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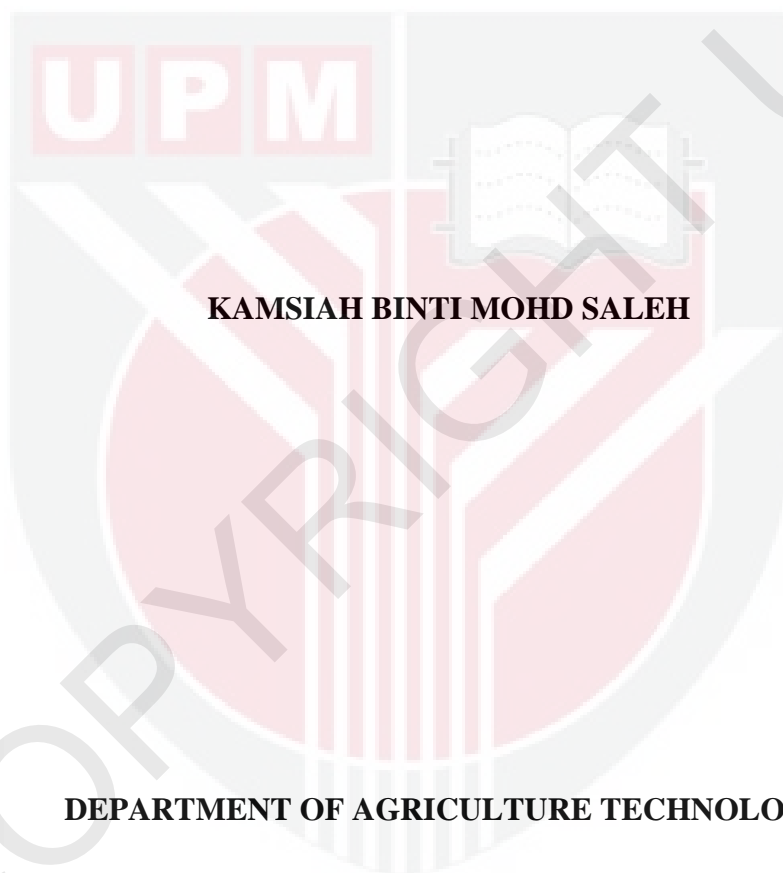
**ESTABLISHMENT OF STERILIZATION PROCEDURES IN IN VITRO
CULTURE FOR NODE OF DUKUNG ANAK (*Phyllanthus niruri* L.)**

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By

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
Cm	Centimetre
g	Gram
g/L	Gram per liter
SGM	Seed germination medium
HSD	Honestly Significant Difference (Tukey's)
Kg	Kilo
m	Meter
mg/L	Milligram per liter
mL	Milliliter
MS	Murashige and Skoog
MSO	Non-hormone Murashige and Skoog
NaOCl	Sodium hypochlorite
RCBD	Randomized Complete Block Design
SAS	Statistical Analysis System
pH	Hydrogen ion concentration/ $-\log(H^+)$
PPM TM	Plant Preservative Mixture
v/v	Volume per volume
°C	Degree centigrade
<i>et al.</i>	Et alia
%	Percentage

ABSTRACT

An experiment was conducted to determine the best sterilization procedure for *in vitro* culture of dukung anak (*Phyllanthus niruri* Linn) using node explant. Different concentrations of Clorox at different immersion time were used to optimise the sterilization procedure. Using 15% (2X) concentration of Clorox with 10 minutes immersion time a decrease in percentage of contamination was observed compared to the other treatment. In another experiment the best sterilisation treatment from previous experiment was used to sterilise *Phyllanthus niruri* node explant. After sterilisation this explant was inoculated in MS medium supplemented with different plant preservative mixture (PPM) concentration. Addition of 2 ml/L PPM into the MS media resulted in a high survival percentage (75%) of node explant while reducing the fungal contamination to 6.7%. Despite that, the contamination percentage by bacteria still exists. Further studies need to be carried out to obtain 100% contamination free explants while increasing its survival percentage.

ABSTRAK

Kajian telah dijalankan bagi menentukan teknik pensterilan yang terbaik untuk pengkulturan eksplan buku dukung anak (*Phyllanthus niruri* Linn.) secara *in vitro*. Proses pensterilan buku ini menggunakan pelbagai paras kepekatan larutan Clorox serta jangka masa rendaman yang berbeza bagi mendapatkan kaedah pensterilan yang terbaik. Larutan Clorox pada kepekatan 15% (2X) dan masa rendaman 10 minit menunjukkan sedikit pengurangan tahap pencemaran berbanding rawatan lain. Pada kajian seterusnya, rawatan agen pensterilan yang terbaik daripada kajian sebelumnya digunakan untuk mensteril eksplan buku *Phyllanthus niruri*. Selepas rawatan pensterilan, eksplan diinokulasi didalam media MS yang mengandungi PPMTM pada paras kepekatan yang berbeza. Penggunaan 2ml/l rawatan PPMTM menyebabkan peratus eksplan hidup meningkat (75%) dan pengurangan pencemaran kulat iaitu hanya 6.7%. Walaubagaimanapun, pencemaran bakteria masih wujud. Kajian selanjutnya perlu dijalankan bagi mendapatkan 100% bebas dari semua pencemaran dan tanpa sebarang kematian pada ekplan.

CHAPTER 1

INTRODUCTION

1.1 General introduction

Dukung anak or its scientific name *Phyllanthus niruri* belongs to the Euphorbiaceae family. It is an annual herbaceous plant grown widespread in place with temperate and tropical climates. *Phyllanthus niruri* is one of the most important herbs because it has been found to be useful for alternative treatment of diseases (Lizuka *et al.* 2006). *P. niruri* have small leaves, arranged in two rows and phyllanthoid branching with leaves. It can grow up to 60 cm tall. It has male and female flower which are pale green in colour. The fruits are green when unripe and turned red and dehisced upon maturity (Bagalkotkar *et al.*, 2006).

In Malaysia, this species is frequently used as traditional medicine to treat diarrhoea, kidney disorder, gonorrhoea and cough. Under natural condition, this herb is considered as weed but well adapted and capable of growing almost everywhere including along the roadside or grows with other plants in pot.

Currently, many scientific research has been carried out on dukung anak due to its ability to treat and cure hepatitis B virus (Wang *et al.* 1995). Based on World Health Organization, this virus caused a major global health problem and its infection can attacks the liver and caused chronic disease. More than 780 000 people die every year due to the consequences of hepatitis B virus. Locally the demand for these plants is expected to increase significantly as it is being used in commercial production of herbal preparation for treating hepatitis B.

Currently, dukung anak is one of 5 herbs focussed under the Malaysian National Key Economic Areas (NKEA) to be developed. Malaysia's herbal industry has identified this plant as one of the agriculture Entry Point Projects (EPPS) under the NKEAs in Malaysia Economic Transformation Programme (ETP). It is targeted that a gross income of RM3.25bil be achieved by 2020 from this high value herbal production (Ministry of Agriculture, 2011). The current market price of dry dukung anak is RM 75/kg (personal communication). Therefore, it is a good potential for developing *in vitro* propagation technique of dukung anak for large scale cultivation in Malaysia

Currently not much has been reported on the success of *in vitro* culture of *Phyllanthus niruri*. Therefore this experiment was conducted with the objective to establish a sterilisation procedure for *in vitro* node culture of dukung anak (*Phyllanthus niruri*) with emphasis on determining the Clorox concentration and immersion period of the explants in the Clorox solution to reduce microbial contamination. Another experiment was conducted using the best sterilisation protocol from previous experiments and the addition of different concentration of PPM in culture medium to further reduce the microbial contamination and increasing explants survivability.

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