Effect of different growing media and media water status on leaf biomass of *Andrographis paniculata*

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Abstract. *Andrographis paniculata* is a medicinal plant that widely distributed in a variety of habitats throughout the world. It has a wide range of medicinal and pharmacological application. This study is aims to investigate the effect of different growing media and media water status on the leaf biomass of *A. paniculata*. An experiment was conducted at the greenhouse Institute of Sustainable Agrotechnology (INSAT), Universiti Malaysia Perlis (UniMAP). The experiment was laid out in randomized complete block design (RCBD) of two factors consisting of 9 treatments with 3 replications. Three different growing media were applied, namely compost, topsoil and sand. The growing media were combined in different ratios (1:2:3, 2:2:1 and 3:2:1) with three different soil water potential; -20 kPa (high), -60 kPa (medium) and -100 kPa (low). The crops were harvested 115 Days after transplanting at maturity. There were significant increases on leaves biomass in each plant that had been planted in media mixture contained compost: topsoil: sand (3:2:1) under high soil water potential compared to other treatments. Besides, there were significant interaction effects between growing media and soil water potential were observed for this plant part. The study concluded that the application of potting mixture that contained compost, topsoil and sand in a ratio 3:2:1 with well water supplied are recommended for *A. paniculata* grown under greenhouse condition.

1. Introduction

Hempedu bumi or scientifics name as *Andrographis paniculata* or King of Bitter is one of the medicinal plants that can be cultivated in Taiwan, China and Malaysia. It is supposed to originate from India and Sri Lanka. It is an herb or medicinal plant with an extensive ethnobotanical uses which has been used for centuries in Asia. Andrographis (Acanthaceae) comprises of about 40 species which play an important part of the biodiversity in the ecosystem [1]. *A. paniculata* is an important and most popular member of the genus Acanthaceae that contains medicinal properties. It thrives well in moist and shaded places with providing sufficient sunshine. It is grown in wide variety of habitats for instances hill slopes, wastelands, plains, sea shores, farms dry or wetlands and even road sides. *A. paniculata* is called as ‘King of Bitter’ due to its remarkably bitter taste. This plant is an annual, branched, herbaceous plant and can erecting to a height of around 90–110 cm. The leaves are small in size (3–7 X 1–2.5 cm), simple, green in colour, with the stems are dark green in colour and 2–6 mm in diameter with presence of...
longitudinal furrows and wings on the angles of young plants. The flowers possess white in colour with reddish-purple spots on the petals. Additionally, seeds are small, abundant, numerous and yellowish brown in colour. *Andrographis paniculata* is generally grown as rainy season crop. The plants grows ideally with the beginning of monsoon and starts flowering with the suitable temperature. The flowering and fruiting phase begins about 2–3 months from phase of planting. The leaves start shedding in 90 days and above then the greatest or maximum biomass can be achieved within this period. The main active constituent which is andrographolide is high in leaves especially at the time of flowering. Commonly, the entire harvested material is dried in sheltered or shade area and powdered due to whole plant parts consist of many bioactive ingredients [2].

*A. paniculata* has been traditionally used for a long time because it has plenty of medicinal benefits especially in Unani, Ayurvedic, Chinese and Thai medicine systems in order to preserve health and treat infectious diseases [3]. The leaves, stem and roots of this plant are used in native medicinal system to treat various disorder such as the aerial part of *A. paniculata* is commonly used in Chinese medicine. Sudhakaran [4] explained that based on Chinese medicine theory, since ancient time the plant carried out a significant cold property to get rid of internal body, remedy for cold, fever, pain, inflammation, detoxification and dispose toxins. The authors also further stated that the leaves and root of the plant are regularly used in the traditional system of Indian medicine for extensive treatments of ailments, bitter tonic, using as febrifuge, flatulence, stomachic, ulcer, wounds, leprosy, skin diseases, dysentery, diarrhea, cardiotonic and althalmetic.

Phytochemical studies of *A. paniculata* have revealed that this plant is having wide range of interesting pharmacological effects. It is also captivating to note that the pharmacological effects reported centered on antiretroviral [5], antioxidative [6], antimalarial [7], anticancer [8][9] immunomodulatory [10], antihypertensive [11][12], anti-psychotic[13,] anti-inflammatory [14][15], anti-thrombotic [16], antidiabetic [17][18][19], antiatherosclerotic [20], cholestatic [21], respiratory conditions [22], analgesic, anti-inflammatory and antiulcerogenic [23] studies. The pharmaceutical value of *A. paniculata* is often related with four major active diterpene lactones which are andrographolide (AG), neoandrographolide, 14-deoxy-11,12-didehydroandrographolide (DDAG), and 14 deoxyandrographolide [24][25][26]. Thoroughly, the major active principle andrographolide is present in larger quantities in the aerial parts so the aboveground parts of the plant which including leaves and stems are used as traditional medicine. However, roots have also been used but with insufficient attainment [27].

The term of growing medium can be described as a substance through which roots grow and develop to extract nutrients and water. According to Landis [28] a growing medium provides various functions which are the main things is it physically supports the plant, followed by promoting oxygen exchange by having large pores for root respiration, then, having small pores to hold water and mostly, assist mineral nutrients to be carried by the water to plant roots. In addition, Cantliffe [29] also Verdonck and Demeyer [30] mentioned that growing media characteristics or properties including proper drainage, high water holding capacity, porosity (n) while being free from weed, pests, disease and toxins. Furthermore, the media should be easy to handle, inexpensive and accessible. The capability of the growing media to make water and gaseous balance was significant for managing and maintaining quality of plants [31]. There are two basic forms of growing media applicable for use in container which are common soil and organic based media. Generally, common soil consists of field soil as a major substrate. However, organic media which is a base of organic components such as peat, compost, coconut coir, or other materials mixed with inorganic ingredients would enhances greater root development for the plant [28]. The use of various organic and inorganic media or combination of both substrates allows the optimum nutrient uptake for the plant and provide ample water and oxygen holding capacity for satisfactory growth and development of the plant. Commonly, plant growth and development could be affected directly or indirectly by different substrates that have several material [32]. Therefore, it becomes pertinent to formulate a growing mixtures amended with optimum proportions media instead of soil alone for growing of plants in limited volume in the containers [31].
A lot of studies have stated the advantageous effects of using compost as the amendment in growing media such as decreased in bulk density and strength, increased porosity and enhanced water retention and availability of water of plants. In addition, compost utilization may lessen phytotoxicity, kills plant and zoonotic pathogen as well as weed seeds. Besides, the material will be stabilises with due to nitrogen and oxygen demand of microorganisms and hence improves their availability to plant roots. For use in container media, composts must be stable and mature to avoid secondary biodegradation, which leads to oxygen and nitrogen deficiencies and phytotoxic compounds in the rhizosphere [33]. For instance, a research conducted by Abdou and Mohamed [34] on the application of compost on Mentha piperita L. plants. The researchers reported that plants applied with 48 t ha⁻¹ of plant compost obtained fresh and dry weights about 43% and 29% higher compared to plants fertilized with NPK and treated with tap water. Gupta, Yadav and Garg [35] studied the effect application of vermicompost in container on growth of marigold plant. The results observed that application of vermicompost (100 % cow dung) with soil gave the highest fresh shoot biomass of marigold plant compared to other ratio of vermicompost. This results indicated that utilization vermicompost as an amendment in the container gave positive influence to shoot biomass of the plants.

The physical and chemical characteristics of top soil and sand as inorganic media has been chosen by nurserymen as a part of growing medium. A study done by Egharevba, Ikhatau and Kalu [36] found that growing African walnut (Plukenetia conophorum) yielded remarkable plant growth and development in terms of leaf number, plant height, collar girth of seedling grown on top soil. The researchers also suggested that other combination of amendments should be applied in order to produce better crop growth performances. Abo-Reqz, Albaho and Thomas [32] studied the comparison of different substrates (sand, peatmoss and perlite) in different percentages on growth and development of Chlorophytum comosum and Lycopersicon esculentum cv. Tyerede (tomato). The growth performance observed in term of plant height and leaves number per plant showed significant increase when the rate of sand higher in the growing medium mixture.

Soil water is among the environmental factors that may hinder the crop production. Growing plant in unsuitable condition may hamper the growth of the plant. Crop yield will be affected by insufficient irrigation and over irrigation. Drainage and loss of nutrients due to too high soil water potential in the growing media gave adverse effect on crop performances. Root growth and function at high soil water potential condition will cause inadequate oxygen diffusion into the soil. Meanwhile, reduction in soil water potential consequently gave negative effects on crop growth, for instance, stoma resistance increases, decreasing photosynthesis and increased soil strength impedes root penetration [37]. Previous research conducted by Chen et al. [38] reported that tea plants (Camellia sinensis) responded to the increasing water stress treatments with a decrease in leaf dry matter and tea quality which was indicated by a significant decrease in tea polyphenolic compound (TP). Other study by Karimi et al. [39] on the effect of soil moisture depletion on Stevia (Stevia rebaudiana) grown in greenhouse conditions has concluded that significant growth reduction occurred at 45 % field capacity (FC) compared to 90 %, 75% and 60 % FC.

Optimum water and growing media is required to increase the leaf biomass of A. paniculata especially for commercial and extensive production of the plant [40]. Choosing the right growing media to grow plant is significant to ensure maximization of plant production. Besides, in order to maintain a decent crop growth and promoted efficient uptake of nutrients, an optimum soil water supplies are indispensable as water supply is pivotal for plant growth and development. Till date there is lack of information on the effect of different growing media and media water status on biomass of leaves in A. paniculata grown under greenhouse condition. Hence, keeping this in view the objective of the current research was to determine the effect of different growing media and media water status on leaves biomass of A. paniculata. In this research, abiotic factors which were growing media and media water status will be focused as limiting factors that affect biomass of leaves in A. paniculata. This information will help in determining the suitable growing media and media water status to produce the optimum leaves biomass in A. paniculata under greenhouse condition.
2. Materials and methods

2.1. Plant material

_A. paniculata_ seeds were procured from Universiti Malaysia Perlis (UniMAP) Agrotechnology Research Station, Sg. Chucuh, Perlis. The fully matured seeds were collected after one complete life cycle of the plants and germinated in trays before transplanting to the containers in the greenhouse.

2.2. Experimental design

The experiment was performed in the greenhouse of Universiti Malaysia Perlis (UniMAP) Agrotechnology Research Station, Sg. Chucuh, Perlis. The lay out of the experiment took the form of randomized complete block design (RCBD) of two factors with each factor consists of three levels and 3 replications. Three different growing media were used which were compost, topsoil and sand. The components were mixed in different proportions using volume ratio (refer Table 1). Meanwhile, three water regimes were applied during the experiment which were high, medium and low soil potential. The irrigation was applied when soil water potential reached certain value (refer Table 2). No additional fertilizer or manure were added at any stage during the experiment.

| Treatments | Compost | Topsoil | Sand |
|------------|---------|---------|------|
| Mix 1      | 1       | 2       | 3    |
| Mix 2      | 2       | 2       | 1    |
| Mix 3      | 3       | 2       | 1    |

**Table 1.** Growing media composition used during experiment.

| Treatments | Soil water potential (kPa) |
|------------|---------------------------|
| Low        | -100                      |
| Medium     | -60                       |
| High       | -20                       |

**Table 2.** Soil water potential applied during experiment.

2.3. Monitoring soil moisture of growing media

All pots were watered until saturated and allowed to drain freely until there was no change in pot weight. Prior to installing the gypsum blocks to the soil, the blocks were thoroughly soaked into water overnight to improve the block response in the first irrigation. The block were soaked in the water to remove air from the blocks and ensures accurate soil moisture reading. The blocks were used to absorb moisture from the soil and the electrical conductance which increased with the moisture were measured by using electrodes in the blocks. Gypsum blocks were installed immediately after transplanting the seedlings into container in order to allow time for plant roots to grow around the blocks. The indication of available soil moisture would be displayed directly on the meter after the measurement was obtained from the soil. Meter readings were interpreted in terms of soil moisture tension and converted to kPa. The irrigation was begun when the data were below the target soil water potential [41].

2.4. Analysis of physical and chemical properties of growing media
Bulk density were measured by using core method. The core ring were saturated by allowing water to disperse into the media for two days. The samples were oven-dried at 105°C for 24 h after recording the weights [42]. Mechanical analysis was determined by sieve analysis. Meanwhile, organic carbon was analysed by Walkley and Black method. Total N was done by sulphuric acid digestion and Cation exchange capacity (CEC) was measured by ammonium acetate. pH meter was used to measure pH and Electrical conductivity (EC) values was measured by using conductivity meter. Table 3 showed the physical and chemical properties of growing media.

### Table 3. Physical and chemical properties of different growing media combination.

| Properties                  | Mix 1    | Mix 2    | Mix 3    |
|-----------------------------|----------|----------|----------|
| Mechanical analysis (%)     |          |          |          |
| Coarse sand                 | 42.88    | 30.68    | 23.16    |
| Fine sand                   | 41.68    | 39.00    | 36.14    |
| Silt                        | 10.30    | 22.08    | 27.58    |
| Clay                        | 5.14     | 8.24     | 13.12    |
| Bulk density (g/cm³)        | 1.20     | 1.08     | 0.91     |
| pH                          | 7.40     | 7.19     | 6.80     |
| EC (dSm⁻¹)                  | 0.28     | 0.47     | 0.61     |
| Organic carbon (%)          | 1.86     | 4.97     | 7.09     |
| Total N (%)                 | 0.09     | 0.18     | 0.27     |
| C:N                         | 20.07    | 27.60    | 26.30    |
| CEC c mol(+)/kg             | 8.22     | 14.62    | 20.65    |

2.5. Harvesting of *A. paniculata*
The plants samples were collected during harvesting process at 115 days after transplanting (DAT). All the plants were discard carefully from the container. *A. paniculata* leaves were collected and washed under tap water to remove soil.

2.6. Determination of leaf biomass of *A. paniculata*
To measure the leaf biomass of *A. paniculata*, the leaves sample was air dried and weighing with an electronic weighing balance until constant weight was attained and the leaves dry weight data were recorded.

2.7. Statistical analysis
The data were subjected to two-way Analysis of Variance (ANOVA) by using JMP Pro 11 statistical software with two factors and three replicate. For statistical comparison between the treatments, Tukey”s HSD (honest significance difference) test was done to observe significant difference at the P-value<0.05.

3. Results and discussion
3.1. Effect of growing media and media water status on leaf biomass of A. paniculata

Plants have an outstanding capacity to coordinate the growth of their organs, so that there is generally a tight balance between the biomass invested in the shoot and that invested in roots. There are many factors that involved in the allocation of biomass to different plant parts such as species, ontogeny and the environmental factors. Thus, in this research the abiotic factors which were different growing media mixture and water status of the media would be examined on the biomass of A. paniculata.

Table 4. ANOVA for leaf biomass of A. paniculata as influenced by different growing media, media water status and the interaction of both factors.

| Source of variations                     | DF | Dry weight of leaves (g/plant) |
|---------------------------------------|----|--------------------------------|
| Replication                           | 2  | 0.02281                        |
| Growing media                         | 2  | <0.001                         |
| Media water status                    | 2  | <0.001                         |
| Growing media*Media water status      | 4  | <0.001                         |

The results presented in Table 4 showed the significance level for analysis of variance in each of the response variables that were used in this experiment. There were highly significant effect was seen on the dry weight of leaves based on the analysis of growing media, media water status and interactions between the two factors. Additionally, Tukey’s test for post-hoc analysis were performed to compare the means of all treatments to the mean of every other treatment. The values shown in the figures are the mean values of three replicates with standard deviation. The results were shown in the next section.

3.2. Effect of different growing media and media water status interaction on yield of A. paniculata

According to the result shown in figure 1, interaction effects of different growing media and media water status on leaves of A. paniculata was seen in this experiment. The results indicate that the minimum dry weight of leaves (9.62 g/plant) was recorded in Mix 1 with low soil water potential. Meanwhile, the maximum dry weight of leaves (33.51 g/plant) was weighed in Mix 3 with high soil water potential. The production of leaves dry weight under high soil water potential with Mix 2 and Mix 3 showed there were no significant difference between these two treatments at 5% significant level. The values for each treatment were 32.1 g/plant and 33.51 g/plant respectively. Similar results also can be observed from the graph that plants grown in combination mixture 2 with low (20.37 g/plant) and medium soil water potential (21.47 g/plant) produced no significant differences of leaves dry weight.

The minimum dry weight of leaves in Mix 1 with low soil water potential mainly attributed to low organic amendment and available soil water. This result was consistent with report by Ramzjoo, Heydarizadeh and Sabzalian [43] in Matricaria chamomile that had been exposed with water stresses during the growth. The plants produced significant reduction of leaves dry weight compared to well water treatments. Herralde, De Biel and Save [44] stated that reduction in leaf biomass was promoted by water stress and would result in both senescence and death of leaves which had been investigated as an avoiding mechanism that grants minimizing water losses. The researchers also reported a same result that plants imposed to water stress had lack of leaf biomass due to senescence and death of leaves at the end of the experiment.
Further explanation by other researchers mentioned that water deficits would lead to decrease in plant growth due to both reduction rates of photosynthesis and growth of leaf [45]. In addition, Zhao et al. [46] agreed that a typical drawback impact of water stress on crop plants is the reduction in dry biomass production. Plants were adversely affected by water stress whenever water uptake by the roots were less than water loss from canopy. This result is similar with the study conducted by Pizarro and Bisigato [47]. The researchers had imposed six Patagonian Monte species with five variation of irrigation regimes and the result shown that higher biomass were allocated towards leaf in plants grown in the low water supply regime (5%) comparing to the other treatments. The ability of plants to capture resources was indicated by changes in allocation patterns of the plants in response to the environment in particular with the availability of soil nutrients [48] [49].

Another research conducted by Bettaieb et al. [50] found that dry matter weight of sage (Salvia officinalis) grown under severe water deficit was significantly affected by drought and was reduced 17.85% and 26.33% respectively compared to moderate water treatment. The similar trend also can be observed in other growth parameter which were fewer and thinner stem produced with dry and smaller leaves compared to control treatment. Baghalian et al. [51] examined different application of water stress of German chamomile (Matricaria recutita L.) with the focused on agro morphological characters shoot weight. The authors found that water stress decreased plant shoot weight of M. recutita. Furthermore, a study conducted by Laribi et al. [52] also found that leaves dry weight of caraway (Carum carvi L.) was significantly affected by application of water stress to the plants. Previous study done by Petropoulos at al. [53] on the effect of water deficit stress on the yield of parsley (Petroselinum crispum) found that application of water stress would decreased the growth (foliage and root dry weight, leaf number) of the plant.

Figure 1. Dry weight of leaves as affected by different growing media and media water status
The highest dry weight of *A. paniculata* was obtained in Mix 3 with high soil water potential. This result was attributed to the right proportion of compost with suitable soil water applied to the plant. A research done by Ievinsh, Andersone-Ozola and Zeipina [54] compared the effects of soil amendments (compost) in organic production of four herb species which were (*Dracocephalum moldavica* L., *Melissa officinalis* L., *Nepeta cataria* L. and *Thymus vulgaris* L.) as model plants. The study revealed that additions of the organic amendments would gave greater fresh and dry weight of *M. officinalis* and *N. cataria* shoots. Meanwhile, the similar effect can be observed with the addition of vermicompost to *D. moldavica*. Further increased of compost dosage from 20% to 40% did not gave positive result to the biomass of the plant. In contrast, other model plant which was *T. vulgaris* showed that the highest growth was attributed by the amendment of 20% compost and 30% amendment of vermicompost. However, increasing the incorporation rate of the amendments would result in significantly lowered in the plant growth. Additionally, there were no significance difference in both fresh and dry weight was obtained for *M. officinalis* and *N. cataria* planted with amended compost or vermicompost. There were reduction in both fresh and dry weight of the shoots compared to control plants of *T. vulgaris* but the difference were not statistically significant.

Plant growth and development particularly for salt-sensitive species can have detrimental effects with media containing high salt. Thus, less than 20% of compost encompasses greater levels of soluble salts should be included into a soil mixture for the production [55] [56]. Recent studies done by Grigatti, Giorgioni and Ciavatta [57] investigated that media having 25–50% composts gave better or comparable biomass production and ornamental value with respect to the control peat of grown french marigold. Other research by Osaigbovo, Nwaoguala and Falodun [58] observed that maximum growth of seedlings can be produced in plant height, stem girth, leaf number and dry mass of aerial plant parts also belowground parts by using combination of top soil and cow dung as potting medium. Both inorganic and organic mixture help to improved aeration, water holding capacity and enhance nutrient utilization.

Al-Romian [59] have conducted a pot experiment in the greenhouse to assess the impact of three compost types (chicken residues, animal residues, and mixed organic residues) and four rates (1%, 2%, 4%, and 6%) on lettuce growth produced which were added to sandy soil. The results revealed that the dry weight of lettuce showed great variation between the different compost treatments. In contrast from the previous research, the dry weight of lettuce gave negative effect to the increasing rate of compost applied to the plants. The author also specified that type of soil, compost, rate of application and maturity level characterize the effect of composed on the growth and development of the plant [60]. The outcome of the study concluded that the use of suitable organic amendments and mineral soil would directly effects plant biomass of studied plants [59].

4. Conclusion

The assessment of different growing media and media water status on the leaves biomass of *A. paniculata* revealed the importance of proper combination of growing media mixture and soil water potential to enhance production of the plant. The result showed that the maximum leaf biomass was attained when the higher ratio of compost added to the growing media as well as sufficient irrigation was applied in the container. Thus, it is suggested and recommended to use the combination mixture compost: topsoil: sand (3:2:1) with well watered supplied in order to maintain satisfying leaf biomass in *A. paniculata* grown under greenhouse condition. As the conclusion, growing plants in media with adequate nutrient and water supply would give sustainable *A. paniculata* management.

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