# EFFECT OF ORGANIC ADDITIVES ON GROWTH AND DEVELOPMENT OF *Clinacanthus nutans IN VITRO*

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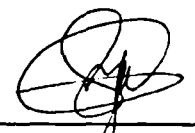

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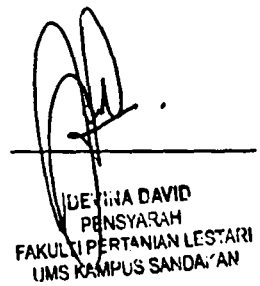

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**THE EFFECT OF ORGANIC ADDITIVES ON GROWTH AND DEVELOPMENT OF *Clinacanthus nutans IN VITRO*.**

## ABSTRACT

An experiment was conducted at the Faculty of Sustainable Agriculture in University Malaysia Sabah to investigate the effect of organic additives on growth and development of *Clinacanthus nutans*. The nodal segment of *C. nutans* was cultured on different MS medium supplemented with different types of organic additives. The media used were MS0 (control), MS + 10% (v/v) coconut water, MS + 10% (v/v) banana homogenate, MS + 10% (v/v) potato homogenate and MS + 10% (v/v) tomato juice. The experiment was designed in a completely randomized designed (CRD) and each of the treatment was replicated 8 times. Data was collected according to number of leaves, length of regenerated shoots and number of roots. After 26 days of culture, results showed that MS medium supplemented with 10% (v/v) of coconut water showed the highest number of leaves (8.13±0.30), and highest length of regenerated shoot (2.71±0.07cm). While, the number of roots produced was (0.75±0.71). The addition of 10% (v/v) potato homogenate showed the highest number (0.88±0.64) of roots. Thus, the presence of coconut water as organic additive in culture medium increase effectiveness of regeneration of nodal segment of *C. nutans*.

UoW ISA

**KESAN ADITIF ORGANIK KE ATAS TUMBESARAN DAN PERTUMBUHAN *Clinacanthus nutans* SECARA *IN VITRO*.**

## ABSTRAK

Satu eksperimen telah dijalankan di Fakulti Pertanian Lestari Universiti Malaysia Sabah untuk mengkaji kesan aditif organic ke atas tumbesaran dan pertumbuhan *Clinacanthus nutans* secara *in vitro.* Bahagian nodal *Clinacanthus nutans* dikultur ke medium MS dengan penambahan aditif organik yang berbeza-beza. Reka bentuk eksperimen yang digunakan ialah CRD di mana MS0 (kawalan), MS + 10% (v/v) air kelapa, MS + 10% (v/v) homogenat pisang, MS + 10% (v/v) homogenat kentang and MS + 10% (v/v) jus tomato. Setiap rawatan direplikasi sebanyak 8 kali. Data dikumpul berdasarkan bilangan daun, panjang pucuk dan bilangan akar. Pada hari ke 26 selepas pengkulturan, medium MS + 10% (v/v) air kelapa menunjukan bilangan daun yang tertinggi (8.13±0.30), panjang pucuk yang tertinggi (2.71±0.07cm). Penambahan 10% (v/v) homogenat kentang telah menunjukan bilangan akar yang tertinggi (0.88±0.64). Secara kesimpulannya, penambahan air kelapa sebagai aditif organic ke dalam medium kultur dapat meningkatkan kesan pertumbuhan semula bahagian nodal *Clinacanthus nutans*. Eksperimen ini boleh dicuba dengan menggunakan tumbuhan herba yang berlainan untuk membandingkan keputusan yang diperoleh dari kajian ini.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF SYMBOLS, UNITS, AND ABBREVIATIONS

| | |
|---|---|
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| ABA | Abscisic acid |
| ANOVA | Analysis of Variance |
| BA | 6-benzyladenine |
| cm | Centi metre |
| CRD | Completely randomized design |
| DAC | Days after culture |
| g | Gram |
| GA | Gibberellic acid |
| HCL | Hydrochloric acid |
| IAA | Indoleacetic acid |
| IBA | Indole-3-butyric acid |
| ml | Mili litre |
| MS | Murashige and Skoog medium |
| NAA | Napthaleneacetic acid |
| NaOH | Sodium hydroxide |
| SPSS | Statistical Package of Social Science |
| v/v | Volume per volume |
| Z | Zeatin |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

A few decades ago, there has been a discovery of the ability of the plant to synthesis secondary metabolites for medical purpose. These metabolites are used for defensive and biological functions in plants. Tapsell (2006) reported that the secondary metabolites produced by plants are able to improve human health and can also be used as pharmaceutical drugs. Edgar (2002) stated that based on World Health Organization (WHO), almost 80% of humankind still depends on herbal medicine for their basic health care.

The tropical rainforest in Malaysia has abundant of herbal plants due to the biological diversity (Yusof, 2002). However, Malaysia still lack of information about the variety of herbs due to few studies and researches conducted on herbs. Herbal plants are famous for medicinal purpose. For example, *Lavandula officinalis* is very useful in relieving pain, *Mentha piperita* helps in relaxing muscle stress. *Clinacanthus nutans* is famous for antioxidant, antiproliferation, antivenum, anti-inflammatory effects as well as containing a high amount of vitamin C (Yoke, 2013).

*Clinacanthus nutans* is a native medicinal plant mostly in South East Asian countries such as China, Indonesia, Thailand and Malaysia (Yuann, 2012). *Clinacanthus nutans* comes from family Acanthaceae. Usually, *C. nutans* known as Belalai Gajah, Dandang Gendis, Sha Ba She Cao and Sabah Snake Grass. According to Malaysia Herbal, *C. nutans* favours countries that have a tropical weather and have a hollow stem structure as well as thin and long leaves with average surface of 1.5 cm by 7 cm. The colour of *C. nutans* is green for seedling stage, and dark green for mature stage.

Due to the ability of *C. nutans* to cure diseases and high nutritional content, undoubtedly *C. nutans* is one of the herbs that need to be included in medicinal research (Yuann, 2012). Furthermore, China Vegetable Research Institute (2009) reported that *C. nutans* is a very good antioxidant and has a potential in curing cancer. Moreover, it is also effective against Hepatitis B, diabetes, bone fracture and food poisoning (Siang, 2012).

Plant tissue culture is a technique of in vitro plant propagation that been used in the investigation of totipotency and role of hormone during plant cell differentiation, as well as organogenesis (Lorraine, 2000). The study regarding plant tissue culture is very important because the applications of tissue culture technique brings a lot of benefits to mankind. By tissue culture, large number of clones from a single explant can be produced. Furthermore, tissue culture also provides rapid propagation and producing a plant that is free from diseases through careful stock selection and sterile techniques (Abdullah, 2012).

Based on a study by Siang (2012) on the investigation of bioactivities of *C. nutans,* showed that *C. nutans* have analgesic properties and anti-viral activities against various diseases. This is due to the composition of *C. nutans* containing secondary metabolites such as C-glycosyl flavones, sulphur - containing glucosides, glycoglycerolipids and monoacylmonogala ctosylglycerol. Malaysia has been using *C. nutans* as an alternative health care for cancer patients.

## 1.2 Justification

The demand for medicinal plant is increasing and might reduce the sustainable supply of medicinal herbs in the future. In managing a sustainable production of medicinal plant, avoiding over-collecting, unsustainable planting practices and pollution are very important. Therefore, the application of plant tissue culture techniques is needed in mass producing *C. nutans* for better propagation and production of valuable secondary metabolites. In addition, this study will provide new information and understanding about the best culture medium for micropropagating *C. nutans.* Moreover, there is only little research that has been done regarding this study. The ability of tissue culture technique to produce *C. nutans* in a large scale, provide an advantage for *C. nutans* to be commercialized. In this study, growth and development of nodal segment of *C. nutans* will be investigated in a period of one month by using a basic MS medium supplemented with different organic additive composition. The suitable composition of media for the growth and development of *C. nutans* will show a responds on length of regenerated shoots, number of leaves and number of roots. Hence, this study was conducted to study the effect of organic additives and to investigate the necessity of medium and nutrient that is suitable for growth and development of *C. nutans* via tissue culture.

## 1.3 Objective

To study the effect of organic additives on growth and development of *C. nutans* via tissue culture.

## 1.4 Hypothesis

Ho : There is no significance difference between the addition of organic additives on growth and development of *C. nutans* via tissue culture.

$H_A$ : There is a significance difference between the addition of organic additives on growth and development of *C. nutans* via tissue culture.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Acanthaceae

*Clinacanthus nutans* comes from the family Acanthaceae, which is native to tropical Asia countries. Usually it is called as Sabah snake plant or Belalai Gajah. *Clinacanthus nutans* stems are terete, striate and glabrescent as well as 1-3 metre high with pubescent branches. The leaves are opposite and lanceolate with a length of 2.5-13cm and wide by 0.5-4cm. *Clinacanthus nutans* has various synonyms, they are *Clinacanthus angustus*, *Clinacanthus burmanni*, *Clinacanthus spirei*, *Clinacanthus siamensis* (Nees, 2012). These four species of *Clinacanthus* are used traditionally as herbs in Southeast Asia and China and able to grow in nearly every habitat such as valleys, scrublands, and wet fields. This family mostly consists of tropical herbs, shrubs or twining vines and some are epiphytes (Sangeetha, 2013).

Table 2.1 Shows the taxonomic classification and nomenclature of *Clinacanthus nutans.*

Table 2.1 Scientific classification of *Clinacanthus nutans* or Sabah snake grass.

| | |
|---|---|
| **Kingdom** | Plantae |
| **Phylum** | Magnoliophyta |
| **Class** | Magnoliopsida |
| **Subclass** | Asteridae |
| **Order** | Lamiales |
| **Family** | Acanthaceae |
| **Genus** | Clinacanthus |
| **Species** | nutans |
| **Scientific name** | *Clinacanthus nutans* |

Source: Gunasekaran, 2014.

Figure 2.1 The leaves of *Clinacanthus nutans*

## 2.2 Uses of *Clinacanthus nutans*

*Clinacanthus nutans* is a type of medicinal plant that provides medicinal uses and products. There are a variety of medicinal products that have been produced based on *C. nutans* such as medicinal cream, drug, lotion, tea, and secondary metabolite products. Furthermore, according to Remedy, (2013) the extracted dried leaves of *C. nutans* help to serve as medicine in relieving pain and healing herpes infection.

*Clinacanthus nutans,* also known as Sabah snake grass is an alternative treatment and cure for cancer using natural therapies. Traditionally it was used as an herb to cure snakebite. It is believed that it able to neutralize the poison from the snake. Recently, it is well known for its healing properties such as the cure for cancer and kidney failure alleviation. According to Ong (2013), *C. nutans* reduces hyperlipidemia, high cholesterol and high blood pressure. Furthermore, based on a research done by experts from Academy of Agricultural Science China, they found out that *C. nutans* contain flavanoids which is quite effective towards cancer (Katrin, 2014).

In Malaysia, *C. nutans* are broadly used to treat uric acid, gout, urinates neuropathies, liver cancer, kidney syndrome, nasal cavity cancer and uterine fibroid. This plant has been certified for treatment of herpes simplex, herpes zoster and skin psoriasis in the Primary Health Care Programme (Prakash *et al.*, 2014). *Clinacanthus nutans* acts as an anti-inflammatory which relieves major skin inflammation and rashes, and insect bites. Apart from these reports and preliminary studies, Malaysian species of *C. nutans* may give potential lead compounds in drug discovery.

Table 2.2 shows the recommended daily dosage of *C. nutans* leaves (mix with cool boiled water and the filtered juice will be drunk without residue once a day), that should be taken by a cancer patient with different level of cancer status.

Table 2.2 The recommended daily dosage of *C. nutans* leaves for cancer patient.

| Cancer Status | Number of Leaves | Weight |
|---|---|---|
| Stage 1 | 50 leaves | 10g |
| Stage 2 | 100 leaves | 20g |
| Stage 3 | 150 leaves | 30g |
| Stage 4 | 300 leaves | 60g |

Source: Ong, 2013.

## 2.3 Plant Tissue Culture

Plant tissue culture refers to growing and multiplying of cells, tissues and organs on defined solid or liquid media under aseptic and controlled environment. According to a research done by Trevor (2006), the theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt in his address to the German Academy of Science in 1902 on his experiments on the culture of single cells.

Through tissue culture methods, plant can be produced in large quantities and time required is short. Plant with high quality and free from any disease is one of the advantages of this technique and made it widely performed across the world. Various quality hybrids can be produced and preservation process for the endangered species could be done. Micro-propagation has superiority over conventional methods of propagation because of the high multiplication rate and disease free plants. Efficient plant regeneration protocol is essential for genetic manipulation of crop species (Samuel *et al.,* 2001).

## 2.4 Micropropagation of medicinal plant

Plants have been an important source of living for mankind and they depend on plant as food sources, flavours, medicinal and many other uses. The demand for a huge raw material of medicinal plant has been incline globally because plant medicine practices has been well established and very popular in many parts of the world. According to World Health Organization (WHO), 80% of peoples living around the globe is using medicinal plant as their primary health care (Stuart, 2010). Due to the high demand of for medicinal plants, plant tissue culture was done to accelerate clonal multiplication and high yielding of medicinal plant. *Nicotiana tabacum* was the first medicinal plant that being propagated through micropropagation, followed by *Daucus carota* (Chaturvedi, 2007). By that, species of some Acanthaceae families such as *Adhatoda beddomei* are vegetatively propagated using *in vitro* culture methods (Jitendriya, 2014).

In addition, medicinal herbs such as *Justicia gendarussa*, was also propagated via tissue culture by using nodal segment as explant and shoot multiplication were obtained by using MS medium supplemented with 1.0 mg/L BAP alone with 10% coconut milk (Sumathi, 2010). Furthermore, in vitro plant regeneration was done by using shoot tip of *Exacum travancoricum Beedi,* which is an endangered herb mainly known for ornamental purposes (Janarthanama, 2010). In addition, Vinod (2003) successfully developed a protocol in micropropagating a medicinal plant, *Leptadenia reticulate*.

There are four stages in micropropagation of most plants. Initially, the first stage is the establishment of axenic explants for producing aseptic culture, where contamination-free of selected explants need to be ensured because it will be used for shoot production stage. The next stage is the shoot multiplication stage, where shoots will be developed. This developed shoots will be separated and sub-cultured several times into a new media to enhance a better quality of shoot production. Then, followed by rooting stage, where root induction could be achieved *in vitro*. Finally, is acclimatization stage, where the cultured plant will be transferred to soil for better survival (Kenneth, 2015).

## 2.5 Factors affecting in plant tissue culture.

### 2.5.1 Explant selection

In tissue culture, explant selection is the main steps that need to be handled properly. A proper selection of explant is very important because the key to a successful micropropagation is by choosing the best explant. Gintonga *et al.* (2010) stated that the explant characteristics such as type source, genotype, and history affect the success and commercial viability of tissue culture systems.

Naphaporn *et al.* (2009) and Prerna (2011) was successful in propagating *Adhatoda vasica* via micropropagation using nodal segment as explant. Koilpillai (2010) also meets a success in the micropropagating nodal segment of *Graptophyllum pictum L.* by using MS media fortified with BAP and the addition of organic additive and have a high rate of survival after being transferred to soil for hardening.

### 2.5.2 Surface sterilization

Explant sterilization is the initial step in culture establishment. Surface sterilization aims to remove microorganism from the surface of the explants. The major contaminant *in vitro* culture is fungus and bacteria. In order to overcome these contaminants, antibiotics and fungicides are used as surface sterilants on and into the medium. Ethyl alcohol, mercuric chloride, chlorine water, bromine water, and other commercial bleaches are some example of surface sterilants that usually used in in vitro culture (Gunasekaran, 2014).

Hartmann (1975) stated that surface sterilized the explants in the series of steps involving a rinse in ethyl alcohol (45% w/r) followed by 10 minutes bleach treatment and finally rinsed in double distilled water (Gunasekaran, 2014). In *Elatteria cardamom* mercuric chloride gave better sterilization that sodium hyperchloride (Raghunath1989). In *Piper Nigrum* also mercuric chloride was found to be best surface disinfectant (Nazeem 1994).

Surface sterilants usually are toxic to explants. Hence, washing off the sterilants from the surface of explant after surface sterilization process is crucial. It is done by washing twice or thrice by using sterilized distilled water. Percentage of contamination can be reduced by pre-treatment of plants with the use of effective fungicides (Mc Grath, 2004).

### 2.5.3 Culture media

The media of plant tissue culture should generally contain macronutrients, micronutrients, vitamins, amino acids or nitrogen supplements, sources of carbon, undefined organic supplements, growth regulators, and solidifying agents. Tissue culture media were first developed from nutrient solutions used for culturing whole plant such as root culture medium of White and callus culture medium of Gautheret (Abobkar, 2009).

Basic media that are frequently used include Murashige and Skoog (MS) medium, Linsmaier and Skoog (LS) medium, Gamborg (B5) medium, and Nitsch and Nitsch (NN) medium (Ahmed, 2009). According to Kartha (2008) different media composition of potato meristem culture was tested and found that basal medium (Murashige and Skoog 1962) performed well than other culture media, in terms of meristem survival and development of shoots. The basal medium has been invariably used in meristem and shoot tip cultures.

Usually, all mediums of plant tissue culture contains sucrose as 1-3% as carbon source, glucose, sorbitol as well as fructose were used as a carbon source in various experiments (Bhagya, 2007).

### 2.5.4 Organic additives

The use of organic additives on plant tissue culture medium helps to reduce the cost of using plant growth regulators. Many undefined supplements such as organic additives were employed in early tissue culture media and now it is a common practice to improve the growth of in vitro (Norhayati, 2011). Organic additives act as a natural source that provide minerals, sucrose, vitamins, and amino acids (Shivani *et al*, 2009).

### a) Coconut Water

According to Steward, 1948, coconut water was used as organic additives at the rate of concentration of 10% to 20% (v/v) (Ying, 2013). In 1941, Van Overbeck introduced the usage of coconut water in culture medium. Young coconut water was supplemented in the media culture. The use of young coconut water acts as a plant growth regulator that gives a better response on plant tissue culture. According to George (2001), coconut water is composed of many amino acids, nitrogenous compounds, inorganic compounds, organic acids, carbon sources, vitamins and growth regulators such as cytokinin and auxin.

At a low concentration, coconut water is a natural source that acts as a growth regulator which plays an important role in the development of the plant physiology (Davies *et al.*, 1997). Liya (2005) stated that young coconut water contains Kinetin which are significantly important in regulating the growth and development of plant tissue culture. According to an analysis done by Zhen *et al.* (2008), there are four classes of phytohormone in coconut water, which are Indoleacetic acid (IAA), Indole-3-butyric acid (IBA), Abscisic acid (ABA), Gibberellic acid (GA), Zeatin (Z), 6-benzyladenine (BA), Napthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D). Based on previous studies on micropropagation of *Adhatoda vasica,* the combination of 15% coconut water (v/v) and BAP helps to induce a high number of shoot development (Sangeeta, 2013; Peixe *et al.,* 2007).

### b) Potato Homogenate

Potato is an important source of vitamins such as vitamins C, vitamins B1, B3, and B6. In addition, potato also rich in minerals such as phosphorus, calcium, magnesium, manganese, iron, and zinc. Potatoes are also known as sources of antioxidant compounds, including polyphenols, carotenoids and vitamins, pointing to their relevance not only as a starchy food, but also as a vegetable (Hannah, 2011). Bin *et al.* (2007) reported that MS media supplemented with 10% potato homogenate (v/v) for micropropagation of *Saussurea involucrate* can achieve high root induction.

## REFERENCES


Abdullah, S. 2012. Somatic Embryogenesis and regeneration of *Polianthes tuberosa* L. *Institute of Biological Sciences* **12(9)**:32-37

Abdul Karim, A.G. and Hairani, H. 2000. Leaf Culture of Some Malaysian Orchids In: Proc. International Conference and Exhibition on Orchids and Ornamental Plants. *Orchid Society Journal* **40(1)**:12-17

Abobkar, I.M. 2009. Plant Tissue Culture Media. *Intech Agricultural Journal* **2(2)**:31-39

Ahmed, M. 2009. Plant Tissue Culture Media and Storing. *Intechnology Agricultural Journal* **4(3)**:53-61

Andre, J. 2007. Evaluation of Gelrite and Numerous Agar Sources for *In Vitro* Regeneration of Sugarcane. *Horticultural Science* **23(2)**:755

Barwick, S. A., Wolcott, M. L., Johnston, D. J., Burrow, H. M. and Sullivan, M. T. 2009. Genetics of Steer Daily and Residual Feed Intake in Two Tropical Beef Genotypes, and Relationships Among Intake, Body Composition, Growth and Other Post-Weaning Measures. *Animal Production Science* **49(6)**: 351-366

Bhagyalakshmi, N., Singh, N.S. 1988. Meristem Culture and Micropropagation of Variety of Ginger (*Zingiber officinale Rose*) with a high yield of Oleoresin. *Journal of Horticultural Science* **63(2)**:321-327

Bin, A.N., Bilton, R. 2007. Development Variation In Plant Tissue Culture. *Environment Botany Journal* **21(3)**:325-332

Bruce, W.R. 2011. Mitochondrial Function and Toxicity: Role of B Vitamins on The One-Carbon Transfer Pathways. *Chem. Biol. Interact* **163(3)**:113–132

Birch, R., Gartland, K.M.A, Gartland, J.S., Bracier, C.M., Fenning, T.M. 2001. Ri-Plasmid Mediated Transformation and Regeneration of *Ulmus procera. Plant Growth Regulator* **30(3)**:513-586

Chaturvedi, A. 2007. The Treatise on India Medicinal Plants. *NISCOM Journal of New Delhi* **3(4)**:109

Daud Ahmad, Dahleen, L.S. and Bregitzer, P. 2011. An Improved Media System for Higher Regeneration Rates From Barley Immature Embryo Derived Callus Cultures of Commercial Cultivars. *Crop Science* **42(2)**:934–938

David, S.B. 2014. The Carbohydrate Nutrition of Tomato Roots. The Promotion and Inhibition of Excised Root Growth by Various Sugars and Sugar Alcohols. *Botany Journal* **22(3)**:513-524.

Davies, F.T., Kester, D.E., Geneve, R.T., Hartmann, H.J. 1997. Plant Propagation Principle and Practices. *Journal Horticultural Science* **6(4)**:48-53

Edgar, J.D., Elias, B. 2002. Biotechnology and The Developing World. *Electronic Journal of Biotechnolgy* **5(1)**:25-29

Faria, J.D., Street, H.E. 2004. The Carbohydrate Nutrition of Tomato Roots. V. The Promotion and Inhibition of Excised Root Growth by Various Sugars and Sugar Alcohols. *Botany Journal* **22(4)**:513-524

George, F.F. 2001. Plant Propagation by Tissue Culture. *Directory of Commercial laboratories* **34(1)**:201-233

Gitonga, L.N., Gichuki, S.T., Ngamau, K., Muigai, A.W.T., Kahangi, E.M., Wasilwa, L.A., Wepukhulu S., Njogu, N. 2010. Effect of Explant Type, Source and Genotype on *In Vitro* Shoot Regeneration in *Macadamia. Journal of Agricultural Biotechnology and Sustainable Development* **2(7)**:129-135

Globinmed. 2010. Medicinal Herbs and Plants Malaysian Herbal Monograph: 118

Gunasekaran, U. 2014. Callus Induction and Plant Regeneration Studies of *Clinacanthus nutans* (Sabah Snake Grass). *Academia Journal* **21(4)**:1-50

Hannah, S. 2011. Metabolism of Myoinositol and Growth In Various Sugars of Suspension Cultured Cells. *Planta Science Journal* **141(4)**:77-82

Janarthama, B., Sumathi, L. 2010. Rapid and Effect Plant Regeneration From Nodal Explant of *Exacum travancoricum Beedi. Plant Tissue Culture and Biotech Journal* **22(1)**:33-39

Jitendriya, P., Vaishili, D.R., Illa, C.P. 2014. Multiple Shoot Regeneration of *Polianthes tuberosa Cultivars Phulerajni* and *Calcutta* double. *Journal of Biotechnology* **12(3)**:23-43

Josh, K. 2010. The Effect of Medium on Growth and Development of *Bulbophyllum lobbii* Orchid Seedlings *In Vitro*. Bachelor of Agricultural Science Dissertation. Universiti Malaysia Sabah.

Kartha, K.K. 2008. Morphogenetic Investigation of *In Vitro* Leaf Culture of Tomato *(Lycopersicon esculentim Mill strarfire)* and a High Frequency of Plant Regeneration. *Agricultural Biotech Journal* **77(1)**:292-301

Katrin, K.M. 2013. Morphogenetic Investigation of *In Vitro* Leaf Culture of Tomato and a High Frequency of Plant Regenaration *z. planzenphys* **77(2)**:292-301

Kenneth, C.T. 2015. Tissue Culture Techniques for Horticultural Crops: Stages of Micropropagation. *International Malaysia Journal* **15(2)**:13-16

Ket, C.Y., Lee, J.H., Bang, D. and Lee, K.H. 2004. Searching for Aging-Related Proteins In Human Dermal Microvascular Endothelial Cells Treated With Anti-Aging Agents. *Proteomics Journal* **6(2)**:1351–1361

Koilpillai, Y.J., Wilson. Y. 2010. *In Vitro* Propagation of *Graptophyllum pictum* A Medicinal Plant. *Journal of Pharmacy Research* **3(9)**:2201

Liya, D.S. 2005. A Compound In Coconut Milk Which Actively Promoted Radish Cotyledon Expansion, but Exhibited Negligible Activity In Tissue Culture Bioassays for Cytokinins. *Plant Science Journal* **26(3)**:241-249

Manawadu, R. 2014. Osmolarity as A Measure of Growth of Plant Cells In Suspension Cultures. *Microb. Technology* **17(2)**:989-991

Mc Grath, B.H. 2004. Commercially Feasible Micropropagation of Mountain *Laural Kalmia latifolia* by Use of Shoot Tip Culture. *International Sciences* **25(3)**:129-135

Mugwe, J., Mugendi, D., Kungu, J. and Muna, M. -M. 2009. Maize Yields Response to Application of Organic and Inorganic Input Under On-station and On-farm Experiments in Central Kenya. *Experimental Agriculture* **45(1)**: 47-59

Murashige, T. and Skoog, F. 1962. A Revised Medium For Rapid Growth and Bioassay With Tobacco Tissue Cultured. *Physiol Plant* **15(2)**:473-497

Murdock, K. 2005. RNA Polymerase From Eukaryotic Cells. Isolation and Purification of Enzymes and Factors From Chromatin of Coconut Nuclei. *Biochemical Journal* **25(4)**:463-470

Naphaporn, U.N., Kantamath, K. 2009. Micropropagation from Cultured Nodal Explants of Rose. *Biology Journal* **31(6)**:583-586

Navarre, S. 2009. The Morphogenic Effects of Various Physical Factors on The Gemmae of *Lunaria*. *Phytomorph Journal* **5(1)**:99-109

Nazeem, P.A. 1994. *In Vitro* Clonning of Black Pepper (*Piper nigrum*) of Plantation Crops. *Biotech Journal* **20(1)**:257-259

Ness, Y.K. 2012. Acute Oral Toxicity Study of *Clinacanthus nutans* In Mice. *International Journal of Pharmaceutical Sciences and Research* **3(11)**:4202

Norhayati, A. 2011. Two new montane species of Acanthosaura Gray, 1831 (Squamata: *Agamidae*) from Peninsular Malaysia Zootaxa. *Agricultural Science Journal* **14(1)**:28-46

Ong, K.M. 2013. Sabah Snake Grass Treating Cancer. *Diet for Cancer Patient Journal* **10(2)**:466

Peixe, P.A., Van der harst, B. and Van der meys, Q.G. 2007. Seed Germination and Further Development of Plantlets of *Paphiopedilum Ciliolare Pfitz In Vitro*. *Science Horticulture* **34(12)**:139-153

Plante, A. F., Six, J., Paul, E. A. and Conant, R. T. 2009. Does Physical Protection of Soil Organic Matter Attenuate Temperature Sensitivity. *Soil Science Society of America Journal* **73(2)**: 1168–1172

Prakash, E., Khan, P.S., Sairam, R.P. and Rao, K.R. 2014. Regeneration of Plant From Seed Derived Callus of *Hybanthus enneaspermus* a Rare Ethobotanical Herb. *Plant Cell* **18(1)**:873-878

Prerna, A.S. 2011. Micropropagation From Cultured Nodal Explants *Rosa hybrida. Genomics and Bioinformatics Journal* **18(2)**:19-33

Ragunath, B.R. 1989. *In Vitro* Studies on The Propagation of Cardamom. *Kerala Agricultural Journal* **22(6)**:24-66

Remedy, K.S. 2013. Herbs Tretments Towards Diseases. *Remedy Herbal Medication* **1(2)**:455-458

Samuel, M.A., Salt, J.N., Shiu, S.H. and Goring, D.R. 2001. Multifunctional Arm Repeat Domains in Plants. *International Review of Cytology* **253(2)**:1-26

Sangeetha, S. 2013. In Vitro Screening of Cytotoxic, Antimicrobial and Antioxidant Activities of *Clinacanthus nutans* Leaf Extracts. *Tropical Journal of Pharmaceutical Research* **13(9)**:1455-1461

Shah, M.I., Jabeen, M., Ilahi. 2003. *In Vitro* Callus Induction, Proliferation and Regeneration in Seed Explants of Wheat. *Pakistan Journal of Botany* **35(2)**:209-217

Shivani, R.D., Paszkowski, J. and Potrykus, I. 2009. Agarose Plating and a Bead Type Culture Technique Enable and Stimulate Development of Protoplast-derived Colonies in a Number of Plant Species. *Plant Cell Rep* **2(1)**:244-247

Siang, Y.S. 2012. Prespective and Insight on *Clinacanthus nutans* In Traditional Medicine. *International Intergrative Biology* **10(3)**:11-18

Sinha, N. 2009. *In Vitro* Propagation of *Coleus forskohlii*. A Threatened Medicinal Plant. *Plant Cell* **10(8)**:67-70

Stuart, A.G. 2010. Use of Medicinal Plants in Monterrey, Mexico. *Mexico: Department Of Agriculture* **23(1)**:3-25

Sumathi, E. 2010. Rapid and Effect Plant Regeneration From Nodal Explant of *Artemisia annua. Plant Tissue Culture and Biotech Journal* **22(1)**:33-39

Tapsell, L.C., Varga, K. 2006. Health Benefits of Herbs and Spices: The Past, The Present, The Future. *The Medical Journal of Australia* **185(4)**:4-24

Thomas, T.D., Massena, E.A. 2006. Callus Induction and Plant Regeneration *Cardiospermum halicacabum* L. Important Medicinal Plant. *Scientia Horticulturae* **108(3)**:332-336

Thulasi, T.A. 2014. Physiological and Biochemical Aspects of Organogenesis *In Vitro*. *Fujiwara Journal* **43(23)**:121-124

Trevor, T.A. 2006. The Current Status of Plant Tissue Culture, in Plant Tissueculture: Applications and Limitations. *Elsevier Journal of Amsterdam* **1(1)**:1-33

Vinod, A. 2003. Micropropagation of *Leptadenia reticulate* A Medical Plant. *In Vitro Biology Journal* **15(1)**:15-18

Van overbeck, J. 1941. Factors In Coconut Milk Essential for Growth and Development of Very Young Datura Embryos. *Science Journal* **94(2)**:350-351

Vince, J. 2011. Micropropagation of Pear, *Pyrus Communis*, In A Double-Phase Culture Medium. *Acta Hortic Journal* **212(4)**:117-124

Wassmann, R., Jagadish, S. V. K., Heuer, S., Ismail, A., Redona, E., Serraj, R., Singh, R. K., Howell, G., Pathak, H. and Sumfleth, K. 2009. Climate Change Affecting Rice Production: The Physiological and Agronomic Basis for Possible Adaptation Strategies. *Advances in Agronomy* **101**: 59-122

Wood, H.N. 2011. Studies on The Regulation of Certain Essential Biosynthetic Systems In Normal and Crown-Gall Tumor Cells. *Proc Natl Acad Sci* **47(4)**:1907-1913

Ying, L.N. 2013. Establishment of Axenic Explants and Callus Culture of *Clinacanthus nutans*. *Resource Science and Technology Journal* **23(3)**:1-23

Yoke, K.Y. 2013. *Clinacanthus nutans* Extracts Are Antioxidant With Antiproliferative Effect on Cultured Human Cancer Cell Lines. *Complementary and Alternative Medicine* **1**:1-8

Yuann, J.P. 2012. Effects of *Clinacanthus nutans* Leaf Extracts on Protection of Plasmid DNA From Riboflavin Photoreaction. *Health Technology and Biotechnology* **4(1)**:45-56

Yusof Zaman. 2002. Potential of Developing Cosmetics and Cosmeceutical Products From Medicinal Plant. *The Aromatic Plant* **10**:1-17

Zoltan, J.A. 2011. A Comparison of Plant Cell Division Inducers From Coconut Milk and Apple Fruitlets. *Nature Journal* **200(4)**:679-680

Zhen, B., Stoltz, L.P. and Snyder, J.C. 2008. In Vitro Proliferation and *In Vivo* Establishment of *Euphorbia Fulgens*. *Hort Science* **21(1)**:859