J. Rubb. Res., **21(1)**, 62-72

Antagonistic Effects of Fungicolous Ascomycetous Cladobotryum Semicirculare *on* Rigidoporus Microporus *White Root Disease in Rubber Trees (*Hevea Brasiliensis) *under* in vitro *and Nursery Experiments*

Y.K. GOH[*#], N.F. MARZUKI[*], Y.A. LIEW[*], Y.K. GOH[*] AND K.J. GOH[*]

White root disease caused by the pathogenic Rigidoporus microporus *(Sw.) Overeem, in rubber trees (*Hevea brasiliensis *Muell. Arg.) could limit latex production by reducing the number of productive rubber trees. Fungicolous ascomycetous* Cladobotryum semicirculare *G.R.W. Arnold, R. Kirschner & Chee J. Chen, was assessed for its capability of suppressing* Rigidoporus microporus *mycelia growth in vitro and its efficacy in reducing white root disease of rubber seedlings. Mycelia growth of* Rigidoporus microporus *was inhibited by* Cladobotryum semicirculare *mycelia (direct antagonism) with 79% inhibition at nine days-after-inoculation through a dual - culture assay and heat stable antifungal* Cladobotryum semicirculare *filtrates (exudates sterilised through either filter-sterilisation or autoclaving - antibiosis) followed by the poison agar test.* Cladobotryum semicirculare *was also found to reduce the regeneration of* Rigidoporus microporus *mycelia. Area under the disease progress curve, calculated using disease severity of white root disease, were reduced by 47 to 50% through application of* Cladobotryum semicirculare *inoculant on two separate scion-rootstocks (PB 350 – RRIM 2025 and PB 347 – RRIM 2025) combinations of rubber clones, respectively. There was no scion-rootstock interaction observed.*

Keywords: Antifungal; ascomycetes; basidiomycetes; disease severity; dual-culture; fungal extract; *hypomyces*; rhizomorph

Basidiomycetous *Rigidoporus microporus* (Rm) (Sw.) Overeem (synonyms *Fomes lignosus* (Klotzsch) Bres. or *R. lignosus* (Klotzsch) Imazeki), the primary causal agent of rubber (*Hevea brasiliensis* Muell. Arg.) white root disease (WRD), has been reported as one of the most serious fungal root diseases in rubber plantations in South East Asian (SEA) countries, including Malaysia, Indonesia, Thailand, and Philippines, as well as Sri Lanka and Africa (West and Central regions)[1–4]. WRD of rubber trees is causing economic losses of up to several millions dollars (USD) annually in SEA and other rubber planting countries through reducing the economical lifespan of rubber trees, as well as costs required for disease detection and treatment[5-6].

Rm possesses highly branched rhizomorphic structures for ectotrophic growth that are capable of attaching and penetrating healthy plant roots and non-host food sources to extract nutrients[4].

[*] Advanced Agriecological Research Sdn Bhd, 11 Jalan Teknologi 3/6, Taman Sains Selangor 1, Kota Damansara, 47810 Petaling Jaya, Selangor Darul Ehsan, Malaysia.
[#] Correspondence author: (e-mail: gohykheng@aarsb.com.my)

This allows it to spread from one infected tree to other healthy trees in a relatively fast pace. Some of the commonly adopted disease management measures are detection (foliar symptoms and collar inspection) and treatment with systemic fungicides[7], as well as digging trenches to minimise contact between infected and healthy trees[4]. Various other control measures have been outlined by Mohammed *et al.*[4] as well.

In the past decade, several common beneficial antagonistic fungal species from the genera of *Trichoderma, Chaetomium,* and *Aspergillus* species, have been studied for their efficacy in suppressing WRD of rubber trees[8–9]. Go *et al.*[10] isolated a few *Trichoderma* species and *Chaetomium cupreum* in soil samples collected from healthy rubber trees and regrowth of trees after WRD infection with a potential to be studied for suppressing Rm in rubber.

In one of the studies on screening for potential biocontrol agents against *Ganoderma boninense* Pat., causal agent of basal stem rot (BSR) disease in oil palm, a fungicolous ascomycetous *Cladobotryum semicirculare* (Cs) G.R.W. Arnold, R. Kirschner & Chee J. Chen, was found to sporulate on the fruiting bodies of *G. boninense*[11]. In the same study, Cs suppressed the growth of *G. boninense* and a few other tested fungal candidates. However, the ability of this fungus in inhibiting Rm is yet to be evaluated. There is limited information related to fungicolous fungi and their association with Rm, as well as the uses of fungicolous fungi for controlling Rm. Presently, only two fungicolous fungal isolates, namely *Hypomycetes chrysostomus* Berkeley and Broome (Anamorph *Acremonium lindtneri* (Kirschst.) and *H. lanceolatus* were isolated from fruiting bodies of Rm[12].

This preliminary study was conducted to evaluate the antagonistic activity of Cs in suppressing Rm in view of WRD in rubber under *in vitro* and nursery conditions. The efficacy of Cs in reducing incidences and severity of Rm in WRD of rubber seedlings was studied with two separate scion-rootstocks (PB 350 – RRIM 2025 and PB 347 – RRIM 2025) combinations of rubber clones in a nursery experiment.

EXPERIMENTAL

Fungal Isolates and Culture Conditions

Cs isolate AAS0114[11] and Rm isolate AA0001 isolates (courtesy of Dr. Murnita from RRIM – Rubber Research Institute Malaysia) were cultured and maintained on malt extract agar (MEA) (Difco, Becton Dickinson Diagnostics, Sparks, Maryland) prior to both *in vitro* and *in vivo* studies.

Molecular and Phylogenetic Analyses of Rm

Genomic DNA of the Rm isolate AA0001 was extracted using a FastDNA Spin Kit (MP Biomedicals, USA) as per manual instructions. Primer set *i.e.* ITS1 and ITS4 were used for targeting internal transcribed spacer (ITS) region[13] in the polymerase chain reaction (PCR). Preparation and processing of PCR mixtures, amplification conditions, purification and sequencing were conducted according to Marzuki *et al.*[11]. ITS sequence from the Rm was submitted to GenBank with an accession number of MF574815. Eighteen Rm and three *R. ulmarius* (Sowerby) Imazeki ITS sequences (accession numbers for the *Rigidoporus* isolates used are outlined in *Figure 1A*)[14] retrieved from GenBank and Rm ITS sequence from the present study

(MF574815) were aligned and edited with the MEGA6 software[15]. Neighbour joining (NJ) analysis was conducted using the MEGA6 software (bootstrap – 1000 repetitions) and a phylogenetic tree was generated with sequences illustrating bootstrap values of higher than 50%. Trees based on ITS sequences were rooted with *Oxyporus corticola* (Fr.) Ryvarden (KC176676) sequence as the out group. Alignment has been deposited and is available on treebase. org at the following link http://purl.org/phylo/treebase/phylows/study/TB2:S21454

In Vitro Dual-Culture, Mycoparasitism Tests and Poison Agar Assay

Percentage inhibition of radial growth (PIRG) (%) of Rm by Cs was assessed using dual-culture assays. Measurements were recorded on five, seven, and nine days-after-inoculation (DAI) according to Marzuki *et al*.[11]. *In vitro* mycoparasitism test of Cs on Rm was performed according to the procedures outlined in Goh *et al*.[16] and Marzuki *et al.*[11]. Two week old Rm-colonised petri dishes were inoculated with a mycelial plug of Cs, and the plates were incubated for an additional two-weeks prior to excising two mycelial plugs from *Rigidoporus* mycelium colonised by Cs for re-isolation of Rm. Fungal filtrates from Cs were prepared according to Sundram[17] and Marzuki *et al.*[11], and incorporated into a malt extract broth (MEB) for the poison agar assay. Percentage inhibition of mycelial growth (PIMG) (%) of Rm on MEB amended with Cs filtrates was recorded and calculated on five DAI. Five replicates of the respective treatments were adopted for both *in vitro* dual-culture and poison agar tests. *In vitro* mycoparasitism test were carried out in six replications.

Nursery Study

Approximately two-month-old rubber seedlings (with two whorls of leaves) from two separate scion-rootstock combinations (PB 350 grafted onto RRIM 2025 and PB 347 budded onto RRIM 2025) of rubber clones, planted in 18 x 38 cm black polythene bags were used to evaluate the ability of Cs in reducing disease incidence (DI) and severity (DSI) caused by Rm in the nursery. Artificial inoculation of Rm onto 6 x 6 x 3 cm rubber wood blocks (RWB) was prepared according to Kok *et al*.[18]. Blended Cs-inoculated maize was prepared according to Goh *et al.*[16]. Five different treatments were selected for this study as follows:

(i) Non-inoculated RWB (+RWB-Rm-Cs);
(ii) Rm-inoculated RWB (+RWB+Rm-Cs);
(iii) Non-inoculated RWB amended with 50 g of Cs (+RWB-Rm+Cs);
(iv) Rm inoculated RWB with 50 g of Cs (+RWB+Rm+Cs); and
(v) Without RWB (-RWB-Rm-Cs)

During the transplanting, the 18 x 38 cm polybag was removed and the rubber seedling and soil core (indicated as Soil A in *Figure 1B*) was then planted into a bigger polythene bag (38 x 51 cm) filled with Bungor series soil (*Typic Paleudult*) (indicated as Soil B in *Figure 1B*) for the treatment of -RWB-Rm-Cs. For treatments with *Rigidoporus*-inoculated (+RWB+Rm-Cs and +RWB+Rm+Cs) or non-inoculated RWB (+RWB-Rm-Cs and +RWB-Rm+Cs), two pieces of *Rigidoporus*-inoculated or non-inoculated RWB (in the size of 6 x 6 x 3 cm) were placed in between Soil A and Soil B (approximately 2 cm from the soil surfaceas as indicated in *Figure 1B*).

50 g of blended Cs inoculant was applied around and on top of RWB, and near the base of rubber seedlings prior to covering back the hole with soil for +RWB+Rm+Cs and +RWB-Rm+Cs treatments, whereas 50 g of non-inoculated blended maize was applied in similar procedures to the treatments of +RWB+Rm-Cs and +RWB-Rm-Cs. Five replicates for the respective treatments were used in the current nursery experiment. Randomised complete block design (RCBD) was adopted in this study.

General observations and disease scorings DI – percentage of infected seedling and DSI for the presence of potential *Rigidoporus* infection symptoms were recorded at two-weekly intervals up to four months-post-inoculation (MPI). However, only monthly results were illustrated in *Table 1*. Disease classes adopted for DSI calculation were outlined in Kaewchai and Soytong [9]. The area under the disease progress curve (AUDPC) based on DSI from one to four MPI was generated using the formula proposed by Shaner and Finner[19] and percent of disease reduction was determined based on AUDPC values.

Statistical Analysis

Differences in means for percent of inhibition in radial growth (PIRG) (%) of Rm challenged with Cs on dual-culture assay among three different time points, namely five, seven, and nine DAI, as well as percent age inhibition of mycelial growth (PIMG) (%) of Rm inoculated on media amended with two different concentrations of Cs's filter-sterilised filtrates (50 and 100%) and autoclaved filtrate (100%) were analysed with ANOVA-Tukey test at $P = 0.05$ using Minitab 16 (Minitab Inc., State College, PA). Means for DSI of rubber seedlings artificially inoculated with Rm at two, three, and four MAT were not normally distributed. Therefore, the differences in means of DSI among the treatments at three separate time points were analysed with the Kruskal-Wallis test and followed by Mann-Whitney U test at $P = 0.05$ using Minitab 16.

RESULTS AND DISCUSSION

ITS sequence of approximately 486 base pairs derived from Rm selected for the current study was used for the phylogenetic analysis. Together with 18 other Rm (isolates from three different regions – Africa, Asia, and South or Central America) and three *R. ulmarius* ITS sequences obtained from GenBank a phylogenetic tree using NJ subjected to 1000 bootstrap replications was produced to illustrate position of the Rm isolate used (*Figure 1A*). Based on the origins of the Rm isolates, Oghenekaro *et al.*[14] showed Rm isolates can be grouped into three clades (Clade I – Africa; Clade II – Asia; and Clade III – South or Central America). Rm isolate AA0001 selected for this study has been grouped together with other Rm isolates originating from Asia (Clade II) (*Figure 1A*).

In vitro dual-culture assays showed approximately 69 to 70% and 79% of Rm radial mycelial growth suppression by Cs, on five to seven and nine DAI, respectively (*Figures 2A* and *3A*). In a study by Marzuki *et al.* [11], Cs was able to inhibit the growth of other phytopathogenic fungal pathogens as well. Furthermore, *in vitro* pathogenicity test of direct inoculation of Cs onto a 14-day old culture plate fully covered with Rm and Cs was allowed to proliferate for an additional 14 days. Rm mycelia plugs were excised and transferred to fresh MEA medium. There was no re-isolation or recovery of Rm and regeneration of Rm was suppressed (0% regeneration; n = 6) (*Figures 3B* and *C*)

TABLE 1. EFFECTS OF Cs ON DEVELOPMENT OF WRD DISEASE BY Rm IN TWO SCION-ROOTSTOCK COMBINATIONS OF RUBBER CLONES

Planting materials ‡	Treatments	DI (%)ǂǂ			DSI (%)††			AUDPC (%)∞	DR (%)Δ
		2 MAT†	3 MAT	4 MAT	2 MAT	3 MAT	4 MAT		
PB 350 – RRIM 2025	+RWB-Rm-Cs	0	0	0	0 a	0 b	0 b	0	
	+RWB+Rm-Cs	20	80	100	16.67 a	50 a	60 a	30	
	+RWB-Rm+Cs	0	0	0	0 a	0 b	0 b	0	
	+RWB+Rm+Cs	20	20	60	3.33 a	10 ab	30 ab	15	50
	-RWB-Rm-Cs	0	0	0	0 a	0	0	0	
PB 347 – RRIM 2025	+RWB-Rm-Cs	0	0	0	0 a	0 b	0 b	0	
	+RWB+Rm-Cs	40	60	80	6.67 a	23.33 ab	50 a	25	
	+RWB-Rm+Cs	0	0	0	0 a	0 b	0 b	0	
	+RWB+Rm+Cs	0	40	40	0 a	13.33 ab	26.67 ab	13.34	46.66
	-RWB-Rm-Cs	0	0	0	0 a	0 b	0 b	0	

* Treatments: +RWB-Rm-Cs – with non-inoculated rubber wood block (RWB) only; +RWB+Rm-Cs – with *Rm* inoculated RWB only; +RWB-Rm+Cs – with non-inoculated RWB and *Cs* inoculum; +RWB+Rm+Cs – with Rm-inoculated RWB and Cs inoculum; and without both RWB and Cs inoculum.

‡ Two planting materials/clones used were PB 350 and PB 347 scions budded onto the RRIM 2025 rootstock: PB 350 – RRIM 2025 and PB 347-RRIM 2025 scion-rootstock combinations.

† MAT refers to month-after-transplanting.

ǂǂ DI (%) refers to percent of infected seedlings.

†† DSI (%) = (number of seedlings in the scoring x scoring number/disease class value)/(total number of seedlings assessed x highest scoring or disease class value). DSI for the respective treatments at 2, 3, and 4 MAT presented as mean of 5 replications, and DSI at different MAT were analysed separately. Means within each column of MAT followed by same letter are not significantly different at $P = 0.05$ after Kruskall-Wallis test followed by Mann-Whitney test.

∞ AUDPC refers to Area Under Disease Progress Curve.

Δ DR refers to Percent Disease Reduction in percent of AUDPC = {[(+RWB+Rm-Cs) - (+RWB+Rm+Cs)]/(+RWB+Rm-Cs)} x 100

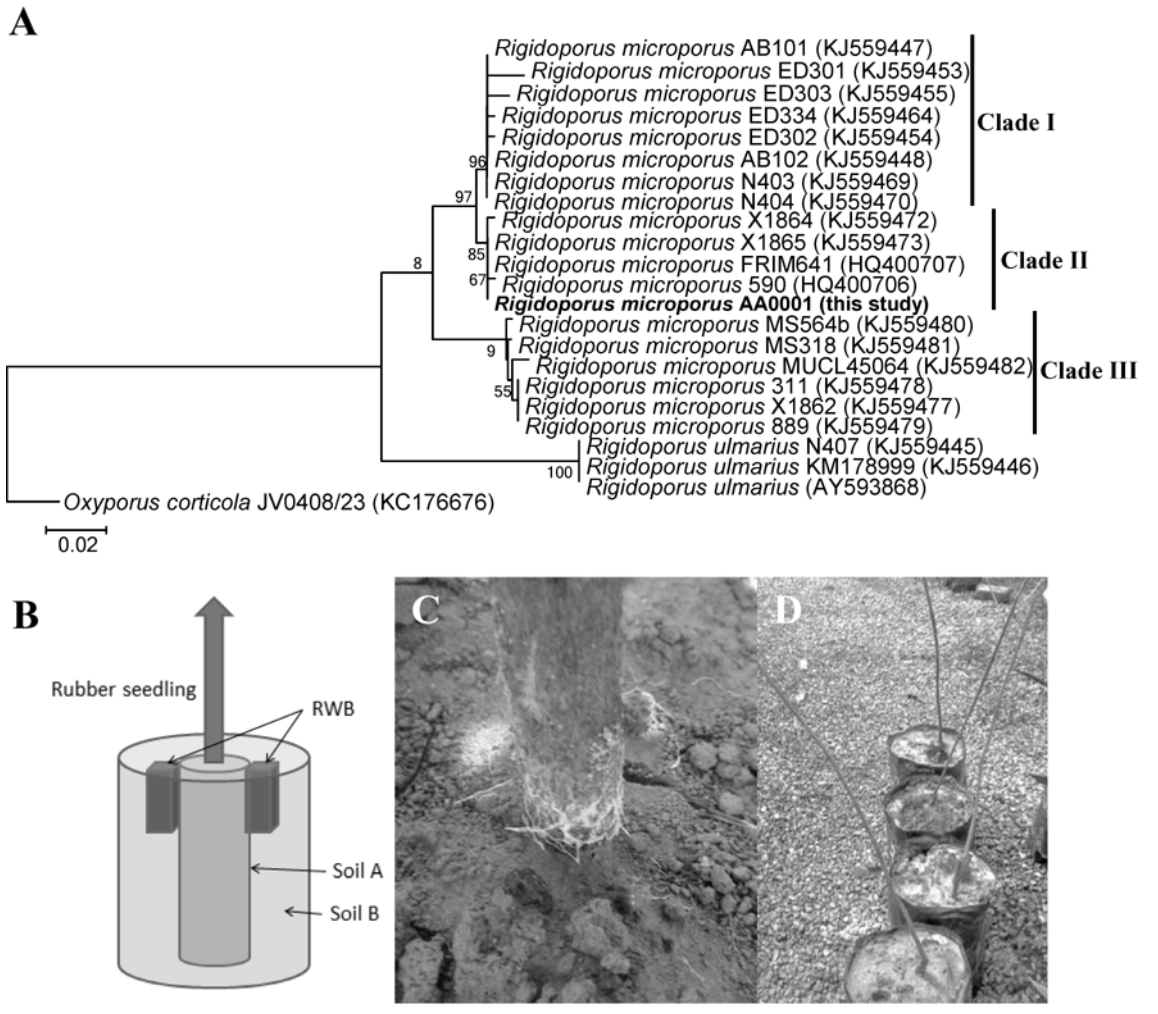

Figure 1. Phylogenetic tree based on ITS sequences illustrating the position of Rm for the current study (in bold) among Rm isolates from Africa (Clade I), Asia (Clade II), and South or Central America (Clade III) (Oghenekaro et al. [14]) using NJ analysis with 1000 bootstrap replications and only bootstrap values of 50% or higher were shown in the tree (A); Transplanting and artificial inoculation practice for in vivo *pathogenicity test with abbreviations of RWB, Soil A, and Soil B indicate rubber wood block (*Rigidoporus*-inoculated or non-inoculated), soil core from pre-nursery, and Bungor series soil, respectively (B); Rhizomorph structures produced by* Rm *and travelled to the stem and roots of rubber seedlings (C); and diseased and dead rubber seedlings with no leaf (D).*

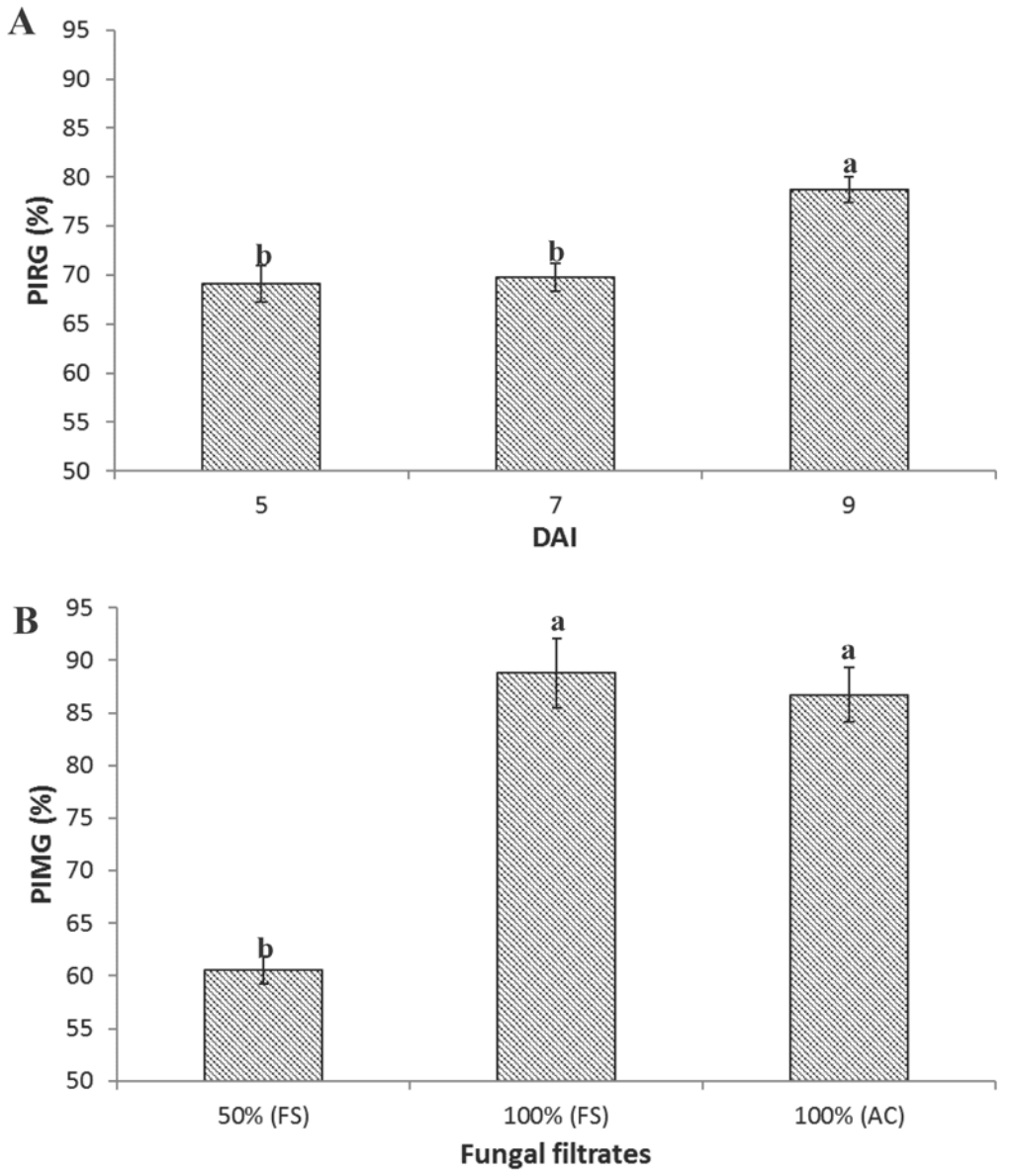

Figure 2. Percent of inhibition in radial growth (PIRG) (%) of Rm co-inoculated with Cs on dual-culture assays on five, seven, and nine days-after-inoculation (DAI) (A); and percent of inhibition in mycelial growth (PIMG) (%) of Rm inoculated on MEA amended with Cs filtrates at two separate concentrations (50 and 100%) obtained through filter-sterilisation (FS) and fungal filtrate achieved through autoclaving (AC) (100%) for poison agar tests (B). PIRG (%) and PIMG (%) (error bars indicate standard errors) for dual-culture and poison agar assays were analysed separately. Means of PIRG (%) at three different time points and PIMG (%) for three separate fungal filtrates (concentrations and method of sterilisation) followed by the same letter are not significantly different at P = 0.05 after ANOVA-Tukey test.

compared to the control (only with Rm) (100% regeneration; n = 6) (*Figure 3D*). On the contrary, in a previous study, regeneration of eight other fungal candidates (*Schizophyllum commune, Pleurotus* sp., *Cochiobolus* sp., *G. lucidum, G. australe,* and three *G. boninense* isolates), ranged from 33 to 100% when the cultures were inoculated with *Cs*[11].

Fungal filtrates from Cs at the concentration of 100% (undiluted), sterilised through filter-sterilisation (FS) and autoclaving (AC) approaches showed approximately 87 to 89% inhibitions in the growth of Rm under poison food assays, and the inhibition levels were significantly higher compared to medium amended with Cs fungal filtrates at the concentration of 50% (filter-sterilised extracts) (Tukey's test, $P = 0.05$) (*Figure 2B*). Similar observations were reported in previous studies where increased concentrations of fungal filtrates from potential biocontrol agents, namely *Cladobotryum, Trichoderma*, and *Scytalidium* species were reported to contribute to an increased in the inhibition levels for pathogenic *G. boninense* [11,16,17]. The fungal filtrates subjected to autoclaving at 121°C for one hour which still had inhibitory effects toward Rm could be further explored in future studies for heat stable antimicrobial compounds. More in-depth studies on the effects of concentrated fungal liquid filtrates or fungal extracts derived from extraction with different solvents could be conducted to find out the minimum dosage for the effective Rm inhibition in both laboratory and field experiments.

Two separate scion-rootstock combinations of rubber clones (PB 350-RRIM 2025 and PB 347 – RRIM 2025) were incorporated into this study to determine the effect of Rm WRD on scion-rootstock

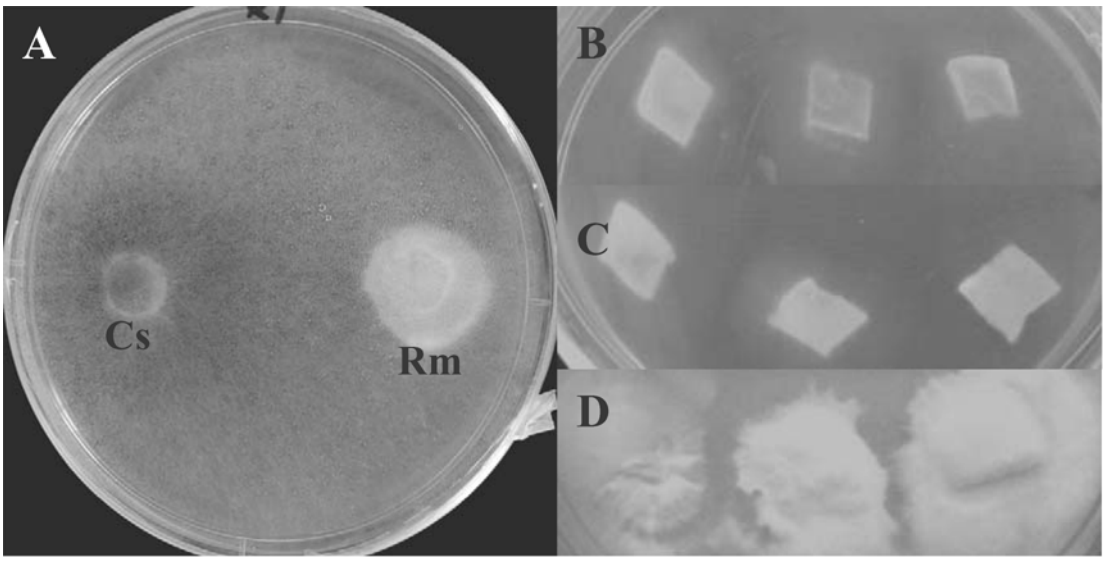

Figure 3. In vitro *dual-culture assay between Cs and Rm on nine day-after-inoculation (A) and* in vitro *mycoparasitism test (B-C).* In vitro *mycoparasitism test: Mycelial plugs excised from Rm culture challenged with Cs (D) and from* Rm *only control plates, and the newly excised plugs were transferred onto fresh malt extract agar (MEA).*

interactions. However, there was no scion-rootstock interaction observed. The results summarised in *Table 1* illustrated both scion-rootstock combinations (PB 350 – RRIM 2025 and PB 347 – RRIM 2025) were found to have relatively high DSI (*Table 1*). Whitish rhizomorphic structures were found on the soil surface and base of the stem or rootstock in the nursery as early as one and a half MAT (*Figure 1C*). Furthermore, one of the PB 350 – RRIM 2025 seedlings died around one and a half MAT. Leaves and stems of diseased seedlings desiccated and resulted in the death of rubber seedlings (*Figure 1D*). At two MAT, more seedlings showed yellowing or chlorotic leaves (data not shown) and they were also detected with rhizomorphic structures at the stem base (*Figure 1C*). Leaf symptoms observed were similar to a few previous reports[8–9]. Both DI and DSI of five separate treatments at four different time points, namely two to four MATs for two separate clones were summarised in *Table 1*. AUDPC (%) was lower in the treatments applied with Cs (+RWB+Rm+Cs) for both PB 350 and PB 347 clones. Disease reductions (based on AUDPC values) were at 50 and 47% for PB 350 and PB 347, respectively (*Table 1*). These results will be useful for more detailed studies on the use of Cs to control rubber WRD caused by Rm in the future.

CONCLUSION

Fungicolous ascomycetous *Cladobotryum semicirculare*, isolated from fruiting bodies of *Ganode Rigidoporus microporus a boninense*, suppressed the growth of *Rigidoporus microporus* in co-culturing assays and completely reduced the regeneration or survival of *Rigidoporus microporus* mycelia during *in vitro* pathogenicity test. Conventional medium supplemented with fungal exudates harvested from *Cladobotryum semicirculare* illustrated antifungal and inhibitory effects towards *Rigidoporus microporus* . Antifungal effect of *Cladobotryum semicirculare* filtrates did not reduce with autoclaving and the antifungal compounds could be heat stable.

In the nursery experiment, *Cladobotryum semicirculare* reduced both the disease incidences and severity caused by *Rigidoporus microporus.* There was no significant difference between the two tested clones of scion-rootstock combinations (PB 347 – RRIM 2025 and PB 350 – RRIM 2025), in terms of area under disease progression curve (AUDPC).

ACKNOWLEDGMENTS

The authors would like to acknowledge the valuable technical assistances of staff from the AAR Crop Protection Lab: En. Ismail Hasim, Rahaizul Rakman, Muhd. Al-Qayyum Hassan Basri, and Muhd. Nazirul Rosli. Furthermore, we would like to thank Dr. Murnita of Rubber Research Institute Malaysia (RRIM), Malaysia for providing us with the *Rigidoporus* culture and Mr. Lim Sey Piew, Manager of Batang Jelai Estate for providing us with the rubber seedlings from PB 347 and PB 350 clones.

We would also like to thank Dr. Tasren Nazir Mahamooth and Ms. Tan Swee Sian for useful assistances and discussion on the topic of molecular works. We express gratitude to both Mr. Tey Seng Heng and Dr. Cheng Cong Rong for the useful discussion on rubber physiology. We are grateful to our Principals, Boustead Plantations Berhad and Kuala Lumpur Kepong Berhad for their permission to publish this paper.

Date of receipt: September 2017
Date of acceptance: November 2017

REFERENCES

1. ALLEN, P.W. AND CRONIN, M.E. (1994) Analysis of the 1993/1994 IRRDB Survey on Severity of Diseases of *Hevea.* Cochin, India, IRRDB Symposium on Diseases of *Hevea*.

2. CHEE, K.H. (1990) Present Status of Rubber Diseases and Their Control. *Rev. Plant Pathol.*, **69(7)**, 423 – 430.

3. JAYASINGHE, C.K. (1999) Pests and Diseases of *Hevea* Rubber and Their Geographical Distribution. *Bull. Rubber Res. Inst. Sri Lanka*, **40**, 1 – 8.

4. MOHAMMED, C.L., RIMBAWANTO, A. AND PAGE, D.E. (2014) Management of Basidiomycete Root – and Stem – Rot Diseases in Oil Palm, Rubber and Tropical Hardwood Plantation Crops. *Forest Pathol.*, **44(6)**, 428 – 446.

5. NANDRIS, D., CHADOEUF, J., PIERRAT, J.C., JOANNES, H., GEIGER, J.P. AND NICOLE, M. (1996) Modelling Rubber-Tree Root Diseases, Simulations of Various Inoculum Rates and Methods of Control. *Eur. J. Forest Pathol.*, **26(1)**, 25 – 44.

6. SOEKIRMAN, P. AND BUDI, S. (2009) Integrated Disease Management of White Root Disease on *Hevea* Rubber using *Trichoderma* and Antagonistic Plants. Getas Research Center. Indonesian Rubber Research Institute, In: Disease Management Strategies in Plantations, Jogjakarta, AusAID sponsored workshop, 4 - 8 May.

7. CHAN, W.H., WONG, C.P. AND WONG, C.C. (1991) Control of White Root Disease in Immature Rubber with Three Systematic Fungicides. *The Planter*, **67(783)**, 251 – 265.

8. JAYASURIYA, K.E. AND THENNAKOON, B.I. (2007) Biological Control of *Rigidoporus Microporus*, The Cause of White Root Disease in Rubber. *Cey. J. Sci. (Bio. Sci.)*, **36(1)**, 9 – 16.

9. KAEWCHAI, S. AND SOYTONG, K. (2010) Application of Biofungicides Against *Rigidoporus Microporus* Causing White Root Disease of Rubber Trees. *J. Agric. Technol.*, **6(2)**, 349 – 363.

10. GO, W.Z., H'NG, P.S., WONG, M.Y., TAN, G.H., LUQMAN CHUAH, A., SALMIAH, U., TOCZYŁOWSKA-MAMIŃSKA, R., SONI, O., WONG, W.Z., CHIN, K.L. AND CHAI, E.W. (2015) Occurrence and Characterisation of Mycoflora in Soil of Different Health Conditions Associated with White Root Disease in Malaysia Rubber Plantation. *J. Rubb. Res.*, **18(3)**, 159 – 170.

11. MARZUKI, N.F., GOH, Y.K., TUNG, H.J., GOH, Y.K. AND GOH, K.J. (2015) Evaluation on the Cultural Characteristics and Antagonistic Activities of *Cladobotryum Semicirculare* Against *Ganoderma Boninense In Vitro. J. Oil Palm Res.*, **27(4)**, 326 – 338.

12. ROGERSON, C.T. AND SAMUELS, G.J. (1993) Polyporicolous Species of *Hypomyces. Mycologia*, **85(2)**, 231 – 272.

13. WHITE, T.J., BRUNS, T., LEE, S. AND TAYLOR, J. (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics, In: INNIS, M.A., GELFAND, D.H., SNINSKY, J.J. AND WHITE, T.J. (Eds.) PCR Protocols: A guide to Methods and Applications. New York: Academic Press, 315 – 322.

14. OGHENEKARO, A.O., MIETTINEN, O., OMORUSI, V.I., EVUEH, G.A., FARID, M.A., GAZIS, R. AND ASIEGBU, F.O. (2014) Molecular Phylogeny of *Rigidoporus Microporus* Isolates Associated with White Rot Disease of Rubber Trees (*Hevea Brasiliensis*). *Fungal Biol.*, **118(5-6)**, 495 – 506.

15. TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. AND KUMAR, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.*, **30(12)**, 2725 – 2729.

16. GOH, Y.K., MARZUKI, N.F., GOH, T.K., TAN, S.Y., GOH, Y.K. AND GOH, K.J. (2016) Mycoparasitic *Scytalidium Parasiticum* as a Potential Biocontrol Agent Against *Ganoderma Boninense* Basal Stem Rot in Oil Palm. *Biocontrol Sci. Technol.*, **26(10)**, 1352 – 1365.

17. SUNDRAM, S. (2013) First Report: Isolation of Endophytic *Trichoderma* from Oil Palm (*Elaeis Guineensis* Jacq.) and their *In Vitro* Antagonistic Assessment on *Ganoderma Boninense. J. Oil Palm Res.*, **25(3)**, 368 – 372.

18. KOK, S.M., GOH, Y.K., TUNG, H.J., GOH, K.J., GOH, Y.K. AND WONG, W.C. (2013) *In Vitro* Growth of *Ganoderma Boninense* Isolates on Novel Palm Extract Medium and Virulence on Oil Palm (*Elaeis Guineensis*) Seedlings. *Malays. J. Microbiol.*, **9(1)**, 33 – 42.

19. SHANER, G. AND FINNER, R.W. (1977) The Effect of Nitrogen Fertilisation on the Expression of Slow-Mildewing Resistance in Knox Wheat. *Phytopathol.,* **77**, 1051 – 1056.