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GRANULAR FORMULATION OF ENDOPHYTIC FUNGUS, *Hendersonia toruloidea* GanoEF1 FOR CONTROLLING *Ganoderma* DISEASE AND PROMOTING OIL PALM GROWTH

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By

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Chair : Prof. Dzolkhifli Omar, PhD
Faculty : Agriculture

Oil palm is one of the important crops in Malaysia and plays an important role in the agricultural and economic development of the country. Basal stem rot (BSR) caused by *Ganoderma boninense* is the biggest threat for oil palm production and has been documented to cause a huge damage to the oil palm industry in Malaysia. There is yet an effective control measure for BSR disease. Endophytic fungi have been previously studied and identified as potential biological control agents of many crop diseases. Isolate *Hendersonia toruloidea* GanoEF1 is a novel and promising biological control agents against *G. boninense*. The use of endophytes in form of formulated product is preferred and sought as such preparations offer many advantages during application in the field. Therefore this study was designed with the specific objectives to (i) determine the compatible carriers for viability and quality of *H. toruloidea* GanoEF1 in the preparation of granular formulation, (ii) investigate the effects of granular formulations of *H. toruloidea* GanoEF1 for controlling BSR in oil palm, (iii) determine the biochemical compounds released in oil palm treated with *H. toruloidea* GanoEF1, and (iv) study the effects of the granular formulations developed of on oil palm growth. *H. toruloidea* GanoEF1 isolated from healthy oil palm roots was cultured on potato dextrose agar (PDA) media. A suspension containing 10^8 CFU ml^{-1} of the conidia cells was prepared as granular formulation by using empty fruit bunch (EFB), rice bran (RB), talc powder, paddy husk (PH) and sawdust (SD) as nutrient supplement mixed with inert ingredient of either kaolin or palm kernel cake powder in the solution containing alginate-pectin as a binder. The best ratio of alginate:pectin for optimum growth of *H. toruloidea* GanoEF1 was 1:3 at temperature 35 ºC with the highest number of conidia cell recorded was log 10^8 CFU g^{-1} at 30 days after storage. Three best nutrient carrier of EFB, RB and SD that succeeded to sustain the viability of *H. toruloidea* GanoEF1 were further formulated with kaolin (K) or palm kernel cake (PKC) and tested for their viability over 12 months storage and efficacy against *G. boninense.* Amongst them, three granular formulation of empty fruit bunch-kaolin (EFB-K), empty fruit bunch-palm kernel cake (EFB-PKC) and rice bran-palm kernel cake (RB-PKC) showed highest conidia viability of *H. toruloidea* GanoEF1 more than 10^5 CFU g^{-1} and recorded more than 50% of percentage inhibition of radial growth (PIRG) values. The effectiveness of *H. toruloidea* GanoEF1 in EFB-PKC, EFB-K and RB-PKC was then evaluated in the glasshouse on their efficacy against *G. boninense*. Disease suppression was highest in the treatment that had the application of *H. toruloidea* GanoEF1 in EFB-PKC with a disease reduction of 65.92% (P<0.05). The percentage of dead seedlings also was significantly lowest in seedlings treated with *H. toruloidea* GanoEF1 in EFB-PKC (26.7%) as compared to the control seedlings (93.3%). The lower percentage of dead seedlings indicates that the lower infection of BSR occurred. Furthermore, the production of POX and PPO were detected in the seedlings pre-inoculated with *H. toruloidea* GanoEF1 and significantly higher than control treatment at the post *G. boninense* challenge inoculation indicating induced resistance is one the mechanism of *H. toruloidea* GanoEF1 to control *Ganoderma* infection. The effect of the formulations on plant growth showed the seedlings treated with *H. toruloidea* GanoEF1 in EFB-PKC gave significantly (P<0.05) highest results on plant height (98.61 cm), girth (38.7 mm), number of frond (11.0), chlorophyll content (60.85 µg/L), root biomass (42.5 g) and leaves biomass (70.1 g) respectively, followed by seedlings treated with granular formulation of EFB-K, RB-PKC and control treatment. The oil palm roots colonized positively to *H. toruloidea* GanoEF1 inoculation with the highest population (between 2.0 x 10^4 cfu g^{-1} to 5.3 x 10^7 cfu g^{-1}) was observed in the treatment of EFB-PKC and EFB-K granular formulations. This phenomenon was supported with the colonization of *H. toruloidea* GanoEF1 within the cortex of the root observed by using transmission electron microscopy (TEM). This study showed the *H. toruloidea* GanoEF1 in granular formulations containing carrier EFB-PKC was the most effective as biological control agent for controlling *Ganoderma* disease and promoting the growth of oil palm seedlings.

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

FORMULASI GRANULAR YANG MENGANDUNGI KULAT ENDOFITIK, *Hendersonia toruloidea* GanoEF1 UNTUK MENGAWAL PENYAKIT *Ganoderma* DAN UNTUK MENGGALAKKAN PERTUMBUHAN POKOK SAWIT

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Pokok sawit merupakan salah satu tanaman penting di Malaysia dan memainkan peranan penting dalam pembangunan pertanian dan ekonomi negara. Penyakit reput pangkal batang (BSR) yang disebabkan oleh kulat *Ganoderma boninense* adalah ancaman terbesar bagi pengeluaran minyak sawit dan telah didokumentasi menyebabkan kerosakan besar kepada industri sawit di Malaysia. Terdapat beberapa langkah kawalan yang berkesan untuk penyakit BSR. Kulat endofitik telah dikenalpasti sebagai agen kawalan biologi yang berpotensi untuk banyak penyakit. Isolat *Hendersonia toruloidea* GanoEF1 adalah merupakan novel strain yang belum pernah dilaporkan, namun menjanjikan strategi kawalan biologi untuk mengawal *G. boninense*. Penggunaan kulat endofitik dalam pembangunan formulasi juga dipilih kerana banyak kelebihan semasa aplikasi di lapangan. Oleh itu, kajian ini telah direka dengan objektif khusus untuk (i) menentukan substrat nutrien pembawa yang sesuai dengan penghasilan konidia dan kualiti *H. toruloidea* GanoEF1 dalam rumusan formulasi granular, (ii) mengkaji kesan rumusan formulasi granular *H. toruloidea* GanoEF1 untuk mengawal penyakit RPB pada pokok sawit, (iii) menentukan tindak balas biokimia dalam anak sawit yang diinokulat dengan *H. toruloidea* GanoEF1, dan (iv) mengkaji kesan-kesan rumusan formulasi granular *H. toruloidea* GanoEF1 pada pertumbuhan vegetatif pokok sawit. Kulat endofitik, *H. toruloidea* GanoEF1 telah dipencilkan daripada akar pokok sawit yang sihat dan dikultur di atas piring agar dektrosa kentang (PDA). Larutan suspensi yang mengandungi 10^8 CFU ml^{-1} sel-sel konidia telah dirumuskan dalam formulasi granular dengan campuran substrat nutrient pembawa seperti buah tandan buah kosong (EFB), dedak beras (RB), serbuk talkum, sekam padi (PH) dan serbuk habuk kayu (SD) bersama ramuan lengai; serbuk kaolin atau hampas isirung sawit (PKC) dalam larutan alginat-pektin sebagai pengikat. Kajian ini mendapati bahawa nisbah terbaik alginat:pektin untuk pertumbuhan optimum *H. toruloidea*

GanoEF1 adalah 1:3 pada suhu 35 ºC dengan jumlah tertinggi sel konidia yang direkodkan adalah 10^8 CFU g^{-1} pada 30 hari selepas penyimpanan. Tiga substrat nutrient pembawa terbaik iaitu EFB, RB dan SD yang berjaya menunjukkan bilangan sel kulat yang tinggi telah dipilih dan diformulasi lagi dengan kaolin (K) atau hampas isirung sawit (PKC) dan ujian kualiti serta aktiviti antagonistik *H. toruloidea* GanoEF1 terhadap *G. boninense* dijalankan setiap bulan selama 12 bulan. Tiga jenis formulasi granular yang mengandungi buah tandan kosong-serbuk kaolin (EFB-K), buah tandan kosong-hampas isirung sawit (EFB-PKC) dan dedak beras-hampas isirung sawit (RB-PKC) berjaya mengekalkan bilangan sel konidia pada 10^5 CFU g^{-1} dan mencatatkan peratusan perencatan pertumbuhan radial (PIRG) terhadap *G. boninense* melebihi 50%. Keberkesanan *H. toruloidea* GanoEF1 dalam formulasi granular EFB-PKC, EFB-K dan RB-PKC telah dipilih bagi menguji keberkesanan setiap satunya dalam mengawal penyakit ke atas anak sawit di tapak semaian. Penindasan penyakit adalah tertinggi dalam rawatan aplikasi *H. toruloidea* GanoEF1 dalam formulasi EFB-PKC dengan pengurangan penyakit sebanyak 65.92% ($P<0.05$). Peratusan anak sawit mati juga jauh lebih rendah dalam anak sawit yang dirawat dengan *H. toruloidea* GanoEF1 dalam EFB-PKC (26.7%) berbanding dengan kawalan (93.3%). Peratusan anak sawit mati disebabkan oleh jangkitan *G. boninense* yang lebih rendah menunjukkan bahawa jangkitan BSR yang lebih rendah berlaku. Di samping itu, aktiviti POX dan PPO dikesan pada anak sawit yang telah dirawat dengan *H. toruloidea* GanoEF1 dan jauh lebih tinggi daripada rawatan kawalan pada sebelum dan selepas jangkitan *G. boninense*. Kesan formulasi pertumbuhan tumbuhan menunjukkan anak sawit yang dirawat dengan *H. toruloidea* GanoEF1 dalam formulasi granular EFB-PKC telah didapati meningkat secara signifikan ($P<0.05$) pada ketinggian pokok (98.61 cm), lilitan diameter batang (38.7 mm), jumlah bilangan pelepah (11.0), kandungan klorofil (60.85 µg / L), serta jumlah biomas akar (42.5 g) dan daun (70.1 g) masing-masing, diikuti oleh anak sawit yang dirawat dengan rumusan EFB-K, RB-PKC dan rawatan kawalan. Kulat *H. toruloidea* GanoEF1 mengkolonisasi akar pokok sawit secara positif dengan populasi tertinggi diperhatikan dalam rawatan formulasi granular EFB-PKC dan EFB-K antara 2.0×10^4 cfu g^{-1} hingga 5.3×10^7 cfu g^{-1}. Fenomena ini disokong dengan pengkolonian *H. toruloidea* GanoEF1 dalam korteks akar yang diamati dengan menggunakan transmisi elektron mikroskop (TEM). Kajian ini menunjukkan *H. toruloidea* GanoEF1 dalam formulasi granular yang mengandungi substrat nutrient pembawa EFB-PKC adalah paling berkesan sebagai agen kawalan biologi untuk mengawal penyakit *Ganoderma* dan meningkatkan pertumbuhan vegetatif pokok sawit.

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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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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSR	Basal Stem Rot
MA	Malt Agar
MEA	Malt Extract Agar
CMA	Corn Meal Agar
CDA	Czapek's Dox Agar
AMF	Arbuscular Mycorrhiza Fungi
MPOB	Malaysian Palm Oil Board
PGPR	Plant Growth Promoting Rhizobacteria
ISR	Induced Systemic Resistance
PO	Peroxidase
PPO	Polyphenol oxidase
EFB	Empty Fruit Bunch
FFB	Fresh Fruit Bunch
GSM	Ganoderma selective medium
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PIRG	Percentage inhibition of radial growth
CFU/g	Colony forming unit per gram
RCBD	Randomized Completely Block Design
DI	Disease Incidence
AUDPC	Area Under the Disease Progress Curve
DS	Dead Seedlings
DSFI	Disease Severity of Foliar Index
DSBI	Disease Severity of Bole Index
DSRI	Disease Severity of Root Index
RWB	Rubber Wood Blocks
ANOVA	One Way Analysis of Variance

CHAPTER 1

INTRODUCTION

1.1 Background

Oil palm is the world's highest oil crops' producer compared to the other oil crops planted on the same size of land with Malaysia and Indonesia contributes up to 90% of world's palm oil production. High demand for palm oil has led to wider cultivation of oil palm i.e. from 54,000 hectares in 1960 to 5.81 million hectares in 2017, with an annual growth of 10.06%. Besides that, in 2016, it was reported that production of palm oil reached up to 17.32 million tonnes (MPOB, 2017; Nambiappan *et al.,* 2018).

Nowadays, one main constraint faced by the palm oil industry is diseases caused by plant pathogens. The most worrying disease is basal stem rot (BSR) caused by *Ganoderma boninense*. BSR is not new to Malaysia whereby its first attack was not long after oil palm was introduced in the country. The first known attack was on oil palms aged 25 years and above, which were not considered as economically important at that time (Thompson, 1931). Nonetheless, in 1960, as oil palm became a plantation crop, occurrence of BSR started to rise whereby plants, as young as 12–24 months, were attacked (Hoong and Idris, 2010). BSR also infected plants aged 4–5 years (Singh, 1990) and 10 to 15 years in replanted areas (Turner, 1981).

Basal stem rot disease have caused the highest economic loss, estimated about RM 1.5 billion per annum, for both Malaysia and Indonesia compared to other palm oil producing countries such as Africa, Papua New Guinea, and Thailand (Idris *et al.,* 2016; Ommelna *et al.,* 2012;). BSR is very hard to eradicate and numerous ways have been tried to overcome the infection such as applying chemical fungicides, soil mounding, and sanitation by removing infected palms (Mohammed *et al.,* 2014; Idris, 2011). Physical methods such as clean clearing and sanitation involve removal of infected plants from the field. This requires mechanised equipment and a high labour cost. Furthermore, physical methods are more towards preventing spread of disease rather than curing of infected palms. High labour cost and need for periodical observation make physical methods relevant to be applied in severe infected fields, however, it is not sustainable.

On the other hand, manipulation of microorganisms such as fungi, bacteria, and actinomycetes as biological controls has been explored for inhibiting the most severe disease of oil palm (Idris *et al.,* 2010). Biological control is introduced as an alternative to minimise impact on non-targeted organisms and improve agricultural sustainability besides reducing high dependency on mechanisation. Disease caused by *Ganoderma* plays a major role in lowering oil palm yield if no control measures are implemented. Advances in biotechnology have led to a significant increase in use of microbes as biological control agents against plant pathogens. For instance, biological properties of several antagonistic fungi, such as *Aspergillus* (Shukla & Uniyal, 1989), *Penicillium* (Dharmaputra *et al*., 1989), and *Trichoderma* (Angel *et al*., 2016; Sundram, 2013; Nur Ain Izzati & Abdullah, 2008;), and endophytic bacteria (Nurrashyeda *et al.,* 2016; Sapak *et al.,* 2008) have been studied and are proven to have antagonistic effect against *G. boninense*.

The application of microbes as biological agents is a good alternative to control *Ganoderma,* especially in reducing cost of source and maintenance management in the field, compared to chemical fungicides. Endophytic microorganisms, specifically endophytic fungi are therefore a new field of study in biological disease control of *Ganoderma*. The use of endophytic fungi are preferable due to its role as internal colonizers and therefore offering protection from diseases caused by pathogens (Miliute *et al.,* 2015). This was successfully demonstrated with *Hendersonia toruloidea* isolate GanoEF1, *Amphinema byssoides* isolate GanoEF2 and *Phlebia radiata* isolate GanoEF3 which effectively suppressed BSR in oil palm seedlings (Idris *et al.,* 2010).

1.2 Justification

Basal stem rot disease (BSR) is the most destructive disease facing by the oil palm industry. In addition to existing disease management tools, new eco-friendly approaches are being explored to suppress the disease. Studies have proven the potential of endophytic fungi in controlling BSR disease. However, Nurrashyeda's (2018) work used only a pure culture of endophytic fungus, *Hendersonia toruloidea* isolate GanoEF1 in controlling BSR in oil palm seedlings while this study looks at the development of granular formulation of *H. toruloidea* GanoEF1 for application in oil palm plantation. While many biological control products of *Ganoderma* are already in the market, a limited number of these are reported as granular formulation. Liquid and powder formulation are not preferred due to handling difficulties, drift and safety to handlers. The hypothesis of this study is pre-inoculation with granular formulation of *Hendersonia toruloidea* isolate GanoEF1 delays the onset and development of BSR disease in oil palm.

1.3 Research Objectives

i. To determine carriers that are compatible with viability and quality of endophytic fungus, *H. toruloidea* isolate GanoEF1 in different types of granular formulation.

ii. To investigate the effects of granular formulations of endophytic fungus, *H. toruloidea* isolate GanoEF1 for controlling BSR in oil palm.

iii. To determine the biochemical compounds released in oil palm treated with endophytic fungus, *H. toruloidea* isolate GanoEF1.

iv. To study the effects of granular formulations of endophytic fungus, *H. toruloidea* isolate GanoEF1 on oil palm growth.

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