UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DIFFERENT INDOLE-3-BUTYRIC ACID (IBA) CONCENTRATIONS AND ROOTING MEDIA ON ROOTING RESPONSE OF ORTHOSIPHON ARISTATUS STEM CUTTINGS

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By

AMINAH BINTI ARBAI

A Project Report Submitted in Partial Fulfilment of the Requirement for the Degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences Universiti Putra Malaysia Bintulu Sarawak Campus

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DEDICATION

Specially dedicated to;

My beloved father and mother,
Arbai b. Hj. Tahir and Siti Esah bt. Mohd Nasir

To all my brothers and sisters,
Aziz, Arif, Azfar, Azniyati, Aniza, and Asmawati.

ABSTRACT

This study was undertaken to evaluate the effects of indole-3-butyric acid (IBA) on induction of root from two-node stem cuttings of *Orthosiphon aristatus*. The study was conducted at the Horticultural Unit, Universiti Putra Malaysia Bintulu Campus using two rooting media, sand medium and water medium. Five different treatments made up of five different levels of IBA at 0, 500, 1000, 1500, and 2000 ppm were investigated in this study in three replications for each of the rooting medium. The treatments were administered by immersing the basal end of the stem cuttings into the IBA solution for 15 minutes prior to rooting. A total of 20 cuttings were administered for every treatment per replication. Root production was evaluated based on the number of root and length of the longest root for each cutting. Evaluation of rooting was done at 7 days after treatment for the water medium and 10 days for the sand medium followed by three evaluations at every five days intervals respectively. It was found that cuttings planted on sand medium and treated with 500 ppm IBA on average had the longest root but cuttings with treatments of higher IBA concentrations tended to produce more roots. Cuttings rooted in water medium with no IBA application produced the longest root while cuttings treated with higher IBA concentration produced the most roots. The overall results indicated the water medium appeared to be more conducive for rooting of *O. aristatus* stem cuttings in term of root length but in term of inducing more roots IBA treatments were very effective especially at the 1500 ppm and 2000 ppm levels in either rooting medium.

ABSTRAK

Kajian ini dijalankan untuk menilai kesan asid indol-3-butirik (IBA) pengakaran keratan batang dua buku *Orthosipon aristatus*. Kajian telah dilakukan di Unit Hortikultur, Universiti Putra Malaysia Kampus Bintulu dengan menggunakan dua media pengakaran iaitu media pasir dan air. Lima rawatan IBA dengan kepekatan berlainan iaitu 0, 500, 1000, 1500, dan 2000 ppm dengan tiga replikasi bagi setiap media pengakaran. Rawatan telah dijalankan dengan mencelup bahagian bawah keratan batang ke dalam larutan IBA selama 15 minit. 20 keratan digunakan bagi setiap rawatan per replikasi. Pengakaran dinilai berdasarkan bilangan akar dan juga panjang akar yang terpanjang. Penilaian dilakukan selepas tujuh hari rawatan bagi media air dan 10 hari bagi media pasir diikuti dengan tiga lagi bacaan setiap lima hari. Keputusan berdasarkan purata mendapati keratan yang ditanam pada media pasir dengan rawatan 500 ppm IBA mempunyai akar yang terpanjang tetapi keratan dengan rawatan kepekatan IBA yang lebih tinggi menghasilkan bilangan akar yang lebih banyak. Keratan pada media air tanpa rawatan IBA menghasilkan akar terpanjang manakala keratan dengan rawatan kepekatan IBA yang lebih tinggi menghasilkan akar yang lebih banyak. Keputusan keseluruhan mendapati media air lebih kondusif sebagai media pengakaran keratan batang *O. aristatus*.

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APPROVAL SHEET

I certified that this research entitled **"Effects of Different Indole-3-Butyric Acid (IBA) Concentrations and Rooting Media on Rooting Response of *Orthosiphon aristatus* Stem Cuttings"** has been examined and approved as a partial fulfilment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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CHAPTER 1

INTRODUCTION

1.1 Background

Orthosiphon aristatus (Blume) Miq. is a herbaceous shrub that can be found growing along the forest edges, roadsides and wastelands. This species belongs to Family Laminaceae and has several scientific names such as *Orthosiphon stamineus, Orthosiphon spicatus, Ocimum aristatum, Ocimum grandiflorum, Clerodendrum spicatum* and *Cleridendronthus spicatus.* This herbaceous plant is commonly called as Misai Kucing, Kumis Kucing, Java-Tea and cat's whiskers. They are always used as ornamental plant and known as popular medicinal herbs in Southeast Asia (Goh *et al.,* 1995).

Orthosiphon aristatus is popular as garden plant besides their medicinal properties. This plant is very attractive with long white (Plate 1), violet or sometimes light blue flowers. Stamens look like cat's whiskers. The leaves are arranged in opposite pairs. There are simple, green, and glabrous with lanceolate leaf blade and margin. The stem is quadrangle, reddish in color, erect, and branches profusely. This plant can grow to a height of 1.5 m (Nusa Herb, 2004). There are 3 major cultivars have been distinguished; one with blue and two with white flowers. The white flowered cultivar with reddish stem, petioles and leaf veins appears to possess the best diuretic qualities (Westphol, 1993).

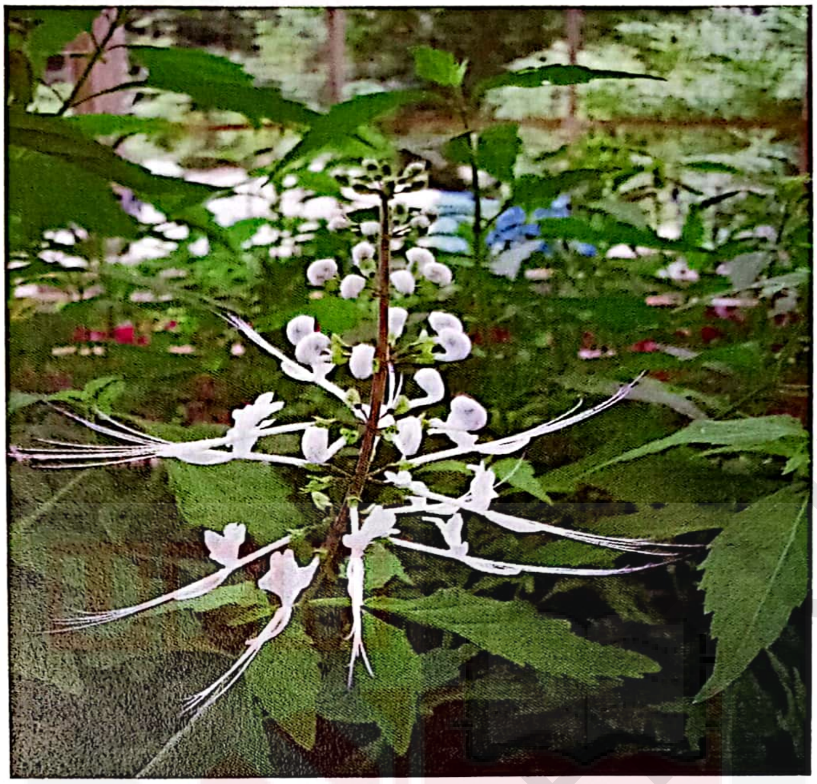

Plate 1: Flower of *Orthosiphon aristatus*

Their leaves are widely used in Europe and Japan as health tea. In Javanese traditional medicine or also known as '*jamu*' has been produced from water decoction of leaves of *O. aristatus*. This herb is used for treatment of diabetes and hypertension (Mat Salleh and Latiff, 2002). Beside that it was used to control urinary system ailments, high blood pressure, treat kidney stone, gout and arthritis (Goh *et al.*, 1995).

1.2 Propagation Method

Orthosiphon aristatus has been cultivated for a long time using stem cutting. This herb is easy to plant by vegetative propagation. Commonly, they are propagated by stem cutting. Besides cutting, they also can be planted using seed (PIER, 2007). After the flowers fade or dry, it will produce seedpod containing small seeds. Small seed has to be handling carefully to maintain seedling quality and avoiding from pest infections.

Due to high market demand, now there are researches were done to propagate this valuable herb. Study has done by Lee and Chan (2004) in order to regenerate new shoot from stem nodal segment through micropropagation method or known as tissue culture. Previously, they also have done some research on modified micropropagation method to culture more plant from limited resources. All this work is to supply enough planting materials for commercial productions. However, this propagation method is complicated and need high cost facility such as laboratory and sterile equipment. So this is not a choice for small budget planters.

Otherwise, cutting techniques as propagation method give many advantages such as low cost with high production of new plant and easier to practices compared to tissue cultures. Besides that they can maintain genetic stability that was gain from uniform planting material. In seed propagation, there sometimes have gene alteration in seedlings. Stem cutting also can provide plant supply throughout the year (Darus and Aminah, 1993).

1.3 Plant Growth Regulator

Plant growth regulators (PGRs) consist of both natural and synthetic types in which they have important role in propagation. Plant hormone is important as regulators in plant function and induce root production. Five important plant hormones are auxin, cytokinin, gibberellin, abscisic acid, and ethylene.

In plant propagation, the most widely used is auxin. Auxin is used to induce adventitious rooting in cutting and to control morphogenesis in macropropagation. The most useful synthetic auxin is indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA). While indole-3-acetic acid (IAA) is synthesize from plant extract and also used in experimental study. Indole-3-butyric acid also has been found to occur naturally (Hartmann *et al.,* 2002).

Cuttings treated with plant growth regulator, especially auxin, show rooting effects such as; percentage roots producing increased, hasten root initiation, and uniformity of rooting also increased. Indole-3-butyric acid is the best auxin for general used because it is non-toxic to plant in a wide concentration range, and is effective in promoting rooting for many plant species. Besides IBA is a stable compound and can extend its shelf-life by darkness and refrigeration. Indole-3-butyric acid usually can be found in commercial hormone product and often combination with NAA. Commercial preparation that available in market is in talc or in liquid form that can easily dilute with water. However, pure IBA powder can be diluted with 50% ethanol or 1N NaOH only (Hartmann *et al.,* 2002).

1.4 Rooting Media

Rooting media is another important factor in cutting propagation. Major function of rooting media includes give support to the cuttings, supply moist and air circulation at cutting base and also provide dark environment for root development. Suitable rooting media is important to make sure cuttings have good condition for root and plant growth development (Hartmann *et al.,* 2002).

Orthosiphon aristatus requires consistent moist root environment to keep them in good condition. In this study, rooting media that has been used are river sand and water. Sand usually used in nurseries, readily available and inexpensive. Sand particles provide good drainage and aeration to the cuttings. But sand has poor ability to retain moisture. Watering needed done regularly to avoid cutting drying. Do not use too coarse sand because of very low water retention (Darus and Aminah, 1993).

Aerated water system has been reported used to root several tropical plant species especially dipterocarp species (UNDP/FAO, 2004). Articles found in Australian Plant Society (2007), stated that *O. aristatus* can root easily in water. Aerated water system and hydroponic system has been considered and modified as a new rooting system. Simple system combination of basin and germination tray is used. Without apply aerator and nutrient solution, cost of production was reduced.

1.5 Objectives

Main objective of this study was to evaluate rooting response of *O. aristatus* cutting due to different IBA concentrations. Rooting response is measured through mean root length and number of root produced per cuttings. Second objective was to observe either sand or water media is more suitable as rooting medium for rooting *O. aristatus* stem cuttings. Suitability of rooting medium was referred to rooting response showed on cuttings.

CHAPTER 2

LITERATURE REVIEW

2.1 Medicinal Values of *Orthosiphon aristatus*

The flavonoid and phenolic compound found in *O. aristatus* were reported to be the active diuretic and anti-bacterial agents (Schut and Zwaving, 1993). It is also used for treatment of diabetes and hypertension (Mat Salleh and Latiff, 2002). Goh *et al.* (1995) has stated that for treating hypertension, 20 g of its leaves were used to prepare as tea. Clinical studies showed that the diuretic effect of *O. aristatus* have no influence on 12 and 24 hours urine output or the sodium excretion but show significant diuretic activity in rats in form of alcoholic and water extracts (Indu Bala, 2000).

A study by Ohashi *et al.* (2002) on antihypertensive substance in *O. aristatus* had shown that major compound known as methylripriochromene was found in the water decoction of *O. aristatus* leaves. This compound has exhibited significant effect in decreasing blood pressure after applied to the hypertensive rats.

A collaboration study between Institute of Medical Research, Kuala Lumpur Hospital and School of Pharmaceutical Sciences, USM has found that this plant is also useful for treatment of kidney stones. Intake of this herb for a long term can slowly reduced size of kidney stone. Other uses of *O. aristatus* included as antiallergic, anti-inflammatory, remedy for arterio-sclerosis (capillary and circulatory disorders) and nephritis, treatment for gout and rheumatism (Goh *et al.*, 1995).

2.2 Efficient Propagation Technique

Reproduction is term of the process when organisms duplicate themselves. Plant reproduction is recognized as a complicated process that results in the multiplication of cells and the organisms they make up. When reproduction is deliberately controlled and manipulated, it becomes propagation (Ingels, 2001).

Plant can be reproduced both sexually and asexually. Sexual reproduction involved the formation of seeds through the fusion of two sex gametes to form zygote. The genetic material of cell form from sexual reproduction is differing in physical and physiological characteristic from their parent plant. While asexual reproduction is vegetative process that eliminates genetic variation. Asexual propagation used vegetative part of plant to grow new plants such as stems, root and leaves. New plants produced asexually is called clone (Ingels, 2001).

There is much technique to propagate plant asexually such as cutting, grafting, budding and tissue culture (Ingels, 2001). Cutting is more preferred by propagators because it more easy to apply for broad category of plants. Hence, cutting method is more easy and cheap compared to other methods. Cutting can be made from the vegetative portion of the plant such as stems, modified stems (rhizomes, tubers, corms, and bulbs), leaves or roots (Hartmann *et al.*, 2002).

In this study, stem cutting was used to propagate *O. aristatus*. Propagation by stem cuttings, segment of shoots containing lateral or terminal buds are obtained with the expectation that under the proper conditions adventitious roots will develop and thus produce independent plant. There are many types of stem cutting such as hardwood, semi-hardwood and softwood cuttings.

According to Hartmann *et al.* (2002), hardwood cutting are those made of matured, dormant firm wood after leaves abscised. While a semi-hardwood cutting differs from softwood cutting only in maturity of the wood. Softwood cutting were taken from woody plants that still soft usually three to five weeks after a new flush of growth. Semi-hardwood cuttings generally are taken from new shoots six to nine weeks after a flush of new growth. *Orthosiphon aristatus* cuttings were taken eight weeks after a new flush of growth. This means it was categorized under semi-hardwood cutting.

Semi-hardwood cuttings are made 7.5 to 15 cm long with leaves retained at the upper end. Big sized of leaves should be trimmed to reduce transpirational water loss and allows closer spacing in the rooting bed. The shoots terminals are often used in making cuttings, but the basal parts of the stem will usually root also. The basal cut is usually just below a node. Extremely fast growing, soft and tender shoots are not desirable because they often deteriorate before rooting. However, mature old woody stems are very slow to root (Hartmann *et al.*, 2002).

Darus and Aminah (1993) reported that mature tree is more difficult to roots. Factors that related to the observations are;

1) increasing production of rooting inhibitors as plant grow older,
2) decreasing phenolic level, which act as an auxin cofactor or synergist in root initiation of stem cutting,
3) lignifications of the stem and,
4) anatomical barriers such as a sclerenchymatous sheath.

2.3 Effectiveness of Plant Growth Regulators

In the 1930's horticultural researchers isolated the plant growth regulators which promote rooting of cuttings and improve the root mass of rooted plants. These regulators even produce rooting of some cuttings which do not self root (Ingels, 2001). Plant growth regulators are any natural and synthetic chemical that shows hormonal effects. The five major phytohormones are auxin, cytokinin, gibberellin, abscisic acid, and ethylene. However, auxin has the greatest effects on root formation in cuttings. Used in high doses, auxin stimulates the production of ethylene. Excess ethylene can inhibit elongation growth, cause leaves to fall, and even kill the plant (Hartmann *et al.*, 2002).

The first hormone to be discovered is auxin that has played essential role in coordination of many growth and behavioral processes in the plant life cycle. Auxin is involved in many plant activities including coleoptiles bending toward light, inhibition of lateral buds by terminal buds, formation of abscission layer on leaves and fruit, and activation of cambial growth. Auxin is most widely used in plant propagation to induce adventitious rooting in cutting, and to control morphogenesis in micropropagation (Hartmann *et al.*, 2002). They can also be used to promote uniform flowering, to promote fruit set and to prevent premature fruit drop.

Auxin family consists of several hormones and the widely used members are indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and α-naphthalene acetic acid (NAA) (Hartmann *et al.*, 2002). Indole-3-acetic acid is the most important member of the auxin family. It generates the majority of auxin effects in intact plants, and is the most potent native auxin. However, molecules of IAA are chemically labile in aqueous solution therefore IAA rarely being applied commercially as a plant growth regulator.

Two synthetic hormones, IBA and NAA were more effective than naturally occurring or synthetic IAA for rooting. It has been proofed IBA and NAA can effectively induced rooting of stem cuttings and tissue-culture-produced micro-cuttings. Indole-3-butyric acid also can be found naturally in plant but less abundant compared to IAA. Combinations IBA with NAA always used in horticultural practices. The best auxin for general use is IBA because it is non toxic to plant over a wide concentration range (Hartmann *et al.*, 2002).

In stem tissue, auxin generally moves in a basipetal direction which is from apex to base. In first time, auxin was applied at the cutting apical end to conform to the natural downward flow. But, basal application was found gave better results. Sufficient movement carried the applied auxin into parts of the cutting where it stimulates root production (Ingels, 2001).

According to the "acid growth theory," auxin may directly stimulate the early phases of cell elongation by causing responsive cells to actively transport hydrogen ions out of the cell, thus lowering the pH around cells. This acidification of the cell wall region activates enzymes known as expansins, which break bonds in the cell wall structure, making the cell wall less rigid. When the cell wall is partially degraded by the action of auxin, this now-less-rigid wall is expanded by the pressure coming from within the cell, especially by growing vacuoles. However, the acid growth theory does not by itself account for the increased synthesis and transport of cell wall precursors and secretory activity in the Golgi systems that accompany and sustain auxin-promoted cell expansion (Hartmann *et al.*, 2002).

CHAPTER 3

METHODOLOGY

3.1 Introduction

This study investigates the effect of different concentrations of indole-3-butyric acid (IBA) to induce rooting on stem cuttings of *O. aristatus*. Five treatments were used consisting of four different concentrations of IBA at, in ppm, 500, 1000, 1500, and 2000, plus 0 IBA as control. The two rooting media used in this study were cleaned river sand and plain tap water. Cleaned river sand was used because it is free from contaminants such as plant remains stones and other debris. Each treatment was made up of 20 cuttings per replicate for three replications except for control. Cuttings were examined for number of roots and root length. This study was conducted at the Horticultural Unit UPMKB.

3.2 Stock Plant

The stock plants of *O. aristatus* was selected from plants grown at the Dean's house yard as shown in Plate 1 as well as from plants grown at the Horticultural Unit, UPM Bintulu Campus. Stock plants were maintained by fertilizing once in two months to encourage new shoot growth before cuttings were obtained for the experiment.

Plate 2: Stock plant

3.3 Rooting media

3.3.1 Sand medium

The sand medium was prepared about a month before planting were scheduled by soaking the sand for four days with plain tap water in an 18 gallon tank to clean any contaminant before use. The cleaned sand was then filled into a sand bed made up of concrete frames of size (70 x 400 cm). Fourteen wheelbarrows of sand were needed to fill up a sand bed.

After letting to dry for a month, the sand bed was subdivided into sections each of size 70 x 95 cm for each of the four IBA levels and at 23 x 95 cm for the control treatment as in Plate 2. Each section of the IBA treatment was further subdivided into three subsections each of size 23 x 95 cm for each of the three replicates.

Plate 3: Sections of prepared sand bed rooting medium

3.3.2 Water medium

The water rooting medium was prepared by using plastic basins of size 18 L which holds the germination trays made up with 104 holes. The germination trays was used for holding the cuttings in place and dipped horizontally inside the plastic basins containing the water medium as shown in Plate 3. Each cutting tray was provided with attached string to take it out of the basin when required during the root counting process. Each tray was only sufficient for two replicates and a total of seven trays in seven basins were prepared to accommodate the entire experimental set up.

Plate 4: Prepared water medium container.

3.4 Hormone Preparation

Two weeks before experiment, stock solution of indole-3-butyric acid (IBA), 98.5 % purity, of 4000 ppm were prepared at first by dissolving 4 g of IBA powder in 25 ml 1N NaOH and then top up to 1 L with distilled water. Dilution of stock solution for the required treatment concentration was done according to the formula $M_1V_1=M_2V_2$ as illustrated below:

Stock solution, M_1=4000 ppm, V_1=?

Hormone solution, M_2= 500 ppm, V_2= 600 mL

$M_1V_1 = M_2V_2$

$(4000)\ V_1 = (500)\ (600)$

$V_1 = 75$ mL

This mean the 75 mL of stock solution needs to be top up with 525 mL of distilled water to make up the 600 mL required to produce 500 ppm of IBA solution. Average pH for all hormones preparations ranged from pH 6.13 to pH 7.06. The prepared solutions were stored in dark bottles and kept in refrigerator until used.

Five different levels of IBA concentrations were evaluated as treatments in this study as indicated below:

a) Control treatment (T0): water only without IBA
b) Treatment 1 (T1): 500 ppm IBA
c) Treatment 2 (T2): 1000 ppm IBA
d) Treatment 3 (T3): 1500 ppm IBA
e) Treatment 4 (T4): 2000 ppm IBA

3.5 Cutting Preparation

Cuttings were prepared in the early morning, to avoid excessive loss of water and to maintain plant turgidity. Only healthy stock plants were selected for preparation of cuttings. Stock plants with symptoms of mineral deficiency, disease and insect damage were avoided. In generally, cutting from young plants will produce higher percentage of root than cutting taken from older plant. So, only cutting from young shoot were taken. A sharp secateurs used while do the cuttings. The base cut at the right angle.

Two nodes stem cutting are used in this study, in which type of cuttings that regularly used for other ornamental plants. Stem diameters of cuttings averaged about 0.4 cm and stem length ranged from 8 cm to 15 cm, depending on nodes position on the plant, see Plate 4. Leaves present on cutting were cuts ½ or ¼ from total leave area to reduce water loss through transpiration and conserve space. A total of 520 cuttings were used with each replicate consisting 20 cuttings.

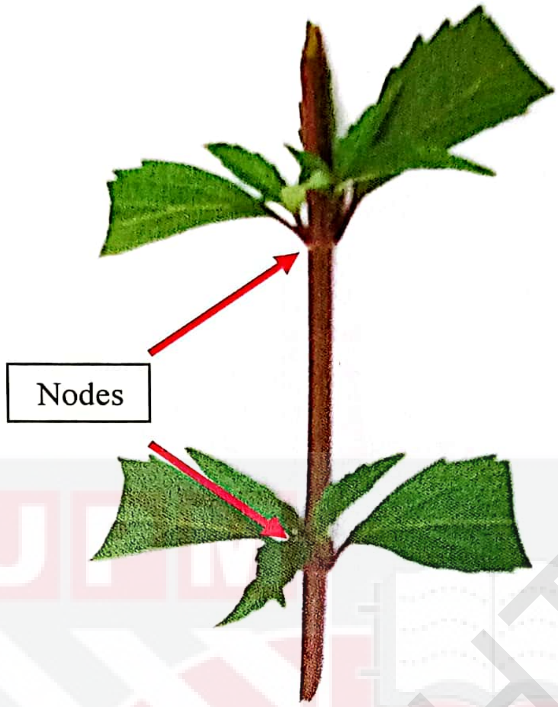

Plate 5: Two nodes stem cutting

3.6 Planting

Before planting, all the cuttings were treated with 5 treatments in different hormone concentration. The base of the cuttings soaked into hormone solution for 15 minutes to allow cuttings absorbed active ingredient.

For sand medium, sand being watered first to keep it moist and planting arrangement were done as shown in Plate 5(a). While for water medium, tap water filled into the container but not exceeding the germination tray level. Put some space between water and germination tray. Cuttings were put into hole of germination tray but make sure they were not too close to each other as shown in Plate 5(b). Cuttings were being watered manually twice a day for sand media cuttings.

Plate 6: Planting arrangement; (a) sand medium; (b) water medium.

3.7 Assessments

Measurement for cuttings planted on water media were carried out once in five days initiated seven days after planting. While for cutting planted on sand media were measured once in a seven days after ten days planting. At each assessment, the number of root produced and root length were recorded. A cutting was scored as rooted when it produced at least 5 mm length of root. Number of roots were count if they has 5 mm length or more and the longest root for each cutting produced was considered as the root length. Reading was taken for a month.

Cuttings planted back on sand and water medium after measurement finish. Non-destructive method was used to prevent root damage. This is easy to apply for water medium cuttings. However, for sand medium, digging and planting back processes have to be done carefully to avoid roots break.

After a month all the lived cuttings were transferred into polyethylene bag to see growth performance of the cuttings. Mixed media 3:2:1 (soil: sand: organic fertilizer) was filled into 6 x 9 polyethylene bags to plant every lived *O. aristatus* saplings for further growth stage.

3.8 Data Analysis

Yield data for all treatment was determined on the mean length and number of roots produced of three replicates for each treatment. Percentage of roots increasing also determined. All data were analyzed using the Statistical Analysis System (SAS) software and all statistical comparisons of data were made in according to the Duncan's New Multiple Range Test (DNMRT) procedure.

CHAPTER 4

RESULTS

4.1 Rooting Response of *Orthosiphon aristatus* Cuttings Treated with IBA in Sand Medium

4.1.1 Effect of IBA treatment on root length of cutting

All cuttings of *Orthosiphon aristatus* treated with IBA treatments result in significantly longer roots than untreated cuttings as shown in Table 1, and the root growth was progressively longer at the fourth week of rooting as shown in Plate 7. However, among the different IBA treatments statistical differences were noted.

The best IBA treatment which produced the longest root was at 500 ppm which by the end of the first week had attained 7.87 cm in length and statistically longer than 7.33 cm of the next longest root from cuttings treated with 1500 ppm IBA. Cuttings treated with 500 ppm IBA also consistently produced the longest root which measured at 12.37 cm long at two weeks of rooting, 15.10 cm long at three weeks of rooting, and 17.53cm long at four weeks of rooting all of which were significantly longer than cuttings treated with other IBA treatments.

The IBA treatment at levels of 1000 ppm, 1500 ppm and 2000 ppm did not show significant difference among them with respect to root length of cuttings and after two weeks of rooting until the final evaluation at four weeks of rooting, none of these treatment levels of 1000 ppm, 1500 ppm and 2000 ppm of IBA showed any difference from untreated cuttings in term of the length of the longest roots.

Table 1: Effects of indole-3-butyric acid (IBA) treatments on length of the longest root of cuttings rooted in sand medium.

IBA Concentration (ppm)	Mean root length of the longest root (cm)			
	Week			
	1	2	3	4
0	6.23^{c}	10.20^{b}	12.33^{c}	15.13^{c}
500	7.87^{a}	12.37^{a}	15.10^{a}	17.53^{a}
1000	6.13^{c}	11.13^{b}	13.87^{b}	16.00^{bc}
1500	7.33^{b}	11.13^{b}	13.70^{b}	16.83^{ab}
2000	7.10^{b}	10.80^{b}	13.43^{b}	16.03^{bc}

Note: Values within column with the same superscripts are not significantly different at P = 0.05 using the DNMRT.

4.1.2 Effects of IBA treatment on the number of root per cutting

The number of root produced by *O. aristatus* cuttings treated with all IBA treatments was significantly higher than cuttings without IBA treatment as shown in Table 2, and the number of root per cutting also increased by the fourth week of rooting as indicated in Plate 7.

Nonetheless, differences were noted among the different levels of IBA treatments with respect to the number of root on cuttings. In the first week of rooting cuttings treated with 1500 and 2000 ppm levels of IBA both produced significantly the most roots with means of 50.77 and 51.03 roots per cutting, respectively.

At week two, both 1500 ppm and 2000 ppm of IBA treatments continued to produce cuttings with the most roots than cuttings treated with 500 ppm and 1000 ppm of IBA treatments with mean of 55.46 and 53.87 roots per cutting, respectively. But at week three of rooting the number of root on cuttings was not significantly different among all levels of IBA treatments. However, at four weeks of rooting, cuttings treated with 1500 ppm and 2000 ppm of IBA treatments were noted to have significantly more roots than cuttings treated with 500 ppm and 1000 ppm of IBA treatments.

Table 2: Effects of indole-3-butyric acid (IBA) treatments on the mean number of root per cutting rooted in sand medium.

IBA Concentration (ppm)	Mean number of root per cutting			
	Week			
	1	2	3	4
0	24.15^{c}	28.25^{c}	29.72^{b}	30.52^{d}
500	40.92^{b}	49.68^{ab}	52.17^{a}	53.07^{b}
1000	41.32^{b}	47.18^{b}	48.57^{a}	49.45^{c}
1500	50.77^{a}	55.46^{a}	57.78^{a}	58.38^{a}
2000	51.03^{a}	53.87^{a}	56.45^{a}	57.32^{a}

Note: Values within column with the same superscripts are not significantly different at P = 0.05 using the DNMRT.

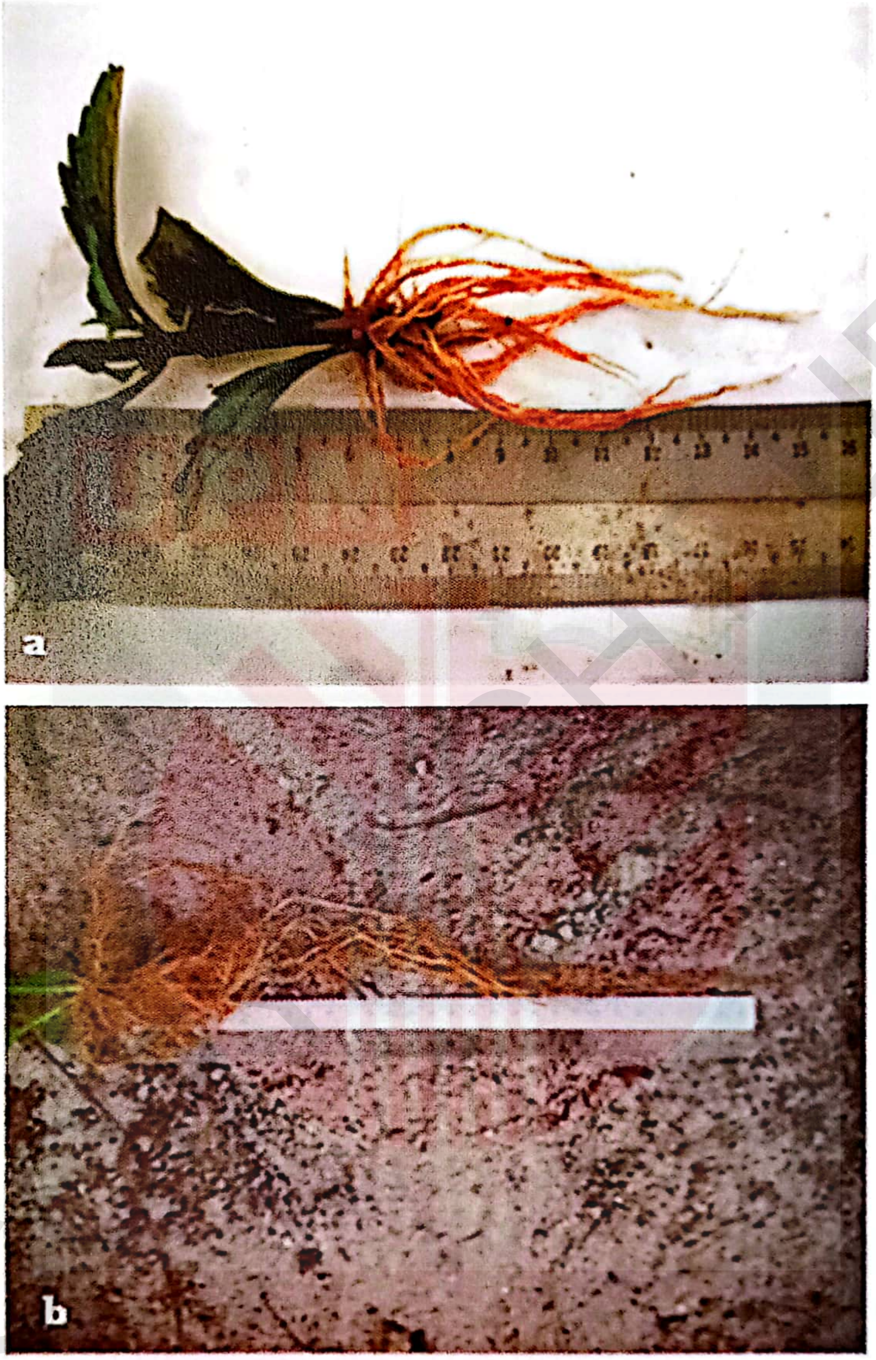

Plate 7: Root system development of *O. aristatus* cutting rooted in sand medium; (a) at first week assessment; (b) at fourth week assessment.

4.2 Rooting Response of *Orthosiphon aristatus* Cuttings Treated with IBA in Water Medium

4.2.1 Effect of IBA treatment on length of the longest root of cuttings

In the first week of rooting, the length of the longest root of *O. aristatus* cuttings treated with all levels of IBA treatments did not show any significant difference from that of untreated cuttings as shown in Table 3. However, at two weeks of rooting, cuttings treated with 500 ppm of IBA treatment produced the longest root with mean length of 9.17 cm long which was significantly longer than other levels of IBA treatments but this was not significantly longer than the length of the longest root of untreated cuttings which was 9.40 cm long.

All cuttings treated with 500 ppm of IBA consistently produced longer roots which at the third week of rooting was 13.57 cm long, and 17.37 cm long at the fourth week of rooting both of which were significantly longer than cuttings treated with other IBA treatments but again this was not significantly better than the longest root of untreated cuttings of 19.70 cm long.

Table 3: Effects of indole-3-butyric acid (IBA) treatments on length of the longest root of cuttings rooted in water medium.

IBA Concentration (ppm)	Mean length of the longest root (cm)			
	Week			
	1	2	3	4
0	2.17[a]	9.40[a]	14.80[a]	19.70[a]
500	3.00[a]	9.17[ab]	13.57[a]	17.37[ab]
1000	3.20[a]	8.03[b]	10.77[b]	13.47[c]
1500	2.93[a]	8.73[ab]	12.77[ab]	16.20[b]
2000	3.03[a]	8.03[b]	12.33[ab]	15.97[cb]

Note: Values within column with the same superscripts are not significantly different at P = 0.05 using the DNMRT.

4.2.2 Effects of IBA treatments on number of root per cutting

All cuttings treated with all four levels of IBA treatments of 500 ppm, 1000 ppm, 1500 pm and 2000 ppm, in the first week of rooting produced the most roots per cutting (Plate 8a), all of which were significantly more than the number of root produced by untreated cuttings but differences among all IBA treated cuttings was not statistically significant as shown in Table 4.

All cuttings treated with IBA treatments evaluated at week two, three and four of rooting consistently produced significantly more roots than untreated cuttings. However, differences among treatments of IBA with respect to the number of roots produced by IBA treated cuttings were observed at two weeks of rooting and continued into the last fourth week of rooting evaluation. It was observed that cuttings treated with 2000 ppm of IBA produced significantly more roots with mean of 39.28 roots per cutting in week two, 47.35 roots in week three, and 48.10 roots in week four (Plate 8b) and within each stage of evaluation each one significantly exceeded the number of root obtained from cuttings treated with other IBA treatments at 500 ppm, 1000 ppm, and 1500 ppm concentrations.

Table 4: Effects of indole-3-butyric acid (IBA) treatments on the mean number of root per cutting rooted in water medium.

IBA Concentration (ppm)	Mean number of root per cutting			
	Week			
	1	2	3	4
0	8.20^{b}	21.10^{c}	26.10^{c}	27.25^{c}
500	33.23^{a}	36.43^{ab}	38.72^{b}	39.27^{b}
1000	25.12^{a}	31.25^{b}	33.73^{b}	34.70^{bc}
1500	28.55^{a}	34.47^{ab}	41.12^{ab}	42.33^{ab}
2000	25.45^{a}	39.28^{a}	47.35^{a}	48.10^{a}

Note: Values within column with the same superscripts are not significantly different at P = 0.05 using the DNMRT.

Plate 8: Root system development of *O. aristatus* cuttings rooted in water medium; (a) at first week assessment; (b) at fourth week assessment.

4.3 Growth of Plants from Cuttings Rooted in Different Rooting Media

4.3.1 Growth of plants from cuttings rooted in sand medium

Shoot growth of *O. aristatus* cuttings between the first and fourth week is shown in Plate 9. In the first week of rooting only the initial leaves were present on cuttings. In the fourth week of rooting young shoots then emerged which produced new leaves as shown in Plate 9b.

4.3.2 Growth of plants from cuttings rooted in water medium

Shoot growth of *O. aristatus* cuttings rooted in water medium is shown in Plate 10. In the first week of rooting no shoot was produced (Plate 10a). However, at fourth week of rooting, a few young shoots began to develop new leaves (Plate 10b).

Plate 9: Shoot growth of *O. aristatus* cuttings on sand medium; (a) at first week assessment; (b) at fourth week assessment.

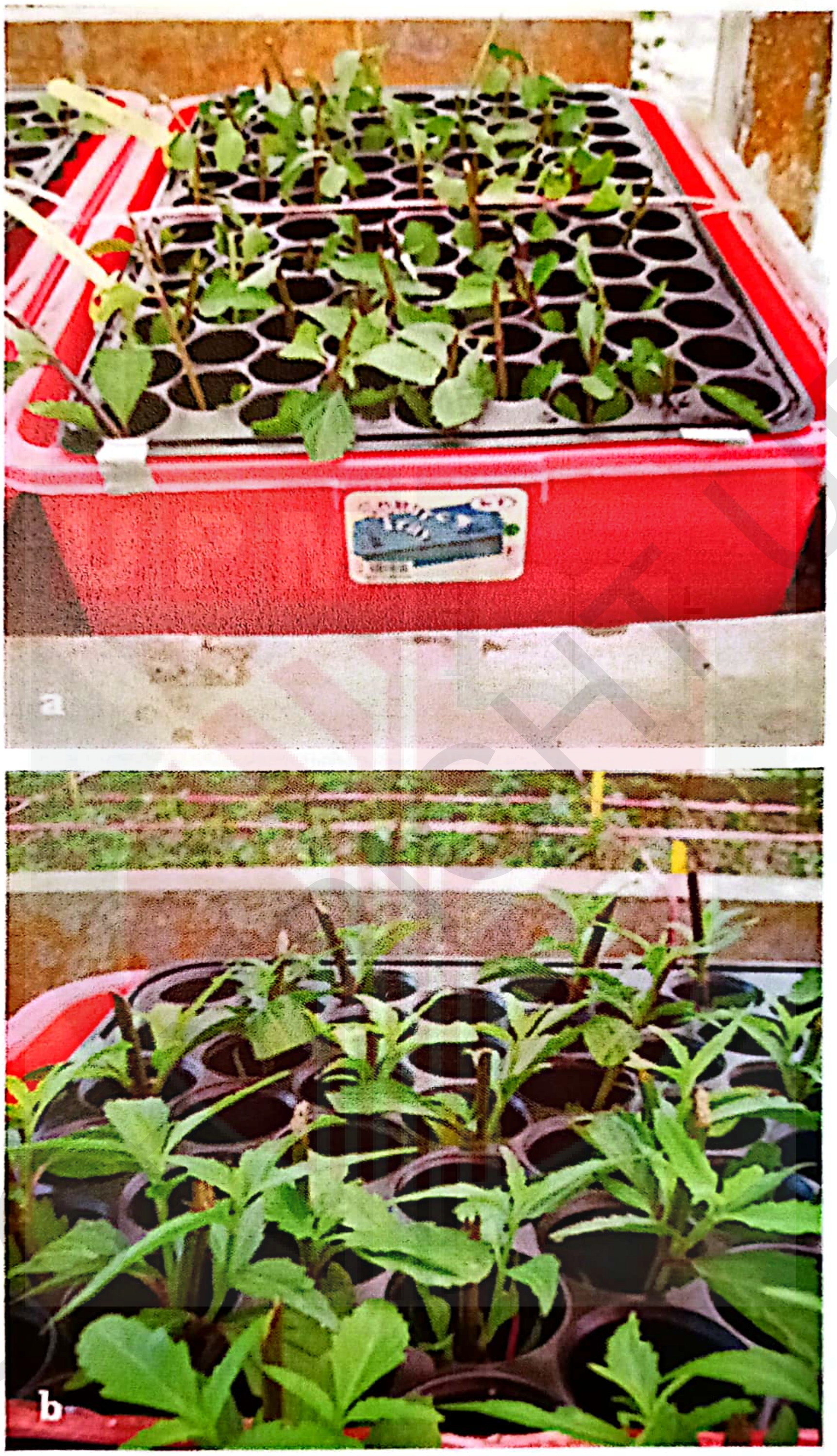

Plate 10: Shoot growth of *O. aristatus* cuttings on water medium (a) at first week assessment; (b) at fourth week assessment.

4.4 Survival of *Orthosiphon aristatus* Plants from Rooted Cuttings

After collection of data which was completed in a month, another observation on plant growth was conducted. Two months of growth performance was observed on plants from rooted cuttings transplanted into polyethylene bags with medium made up of top soil, sand and organic compost in 3:2:1 ratio. All transplanted cuttings of *O. aristatus* survived during the two months of plant growth assessment. Plants grown in polyethylene bags showed good growth producing two to four branches per plant with healthy leaves and well developed root system, Plate 11.

Plate 11: Growth of *O. aristatus* rooted cuttings grown in polyethylene bags in 3:2:1 medium mixture two months after transplanting with (a) well developed branches and shoots and (b) well developed root system.

CHAPTER 5

DISCUSSION

5.1 Comparison of Rooting Response among IBA Treatments

The induction of rooting using IBA treatments on cuttings of *Orthosiphon aristatus* in sand medium was clearly beneficial since all cuttings treated with IBA at all levels of treatment were significantly better than untreated cuttings. This result concurred with the finding of Tchoundjeu *et al.* (2002) and Ofori *et al.* (1996) in which they found the use of auxin had improved rooting of cuttings of many difficult to root species. In this study, the best rooting treatments for rooting of *O. aristatus* cuttings in sand medium was at 500 ppm IBA treatment in term of producing the longest roots. However, in term of the most number of roots produced by cuttings, the best treatments were at 1500 to 2000 ppm of IBA concentrations.

The use of water medium for rooting was not beneficial for rooting *O. aristatus* cuttings treated with IBA treatments in term of root length, as the result indicated all untreated cuttings rooted in water medium produced significantly longer roots than on cuttings at all levels of IBA treatments. The only explanation for this rooting response is in the use of water as rooting medium. Although in this case, cuttings were treated with various levels of IBA treatments, rooting the cuttings in the water medium could have reduced or even nullified the effect of IBA resulting in poorer rooting response than untreated cuttings in term of root length.

In previous study on *Podocarpus polystachyus*, cuttings treated with Indole-3-butyric acid also did not show significant different between control and treated cuttings which was attributed to differences between species (Jun, 2004). However, in term of the number of root produced by cuttings, IBA treatments at 1500 ppm and 2000 ppm of IBA concentrations have produced the most number of roots. In general, in term of the most number of roots produced per cuttings, the best treatments of IBA for rooting of *O. aristatus* cuttings were at 1500 ppm and 2000 ppm concentrations regardless of water or sand rooting medium used.

5.2 Comparison of Rooting Response among Rooting Media

Untreated cuttings of *O. aristatus* rooted in water medium had produced the longest root than cuttings treated with IBA treatments. However, cuttings rooted in sand medium treated with 500 ppm of IBA treatment produced significantly longer roots than other IBA treatments. At treatments of 1500 and 2000 ppm of IBA concentration, cuttings rooted in both sand and water media produced significantly more roots than other IBA treatments. It appears that in term of inducing longer roots from cuttings of *O. aristatus* plants, the use of water as rooting medium is of advantage. Cuttings of *O. aristatus* plants have been reported to root easily in water that requires consistent moist rooting environment for good growing conditions (Australian Plant Society, 2007).

Rooting of cuttings in water rooting medium by using 'bubble bath' method has been found effectives in *Anisoptera marginata, Shorea smithiana, S. laevis, S. leprosula, S. ovalis, S. blanco, and S pauciflora* stem cuttings (UNDP/FAO, 2004).

This method has been commercialized in planting programme at the Wanariset Research Station of the Indonesian Agency for Forestry and Research Development, Samboja, East Kalimantan.

5.3 Effects of IBA Concentrations on Rooting Response

Generally, auxin will stimulate root elongation in low concentration but inhibit at high concentration (Ridge and Emons, 2000). This is because the cell wall is loosening due to acidification of auxin, while at high concentration; auxin will induce ethylene production and inhibits root growth. Ethylene has the effects of either to induce or inhibit root formation of cuttings (Hartmann *et al.*, 2002).

In this experiment, low IBA concentration had induced root elongation but at higher IBA concentration more roots formation was induced on cuttings rooted in sand medium. The effects of IBA treatments on cuttings rooted in water medium result in negative effect with respect to the length of root produced but in term of the number of root, treatments with high IBA concentration had positive effect.

Some research had previously found that low concentrations of IBA treatment were more effective in promoting rooting. Tchoundjeu *et al.* (2002) stated that the application of auxin (IBA) promoted rooting of *Prunus africana* cuttings up to an optimum application of 100-200 μg IBA per cutting. However, different tree species vary considerably in auxin application in that the application of more than 0.2% IBA will decline rooting percentage on *Milicia excelca* cuttings (Ofori *et al.*, 1996).

It has been generally acknowledged, that auxin promotes adventitious root development of stem cutting through their ability to promote the initiation of lateral root primordia and to enhance transport of carbohydrates to the cutting base (Leakey *et al.*, 1982; Hartmann *et al.*, 2002). The application of auxin to attract assimilates to the cutting base and to stimulate merismatic differentiation is probably the best-known means of promoting rooting in all kinds of cuttings.

5.4 Effects of Rooting Medium on Rooting Response

The type of rooting medium used can have a major influence on the rooting ability of cuttings (Hartmann *et al.*, 2002). Since aeration and water holding capacity of the media are often negatively correlated, a balance between these must be achieved to ensure optimal rooting.

In this experiment, the use of water as a rooting medium gave significant result in root length without IBA applications while fine river sand rooting medium gave significant results in root length at 500 ppm of IBA concentration. Obvious differences in medium compaction, aeration and water availability are some of the causes of these results.

Cuttings are easier to root in water because it has less compaction and high water availability for root intake compared to sand medium. Besides, aeration is also readily available in water where as sand rooting medium has small particles and small pores providing it with lower aeration as compared to water rooting medium. However, sand medium is most often used for rooting as it is the most suitable rooting medium for many rooting applications. Study done by Aminah *et al.* (2004) stated that cleansed river sand medium was suitable for most species of Dipterocarparcea compared to coconut husk.

5.5 Other Factor Affecting Rooting of Cuttings

According to Darus and Aminah (1993) as cited from Girouard (1974), the success of rooting mainly depends on species, but there are also some factors such as physiological and environmental factors of cutting materials which can affect rooting success.

5.5.1 Diameter and length of cuttings

The diameter of cutting may have influenced the development of the number of roots on the *O. aristatus* cuttings since the diameter of cuttings used varied among the IBA treatments. Bigger diameter cuttings may have more carbohydrate reserved for root development compared to smaller diameter cuttings. According to Aminah *et al.* (1995) as cited from Mesen (1993), an increase in cutting diameter of *Cordia alliodora* had resulted in significant increased in the number of root.

The length of cuttings used in this experiment was two-node stem cutting method. With long internodes, the length of cuttings gets longer. Longer cuttings rooted better due to the influence of greater carbohydrate reserve and the presence of other essential substances for root formation (Hartmann *et al.*, 2002). However, the varied length of cuttings among the IBA treatments might have influenced some of the results obtained in this study.

5.5.2 Buds and leaves

The presence of buds and leaves can have positive relationship to the formation of roots on cuttings. In this experiment, some of the cuttings were already having buds during the preparation of cutting materials. This might have some effects on the results of the rooting efficiency in addition to the effects of IBA concentrations although each cutting was only having four leaves and each was trimmed by halves.

Buds and leaves have a role in auxin production and also nutritional effects. The leaves while supplying carbohydrates to the stem tissues are also involve in influencing root formation and development (Darus and Aminah, 1993). Trimming leaves can reduce water loss and initiate root formation and as such the presence of buds and leaves can increase root formation.

5.5.3 Environmental factors

Water loss due to high transpiration rate can cause death to cuttings. In this experiment, cuttings were planted under shelter house to maintain humidity to avoid heat stress on cuttings. Watering was also done twice a day on cuttings rooted in the sand rooting medium to ensure adequate moisture at all times.

Light and temperature are also important factors influencing root formation of stem cuttings. These factors could not have been the restraining factors since rooting of cuttings was conducted under shelter house where the temperature ranges between 28 to 32°C with 12 hours of day length which are considered very suitable for rooting of cuttings of tropical species (Darus and Aminah, 1993).

5.6 Growth Performance after Transplant

After four weeks of assessment, all rooted plants were transplanted into polyethylene bags with 3:2:1 medium mixture. Each plant were producing branches two to four with length ranging from 22 to 60 cm. There were some minor problems from attack of some insect pests which laid their eggs on the leaves and causing wrinkled leaves, see Plate 12.

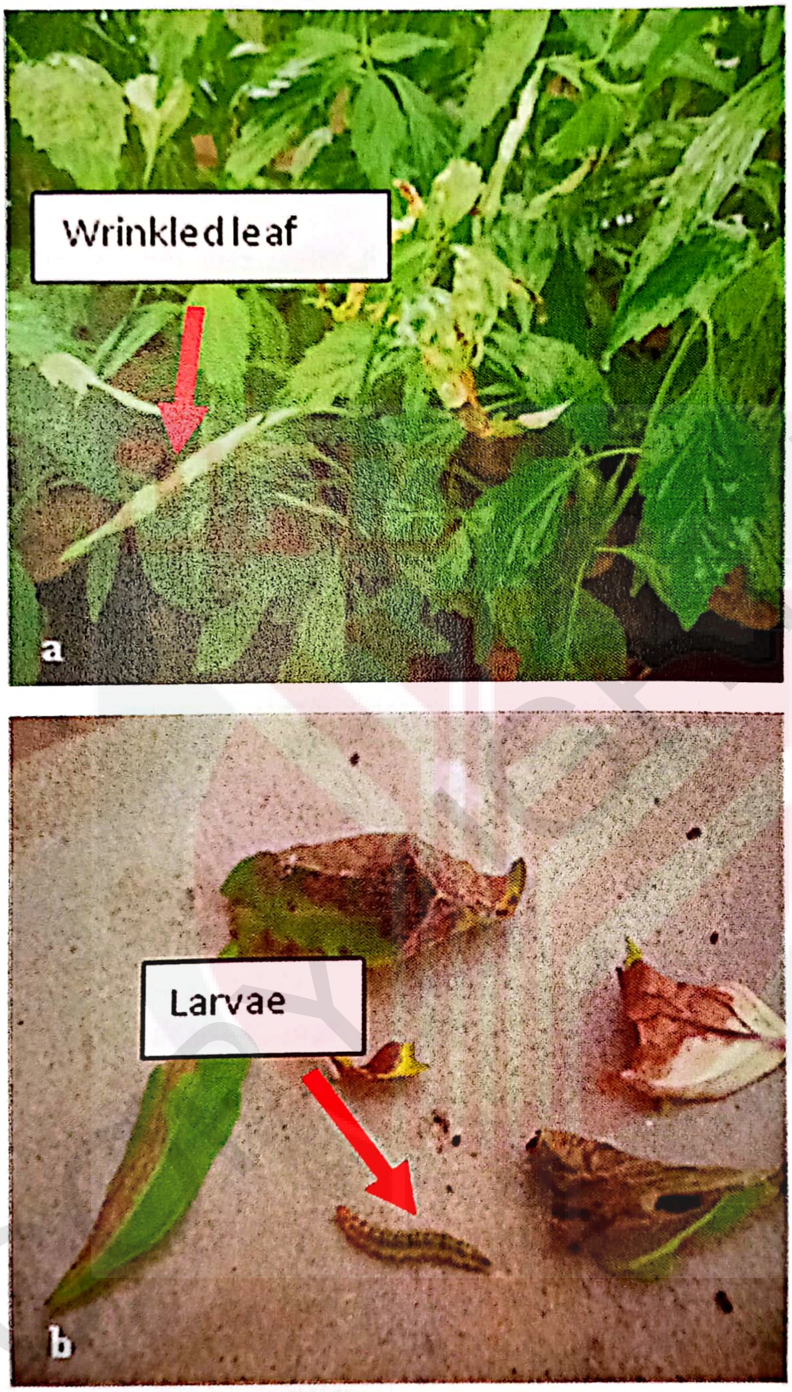

Plate 12: Infected plant; (a) wrinkled leaf cause by larvae; (b) larvae exist on leaves.

CHAPTER 6

CONCLUSION

Orthosiphon aristatus is a species that can root easily in either sand or water rooting medium. Cuttings that were rooted in water rooting medium did not need any treatment of indole-3-butyric acid (IBA) to produce longer roots, but with 2000 ppm of IBA treatment the cuttings of *O. aristatus* produced more roots compared to cutting treated at lower IBA concentration. When rooted in sand medium, cuttings treated with IBA at 500 ppm concentration produced longer roots where as at higher IBA treatment concentrations, treated cuttings produced more roots.

There was no mortality of plants grown from rooted cuttings and these plants showed high adaptation to the environment. In view that *O. aristatus* has high potential in term of health benefit, growing this valuable plant species on commercial scale can be undertaken since it can easily be mass produced through simple stem cuttings procedure to produce the required planting materials needed.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled **"Effects of Different Indole-3-Butyric Acid (IBA) and Rooting Media on Rooting Response of *Orthosiphon aristatus* Stem Cuttings"** by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agricultural and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the faculty.

Aminah bt. Arbai

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