
UNIVERSITI PUTRA MALAYSIA

# DEVELOPMENT OF ENCAPSULATED *Trichoderma harzianum* UPM40 AGAINST *Sclerotium rolfsii* ON CHILI PLANT

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IPTSM 2019 11

# DEVELOPMENT OF ENCAPSULATED *Trichoderma harzianum* UPM40 AGAINST *Sclerotium rolfsii* ON CHILI PLANT

**By**

**FARIZ BIN ADZMI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

**September 2018**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**DEVELOPMENT OF ENCAPSULATED *Trichoderma harzianum* UPM40 AGAINST *Sclerotium rolfsii* ON CHILI PLANT**

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**September 2018**

**Chairman : Professor Mohamed Hanafi Musa, PhD**
**Institute : Tropical Agriculture and Food Security**

*Trichoderma* as a biological control agent is widely used in sustainable agriculture. Commercialisation of this product in large scale with suitable formulation is still remains a problem especially to ensure the viability and efficiency of the formulation upon application in the field. Encapsulation method has emerged as sophisticated technique to develop the formulation of biological control agents. Encapsulation is the process of entrapping an active ingredient to shield it from adverse environmental conditions. Encapsulation of biological control agents (BCA) enables the creation of a microenvironment wherein the viability of the cells is maintained for storage, controlled release, and easy delivery. Encapsulation technique using extrusion method was used to prepare *Trichoderma harzianum* UPM40 beads. Based on the physical characterisation, a formulation with 2% (w/v) alginate, 1% (w/v) montmorillonite clay (MMT), and 5% (w/v) starch was able to produce *T. harzianum* UPM40 beads. This formulation produced almost perfectly shaped beads with SF 0.041 ± 0.006. It also offered a good swelling ability (62.11%) and less shrinkage (48.48%) during the drying process. Chemical characterization using the Fourier Transform Infrared Spectroscopy (FTIR) showed the interaction between the functional groups of alginate, MMT, and starch in the alginate-MMT-starch beads. It was shown by the shifting characteristic peaks of $COO^-$ from starch at 1602 to 1610 $cm^{-1}$. Next C-O-C of alginate shifted from 1024 to 1002 $cm^{-1}$. Lastly, Si-O bending of MMT shifted from 405 to 453 $cm^{-1}$. Thermogravimetric analysis (TGA) showed an improvement in the thermal stability of the alginate-MMT-starch compared to alginate-MMT beads. X-ray Diffraction Analysis (XRD) shows the intercalation and exfoliation between starch and MMT. Peak for starch at 2θ° (15.06°) was shifting to 2θ° (16.64°) while peak at 2θ° (8.8°) for MMT was disappear in alginate-MMT-starch. Scanning electron microscopy (SEM) revealed a homogeneous distribution of MMT and starch particles throughout the alginate linkage. The surface area

and pore diameter were 4.46 $m^2/g$ and 38.2 Å, respectively. Thus, *T. harzianum* UPM40 was successfully encapsulated in the alginate–MMT–starch beads. Storage analysis of the encapsulated *T. harzianum* UPM40 showed that low storage temperature (5 °C) was significantly better ($p < 0.05$) compared to at room temperature (30 °C). At low temperature, *T. harzianum* UPM40 beads maintained its viability of 6.59 log cfu/g up to seven months. The *T. harzianum* UPM40 beads were tested as a biological control agent against a soil-borne pathogen, *S. rolfsii*. In a dual culture, *T. harzianum* UPM40 displayed strong antagonistic activity against *S. rolfsii*. The percentage of inhibition of radial growth (PIRG) was 58.12%. Efficacy test of *T. harzianum* UPM40 for suppressing soil-borne diseases caused by *S. rolfsii* was conducted on chilli plants. Disease assessment results showed significant differences in disease incidence and disease severity index. *T. harzianum* UPM40 beads suppressed *S. rolfsii* with disease reduction of DI at 88.46% and DSI at 72.80%.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PEMBANGGUNAN PENGKAPSULAN *Trichoderma harzianum* UPM40 KESAN TERHADAP *Sclerotium rolfsii* PADA POKOK CILI**

Oleh

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Trichoderma sebagai agen kawalan biologi merupakan salah produk yang diguanakan dalam sektor pertanian mampan. Pengkomersilan produk ini pada sekala yang lebih besar dengan formulasi yang tebaik adalah menjadi salah satu masalah utama, terutamanya dalam memastikan kadar penyimpanan dan efisensi dalam penggunaan di lapangan. Kaedah pengkapsulan muncul sebagai satu teknik yang terbaik dalam pembanggunan formulasi agen kawalan biologi. Pengkapsulan adalah proses memerangkap suatu bahan aktif untuk melindunginya daripada persekitaran yang tidak diingini. Pengkapsulan agen kawalan biologi membolehkan pembentukan mikropersekitaran yang boleh mengekalkan keboleh hidupan sel-sel untuk penyimpanan, pelepasan terkawal, dan memudahkan penghantaran. Teknik pengkapsulan yang dilakukan melalui proses penyemperitan digunakan untuk menyediakan manik *Trichoderma harzianum* UPM40. Berdasarkan pencirian fizikal, formulasi dengan 2% (w/v) alginat, 1% (w/v) tanah liat montmorilonit (MMT), dan 5% (w / v) kanji merupakan formulasi terbaik untuk menghasilkan manik *T. harzianum* UPM40. Formulasi ini menghasilkan bentuk manik yang hampir sempurna dengan SF 0.041±0.006. Ia juga menunjukkan keupayaan mengembang sebanyak 62.11% dan kadar pengucupan 48.48%. Pencirian kimia menggunakan Fourier Transformasi Spektroskop Inframerah (FTIR) menunjukkan interaksi antara kumpulan-kumpulan berfungsi alginat, MMT, dan kanji dalam biji-biji kapsul alginat-MMT-kanji. Ini telah ditunjukkan pada pencirian perubahan puncak-puncak pada kumpulan COO- daripada kanji dari 1602 kepada 1610 $cm^{-1}$. Seterusnya, kumpulan berfungsi C-O-C daripada alginat dari 1024 to 1002 $cm^{-1}$. Akhir sekali kumpulan berfungsi Si-O lengkokan dari 405 to 453 $cm^{-1}$. Analisis Termogravimetrik (TGA) menunjukkan peningkatan dalam kestabilan terma manik alginat-MMT-kanji adalah lebih baik daripada manik alginat-MMT. Analisis Difraksi Sinar-X menunjukkan berlakunya interkelasi dan pengulupasan di antara zarah kanji dan MMT. Puncak untuk zarah kanji pada

2θ° (15.06°) telah berubah, kepada 2θ° (15.64°) sementara puncak 2θ° (8.8°) untuk MMT telah hilang. Mikroskopi Elektron Pengimbas (SEM) mendedahkan taburan secara sekata zarah-zarah MMT dan kanji di dalam jaringan alginat. Keluasan permukaan dan diameter adalah 4.46 $m^2$/g and 38.2 Å. Maka, *T. harzianum* UPM40 berjaya diperangkap di dalam biji-biji manik alginat-MMT-kanji. Analisis penyimpanan manik *T. harzianum* UPM40 menunjukkan bahawa suhu penyimpanan yang rendah pada 5 °C adalah lebih baik ($P<0.05$) berbanding pada suhu bilik (30 °C). Pada suhu rendah keupayaan hidup *T. harzianum* UPM40 dapat dikekalkan pada 6.59 log cfu/ g selama 7 bulan. Manik *T. harzianum* UPM40 diuji sebagai agen kawalan biologi untuk melawan patogen bawaan tanah, *S. rolfsii*. *T. harzianum* UPM40 menunjukkan aktiviti antagonistik yang kuat terhadap *S. rolfsii* di dalam kultur berganda. Analisis Peratusan Kerencatan Jejari (PIRG) dicatatkan pada 58.12%. Ujian keberkesanan *T. harzianum* UPM40 untuk merencat penyakit bawaan tanah oleh *S. Rolfsii* terhadap pokok cili. Keputusan penilaian penyakit menunjukkan perbezaan yang ketara dalam insidens penyakit dan indeks keterukan penyakit. Biji kapsul *T. harzianum* UPM40 boleh merencatkan *S. rolfsii* dengan pengurangan penyakit pada DI pada 88.46% dan DSI pada 72.80%.

**ACKNOWLEDGEMENTS**

Alhamdulillah to Almighty God

First and foremost, I would like to express my deepest appreciation to my supervisor, Prof. Dr. Mohamed Hanafi Musa, my previous supervisor Prof. Dr. Sariah Meon and members of the committee, Assoc. Prof. Dr. Radziah Othman and Prof. Dr. Nor Azah Yusuf for their guidance, advice and suggestion throughout the duration of this study.

I would like to extend my tremendous thanks and greatest gratitude to the lecturers and supported staff at Universiti Putra Malaysia who have directly or indirectly contributed their part. My sincere thanks also to my office and laboratory mate. I'm also very thankful to the financial support from Universiti Putra Malaysia.

My special thanks to my family especially my parent Tuan Haji Adzmi Othman and Hajjah Maznah Yaacob and also my wife Norhafeza Hamzah together with my children Fara Ellysha, Muhammad Iman and Muhammad Anas for their support and encourage.

Last but not least, I'm also indebted to all my beloved friend around the world, whose friendship I'll forever treasure.

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

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysed One-way of Variance |
| ASAP | Surface Area and Porosity Analysis |
| AUDPC | Area Under the Disease Progressive |
| BCA | Biological Control Agent |
| DAT | Day After Transplant |
| DI | Disease Incidence |
| DSI | Disease Severity Index |
| EPA | Environmental Protection Agency |
| FTIR | Fourier Transfrom Infared |
| LSD | Least Significant Different |
| MMT | Montmorillonite Clay |
| PDA | Potato Dextrose Agar |
| PIRG | Percentage of Inhibition Radial Growth |
| RCBD | Randomized Complete Block Design |
| SEM | Scanning Electron Microscopy |
| SF | Sphericity Factor |
| TGA | Thermal Gravimetric Analysis |
| XRD | X-ray Diffraction |

# CHAPTER 1

## INTRODUCTION

Plant disease is a serious problem worldwide and has been a major factor that influences food production and human society development over thousand years (Palmgren et al., 2015). On the economic side, plant diseases cause an estimated 40 billion dollars losses worldwide every year, either directly or indirectly. At least 20%–40% of losses in crop yield are caused by pathogenic infections (Savary et al., 2012).

In Malaysia, the agriculture sector is the backbone of the economic strength and contributed 8.1% (RM89.5b) to the gross domestic product in 2016 (Department of Statistics Malaysia, 2016). However, Malaysia's agriculture is in an unsatisfactory condition with low productivity and quality. Several factors have been identified such as the quality of soil and seed, unpredictable weather, limitations in the use of new technologies, labour shortage, and poor maintenance of agriculture infrastructures. Disease outbreaks and insect pests are other contributing factors that cause the low production of yield. Infectious plant diseases are often caused by pathogenic organisms, such as fungi, bacteria, mycoplasma, viruses, viroids, and nematodes. Examples of plant disease include leaf spot, bacterial wilt, fusarium wilt, mosaic virus infection, and soil-borne disease (Nasehi et al., 2012; Vos et al., 2014; Ben-Jabeur et al., 2015).

The current agricultural practice to control plant diseases is by using pesticide. The U.S. Environmental Protection Agency (EPA) defines pesticide as 'any substance intended for preventing, destroying, repelling, or mitigating any pest'. Pesticides cover a wide range of compounds, including fungicides, insecticides, herbicides, rodenticides, molluscicides, nematicides, and plant growth regulators. This practice is uneconomical in the long run because pesticide use can damage agricultural land by harming beneficial insect species, soil microorganisms, and worms that naturally limit pest populations and maintain soil health; weakening plant root systems and immune systems; and reducing concentrations of essential plant nutrients in the soil such as nitrogen and phosphorus.

Biological control agents (BCA) is sociologically, commercially, and environmentally accepted as a legitimate tool to control plant pathogen. It can be enhanced by cultural practices such as crop rotation and soil amendments, priming plants to be disease-resistant, or genetically altering them to control pathogens (Lazarovits et al., 2014).

Nowadays, there are several commercial BCA available in the market, such as *Agrobacteria*, *Pseudomonas*, *Streptomyces*, *Bacillus*, *Gliocladium*, *Trichoderma*, *Ampelomyces*, and *Coniothyrium*. The shelf life and effectiveness are the major difficulties of BCAs to compete with chemical fungicides. Both problems can be solved with scientific development of BCA formulations. The advantages of formulation include greater efficacy, increased shelf life, ease of handling, increased safety, lower production costs, and compatibility with agricultural practices (Vemmer & Patel, 2013).

It is well established that encapsulation technique has emerged as a sophisticated method in the formulation of biological control agents. Encapsulation within a matrix protects the microbial agents from biotic and abiotic stress factors (contaminations, soil antagonist, temperature, dryness, UV light, or mechanical stress) by providing a beneficial microenvironment (Rathore et al., 2013). This leads to extended shelf-life and maintains the metabolic activity for extended periods of time, not only during storage, but also after application, which results in fewer applications, dose reduction, as well as reduced biomass content (Szczech & Maciorowski, 2016).

Furthermore, one of the properties of encapsulated beads is the controlled release of the entrapped or encapsulated cell. The cells are released slowly by the factor of osmosis from the bead matrix (He at al., 2015). Cells survive longer in the soil and have extended persistence, which results in a reduced number of applications.

This study adopted an encapsulation technique to improve the viability and shelf life of *Trichoderma harzianum* UPM40, which was used as a biocontrol agent. Depending on the strain, the use of *Trichoderma* in agriculture provides numerous advantages: (i) colonisation of the rhizosphere by the BCA (rhizosphere competence) and allowing rapid establishment within the stable microbial communities in the rhizosphere, (ii) controlling pathogenic and competitive/deleterious microflora by using a variety of mechanisms, (iii) improving plant's health, and (iv) stimulating root growth (Soresh & Harman, 2008). For the encapsulated *Trichoderma* to be a success, the choice of carrier for the bead matrix is an important factor. Biodegradable polymer materials are usually used as a capsule matrix, as well as more specific, natural polysaccharides.

The characteristics of alginate, such as its biodegradability, non-toxicity, and biocompatibility, make it a preferred carrier material (Lee et al., 2012). It consists of α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues, linearly linked by 1,4-glycosidic linkages (Paques et al., 2014).

However, the physical and chemical properties of alginate as a carrier are insufficient to achieve long-term stability of the encapsulated cells. Thus, to overcome this limitation, a combination carrier with different characteristics should be added into the formulation. It can be soluble, insoluble, or a combination of both. Several carriers, such as clay and starch, have been used in the formulation of encapsulated beads (Roy et al., 2009). The incorporation of clay as a carrier attracted great attention because it entrapped higher amount and increased encapsulation efficiency (Bušić et al., 2016). Moreover, the starch acted as a structural support to control the extent of shrinkage and maintain the shape of the alginate backbone during the drying process.

This study reports the preparation of alginate–MMT–starch beads through the extrusion technique. The resulting beads were characterised using Fourier transform infrared spectroscopy, thermogravimetric analysis, X-ray diffraction analysis, and scanning electron microscopy. Then, the encapsulation technique was used for *T. harzianum* UPM40. The viability and stability of *T. harzianum* UPM40 in different storage conditions were tested. The efficiency of *T. harzianum* UPM40 beads as a biological control agent against *S. rolfsii* was studied in a dual culture analysis and a shelter house condition.

**Objectives:**

1. To prepare and characterise encapsulated *T. harzianum* UPM40 in alginate–MMT formulation.
2. To evaluate the viability of encapsulated *T. harzianum* UPM40 in alginate–MMT during storage period.
3. To study the optimum content of starch in the alginate–MMT–starch formulation.
4. To investigate the bio-efficacy of the encapsulated *T. harzianum* UPM40 beads in alginate–MMT–starch as a biological control agent against *S. rolfsii* in chilli plants.

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## PUBLICATION

Fariz A., Sariah, M., Hanafi, M. M., Azah, Y. (2012). Preparation, characterisation and viability of encapsulated *Trichoderma harzianum* UPM40 in alginate-montmorillonite clay. *Journal of Microencapsulation* 29 (3): 205-210.

**Patent**

Encapsulation of Bio-control Agent, PI2013700219, Malaysia Patent

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