EFFECTS OF GREEN SILVER NANOPARTICLES ON BACTERIAL-INFECTED *In Vitro Eucalyptus pellita* F.Muell PLANT

By

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Chairman : Associate Professor Siti Aqlima binti Ahmad, PhD
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Nanotechnology has emerged as a vibrant field of research in several modern scientific and engineering fields. The application and development of silver nanoparticles (AgNPs) have remarkably increased due to their distinctive features, including size (1-100 nm), shape, and antimicrobial properties. Diverse biomolecules and biological entities (prokaryotes and eukaryotes) are widely used as bio-stocks to create nanostructured materials. Green AgNPs provide non-toxic and sustainable materials to be used as antimicrobial agents. The growing demand for *Eucalyptus* species for industrial applications requires an efficient propagation strategy for rapidly and genetically producing improved trees. However, various microbial pathogens affecting the growth of *Eucalyptus*, which are considered the main concerns since they can cause long-term damage to *Eucalyptus* plantation. This study examined the effect of green AgNPs on the pathogen-infected *in vitro Eucalyptus pellita (E. pellita)* plants. Shoot regenerated *in vitro* from cotyledonary leaf explants of *E. pellita* were induced through direct organogenesis using (Murashige and Skoog) MS media supplemented with different concentrations (0.4, 1.3, 2.2, 3.1, 3.9 µM) of thidiazuron (TDZ), 6-benzylaminopurine (BAP) and kinetin (KIN). The elongated shoots were rooted *in vitro* using ½ MS media supplemented with different concentrations (0.5, 1, 1.5, 2, 2.5 µM) of indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (1-NAA), respectively.The highest shoot formation from cotyledonary leaves was observed in the MS media supplemented with 2.2 µM BAP, followed by 3.1 µM BAP, giving a mean of 7.4 and 6 shoots per explant, respectively. The highest root formation was observed in the ½ MS media supplemented with 1.5 µM IBA averaging 17.47 roots per explant. The regenerated plantlets were infected with three bacterial strains (*Bacillus sp.* EU_UPM1, *Pntoea dispersa* EU_UPM2 and *Pntoea dispersa* EU_UPM3). The results showed that the antibacterial effect of green AgNPs on infected plants was evaluated *in vitro* by the direct inoculation of 100 ppm of green AgNPs to the bacterial infected *E. pellita* plantlets. This research will create awareness of new aspirations to mass-produce the free pathogen-infected *E. pellita* plants.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN NANOPARTICLES PERAK HIJAU TERHADAP PATOGEN YANG DIJANGKIT DALAM VITRO *Eucalyptus pellita F. Muell* PLANT

Oleh

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Nanoteknologi telah muncul sebagai bidang penyelidikan yang rancak dalam beberapa bidang saintifik dan kejuruteraan moden. Aplikasi dan pembangunan nanopartikel perak (AgNPs) telah meningkat dengan ketara disebabkan oleh ciri-ciri tersendiri, termasuk saiz (1-100 nm), bentuk, dan sifat antimikrob. Biomolekul dan entiti biologi yang pelbagai (prokariot dan eukariot) digunakan secara meluas sebagai stok bio untuk mencipta bahan berstruktur nano. AgNPs hijau menyediakan bahan tidak toksik dan mampan untuk digunakan sebagai agen antimikrob. Permintaan yang semakin meningkat untuk spesies *Eucalyptus* untuk aplikasi perindustrian memerlukan strategi pembiakan yang cekap untuk menghasilkan pokok yang lebih baik secara pantas dan genetik. Walau bagaimanapun, pelbagai patogen mikrob yang menjejaskan pertumbuhan *Eucalyptus*, yang dianggap sebagai kebimbangan utama kerana ia boleh menyebabkan kerosakan jangka panjang kepada ladang *Eucalyptus*. Kajian ini mengkaji kesan AgNPs hijau pada tumbuhan *in-vitro Eucalyptus pellita* (*E. pellita*) yang dijangkiti patogen. Pucuk *in-vitro* yang dijana semula daripada eksplan daun kotiledon *E. pellita* diinduksi melalui organogenesis langsung menggunakan media MS ditambah dengan kepekatan berbeza (0.4, 1.3, 2.2, 3.1, 3.9 µM) thidiazuron (TDZ), 6-benzylaminopurine (BAP) dan kinetin (KIN). Pucuk memanjang telah diakarkan secara *in-vitro*, masing-masing dengan menggunakan media ½ MS ditambah dengan kepekatan berbeza (0.5, 1, 1.5, 2, 2.5 µM) asid indole-3-butyric (IBA) dan asid 1-naphthaleneacetic (1-NAA) yang berbeza. Pembentukan pucuk tertinggi daripada daun kotiledon diperhatikan dalam media MS ditambah dengan 2.2 µM BAP, diikuti oleh 3.1 µM BAP, memberikan min masing-masing 7.4 dan 6 pucuk setiap eksplan. Pembentukan akar tertinggi diperhatikan dalam media ½ MS ditambah dengan 1.5 µM IBA dengan purata 17.47 akar setiap eksplan. Planlet yang dijana semula telah dijangkiti dengan tiga strain bakteria (*Bacillus* sp. EU_UPM1, *Pntoea dispersa* EU_UPM2 dan *Pntoea dispersa* EU_UPM3). Keputusan menunjukkan bahawa kesan antibakteria AgNP hijau ke atas tumbuhan yang dijangkiti telah dinilai secara in vitro dengan inokulasi terus 100 ppm AgNP hijau kepada tumbuhan *E. pellita* yang dijangkiti bakteria. Penyelidikan ini akan mewujudkan

kesedaran tentang aspirasi baharu untuk menghasilkan besar-besaran tumbuhan *E. pellita* yang dijangkiti patogen bebas.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of the Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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LIST OF ABBREVIATIONS

1-NAA	1-Naphthaleneacetic acid
2,4-D	2,4-dichlorophenoxyacetic acid
BAP	6 Benzylaminopurine
$Ca(OCl)_2$	Calcium hypochlorite
E.pellita	*Eucalyptus pellita*
AuNPs	Gold nanoparticles
g	Gram
HCL	Hydrochloric acid
EU_UPM2O_2	Hydrogen peroxide
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
FeNPs	Iron nanoparticles
KIN	Kinetin
LS	Linsmaier and Skoog
L	Liter
$HgCl_2$	Mercuric chloride
µM	Micromolar
mm	Millimeter
MBC	Minimum bacterial concentration
MIC	Minimum inhibitory concentration
MS	Murashige and Skoog
NPs	Nanoparticles
NA	Nutrient agar
OD	Optical density
PGRs	Plant growth regulators

AgNPs	Silver nanoparticles
NaOH	Sodium hydroxide
NaClO	Sodium hypochlorite
sp.	Species
TDS	Thidiazuron
WPM	Woody plant medium
ZnSNPs	Zinc sulphide nanoparticles
ZOI	Zone of inhibition

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Nanotechnology has linked engineering, chemistry, biological sciences and materials science to create novel nanomaterials (Jadoun *et al.*, 2020). It has great promise in biotechnology, food technology, agriculture, as well as environmental and medical activities (Thakur *et al.*, 2018). It entails molecular-scale structures less than 100 nm in at least one dimension with a tiny size and large surface area to volume ratio imparting specific features that differ from their bulk materials (Thakur *et al.*, 2018). Bacterial resistance to antibiotics has become a significant concern in environmental and public health; therefore, there is a considerable incentive to create novel bactericides (Manyi-Loh *et al.*, 2018). Bacteria have different structural membranes, allowing them to be generally categorised as Gram-negative or Gram-positive (Megrian *et al.*, 2020). The structural variations are came from the arrangement of the principal constituent of the membrane, peptidoglycan (Silhavy *et al.*, 2010). Gram-negative bacteria have a thin layer of peptidoglycan (~2–3 nm) between the cytoplasmic and the outer membrane (Murray *et al.*, 1965). In comparison, Gram-positive bacteria have a thicker layer of peptidoglycan (~30 nm) in the absence of the outer membrane (Shockman and Barren, 1983).

Since a long time ago, silver has been regarded as a strong toxic element to a broad range of microorganisms (Fernando *et al.*, 2018). In light of this, silver-based derivatives have been widely employed in a vast range of bactericidal applications (Jiang *et al.*, 2022) including biosensors (Karimi-Maleh *et al.*, 2021), health industries (Olmos *et al.*, 2018), drug delivery (Dhayalan *et al.*, 2018), antibacterial agents (Hamad *et al.*, 2020) as well as textile and wastewater treatment (Ahmed *et al.*, 2020). Nevertheless, limited studies have been conducted to study the potential of nanotechnology in agriculture (Ndaba *et al.*, 2022).

Nanotechnology opens a plethora of emerging applications in the field of biotechnology and agriculture, owing to the unique physicochemical properties of NPs (Mahendran *et al.*, 2019). Plant tissue culture is a technique in which plant tissues, organs or cells are *in-vitro* cultured under aseptically nutritional and controlled environmental conditions (Oseni *et al.*, 2018), commonly for plant cloning. These clones represent true to the type of chosen genotype (Entuni *et al.*, 2022). Under such a properly monitored environment, the culture can develop and multiply (Suman, 2017). An aseptic environment includes an adequate supply of nutrients, pH value of the medium, suitable temperatures and a balanced gaseous and liquid environment (Oseni *et al.*, 2018). The plant tissue culture technique is mainly exploited for large-scale plant reproduction. Besides their use as a research tool, they have acquired considerable industrial relevance in plant improvement, plant propagation, production of secondary metabolites and disease control (Jain *et al.*, 2019). Small fragments of tissue (called explants) can be used to mass produce of plants continuously (Oseni *et al.*, 2018). Endangered and rare species have been established and preserved by micropropagation owing to its high number of multiplications and low starting materials needed (Singh, 2022).

In recent forestry breeding programs and biotechnology research, micropropagation opens up new promises for increasing the multiplication coefficient (Abiri *et al.*, 2020). *Eucalyptus* (a group of the Myrtaceae family) is a widely distributed evergreen genus. *Eucalyptus* has a fast growth rate including 900 species native to Chile, Australia, Brazil, Portugal, Indonesia and South Africa (Batista *et al.*, 2018). *Eucalyptus* has long been known for its economic value and commercial importance owing to its rapid growth, hard wood, high profitability and resistance to biotic stresses (Nogueira *et al.*, 2020). *E. pellita* has been suggested as a leading resource in various regions worldwide, mainly because of its rapid growth. Furthermore, this species possesses other favorable features such as strong environmental adaptability, vertical stem growth, a tall and unbranched trunk, high resistance to pests and diseases, and easy cultivation.

1.2 Problem Statement

Despite the wide availability of wild *Eucalyptus* trees, seed propagation became a traditional cultivation technique with varying levels of success and achievement. Clonal propagation is one of the most effective *in vitro* methods for increasing the multiplication of *Eucalyptus* sp. (Bhandari *et al.*, 2022). Despite the advancement in the micropropagation strategy, the obstacles due to microbial contamination during early and late growth stages are still the major barriers to clonal propagation of *Eucalyptus* species. The nutrient medium in which plant tissue is grown is an ideal source of nutrients for microbial development. These microbes are adversely competing with plant tissues for nutrient. As a result, the existence of these microbes in plant cultures is linked to the explants mortality, tissue necrosis and latent infections which leads to the shoot proliferation and rooting (Mustafa *et al.*, 2021). On another hand, Antibiotic resistance refers to the capability of microorganisms to resist the effects of antimicrobial agents, which happens when an antibiotic becomes ineffective in preventing the growth of bacteria (Pulingam *et al.*, 2022). This problem has emerged as a significant concern in different agricultural sectors (Nadeem *et al.*, 2020). Thus, there is a need to develop new eco-friendly antimicrobials to avoid the antimicrobial resistance in various agricultural applications.

1.3 Objectives

The main objective of this study was to optimise the mass production of *in vitro E. pellita* plant and to examine the influence of green AgNPs on the pathogen-infected *in vitro E. pellita* plant. Therefore, the sub-objectives of this study were:

1. To induce shoots and roots from leaf explants of *E. pellita* using cytokinin and auxin as plant growth regulators (PGRs).

2. To evaluate the antibacterial effect of green AgNPs on *in vitro* cultured *E. pellita* plantlets.

1.4 Scope of Research

This study was conducted under *in vitro* aseptic conditions. The first part of the study followed the plant tissue culture techniques to achieve the healthy *E. pellita* plantlets to better observe whether the bacterial strains have significant effect on the plantlets or not. The tissue culture techniques started from germination of seeds to obtain the cotyledonary leaves which used as explants for shoots regeneration and finished in the rooting of the regenerated plantlets. The second part of the study used the inoculation technique of identified bacterial isolates alone and with green AgNPs to find the difference of morphological traits of *E. pellita* plantlets. To conclude, the results of this study found that the green AgNPs can control the bacterial infection on the *in vitro* plantlets caused by *Bacillus* sp. And *Pantoea dispersa*.

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