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Improvement in *in vitro* growth rates of *Ganoderma* species with industrial wood waste supplements

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The bracket-like polypore fungi of the genus *Ganoderma*, as a pathogen of oil palm (*Elaeis guineensis* Jacq.), is a major concern primarily because it plays a significant role in the economy of many countries in South-East-Asia. Growth of several *Ganoderma* isolates was examined on various culture media to develop a medium for rapid growth. Growth analyses revealed that growth of the different isolates was different on various culture media which differed in their nutrient composition and their growth levels were influenced by industrial wood waste. High rate of growth was achieved on rubber/oil palm wood extract agar. Potato dextrose agar supplemented with wood powder and peptone also favoured mycelial growth. Malt extract agar was the poorest among the media investigated for obtaining rapid mycelial growth of *Ganoderma* isolates. *G. boninense* PER 71, *G. tornatum* POR 57 and *G. subamboinense* var. *laevisporium* (ATCC 52419) presented higher growth rate on the different culture media. The *G. boninense* PER 71 showed the fastest growth on the different media, while the minimum growth was achieved for *G. tornatum* POR 54. Rubber/oil palm wood extract agar media appears to increase growth rate of the different *Ganoderma* isolates. Increase growth rate of different *Ganoderma* isolates by industrial wood waste suggested that industrial wood waste is sufficient to improve mycelial growth of *Ganoderma* isolates. Supporting the assumption, industrial wood waste could be a useful renewable source for the early detection of *Ganoderma* disease that will improve efforts to prevent economic losses in oil palm. Our rapid and less expensive culture media may also have commercial potential and novel biotechnological applications.

Keywords: *Ganoderma*, mycelial growth rate, oil palm, wood waste.

INTRODUCTION

Ganoderma species, the white rot basidiomycete have been associated with the devastating basal stem rot (BSR) disease of oil palm in various South-East-Asian countries. At present the number of *Ganoderma* species involved in the disease has not been resolved. The species was described originally as *G. lucidum* in Malaysia (Ho and Nawawi, 1985). *G. boninense* Pat. is the main causal agent of oil palm diseases in Malaysia (Ho and Nawawi, 1985; Pilotti, 2005). In addition, several *Ganoderma* species have been isolated and characterized from infected oil palm in Malaysia, including *G. miniatocinctum*, *G. zonatum* and *G. tornatum* (Idris et

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al., 2006; Paterson et al., 2009). White rot basidiomycetes are unique in their ability to degrade most components of wood due to their capability to synthesize the relevant hydrolytic and oxidative extracellular enzymes including the endo-1,4-ß-D-glucanase, exo-1,4-ß-D-glucanase and xylanase. The fungi also secrete extracellular non-specific and non-stereoselective lignin degrading enzymes essential for the fungal survival. The lignin-degrading enzymes associated with white rot fungi are lignin peroxidase, manganese peroxidase and laccase. The white rot basidiomycetes have been classified with respect to the potential to express ligninolytic enzymes (Leonowicz et al., 1999; Arora and Gill, 2000; Paterson, 2007; Elisashvili et al., 2009). Like other white rot basidiomycetes, *Ganoderma* species are capable of selective delignification of wood leaving white cellulose exposed. *Ganoderma* species are capable to produce hemicellulase, ligninase and amylase. The lignin-degrading enzymes consist of lignin peroxidase, manganese peroxidase and laccase. They gain energy from carbohydrates such as cellulose, starch and pectin (Leonowicz et al., 1999; Paterson et al., 2000; Paterson, 2007).

Although the genus *Ganoderma,* a mushroom-like fungus is an economically important plant pathogen*;* some of these species have been used in traditional Asian medicines (Seo and Kirk, 2000) and for industrial applications such as biopulping (Mendonca et al., 2008), bioremediation (Rigas et al., 2007), bioconversion (Elisashvili et al., 2009), waste water treatment and development of animal food sources from lignocellulose (Adaskaveg et al., 1991).

Growth and development of fungi *in vitro* depend on successful cultivation that satisfies their nutritional requirement. Although it is relatively easy to culture fungi on complex synthetic media such as malt extract agar (MEA) or potato dextrose agar (PDA) but they can also be grown on different special substrates depending on the main provincial availability (Walker and White, 2005). Undoubtedly, many factors including type of fungi, age of the culture and composition of the medium, as well as chemical, physical and environmental factors play pivotal roles in mycelial growth rates of fungi (Kalm and Kalyoncu, 2008).

The accumulation of industrial wood wastes and the efficient capture of their bioconversion potential are widespread concerns. Industrial wood wastes from wood processing constitute vast available renewable energy resource. The structural components of industrial wood wastes are suitable for bioproduct development (Van Wyk, 2001). Several lines of study demonstrate that wood blocks from rubber (*Hevea brasiliensis*) tree and sawdust provide a suitable growth environment for *Ganoderma* species (Sariah et al., 1994; Susanto et al., 2005).

Studying the genotypic and phenotypic diversity and the dynamics of pathogen populations can be achieved by recovery and isolation of plant pathogens (Amiri et al., 2009). However, the most obvious consequence of culture media contamination is the presence of bacterial, yeast, and several other fungal contaminants which can make such efforts difficult. Isolation of *Ganoderma* species is difficult due to the presence of bacterial, yeast, and several fast-growing fungal species. It can take at least seven days to obtain a result using commercial media. The nature of a particular culture medium has a major role to play in the growth of fungi (Zhao and Shamoun, 2006). For this reason, it is possible to develop a substantial number of alternative rapid culture media to produce results more quickly for various biotechnological applications.

The overall objective of this study was to improve culture media for *Ganoderma* species in order to find a medium that would allow for rapid colony growth. For this purpose, the influence of industrial wood waste on growth rate of *Ganoderma* isolates was investigated by studying the mycelial growth area, relative density and relative growth rate of colony. We selected rubber and oil palm wood powder as the major substance in the media because they are the most abundant industrial wood waste in Malaysia.

MATERIALS AND METHODS

Experimental design and statistical analysis

The two-factorial experiment of eight *Ganoderma* (G) isolates and 14 culture media (M) was conducted in which the replications were nested within each G × M combination. The statistical analysis that is the analysis of variance (ANOVA) was performed by the following liner additive model (LAM): $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$ wherein Y_{ijk} = ijk_{th} observations; α_i = i_{th} *Ganoderma* effects; β_j = j_{th} media effects; $(\alpha\beta)_{ij}$ = ij_{th} G× M interactions effects; and ε_{ijk} = ijk_{th} error term.

In the above LAM, ε_{ijk} was the pooled error term of experimental and sampling errors. The experimental error was the biological replicate nested to G× M combinations, while the sampling error was the software readings nested within biological replicate × G × M. The protected least-significant difference (LSD) test was used for multiple mean comparisons. Statistical analysis was performed using SAS software (version 9.1; SAS Institute Inc.). All experiments were repeated two times to compensate for possible errors. As the results of analysis obtained in the second repeat were the same as the first, results were only shown for the data of the first experiment.

Fungi and culture media preparation

Isolates of *G. subamboinense* var. *laevisporium* (ATCC 52419), *G. boninense* PER 71, *G. zonatum* POR 67, *G. zonatum* POR 69, *G. miniatocinctum* 331035, *G. miniatocinctum* 331037, *G. tornatum* POR 54 and *G. tornatum* POR 57 were obtained from the culture collection of Malaysian Palm Oil Board (MPOB) and Mycology Laboratory, Biology Department, Faculty of Science, Universiti Putra Malaysia.

The culture media compositions used in this study are presented in Table 1. The wood powder (WP, 20 mesh); from either rubber (*H.*

Table 1. Candidate culture media.

Annotation culture media*	pH
RWEA	3.5
OPWEA	3.5
PDA-P-RWP	3.5
PDA-P-OPWP	3.5
PDA-RWP	3.5
PDA-OPWP	3.5
MEA-P-RWP	4.7
MEA-P-OPWP	4.7
MEA-RWP	4.7
MEA-OPWP	4.7
PDA-P	3.5
PDA	3.5
MEA-P	4.7
MEA	4.7

*R, rubber; WEA, wood extract agar; OP, oil palm; P, peptone; WP, wood powder; PDA, potato dextrose agar; MEA, malt extract agar.

brasiliensis) or oil palm (*E. guineensis* Jacq.) wood used as industrial wood waste was obtained from Huot Hing Factory in Semenyih, Selangor, Malaysia. The ingredients for novel culture media, oil palm/rubber wood extract agar (OP/RWEA) were 500 mL WP extract, 30 g Bacto™ malt extract, 5 g dextrose, 5 g peptone, 0.25 g KH_2PO_4 and 15 g agar made up to 1 L volume with distilled water. For WP extract, 65 g of WP was infused in 1 L of tap water and autoclaved at 121°C for 25 min. The WP extract was filtered through cotton and filter paper (no. 42, Whatman). PDA (Difco Laboratories, Detroit, Michigan, USA) and MEA (Difco Laboratories, Detroit, Michigan, USA) were made according to the manufacturer's directions. The basal culture media PDA and MEA were supplemented with 5 g/L of peptone or 20 g/L WP or both peptone and WP. The culture media were amended with streptomycin sulfate (100 mg/L).

Inoculum preparation

Ganoderma isolates were cultivated in freshly made culture media for three days and used as inoculum. Inoculum for different culture media was provided from identical (Table 1) media. 5 mm diameter mycelial discs were aseptically transferred upside-down from the periphery of each *Ganoderma* colony to the centre of a 90 mm Petri plates containing 25 mL of medium. The Petri plates were wrapped in aluminum foil and incubated in a plastic bag at 28°C. *Ganoderma* inoculum plated on *Ganoderma* selective medium (GSM) and mycelia morphology observed under a light microscope was used to confirm the identity of *Ganoderma*. All experiments were performed two times with five independent replicates to compensate for possible errors.

Image acquisition and determination of growth profile

Growth profiles of *Ganoderma* isolates were estimated by image analysis using UTHSCSA ImageTool software 3.0 (http://ddsdx.uthscsa.edu/dig/itdesc.html). The growth of *Ganoderma* isolates colony was daily photographed with a digital camera at zero time until the colony covered the entire area of Petri plate. The colonies were photographed under similar condition (position of camera, distance, background and light) by Canon digital camera (model power shot SD1000/Digital ELPH/Digital IXUS70). Images were expressed in gray-scale and objects were found manually. Each image was threshold manually.

The relative density of colony was determined. The optical density step tablet standard was used to generate calibration of gray-scale values according to the UTHSCSA ImageTool manufacturer's instruction. At least three replicate were performed for each colony.

The relative growth rate (RGR) of *Ganoderma* isolates was determined using standard formula (Sundari and Adholeya, 2003):

$$\mathrm{RGR} = \frac{A_t - A_0}{A_0} \times t$$

Where, A_t is the area of the colony at time t; A_0 is the area of the colony at zero time and t is the time period. Identification of differential relative growth rate was based on consistent fold change across experimental replicates relative to commercial media, PDA and MEA. Fold changes of ≥2-fold or ≤0.5-fold were considered as significant.

RESULTS

Mycelial growth area, relative density and relative growth rate of *Ganoderma* colony was analyzed on various culture media. Different culture media had a significant effect on *Ganoderma* isolates. The interaction of *Ganoderma* isolates × culture media was significant ($p \leq 0.0001$) for all *Ganoderma* isolates because of the different mycelial growth area, relative density and relative growth rate of *Ganoderma* colony on various culture media.

Mycelial growth area of *Ganoderma* isolates

Mycelial growth area of colonies on various culture media demonstrated that the growth of *Ganoderma* isolates was significantly different in gray-scale display and their levels are influenced by wood powder (Figure 1 and Supplementary Table 1). The mycelial growth area of *Ganoderma* isolates on media supplemented with rubber WP was higher than those growing on oil palm WP containing media. The results of mycelial growth area of different *Ganoderma* isolates were in the order of *G. boninense* PER 71> *G. tornatum* POR 57> *G. zonatum* POR 69> *G. subamboinense* var. *laevisporium* (ATCC 52419)> *G. miniatocinctum* 331035> *G. miniatocinctum* 331037> *G. zonatum* POR 67> *G. tornatum* POR 54 on R/OPWEA and PDA-P-R/OPWP at four days post-inoculation (dpi). Our results demonstrate that the pattern of mycelial growth area of colonies was different in *G. tornatum* and *G. zonatum* species on various culture media. *G. tornatum* POR 57 and *G. zonatum* POR 69 isolates presented high mycelial growth area than *G. tornatum* POR 54 and *G. zonatum* POR 67 isolates. The *G. miniatocinctum* species showed similar mycelial growth area of colonies on various culture media. *G. boninense* PER 71, *G. tornatum* POR 57, *G. zonatum*

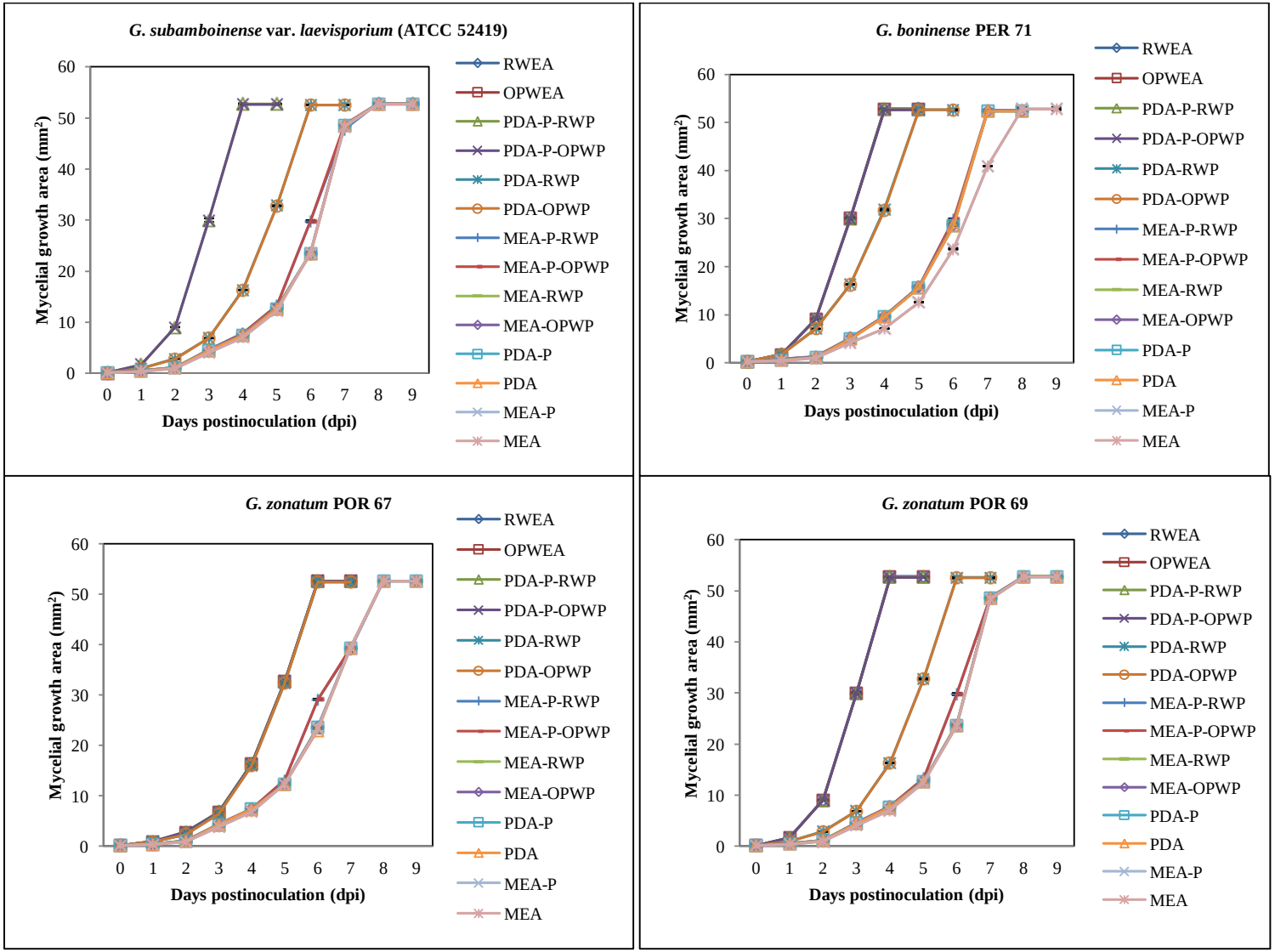


Figure 1. Time profiles of mycelial growth area of *Ganoderma* isolates on various culture media. R/OPWEA, rubber/oil palm wood extract agar; PDA-P-R/OPWP, potato dextrose agar-peptone-rubber/oil palm wood powder; PDA-R/OPWP, potato dextrose agar-rubber/oil palm wood powder; MEA-P-R/OPWP, malt extract agar-peptone-rubber/oil palm wood powder; MEA-R/OPWP, malt extract agar-rubber/oil palm wood powder; PDA-P, potato dextrose agar-peptone; PDA, potato dextrose agar; MEA-P, malt extract agar-peptone; MEA, malt extract agar. Data represent the mean ± S.E of five biological replicates and three technical replicates. Error bars indicate S.E.

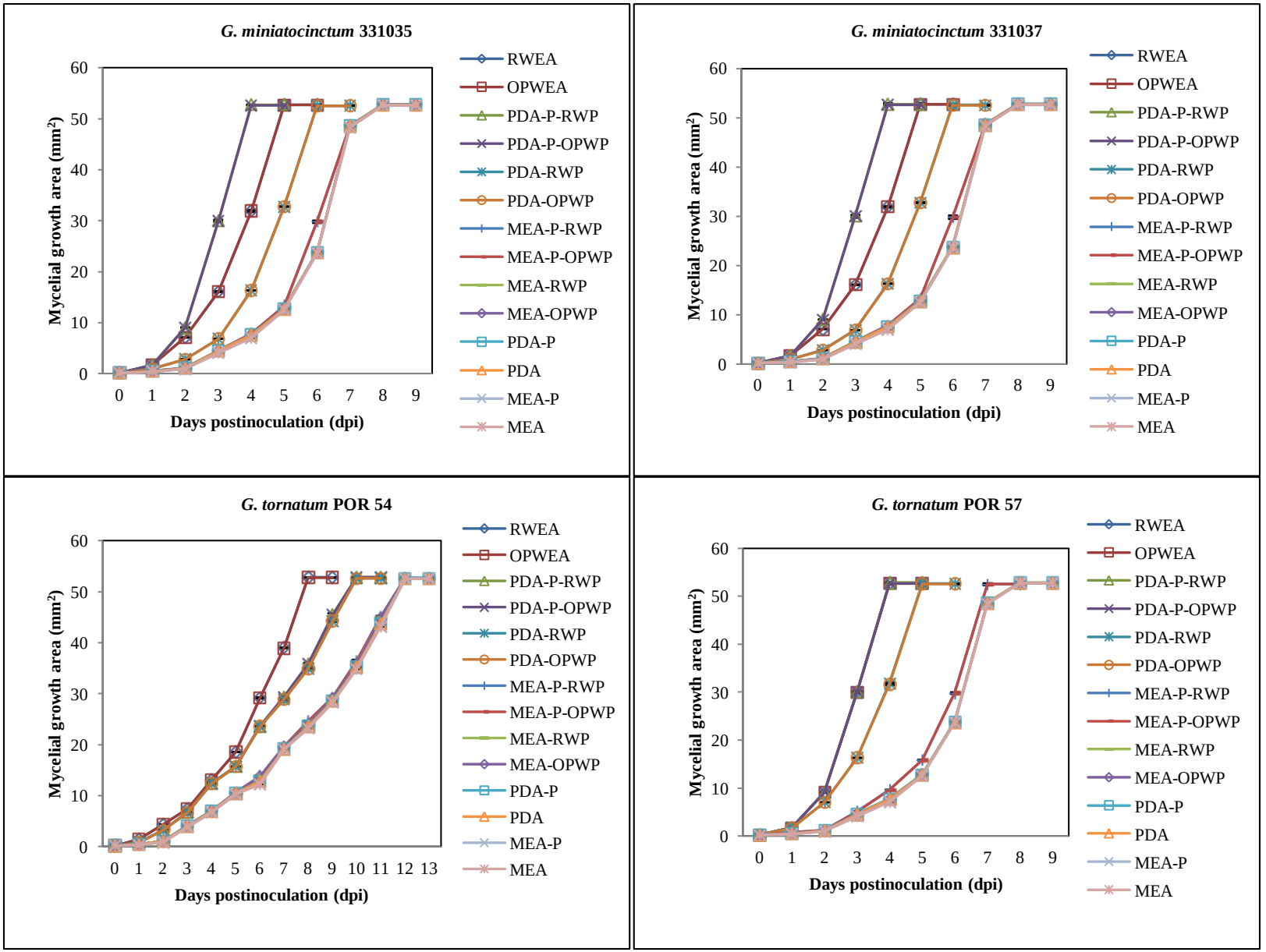


Figure 1. Contd.

POR 69 and *G. subamboinense* var. *laevisporium* (ATCC 52419) showed higher mycelial growth area on the RWEA, PDA-P-RWP, OPWEA and PDA-P-OPWP media at 4 dpi in comparison to the other *Ganoderma* isolates. The highest mycelial growth area (52.84 ± 0.53 mm^2) was observed on RWEA media in *G. boninense* PER 71 at 4 dpi. High mycelial growth area of *G. boninense* PER 71 (52.83 ± 0.56 mm^2) and *G. tornatum* POR 57 (52.83 ± 0.61 mm^2) was observed on PDA-P-RWP media at 4 dpi. *G. boninense* PER 71 and *G. tornatum* POR 57 achieved significantly bigger mycelial growth area on PDA-R/OPWP media at 5 dpi compared to the other *Ganoderma* isolates. The mycelial growth area for *G. boninense* PER 71 isolates were completed on MEA-P-R/OPWP, MEA-R/OPWP, PDA-P and PDA media at 7 dpi. In addition, on MEA-P and MEA media, the mycelial growth of *G. boninense* PER 71 was completed at 8 dpi. Mycelial growth area increased on MEA-P-R/OPWP at 7 dpi in *G. tornatum* POR 57. At 8 dpi, *G. tornatum* POR 57 was able to complete mycelial growth area on PDA-P, PDA, MEA-P and MEA media. Moreover, *G. subamboinense* var. *laevisporium* (ATCC 52419) and *G. zonatum* POR 69 completed growth on the PDA-R/OPWP media at 6 dpi. The mycelial growth area of these isolates on the other culture media was completed at 8 dpi. Significant mycelial growth of *G. zonatum* POR 67 was observed on PDA-P-R/OPWP, R/OPWEA and MEA-P-R/OPWP at 6 dpi. After 8 days of incubation, the growth of *G. zonatum* POR 67 was completed on the other media. Furthermore, both isolates of *G. miniatocinctum* reached complete mycelial growth on PDA-P-R/OPWP and R/OPWEA media at 4 and 5 dpi, respectively. Significant mycelial growth was observed on MEA-P-R/OPWP media in *G. miniatocinctum* isolates at 6 dpi, while the growth was completed on the other media at 8 dpi. However, the lowest increase in the colony growth area was apparent in *G. tornatum* POR 54. The isolate of *G. tornatum* POR 54 took 8 days to attain full growth on R/OPWEA, 10 days on PDA-P-R/OPWP and MEA-P-R/OPWP and 12 days on the other media. The lowest mycelial growth area was recorded in *G. tornatum* POR 54 isolates on MEA media in comparison to the other *Ganoderma* isolates.

Relative density of *Ganoderma* isolates

Our results reveal significant differences in relative density of the colony of *Ganoderma* isolates on various culture media. The pattern of the relative density was similar for all *Ganoderma* isolates (Figure 2 and Supplementary Table 2). The relative density significantly increased in the mean values on different media on the first day after inoculation. This was followed by a gradual increase in the mean values over a time course when fungal growth occurred. After the maximum relative density level was achieved, there was a decline until the last day. Our results indicate that the time profiles of relative density of *Ganoderma* isolates on 14 different culture media at 4 dpi can be categorized into three groups. The highest relative density presented by R/OPWP and PDA-P-R/OPWP were classified as group I. The relative density recorded by PDA-R/OPWP and PDA-P belonged to group II. The other media which presented the lowest relative density was classified as group III. Interestingly, in contrast to mycelial growth area, the relative density of *Ganoderma* species was similar on all culture media.

This data demonstrates that the significantly highest relative density was observed for *G. tornatum* POR 54 on all culture media. *G. boninense* PER 71, *G. tornatum* POR 57 and *G. subamboinense* var. *laevisporium* (ATCC 52419), respectively presented higher relative density on all culture media in comparison to the other *Ganoderma* isolates. The lowest relative density was observed for *G. miniatocinctum* 331035. The maximum relative density was recorded on R/OPWEA for all *Ganoderma* isolates except for both isolates of *G. miniatocinctum*, which presented maximum relative density on PDA-P-R/OPWP. Moreover, all *Ganoderma* isolates achieved the minimum relative density on MEA media. The results on relative density of *Ganoderma* isolates were in the order of *G. tornatum* POR 54> *G. boninense* PER 71> *G. tornatum* POR 57> *G. subamboinense* var. *laevisporium* (ATCC 52419)> *G. zonatum* POR 67> *G. zonatum* POR 69> *G. miniatocinctum* 331037> *G. miniatocinctum* 331035 on all culture media. The relative density of *G. boninense* PER 71, *G. tornatum* POR 57 and *G. subamboinense* var. *laevisporium* (ATCC 52419) isolates correlated with mycelial growth area.

Relative growth rate of *Ganoderma* isolates

Our results reveal significant ($p \leq 0.0001$) differences in relative growth rate of *Ganoderma* isolates on the various culture media. The results illustrated in Figure 3, Table 2 and Supplementary Table 3 indicates that the relative growth rate of *Ganoderma* isolates is dependent on the culture media. In addition, the R/OPWEA and PDA-P-R/OPWP media were found to be the best with respect to relative growth rate of *Ganoderma* isolates. Relative growth rate of *G. subamboinense* var. *laevisporium* (ATCC 52419), *G. zonatum* POR 69 and *G. tornatum* POR 57 significantly ($p \leq 0.0001$) increased on R/OPWEA and PDA-P-R/OPWP media. Compared to the commercial media (PDA and MEA), the change in terms of relative growth rate of *G. subamboinense* var. *laevisporium* (ATCC 52419), *G. zonatum* POR 69 and *G. tornatum* POR 57 on R/OPWEA and PDA-P-R/OPWP was approximately 2.00-fold. Our findings demonstrate that the fold change relative to MEA and PDA for *G. boninense* PER 71 on R/OPWEA and PDA-P-R/OPWP were 2.01 and 1.77-fold, respectively. The relative growth

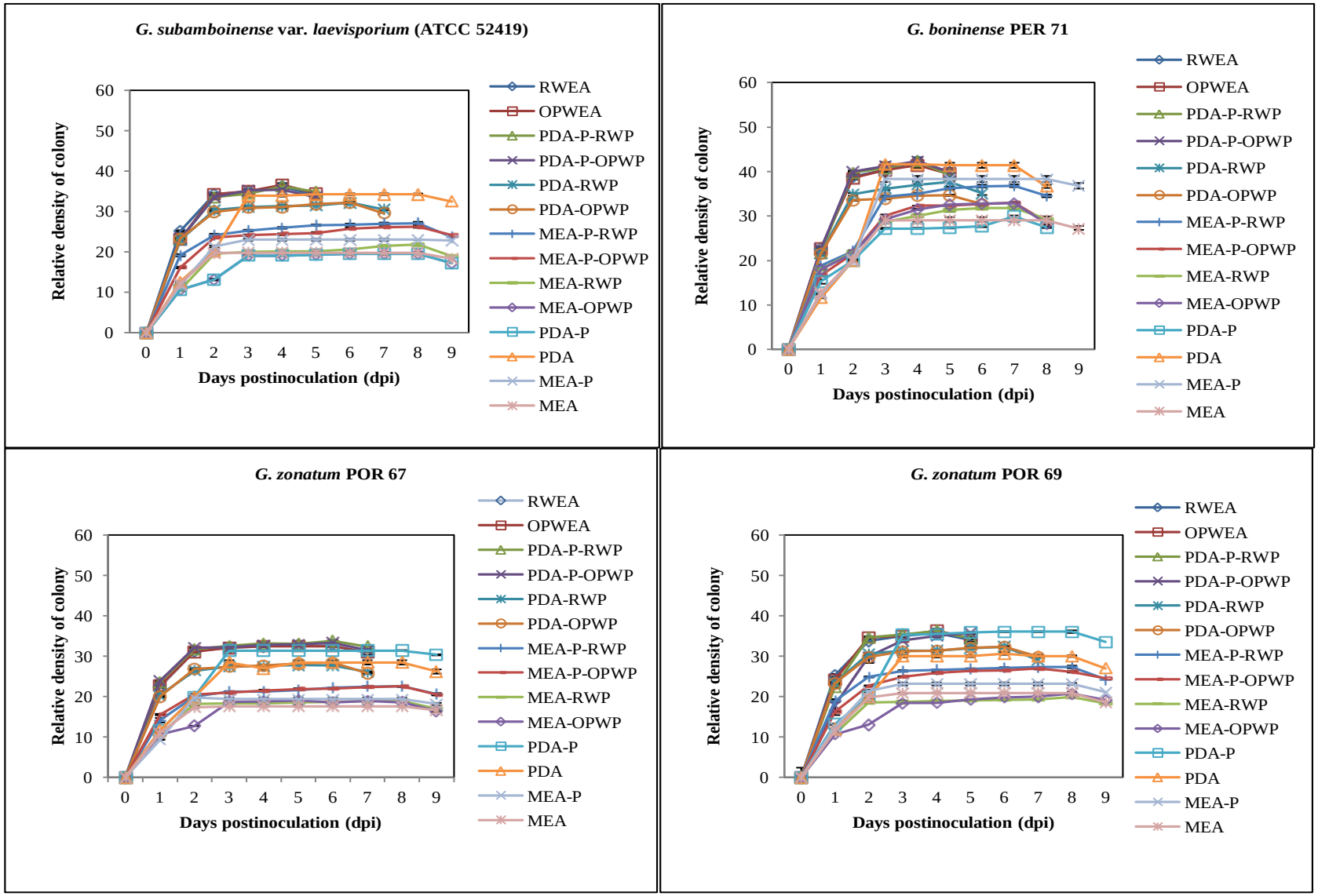
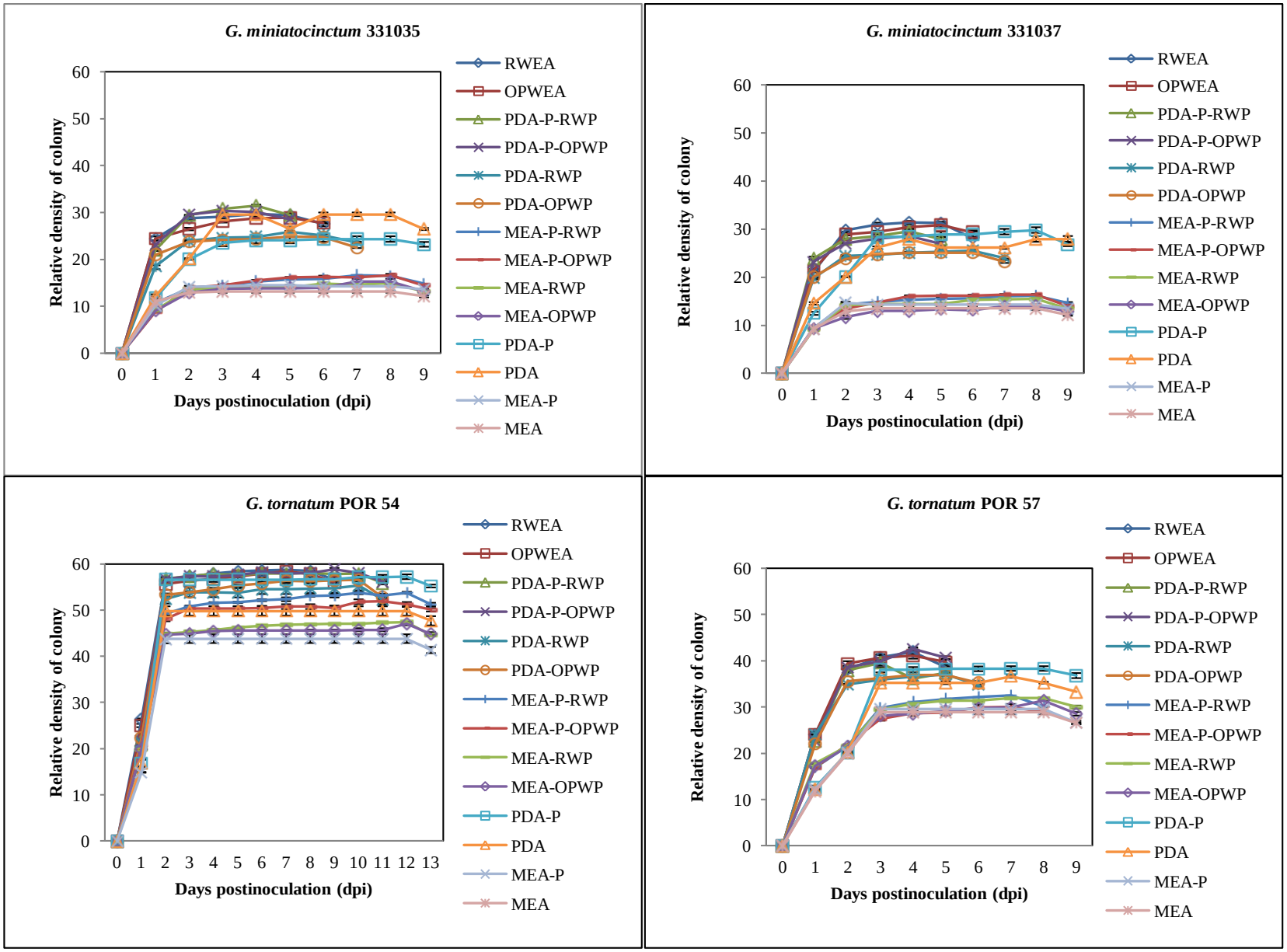


Figure 2. Time profiles of relative density of *Ganoderma* isolates on various culture media. R/OPWEA, rubber/oil palm wood extract agar; PDA-P-R/OPWP, potato dextrose agar-peptone-rubber/oil palm wood powder; PDA-R/OPWP, potato dextrose agar-rubber/oil palm wood powder; MEA-P-R/OPWP, malt extract agar-peptone-rubber/oil palm wood powder; MEA-R/OPWP, malt extract agar-rubber/oil palm wood powder; PDA-P, potato dextrose agar-peptone; PDA, potato dextrose agar; MEA-P, malt extract agar-peptone; MEA, malt extract agar. Data represent the mean ± S.E of five biological replicates and three technical replicates. Error bars indicate S.E.



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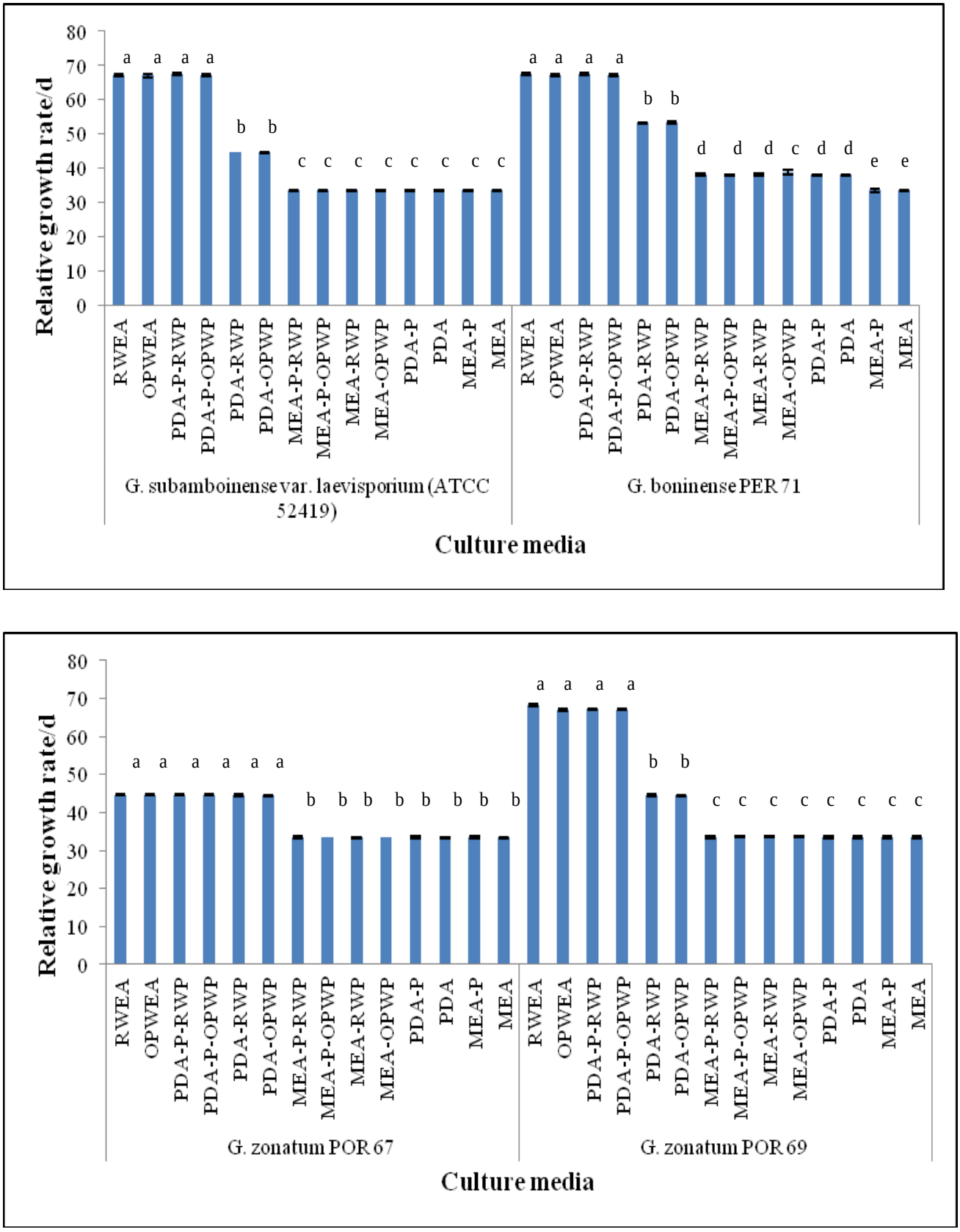

Figure 3. Relative growth rate of *Ganoderma* isolates on various culture media. Data represent the mean ± S.E of five biological replicates and three technical replicates and two times repeat. Error bars indicate S.E. Means with different letters indicate statistically significant differences at $p \leq 0.0001$ for each *Ganoderma* isolate.

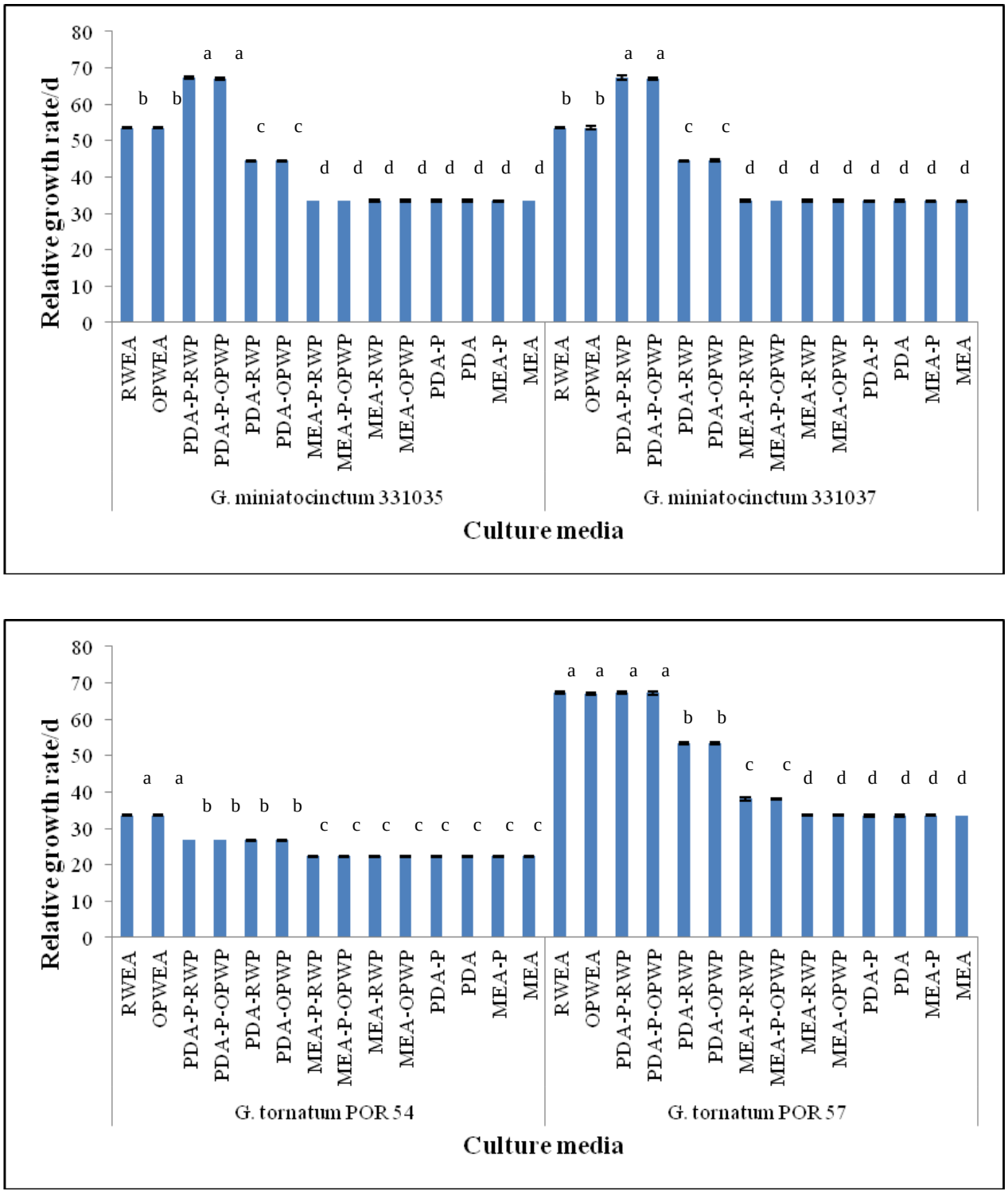

Figure 3. Contd.

rate of *G. zonatum* POR 67 did not significantly increase on different culture media. The relative growth rate of *G. zonatum* POR 67 achieved approximately 1.33-fold increase in the mean values on R/OPWEA, PDA-P-R/OPWP and PDA-R/OPWP media. In the case of *G. miniatocinctum* isolates, the relative growth rate significantly ($p \leq 0.0001$) increased by 2.00-fold on PDA-P-R/OPWP. *G. tornatum* POR 54 did not show a significant

Table 2. Comparison of relative growth rate of *Ganoderma* isolates based on consistent fold change across experimental replicates relative to commercial media, PDA and MEA.

***Ganoderma* isolates /cultures media**	**Mean/fold change**	***G. subamboniase* var. *laevisoporum* (ATCC52419)**	***G. boninense* PER 71**	***G. zonatum* POR 67**	***G. zonatum* POR 69**	***G. miniatocinctum* 331035**	***G. miniatocinctum* 331037**	***G. tornatum* POR 54**	***G. tornatum* POR 57**
RWEA	Mean RGR/d	67.09 ± 0.47	67.49 ± 0.68	44.62 ± 0.40	67.32 ± 0.64	53.56 ± 0.26	53.52 ± 0.49	33.59 ± 0.39	67.33 ± 0.81
	Fold change to PDA	2.00 ± 0.02*	1.78 ± 0.02	1.34 ± 0.01	2.01 ± 0.04*	1.60 ± 0.03	1.60 ± 0.02	1.51 ± 0.01	2.00 ± 0.06*
	Fold change to MEA	2.00 ± 0.03*	2.01 ± 0.04*	1.34 ± 0.02	2.01 ± 0.04*	1.60 ± 0.02	1.60 ± 0.02	1.51 ± 0.02	2.01 ± 0.03*
OPWEA	Mean RGR/d	66.96 ± 1.02	67.31 ± 0.61	44.57 ± 0.34	67.09 ± 0.79	53.51 ± 0.37	53.56 ± 0.78	33.56 ± 0.24	67.03 ± 0.79
	Fold change to PDA	2.00 ± 0.05*	1.77 ± 0.03	1.33 ± 0.02	2.00 ± 0.04*	1.60 ± 0.03	1.60 ± 0.02	1.51 ± 0.02	2.00 ± 0.04*
	Fold change to MEA	2.00 ± 0.04*	2.00 ± 0.02*	1.33 ± 0.01	2.00 ± 0.03*	1.60 ± 0.02	1.60 ± 0.02	1.51 ± 0.01	2.00 ± 0.03*
PDA-P-RWP	Mean RGR/d	67.05 ± 0.63	67.49 ± 0.70	44.57 ± 0.19	67.30 ± 0.25	67.21 ± 0.73	67.27 ± 1.42	26.84 ± 0.20	67.31 ± 0.90
	Fold change to PDA	2.00 ± 0.03*	1.77 ± 0.02	1.33 ± 0.01	2.01 ± 0.04*	2.00 ± 0.04*	2.01 ± 0.02*	1.21 ± 0.01	2.00 ± 0.06*
	Fold change to MEA	2.00 ± 0.04*	2.01 ± 0.02*	1.33 ± 0.01	2.01 ± 0.03*	2.01 ± 0.03*	2.01 ± 0.05*	1.21 ± 0.02	2.01 ± 0.04*
PDA-P-OPWP	Mean RGR/d	66.92 ± 0.54	67.23 ± 1.07	44.58 ± 0.42	67.07 ± 0.43	67.06 ± 0.37	67.01 ± 0.80	26.85 ± 0.27	67.17 ± 1.31
	Fold change to PDA	2.00 ± 0.02*	1.77 ± 0.04	1.33 ± 0.02	2.00 ± 0.03*	2.00 ± 0.04*	2.00 ± 0.02*	1.21 ± 0.01	2.00 ± 0.07*
	Fold change to MEA	2.00 ± 0.03*	2.00 ± 0.04*	1.33 ± 0.01	2.00 ± 0.03*	2.01 ± 0.01*	2.00 ± 0.02*	1.21 ± 0.02	2.01 ± 0.05*
PDA-RWP	Mean RGR/d	44.68 ± 0.25	53.29 ± 0.48	44.44 ± 0.66	44.52 ± 0.46	44.47 ± 0.34	44.50 ± 0.37	26.79 ± 0.27	53.44 ± 0.65
	Fold change to PDA	1.33 ± 0.02	1.40 ± 0.02	1.33 ± 0.02	1.33 ± 0.02	1.33 ± 0.01	1.33 ± 0.02	1.20 ± 0.02	1.60 ± 0.03
	Fold change to MEA	1.33 ± 0.02	1.60 ± 0.01	1.33 ± 0.01	1.33 ± 0.01	1.33 ± 0.01	1.33 ± 0.01	1.20 ± 0.02	1.60 ± 0.02
PDA-OPWP	Mean RGR/d	44.61 ± 0.33	53.38 ± 0.97	44.40 ± 0.52	44.41 ± 0.59	44.43 ± 0.42	44.51 ± 0.51	26.74 ± 0.21	53.30 ± 0.80
	Fold change to PDA	1.33 ± 0.02	1.40 ± 0.03	1.33 ± 0.01	1.33 ± 0.01	1.33 ± 0.02	1.33 ± 0.03	1.20 ± 0.01	1.60 ± 0.02
	Fold change to MEA	1.33 ± 0.02	1.60 ± 0.04	1.33 ± 0.02	1.33 ± 0.03	1.33 ± 0.01	1.33 ± 0.01	1.20 ± 0.01	1.60 ± 0.03
MEA-P-RWP	Mean RGR/d	33.56 ± 0.49	38.16 ± 0.73	33.44 ± 0.41	33.53 ± 0.80	33.49 ± 0.34	33.49 ± 0.44	22.32 ± 0.21	38.13 ± 0.76
	Fold change to PDA	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.13 ± 0.02
	Fold change to MEA	1.00 ± 0.03	1.14 ± 0.03	1.00 ± 0.01	1.00 ± 0.04	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.14 ± 0.01

Table 2 Contd.

MEA-P-OPWP	Mean RGR/d	33.58 ± 0.29	38.15 ± 0.39	33.47 ± 0.13	33.51 ± 0.37	33.52 ± 0.29	33.50 ± 0.24	22.33 ± 0.28	38.11 ± 0.42
	Fold change to PDA	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.01	1.13 ± 0.02
	Fold change to MEA	1.00 ± 0.01	1.14 ± 0.02	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.03	1.13 ± 0.01
MEA-RWP	Mean RGR/d	33.53 ± 0.39	38.06 ± 0.64	33.43 ± 0.43	33.53 ± 0.32	33.48 ± 0.57	33.48 ± 0.55	22.29 ± 0.38	33.57 ± 0.53
	Fold change to PDA	1.00 ± 0.02	1.00 ± 0.03	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.03
	Fold change to MEA	1.00 ± 0.02	1.14 ± 0.02	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.01
MEA-OPWP	Mean RGR/d	33.54 ± 0.42	38.93 ± 2.44	33.44 ± 0.25	33.57 ± 0.43	33.48 ± 0.40	33.45 ± 0.68	22.30 ± 0.17	33.56 ± 0.57
	Fold change to PDA	1.00 ± 0.01	1.00 ± 0.06	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.03	1.00 ± 0.01	1.00 ± 0.02
	Fold change to MEA	1.00 ± 0.01	1.17 ± 0.06	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02
PDA-P	Mean RGR/d	33.53 ± 0.34	38.02 ± 0.50	33.43 ± 0.39	33.52 ± 0.67	33.49 ± 0.41	33.47 ± 0.38	22.29 ± 0.27	33.59 ± 0.69
	Fold change to PDA	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.01
	Fold change to MEA	1.00 ± 0.01	1.14 ± 0.02	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02
PDA	Mean RGR/d	33.5 ± 0.34	37.99 ± 0.39	33.41 ± 0.30	33.51 ± 0.51	33.46 ± 0.54	33.48 ± 0.48	22.27 ± 0.21	33.59 ± 0.62
	Fold change to PDA	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	Fold change to MEA	1.00 ± 0.01	1.14 ± 0.02	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02
MEA-P	Mean RGR/d	33.49 ± 0.34	33.53 ± 1.09	33.41 ± 0.54	33.38 ± 0.58	33.44 ± 0.42	33.43 ± 0.40	22.27 ± 0.40	33.51 ± 0.34
	Fold change to PDA	1.00 ± 0.02	0.88 ± 0.03	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02
	Fold change to MEA	1.00 ± 0.02	1.00 ± 0.03	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.03	1.00 ± 0.01
MEA	Mean RGR/d	33.49 ± 0.39	33.51 ± 0.33	33.41 ± 0.21	33.47 ± 0.44	33.44 ± 0.24	33.40 ± 0.22	22.27 ± 0.21	33.50 ± 0.21
	Fold change to PDA	1.00 ± 0.01	0.88 ± 0.02	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02
	Fold change to MEA	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

RGR/d, relative growth rate/day; R/OPWEA, rubber/oil palm wood extract agar; PDA-P-R/OPWP, potato dextrose agar-peptone- rubber/oil palm wood powder; PDA-R/OPWP, potato dextrose agar-rubber/oil palm wood powder; MEA-P-R/OPWP, malt extract agar-peptone-rubber/oil palm wood powder; MEA-R/OPWP, malt extract agar-rubber/oil palm wood powder; PDA-P, potato dextrose agar-peptone; PDA, potato dextrose agar; MEA-P, malt extract agar-peptone; MEA, malt extract agar. Data represent the mean ± S.E of five biological replicates and three technical replicates.
*,Significant relative growth rate compared to commercial media.

increase of relative growth rate on the various culture media. Furthermore, relative growth rate of *G. tornatum* POR 54 demonstrates 1.51- and 1.21-fold increase on R/OPWEA and PDA-P-R/OPWP media, respectively.

DISCUSSION

A typical growth rate of the *Ganoderma* isolates is dependent on the media composition and modified by other environmental factors. Mycelial growth of the fungi was found to be strongly influenced by the different complex media (Walker and White, 2005; Kalm and Kalyoncu, 2008). In this study, we succeeded to optimize a novel culture media with industrial wood waste and improved commercial synthetic media in our efforts to identify the best culture media for rapid colony growth of *Ganoderma* isolates.

Data from our analyses indicate that growth rate of *Ganoderma* is dependent on isolates as well as culture media. In this study, it was observed that the different *Ganoderma* isolates could grow rapidly on four out of the 14 culture media. The R/OPWEA and PDA-P-R/OPWP culture media favoured growth of the *Ganoderma* isolates. A relatively high level of growth was achieved on R/OPWEA media. MEA and MEA supplemented with peptone were poor media, resulting in low mycelial growth for all *Ganoderma* isolates. Studies with different fungi on agar surface demonstrated that the nutrient composition in culture media influenced the growth rate of fungi (Xiao and Sitton, 2004).

Growth requirements for fungi include carbon and nitrogen sources, vitamins, minerals, air and water (Booth, 1971). The R/OPWEA culture media contains several carbon sources such as dextrose, WP (cellulose, hemicelluloses and lignin) and malt extract. Protein is the most abundant source of nitrogen from wood (Venables and Watkinson, 1989). The culture media supplemented with WP and peptone demonstrated high level of growth rate for all *Ganoderma* isolates. Increase in the growth rate of *Ganoderma* species is related to hydrolytic and oxidative enzymes produced by *Ganoderma* (Leonowicz et al., 1999; Paterson, 2007; Elissetche et al., 2007). The ability of the *Ganoderma* species to degrade a wide variety of carbon compounds has been reported. The wood-degrading organisms secrete a large quantity of cellulase and hemicellulase complexes. Several lines of evidence documented that the lignin degrading enzymes are essential for the fungal survival which employs an oxidative process (Adaskaveg et al., 1991; Paterson, 2007; Rigas et al., 2007). Malt extracts are of high nutritional value with notable amounts of several of the vitamin B complexes, minerals and amino acids. The R/OPWEA cultures media also contains peptone and KH_2PO_4 as nitrogen and phosphorus source, respectively. The genus *Ganoderma* needs inorganic nutrients such as nitrogen and phosphorus to support cell growth (Rigas et al., 2007). An invading fungal pathogen will encounter a range of different forms of nitrogen in the apoplast and symplast of the plant tissue, ranging from inorganic N like nitrate through to organic N like glutamine (Walters and Bingham, 2007). Nitrogen is accumulated in the basidiomycete mycelium as free amino acids and an ethanol-insoluble protein fraction yielding amino acids on hydrolysis (Wadekar et al., 1995). Peptone seems to enhance the density of *Ganoderma* isolates. Recent findings demonstrate that the supplementation of media with additional nitrogen source in some cases significantly affected the extracellular enzyme yield in *Ganoderma* species (Elisashvili et al., 2008). Wood-decaying basidiomycetes are able to regulate proteinase activity. The proteinase activity is part of the physiological processes of morpho-genesis, because of the close relationship between fungal nutrition and morphogenesis. The composition and spatial arrangement of the nutrient substrate affects not only the rate of growth of the colony but also the differen-tiation of structures such as mycelial strands (Wadekar et al., 1995). In this study, the culture media supplemented with rubber wood was found to exhibit higher growth rate of *Ganoderma* isolates than media supplemented with oil palm wood. The better growth may be related to the chemical composition of rubber wood compared to oil palm wood (Simatupang et al., 1994; Ratnasingam et al., 2008; Chin et al., 2010).

Our results demonstrate that growth rate of different *Ganoderma* isolates were significantly different on various culture media. These results indicate that *G. boninense* PER 71, *G. tornatum* POR 57 and *G. subamboinense* var. *laevisporium* (ATCC 52419) presented higher mycelial growth area, relative density and relative growth rate of colonies on different culture media. The *G. boninense* PER 71 showed the fastest growth on media, while the minimum growth was achieved by *G. tornatum* POR 54. *Ganoderma* metabolite mixtures may have a role in growth rate, which is essential for the fungal survival (Paterson, 2007; Rigas et al., 2007). The production of extracellular enzymes varies in the *Ganoderma* genus and depends on substrate type and culture conditions (Teerapatsakul et al., 2007; Elisashvili et al., 2008). The rapid growth of *Ganoderma* isolates using wood improved culture media would facilitate significantly the studies on white rot fungi action on woody materials.

Here we report a novel, rapid and less expensive culture media, which may also have commercial potential. The results suggest that industrial wood waste with supplements on N and P is sufficient to improve mycelial growth rate of *Ganoderma* isolates. The Indus-trial wood waste could be a useful renewable source for enhancement of mycelial growth for the early detection of *Ganoderma* disease to reduce economic losses in oil palm. This culture media may be useful to isolate *Ganoderma* species for biotechnological applications.

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Supplementary Table 1. Summarized ANOVA procedure for time profiles of mycelial growth area of *Ganoderma* isolates on various culture media.

Source	Days postinoculation (dpi)											
	0			1			2			3		
	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Ganoderma	1.37	4.07	0.0002	4.94	7272.18	<.0001	179.58	9351.74	<.0001	2361.55	12613.3	<.0001
Media	15.12	1.16	<.0001	29.9	43981.1	<.0001	988.27	51466.1	<.0001	8190.66	43747	<.0001
Ganoderma * Media	105.77	1.16	<.0001	0.49	715.97	<.0001	27.22	1417.34	<.0001	381.55	2037.88	<.0001

Source	Days postinoculation (dpi)														
	4			5			6			7			8		
	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Ganoderma	6884.53	46397.4	<.0001	7207.37	39267	<.0001	6086.82	14369	<.0001	14388.94	27311.1	<.0001	10695.82	7765.1	<.0001
Media	26939.71	181557	<.0001	29068.73	158371	<.0001	12726.86	30043.9	<.0001	455.42	864.42	<.0001	927.59	673.43	<.0001
Ganoderma * Media	1006.39	6782.42	<.0001	3528.7	3528.7	<.0001	340.38	803.53	<.0001	484.68	919.95	<.0001	422.32	306.6	<.0001

Source	Days postinoculation (dpi)														
	9			10			11			12			13		
	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Ganoderma	5665.6	2120.39	<.0001	-	-	-	-	-	-	-	-	-	-	-	-
Media	188.65	70.6	<.0001	1073.58	2890.65	<.0001	270.56	924.96	<.0001	0.05	0.14	0.99	0.05	0.14	0.99
Ganoderma * Media	577.81	216.25	<.0001	-	-	-	-	-	-	-	-	-	-	-	-

Supplementary Table 2. Summarized ANOVA procedure for time profiles of relative density of *Ganoderma* isolates on various culture media.

Source	Days postinoculation (dpi)											
	0			1			2			3		
	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean square	F Value	Pr > F	Mean Square	F Value	Pr > F
Ganoderma	0	.	.	593.63	278.48	<.0001	21015.93	15518	<.0001	20440.43	13625.8	<.0001
Media	0	.	.	2661.69	1342.44	<.0001	5335.11	3939.41	<.0001	3933.7	2622.24	<.0001
Ganoderma * Media	0	.	.	70.94	33.28	<.0001	92.01	67.94	<.0001	77.75	51.83	<.0001

Source	Days postinoculation (dpi)														
	4			5			6			7			8		
	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Ganoderma	20053.23	8414.34	<.0001	20851.45	12122.5	<.0001	21248.09	14611.8	<.0001	22381.05	15285.5	<.0001	22270.91	13783.6	<.0001
Media	4254.1	1785.02	<.0001	3466.57	2015.38	<.0001	3301.36	2266.75	<.0001	3532.08	2428.07	<.0001	6647.89	4141.3	<.0001
Ganoderma * Media	128.03	53.72	<.0001	83.98	48.83	<.0001	0	0	1	0	0	1	0	0	1

Source	Days postinoculation (dpi)														
	9			10			11			12			13		
	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F	Mean Square	F Value	Pr > F
Ganoderma	24479.5	16419.3	<.0001	-	-	-	-	-	-	-	-	-	-	-	-
Media	188.65	70.6	<.0001	364.38	144.79	<.0001	258.85	93.14	<.0001	262.28	83.14	<.0001	288.55	142.58	<.0001
Ganoderma * Media	7171.99	4810.53	<.0001	-	-	-	-	-	-	-	-	-	-	-	-

Supplementary Table 3. Summarized ANOVA procedure for relative growth rate of *Ganoderma* isolates on various culture media.

Source	Mean Square	F Value	Pr > F
Ganoderma	11099.31	35089.60	<.0001
Media	11113.09	50940.3	<.0001
Ganoderma * Media	408.67	1291.98	<.0001
G. subamboinense var. *laevisporium* (ATCC 52419)			
Media	3501.22	12472.1	<.0001
G. boninense PER 71			
Media	2944.92	4653.94	<.0001
G. zonatum POR 67			
Media	487.66	2547.34	<.0001
G. zonatum POR 69			
Media	3492.24	10601.00	<.0001
G. miniatocinctum 331035			
Media	2441.11	13240.7	<.0001
G. miniatocinctum 331037			
Media	2444.73	6453.73	<.0001
G. tornatum POR 54			
Media	251.11	3124.66	<.0001
G. tornatum POR 57			
Media	3410.78	7535.98	<.0001