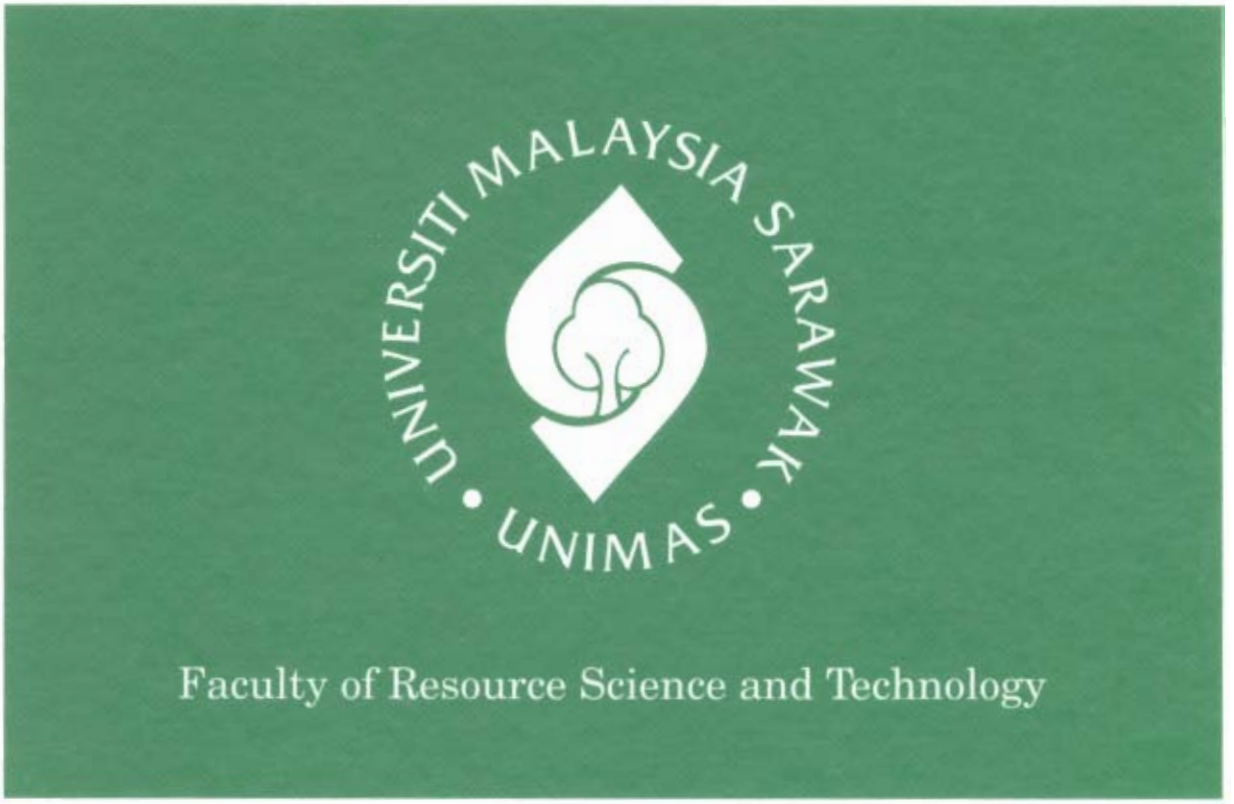

EFFECTS OF *PENICILLIUM* SPP. ON GROWTH AND CELLULOSE DEGRADATION OF *GANODERMA* SPP. THAT ARE PATHOGENIC TO PALMS

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Bachelor of Science with Honours
(Plant Resource Science and Management)
2006

ACKNOWLEDGEMENTS

Alhamdulillah…

First and foremost, I would like to acknowledge my parents, who give me so much support and encouragement during my studies and to complete my project.

I also would like to thank my supervisor, Assoc. Prof. Dr. Sepiah Muid who has been patiently guiding and spending her time upon completing this project. Without her cooperation and assistance, this project would not complete successfully.

I would like to extend my gratitude to master students who really helping and guidance me during the project.

Last but not least, I would like to thanks to everyone especially my friends and siblings for their assistance and support to complete the project.

Thank you.

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EFFECTS OF *PENICILLIUM* SPP. ON GROWTH AND CELLULOSE DEGRADATION OF *GANODERMA* SPP. THAT ARE PATHOGENIC TO PALMS

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

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2006

Effects of *Penicillium* spp. on Growth and Cellulose Degradation of *Ganoderma* spp. that are Pathogenic to Palms

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ABSTRACT

The effects of *Penicillium* spp. on growth and cellulose degradation of *Ganoderma* spp. that are pathogenic to palms were determined by the application of three types of tests. These were dual culture interaction test, effect of crude extracts of *Penicillium* spp. on *Ganoderma* spp. and effect of *Penicillium* spp. on cellulose degradation by *Ganoderma* spp.. Twenty strains of *Penicillium* spp. and two strains of *Ganoderma* spp. were used in this study. From the dual interaction test and effect of crude extract of *Penicillium* spp. on the growth of *Ganoderma* spp., it was found that almost all strains of the *Penicillium* spp. caused significant reduction of growth of *Ganoderma* spp.. It was also found that almost all *Penicillium* spp. had significant effect on cellulose degradation by *Ganoderma* spp.. From the result obtained, there was significant reduction in the growth performance of *Ganoderma* spp. when *Penicillium* spp. was used as an antagonist and this indicating *Penicillium* as a potential biological control agent.

Key words: *Penicillium* spp., *Ganoderma* spp. and biological control agent.

ABSTRAK

Kesan-kesan Penicillium spp. terhadap pertumbuhan dan degradasi selulosa oleh Ganoderma spp. yang patogenik terhadap palma ditentukan dengan mengguna tiga jenis ujian. Antaranya ialah ujian interaksi dwikultur, kesan ekstrak mentah Penicillium spp. terhadap Ganoderma spp. dan kesan Penicillium spp. terhadap degradasi selulosa oleh Ganoderma spp.. Dua puluh strain Penicillium spp. dan dua strain Ganoderma spp. digunakan dalam kajian ini. Daripada ujian interaksi dwikultur dan kesan ekstrak mentah Penicillium spp. terhadap pertumbuhan Ganoderma spp., didapati hampir semua jenis Penicillium spp. menyebabkan pengurangan yang signifikan terhadap pertumbuhan Ganoderma spp. Didapati juga, Penicillium spp. mempunyai kesan signifikan terhadap degradasi selulosa oleh Ganoderma spp..Daripada keputusan yang diperolehi, terdapat pengurangan yang signifikan dalam pertumbuhan Ganoderma spp. apabila Penicillium spp. digunakan sebagai antagonis dan ini menunjukkan potensi Penicillium spp. sebagai agen kawalan biologi.

Kata kunci: Penicillium spp., Ganoderma spp. and agen kawalan biologi.

CHAPTER 1

INTRODUCTION

Ganoderma spp. is known as higher fungus which falls in family of Polyporaceae, order of Aphyllophorales, subclass of Hymenomycetes, and class of Basidiomycetes (Idris *et al.*, 2000). Alexopoulos and Mims (1979) reported that some authors have removed *Ganoderma* spp. from the Polyporaceae and placed it in a separate family, the Ganodermataceae. Most of members in the family of Polyporaceae can cause diseases of trees and is responsible for most of the wood rot (Alexopoulos and Mims, 1979).

Basal Stem Rot (BSR) disease caused by *G. boninense* (Idris *et al.*, 2003; Sariah, 2003 and Ariffin and Idris, 1992) is the most destructive and serious disease occurred in oil palm plantation. BSR of oil palm was recorded in this country as early as 1928 and until now there are no effective control measures that have been identified (Sariah, 2003).

The incidence of BSR disease of oil palm is seriously occurred and caused great loss and economic importance of oil palm plantation in Southeast Asia, Malaysia, which include Peninsular Malaysia, Sabah and Sarawak (Idris *et al.*, 2002) and Indonesia (Idris *et al.*, 2000 and Susanto *et al.*, 2005). Recently, BSR has caused most prevalent and devastating disease in oil palm cultivation, especially in mature palm areas in Malaysia (Sariah, 2003). Experience gained in the Solomon Islands and Papua New Guinea (PNG) over the past 6 years of intensive

research, suggests that control of *Ganoderma* stem rot can only be achieved if all sources of infection are controlled (Griffiths *et al.*, 2001).

Because of the problem arise especially in economics importance of oil palm, therefore an acceptable control measures incorporated with environmental friendly method have to be obtain. Therefore, immediate short-term measures have to be taken to control the disease (Idris *et al.*, 2002). The research is also done to improve or enhance the productivity and quality of the oil palm production.

The objectives of this study were to evaluate the effectiveness of various types of *Penicillium* spp. to inhibit the growth of *Ganoderma* spp., to determine the action of the crude extracts contained in the *Penicillium* spp. on growth of *Ganoderma* spp. and to determine the effects of *Penicillium* spp. on cellulose degradation caused by *Ganoderma* spp..

CHAPTER 2

LITERATURE REVIEW

2.1 Plant diseases caused by *Ganoderma* spp.

Ganoderma species are wood rotting fungi. A number of *Ganoderma* spp. being pathogenic and thus harmful on economically important trees and perennial crops (Idris and Ariffin, 2003). *Ganoderma* spp. have been observed as the causal agent of root and stem rot in plantation crops such as oil palm, coconut, rubber, betel nut, tea, cocoa, peaches, par, guavas, grapevines and forest trees such as *Acacia, Populus and Macadamia* (Idris *et al.*, 2003).

Some *Ganoderma* species that have been identified as pathogenic fungi such as *G.lucidum, G. zonatum, G. encidum, G. colossus, G. boninense*, and *G. applanatum* to oil palm (Wakefield, 1920 cited in Idris *et al.*, 2000). Pathogenicity test done by Idris *et al.* (2000) and Idris *et al.* (2003) showed that *G. boninense, G. zonatum* and *G. miniatocinctum* were pathogenic to palm and the diseased symptoms induced by isolates identified *G. boninense* were more severe than those cause by *G. zonatum* and *G. miniatocinctum*.

G. boninense is among the common species has been reported to cause diseases on plants. The fungus caused disease among crops such as rubber tree, fruit tree, cover crop which is planted in the oil palm plantation, oil palm and ornamental palm such as 'pinang', royal palm, 'pinang alice', 'lontar and 'serdang' (Idris *et al.*, 2003).

2.2 Control of Basal Stem Rot (BSR) disease

Various methods to control BSR disease have been used, it was however no entire satisfactory result has been obtained in reducing the disease incidence (Ariffin and Mohd Basri, 2000 and Idris *et al.*, 2004). Susanto *et al.* (2005) reported that the available control measures for BSR disease such as cultural practices, mechanical and chemical treatment have not proved satisfactory due to the fact that *Ganoderma* has various resting stages such as melanised mycelium, basidiospores and pseudosclerotia.

There are some practices that have been tested for controlling this disease consist of surgical, clean clearing, windrowing, underplanting, removal of infected palms, sanitation during replanting, soil mounding, fungicide treatment or combination of these methods (Idris *et al.*, 2002 and Khairudin, 1990 cited in Idris *et al.*, 2004). Idris *et al.* (2003) suggested that legumes act as an alternative host for *G. boninense* to survive in the fields.

2.2.1 Biological Control

According to Fry (1982), biological control is defined as any condition under which or practice whereby, survival or activity of a pathogen is reduced through the agency of any other organism (except man himself), with the result that there is the reduction in the incidence of the disease caused by the pathogen . Antagonists are biological agents with the potential to interfere in the life processes of plant pathogens (Cook and Baker, 1996). Antagonism can provide biological protection of plant surface through exclusion, displacement, or inhibition of the pathogen on root, leaves or their plant parts (Cook and Baker, 1996)

Biological control practices for direct protection of plants from pathogens involve the deployment of antagonistic microorganisms at the infection court before or after the infection take place (Agrios, 1988).

In developing effective preparations for the biological control of soilborne plant pathogens, one should take into account is the antagonistic activity, inoculum potential, inoculum formulation, mode of application and the site of interaction between the pathogen and its antagonist (Fahima and Henis,1993). The targeted strain for biocontrol agents should comprise the important characteristic or appropriate mechanisms for biocontrol which can prevent the occurrence of basal stem rot (BSR) disease such as antibiosis and mycoparasitism, incorporated with strong competitive ability of antagonistic microorganisms to displace the *Ganoderma* spp. so as to minimize the pathogen's opportunity for colonization, whereby it must able to protect the host plant through exclusion, displacement, inhibition, or colonize the existing pathogen (Cook and Baker, 1996 and Sariah, 2003).

There are several fungus that have been tested to treat the infection of *G. boninense* such as *Trichoderma harzianum* (Shamala *et al.,* 2003), *Aspergillus* spp. (Shukla and Uniyal, 1989 cited in Ariffin and Mohd Basri, 2000) and *Penicillium* spp. (Dharmaputra *et al.*, 1989 cited in Ariffin and Mohd Basri, 2000). However, none of these agents has been used in practice successfully to control BSR and there still no effective biological control methods have been reported (Ariffin and Mohd Basri, 2000).

According to Dharmaputra (1989), *T. harzianum* and *T. viride* can repressed the growth of the *G. boninense* and caused lysis of the hyphae, and the colony was totally overgrown by the antagonists. However, studies conducted in Indonesian Oil Palm Research Institute (IOPRI) showed that a glasshouse and field trial for *Ganoderma* control indicated that treatment with *T. harzianum* and *T. viride* was superior to *Bacillus* sp. (Susanto, 2003). Recent study by Nor Hamirah (2004) using *Penicillium* spp., *Aspergillus* spp. and *Trichoderma* spp. showed that the ability of antagonist to control *G. boninense* also depending on the time of inoculation when both, antagonist and *G. boninense* inoculated simultaneously or antagonist is inoculated earlier than *G. boninense*.

One of the most well known antibiotic produce by *Penicillium* spp. is penicillin. Penicillin was formed by the species *P. notatum* and *P. chrysogenum* which was discovered by Professor Alexander Fleming in 1928 (Alexopoulos and Mims, 1979). Beside that, griseofulvin also identified as an important antibiotic, formed by the species of *Penicillium* (Alexopoulos and Mims, 1979). In 1939, Griseofulvin was first isolated as a metabolic product from *P. griseofulvum* which act as an antifungal antibiotic with excellent transport in the plant system without phytotoxicity. It was greatly marketed in 1950's for control of plant disease, especially *Botrytis* disease and powdery mildew. Griseofulvin play role in inhibiting mitosis in fungi by interference with the functioning microtubules (Altman, 1993).

The ability of *Penicillium* to produce antibiotic may be the main factor that can prevent or control the existence of pathogenic fungi. Beside that, the production of antibiotic substances by

P. chrysogenum in soil and the rhizosphere inhibited the normal growth of *Verticillium albo-atrum* (Agrios, 1988). Raaijmakers (2002) reported that antibiotic play a key role in the suppression of various soilborne plant pathogens by antagonistic microorganisms. This makes *Penicillium* spp. as a potential biological control agent to control plant diseases especially to control diseased oil palm that caused by soilborne fungi.

The interest in biological control of plant pathogens has been stimulated in recent years by trend in agriculture toward greater sustainability and public concern about the use of hazardous pesticides (Raaijmakers *et al.*, 2002) and the impact of the application of chemical pesticides on the environment, non target organism and pollution such as contamination of groundwater which will enter the food chains (Sariah and Ooi, 1999 and Butt *et al.*, 2001). Besides, public concern over the presence of chemicals residues in food has resulted in cancellation of some of the most effective fungicide (Qing and Shiping, 2000). Mukerji and Garg (1988) suggested that it has become essential to do more work on the biological control of disease and to avoid the use of the fungicides and other chemicals, considering the ecological damage which may result.

2.2.2 Chemical control

While in chemical method, the application of chemical pesticide is widely being used in the field to prevent and to eradicate the pathogens that have been infected the plant, especially in the plant surfaces (Agrios, 1988).

Several fungicide have been tested by Malaysian Palm Oil Berhad (MPOB) by treated the fungicide to the infected oil palm for field observation. Fungicides that usually used are such as bromoconazole, hexaconazole, benomyl, thiram, triadimefon, triadimenol and tridemorph (Idris *et al.*, 2004). Khairudin (1990) reported that in a comparison of methods of applying triadimenol and carboxin by trunk injection, soil drenching and a combination of the two, results after 30 months (4 applications at 4-monthly intervals) were inconclusive. However, the use of fungicide is not very effective (Sariah, 2003) due to the failure of the fungicide to reach the disease margin and within disease lesion (Idris *et al.*, 2002). As a result, MPOB have developed a pressure injection apparatus which is enable the delivering of chemical to the target sites quickly and efficiently (Idris *et al.*, 2002). Thereafter, Idris *et al.* (2004) have identified that bromoconazole and hexaconazole as the most effective fungicide compared to others, whereas it is effective in prolonging the productive life of infected palms

2.2.3 Clean clearing

Clean clearing is also practiced during replanting of oil palm. But seem that, not entirely satisfactory reduction in the disease incidence (Idris *et al.*, 2004) and the infection of the disease can still become established progressively earlier with each planting cycle (Sariah, 2000).

2.3 *Penicillium* spp. as Biological Control Agents (BCAs)

Recently, researches on the potential of *Penicillium* spp. as an antagonist are widely being done. *Penicillium* spp. is a fungus which belongs to the class of Ascomycetes, under order of Eurotiales, family of Eurotiaceae, and genus of *Penicillium.*

P. citrinum inhibited the growth of *G. boninense* and formed a zone of inhibition on the agar media (Dharmaputra, 1990). Cook and Baker (1996) reported that seed treatment with *P. oxalicum* were effective against damping off of garbanzo beans caused by *Pythium* spp.. *P. oxalicum* also effective to control *Fusarium* and *Verticillium* wilt of tomato under greenhouse and field conditions (Cal *et al.*, 2003). *Penicillium* operates mainly as a competitor of pathogen on the host and as a secondary colonist of lesions or infested crop residues (Dharmaputra, 1990).

Some *Penicillium* species such as *P. notatum* and *P. chrysogenum* have been widely known as an important sources for the production of antibiotic called penicillin (Alexopoulos and Mims, 1979), whereas *P. griseofulvum* also known as an important for the production of antibiotic called griseofulvin (Alexopoulos and Mims, 1979 and Altman, 1993).

2.4 Modes of antagonism

There are three types of modes of antagonism activity which result in the suppression of plant disease. These are in competition, parasitism and antibiosis activities (Tronsmo, 1996). These make the application of biological control effective and successful.

Competition occurs when there is demand by two or more microorganisms for the same resource in excess of the immediate supply (Mukerji and Garg, 1988). For example, there is the need among the microorganism for nutrients, oxygen, space and others (Tronsmo, 1996). Therefore, the microorganisms have to compete between each other to get the entire requirement needed.

Competition between the biological control agent and the pathogen may lead to disease control (Tronsmo, 1996)

There are four types of phenomenon in fungal parasitism which is mycoparasitism, hyperparasitism, direct parasitism and interfungal parasitism (Tronsmo, 1996). Hyperparasitism activity cover wide range of interaction that usually occur include minor or major morphological disturbances, the overgrowth of hyphae of fungus by another, penetration and direct parasitism by the production of haustoria and the lysis of one hyphae by another (Mukerji and Garg, 1988).

Antibiosis occurs when the production of antibiotic or toxic metabolites by microorganisms has a direct effect on another microorganism (Tronsmo, 1996 and Mukerji and Garg, 1988). Antibiotic is a chemical compound which can inhibits or kill other microorganisms (Agrios, 1988). Many fungi have been shown to be capable of producing volatile and nonvolatile antibiotics (Mukerji and Garg, 1988). Even though many antagonists are able to produce antibiotic or toxin in the pure culture, there is little proof of the effect of such compound in biological control (Papavizas and Lumsden, 1980 cited in Tronsmo, 1996).

Modes of antagonism play an important role in the biological control of plant pathogen in the field. It is very difficult to estimate the relative importance of each of the three mechanisms, because the importance of the different biological control mechanisms is dependent on the isolates used, the target organism, and the environmental conditions (Tronsmo, 1996). Mukerji

and Garg (1988) stated that understanding of the interaction that occurs between microorganisms is insufficient.

2.5 Cellulose degradation

Ganoderma spp. has been observed as the causal agent of root and stem rot in rubber, plantation (Idris *et al.*, 2003). *G. applanatum* caused cellulose degradation on rubber wood. Hong (1982) reported the maximum weight loss and degraded the largest amount of lignin, holocellulose and alpha-cellulose.

Interaction between fungi affects the ability of pathogenic fungi to degrade cellulose. Some antagonist fungi will inhibit, enhance and some may not have any effects on cellulose degradation. Lundborg (1988) reported that co-cultivation of antagonist fungi, isolate D37 with *Heterobasidion annosum* on agar plates containing crystalline cellulose inhibited cellulose degradation caused by *H. annosum* and the formation of clearing zone caused by *H. annosum* was stable for several months although relatively high amount of cellulases were detected. The exudates from the antagonistic fungus D37 inhibited and prevented cellulose degradation caused by *H. annosum*.

CHAPTER 3

MATERIALS AND METHODS

3.1 Cultures

For this research, the cultures of *Penicillium* spp. were obtained from Unimas culture collection and freshly collected samples. Cultures of fresh specimen were obtained from plant materials collected in Sungai Rayu, Matang Wildlife Centre and Peat Swamp Forest. The plant materials such as leaves and stems were cut into 5mm x 5mm. The cutting of leaves and stem were washed thoroughly using sterilized water and then dried with filter paper. Then, the cutting of leaves and stem were plated onto Petri dishes containing water agar. Each Petri dish contained 6 to 7 cuttings. The inoculated plates were incubated at room temperature (26°-30°C). Occurrences of *Penicillium* spp. were observed under microscope. The *Penicillium* spp. were transferred onto new PDA in Petri dishes. Twenty strains of *Penicillium* spp. and two strains of *Ganoderma* spp. used in this study were listed in Table 1. Cultures of *Ganoderma* spp. were also obtained from Unimas culture collections. They were *Ganoderma boninense* (isolate SOPK1P) and *Ganoderma* sp. (isolate PG03). The strains were obtained from diseased ornamental palm (isolate PG03) in Unimas area and diseased oil palm (isolate SOPK1P). *Penicillium* spp. and *Ganoderma* spp. which were obtained from the stock culture were recultured on PDA.

Table 1. Strains of *Penicillium* spp. and *Ganoderma* spp. used in this study

No.	Unimas culture collection	Species
1	702	*Penicillium* sp.
2	684	*Penicillium* sp.
3	666	*Penicillium* sp.
4	577	*Penicillium* sp.
5	477	*Penicillium* sp.
6	432	*Penicillium* sp.
7	537	*Penicillium* sp.
8	515	*Penicillium* sp.
9	586	*Penicillium* sp.
10	776	*Penicillium* sp.
11	552	*Penicillium* sp.
12	494	*Penicillium* sp.
13	760	*Penicillium* sp.
14	1316	*Penicillium* sp.
15	1317	*Penicillium* sp.
16	1318	*Penicillium* sp.
17	1319	*Penicillium* sp.
18	1320	*Penicillium* sp.
19	1321	*Penicillium* sp.
20	1322	*Penicillium* sp.
21	SOPK1P	*Ganoderma boninense*
22	PG03	*Ganoderma* sp.

3.2 Interaction tests between *Ganoderma* spp. and *Penicillium* spp.

The test was done by using three types of dual culture inoculations. All strains of *Penicillium* spp. and *Ganoderma* spp. in Table 1 were used. Potato dextrose agar (PDA) was used as the fungal growth media in the tests. Block of agar (5mmx5mm) containing mycelium of the tested *Ganoderma* spp. and *Penicillium* spp. were inoculated on agar in the same petri dish. The distance between the two different fungus is approximately 4 cm. The tested *Penicillium* strains were inoculated at three different periods regarding to the *Ganoderma* spp.

i) *Penicillium* spp. was inoculated simultaneously with *Ganoderma* spp.

ii) *Penicillium* spp. was inoculated 5 days later than the *Ganoderma* spp.

iii) *Penicillium* spp. was inoculated 4 days earlier than the *Ganoderma* spp.

Ganoderma spp. that was inoculated individually was used as control. All the inoculated plates were incubated at room temperature (26°-30°C). Average colony diameters of *Ganoderma* spp. were observed every two days. Triplicates were prepared for each test.

3.3 Effects of crude extracts from *Penicillium* spp. on *Ganoderma* spp.

Preparation of crude extracts from *Penicillium* spp. was done by growing the *Penicillium* spp. in liquid media of potato dextrose. Two isolates of *Ganoderma* spp. (PG03 and SOPK1P) and five strains of *Penicillium* spp. (494, 1317, 1319, 1320 and 1321) were used. The potato dextrose broth was prepared using 200g potato which was boiled for 30 minutes. Then, this solution was filtered. 20g dextrose and distilled water was added in the solution until the volume reach 1000ml. The solution was transferred into 200ml of conical flask and each flask contains 100ml of media solution. The media was autoclaved at 15 psi for 20 minutes. The tested *Penicillium* sp.

was inoculated into the potato dextrose broth and incubated at room temperature (26°-30°C). After one week, the solution was filtered. The aqueous solution which contain crude extracts of the *Penicillium* sp. was added into PDA at three different concentrations, which were 10%, 20% and 30%. The *Ganoderma* spp. were inoculated in the Petri dish containing PDA incorporated with the crude extracts. The changes in the growth of *Ganoderma* spp. were observed for every two days for two weeks. The control will be prepared by using PDA without the addition of crude extracts. Triplicates were prepared for each test.

3.4 Effects of *Penicillium* spp. on cellulose degradation by *Ganoderma* spp.

The Carboxyl Methyl Cellulose (CMC) agar was prepared and pours in the Petri dish. All strains of *Penicillium* spp. and *Ganoderma* spp. in Table 1 were used. Block of agar (5mmx5mm) containing mycelium of the tested *Ganoderma* spp. were inoculated onto CMC agar and allowed to grow for three days. The spore suspensions of *Penicillium* spp. were prepared using mature spores from seven to ten days old pure culture. The amount of spores was calculated using haemocytometer. The spore suspension containing 1 x 10^7 spore/ml was inoculated onto the top of the *Ganoderma* spp. colony on the same Petri dish. Triplicates were prepared for each test. The inoculated plates were incubated for one week at room temperature (26°-30°C). After the incubation period, congo red was added on the surface of CMC agar to facilitate the observation of the effects of the *Penicillium* spp. on cellulose degradation by *Ganoderma* spp.. After 15 minutes, the congo red was washed thoroughly with 1 M NaCl to maintain the colour. Averages colony size of the *Ganoderma* spp. and the colony growth pattern were observed for every two days. The growths of *Ganoderma* spp. were compared with the control experiment which was