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Early Growth, Biomass and Phenolics Response Of *Labisia pumila* var. *alata* Seedlings to Fertilisation and Soil Amendments

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ABSTRACT

Labisia pumila is an important herbal plant belonging to the Myrsinaceae family. Water decoction of this herb is traditionally used for pre- and post-partum treatments to regulate body weight, prevent photo-aging and has anti-bacterial and anti-fungal effects. A study was conducted to determine the effects of fertilisers and soil amendments on 8-month tissue cultured *L. pumila* at nursery stage. Six treatments were used in this study which were inorganic fertiliser (NPK Green 15:15:15) at 90 kg N/ha (T1) which acted as control, inorganic fertiliser with biochar (5%) (T2), controlled release fertiliser (CRF) (NPK granular 10:15:17:2:5) (T3), CRF with biochar (5%) (T4), CRF with compost (5%) (T5), and CRF with compost (5%) and biochar (5%) (T6). The experiment was arranged in a randomised complete block design and replicated 16 times. At the end of 6 months after transplanting, our findings showed that T4 and T5 gave the best growth performances in terms of stem height, leaf number, leaf width, leaf length, collar diameter and leaf dry weight. T6 gave the highest value for total phenolic content of 310 mg/100 g GAE followed by T5 with 202 mg/100 g GAE. The elevated levels of soil N, C, P, Ca and Mg in the T5 and T6 caused increased seedling yield and enhanced TPC values. Therefore, mass production of *L. pumila* may consider the utilisation of controlled release fertilisers with organic compost and biochar.

Keywords: Soil, biochar, total phenolic content, growth performance, medicinal plants

INTRODUCTION

The utilisation of medicinal plants in the treatment of ailments dates back a few centuries and pioneered the medicine world before the emergence of commercialised drugs. Prior to the emergence of commercialised medicine, traditional remedies were often sought after especially by those who did not have access to hospitals. Medicinal plants are diverse but specific in nature with certain parts being used to treat targeted areas in the body. Currently, traditional medicine is gaining momentum as the vast majority is concerned with the side effects from synthetically derived drugs (Karimi et al., 2013).

Labisia pumila or better known as '*Kacip Fatimah*' is a medicinal plant from the Myrsinaceae family. It is commonly found in Malaysia, Thailand and Indochina. A decoction of the leaves and roots is believed to expedite labour and as a protective medicine after child birth (Jaganath and Ng, 2000). *L. pumila* is also used to treat menstrual cramps as well as irregular periods. *L. pumila* which is customarily referred to as the lady's herb, is used for women's health because of its phytoestrogen effects and chemical content having almost identical structures to that of estrogen (Jamia et al., 2006). There are three varieties of Kacip Fatimah in Peninsular Malaysia namely *L. pumila* var. *alata*, *L. pumila* var. *pumila* and *L. pumila* var. *lanceolata.* Extracts of *L. pumila* have been known to contain flavonoids, phenolics and various bioactive compounds (Karimi et al., 2011, 2013) that assist in treating illnesses.

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Nutrients play a crucial role in the growth and viability of a plant. Fertilisers, be it organic or inorganic, are soil amendments added for the supply of essential elements which assist plant growth and production (Siti Naimah et al., 2015). Past studies have shown that the incorporation of fertilisers into media resulted in the increase of secondary metabolites. Ibrahim et al. (2013) reported that organic fertilisers increased the production of total phenolics and total flavonoids. Organic fertilisers such as compost are derived from biological decomposition of organic material. Compost incorporated into the media usually has overall positive response on plant growth as it has the potential to improve water holding capacity, soil structure, improve cation exchange capacity and stabilise soil pH (El Sayed et al., 2015; Lehmann et al., 2003). Likewise, composts and biochar which are derived from plant or animal residues have also been found to improve soil fertility, nutrient holding capacity, mitigate climate change as well as reduce nutrient loss (El Sayed et al., 2015). Biochar applied to *L. pumila* at a rate of 20 tons/ha produced favourable outcomes in terms of plant growth and chlorophyll content compared to its non-biochar counterparts (Siti Norayu et al., 2012).

Most fertiliser trials elucidate the effects of nutrition on the physical growth of *L. pumila* (Siti Norayu et al., 2012; Farah Fazwa et al., 2017) but rarely on secondary metabolites such as the total phenolic content. In line with the country's vision in commercialising medicinal herbs such as *L. pumila*, it is pertinent to select appropriate agronomic practices which not only have profitable yield but incorporate good management practices that reduce environmental stress, health hazards and potential pollution of inorganic fertilisers. Testing organic based soil amendments such as biochar and compost derived from waste and the utilisation of controlled release fertilisers that control nutrient supply according to plant uptake is one of such approaches. Dwindling supply of *L. pumila* from the natural forests is also a timely alarm, instigating the need to carry out suitable agronomic practices that may complement sustainable mass production of this medicinal herb for drug discovery applications. Thus, the aim of this study was to investigate the effects of various soil amendments and fertilisation on the vegetative growth and total phenolic content of *L. pumila*. We hope to elucidate a suitable agronomic practice that can increase the production of phytochemical compounds in *L. pumila*.

MATERIALS AND METHODS

This study was conducted at Forest Research Institute Malaysia (FRIM) nursery (GPS N 03 13' 57.4" E 101 38' 07.0") from September 2016 until February 2017. Polythene bags were filled with 3.8 kg of 3:1:1:1 media comprising soil, sand, coco-husk and peat prior to transplanting 8-month old tissue cultured seedlings of *L. pumila* var. *alata*. Prior to the experiment, the tissue cultured seedlings underwent acclimatisation process in an acclimatisation chamber for 4 weeks. After 4 weeks, the plantlets were potted in a growing media consisting of topsoil, compost and sand in the ratio of 2:3:1 before transplanting were done for the experiment.

The various treatments applied on the transplanted seedlings consisted of NPK Green 15:15:15 fertiliser, controlled release fertiliser (CRF NPK Mg + TE 10:15:17:2:5), compost (95% matured agricultural waste of oil palm empty fruit bunches) and rice husk biochar which underwent pyrolysis. The rice husk biochar was produced using the rotary husk furnace method whereby it underwent pyrolysis (less than 10s) at 550 - 600^{o}C in a rotary kiln with limited oxygen following previously published method (Siti Norayu et al., 2012). The rice husk biochar had a pH of 7.5. The experiment was arranged in a randomised complete block design consisting of 96 seedlings from 4 blocks which were subjected to six different treatments as follows:

i. T1: NPK (standard fertiliser, control)
ii. T2: NPK+BC (standard fertiliser + biochar)
iii. T3: CRF (controlled release fertiliser)

iv. T4: CRF+BC (controlled release fertiliser + biochar)
v. T5: CRF+C (controlled release fertiliser + compost)
vi. T6: CRF+C+BC (controlled release fertiliser + compost+ biochar)

Each treatment had 16 replicates (4 blocks x 4 replicates) which were subjected to overhead misting twice daily at 1000 and 1600 hours (6 treatments x 4 blocks x 4 replicates). Each of the biochar and compost made up 5% of the planting media (Hunt et al., 2010) and the fertiliser application was 90 kg N/ha (Ibrahim et al., 2013). Biochar and compost were readily incorporated into the media prior to transplanting. We did not analyse the biochar for nutrient concentration but the properties of compost were as follows: 3.2% N, 2.8% P, 1.6 % K, 1.0% Mg. Application of NPK Green fertiliser (15:15:15) and CRF (10:15:17:2:5) were done at 1 week after transplanting to avoid transplanting shock. Variables such as collar diameter, plant height, leaf width, leaf length and number of leaves were taken once every 3 months to monitor the effects of treatments for up to 6 months after transplanting. Weeding procedures were carried out monthly to eradicate weeds which may interfere with plant growth. At the end of the experiment, leaves were harvested in triplicates out of the total number of replicates for the determination of total phenolic content (TPC) while whole plants (triplicates) were harvested for plant dry biomass determination. The whole plant was washed under running water to remove soil and separated into roots, shoots and leaves, weighed and placed in the oven at 60˚C for 3 days after which the dry biomass was determined. For TPC, the triplicates from each treatment were harvested, washed, dried with a kitchen towel and cut into small pieces of 2 cm lengths. The samples were then weighed and sent to the FRIM Biology Laboratory for TPC determination using Folin Ciocalteu method (Singleton and Rossi, 1965). Representative soil samples from each treatment in duplicates were collected and air dried at room temperature (27°C) until constant weight and sieved thoroughly using a 2 mm sieve. The soil pH, electrical conductivity (EC), concentrations of carbon (C), nitrogen (N), phosphorus (P), exchangeable potassium (K), calcium (Ca) and magnesium (Mg) of the growth media were carried out based on standard soil chemistry laboratory practice. The dry pH of the soil was determined in a 1:2.5; soil: water slurry. Electrical conductivity was determined using an EC meter (Mettler-Toledo S30-K, United States). Organic carbon was analysed according to the Walkley and Black (1934) method and soil nitrogen was determined using Kjeldahl digestion. Available P was determined by Bray & Kurtz Method No.2 (Sharifuddin and Dynoodt, 1981). Exchangeable K, Ca and Mg were extracted using 1 M ammonium acetate (NH_4OAc) calibrated at pH 7. The growth and dry biomass data were analysed using a one-way Analysis of Variance (ANOVA) in Statistical Analysis System version 11.2 whereby means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Our results showed that the values for collar diameter, seedling height, leave width and leaf length were 19%, 17%, 67% and 73% significantly higher for CRF+C treatment compared to control (NPK) (Table 1). Similarly, the growth parameters for CRF+C+BC were comparable to CRF+C. However, values for CRF+C were significantly higher (17%) for leaf width and length compared to CRF+C+BC. Significantly lower values were found for leaf number, collar diameter, seedling height and leaf width for NPK+BC treatments compared to all treatments.

The combination of controlled release fertiliser and compost was found to be effective in increasing vegetative growth since organic compost assists in the efficiency of chemical fertilisers. Composts alone increase water holding capacity and water availability, and improve soil physical and biological properties. The controlled release fertiliser was an important nutrient source releasing nutrients periodically to avoid losses due to leaching (Sempeho et al., 2014). It also contained micronutrients that increased the physical yield of the seedlings. Biochar has the ability to increase nutrient availability, improve soil fertility and quality due to its structure enabling it to retain nutrients more efficiently

(Lehmann and Joseph, 2009). Apart from this, its resistance towards microbial decay (Shindo, 1991; Cheng et al., 2008) also accounts for its persistence in soil for prolonged periods compared to other soil amendments, giving the enhanced effects. Biochar has been widely accepted to sequester long term carbon pools (Lorenz and Lal, 2014; Bouqbis et al., 2017). Based on the treatment effects, biochar was more effective when combined with compost and controlled release fertiliser compared to a compound fertiliser alone (NPK only) as CRF+C+BC reduced the leaching effect of a NPK only treatment (Ibrahim et al., 2013; Helliwell, 2015). Although the effects of biochar were enhanced while combined with CRF and C, its poor effects were noticeable for leaf number, height, leaf width and leaf length when combined with NPK alone. We believe that the negative effects in our study may be attributed to the poor root growth (Table 2) in NPK+BC which showed reduced dry weights compared to CRF+C. Since roots are involved in the mobilisation of nutrients to plant parts, the impeded root growth may have affected the overall growth of the seedlings treated with NPK+BC.

CRF+C had the highest dry weight for leaves (2-fold) compared to control (Table 2). CRF+C+BC had similar values to CRF+C which was at least 1-fold higher compared to control. All treatments were not significantly different for root dry weight except for CRF treatment which was 61% higher compared to control. Dry weights of stem for all treatments were the same but the values for CRF were the highest compared to all treatments. Effects of controlled release fertiliser combined with compost showed that there were positive interactions of these two sources in increasing the dry weights of *L. pumila*. This could be due to the high values of soil chemical properties such as C, N, P, K, Ca, Mg and micronutrients cited in the soil analytical data for CRF+C (Table 3). Higher values for root and shoot weights for CRF further confirmed the improved nutrient use efficiency of the seedlings in increasing biomass (Sempeho et al., 2014).

Table 1. Effects of fertiliser and soil amendments on the vegetative growth of *L. pumila* seedlings at 6 months after transplanting

Treatment	Leaf number	Collar diameter (mm)	Height (cm)	Leaf width (cm)	Leaf length (cm)
NPK(control)	9.35 ab (0.39) ¥	4.15 bc (0.12)	8.39 bcd (0.36)	4.14 d (0.12)	8.01 d (0.28)
NPK + BC	8.57 b (0.72)	4.12 bc (0.16)	6.98 e (0.49)	4.59 cd (0.32)	9.00 cd (0.75)
CRF	9.57 ab (0.52)	4.57 ab (0.20)	8.90 abc (0.46)	5.39 bc (0.26)	10.72 bc (0.58)
CRF + BC	8.57 b (0.40)	4.54 ab (0.12)	8.07 cde (0.54)	5.30 bc (0.32)	10.44 bc (0.86)
CRF + C	10.14 a (0.31)	4.95 a (0.22)	9.86 a (0.48)	6.90a (0.34)	13.85 a (0.79)
CRF + C + BC	10.14 a (0.38)	4.68a (0.18)	9.48 ab (0.47)	5.91b (0.34)	11.78 b (0.66)

Mean values followed by different letters within each column are significantly different for treatments by DMRT ($p \leq 0.05$)

¥ Values in parentheses represent standard error

Table 2. Effects of fertiliser and soil amendments on the biomass of *L. pumila* seedlings 6 months after transplanting

Treatments	Dry weight (g)		
	Leaves	Root	Stem
NPK	1.18d	0.44b	0.69a
	(0.03) †	(0.04)	(0.11)
NPK + BC	1.51c	0.41b	0.48a
	(0.10)	(0.04)	(0.08)
CRF	1.72c	0.71a	0.76a
	(0.06)	(0.09)	(0.14)
CRF + BC	1.53c	0.53ab	0.60a
	(0.12)	(0.08)	(0.05)
CRF + C	2.78a	0.45b	0.70a
	(0.14)	(0.05)	(0.10)
CRF + C + BC	2.33b	0.47b	0.71a
	(0.12)	(0.06)	(0.19)

Mean values followed by different letters within each column are significantly different for treatments by DMRT ($p \leq 0.05$)

† Values in parentheses represent standard error

Based on our soil analysis results, the soil pH for the growth of *L. pumila* ranged from 4.6 to 5.7 (Table 3). The highest amount of N, organic C, P, Cu, Zn and CEC were found in CRF+C+BC and CRF+C. Values recorded ranged from 0.14 to 0.16% N, 0.98 to 1.39% C, 9.95 to 11.33 Cu mg/kg, and 440 to 561 mg/kg. CRF+C also showed higher amount of exchangeable K, Ca, Mg, all ranging from 1 to 2.5 cmol/kg. It was noted that values for trace elements such as Fe, Cu, Zn and Mn for the CRF and the CRF + C treatments were all higher than those in control. The elevated performance of *L. pumila* for vegetative growth, dry weights and total phenolic content correspond closely with the CRF+C and CRF+C+BC treatments. This is due to the added advantage of CRF which included Mg and micronutrients that are vital for the viability of *L. pumilla* seedlings. Magnesium is highly involved in photosynthesis and metabolic processes and synthesis of phospholipids (Taiz and Zeiger, 2002; Marschner, 1995). The increased values of P in CRF+C and CRF+C+BC were also a major factor in the improved growth. Phosphorus is essential for the energy storage and transfer processes as adenosine di- and triphosphates (ADP and ATP) (Havlin et al., 1999). Phosphorus is associated with increased root growth, encouraging nutrient and moisture uptake. Micronutrients as discussed earlier were crucial for the production of total flavonoids and phenolic contents (Sampaio et al., 2011). Another reasonable explanation for the superior effects of CRF is that it is encapsulated in a protective coating, controlling water penetration and the rate of dispersion thus ensuring nutrients are released in small quantities according to plant uptake over a long period (Trenkel, 1997). NPK only treatment allows rapid solubility of fertilisers when in contact with water and is more useful to produce vigorous growth in shorter periods as it allows release of large quantities of nutrients. This further confirms the combined effects of macro- and micronutrients in improving the vitality of *L. pumila*. This nursery study recommends the sufficient levels of nutrients needed for the increased growth of *L. pumila* mainly N (> 0.1%), C (> 1%), available P (> 100 mg/kg), Ca and Mg (>1.0 cmol/kg). Micronutrients such as Cu (10-11 mg/kg) and Zn (> 34 mg/kg) gave an added advantage. However, more experiments with the inclusion of plant nutrient analysis are necessary in order to determine conclusive levels of nutrient uptake of *L. pumilla* at nursery stage.

From our observations, the TPC content in CRF+C+BC was almost 2-fold significantly higher compared to NPK (Table 4). The lowest TPC was recorded for CRF+BC which was 6% lower than control. Total Phenolic Content (TPC) values for NPK, NPK+BC, CRF and CRF +BC were all not significantly different, ranging from 157.2 to 182.0 mg/ 100g GAE. Micronutrients such as copper (Cu) and iron (Fe) are known to assist in the phenol metabolism of plants whereby copper enhances the polyphenol oxidase activity in plants and phenols may complex with Fe (Fe^{3+}) to be mobilized to different tissue types, aiding reductase-type enzymes (Marschner, 1995). Panhwar et al. (2015) have shown that the total phenolic acid of rice grains was increased to 326 mg/100 g GAE when micronutrients such as zinc, copper and molybdenum were incorporated with a NPKS fertiliser. Our results for TPC were slightly lower than the results reported by Syafiqa et al. (2016) who reported total phenolic content in tissue culture plants of *L. pumila* at 326.21 ± 13.37 mg/100 g GAE at 9 months of age probably due to inherent properties of the seedlings.

Table 3. Soil chemical properties of the nursery potting media

Treatments	Dry pH	N	OC	Fe	Cu	Mn	Zn	Av. P	Ex. K	Ex. Ca	Ex. Mg	Ex. Na	CEC
			%			mg/kg					$cmol_c$/kg		
Growing media*	4.47	0.61	0.13	nd	nd	nd	nd	0.04	0.02	0.05	0.02	nd	-
NPK	5.13	0.03	0.50	3.19	6.51	89.03	31.84	0.98	0.03	0.40	0.04	0.01	7.58
NPK + BC	5.63	0.06	0.47	3.06	6.28	91.18	29.35	15.80	0.60	0.66	0.35	0.01	5.38
CRF	4.58	0.04	0.52	3.46	6.38	99.96	32.78	2.05	0.09	0.44	0.07	0.01	8.88
CRF + BC	5.66	0.06	0.41	3.00	7.43	96.88	32.65	20.90	0.91	0.69	0.49	0.02	5.79
CRF + C	5.21	0.16	1.39	3.17	11.33	100.23	36.19	106.50	1.11	2.51	1.69	0.01	8.79
CRF + C + BC	5.35	0.14	0.98	3.01	9.95	95.60	34.11	120.37	0.89	1.53	1.06	0.01	7.81

* : initial concentration of growing media before transplanting was done
nd: not detectable

Table 4. Effects of fertilisation and soil amendments on total phenolic content (TPC)

Treatments	Total phenolic content (TPC) mg/100 g GAE Sample concentration: 200 µL/mL
	Standard: Gallic acid (GAE) standard curve
NPK	166.7 ± 3.0bc
NPK+BC	182.0 ± 6.5bc
CRF	163.7 ± 11.5c
CRF+BC	157.2 ± 7.4cd
CRF+C	202.8 ± 13.4b
CRF+C+BC	310.2 ± 12.8a

CONCLUSIONS

In summary, *L. pumila* requires sufficient levels of mainly N (> 0.1%), C (> 1%), available P (> 100 mg/kg), Ca and Mg (>1.0 cmol/kg) for the continuous viability and the enhancement of TPC values. A combination of CRF+C+BC is suggested for mass production of these herb as it is able to significantly increase vegetative growth, biomass and TPC values compared to NPK fertilisation alone, providing staggered nutrition according to plant growth. NPK+BC treatments had negative effects on vegetative growth mainly due to reduced root biomass. Application of controlled release fertiliser, compost and biochar is recommended mainly to reduce wastage, promote cost savings and reduce environmental pollution.

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