

Endophytic *Trichoderma* in the disease management of *Ganoderma* – TrichoTec



Oil palm

- ✓ *Elaeis guineensis* Jacq.
- ✓ An important **commodity crop** in Malaysia
- ✓ Planted **5.64 million hectare**
- ✓ Contribute approximately **64.92 billion export revenue** (Parveez et al., 2020)
- ✓ Vulnerable to several diseases
- ✓ **Thirty-two** diseases and disorders affects the oil palm in Africa, Southeast Asia, and South America
- ✓ Confined to the region planted



Basal Stem Rot



01

Symptoms

- Flattening of the crown and unopened spear leaves. (daun pucuk tidak kembang)
- Skirting look-alike (tergantung bawah)
- Decay of the lower stem and root system of the oil palm.
- Collapse of palms in final stage.

02

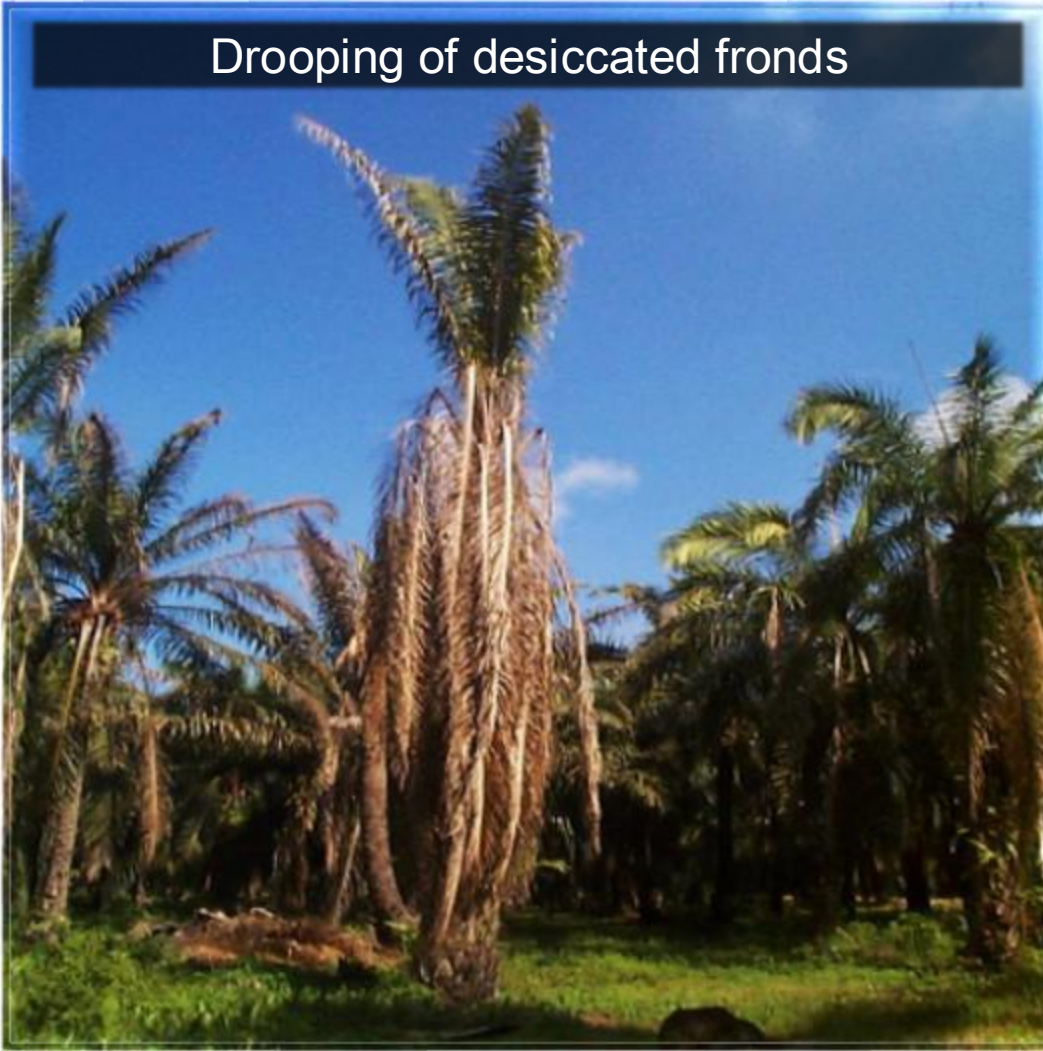
Threat

- BSR causes death of more than 80% of plantings midway through their economic life.



Basal Stem Rot (BSR) disease

Drooping of desiccated fronds



Dry rot of stem base

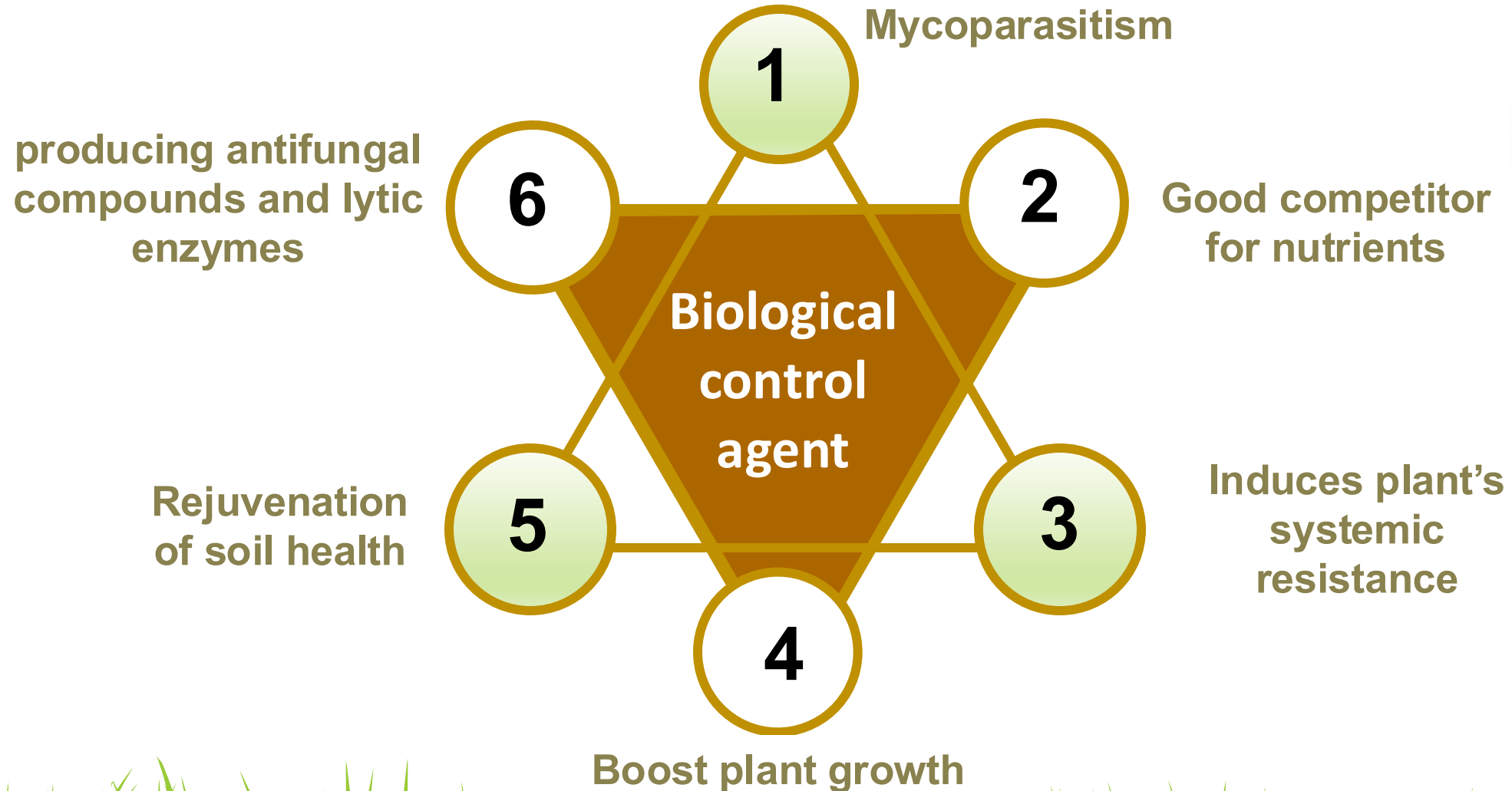


Fruiting body

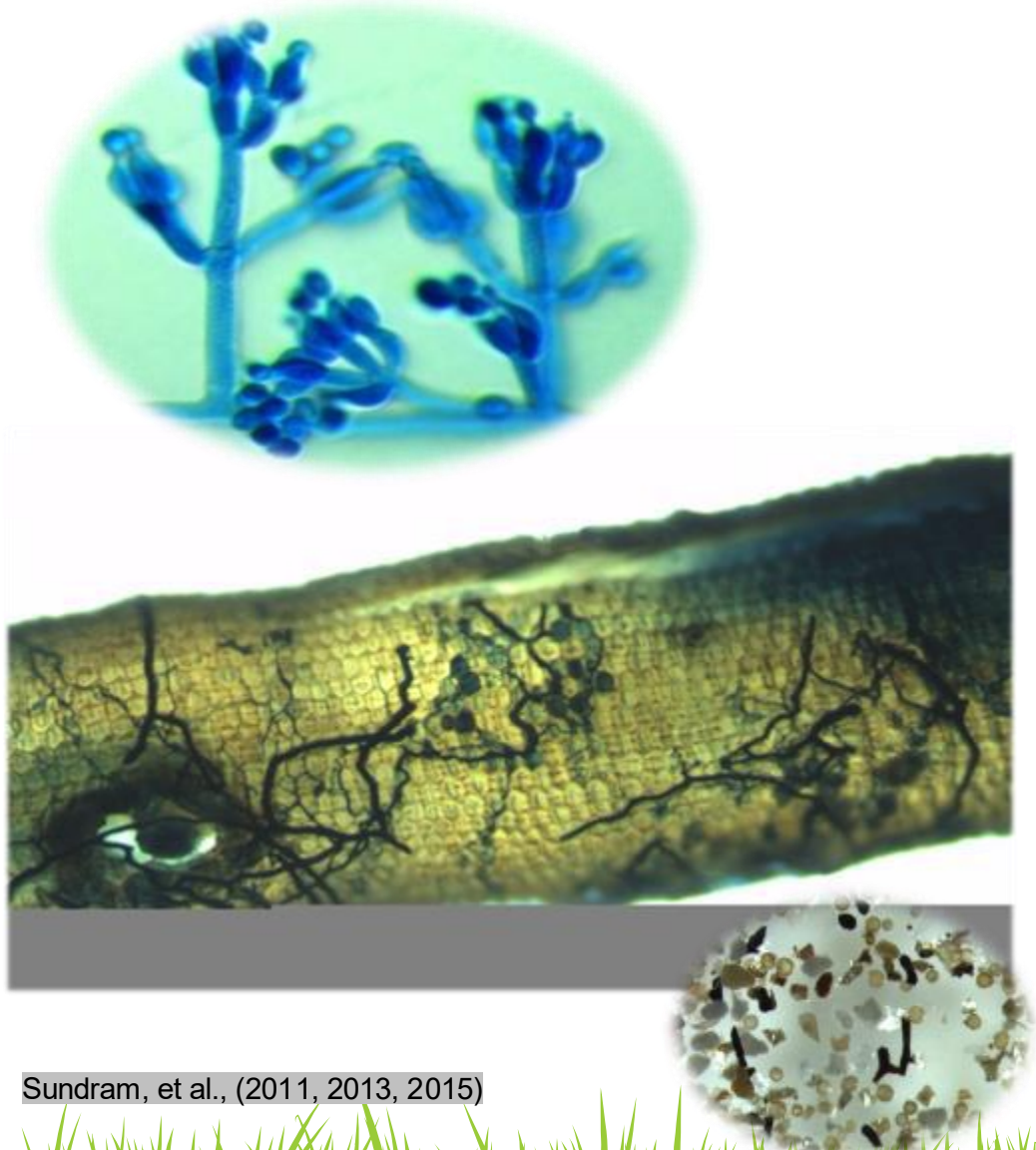


Trichotec (AI: **Trichoderma Viren 159C**)

A Powerful Biocontrol Agent



Biocontrol Agents

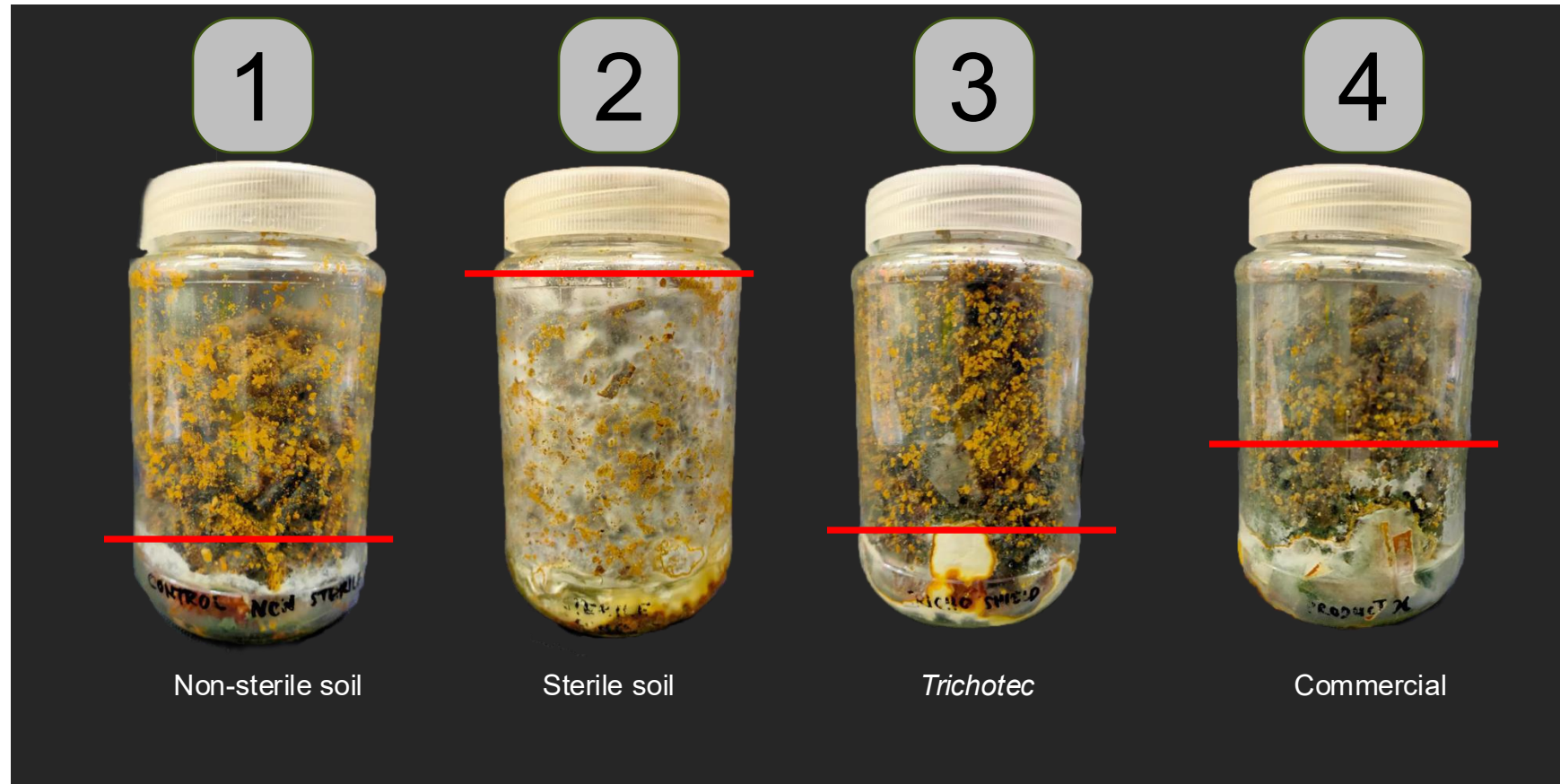


Sundram, et al., (2011, 2013, 2015)

Potential biocontrol agent	Microbial group	References
<i>Pseudomonas fluorescens</i> , <i>Bacillus</i> species	Bacteria	Susanto et al. (2005); Alexander et al. (2017)
<i>Burkholderia cepacia</i> , <i>B. vietnamiensis</i> , <i>Burkholderia</i> species	Bacteria	Zaiton et al. (2008); Buana et al. (2014); Nurrashyeda et al. (2016)
<i>Serratia marcescens</i>	Bacteria	Zaiton et al. (2008)
<i>Nocardiosis alba</i>	Bacteria	Widada et al. (2021)
<i>Pseudomonas aeruginosa</i> , <i>P. syringae</i> , <i>Pseudomonas</i> species	Bacteria	Nurrashyeda et al. (2016); Muniroh et al. (2019)
<i>Bacillus</i> and <i>Enterobacter</i> species	Bacteria	Suryanto et al. (2011); Suryanto et al. (2012)
<i>Streptomyces</i> species	Actinobacteria	Shariffah-Muzaimah et al. (2017); Lim et al. (2018); Shariffah-Muzaimah et al. (2020)
<i>Streptomyces palmae</i>	Actinobacteria	Sujarit et al. (2020b)
<i>Streptomyces philanthi</i>	Actinobacteria	Boukaew et al. (2022)
<i>Streptomyces gelaticus</i>	Actinobacteria	Budi et al. (2022)
<i>Chaetomium lucknowense</i> , <i>C. cochiliodes</i>	Fungi	Soytong (2014)
<i>Hendersonia</i> species	Fungi	Idris et al. (2012); Ismail et al. (2022)
<i>Amphinema</i> species	Fungi	Nurrashyeda et al. (2012a)
<i>Phlebia</i> species	Fungi	Nurrashyeda et al. (2012b)
<i>Scytalidium parasiticum</i>	Fungi	Goh et al. (2016)
<i>Pycnoporus sanguineus</i> , <i>Trametes lactinea</i> , <i>Grammothele fuligo</i>	Fungi	Naidu et al. (2015)
<i>Muscador padawan</i> , <i>M. sarawak</i>	Fungi	Mahidi (2015)
<i>Diaporthe miricidae</i> , <i>T. asperellum</i>	Fungi	Sim et al. (2019)
<i>Clonostachys rosea</i>	Fungi	Goh et al. (2020a)
<i>Trichoderma harzianum</i> , <i>T. viride</i> , <i>Gliocladium viride</i> , <i>Trichoderma</i> species	Fungi	Susanto et al. (2005); Sundram et al. (2008); Alexander et al. (2017)
<i>T. asperellum</i>	Fungi	Muniroh et al. (2019)
Mycorrhizae	Fungi	Sundram et al. (2011); Rini et al. (2022)
<i>Fomes</i> sp., <i>Trametes elegans</i> , <i>T. lactinea</i>	Fungi	Darlis et al. (2023)

Suppressive Soil Concept

- ✓ *Ganoderma* poor competitor
- ✓ Limitation in space, nutrient and food substrate
- ✓ Introducing a beneficial microbe increase the challenge in survival



Result after 1 week

Mycoparasitism: Endophytic *Trichoderma* vs *G. boninense*

Dual Culture assay



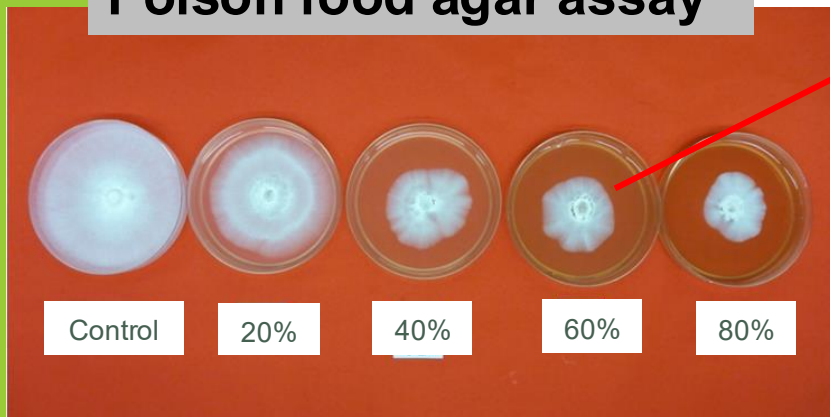
Mycoparasitic interaction of endophytic *Trichoderma* strains with *G. boninense*:

- *Trichoderma* - **fast-growing fungi**
- **Suppressed** the growth of *G. boninense*

Antibiosis involved:

- accompanied by the released of **secondary metabolites (antifungal compounds)** and secrete lytic enzymes (Atanasova *et al.* 2013)
- **Scanning electron microscopy (SEM)** imaging showed severe deformation on the *Ganoderma* hyphal network

Poison food agar assay

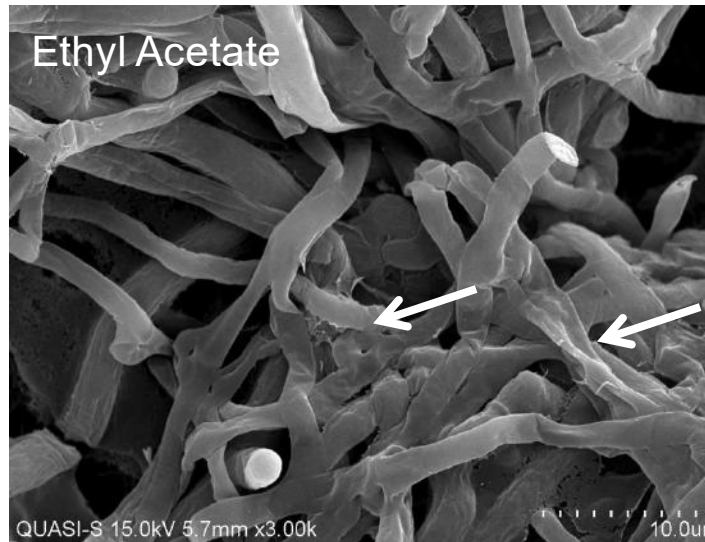
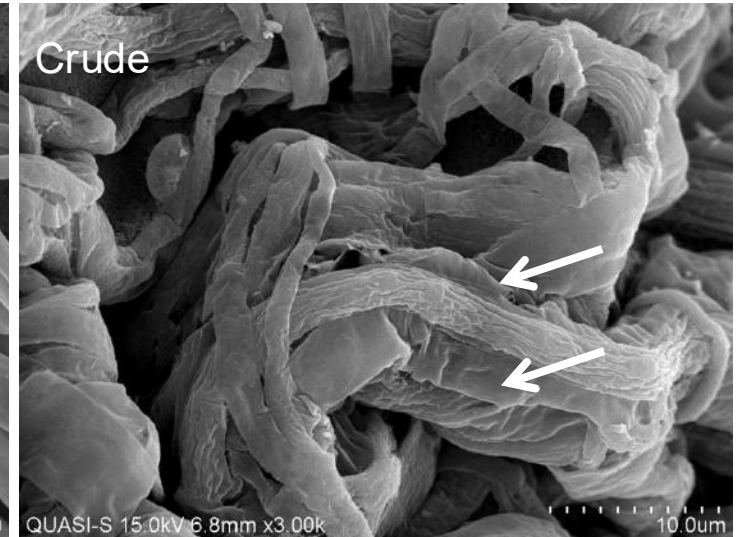
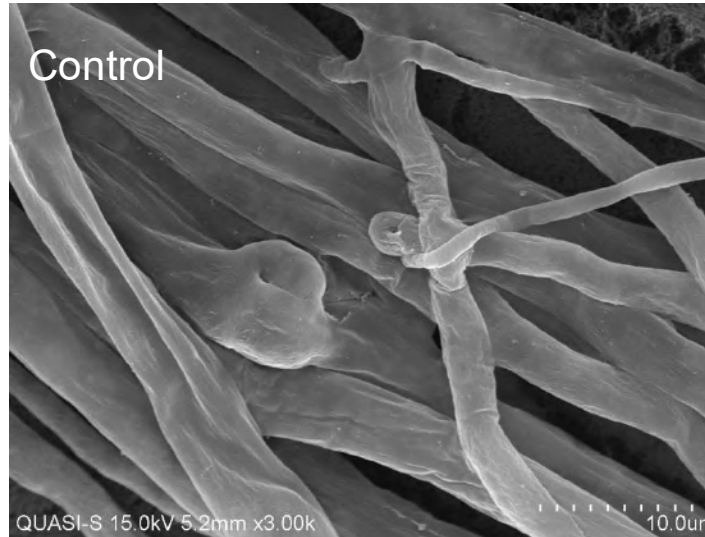


Antibiosis: Scanning electron microscope (SEM) imaging

SEM imaging of
G. boninense
hyphae at the
inhibition region
(10.0µm) by *T.*
virens 159c.

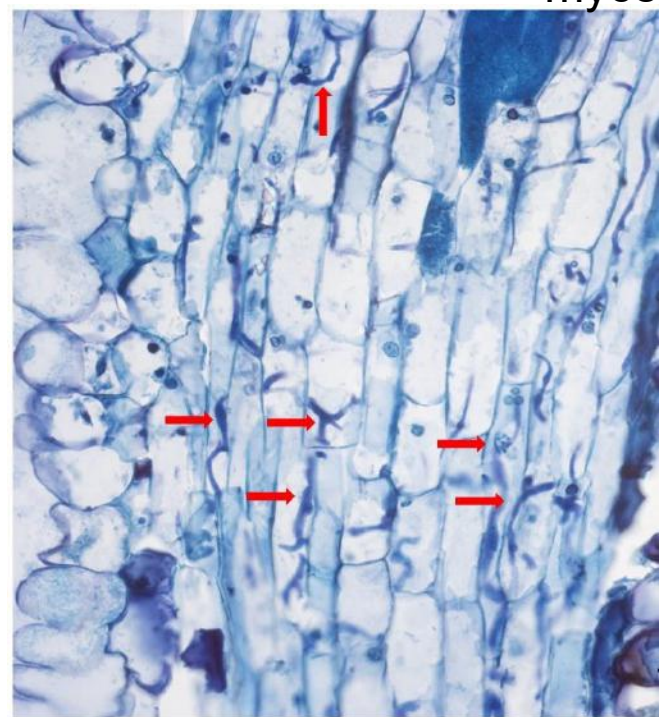
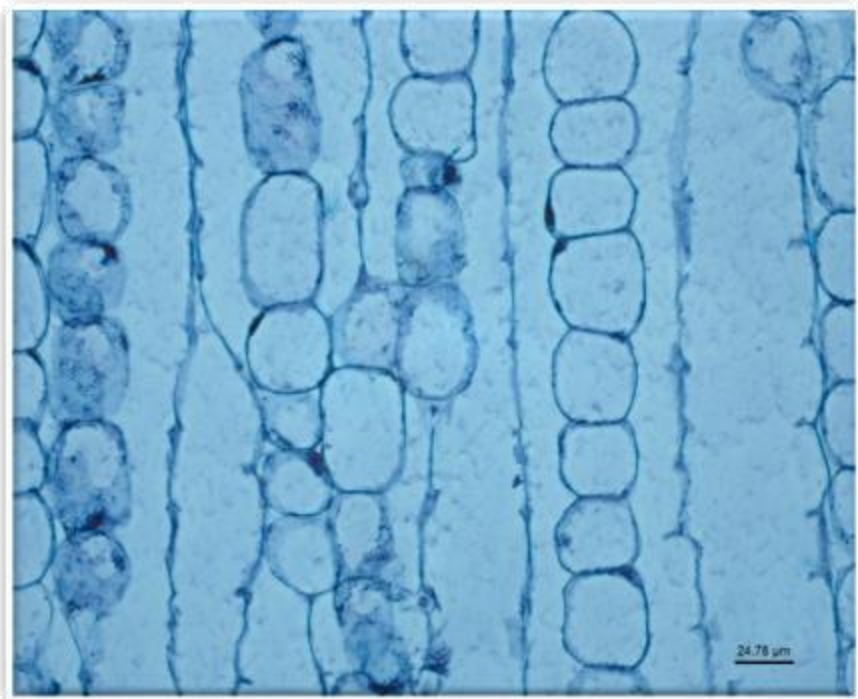
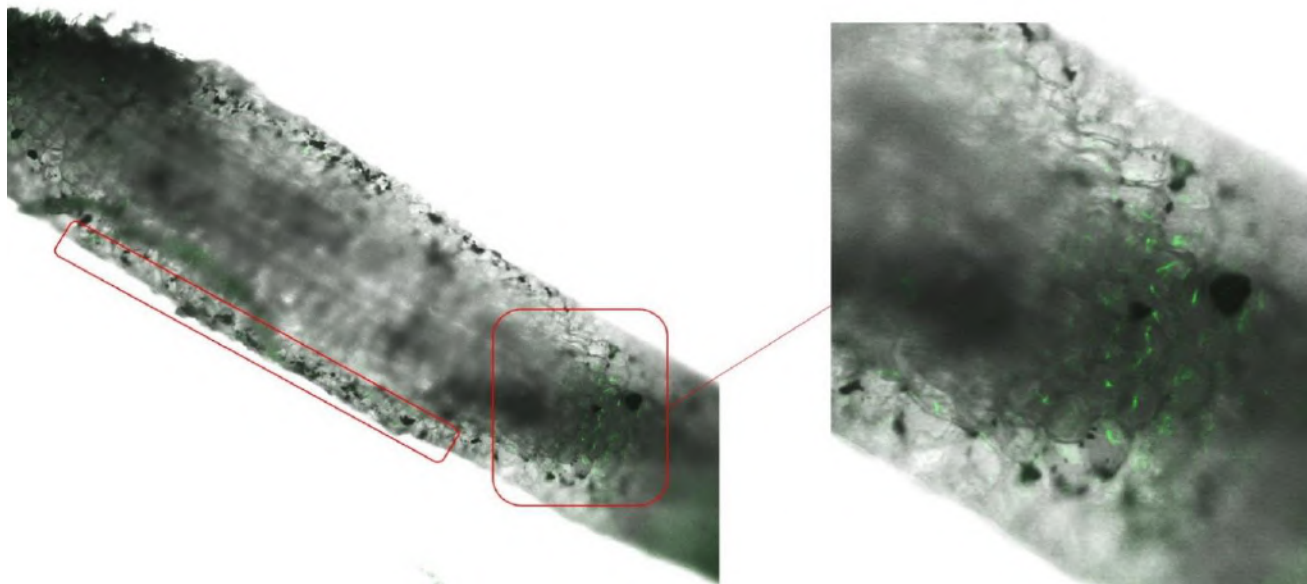
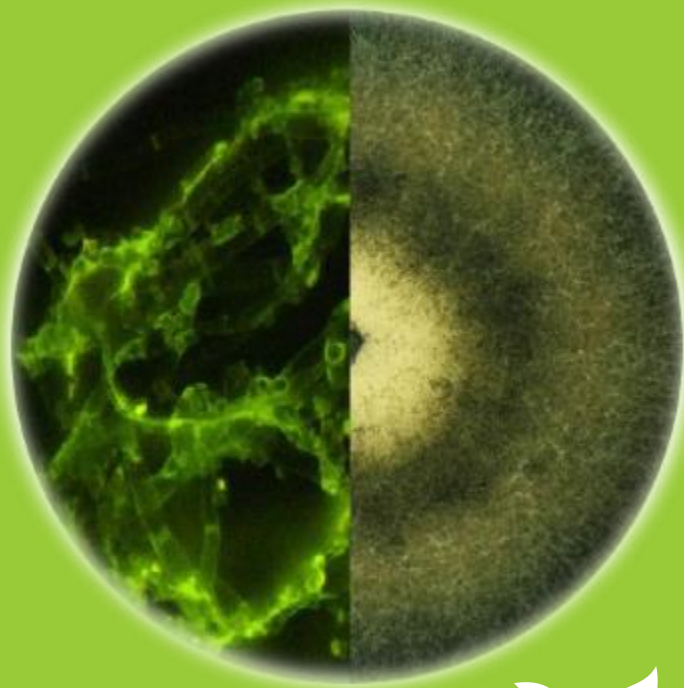


Angel et al., 2017 and 2018



Ethyl acetate extract (50 mg/ml) caused hyphae of *G. boninense* wrinkled, folded and collapse, leading to deformation of mycelia network.

Oil palm root colonization
study using
T. virens GFP



mycelium

Biocontrol Efficiency

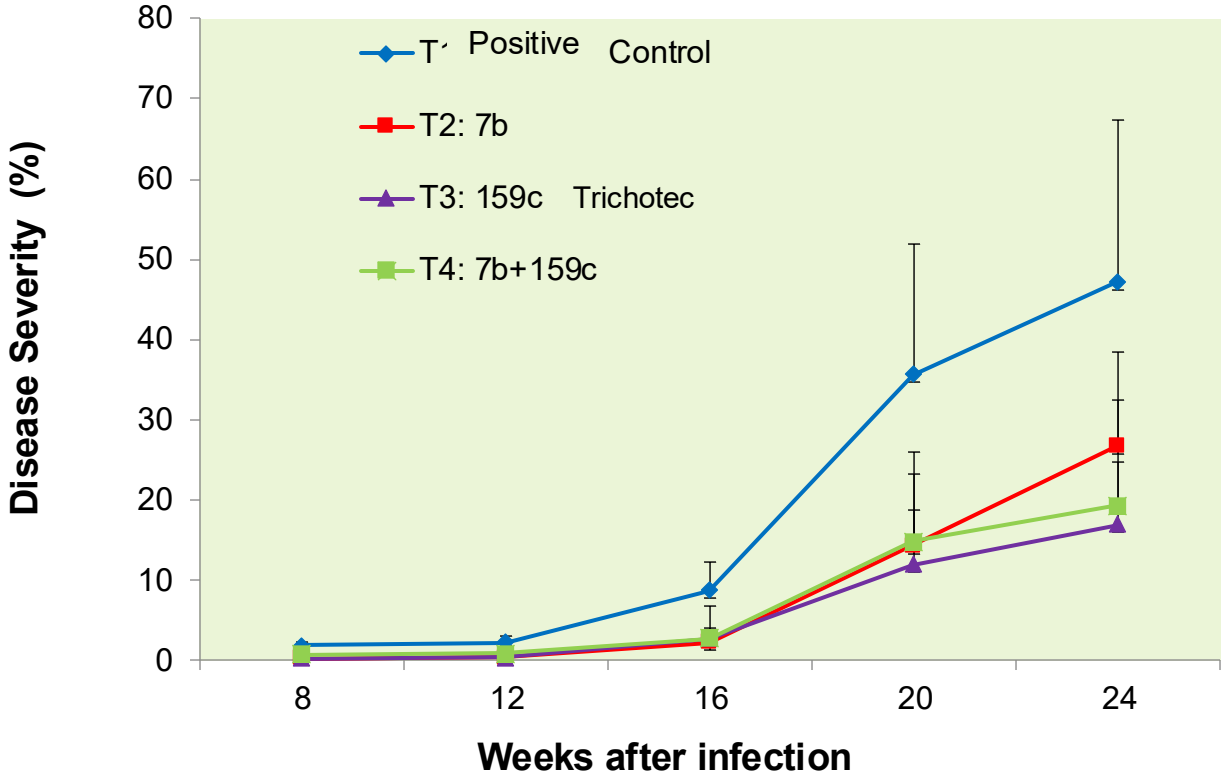
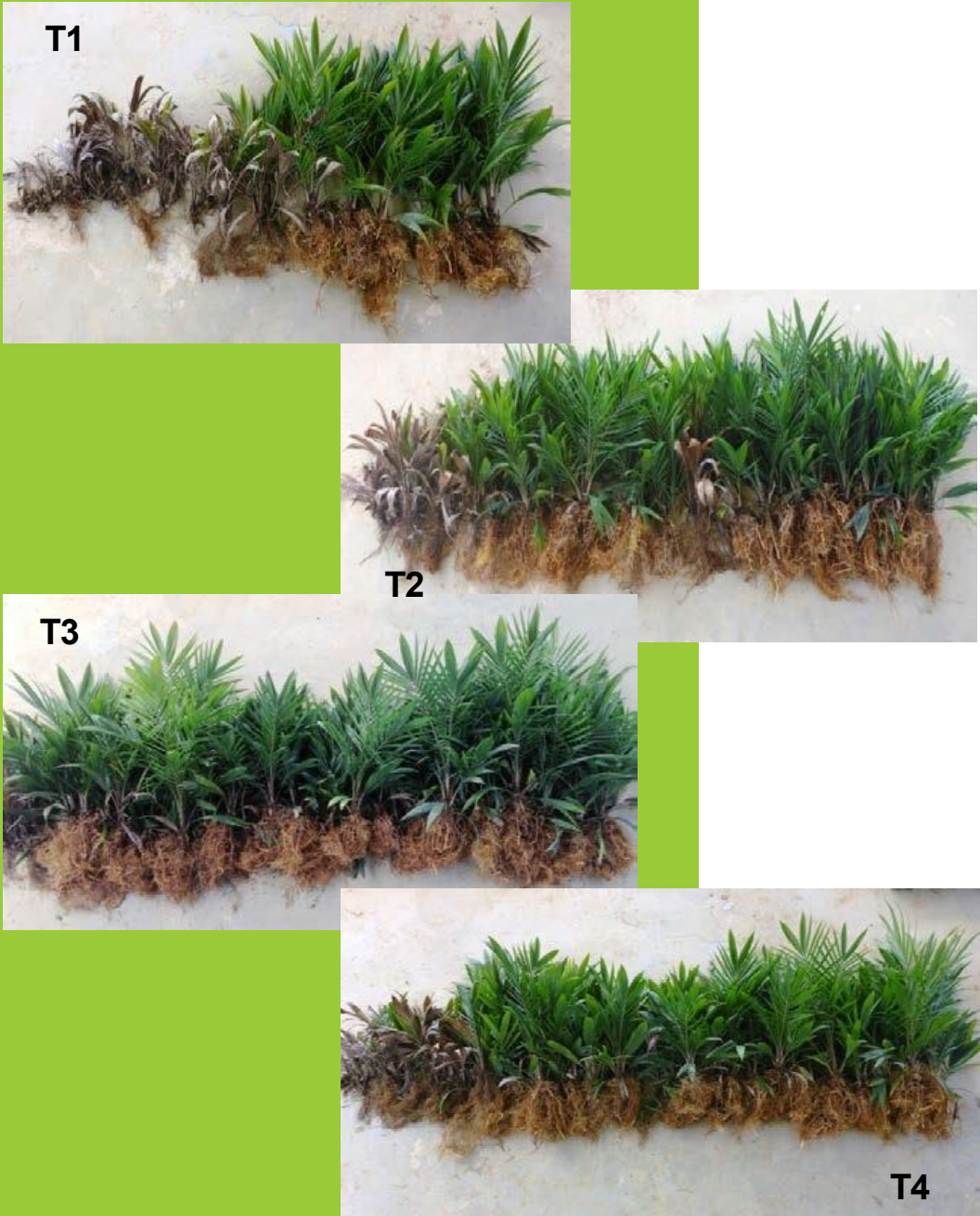


Table 3: The disease development of basal stem rot in bole of oil palm seedlings pre-treated with endophytic *Trichoderma* after infection of *Ganoderma boninense* PER 71

Treatment	Disease severity (%)
T1 (Control)	85.49 ^a
T2 (7b)	53.43 ^b
T3 (159c) Trichotec	41.17 ^c
T4 (7b+159c)	55.88 ^b

Seedling bait trial treated by Trichotec







Treated



Untreated

Seedling bait trial



Basal stem rot disease development in oil palm seedlings treated with *Trichoderma virens*

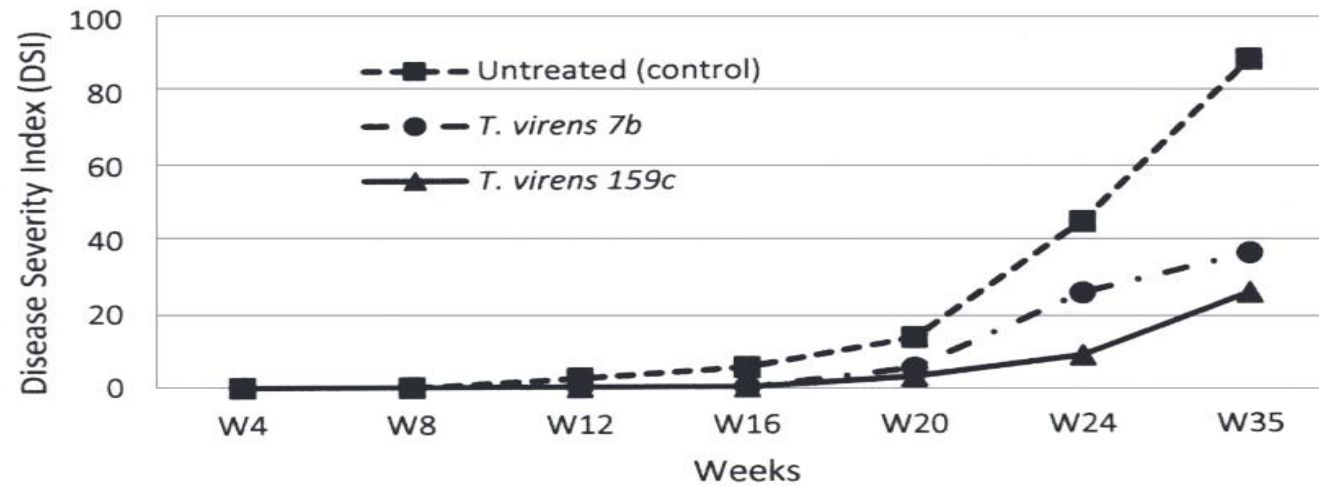


Figure 4. Basal stem rot disease development in oil palm seedlings treated with *Trichoderma virens* 7b and 159c assessed via disease severity index (DSI).

Disease progression of basal stem rot with the application of *Trichoderma virens* 159C (T2) as compared to positive control (T1).

Sundram et al., 2016

Trichotec

1. Mycoparasitism

2. Excellent competitor for nutrients

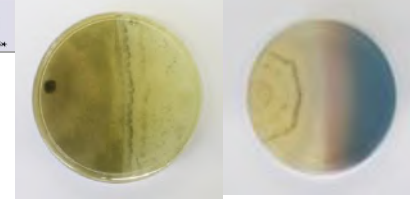


Endophytic *Trichoderma* isolation

Defense mechanism by TrichoTec®

An *in vitro* study of the antifungal activity of *Trichoderma virens* 7b and a profile of its non-polar antifungal components released against *Ganoderma boninense*⁵

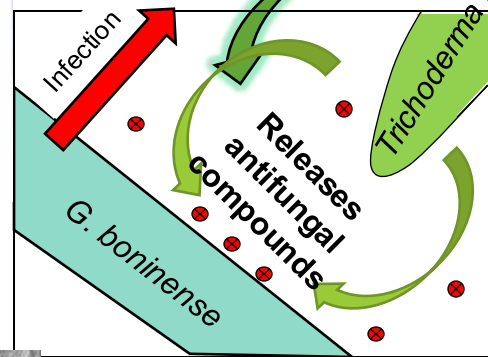
Lee Pei Lee Angel^{1,5}, Mohd Termizi Yusof¹,
Intan Safinar Ismail^{2,3}, Bonnie Tay Yen Ping⁴,
Intan Nur Ainni Mohamed Azni¹,
Norman Hi Kamarudin⁵ and Shamala Sundram^{5*}



T. virens 159c *G. boninense*

Fast release of iron chelators (siderophore) for uptake of irons

4. Antibiosis



3. Induces systemic resistance



Trichoderma spp. has been commercially introduced as biocontrol agent in the agricultural industry to enhance plant growth and defence mechanism (Chacón *et al.*, 2007; Angel *et al.*, 2016; Węgrzyn and Górzyska 2019), to provide a root-knot resistant tobacco plant (Pérez *et al.*, 2001). *T. virens* was mediated by *A. tumefaciens* AGL1 strain through T-DNA transfer. The experiment involved the transformation of plasmid containing SCGP (pCambiaGfp) into chemically competent *A. tumefaciens* AGL1, via heat shock method. Successful transformation of

Journal of Oil Palm Research Vol. 31 (4) December 2019 p. 573-581
DOI: <https://doi.org/10.21894/jopr.2019.0031>

INDUCED SYSTEMIC RESISTANCE AND PROMOTION OF PLANT GROWTH IN OIL PALM SEEDLINGS BY ENDOPHYTIC *Trichoderma virens*

FARAH AMIRA MD PAUDZAI¹; SHAMALA SUNDARAM^{1*}; MOHD TERMIZI YUSOF¹;
LEE PEI LEE ANGEL¹; AMALIA MOHD HASHIM¹ and SITI NOR AKMAR ABDULLAH^{1†}



Plants treated with *Trichoderma* (A) had better survival compared to plants without any treatment (B)

FIRST REPORT: ISOLATION OF ENDOPHYTIC *Trichoderma* FROM OIL PALM (*Elaeis guineensis* Jacq.) AND THEIR *in vitro* ANTAGONISTIC ASSESSMENT ON *Ganoderma boninense*

SHAMALA SUNDARAM*



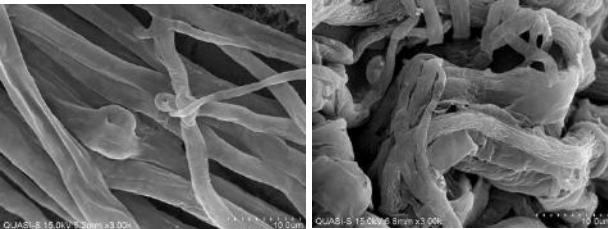
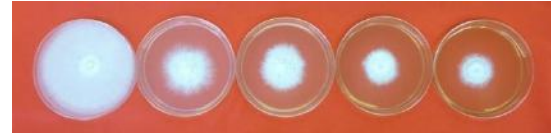
First reported!

PERSADANAN HARTA INTELEK MALAYSIA
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No. 5, Jalan Bersejarah, Tingkat 3,
50000 KUALA LUMPUR, MALAYSIA

CERTIFICATE OF FILING

APPLICANT : MALAYSIAN PALM OIL BOARD
APPLICATION NO : PI 2016700979
REQUEST RECEIVED ON : 18 MARCH 2016
FILING DATE : 18 MARCH 2016
AGENT'S/APPLICANT'S FILE REF. : P/MP/08/16MY06/TP

Please find attached, a copy of the Request Form relating to the above application, with the filing date and application number marked thereon in accordance with Regulation 26(1).



Trichoderma virens 159c

A novel antimicrobial compound identified and effective against *Ganoderma boninense* (PI2016700979)

Phenylethyl Alcohol (PEA)



AWARDS AND PATENTS



**NPS 2014
2nd Prize**



**ITEX 2015
Silver Medal**



**Patent (2016)
PI201670097**



**i-inova 2016
Silver Medal**



**ITEX 2017
Silver Medal**

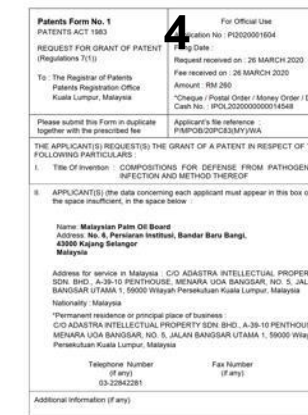


**ITEX 2020
MIPA**

**ITEX 2020
Silver Medal**

**MTE 2020
Gold Medal**

**Patent (2020)
PI202000160**



2013 2016 2018 2018 2018 2019

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Intellectual Property (IP)

Patent 2016

PI2016700979

	PERBADANAN HARTA INTELEK MALAYSIA INTELLECTUAL PROPERTY CORPORATION OF MALAYSIA Agensi di bawah KPDNKK Unit 1-7 & Mezzanine, Arah 12-13 Tower B, Menara UOA Bangsar No. 5, Jalan Bangsar Utama 1 59000 KUALA LUMPUR MALAYSIA	
CERTIFICATE OF FILING		
APPLICANT	: MALAYSIAN PALM OIL BOARD	
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AGENT'S/APPLICANT'S FILE REF.	: P/MPOB/16MY66/TP	
Please find attached, a copy of the Request Form relating to the above application, with the filing date and application number marked thereon in accordance with Regulation 25(1).		
Date	: 29 JUNE 2016	
 (NOORHISHAM BIN OTHMAN) For Registrar of Patents E: noorhisham@myipo.gov.my ☎ 03 - 22968612		
To	: MOHAN K. C/O ADASTRA INTELLECTUAL PROPERTY SDN. BHD., A-28-10, MENARA UOA BANGSAR, NO. 5, JALAN BANGSAR UTAMA 1 59000 KUALA LUMPUR MALAYSIA	
ff 02/02/2016		
(Agensi di bawah Kementerian Perdagangan Dalam Negeri, Koperasi dan Kepenggunaan)		
		

Patent 2020

PI2020001604

Patents Form No. 1 PATENTS ACT 1983 REQUEST FOR GRANT OF PATENT (Regulations 7(1)) To : The Registrar of Patents Patents Registration Office Kuala Lumpur, Malaysia	For Official Use Application No : PI2020001604 Filing Date : Request received on : 26 MARCH 2020 Fee received on : 26 MARCH 2020 Amount : RM 260 *Cheque / Postal Order / Money Order / Draft Cash No. : IPOL202000000014548 Please submit this Form in duplicate together with the prescribed fee
THE APPLICANT(S) REQUEST(S) THE GRANT OF A PATENT IN RESPECT OF THE FOLLOWING PARTICULARS :	
I. Title Of Invention : COMPOSITIONS FOR DEFENSE FROM PATHOGEN INFECTION AND METHOD THEREOF	
II. APPLICANT(S) (the data concerning each applicant must appear in this box or, if the space insufficient, in the space below :	
Name: Malaysian Palm Oil Board Address: No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang Selangor Malaysia Address for service in Malaysia : C/O ADASTRA INTELLECTUAL PROPERTY SDN. BHD., A-39-10 PENTHOUSE, MENARA UOA BANGSAR, NO. 5, JALAN BANGSAR UTAMA 1, 59000 Wilayah Persekutuan Kuala Lumpur, Malaysia Nationality : Malaysia *Permanent residence or principal place of business : C/O ADASTRA INTELLECTUAL PROPERTY SDN. BHD., A-39-10 PENTHOUSE, MENARA UOA BANGSAR, NO. 5, JALAN BANGSAR UTAMA 1, 59000 Wilayah Persekutuan Kuala Lumpur, Malaysia Telephone Number (if any) 03-22842281 Fax Number (if any)	
Additional Information (if any)	



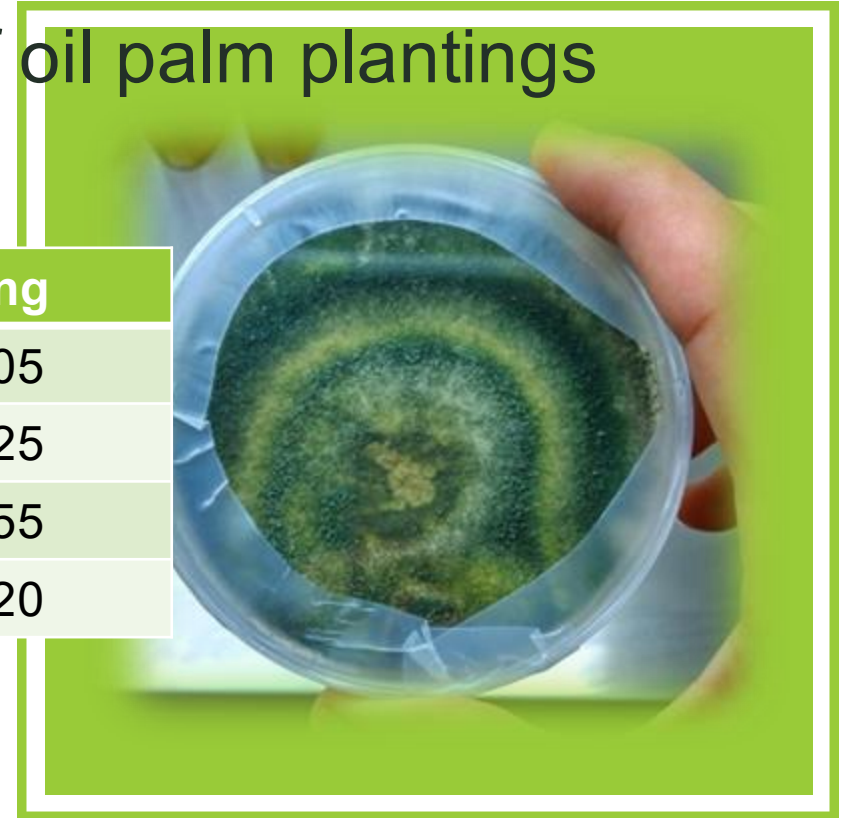
Trichotec

- ✓ is able to protect the roots from being infected by the previous stand's root fragments
- ✓ strengthens the palms – induce defense mechanisms (ISR).
- ✓ releases metabolites that promote increased root and vegetative growth.
- ✓ remediates poor soil condition in and around the rhizosphere. Able to increase soil ecosystem via microbial diversity

Recommendation

- An effective management of *Ganoderma* disease requires the application of TrichoTec®
- TrichoTec® application at different stages of oil palm plantings

Stages	Application	Concentration (cfu)	Costing
Pre-nursery	20 g	10^7	RM0.05
Nursery	100 g	10^7	RM0.25
Planting hole	1 kg	10^7	RM2.55
Existing planting	3 kg	10^4	RM4.20



Germinated Seed

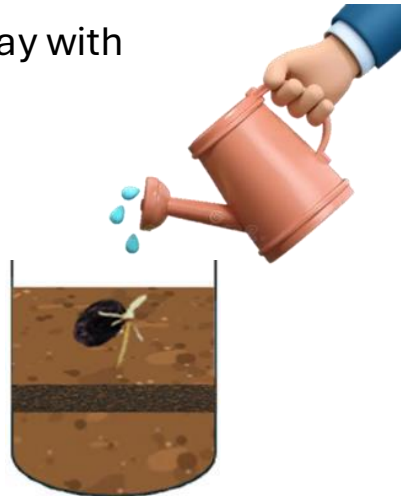


Add 20g of
TrichoTec



Fill the polybag halfway with
soil

Mix TrichoTec with soil, then add
another layer of soil



Cover the seed by adding a little soil
on top. Then water the polybag.



Using finger, poke a hole in the soil to make
space for the germinated seed. Place the seed in
the correct position.

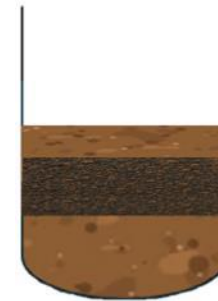
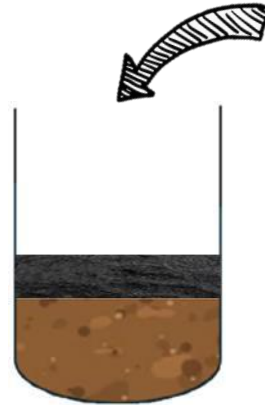
Main Nursery



Add 100g of
TrichoTec



Fill the polybag
halfway with soil



Mix TrichoTec with the soil, then add
another layer of soil on top.



Main Nursery



Place the seedling in the center
of the polybag



Fill it with soil until full
and water the polybag



1 Year Replanting Field

**The total usage of TrichoTec is 1kg, divide it into 3 parts.*



Make a hole in the soil



Add 250g of
Rock Phosphate



Add soil and apply one-third of
the TrichoTec product.

1 Year Replanting



Transplant the one-year-old oil palm seedling into the prepared hole.
Then, apply another one-third of the TrichoTec product around the hole

Cover the hole with soil.
The remaining one-third of the product should be
spread on top of the soil, close to the base of the seedling

Demonstration video



Field application at 6 monthly interval up to 3 years



Thank You

- A Collaborative R&D project between MPOB and Mygro Trading Sdn Bhd.
- Active Ingredient: *Trichoderma Viren 159C*

