EVALUATION OF THE GROWTH PERFORMANCES, BIOMASS AND CHEMICAL COMPOUNDS OF FIVE HERBACEOUS SPECIES GROWN IN PARTIAL SHADE AND DIRECT SUNLIGHT

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ABSTRACT

Five herbal species, namely Clinacanthus nutans (belalai gajah), Andrographis paniculata (hempedu bumi), Orthosiphon stamineus (misai kucing), Gynura procumbens (sambung nyawa), and Strobilanthes crispus (pecah beling), were planted in two different environments at the R&D Centre, Felcra Nasarudin Belia, Seri Iskandar, Perak. The main objective of this study was to evaluate the potential growth of the herbal species under partial shade and direct sunlight. At the partial shade plot, an intercropping system was applied in which the herbal species were intercropped with oil palm trees, while at the direct sunlight plot, herbal plants were planted in an open field. Monthly measurements were taken to assess the plant growth performances, while the biomass and chemical compounds were examined after the fourth month. The findings indicated that all five herbal species exhibited superior growth and biomass in the open field plantation area. Nevertheless, certain herbal species, like Orthosiphon stamineus, Gynura procumbens, and Strobilanthes crispus, exhibit increased leaf length and width when cultivated in shaded fields. Chemical substances were assessed in each species throughout this study. The study's findings indicate that all five herbal species are recommended for cultivation in open-field plantations to achieve high yield production.

INTRODUCTION

Malaysia's herbal sector has been identified as a key source of economic development. It has been designated as one of the Entry Point Projects (EPP1) in the Economic Transformation Programme (ETP), which was launched in early 2011. This corresponds to a rise in worldwide market demand for herbal goods. According to the WHO, the worldwide market for herbal goods was worth USD 60 billion in 2000 and is anticipated to expand at a 7% annual rate to USD 5 trillion by 2050 (Ahmad and Othman, 2013). In Malaysia, the herbal-related industry is expected to develop at a 15% annual pace, with market value growing from USD 1.6 billion in 2010 to USD 6.9 billion by 2020 (Jaafar, 2010).

Despite being rich in agro-biodiversity, Malaysia has constraints in terms of its raw material sources. This is substantiated by the annual increase in the volume of imported raw herbs. According to the report, there has been a notable rise in the value of imported herbs, which has escalated from US$ 381.7 million in 2013 to US$ 507.2 million in 2017 (Comtrade, 2018). According to a study done by the Malaysian Agricultural Research and Development Institute (MARDI) in 2002, around 50% of Small and Medium-sized Enterprises (SMEs) were found to depend on imported raw herbs in order to fulfil the industry needs (Zakaria et al., 2019). Therefore, in order to boost the growth of the herbal sector, it is crucial to establish a sustainable source of high quality raw materials. This would enable Malaysia to effectively compete with other nations involved in herbal production.

In order to guarantee a sustained supply of raw materials to the industry, it is it is important to cultivated the herbal species in commercial plantations. The availability of land for agricultural purposes in Malaysia is becoming constrained as a result of its conversion for other uses, including industrial, residential, and urban applications (Olaniyi, 2013). Hence, it is essential to optimize the use of land. In recent years, the practice of integrated farming has gained traction among Malaysian farmers, whereby short-term crops are combined with permanent crops and forest trees. The identification of appropriate crop selection for integrated farming is crucial in order to enhance output per hectare and cultivate plants of superior quality.

In the Agriculture EPP-1, the government has highlighted several high value herbal products to penetrate the global markets. *Clinacanthus nutans*, *Andrographis paniculata*, *Orthosiphon stamineus*, *Gynura procumbens* and *Strobilanthes crispus* were among the species listed. Each of these species has distinct therapeutic properties that are attributed to the presence of plant secondary metabolites. Secondary metabolites have a crucial role in human society as they provide as valuable sources for food additives, flavours, and medications (Ravishankar & Rao, 2000). The levels of secondary plant products are significantly influenced by the environmental circumstances and physiological factors, which in turn affect the metabolic pathways responsible for the synthesis of these compounds (Pradhan et al., 2017). Consequently, this research was undertaken with the objective of assessing the adaptability and productivity of five herbal species cultivated in both open field (direct sunlight) and shadow field (partial shade) conditions inside an oil palm plantation.

MATERIALS AND METHODS

Plant Material

The plant materials of *C. nutans. A. paniculata, O. stamineus, G. procumbens* and *S. crispus* were obtained from Herbal Plot at Tangkak, Johor. The plant materials were brought to Forest Research Institute Malaysia (FRIM) for propagation. The herbal plants were propagated using stem cuttings. The stem of each herbal species were cut into 10 cm length and commercial rooting hormone (Serdix 1) were applied at each base of cuttings to enhance the rooting production. A total of 60 stem cuttings were produced for each herbal species. The propagation was carried out in an enclosed mist propagation chamber at FRIM. Coarse river sand with the size of particles 0.5 – 2 mm was used as the rooting media. The propagation bed was kept moist with automatic mist sprinkler system. The misting frequency used for irrigation was three minute for three times daily at 9 am, 12 pm and 4.00 pm. The propagation bed was enclosed with a layer of transparent plastic to maintain high relative humidity. The plastic enclosure was also shaded with black plastic netting to provide 50% light intensity (Figure 3.3). The relative humidity (RH), air temperature and light intensity (Light scout, Spectrum Technologies) were measured daily from 8.00 am to 5.00 pm. The average RH of propagation bed was 70% and average temperature was 28°C. The average light intensity recorded was 59 µmol/ $m^{2/}$s. After 8 weeks, the rooted cuttings were planted in 6'x8' size of polyethylene bag filled with 2 topsoil: 1 sand. The rooted cuttings were hardened at FRIM nursery for 16 weeks.

Field plantation

A total of 300 plantlets from five herbal species were transferred to the Research and Development Centre located at Felcra Nasarudin Belia in Seri Iskandar, Perak for field planting. The herbal plants were planted in two different environments: i) Plot 1-Partial shade (a shaded area located under an oil palm plantation) and ii) Plot 2-Direct sunlight (an open field). The establishment of the field plantings was conducted in December 2018.

i) Plot 1 (Partial shade)
The experiment was conducted under the ten years of oil palm plantation. The oil palm plantation used triangular system of planting with 8m x 8m x 8m spacing. The five herbal species were planted along the fronds row (composted) which involved 22.8m x 17.6m oil palm area. The experimental area was plough prior to plant and mixture of leaf compost were applied on each planting holes. A total of 150 plants were planted in one meter by one planting distance.

ii) Plot 2 (Direct Sunlight)
A 10 m by 15 m area was ploughed using a plough machine, and two sets of planting beds, each measuring 1 m by 15 m, were made for each type of herb. A randomized complete block design was used to plant 150 herbal plants in total (RCBD). To improve the soil's condition, leaf compost was mixed into each planting hole at a distance of one meter by one meter.

Plant maintenance
The plants were irrigated manually on a daily basis in each field. Each plant was fertilized monthly with a 10g application of NPK fertilizer (15:15:15). Weeding activities were performed as necessary.

Data collection
The obtained data for plant growth performance included measurements of plant height, leaf length, leaf width, and collar diameter. Measurements of growth were taken on a monthly basis until the plant reached its full development after 16 weeks. The herbal plants were taken indiscriminately from each field after they reached maturity in order to acquire data on their biomass. Prior to the drying process, the fresh samples were weighed. The plant sample was subjected to oven-drying at a temperature of 60 °C until it achieved a consistent weight, which took roughly 72 hours. The environmental variables, including temperature, humidity percentage, and light intensity, were observed and recorded for each plot. The collected data is presented in Table 1. The data were examined using IBM SPSS Statistics version 22 to perform an analysis of variance (ANOVA).

Table 1: The minimum and maximum values for light intensity, temperature, and humidity percentage were measured from December 2018 to March 2019 at two distinct plots

Parameters	Plot 1 (Partial shade)		Plot 2 (Direct Sunlight)	
	Min	Max	Min	Max
Light intensity (lux)	322	782	516	1165
Temperature (°C)	28.6	33.5	31.8	37.5
Humidity (%)	84	89	61.2	67.5

Analysis of chemical compounds

A total of 20 g fresh leaves were collected from each herbal plant, sourced from two distinct plots. The leaf samples were rinsed with flowing tap water and thereafter subjected to oven drying at 60 °C for a duration of 48 hours. The dried leaf samples were ground into a powdered form. About 0.5 g of powdered material (sieved to a size of 500 µm) were combined with 5 ml of methanol. The combination was then subjected to ultrasonic waves for a duration of 15 minutes. Prior to analysis, the resultant solution was filtered using a 0.45 µm syringe filter. The samples were analysed using an HPLC system consisting of a WATERS 2535 quaternary gradient pump, WATERS 2707 auto sampler, and WATERS 2998 PDA. Two gradient systems were employed, each comprising two distinct solvents: A (0.1% formic acid in water) and B (acetonitrile). The flow rate employed was 1.0 mL/min, while the injection volume amounted to 10 µL. Analysed at a wavelength of 220 nm, the retention durations and UV spectra of the selected compounds were examined. Each herbal plant utilised a distinct marker for quantitative measurement such as shaftoside in *Clinacanthus nutans, andrographolide* in *Andrographis paniculata,* sinensetin in *Orthosiphon stamineus,* chlorogenic acid in *Gynura procumbens* and caffeic acid in *Strobilanthes crispus.*

RESULTS AND DISCUSSION

Effects of different environmental condition on the growth performances and biomass of five herbal species

The growth (Table 3) and yield (Table 4) of the five herbal species were significantly influenced by environmental conditions ($p<0.05$). The results indicate that varying environmental conditions have a notable impact on the height of *Clinacanthus nutans* and *Andrographis paniculata* plants. Specifically, these plants exhibit increased height when exposed to high light intensity in plot 2. Meanwhile, the remaining herbal plants exhibit comparable plant height under both environmental conditions. The plant in plot 2 exhibited increased collar diameter and a greater number of branches, resulting in a larger crown width. The increased photosynthetic rate and subsequent plant development and production may be attributed to the greater absorption of photosynthetically active radiation (PAR) in sunny conditions (Kumar et al., 2014). Under conditions of low light intensity, herbal plants exhibit characteristics similar to etiolated plants, such as an undesirably tall look and increased apical dominance, as no measurements of crown width were taken. The results were consistent with previous research on other medicinal plants, such as sage (Zervoudakis et al., 2012) and damask rose (Thakur et al., 2019). The leaf size was modified by varying light intensity, with plot 1 plants exhibiting higher leaf length and width compared to plot 2 plants. According to Lambers et al. (1998), plants that grow in shaded areas produce larger and thinner leaves in order to capture more light. This is because when there is a lack of light, the ability of plants to convert light energy into photosynthesis depends on the efficiency of their light-capturing pigments and how well they carry this energy to the reaction centers (Ruban, 2009).

Table 3: Comparison of growth performances of five different species cultivated in plot 1 and plot 2

Species	Treatment	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Collar diameter (mm)	Crown width- *X* axis (cm)	Crown width- Y axis (cm)
Clinacanthus nutans	Plot 1	24.4±1.86b	9.77±0.39a	2.55±0.10a	3.49±0.23b	NA	NA
	Plot 2	43.4±1.93a	10.1±0.28a	2.20±0.07b	6.35±1.39a	58.4±3.52	55.4±3.49
Andrographis paniculata	Plot 1	22.7±2.91b	5.68±0.47a	1.53±0.11a	2.07±0.11b	NA	NA
	Plot 2	40.6±1.67a	6.13±0.13a	1.51±0.04a	7.26±0.25a	43.0±1.97	41.6±1.70
Orthosiphon stamineus	Plot 1	51.5±1.95a	8.06±0.20a	3.69±0.10a	3.99±0.13b	NA	NA
	Plot 2	52.2±1.28a	4.47±0.09b	2.33±0.05b	6.99±0.35a	86.2±3.21	81.6±2.26
Gynura procumbens	Plot 1	20.7±1.76a	9.87±0.50a	4.43±0.19a	5.75±0.19b	NA	NA
	Plot 2	25.6±1.54a	10.2±0.45a	3.93±0.14b	8.69±0.56a	32.9±4.73	43.8±6.35
Strobilanthes crispus	Plot 1	28.1±4.78a	17.2±0.41a	7.27±0.17a	4.05±0.36a	NA	NA
	Plot 2	29.3±0.84a	11.9±0.20b	5.04±0.08b	8.58±0.34b	41.3±1.22	38.6±1.21

NA: not available

Table 4: Mean value of fresh biomass, dry biomass and moisture content per plant of five different species cultivated in plot 1 and plot 2

Species	Plot 1			Plot 2		
	Fresh biomass (kg)	Dry biomass (kg)	Moisture content (%)	Fresh biomass (kg)	Dry Biomass (kg)	Moisture content (%)
Clinacanthus nutans	0.08 ± 0.03b	0.02 ± 0.002b	75.8±0.13a	0.11 ± 0.01a	0.03 ± 0.003a	73.1±0.11b
Andrographis paniculata	0.008 ± 0.00b	0.001 ± 0.00b	88.3±0.11a	0.18 ± 0.01a	0.08 ± 0.03a	60.9±0.10b
Orthosiphon stamineus	0.24 ± 0.10a	0.06 ± 0.006b	75.4±0.10a	0.25 ± 0.02a	0.09 ± 0.01a	60.0±0.13b
Gynura procumbens	0.05 ± 0.01b	0.004 ± 0.00b	92.0±0.11a	0.59 ± 0.09a	0.09 ± 0.01a	85.0±0.12b

Strobilanthes crispus	0.09 ± 0.01b	0.02 ± 0.004b	77.5±0.09a	0.23 ± 0.13a	0.08 ± 0.005a	65.2±0.11b

Means followed by same value were not significant at p<0.05

The data shown in Table 4 illustrates the productivity of herbal plants in terms of both fresh and dry weight, comparing their growth in plot 1 and plot 2. Varying levels of light intensity had a significant impact on the yield of herbal plants, with greater amounts of fresh and dry biomass being recovered in plot 2. Diminishing the intensity of light decreases both the rate of photosynthesis and the pace of growth, which directly correlates with the biomass of the plant (Baroli et al., 2008). According to the findings, herbal plants cultivated under direct sunlight have a lower moisture content percentage compared to plants grown under shade. The plants in plot 2 may undergo water stress as a result of their elevated transpiration rate. No notable disparity was detected in the fresh biomass of *Orthosiphon stamineus* between the two plots. In plot 2, the plants may exhibit increased leaf production, but with reduced individual leaf size, indicating a lower biomass yield.

Effects of different environmental condition on the chemical compounds of five herbal species

Various factors, including the environment, have an impact on the quality and amount of chemical metabolites in plants. The concentration of phenolic compounds in five herbal species was significantly influenced by various climatic conditions ($p<0.05$), as shown in Table 5. Nevertheless, varying plants exhibited distinct reactions to changes in light intensity, leading to varying concentrations of secondary metabolites. The study found that the average concentration of the reference compound was greater in *Clinacanthus nutans*, *Orthosiphon stamineus*, *Gynura procumbens*, and *Strobilanthes crispus* in plot 2. Conversely, the reference chemical in *Andrographis paniculata* had greater levels in plot 1.

Table 5: Mean concentration of chemical compounds in five herbal species cultivated in plot 1 and plot 2

Species	Reference compound	Treatment	Mean concentration ± SEM
Clinacanthus nutans	Shaftoside	Open field	308.4 ± 2.92a
		Shade field	39.3 ± 2.52b
Andrographis paniculata	Andrographolide	Open field	1073.5 ± 1.18b
		Shade field	1875.1 ± 1.17a
Orthosiphon stamineus	Sinensetin	Open field	653.7 ± 1.86a
		Shade field	317.5 ± 0.46b
Gynura procumbens	Chlorogenic acid	Open field	92.4 ± 0.43a
		Shade field	43.3 ± 0.36b
Strobilanthes crispus	Caffeic acid	Open field	399.1 ± 3.41a
		Shade field	351.3 ± 0.74b

Species	Reference compound	Treatment	Mean concentration ± SEM
Clinacanthus nutans	Shaftoside	Plot 1	39.3 ± 2.52b
		Plot 2	308.4 ± 2.92a
Andrographis paniculata	Andrographolide	Plot 1	1875.1 ± 1.17a
		Plot 2	1073.5 ± 1.18b
Orthosiphon stamineus	Sinensetin	Plot 1	317.5 ± 0.46b
		Plot 2	653.7 ± 1.86a
Gynura procumbens	Chlorogenic acid	Plot 1	43.3 ± 0.36b
		Plot 2	92.4 ± 0.43a

Strobilanthes crispus	Caffeic acid	Plot 1	351.3 ± 0.74b
		Plot 2	399.1 ± 3.41a

Light and temperature differences could be influences in the concentration of secondary metabolites in herbal plants. According to Molmann et al. (2005), the concentrations of quercetin and kaempferol in Alfalfa plant were higher at warmer temperatures, while putrescine content was higher at lower temperatures. Ghasemzadeh et al. (2010), on the other hand, observed that light increased the production of secondary metabolites in *Zingiber officinale*. Total flavonoid biosynthesis was shown to be highest under moderate light intensity (310 µmol m^{-2} s^{-1}), while total phenolic biosynthesis was highest under high light intensity (790 µmol m^{-2} s^{-1}).

CONCLUSIONS

The study determined that the five herbal species exhibited superior development and yield in plot 2 (direct sunlight). These species have the potential to be incorporated into areas with non-shade plants, such as coconut plantations. The synthesis of secondary metabolites in each medicinal plant is influenced by environmental stressors, such as high light intensity and temperature. Therefore, it is recommended to cultivate these species in an open field to promote their vigorous growth and the optimum concentration of bioactive compounds.

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