accumulation of phenolic and flavonoid compounds in plants (Zivcak *et al*., 2017). The higher key metabolic enzymes triggered the synthesis of pigment in order to avoid UV light by accumulating UV-filtering compounds. This reaction called a plastic response of plants toward environmental stimuli of biotic and abiotic stressors by producing its secondary metabolites (Dou *et al*., 2017).

In Figure 3, all phytochemical properties examined in *V. amygdalina* planted under 50% light intensity within EFB growing media were found to be dependent on harvesting times. During maturation, the plant oxidative metabolism, as well as the accumulation of phytochemicals, can change throughout leaf development (Thi & Hwang, 2014). For instance, the maximum total phenolic content was the highest, i.e. 91 mg GAE g^{-1} DW at 9 WAT and followed by 84 mg GAE g^{-1} DW at 18 WAT. At 3 WAT, total phenolic content was 32% and 27% lower than plants harvested at 9 and 18 WAT. However, there was no significant difference observed between total phenolic content in plants harvested at 3, 6 and 15 WAT (Figure 3(a)). The findings of this study are in agreement with Zhang *et al.* (2010) who found that the total phenolic and flavonoid content in pomegranate leaves decreased at the early growth stage.

Total flavonoid content showed a similar pattern to total phenolic content with significant differences observed between plants harvested at different WATs (Figure 3(b)). Plants harvested at 9 and 18 WAT resulted in the highest mean total flavonoid content, i.e. 103 mg QE g^{-1} DW and 90.5 mg QE g^{-1} DW respectively. The total flavonoid content in *V. amygdalina* increased gradually from 3 to 9 WAT, something that may be due to the phenolics functioning as protection, a process that is also found in olive leaves (Brahmi *et al.,* 2017). It is well known that phenolic and flavonoid act as defensive agents (Dixon & Paiva, 1995). It could also be attributed to the ecological roles of these compounds to protect the plants from microbial pathogens and animal herbivores (Kasote *et al.,* 2015). Furthermore, a lower temperature is another factor for the increase in TPC and TFC in plants grown under shade (Nur Faezah *et al.,* 2016). Lower temperatures can cause photo-oxidative stress by limiting the activity of photosynthetic enzymes, therefore, increasing phytochemical levels in plants (Close & McArthur, 2002).

Figure 3(c) shows that harvest time significantly influences the antioxidant activity of *V. amygdalina*. DPPH free-radical scavenging activity fluctuated until 12 WAT, then started to increase slowly until the final harvest at 18 WAT. There were no significant changes among DPPH activities between plants harvested at 9, 15 and 18 WAT. Additionally, *V. amygdalina* harvested at 15 WAT showed no significant difference in DPPH radical scavenging activity compared to plants harvested at 3, 6 and 12 WAT. Plants that were harvested at 3 WAT showed a decline of 23%, 19% and 27% in DPPH free radical scavenging activity compared to those harvested at 9, 15 and 18 WAT. With increased phenolic and flavonoid accumulation, the free radical scavenging power increases in *V. amygdalina.* Most of the flavonoids are phenolic constituents that are responsible for the antioxidants in many plants (Larson, 1998) and antioxidant activity is known to decrease with increased harvest times. It seems that the roles of antioxidants, specifically TPC and TFC, in *V. amygdalina* are important for scavenging of free radicals. The present findings are consistent with Ibrahim and Jaafar (2012) who stated that the higher DPPH activity of *Labisia pumila* was due to high levels of phenolics and flavonoids in *L. pumila* extracts. These results explain the relationship between antioxidants and free-radical scavenging in *V. amygdalina* leaves.

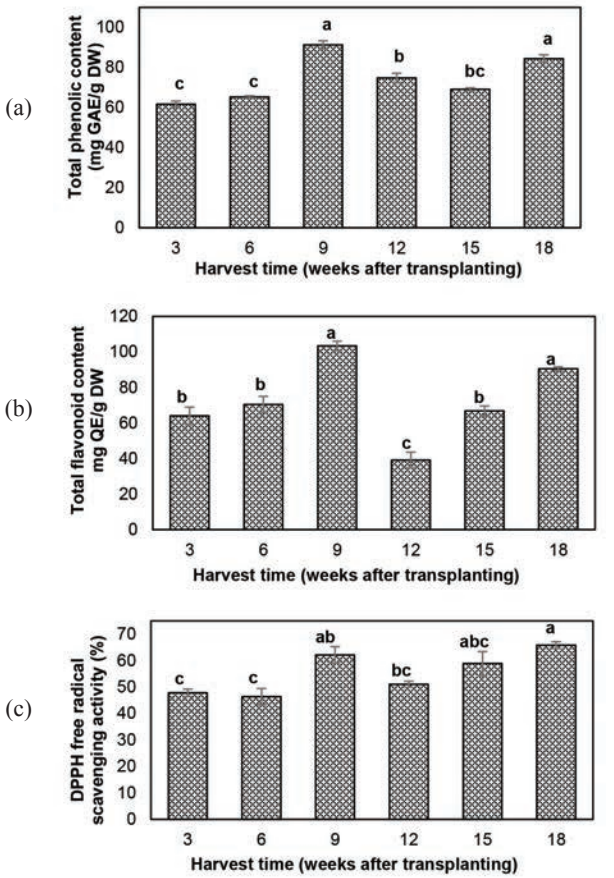

Figure 3: (a) Total phenolic content, (b) Total flavonoid content and (c) DPPH free radical scavenging activity in *V. amygdalina* leaves harvested at 3, 6, 9, 12, 15 and 18 weeks after transplanting. Different letters above each bar indicate significant differences at $P \leq 0.05$ using the Least Significant Difference (LSD) test

Conclusion

Based on the evidence, it is apparent that the growth, physiology and yield of *V. amygdalina* are influenced by light intensity, growing media and harvest time. Changes to light levels and growing media result in significant changes in plant development. *V. amygdalina* plants grown under 30% and 50% light intensity are more likely to achieve higher biomass yield and chlorophyll content compared to those produced in an open field. A higher rate of photosynthesis is shown by plants exposed to 50% light intensity, and those grown in empty fruit bunch-based media show an increase in biomass. Accordingly, *V. amygdalina* plants grown under 50% light intensity and in EFB growing media reach an optimal harvesting point at 18 WAT. After 18 weeks they are more likely to produce higher levels of phytochemicals and flavonoid content, as well as antioxidant activity.

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References

Abdulrahman, D. K., & Othman, R. (2016). Effects of empty fruit bunch biochar and nitrogen-fixing bacteria on soil properties and growth of sweet corn. *Journal of Soil Science*, *20*, 177-194.

Adaramoye, O. (2008). Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Journal of Vascular Health and Risk Management*, *4*(1), 235-241.

Ademola, I., & Eloff, J. (2011). Anthelminthic activity of acetone extract and fractions of *Vernonia amygdalina* against *Haemonchus contortus* eggs and larvae. *Tropical Animal Health and Production*, *43*(2), 521-527.

Adiukwu, P., Kayanja, F., Nambatya, G., Rugera, S., Ezeonwumelu, J., & Tanayen, J., (2013). Antipyretic and antinociceptive properties of the aqueous extract and saponin from an edible vegetable: *Vernonia amygdalina* leaf. *African Journal of Food, Agriculture, Nutrition and Development*, *13*(2), 7588-7589.

Affendy, H., Aminuddin, M., Azmy, M., Amizi, M., Assis, K., & Tamer, A. (2011). Effect of organic fertilizers application to the growth of *Orthosiphon stamineus* Benth. intercropped with *Hevea brasiliensis* Willd. and *Durio zibethinus* Murr. *International Journal of Agricultural Research*, *6*(2), 180-187.

Audu, S. A., Alemika, E. T., Abdulraheem, R. O., Abdulkareem, S. S., Abdulraheem, R. B., & Ilyas, M. (2012). A study review of documented phytochemistry of *Vernonia amygdalina* (Family Asteraceae) as the basis for pharmacologic activity of plant extract. *Journal of Natural Sciences Research*, *2*(7), 1-9.

Bagchi, G. D., Dwivedi, P. D., Haider, F., Singh, S., Srivastava, S., & Chattopadhyay, S. (2003). Seasonal variation in vasicine content in Adhatoda species grown under north Indian plain conditions. *Journal of Medicinal and Aromatic Plant Sciences*, *25*, 37-40.

Bote, A. D., & Struik, P. (2011). Effects of shade on growth, production and quality of coffee (*Coffea arabica*) in Ethiopia. *Journal of Horticulture and Forestry*, *3*(11), 336-341.

Brahmi, F., Mechri, B., Dhibi, M., & Hammami, M. (2015). Effect of growth stage and solvent extract on the antioxidant potential of olive leaves. *Journal of Plant Sciences*, *3*(31), 1-7.

Chua, I. Y. P., King, P. J. H., Ong, K. H., Sarbini, S. R., & Yiu, P. H. (2015). Influence of light intensity and temperature on antioxidant activity in *Premna serratifolia* L. *Journal of Soil Science and Plant Nutrition*, *15*(3), 605-614.

Close, D. C., & Mc Arthur, C. (2002). Rethinking the role of many plant phenolics-protections from photodamage not herbivores? *Journal of Oikos*, *99*, 166-172.

Coombs, J., Hind, G., & Leegood, R. C. (1986). Analytical techniques. In Coombs, J., Hall, D. O., Long, D. O., &Scurlock, J. M. O. (Eds.), *Techniques in Bioproductivity and Photosynthesis* (2nd ed., p. 223-240).

de Carvalho Goncalves, J. F., de Sousa Barreto, D. C., dos Santos Jr, U. M., Fernandes, A. V., Barbosa Sampaio, P. D. T., & Buckeridge, M. S. (2005). Growth, photosynthesis and stress indicators in young rosewood plants (*Aniba rosaeodora* Ducke) under different light intensities. *Brazilian Journal of Plant Physiology*, *17*, 325-334.

Demir, Z., & Gulser, C. (2015). Effects of rice husk compost application on soil quality parameters in greenhouse conditions. *Journal of Soil Science*, *4*(3), 185-190.

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenyl propanoid metabolism. *Plant Cell*, *7*, 1085-1097.

Dou, H., Niu, G., Gu, M., & Masabni, J. G. (2017). Effects of light quality on growth and phytonurient accumulation of herbs under controlled environments. *Horticulturae*, *3*(360).

Du, L., Liu, H., Yan, M., Li, J., & Li, J. (2017). Individual plasticity of the shade response of the invasive *Solidago canadensis* in China. *PLoS ONE*, *12*(1), 1-11.

Elizabeth, A. O. (2013). Shade conditions prevents anthraquinone production in leaves of *Vernonia amygdalina* plants. *International Journal of Science and Nature*, *4*(4), 713-715.

Feng, Y., & van Kleunen, M. (2014). Responses to shading of naturalized and non-naturalized exotic woody species. *Annals of Botany*, *114*, 981-986.

Fernandes, V., de Almeida, L. B., da S. Feijo, E. V. R., da C. Silva, D., de Oliveira, R. A., & Mielke, M. S. (2013). Light intensity on growth, leaf micromorphology and essential oil production of *Ocimum gratissimum*. *Brazilian Journal of Pharmacognosy*, *23*(3), 419- 424.

Gezahen, A. M. (2016). *Integrated nutrient management for maize-soybean cropping system*. (PhD Thesis). Universiti Putra Malaysia, Serdang, Malaysia.

Ghasemzadeh, A., Jaafar, H. Z. E., Rahmat, A., Wahab, P. E. M., & Halim, M. R. A. (2010). Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties (*Zingiber officinale* Roscoe). *International Journal of Molecular Sciences*, *11*(10), 3885-3897.

Gulcin, İ. (2006). Antioxidant activity of Caffeic Acid (3,4-Dihydroxycinnamic Acid). *Toxicology*, *217*(2), 213-220.

Hemm, M. R., Rider, S. D., Ogas, J., Murry, D. J., & Chapple, C. (2004). Light induce phenylpropanoid metabolism in Arabidopsis roots. *The Plant Journal*, *38*, 765-778.

Ibrahim, M. H., & Jaafar, H. Z. E. (2012). Reduced photoinhibition under low irradiance enhanced kacip fatimah (*Labisia pumila* benth) secondary metabolites, phenyl alanine lyase and antioxidant activity. *International Journal of Molecular Sciences*, *13*(5), 5290-5306.

Imaga, N. O. A., &Bamigbetan, D. O. (2013). In vivo biochemical assessment of aqueous extracts of *Vernonia amygdalina* (Bitter leaf). *International Journal of Nutrition and Metabolism*, *5*(2), 22-27.

Iwu, M. (2015). *Handbook of African medicinal plants* (2nd ed.). Hoboken: Taylor and Francis.

Kaewseejan, N., & Siriamornpun, S. (2015). Bioactive components and properties of ethanolic extract and its fractions from *Gynura procumbens* leaves. *Industrial Crops and Products*, *74*, 271-278.

Kasote, D. M., Katyare, S. S., Hedge, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences*, *11*(8), 982-991.

Kumar, R., Sharma, S., & Pathania, V. (2013). Effect of shading and plant density on growth, yield and oil composition of clary sage (*Salvia sclarea* L.) in North Western Himalaya. *Journal of Essential Oil Research*, *25*(1), 23-32.

Larson, R. A. (1998). The antioxidants of higher plants. *Journal of Phytochemistry*, *27*, 969-978.

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: Mechanisms are the key to understanding production systems. *Journal of Experimental Botany*, *53*, 773-787.

Li, A., Li, S., Wu, X., Lu, H., Huang, M., Gu, R., & He, A. (2015). Influence of light intensity on the yield and quality of *Houttuynia cordata*. *Plant Production Science*, *18*(4), 522-528.

Liu, Y., Dawson, W., Prati, D., Haeuser, E., Feng, Y., & van Kleunen, M. (2016). Does geater spesific leaf area plasticity help plants to maintain a high performance when shaded. *Journal of Business Ethics*, *118*(7), 1329-1336.

Ma, X., Song, L., Yu, W., Hu, H., Liu, Y., & Wu, J. (2015). Growth, physiological, and biochemical responses of *Camptotheca acuminata* seedlings to different light environments. *Frontiers in Plant Science*, *6*(5), 1-12.

Mattana, R. S., Vieira, M. A. R., Marchese, J. A., Ming, L. C., & Marques, M. O. M. (2010). Shade level effects on yield and chemical composition of the leaf essential oil of *Pothomorphe umbellata* (L.) Miquel. *Scientia Agricola*, *67*(4), 414-418.

Moniruzzaman, M., Islam, M. S., Hossain, M. M., Hossain, T., & Miah, M. G. (2009). Effects of shade and nitrogen levels on quality Bangladhonia production. *Bangladesh Journal of Agricultural Research*, *34*(2), 205-213.

Mutuchelian, K., Paliwal, K., & Gnanam, A. (1989). Influence of shading on net photosynthesis and transpiration rates, stomatal diffusive resistance, nitrate reductase and biomass productivity of a woody legume tree species (*Erythrina variegate* Lam.). *Journal of Plant Science*, *99*(6), 539-546.

Nur Faezah, O., Siti Aishah, H., Mohd Fauzi, R., Puteri Edaroyati, M. W., & Mohd Puad, A. (2016). Growth and phytochemical responses of *Andrographis paniculata* as influenced by different shade levels and prunings. *Journal of Tropical Plant Physiology*, *8*, 61-69.

Nur Faezah, O., Siti Aishah, H., Puteri Edaroyati, W. M., Ramlan, M. F., & Puad, M. A. (2015). Growth and dry matter partitioning of *Andrographis paniculata* to different light intensities and pruning. *Global Advance Research Journal of Agricultural Science*, *4*(12), 851-857.

Poorter, H., Niklas, K. J., Reich, P. B., Oleksyn, J., Poot, P., & Mommer, L. (2012). Biomass allocation to leaves, stems and roots: meta-analyses of interspecific variation and environmental control. *Journal of New Phytologist*, *193*(1), 30-50.

Ramachendrin, A. D. (2015). *In vitro activity of local plants from Malaysia against chikungunya virus.* (Degree Thesis). Universiti Tunku Abdul Rahman, Malaysia.

Riaz, A., Younis, A., Ghani, I., Tariq, U., & Ahsan, M. (2015). Agricultural waste as growing media component for the growth and flowering of *Gerbera jamesonii* cv. hybrid mix. *International Journal of Recycling of Organic Waste in Agriculture*, *4*(3), 197-204.

Silva, F. G., Pereiro Pinto, J. E. B., das Gracas Cardoso, M. A., Nascimento, E., Nelson, D. L., & de Fatima Sales, J. (2006). Influence of radiation level on plant growth, yield anhd quality of essential oil in Carqueja. *Journal of Science and Agrotechnology*, *30*(1), 52-57.

Suvitha, K., & Babu, N. G. R. (2017). Nutritional analysis of various compost for agriculture purposes. *Journal of Interdisciplinary Research*, *3*(1), 45-48.

Tajidin, N. E. (2017). *Effects of plant harvest age and plant parts on phytochemical compounds of Andrographis paniculata (Burm. F.) Nees.* (PhD Thesis). Universiti Putra Malaysia, Serdang, Malaysia.

Thi, N. D., & Hwang, E. (2014). Bioactive compound contents and antioxidant activity in Aronia (*Aronia melanocarpa*) leaves collected at different growth stages. *Nutrition and Food Science*, *19*(3), 204-212.

Udochukwu, U., Omeje, F. I., Uloma, I. S., & Oseiwe, F. D. (2015). Phytochemical analysis of *Vernonia amygdalina* and *Ocimum gratissimum* extracts and their antibacterial activity on some drug resistant bacteria. *American Journal of Research Communication*, *3*(5), 225-235.

Wong, F. C., Woo, C. C., Hsu, A., & Tan, B. K. H. (2013). The anti-cancer activities of *Vernonia amygdalina* extract in human breast cancer cell lines are mediated through caspase-dependent and p53-independent pathways. *PLoS ONE*, *8*(10).

Xu-yang, Y., Xiao-ying, L., Zhi-gang, X., & Xue-lei, J. (2017). Effects of light intensity on leaf microstructure and growth of rape seedlings cultivated under a combination of red and blue LEDs. *Journal of Integrative Agriculture, 16*(1), 97-105.

Yang, M., Shen, Q., Li, L.Q., Huang, Y. Q., & Cheung, H. Y. (2015). Phytochemical profiles, antioxidant activities of functional herb *Abrus cantoniensis* and *Abrus mollis*. *Food Chemistry*, *177*, 304-312.

Yao, X., Liu, X., Xu, Z., & Jiao, X. (2017). Effects of light intensity on leaf microstructure and growth of rape seedlings cultivated under a combination of red and blue LEDs. *Journal of Integrative Agriculture*, *16*(1), 97-105.

Zhang, L., Gao, Y., Zhang, Y., Liu, J., & Yu, J. (2010). Changes in bioactive compounds and antioxidant activities in pomegranate leaves. *Journal of Science Horticulture*, *123*, 543-546.

Zivcak, M., Bruckova, K., & Allakhverdiev, S. I. (2017). Lettuce flavonoids screening and phenotyping by chlorophyll fluorescence excitation ratio. *Planta*, *245*, 1215-1229.

Zulkarimi, B., Ashrafuzzaman, M., & Mohd Razi, I. (2010). Morpho-physiological growth, yield and fruit quality of rock melon as affected by growing media and electrical conductivity. *Journal of Food, Agriculture and Environment*, *8*(1), 249-250.