Laporan Akhir Projek Penyelidikan IRPA
Kategori 'EAR'

Tajuk Projek	:	Collar Rot in CitrusReticulata and the Development of Bio-Fertilizers for Control Against The Disease
No. Projek	:	01-02-05-2047 EA004
Penyelidik	:	Prof. Madya Hasnah Md Jais

END OF PROJECT & BENEFITS REPORT

Please fill in the relevant details in the space provided.
Please tick (/) where appropriate and provide further description, if required.

I PROJECT IDENTITY

A. PROJECT DESCRIPTION

1 Project Number: **01-02-05-2047 EA 004**

2 Project Title: **Collar rot in *Citrus reticulata* and the development of bio-fertilizers for control against the disease.**

3 Project Leader's Details

Name: **Dr. Hasnah Md. Jais**

Position & Institution: **Lecturer**
Universiti Sains Malaysia, Pulau Pinang

Email: **mjhasnah@usm.my**

Contact No: **04-6534009**

Address *(if differ from RMC/HQ)*:

4 Total Budget Approved: RM **238,000.00**

5 Total Project Expenses: RM **238,000.00**

6 Project Duration: **36** months

Start Date: **1 Sept 2002** End Date: **31 Aug 2005**

B. OBJECTIVES OF THE PROJECT

1 Socio-economic Objectives (SEO)

1.1 What are the SEO addressed by your project?
(Please refer to the Malaysian R&D Classification System, 5th Edition.)

Research Priority Area (priority area): **BIOTECHNOLOGY**
SEO Category: **S5010000**
SEO Group: **S5011400**

2 Fields of Research (FOR)

2.1 What are the two main FOR of your project?
(Please refer to the Malaysian R&D Classification System, 5th Edition)

a. Primary FOR
FOR Category: **F1150401**
FOR Group: **F1150400**
FOR Area: **F1150403**

b. Secondary FOR
FOR Category: **F1150401**
FOR Group: **F1150400**
FOR Area: **F1150401**

C. OBJECTIVES ACHIEVEMENT

1 Original Project Objectives
(Please state the specific project objectives as described in Section II of the Application Form)

- To isolate and identify the causal agents of collar rot and the beneficial residential fungus and bacteria in the rhizosphere of Citrus reticulata.
- To develop healthy seedlings capable of resisting collar rot through the use of residential beneficial soil fungus and bacteria as a biocontrol.
- To develop a biofertilizer containing the beneficial microorganisms isolated from the residential soils.

2 Objectives Achieved
(Please state the extent to which the project objectives were achieved)

- The causal agent of collar rot was successfully isolated and identified as *Phytophtora sp.*
- Prepared bio-fertilizer inoculum which mainly consist of mycorrhiza was found to be capable of protecting inoculated plants from showing symptom throughout the period of this experiment while plants not inoculated with bio-fertilizer showed some mild symptoms of collar rot.
- A bio-fertilizer was hence successfully developed for future use in testing for effectiveness on other fruit crops.

3 Objectives Not Achieved
(Please state the objectives that were not achieved and provide reasons)

All three objectives were achieved however, development of symptom was mild in the control. This could be due to the watering conditions of the experiment where flooding is needed for the establishment of the disease. Full water logged conditions on the plants was not possible because in earlier preliminary experiment with water logged conditions, plants died due to the anaerobic condition of the roots.
The bio-fertilizer consists of mainly mycorrhizal fungus which is the main emphasis. Bacterial inoculum was not considered in the inoculum preparation.

D. TECHNOLOGY TRANSFER/COMMERCIALISATION APPROACH, IF ANY

(Please describe the approach planned to transfer/commercialize the results of your project)

More tests are required to confirm the effectiveness of the bio-fertilizer inoculum to resist other diseases especially on other crops beside *Citrus reticulata*

E. ASSESSMENT OF RESEARCH APPROACH

(Please highlight the main steps actually performed and indicate any major departure from the planned approach or any major difficulty encountered)

The following are the main steps carried out in the project:-

1. Preparation of biofertilizer
2. Microbial enrichment
3. Selection of the microbes
4. Isolation and identification of the pathogen
5. Bio-control experiment

There was no difficulty in achieving the first 4 steps of the project, however, the last step was the most difficult to achieve because the conditions set forth for the disease to occur also caused the plant i.e. the root region to suffocate due to the water logged condition. The last step is actually the most important step and we did not succeed in showing that bio-control occur due to mycorrhizal inoculation.

F. ASSESSMENT OF PROJECT SCHEDULE

(Please make any relevant comment regarding the actual duration of the project and highlight any significant variation from plan)

This project took 3 years (2002-05) to complete. Several difficulties encountered include the difficulty in obtaining diseased/infected plant *(Citrus reticulate)* i.e. plants infected with *Phytophtora.* Most of the plantations are individually owned by farmers and destructive sampling was not allowed on these farms. This is because the disease did not kill the plant.

G. ASSESSMENT OF PROJECT COSTS
(Please comment on the appropriateness of the original budget and highlight any major departure from the planned budget)

The budget was sufficient to buy all equipments etc as originally stated in the proposal.

H. ADDITIONAL PROJECT FUNDING OBTAINED
(In case of involvement of other funding sources, please indicate the source and total funding provided)

None

II BENEFITS OF THE PROJECT

A. Direct Outputs of the Project

1 Technical Contribution of the Project

1.1 What was the achieved direct output of your project?

a. Basic Research Project

☐ Algorithm
Please describe:

☐ Structure
Please describe:

☐ Data
Please describe:

☐ Others, please specify: _____

b. Applied Research Project

☒ Method/technique /process
Please describe: The project has succeeded in producing a protocol for mycorrhizal rich inoculum with coconut coir as the carrier having infective propagules of 784-3584 /100g soil by MPN count and spore count of about 100 spores/10g inoculums.
The product prepared in this project has won a silver medal in the Expo Science, Technology & Innovation 2004 27-29 August Kuala Lumpur for the invention entitled "MYCO-RICH an innovative smart bio-fertilizer, rich with locally isolated mycorrhiza"

☐ Demonstrator/laboratory prototype
Please describe:

☐ Product/component
Please describe:

☐ Software
Please describe:

☐ Others, please specify: _____

1.2 How would you characterize the quality of this output?

☐ Significant breakthrough

☐ Major improvement

☐ Minor improvement

2 Contribution of the Project to Knowledge

2.1 How has the output of your project been documented?

☐	Detailed project report	
☐	Product/process specification documents	
/	Others, please specify:	2 PhD thesis, 3 MSc thesis, 2 paper submitted to J Tropical Forest Science

2.2 What intellectual property (IP) did your project acquire?

		International	National
☐	Patent granted Please state Invention Title & Number: Inventor:	☐ quantity	☐ quantity
☐	Patent pending Please state Invention Title & Number: Inventor:	☐ quantity	☐ quantity
☐	Potential patent to be filed Please state Invention Title & Number: Inventor:	☐ quantity	☐ quantity
☐	Copyright Please state Invention Title & Number: Inventor:	☐ quantity	☐ quantity
☐	Trade Mark Please state Invention Title & Number: Inventor:	☐ quantity	☐ quantity
☐	Industrial Design Please state Invention Title & Number: Inventor:	☐ quantity	☐ quantity
/	Others, please specify:	Do not acquire anything yet	

2.3 What are the available publications of your project?

		International	National
☐	Article(s) in scientific publications Please state Title: Publication: Author(s): Year: Title: Publication: Author(s): Year:	☐ quantity	☐ quantity
☐	Paper(s) delivered at conference/seminar Please state Title: Publication: Author(s): Year: Title: Publication: Author(s): Year:	☐ quantity	☐ quantity
☐	Book(s) Please state Title: Publication: Author(s): Year:	☐ quantity	☐ quantity
☐	Others, please specify:		

Submitted to:

1. Manuscript (code 2009-0123) entitled: Genus of Vesicular-Arbuscular Mycorrhiza Within the Rhizospheres of *Octomeles sumatrana* and *Anthocephalus chinensis* in Niah, Sarawak, Malaysia by John Keen Chubo, Ong Kian Huat, **Hasnah Md. Jais,** Noor Faiqoh Mardatin, Nik Muhamad Nik Abdul Majid, submitted on April 8, 2009 for consideration for publication in *ScienceAsia*,
2. Ref.: Ms. No. BFSO-D-09-00016Chubo JK, Huat OK and **Jais HM** Morphological types of arbuscular mycorrhizal fungi in *Azadirachta excelsa* seedlings. Submitted to Journal: Biology and Fertility of Soils

2.4 How significant are citations of the results of your project?

		International	National
☐	Citations	☐ quantity	☐ quantity

Please state
Title:
Publication:
Author(s):
Year:

☐ None yet

☐ Not known

B ORGANIZATIONAL OUTCOMES OF THE PROJECT

1 Contribution of the Project to Expertise Development

1.1 How did the project contribute to expertise?

☒ PhD degrees

Please state
Name: John Kean Chubo
Nationality: Malaysian
Area of Specialisation: Specialization micorrhiza of forest plans
Duration: (2005 - 2009)

Name: Najihah Farhan Sabri
Nationality: Malaysian
Area of Specialisation: Mycorrhiza on *Durio*
Duration: (2005 - 2009)

☒ MSc degrees

Please state
Name: Norman Kasiran
Nationality: Malaysian
Area of Specialisation: mycorrhiza and bud-grafted nangka madu Mastura
Duration: (2006)

Name: Ng Lee Chu
Nationality: Malaysian
Area of Specialisation: Utilization of mycorrhiza on golf course grasses
Duration: (2006)

Name: Suhaizan Lob
Nationality: Malaysian
Area of Specialisation: Utilization of mycorrhiza on chili
Duration: (2009)

[/] Support staff with new specialty
Please state
Name: Encik Kamaruddin Maideen
Nationality: Malaysian
Area of Specialisation: Mycorrhiza internal identification enumeration
Duration: (Date Started - Date Completed)

[ ] Others, please specify: _____
Please state
Name:
Nationality:
Area of Specialisation:
Duration: (Date Started - Date Completed)

1.2 How significant is the expertise?

[ ] One of the key areas of priority for Malaysia

[ ] An important area, but not a priority one

[ ] Others, please specify: _____

C. INFRASTRUCTURAL CONTRIBUTION OF THE PROJECT

1 What are the infrastructure contributions of your project?

[/] New equipment
Please state:
The equipments are still in the lab (G-09 106) and still being used till present for other projects.

1. Image analyser-including the microscope (stereo and compound) and CCD camera
2. Lap top: twinhead
3. Printer: Cannon S6300- in good functioning condition
4. Scanner: Epson perfection 1650
5. Kjeldhal hot plate
6. Titrator UDK 126D
7. Refrigerator
8. Shredder

[ ] New/improved facility
Please state:

[ ] New information networks
Please state:

[ ] Others, please specify: _____

2 How significant is the infrastructural contribution to your Organization?

[ ] Very significant/significantly leverage other projects

[/] Moderately significant

[ ] Not significant/does not leverage other projects

D. CONTRIBUTION OF THE PROJECT TO THE ORGANIZATION'S REPUTATION

1 How has the project contributed in increasing the reputation of your Organization?

[ ] Recognition as a Center of Excellence

[/] National award
Please state:
The product prepared in this project has won a silver medal in the Expo Science, Technology & Innovation 2004 27-29 August Kuala Lumpur for the invention entitled "MYCO-RICH an innovative smart bio-fertilizer, rich with locally isolated mycorrhiza"

[ ] International award
Please state

[ ] Demand for advisory services

[ ] Invitations to give speeches on conferences

[ ] Visits from other organizations

[ ] Others, please specify: _____

E. CONTRIBUTION OF THE PROJECT CONTRIBUTED TO NATIONAL/INTERNATIONAL LINKAGES

1 What are the linkages created by your project?

[/] Domestic industry linkages
Please state: Sime Darby Plantation

[ ] International industry linkages
Please state:

[/] Linkages with domestic research institutions, universities
Please state: UPM, UiTM, UMT

[/] Linkages with international research institutions, universities
Please state: National Chung Hsing University, Taicung Taiwan

[ ] Others, please specify: _____

2 What is the nature of the linkages?

- ☐ Staff exchange
- ☐ Inter-organizational project team
- ☐ Research contract with a commercial client
- ☐ Informal consultation
- ☐ Others, please specify: _____

III PROPOSED FUTURE WORK

What are the proposed future work(s) of your project?

- ☐ Up scaling of laboratory prototype
- ☐ Development of commercial ready prototype
- ☐ Development of pilot plant
- ☐ Pre-clinical or Clinical trials/field trials
- ☐ Acquisition of foreign/local technology
- ☐ Others, please specify: _____

IV OTHER REMARKS

(Please include any other remarks which you feel is relevant to the evaluation of your project)

Date: 28.05.09 **Signature:** Hasnah Md. Jais

END OF PROJECT & BENEFITS REPORT

Please fill in the relevant details in the space provided.
Please tick (/) where appropriate and provide further description, if required.

I PROJECT IDENTITY

A. PROJECT DESCRIPTION

1 Project Number: 01-02-05-2047 EA 004

2 Project Title: **Collar rot in *Citrus reticulata* and the development of bio-fertilizers for control against the disease.**

3 Project Leader's Details

Name: Dr. Hasnah Md. Jais

Position & Institution: Lecturer Universiti Sains Malaysia 11800 Penang

Email: mjhasnah@usm.my

Contact No: 04 653 4009

Address *(if differ from RMC/HQ)*:

4 Total Budget Approved: RM 238 000.00

5 Total Project Expenses: RM 238 000.00

6 Project Duration: 36 months

Start Date: 1 Sept 2005 End Date: 31 Aug 2005

B. OBJECTIVES OF THE PROJECT

1 Socio-economic Objectives (SEO)NATURAL SCIENCES, TECHNOLOGIES AND ENGINEERING

1.1 What are the SEO addressed by your project?
(Please refer to the Malaysian R&D Classification System, 5th Edition.)

Research Priority Area (priority area):		BIOTECHNOLOGY
SEO Category:	S5010000	
SEO Group:	S5011400	

2 Fields of Research FOR) BIOTECHNOLOGY

2.1 What are the two main FOR of your project?
(Please refer to the Malaysian R&D Classification System, 5th Edition)

a. Primary FOR

FOR Category:	F1150401
FOR Group:	F1150400
FOR Area:	F1150403

b. Secondary FOR

FOR Category:	F1150401
FOR Group:	F1150400
FOR Area:	F1150401

C. OBJECTIVES ACHIEVEMENT

1 Original Project Objectives
(Please state the specific project objectives as described in Section II of the Application Form)

- To isolate and identify: the causal agents of collar rot and the beneficial residential fungus and bacteria in the rhizosphere of *Citrus reticulata*
- To develop healthy seedlings capable of resisting collar rot through the use of residential beneficial soil fungus and bacteria as a biocontrol
- To develop a biofertilizer containing the beneficial microorganisms isolated from the residential soils.

2 Objectives Achieved
(Please state the extent to which the project objectives were achieved)

- The causal agent of collar rot was successfully isolated and identified as *Phytophtora sp.*
- Prepared bio-fertilizer inoculum which mainly consist of mycorrhiza was found to be capable of protecting inoculated plants from showing symptom throughout the period of this experiment while plants not inoculated with bio-fertilizer showed some mild symptoms of collar rot
- A bio-fertilizer was hence successfully developed for future use in testing for effectiveness on other fruit crops

3 Objectives Not Achieved

(Please state the objectives that were not achieved and provide reasons)

All three objectives were achieved however, development of symptom was mild in the control. This could be due to the watering conditions of the experiment where flooding is needed for the establishment of the disease. Full water logged conditions on the plants was not possible because in earlier preliminary experiment with water logged conditions, plants died due to the anaerobic condition of the roots.
The bio-fertilizer consists of mainly mycorrhizal fungus which is the main emphasis. Bacterial inoculum was not considered in the inoculum preparation.

D. TECHNOLOGY TRANSFER/COMMERCIALISATION APPROACH, IF ANY

(Please describe the approach planned to transfer/commercialize the results of your project)

More tests are required to confirm the effectiveness of the bio-fertilizer inoculum to resist other diseases especially on other crops beside *Citrus reticulata*

E. ASSESSMENT OF RESEARCH APPROACH

(Please highlight the main steps actually performed and indicate any major departure from the planned approach or any major difficulty encountered)

The following are the main steps carried out in the project:-

1. Preparation of biofertilizer
2. Microbial enrichment
3. Selection of the microbes
4. Isolation and identification of the pathogen
5. Bio-control experiment

There was no difficulty in achieving the first 4 steps of the project, however, the last step was the most difficult to achieve because the conditions set forth for the disease to occur also caused the plant i.e. the root region to suffocate due to the water logged condition. The last step is actually the most important step and we did not succeed in showing that bio-control occur due to mycorrhizal inoculation.

F. ASSESSMENT OF PROJECT SCHEDULE

(Please make any relevant comment regarding the actual duration of the project and highlight any significant variation from plan)

This project took 3 years (2002-05) to complete. Several difficulties encountered include the difficulty in obtaining diseased/infected plant *(Citrus reticulate)* i.e. plants infected with *Phytophtora*. Most of the plantations are individually owned by farmers and destructive sampling was not allowed on these farms. This is because the disease did not kill the plant.

G. ASSESSMENT OF PROJECT COSTS

(Please comment on the appropriateness of the original budget and highlight any major departure from the planned budget)

The budget was sufficient to buy all equipments etc as originally stated in the proposal.

H. ADDITIONAL PROJECT FUNDING OBTAINED

(In case of involvement of other funding sources, please indicate the source and total funding provided)

None

II BENEFITS OF THE PROJECT

A. Direct Outputs of the Project

1 Technical Contribution of the Project

1.1 What was the achieved direct output of your project?

a. Basic Research Project

Algorithm
Please describe:

Structure
Please describe:

Data
Please describe:

Others, please specify:

b. Applied Research Project

Method/technique /process
The project has succeeded in producing a protocol for mycorrhizal rich inoculum with coconut coir as the carrier having infective propagules of 784-3584 /100g soil by MPN count and spore count of about 100 spores/10g inoculums.

The product prepared in this project has won a silver medal in the Expo Science, Technology & Innovation 2004 27-29 August Kuala Lumpur for the invention entitled "MYCO-RICH an innovative smart bio-fertilizer, rich with locally isolated mycorrhiza"

Demonstrator/laboratory prototype
Please describe:

Product/component
Please describe:

Software
Please describe:

Others, please specify:

1.2 How would you characterize the quality of this output?

Significant breakthrough

Major improvement

Minor improvement

2 Contribution of the Project to Knowledge

2.1 How has the output of your project been documented?

Detailed project report

Product/process specification documents

Others, please specify: 2 Ph D thesis and 3 M Sc thesis plus 2 paper submitted to J Tropical Forest Science

2.2 What intellectual property (IP) did your project acquire?

	International	National
Patent granted Please state Invention Title & Number: Inventor:	quantity	quantity
Patent pending Please state Invention Title & Number: Inventor:	quantity	quantity
Potential patent to be filed Please state Invention Title & Number: Inventor:	quantity	quantity
Copyright Please state Invention Title & Number: Inventor:	quantity	quantity
Trade Mark Please state Invention Title & Number: Inventor:	quantity	quantity
Industrial Design Please state Invention Title & Number: Inventor:	quantity	quantity

Others, please specify: Do not acquire anything yet.

2.3 What are the available publications of your project?

	International	National
Article(s) in scientific publications	quantity	quantity
Please state		
Title:		
Publication:		
Author(s):		
Year:		
Title:		
Publication:		
Author(s):		
Year:		
Paper(s) delivered at conference/seminar	quantity	quantity
Please state		
Title:		
Publication:		
Author(s):		
Year:		
Title:		
Publication:		
Author(s):		
Year:		
Book(s)	quantity	quantity
Please state		
Title:		
Publication:		
Author(s):		
Year:		

Others, please specify: Submitted to.....

1. Manuscript (code 2009-0123) entitled: Genus of Vesicular-Arbuscular Mycorrhiza Within the Rhizospheres of *Octomeles sumatrana* and *Anthocephalus chinensis* in Niah, Sarawak, Malaysia by John Keen Chubo, Ong Kian Huat, **Hasnah Md. Jais,** Noor Faiqoh Mardatin, Nik Muhamad Nik Abdul Majid, submitted on April 8, 2009 for consideration for publication in *ScienceAsia,*
2. Ref.: Ms. No. BFSO-D-09-00016Chubo JK, Huat OK and **Jais HM** Morphological types of arbuscular mycorrhizal fungi in *Azadirachta excelsa* seedlings. Submitted to Journal: Biology and Fertility of Soils

2.4 How significant are citations of the results of your project?

Citations	International quantity	National quantity
Please state Title: Publication: Author(s): Year:		
None yet		
Not known		

B ORGANIZATIONAL OUTCOMES OF THE PROJECT

1 Contribution of the Project to Expertise Development

1.1 How did the project contribute to expertise?

PhD degrees
Please state
The project has benefited 2 Ph D student who are still on-going

1. John Kean Chubo-Malaysian- specialization micorrhiza of forest plants-he has submitted his thesis and waiting for viva; 2005-2009
2. Najihah Farhan Sabri- a Malaysian- mycorrhiza on *Durio*- has submitted the notice for thesis submission; 2005-2009

MSc degrees
Please state The project has produced 3 M Sc graduates, namely:

1. **Norman Kasiran- Malaysian-working with mycorrhiza and bud-grafted nangka madu Mastura (2006)**
2. **Ng Lee Chu- Malaysian-thesis prepared was on utilization of mycorrhiza on golf course grasses (2007)**
3. **Suhaizan bt Lob- Malaysian- thesis prepared was on utilization of mycorrhiza on chili.(2009)**

Support Staff : Lab assistant
En. Kamaruddin Maideen- Malaysian-mycorrhiza internal identificationand enumeration

Others, please specify:
Please state
Name:
Nationality:
Area of Specialisation:
Duration: (Date Started - Date Completed)

1.2 How significant is the expertise?

One of the key areas of priority for Malaysia

An important area, but not a priority one

Others, please specify:

C. INFRASTRUCTURAL CONTRIBUTION OF THE PROJECT

1 What are the infrastructure contributions of your project?

New equipment
Please state: The equipments are still in the lab (G-09 106) and still being used till present for other projects.

1. Image analyser-including the microscope (stereo and compound) and CCD camera
2. Lap top: twinhead
3. Printer: Cannon S6300- in good functioning condition
4. Scanner: Epson perfection 1650
5. Kjeldhal hot plate
6. Titrator UDK 126D
7. Refrigerator
8. Shredder

New/improved facility
Please state:

New information networks
Please state:

Others, please specify:

2 How significant is the infrastructural contribution to your Organization?

Very significant/significantly leverage other projects

Moderately significant

Not significant/does not leverage other projects

D. CONTRIBUTION OF THE PROJECT TO THE ORGANIZATION'S REPUTATION

1 How has the project contributed in increasing the reputation of your Organization?

Recognition as a Center of Excellence

National award
Please state
The product prepared in this project has won a silver medal in the Expo Science, Technology & Innovation 2004 27-29 August Kuala Lumpur for the invention entitled "MYCO-RICH an innovative smart bio-fertilizer, rich with locally isolated mycorrhiza"

International award
Please state
Type:
Category:

Demand for advisory services

Invitations to give speeches on conferences

Visits from other organizations

Others, please specify:

E. CONTRIBUTION OF THE PROJECT CONTRIBUTED TO NATIONAL/INTERNATIONAL LINKAGES

1 What are the linkages created by your project?

Domestic industry linkages
Sime Darby Plantation

International industry linkages
Please state:

Linkages with domestic research institutions, universities
UPM, UITM, UMT

Linkages with international research institutions, universities
National Chung Hsing University,
Taicung Taiwan

Others, please specify:

2 What is the nature of the linkages?

Staff exchange

Inter-organizational project team

Research contract with a commercial client

Informal consultation

Others, please specify:

III PROPOSED FUTURE WORK

What are the proposed future work(s) of your project?

Up scaling of laboratory prototype

Development of commercial ready prototype

Development of pilot plant

Pre-clinical or Clinical trials/field trials

Acquisition of foreign/local technology

Others, please specify:

IV OTHER REMARKS

(Please include any other remarks which you feel is relevant to the evaluation of your project)

Date: 28.05.09 **Signature:** Hasnah Md. Jais