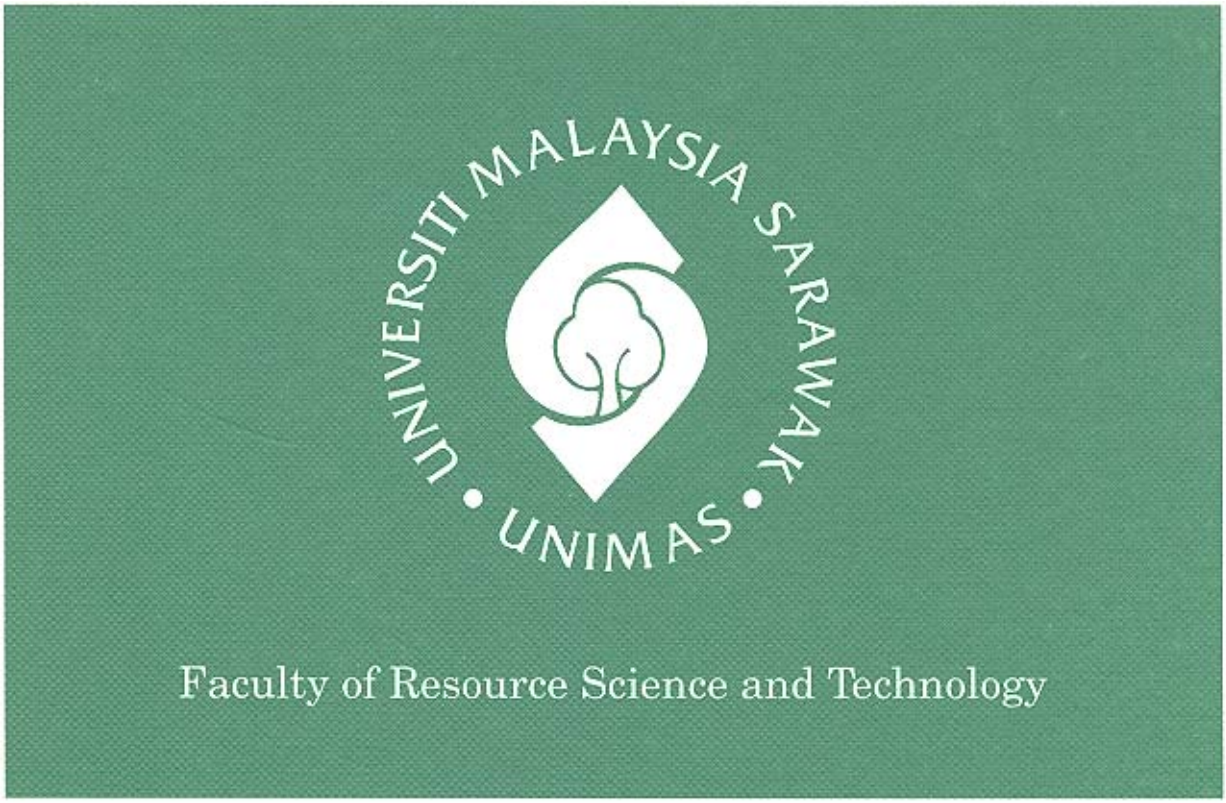

EFFECT OF SAPROPHYTIC FUNGI ON THE GROWTH OF *GANODERMA* SP. ISOLATED FROM ORNAMENTAL PALM BUTT ROT.

Nor Hamirah binti Mahayudin

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This project is submitted in partial fulfillment of
the requirements for the Degree of Bachelor in Science with Honours
(Plant Resource Science and Management Program)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2004

Effect of Saprophytic Fungi on Growth of *Ganoderma* sp. Isolated from Ornamental Palm Butt Rot.

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ABSTRACT

Ganoderma sp. is a pathogenic fungi which caused butt rot disease in palms. In this study, saprophytic fungi were used to look at their effect on growth of *Ganoderma* sp. Mycelia of *Ganoderma* sp. was inoculated together with the antagonist on media in the same Petri dish. Both, spores suspension and mycelia of antagonists were used as inoculum. From the study, it was found that *Gliocladium* sp. was the antagonist that most effective to inhibit the growth of *Ganoderma* sp. in each method. *Aspergillus* sp. *Trichoderma* sp. and *Penicillium* sp. also could inhibit growth of *Ganoderma* sp. The ability of antagonists to control *Ganoderma* sp. was depending on the time of inoculation when both, antagonist and *Ganoderma* sp. inoculated simultaneously or antagonist was inoculated earlier than *Ganoderma* sp.. Further research on using fungi as a biological control should be conducted due to its potential in the future.

Key words: antagonist, pathogenic, saprophytic.

ABSTRAK

Ganoderma sp. adalah kulat yang menyebabkan penyakit pada pangkal pokok palma. Dalam kajian ini, kulat saprofit digunakan untuk menguji kesannya terhadap pertumbuhan Ganoderma sp. Miselia kulat Ganoderma sp. diinokulatkan bersama kulat antagonis. Spora kulat antagonis dan miselia digunakan sebagai inokulum. Hasil kajian menunjukkan Gliocladium sp. adalah antagonis yang paling berkesan menghalang pertumbuhan Ganoderma sp. dalam semua kaedah yang digunakan. Walau bagaimanapun, kulat lain seperti Aspergillus sp., Trichoderma sp. dan Penicillium sp. juga berjaya menghalang pertumbuhan Ganoderma sp. Kebolehan sesuatu antagonis untuk menghalang pertumbuhan Ganoderma sp. bergantung kepada masa sesuatu antagonis atau Ganoderma sp. diinokulatkan. Kajian lanjut tentang penggunaan kulat sebagai kawalan biologi perlu dilakukan kerana potensinya yang besar di masa hadapan.

Kata kunci : antagonis, patogen, saprofit.

INTRODUCTION

Ganoderma sp. is a polyporoid fungi which belong to class Basidiomycetes and family Polyporacea. However, some authors have removed the genus to another family Ganodermataceae (Alexopoulos and Mims 1979). The basidiocarp have a soft and pliable texture when young, but at maturity most are tough, leathery or woody. It is known as the 'mushroom of immortality' in Chinese traditional medicine for its ability to cure certain disease such as headache, depression and sleeplessness (Chiu and Moore 2002). According to Tong (in Tong and Chong 1995), the species which is known as *Ganoderma lucidum*, have medicinal value because of the presence of compound such as organic-Ge, polysaccharides, triterpenoids and adenosine.

Despite for its potential as a medicinal product, *Ganoderma* sp. has been known as a group of wood decaying fungi and as a plant pathogen which cause root and stem rot and causes a great losses for many tropical crops including palms (Anonymous in Bridge *et al.* 2000). The fungus produce enzymes which could degrade woody tissue, primarily consist of lignin and cellulose. The fungus destroy the wood internally and xylem is the affected part.

Basal stem rot (BSR) or palm butt rot is the most significant disease associated with *Ganoderma* sp. Palm which is attacked by *Ganoderma* sp. often cannot be detected by just looking for the existence of its fruiting bodies. Bridge (2000) then reported that the palm butt rot disease initiated when the root come in contact with *Ganoderma* sp. The

young palm plant which is infected by this species often comprise a one-sided yellowing or mottling of the lower frond, followed by necrosis (Singh 1991 in Ariffin *et al.* 2000).

Until now, 15 species of *Ganoderma* sp. have been recorded as probable causal agents for this disease (Turner in Bridge 2000).

So far, several conventional techniques have been used to control this disease, such as using fungicide and fumigants. However, the need to have an environmental friendly in agricultural management, a lower cost and effective solution of controlling this disease enforce people to find other way out (Butt *et al.* 2001). This lead to the biological control where it has also received attention from lots of researchers. For instance, research related with *Trichoderma* sp. as biocontrol agent has been discussed in more than 1700 scientific paper for the past ten years (Wells 1990). The biological control, which is using the mode of antagonism as a controlling agent are based on interaction of two different species, whether as a competition, hyperparasitism or antibiosis (Faull and Singh 1988).

This paper is a report on studies of effect of saprophytic fungi as a potential organism to be used in biocontrol of *Ganoderma* sp.

MATERIALS AND METHOD

The strain of *Ganoderma* sp. which was used in this experiment was obtained from Unimas culture collection. The fungus was already isolated from ornamental palm in Unimas area by Herman (2003). The saprophytic fungi were also obtained from Unimas culture collection . A total of 14 isolates of the saprophytic fungi were used. (Table 1)

Table 1. Saprophytic fungi used as the antagonist.

No	Collection no	Species	Origin
1	787	*Aspergillus nidulans*	Mangrove forest, Samunsam
2	508	*Aspergillus speciso*	Mangrove forest, Samunsam
3	745	*Aspergillus* sp.	Mangrove forest, Samunsam
4	777	*Aspergillus* sp.	Mangrove forest, Samunsam
5	697	*Trichoderma* sp.	Mangrove forest, Samunsam
6	713	*Trichoderma* sp.	Riverine forest, Samunsam
7	703	*Gliocladium* sp.	Riverine forest, Samunsam
8	701	*Gliocladium* sp.	Riverine forest, Samunsam
9	797	*Gliocladium* sp.	Riverine forest, Samunsam
10	676	*Gliocladium* sp.	Riverine forest, Samunsam
11	537	*Penicillium* sp.	Mix dipterocarp forest
12	552	*Penicillium* sp.	Mix dipterocarp forest
13	478	*Penicillium* sp.	Mix dipterocarp forest
14	785	*Penicillium* sp.	Mix dipterocarp forest

All of these fungi were grown in different Petri dishes contains potato dextrose agar (PDA).

Interaction study on Ganoderma species

Method 1

Block of agar (5 mm x 5 mm) containing mycelium of *Ganoderma* sp. was inoculated onto PDA media. The fungus was left for two days on the media to grow. Then, a block of agar containing the test antagonist mycelia was inoculated on the agar at about 1.5 cm apart from the *Ganoderma* sp. inoculum. Three replicates were prepared for each tested fungus. The inoculated plates were incubated at room temperature (25 – 30 °C). Average colony size of both *Ganoderma* sp. and the antagonists was obtained everyday and the colony growth pattern was recorded. The growth of *Ganoderma* sp. was compared with the control where the strain of *Ganoderma* sp. was grown alone on media in Petri dish.

Method 2

Block of agar, (5 mm x 5 mm) containing mycelium of *Ganoderma* sp. was inoculated onto PDA media and allow to grow for two days. Then 0.5 ml of 1x 10^5 spores/ml of spore suspension of antagonist was inoculated onto the *Ganoderma* sp. colony. The spore suspension was prepared using spores from seven to ten day old pure culture and the spore concentration was calculated using haemocytometer. Three replicates were prepared for each tested fungus. The inoculated plates were incubated at room temperature (25 – 30 °C). Average colony size of *Ganoderma* sp. was obtained everyday and the colony growth pattern of both *Ganoderma* sp. and antagonist were recorded. The growth of *Ganoderma* sp. was compared with the control where prepared strain of *Ganoderma* sp. was grown alone on media in Petri dish.

Method 3

Both the spore suspension, 0.5 ml of 1x 10^5 spores/ml of the antagonist and block of agar, (5 mm x 5 mm) containing mycelium of *Ganoderma* sp. were inoculated simultaneously on the PDA. Three replicates were prepared for each tested fungus. The inoculated plates were incubated at room temperature (25 – 30 °C). The growth of *Ganoderma* sp. was compared with the control where prepared strain of *Ganoderma* sp. was grown alone on media in Petri dish.

Method 4

Spore suspension of antagonist containing 0.5 ml of 1x 10^5 spores/ml was inoculated first onto the PDA media and allow to grow for two days. Then, the block of agar, (5 mm x 5 mm) containing mycelium of *Ganoderma* sp. was inoculated onto the saprophytic fungi colony. Three replicates were prepared for each tested fungus. The inoculated plates were incubated at room temperature (25 -30 °C). The growth of *Ganoderma* sp. was compared with the control where prepared strain of *Ganoderma* sp. was grown alone on media in Petri dish.

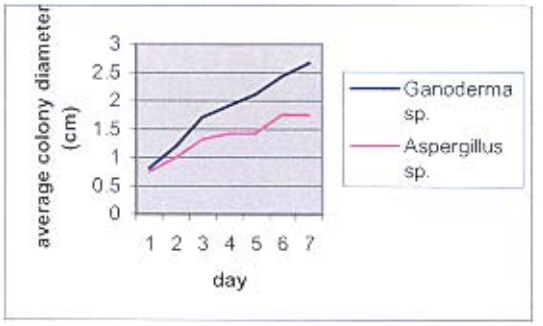
RESULTS

Method 1

Figure 1A-N shows the average colony diameter of each antagonist and *Ganoderma* sp. within the same Petri dishes. Eight of fourteen antagonist used in this study grew faster compared to *Ganoderma* sp. They were *Aspergillus* spp. (787 and 508), *Gliocladium* spp. (701,703,697 and 797), *Penicillium* sp. (552) and *Trichoderma* sp. (676).

The *Trichoderma* sp. (713) and all isolates of the *Pennicillium* sp. and *Aspergillus* sp. did not show much contact with mycelia of *Ganoderma* sp. and grew next to each other without mixing.

Statistical analysis using one way ANOVA shows that there was significant different at $p=0.05$ colony size of *Ganoderma* sp. which was inoculated with the antagonist and *Ganoderma* sp. alone of all the antagonists except for *Trichoderma* sp. (713).



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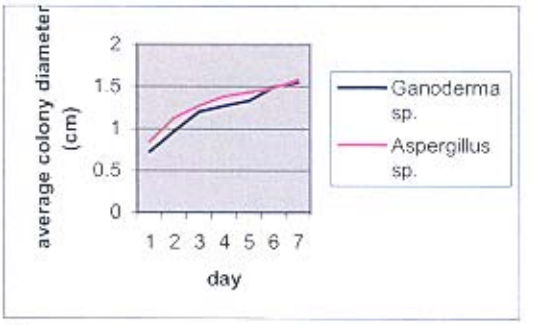

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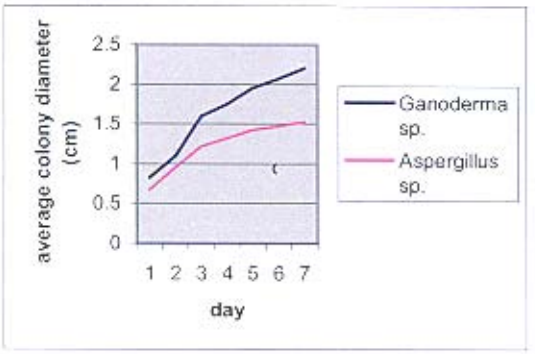

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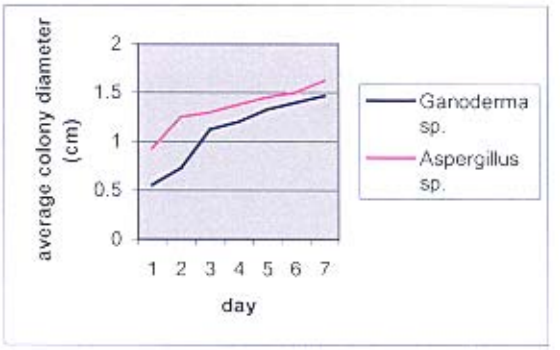

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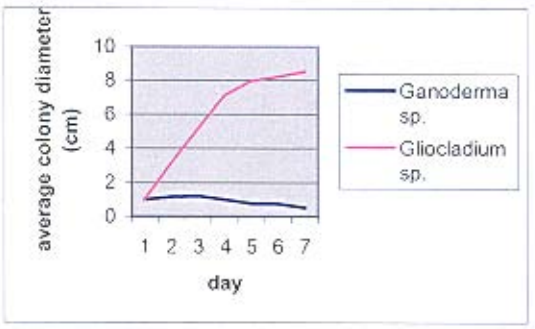

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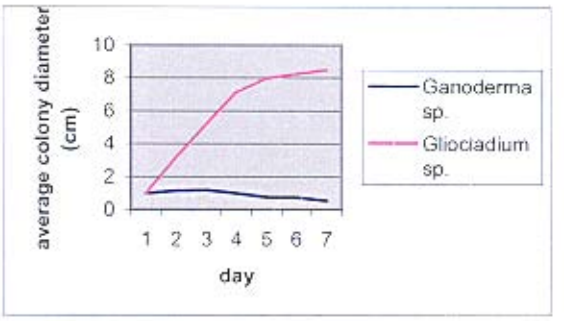
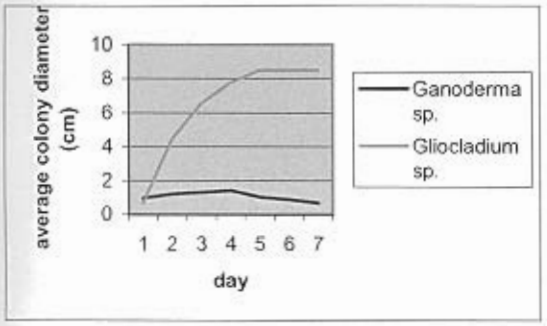


F

Figure 1(A-F). Average colony diameter of *Ganoderma* sp. and the antagonists. A (*Aspergillus* sp.745), B (*Aspergillus* sp. 777), C (*Aspergillus* sp.787), D (*Aspergillus* sp. 508), E (*Gliocladium* sp. 797) and F (*Gliocladium* sp. 701),



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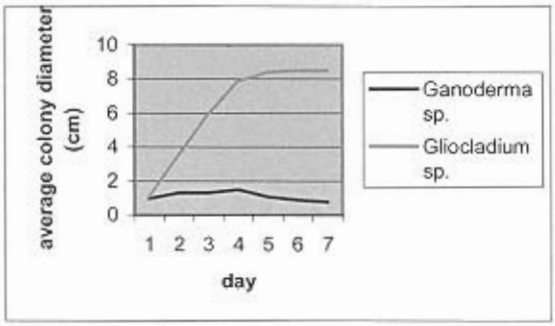

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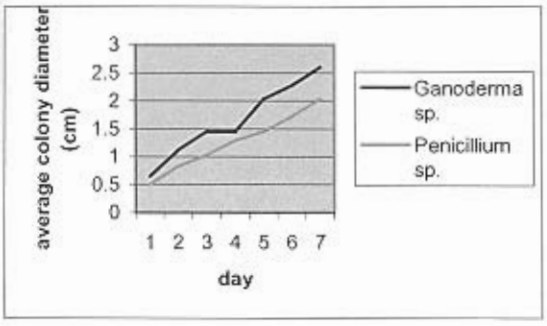

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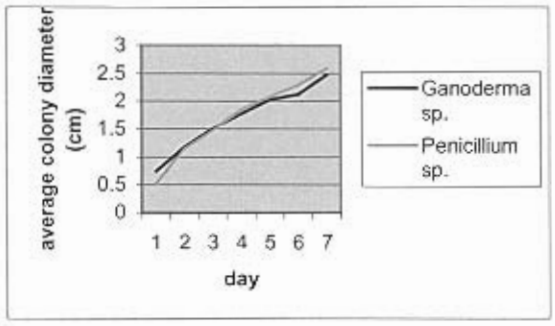

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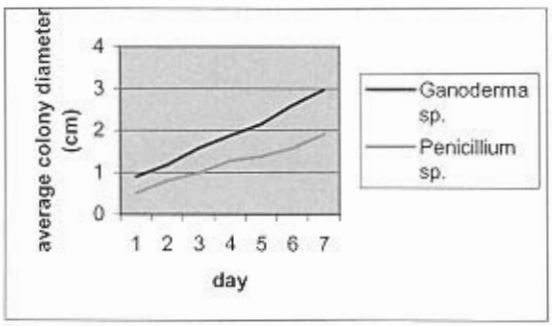

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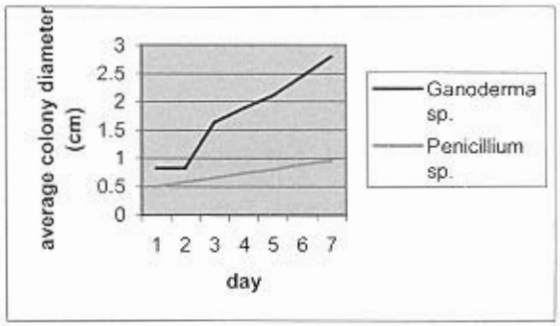
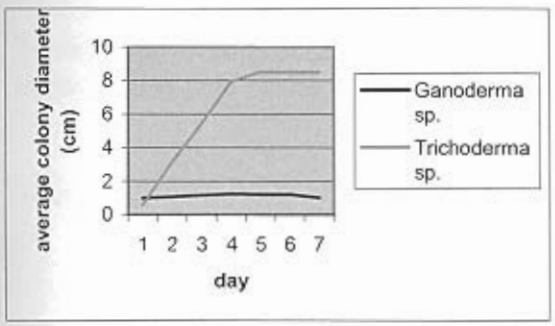


L

Figure 1 (G-L). Average colony diameter of *Ganoderma* sp. and the antagonists. G (*Gliocladium* sp. 697), H (*Gliocladium* sp. 703), I (*Penicillium* sp. 537), J (*Penicillium* sp. 552), K (*Penicillium* sp. 785) and L (*Penicillium* sp. 478),



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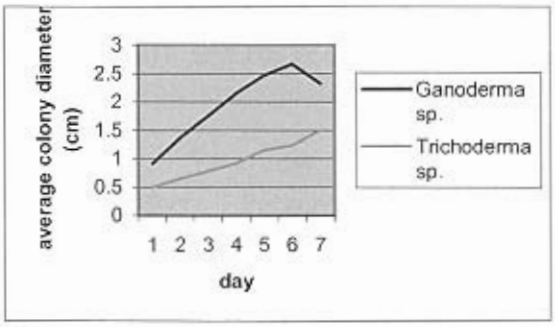

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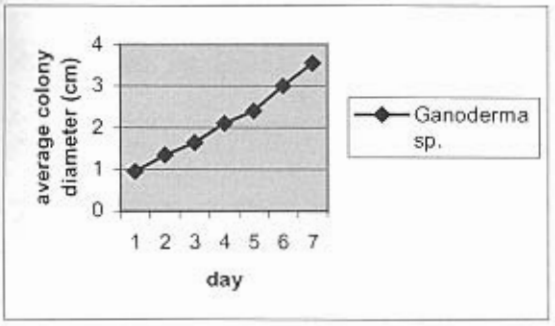

O

Figure 1 (M-N). Average colony diameter of *Ganoderma* sp. and the antagonists. M (*Trichoderma* sp. 676), N (*Trichoderma* sp. 713) and O is control (*Ganoderma* sp. alone).

The colony sizes of the *Ganoderma* sp. which was inoculated together with the antagonists were smaller than of the control. From the result, the percentage of reduction of the colony sizes in this parasitic *Ganoderma* sp. were different from one antagonist to another. The *Gliocladium* spp. were the best inhibitor where the percentage of reductions of colony sizes of the Ganoderma sp. were in a range of 78.8% to 85.07% compared to the control (Table 2). While the least percentage of colony size reduction were by antagonist of *Penicillium* spp. where the range were between 30.42% to 16.34% only.

Table 2. The colony size of *Ganoderma* sp. (mean ±s.d) and the percentages of reduction of the colony size of the fungus when inoculated with the antagonists in Method 1.

Antagonist	Colony diameter (cm) after 7 days of inoculation	Percentage of reduction of colony size
Ganoderma sp. control	3.55±0.13	-
Aspergillus sp. (787)	1.55±0.13	56.34
Aspergillus sp. (508)	1.47±0.30	58.59
Aspergillus sp. (777)	2.20±0.50	38.03
Aspergillus sp. (745)	2.67±0.37	24.79
Gliocladium sp. (797)	0.53±0.16	85.07
Gliocladium sp. (697)	0.68±0.14	80.85
Gliocladium sp. (701)	0.67±0.60	81.13
Gliocladium sp. (703)	0.75±0.05	78.87
Penicillium sp. (478)	2.80±0.10	21.13
Penicillium sp. (785)	2.97±0.27	16.34
Penicillium sp. (537)	2.60±0.30	26.76
Penicillium sp. (552)	2.47±0.33	30.42
Trichoderma sp. (713)	2.33±0.37	34.37
Trichoderma sp. (676)	1.33±0.16	62.54

Compared to other genus, the *Trichoderma* sp. and *Gliocladium* sp. grew faster. On the fifth and sixth day of the inoculation, the mycelia have fully covered surface of the Petri dishes. The colony sizes were larger than of the *Ganoderma* sp. During the colonization, both the *Gliocladium* sp. and *Trichoderma* sp. grew on and surrounded the edges of colonies of *Ganoderma* sp. and this restricted and inhibited the mycelia growth of *Ganoderma* sp. The mycelia of the antagonists contacted the mycelia of *Ganoderma* sp. caused changes of colour of *Ganoderma* sp. from white to yellowish. There was no change of colour of the *Ganoderma* sp. mycelia when they were in contact with the mycelia of *Trichoderma* sp.

Figure 2. Myselia of antagonist *Trichoderma* sp. (797), (green) inhibiting growth of *Ganoderma* sp. (white).

Method 2

Statistical analysis using one way ANOVA shows that there were significant different at p=0.05 between the colony sizes of *Ganoderma* sp. which was inoculated with the antagonists and the control except for *Trichoderma* sp. (713).

Table 3 shows the colony diameter sizes and the percentages of reduction of *Ganoderma* sp. compared to control when the *Ganoderma* sp. was inoculated two days earlier than the antagonists. From the table, it shows that *Gliocladium* sp. (697) caused the highest percentage of colony size reduction of *Ganoderma* sp. followed by *Aspergillus* sp. (777) and *Penicillium* sp. (537). The reduction of colony size after seven days of inoculation of *Ganoderma* sp. caused by the *Gliocladium* sp. (697) was 71.23% while the reduction caused by both *Aspergillus* sp. and *Penicillium* sp. were 69.3%. The least percentage of reduction of colony size of *Ganoderma* sp. was caused by for *Trichoderma* sp. (713), which was only by 9.60%. Compared to the first method, the colony size for *Ganoderma* sp. were larger in second method. This is be due to different type of inoculum used in this second method where *Ganoderma* sp. was allowed to grow freely on the media before the antagonist were introduced.

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Table 3. The colony sizes of *Ganoderma* sp. (mean ±s.d) and the percentages of reduction of the colony size of the fungus when inoculated with the antagonists in Method 2.

Antagonist	Colony diameter (cm) after 7 days of inoculation	Percentage of reduction of *Ganoderma* sp. colony size
Ganoderma sp. control	3.65±0.26	-
Aspergillus sp. (787)	1.67±0.30	54.25
Aspergillus sp. (508)	1.55±0.00	58.33
Aspergillus sp. (777)	1.12±0.08	69.32
Aspergillus sp. (745)	1.65±0.73	54.79
Gliocladium sp. (797)	1.43±0.03	60.82
Gliocladium sp. (697)	1.05±0.08	71.23
Gliocladium sp. (701)	1.82±0.06	50.14
Gliocladium sp. (703)	1.32±0.08	63.83
Penicillium sp. (478)	1.53±0.14	58.08
Penicillium sp. (785)	1.68±0.08	53.70
Penicillium sp. (537)	1.12±0.15	69.32
Penicillium sp. (552)	1.95±0.30	46.58
Trichoderma sp. (713)	3.30±0.33	9.59
Trichoderma sp. (676)	1.02±0.08	63.56

Mycelia of the *Trichoderma* sp. (697) and *Gliocladium* spp. (701,703,697 and 797) can grow on the *Ganoderma* sp. These antagonists covered the upper colony surface of *Ganoderma* sp. for about one quarter of the colony size of *Ganoderma* sp. However, *Trichoderma* sp. (713) did not cause reduction of the colony size of *Ganoderma* sp. In fact the colony diameter nearly the same size as of the control.

However, when the isolates of *Penicillium* sp. were used, this antagonist could inhibit the growth of *Ganoderma* sp. better in second method, compared to the Method 1.

Figure 2. Myselia of antagonist *Aspergillus* sp. (787), (brown) inhibiting growth of *Ganoderma* sp. (white).

Method 3

The growths of *Ganoderma* sp. were strictly inhibited by all the antagonists fungi when they were inoculated simultaneously. The *Ganoderma* sp. survived but the colony could not spread widely because the media were fully covered by the antagonists. The antagonists, especially for *Gliocladium* sp. not just inhibit the growth of *Ganoderma* sp., but also covered the colony of the *Ganoderma* sp.

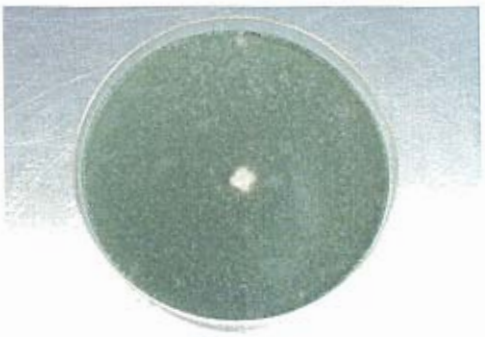

Figure 3. Myselia of antagonist, *Trichoderma* sp. (701), (green) inhibiting growth of *Ganoderma* sp. (white)

Method 4

When the antagonists fungi were inoculated earlier on the media, the *Ganoderma* sp. succeed to develop colony. Spore suspension of the antagonist germinated and grew well on the media. So, the surface area of the media were fully covered by the antagonists and mycelia of the *Ganoderma* sp. could not reach the media for nutrients.

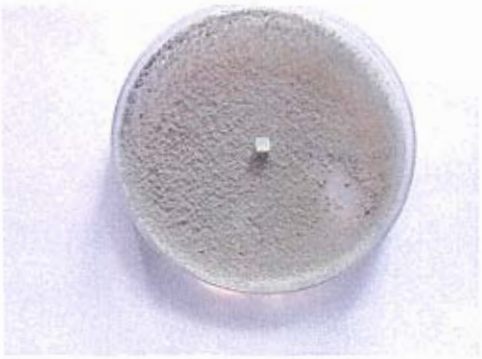

Figure 4. Mycelia of *Ganoderma* sp. (white cube) could not grow on antagonist colony, *Penicillium* sp. (552)

Correlation Analysis

In order to find whether there was relationship between different time of inoculation with the colony size *Ganoderma* sp., statistical analysis using correlation was used. From the analysis, the result shows that there were significant correlation between the two factor at $p=0.01$ level of confidence. This proved that there were effect of different time of inoculation on the colony size of *Ganoderma* sp.

DISCUSSION

All the *Gliocladium* sp. isolates were able to reduced the growth of *Ganoderma* sp. as indicated in the present study. The reductions of growth of the *Ganoderma* sp. were greater than those shown by the other antagonist fungi. This might be due to the ability of the *Gliocladium* sp. to grow faster than the other antagonists. The previous report by Howell in Sundheim and Tronsmo (1988) had indicated the ability of *Gliocladium* sp. to control plant disease. Treatment of soil with *G. virens* resulted in a 63 % reduction in the number of *Rhizoctonia solani*.

When the *Ganoderma* sp. colony was allowed to develop before the antagonist was introduced as in the second method, *Gliocladium* sp. was still the best inhibitor to restrict the growth of the *Ganoderma* sp.. The percentage of colony size reduction caused by *Gliocladium* sp. (697) was 71.23% and *Gliocladium* sp. (703) were 63%. *Aspergillus* sp. (777) and *Penicillium* sp. (737) also caused a high percentage of colony size reduction of *Ganoderma* sp. by 69.32%. *Trichoderma* sp. (676) also caused less diameter size of *Ganoderma* sp. by 63%. This result indicated that other antagonists such as *Aspergillus* sp., *Penicillium* sp. and *Trichoderma* sp. could also be used to control the growth of *Ganoderma* sp. Cooksey and Moore in Aggarwal *et al.* (1988) found that the incident of galling on mazzard and cherry seedling caused by some fungal pathogens was reduced when isolates of *Penicillium* sp. and *Aspergillus* sp. were tested in a field. *Trichoderma* sp. have also been used to control *Rhizoctonia solani* which caused damping-off bean, tomato and eggplant seedling as reported by Hader *et al.* cited by Aggarwal *et al.* (1988).

Results in method 3 and 4, where the antagonists were inoculated simultaneously or earlier than *Ganoderma* sp. showed a better growth inhibition of pathogen than the first two methods. In method 3, although there were growths of *Ganoderma* sp. but the colony could not expand. While in method 4, where the antagonist had established, there was no growth at all of the *Ganoderma* sp.

The differences between the results obtained for each methods due to some factors. The first factor was the type of inoculum. In method 1, mycelia of the antagonist were used and in this condition, *Gliocladium* sp. was shown to be the best inhibitor fungi compared to the other antagonists on *Ganoderma* sp.. While in the second method, spores suspension of the antagonist were used as inoculum, the percentage of colony size reduction of *Ganoderma* sp. were decreased when *Gliocladium* sp. were used as antagonist compared to in method 1, but the percentage of colony size reduction were higher for other antagonists such as *Aspergillus* sp. and *Penicillium* sp. The second factor which contribute to the different percentage of reduction was time of inoculation. For the first two method, strain of *Ganoderma* sp. were inoculated two days earlier than the antagonists. Thus, the colonies could still grow even though they were restricted. However, in method 3 and 4, the *Ganoderma* sp. was inoculated later or simultaneously with the antagonist, which made the *Ganoderma* sp. colony could not develop and expand. This means that the *Ganoderma* sp. growth were strictly inhibited in these two methods.