CHAPTER 4
RESULTS AND DISCUSSION

4.1 Screening of pathogenic fungi from shallot and onion

The sheath of shallot and onion was shelled and soaked in the Clorox solution for 1 min. Then, it was put on PDA medium plate. After 3 days of incubation, the fungi grow on PDA plate were isolated and purified by point method.

The colony morphology of six isolates found on shallot and onion were shown in Fig 4.1. Isolate CMU1, CMU2 and CMU3 were isolated from onion. Isolate CMU4, CMU5 and CMU6 were isolated from shallot. The characteristics of six isolates were shown in Table 4.1. After isolation, they were identified from the characteristics of mycelium and spores (Larone, 1993; Alexopoulos *et al.*, 1996; Vanittanakom, 1999). The results are shown in Table 4.2 in which six isolates were identified as 2 isolates of *Aspergillus niger* and 4 isolates of *Penicillium* sp. which found from both onion and shallot. Microscopic morphology is also shown in Fig. 4.2.

Table 4.1 The characteristics of six isolates

Isolate	Characteristics of isolated fungi
CMU1	white mycelium and green spores
CMU2	white mycelium and black spores
CMU3	white mycelium and green spores
CMU4	white mycelium and black spores
CMU5	yellow mycelium that later changed to green mycelium and green spores
CMU6	white mycelium and green spores

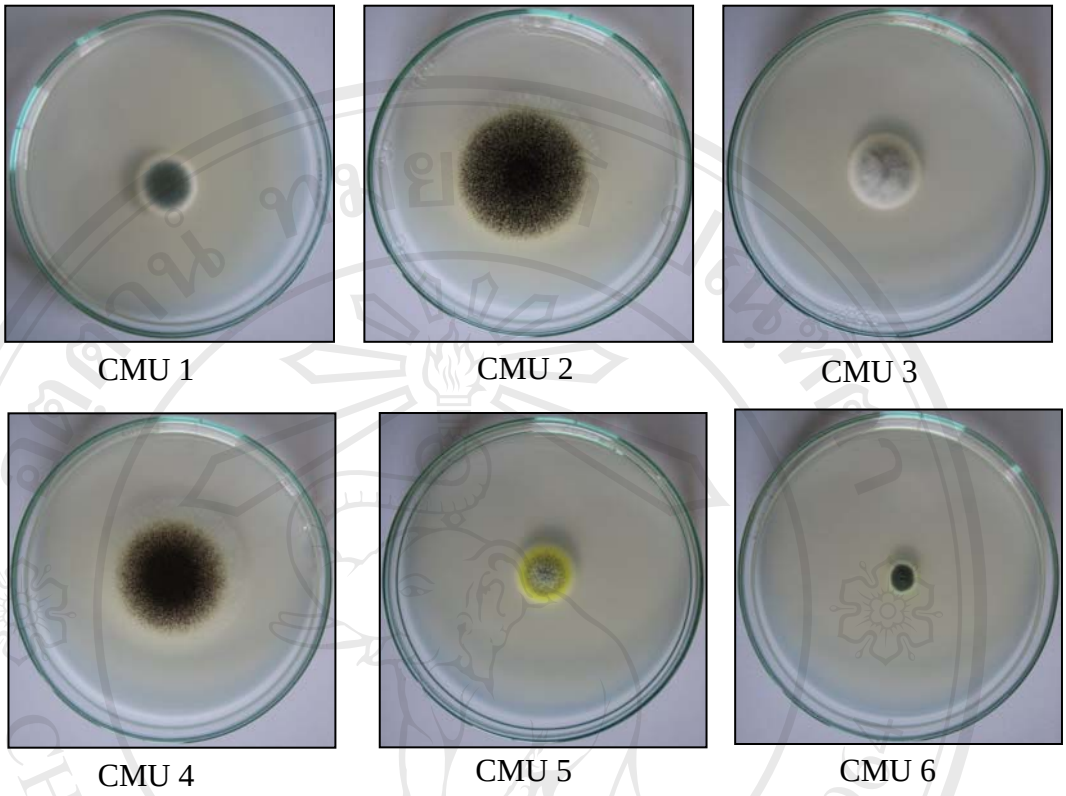
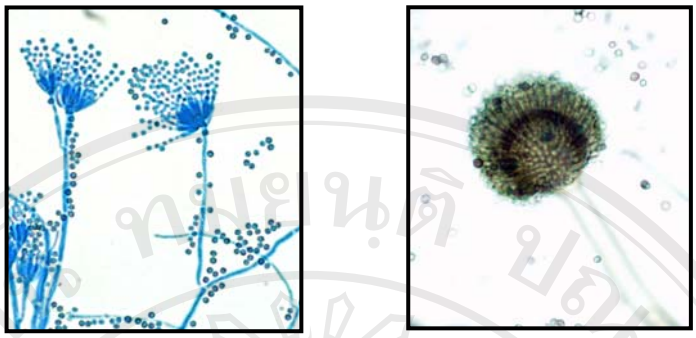


Fig. 4.1 Colony morphology of six isolates on PDA plates

Table 4.2 The species of fungi from the isolation

Isolate	Species
CMU1	*Penicillium* sp.1
CMU2	*Aspergillus niger*1
CMU3	*Penicillium* sp.2
CMU4	*Aspergillus niger*2
CMU5	*Penicillium* sp.3
CMU6	*Penicillium* sp.4



(A) **(B)**

Fig. 4.2 Characteristic of *Aspergillus niger* **(A)** and *Penicillium* sp. **(B)** under microscope, × 400

Aspergillus niger are frequently and commonly blemished stored bulbs which cause black mold disease. For *Penicillium* sp., this fungus was found during storage and caused various diseases. *A. niger* and *Penicillium* sp. have also been reported on disease causing and destroyed the product during storage of shallot and onion (Brewster, 1994).

4.2 Antifungal activity test

The antifungal activity of garlic, galangal, ginger, lemon grass, shallot and onion extracts against the growth of *A. niger*1, *A. niger*2, *Penicillium* sp.1, *Penicillium* sp.2, *Penicillium* sp.3 and *Penicillium* sp.4 was determined. All plants were separately extracted by distilled water, ethanol and methanol. *In vitro* study was performed by the agar well method. The plate was incubated at room temperature and daily observed for 5 days. The inhibition zone was recorded as the mean diameter of triplicate.

4.2.1 Effect of aqueous plant extracts on fungal growth

The effect of aqueous plant extracts on fungal growth was investigated. All plants were extracted by using the distilled water. Fig. 4.3 shows the effect of each aqueous fresh plant extract on isolate *Penicillium* sp.1. Antifungal activity was evaluated from the inhibition zone diameter. The inhibition zone can be seen in Fig. 4.3 (B) and (G). Effect of plant extract on each isolated fungi were presented in Fig. 4.4 to 4.9.

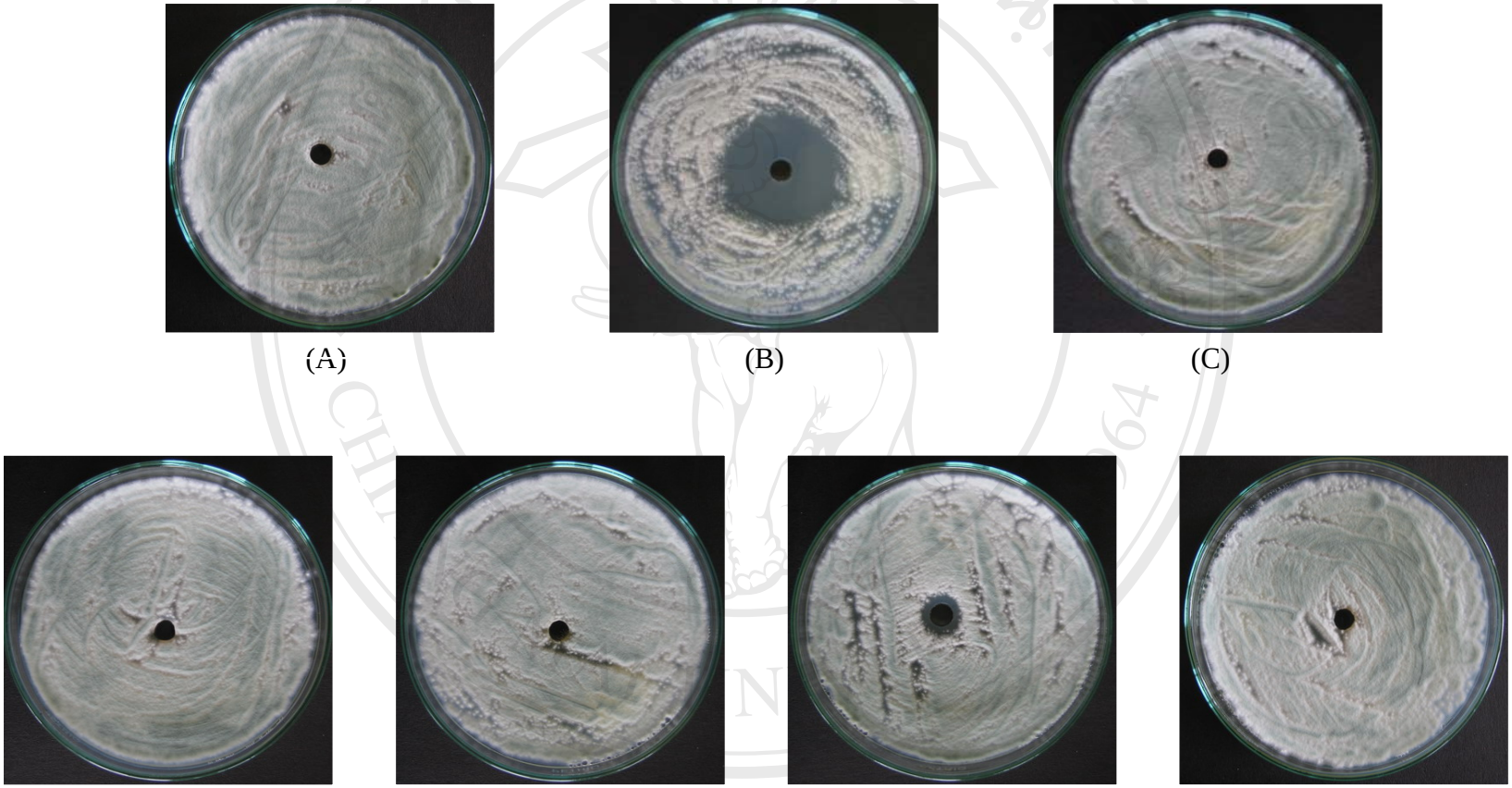


Fig. 4.3 Effect of each aqueous fresh plant extract on the growth of *Penicillium* sp.1 after 5 days of incubation (A) control (water); (B) garlic extract; (C) galangal extract; (D) ginger extract; (E) lemon grass extract; (F) shallot extract and (G) onion extract

Fig. 4.4 presents the inhibitory effect of six aqueous fresh plant extracts on *A. niger*1 growth for 5 days. It was found that aqueous fresh garlic extract could more effectively inhibit *A. niger*1 than other aqueous fresh plant extracts. The largest inhibition zone was obtained from aqueous fresh garlic extract (19.67 mm in diameter on the first day). Aqueous fresh garlic extract could inhibit *A. niger*1 for 3 days. Aqueous fresh shallot and onion extracts showed inhibitory effect on the first day which the inhibition zone diameter of 9.33 mm and 8.00 mm, respectively. In addition, no inhibition zone diameter was observed from other fresh plant extracts when compared to control.

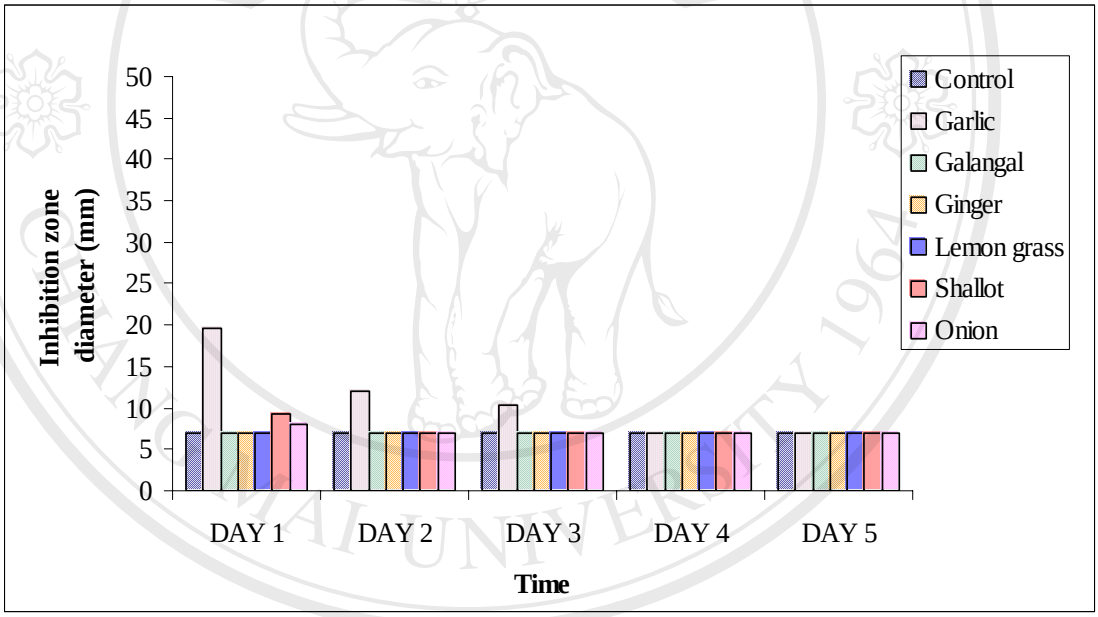


Fig. 4.4 The effect of six aqueous fresh plant extracts on growth inhibition of *A. niger*1 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.5 presents the inhibition zone diameters obtained from six aqueous fresh plant extracts against *A. niger*2 growth for 5 days. The result showed that the biggest inhibition zone diameter was obtained from aqueous fresh garlic extract (30.00 mm) on the first day. Aqueous fresh garlic extract could against this isolate for 3 days. After that, inhibition zone diameter was slightly decreased. The biggest inhibition zone diameter of aqueous fresh shallot extract was 11.00 mm on the first day and could inhibit this isolate for 2 days. Whereas, aqueous fresh onion extract could inhibit this isolate on the first day after that inhibition zone disappeared. When compared to the control, aqueous fresh galangal, ginger and lemon grass extracts did not show antifungal activity.

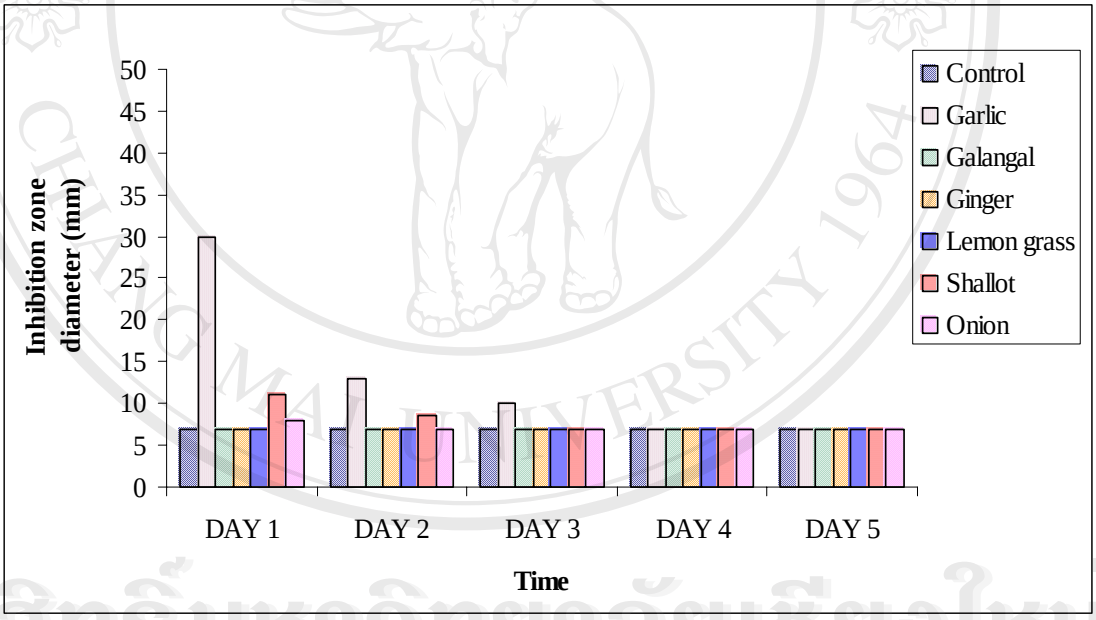


Fig. 4.5 The effect of six aqueous fresh plant extracts on growth inhibition of *A. niger*2 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.6 shows the inhibition zone diameters from six aqueous fresh plant extracts against *Penicillium* sp.1 growth for 5 days. It was found that aqueous fresh garlic extract could more effectively inhibit *Penicillium* sp.1 than other aqueous fresh plant extracts. The largest inhibition zone diameter of aqueous fresh garlic extract was 36.33 mm on the first day. After that, inhibition zone was slightly decreased. However, aqueous fresh garlic extract could inhibit *Penicillium* sp.1 growth for 5 days. Whereas, aqueous fresh shallot extract could inhibit *Penicillium* sp.1 for 4 days and the largest inhibition zone diameter was 11.33 mm on the first day. In addition, fresh onion extract could inhibit *Penicillium* sp.1 for 2 days and the largest inhibition zone diameter was 8.00 mm on the first day. Aqueous fresh galangal, ginger and lemon grass extracts could not inhibit this isolate.

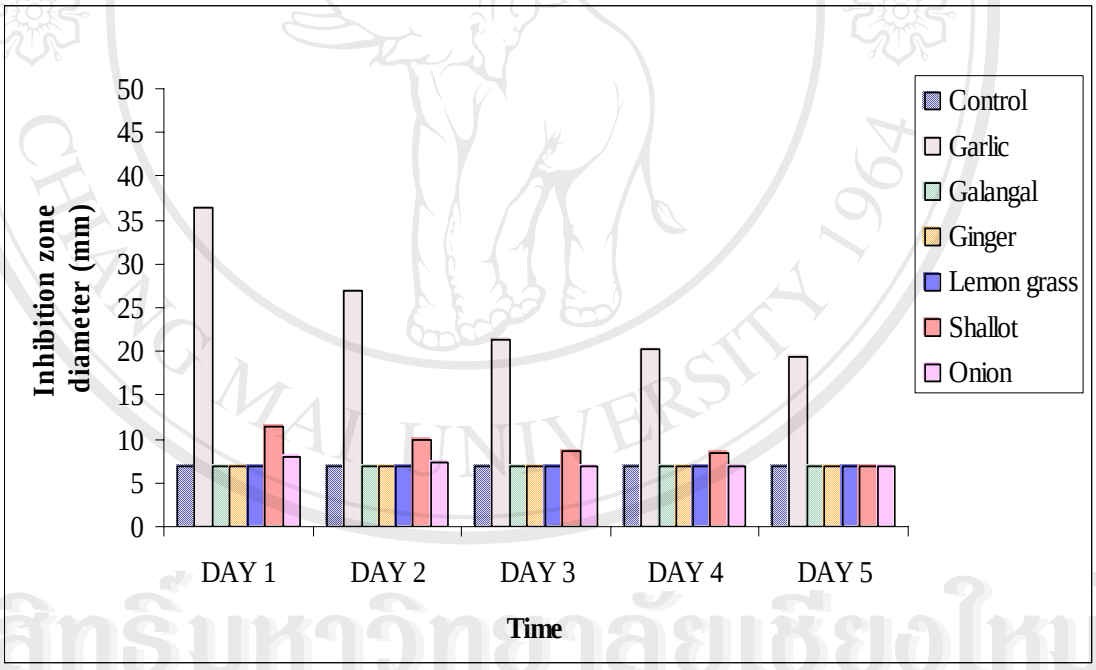


Fig. 4.6 The effect of six aqueous fresh plant extracts on growth inhibition of *Penicillium* sp.1 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.7 shows the inhibitory effect of six fresh plant extracts on the growth of *Penicillium* sp.2 for 5 days. It can be seen that the largest inhibition zone diameter was obtained from aqueous fresh garlic extract which the inhibition zone diameter of 36.67 mm on the first day and its inhibitory effect was remained for 5 days. Whereas, aqueous fresh shallot and onion extracts could inhibit this isolate for 4 days. The inhibition zone diameter was not observed from aqueous fresh galangal, ginger and lemon grass extracts.

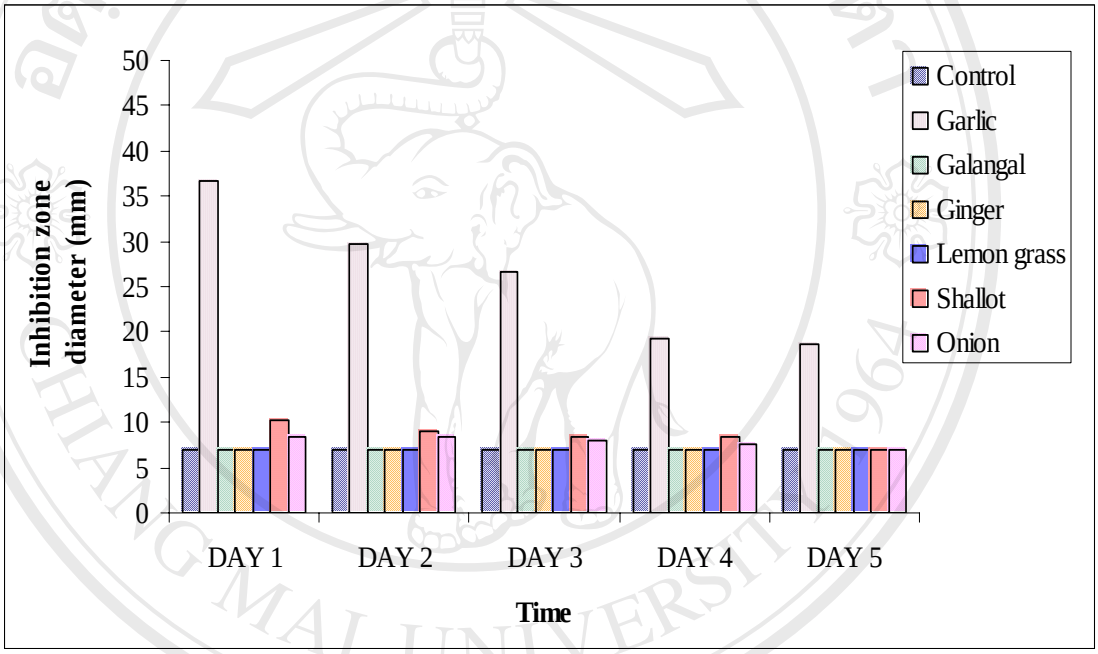


Fig. 4.7 The effect of six aqueous fresh plant extracts on growth inhibition of *Penicillium* sp.2 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.8 displays the inhibition zone diameters obtained from six aqueous fresh plant extracts against *Penicillium* sp.3 growth for 5 days. The result showed that the largest inhibition zone diameter was 50.00 mm which was obtained from aqueous fresh garlic extract on the first day. In addition, aqueous fresh garlic extract could still inhibit *Penicillium* sp.3 for 5 days. However, aqueous fresh shallot and onion extracts could inhibit this isolate for 4 days and 2 days, respectively. Whereas, aqueous fresh galangal, ginger and lemon grass extracts were not able to inhibit this isolate.

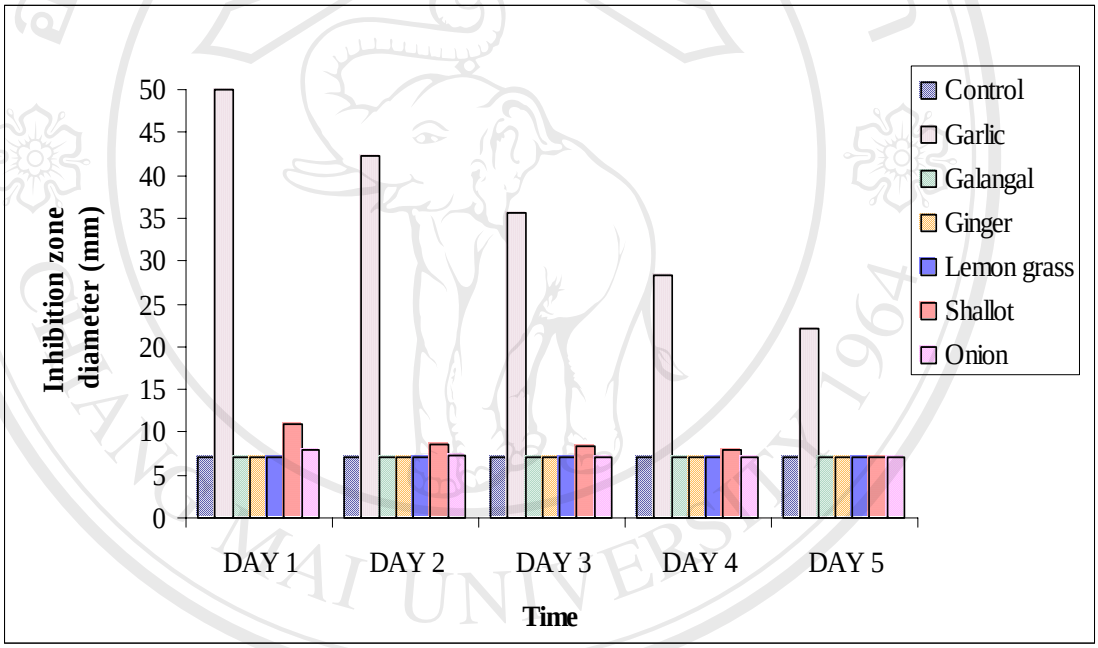


Fig. 4.8 The effect of six aqueous fresh plant extracts on growth inhibition of *Penicillium* sp.3 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.9 shows the inhibitory effect of six aqueous fresh plant extracts on *Penicillium* sp.4 growth for 5 days. It can be seen that the largest inhibition zone diameter was 35.00 mm which was obtained from aqueous fresh garlic extract on the first day. After that, inhibition zone diameter was slightly decreased. Whereas, aqueous fresh shallot and onion extracts could inhibit *Penicillium* sp.4 for 4 days and 2 days, respectively. When compared to the control, aqueous fresh galangal, ginger and lemon grass extracts could not inhibit this isolate.

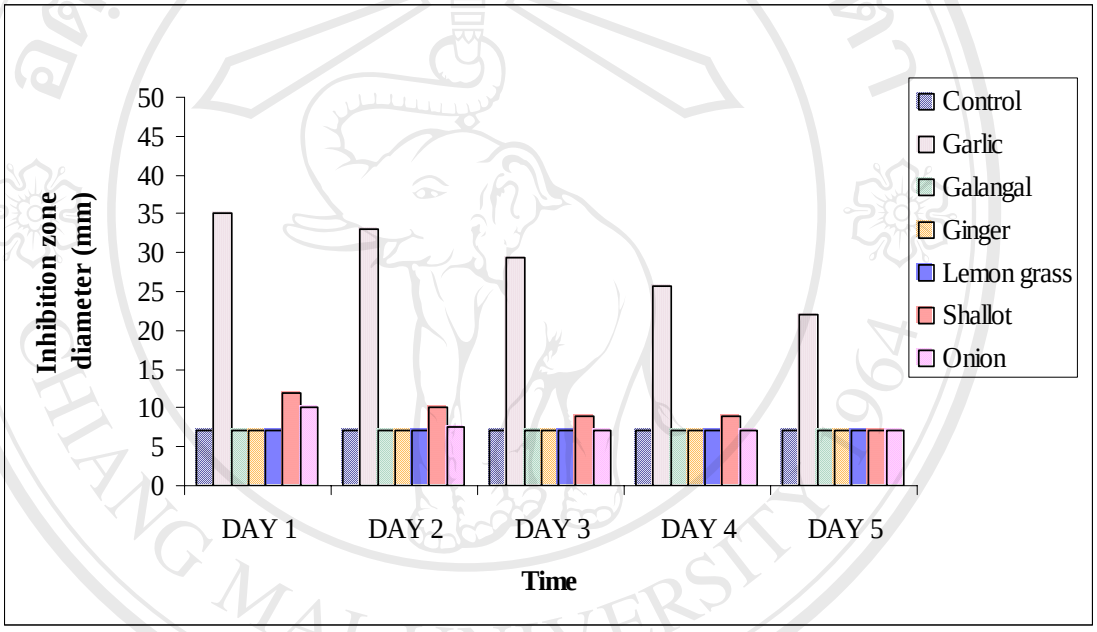


Fig. 4.9 The effect of six aqueous fresh plant extracts on growth inhibition of *Penicillium* sp.4 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

From the inhibitory effect of six aqueous fresh plant extracts on these six isolates, the results showed that aqueous fresh garlic extract had the most effective inhibition against all six isolates because garlic has allicin (diallylthiosulfinate) known to pose a vast variety of biological effects. Its antimicrobial, anti-inflammatory, antithrombotic, anticancer and antiatherosclerotic activities are known (Miron *et al*., 2002). Since, garlic extracts were shown to decrease the oxygen uptake, inhibit the synthesis of lipids, proteins and nucleic acids and damage membranes (Harris *et al*., 2001). Many researchers reported that aqueous garlic demonstrated a good antifungal activity against the pathogenic fungi (Bianchi *et al*., 1997; Ke-Qiang and Bruggen, 2001; Khallil, 2001). However, inhibition zone diameters gradually decreased when the incubation time increased because the compounds in those extracts may not stable. In the case of garlic extract, allicin is an unstable product and will undergo additional reaction of form other derivatives because it is provided in volatile flavor compounds (Brewster, 1994; Miron *et al*., 2002).

4.2.2 Effect of ethanolic dry plant extracts on fungal growth

The effect of ethanolic dry plant extracts on fungal growth was investigated. All plants were extracted by ethanol. Antifungal activity was evaluated from the inhibition zone diameter. Fig. 4.10 shows the effect of each ethanolic dry plant extract on isolate *Penicillium* sp.1. The inhibition zone can be seen in Fig. 4.10 (B) and (C).

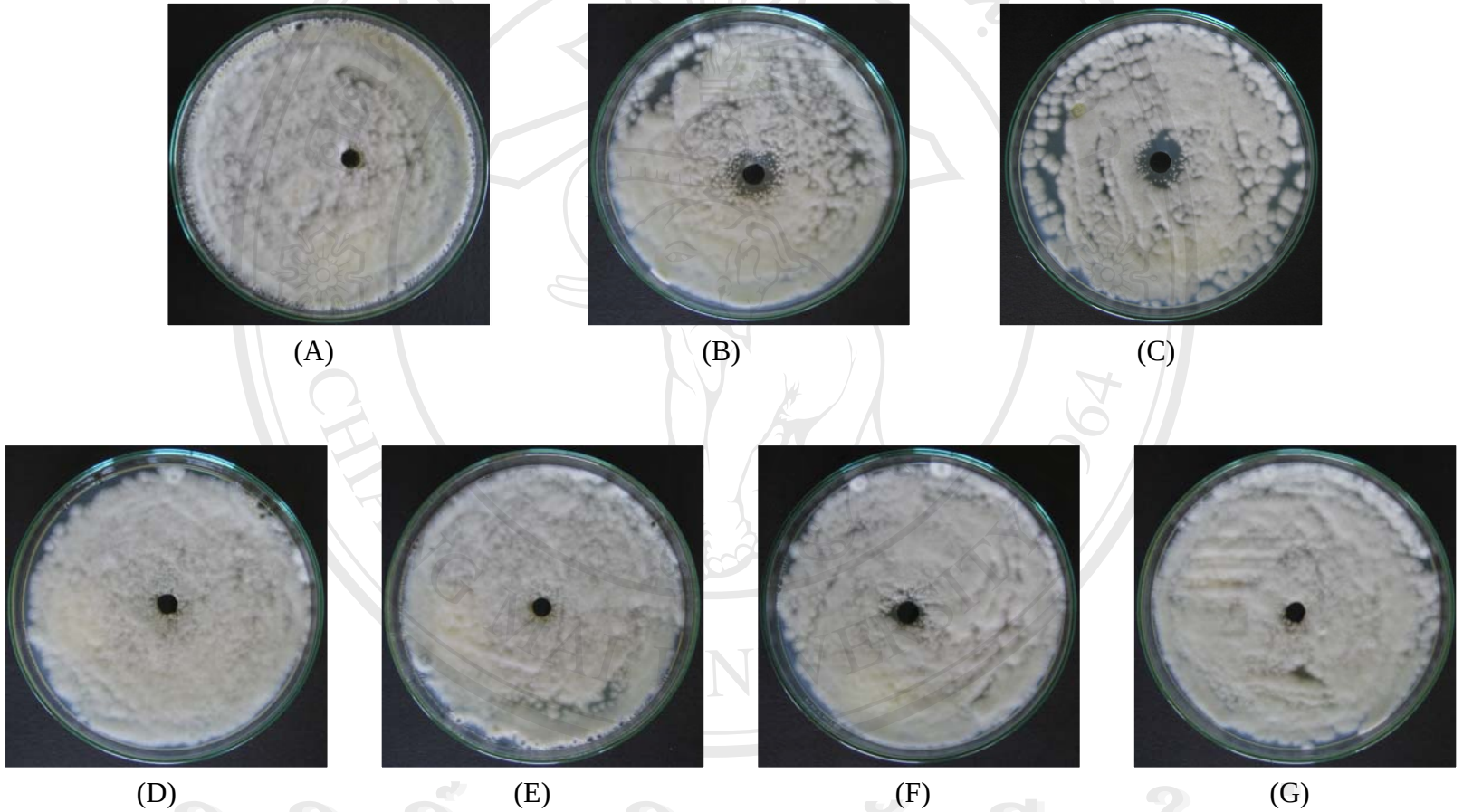


Fig. 4.10 Effect of ethanolic dry plant extracts on the growth of *Penicillium* sp.1 after 5 days of incubation (A) control (ethanol and 0.1% Tween 80); (B) garlic extract; (C) galangal extract; (D) ginger extract; (E) lemon grass extract; (F) shallot extract and (G) onion extract

Fig. 4.11 presents the inhibition zone diameters obtained from six ethanolic dry plant extracts against *A. niger*1 growth for 5 days. The result showed that the biggest inhibition zone diameter was obtained from ethanolic dry galangal extract which was 12.67 mm on the first day. Ethanolic dry garlic and shallot extracts showed inhibition zone diameter (11.67 mm and 10.33 mm, respectively) on the first day. After that, these inhibition zones disappeared. Ethanolic dry ginger, lemon grass and onion extracts showed no inhibition zone at all.

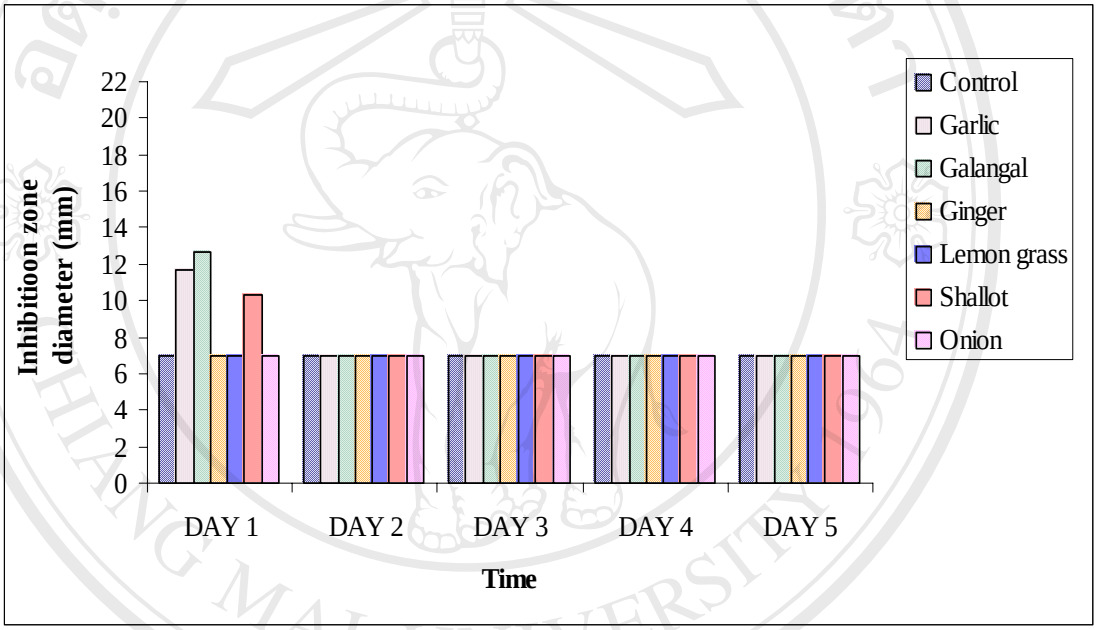


Fig. 4.11 The effect of six ethanolic dry plant extracts on growth inhibition of *A. niger*1 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.12 presents the inhibition zone diameters obtained from six ethanolic dry plant extracts against *A. niger*2 growth for 5 days. The result showed that the biggest inhibition zone diameter was obtained from ethanolic dry galangal extract which was 13.33 mm on the first day and had effect against this isolate for 5 days. However, ethanolic dry garlic and shallot extracts showed smaller inhibition zone diameter (12.33 mm and 11.00 mm, respectively) on the first day. Whereas, ethanolic dry ginger, lemon grass and onion extracts were not able to inhibit this isolate when compared to the control.

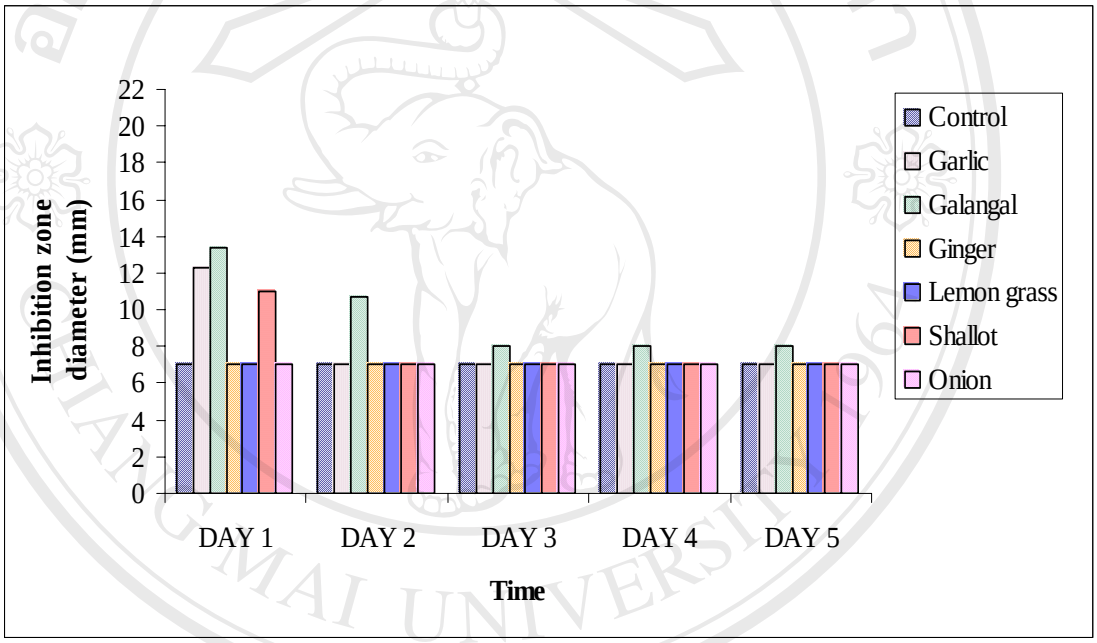


Fig. 4.12 The effect of six ethanolic dry plant extracts on growth inhibition of *A. niger*2 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.13 shows the inhibition zone diameters of six ethanolic dry plant extracts on *Penicillium* sp.1 growth for 5 days. It was found that ethanolic dry galangal extract inhibited *Penicillium* sp.1 more than other ethanolic dry plant extracts. The largest inhibition zone diameter of ethanolic dry galangal extract was 20.33 mm on the first day. After that, inhibition zone diameter was gradually decreased. The inhibition zone diameter of ethanolic dry garlic extract was 13.67 mm on the first day. However, ethanolic dry garlic extract could inhibit *Penicillium* sp.1 for 5 days but its inhibitory effect was less than ethanolic dry galangal extract. Ethanolic dry shallot was most effective (16.00 mm) on the first day and could inhibit this isolate for 4 days, whereas, ethanolic dry ginger, lemon grass and onion extracts could inhibit this isolate showing 13.00 mm, 9.00 mm and 9.67 mm inhibition zone diameters, respectively. After that, these three ethanolic dry plant extracts could not inhibit this isolate. In addition, ethanolic dry garlic and galangal extracts had similarly effect on this isolate on day 5 as observed from the inhibition zone diameter.

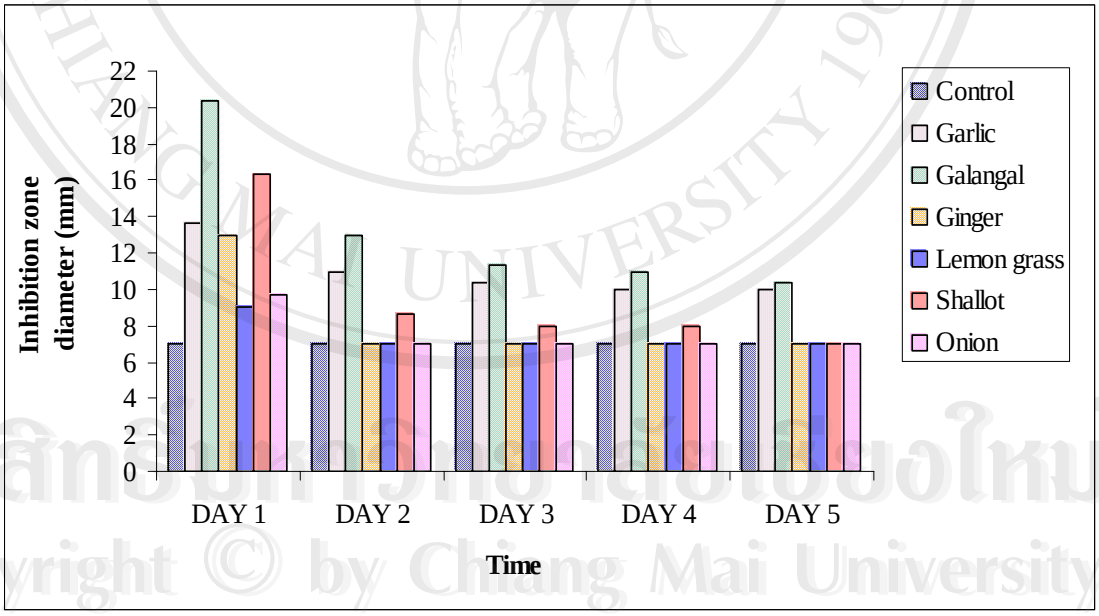


Fig. 4.13 The effect of six ethanolic dry plant extracts on growth inhibition of *Penicillium* sp.1 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.14 displays the inhibitory effect of six ethanolic dry plant extracts on *Penicillium* sp.2 growth for 5 days. It was found that the largest inhibition zone diameter of ethanolic dry galangal extract was 12.33 mm on the first day. After that, inhibition zone diameter was decreased. Whereas, ethanolic dry ginger and shallot extracts showed inhibition zone on the first day which were 11.33 mm and 10.67 mm, respectively. No inhibition zone was observed from other dry plant extracts when compared to the control.

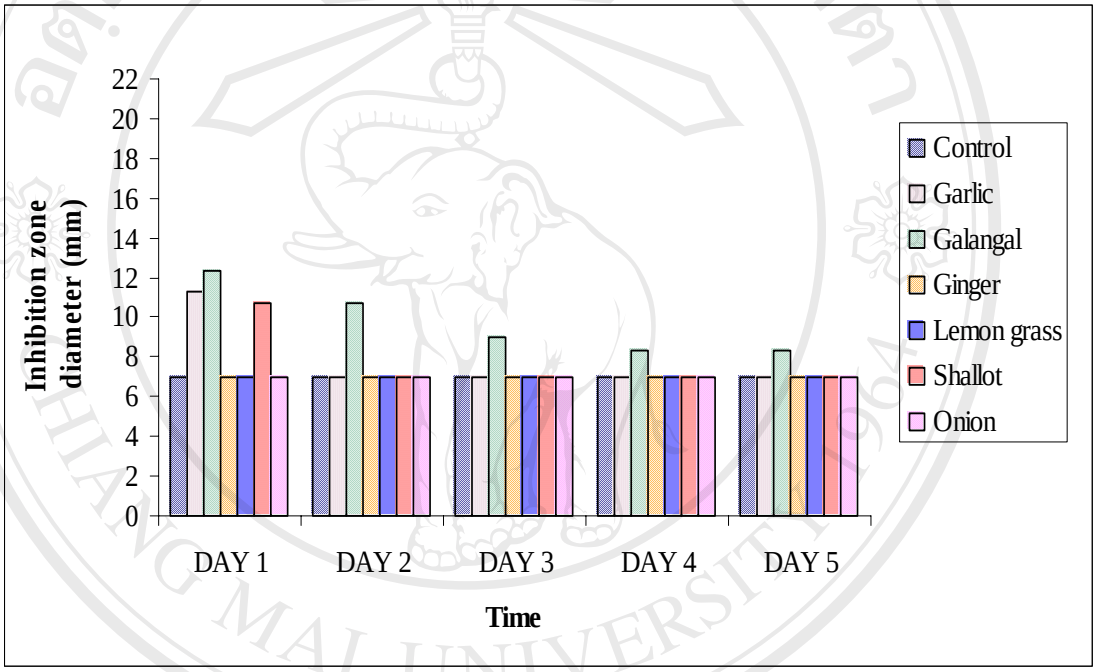


Fig. 4.14 The effect of six ethanolic dry plant extracts on growth inhibition of *Penicillium* sp.2 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.15 presents the diameters of inhibition zone obtained with six ethanolic dry plant extracts against *Penicillium* sp.3 growth for 5 days. It can be seen that inhibition zone diameter of ethanolic dry galangal extract was 19.67 mm on the first day. After that, inhibition zone diameter was slightly decreased. However, ethanolic dry galangal extract could inhibit this isolate for 5 days. Whereas, ethanolic dry garlic extract showed inhibition zone diameter which was 9.00 mm on the first 2 day, ethanolic dry garlic extract could not inhibit this isolate. Moreover, ethanolic dry ginger, lemon grass and onion extracts were not able to inhibit this isolate.

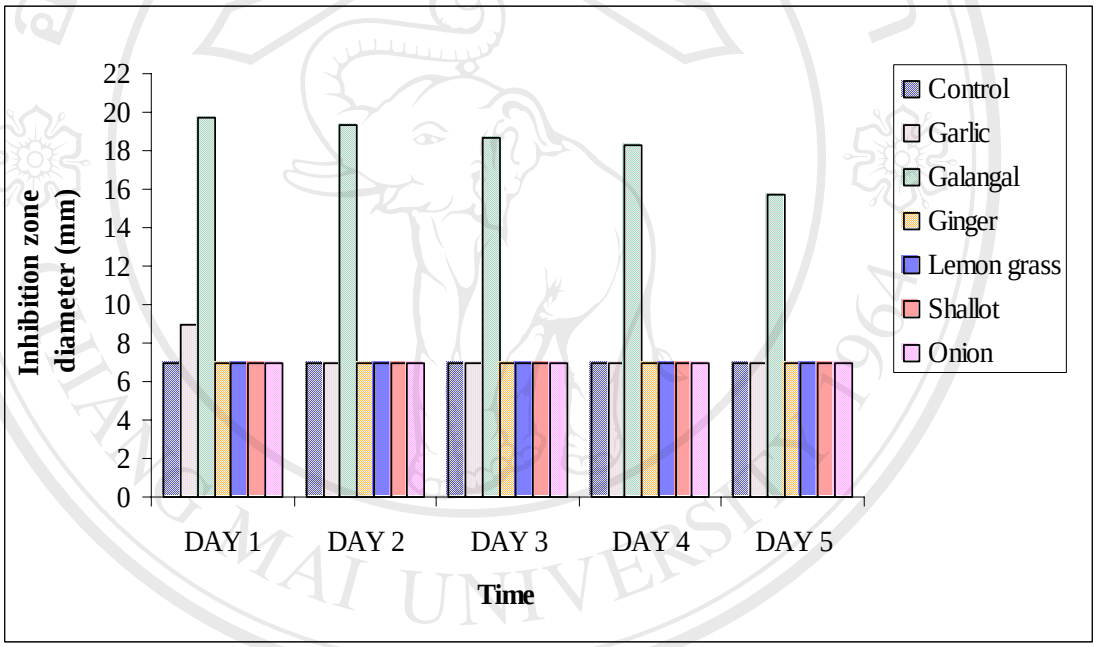


Fig. 4.15 The effect of six ethanolic dry plant extracts on growth inhibition of *Penicillium* sp.3 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.16 shows the inhibitory effect of six ethanolic dry plant extracts on *Penicillium* sp.4 growth for 5 days. It can be seen that the largest inhibition zone diameter was obtained from ethanolic dry galangal extract which was 15.33 mm on the first day and its inhibitory effect still remained for 5 days. Whereas, ethanolic dry garlic, ginger and shallot extracts could repress *Penicillium* sp.4 on the first day which the inhibition zone diameter of 15.33 mm, 9.33 mm and 13.33 mm, respectively. In addition, no inhibition zone diameter was observed from lemon grass and onion extracts when compared to the control.

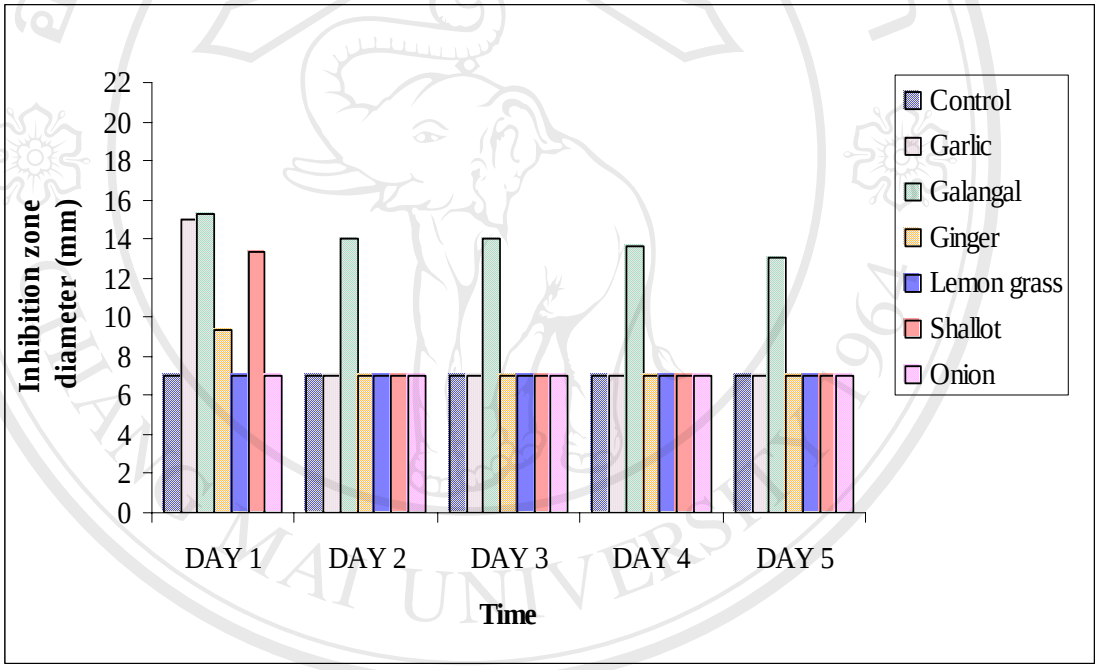


Fig. 4.16 The effect of six ethanolic dry plant extracts on growth inhibition of *Penicillium* sp.4 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

From the effect of six ethanolic dry plant extracts on these six isolates, the results showed that ethanolic dry galangal extract was the most effective inhibit growth of all six isolates. The inhibitory activity may due to the action of compound in the rhizome. Haraguchi *et al*. (1996) reported that antimicrobial diterpene isolated from galangal. This diterpene synergistically enhanced the antifungal activity of quercetin and chalcone against *C. albicans*. Protoplasts of *C. albicans* were lysed by the diterpene. These results suggested that the antifungal activity of this compound is due to a change of membrane permeability arising from membrane lipid alteration. In other words, the used solvent extract (ethanol) affects on the concentration level of diterpene due to it is a constituent in essential oil (Tan and Vanitha, 2004) which can be effectively soluble in alcohol (ethanol, methanol, etc.). Therefore, diterpene may be found in ethanol solvent in high level which it influenced on the effective inhibition on the fungal growth. Kwon *et al.* (2003) reported that saponin structures, the major effective component of ginseng, consisted of aglycones of the dammarane-type of triterpene. Its content of extract noticeably increased with ethanol concentration up. However, inhibition zone diameter decreased when the incubation time increased. It maybe caused from the reduction of concentration level of the extract from the evaporation of ethanol when maintaining the plates for a long period.

4.2.3 Effect of methanolic dry plant extracts on fungal growth

The dry garlic, galangal, ginger, lemon grass, shallot and onion were extracted by methanol, after that these extracts were tested antifungal activity. Antifungal activity was evaluated from the inhibition zone diameter. Fig. 4.17 shows the effect of methanolic dry plant extracts on the growth of *Penicillium* sp.1. The inhibition zone can be seen in Fig. 4.17 (B), (C) and (D).

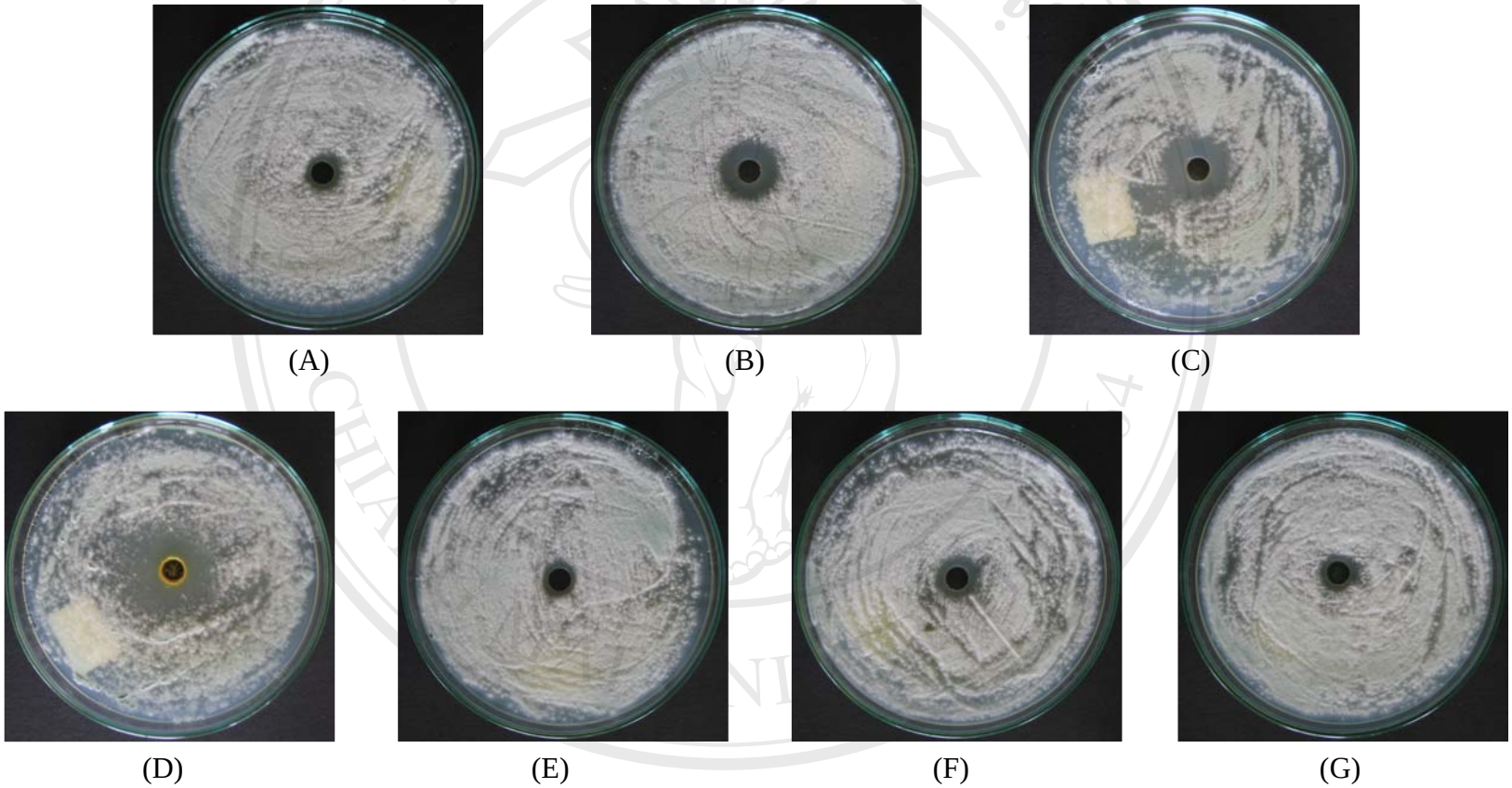


Fig. 4.17 Effect of methanolic dry plant extracts on the growth of *Penicillium* sp.1 after 3 days of incubation

(A) control (methanol); (B) garlic extract; (C) galangal extract; (D) ginger extract; (E) lemon grass extract; (F) shallot extract and (G) onion extract

Fig. 4.18 presents the inhibition zone diameters obtained from six methanolic dry plant extracts against *A. niger*1 growth for 5 days. The result showed that large inhibition zone diameter was obtained from methanolic dry galangal and ginger extracts which were 11.33 mm and 11.67 mm, respectively on the first day. However, methanolic dry ginger extract could more effectively inhibit than other methanolic dry galangal extract because its inhibitory effect was occurred for 4 days, whereas, methanolic dry galangal extract had effect for 2 days. However, methanolic dry shallot extract showed inhibition zone diameter of 8.33 mm on the first day. Whereas, methanolic dry garlic, lemon grass and onion extracts showed no inhibition.

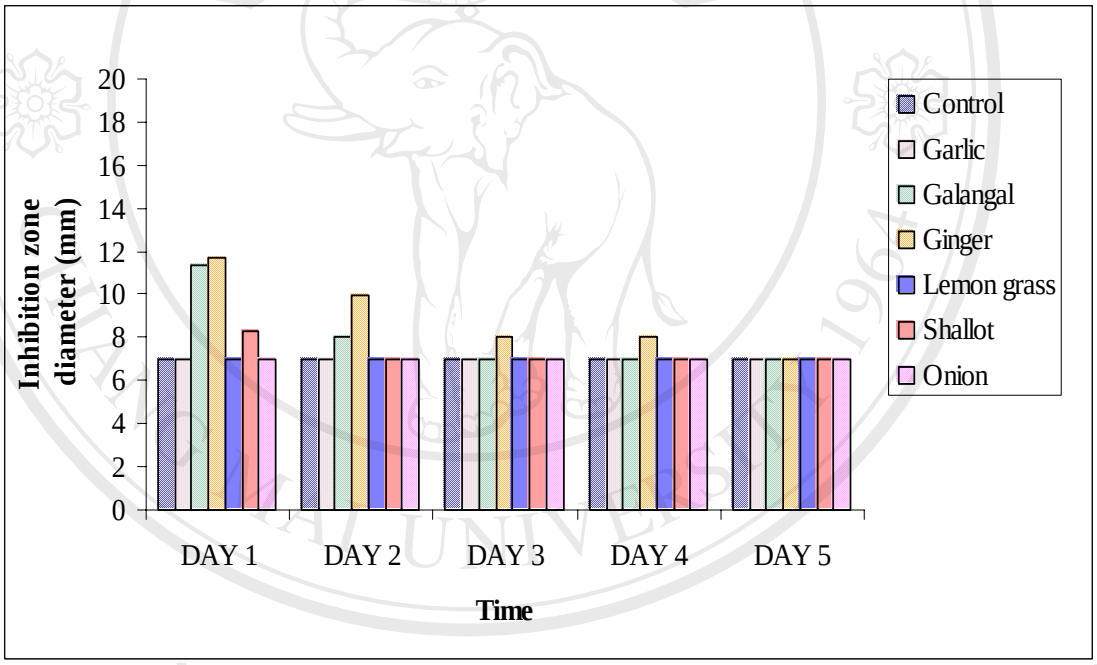


Fig. 4.18 The effect of six methanolic dry plant extracts on growth inhibition of *A. niger*1 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.19 shows the inhibitory effect of six methanolic dry plant extracts on the growth of *A. niger*2 for 5 days. It was found that the largest inhibition zone diameter was obtained from methanolic dry galangal and ginger extracts (11.00 mm) on the first day. However, methanolic dry ginger extract had more effectively inhibit than methanolic dry galangal extract because its inhibitory effect was occurred for 3 days, whereas, methanolic dry galangal extract inhibited this isolate on the first day. For both methanolic dry lemon grass and onion extracts, inhibition zone diameters were 8.00 mm on the first day and had effect against this isolate for 2 days. In addition, methanolic dry garlic and shallot extracts could not inhibit this isolate.

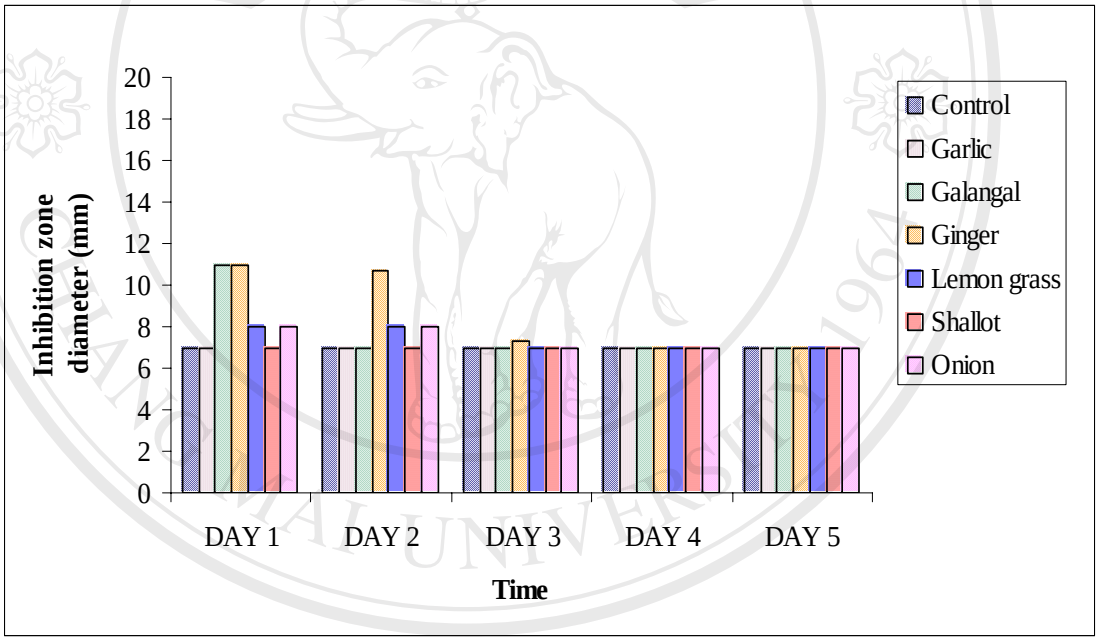


Fig. 4.19 The effect of six methanolic dry plant extracts on growth inhibition of *A. niger*2 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.20 shows the effect of six methanolic dry plant extracts on *Penicillium* sp.1 growth for 5 days. It was found that methanolic dry galangal and ginger extracts could more effectively inhibit the growth of *Penicillium* sp.1 than other methanolic plant extracts. The large inhibition zone diameters of methanolic dry galangal and ginger extracts were 14.33 mm and 12.33 mm, respectively on the first day. After that, methanolic dry galangal and ginger extracts could inhibit this isolate for 5 days. For methanolic dry garlic and shallot extracts, inhibition zone diameters were similar. However, methanolic dry galangal and ginger extracts showed stronger antifungal activity than methanolic dry garlic and shallot extracts. Whereas, methanolic dry lemon grass and onion extracts showed inhibition zone diameter of 10.67 mm and 9.33 mm, respectively on the first day. After that, methanolic dry lemon grass and onion extracts were not able to inhibit this isolate when compared to the control. In addition, all methanolic dry plant extracts had effect against this isolate on the first day. But there were two methanolic dry plant extracts (lemon grass and onion) could not inhibit *Penicillium* sp.1 growth until the 5th day.

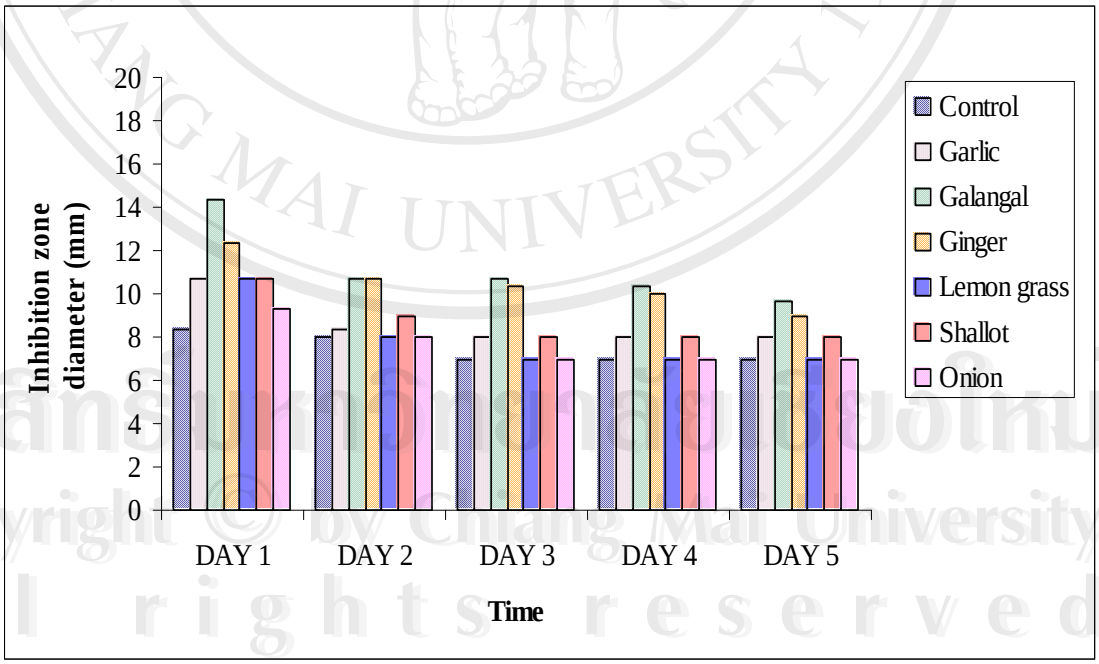


Fig. 4.20 The effect of six methanolic dry plant extracts on growth inhibition of *Penicillium* sp.1 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.21 displays the inhibitory effect of six methanolic dry plant extracts on the growth of *Penicillium* sp.2 for 5 days. The result showed that large inhibition zone diameter of methanolic dry galangal and ginger extracts were 14.00 mm and 13.33 mm, respectively on the first day. After that, inhibition zone diameter was decreased. Methanolic dry galangal extract had biggest inhibition zone in the first day but methanolic dry ginger extract had highest effect on day 5. In the first day, methanolic dry garlic and shallot extracts were 11.67 mm and 11.00 mm, respectively. However, methanolic dry garlic and shallot extracts could inhibit *Penicillium* sp.2 growth for 5 day but methanolic dry galangal and ginger extracts showed higher antifungal activity than methanolic dry garlic and shallot extracts. Methanolic dry onion extract showed inhibition zone diameter of 10.00 mm on the first day and methanolic dry lemon grass extract was not able to inhibit this isolate.

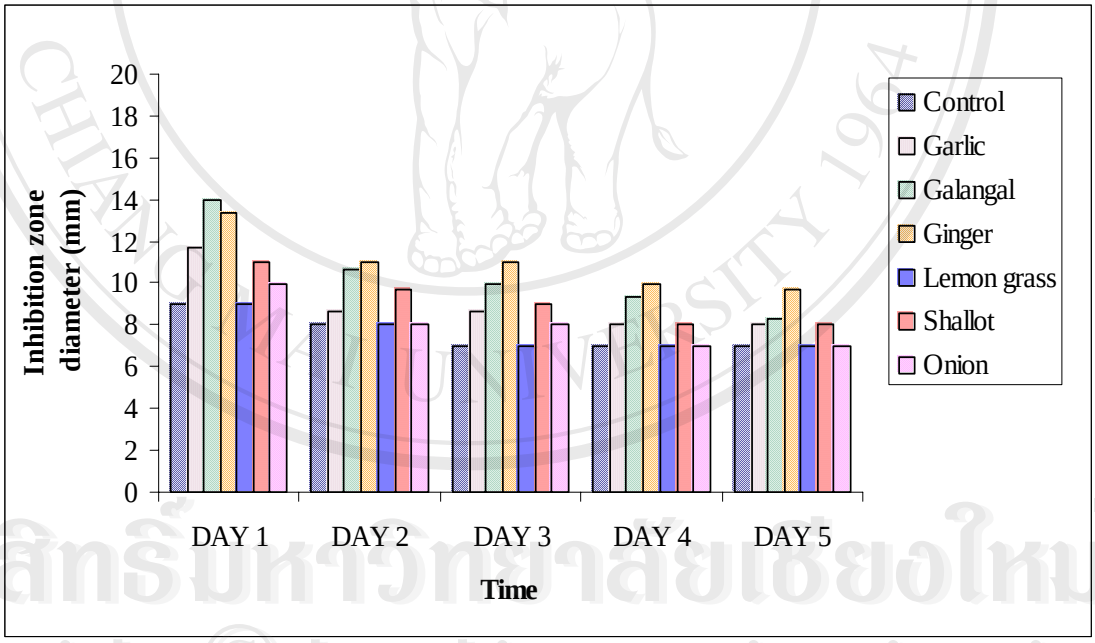


Fig. 4.21 The effect of six methanolic dry plant extracts on growth inhibition of *Penicillium* sp.2 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.22 shows the inhibition zone diameters obtained from six methanolic dry plant extracts against *Penicillium* sp.3 growth for 5 days. It was found that the large inhibition zone diameter of methanolic dry galangal and ginger extracts were 14.33 and 13.67 mm, respectively on the first day. However, methanolic dry galangal extract had more effectively inhibit than methanolic dry ginger extract on day 1-3, after that, methanolic dry ginger extract had higher inhibition zone diameter on day 4 and 5. Whereas, no inhibition zone diameter was observed from other plant extracts.

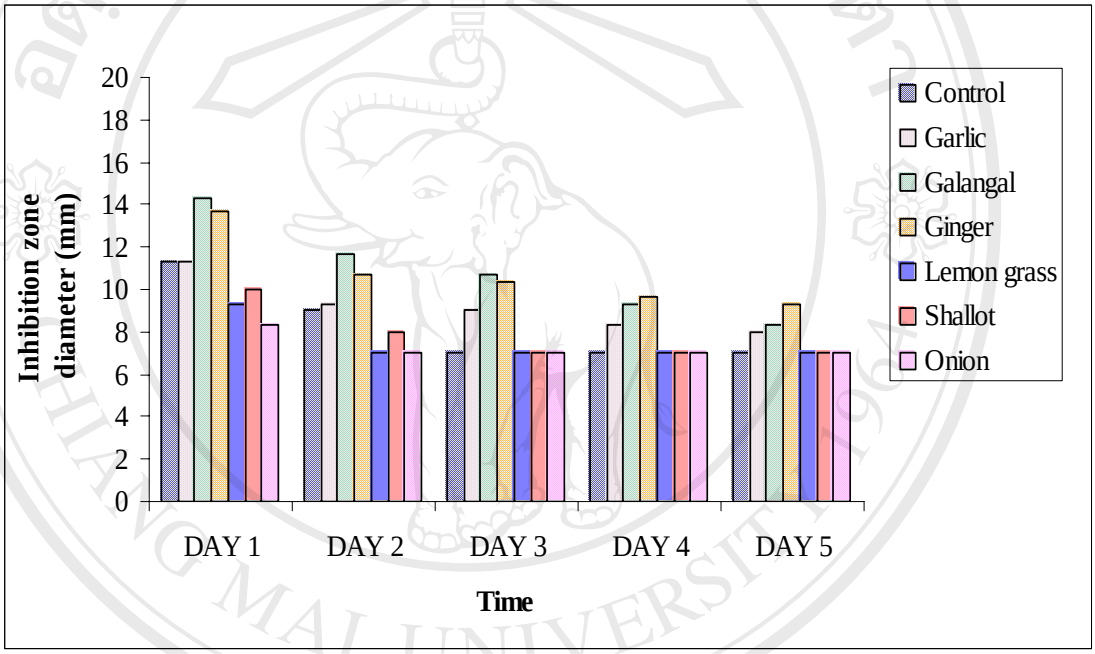


Fig. 4.22 The effect of six methanolic dry plant extracts on growth inhibition of *Penicillium* sp.3 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

Fig. 4.23 presents the effect of six methanolic dry plant extracts on the growth of *Penicillium* sp.4 for 5 days. It can be seen that the large inhibition zone diameter of methanolic dry galangal and ginger extracts were 18.00 mm and 17.67 mm, respectively on the first day. Methanolic dry galangal extract had most effective against *Penicillium* sp.4 and could inhibit this isolate for 5 days. Whereas, methanolic dry ginger and shallot extracts could inhibit this isolate for 2 days. When compared to the control, methanolic dry garlic and onion extracts showed inhibition zone diameter of 11.67 mm on the first day and methanolic dry lemon grass extract was not able to inhibit this isolate.

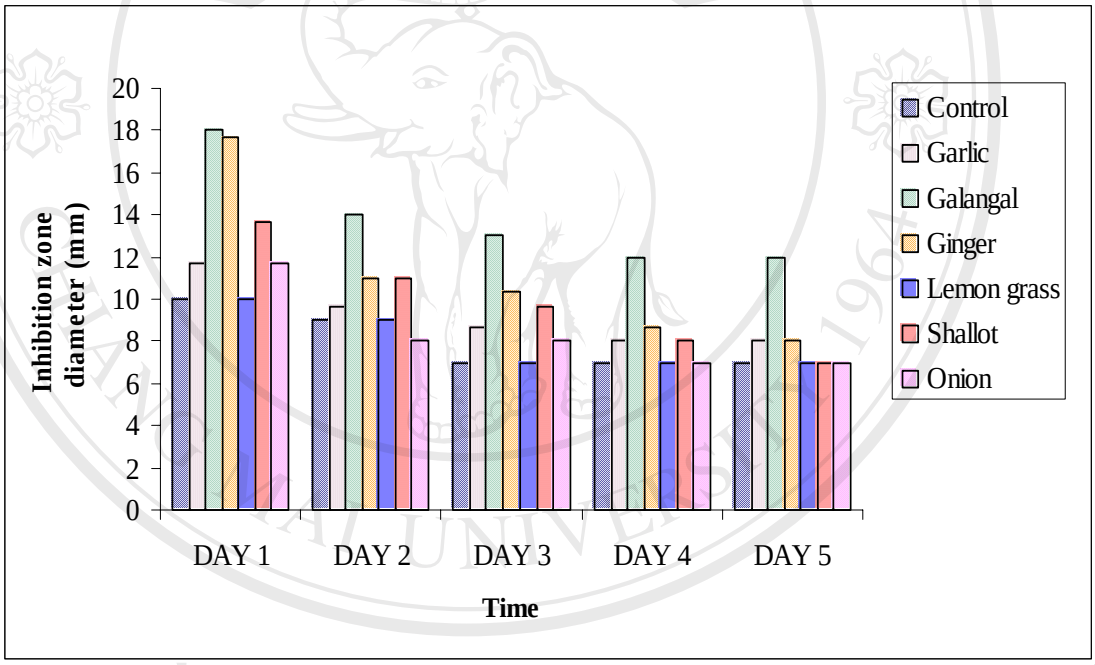
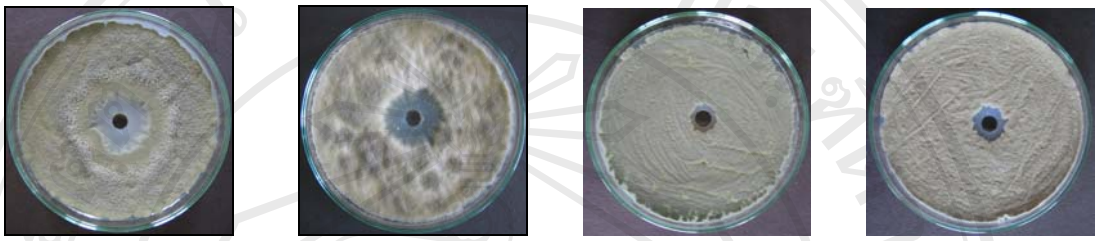


Fig. 4.23 The effect of six methanolic dry plant extracts on growth inhibition of *Penicillium* sp.4 (evaluated in term of inhibition zone diameter) in a range of 1 to 5 days

From the effect of six methanolic dry plant extracts on these six isolates, the results showed that methanolic dry ginger and galangal extracts had more inhibitory effect than other methanolic dry plant extracts. In the case of methanolic dry ginger extract, it showed high antifungal activity due to ginger has strong antibacterial and some other extent antifungal properties (Singh *et al*., 2003; Somchit and Shukriyah, 2003). Purseglove (1972) noted that ginger rhizome contains two classes of constituent: (i) the essential oils which give the aroma, and (ii) the main pungent principles: gingerols and their dehydrated products (shogaol). Martins *et al*. (2001) reported that the constituents in essential oil were found to decrease growth rate of a variety of bacteria and fungi. The essential oil has an antimicrobial compound which are citral, curcumene (sesquiterpene) and dehydrozingerone (Tan and Vanitha, 2004). Gingerols contain an active compound which also exhibit strong antimicrobial and antifungal properties. The methanolic dry galangal extract also gave high antifungal activity due to diterpene which show the same property as sesquiterpene found in ginger. Both of ginger and galangal extracted by methanol gave the high antifungal activity because terpene existed in essential oil (Tan and Vanitha, 2004). Essential oils are usually lipophilic compound so it can be soluble in alcohols such as methanol, ethanol and organic solvents. Therefore, the antimicrobial substances of ginger and galangal were found in high level when using methanol as solvent extraction. However, one of disadvantage of using methanol as solvent extraction is its volatile property which it may caused the reduction of antimicrobial substances level when maintaining the plates for a long period. As the mentioned reason, inhibition zone diameter decreased when the incubation time increased.

4.2.4 Stability of plant extracts

The stability of plant extracts and its ability against fungal growth was determined. The results shown in Fig. 4.24, it can be seen that the inhibition zone still remained after leaving culture plate at room temperature (29-31 ºC) for a month. However, the inhibition zone diameter decreased when the time increased.



(A) **(B)** **(C)** **(D)**

Fig. 4.24 Effect of plant extracts on fungal growth for a month

(A) Inhibition of aqueous fresh garlic extract on the growth of *Penicillium* sp.1,

(B) Inhibition of aqueous fresh garlic extract on the growth of *Penicillium* sp.2,

(C) Inhibition of aqueous fresh garlic extract on the growth of *Penicillium* sp.3,

(D) Inhibition of aqueous fresh garlic extract on the growth of *Penicillium* sp.4

It can be concluded that ethanol and methanol were less effective inhibition than the antifungal substance in plant extract. The stability of aqueous fresh garlic extract was highest because its inhibition zone diameter still remained more than other plant extract.

4.2.5 Study of plant extract concentration and antifungal activity

Aqueous fresh garlic extract, ethanolic dry galangal extract, methanolic dry galangal extract and methanolic dry ginger extract were examined for their dilution on fungal growth. These plant extracts were diluted to 2, 5 and 10 fold. It was found that crude extract of each plant showed the greatest antifungal activity. It can be seen that, crude plant extract could more effectively inhibit than 2, 5 and 10 fold diluted one, respectively. It could be the effect of concentration of active compound existed in the plant extract. This result show the similar to outcome from many previous report (Nwachukwu and Umechuruba, 2001; Selvi *et al*., 2003). However, the inhibition zone diameter of fungal growth decreased when the incubation time increased. The results are shown in Fig. 4.25 to 4.30.

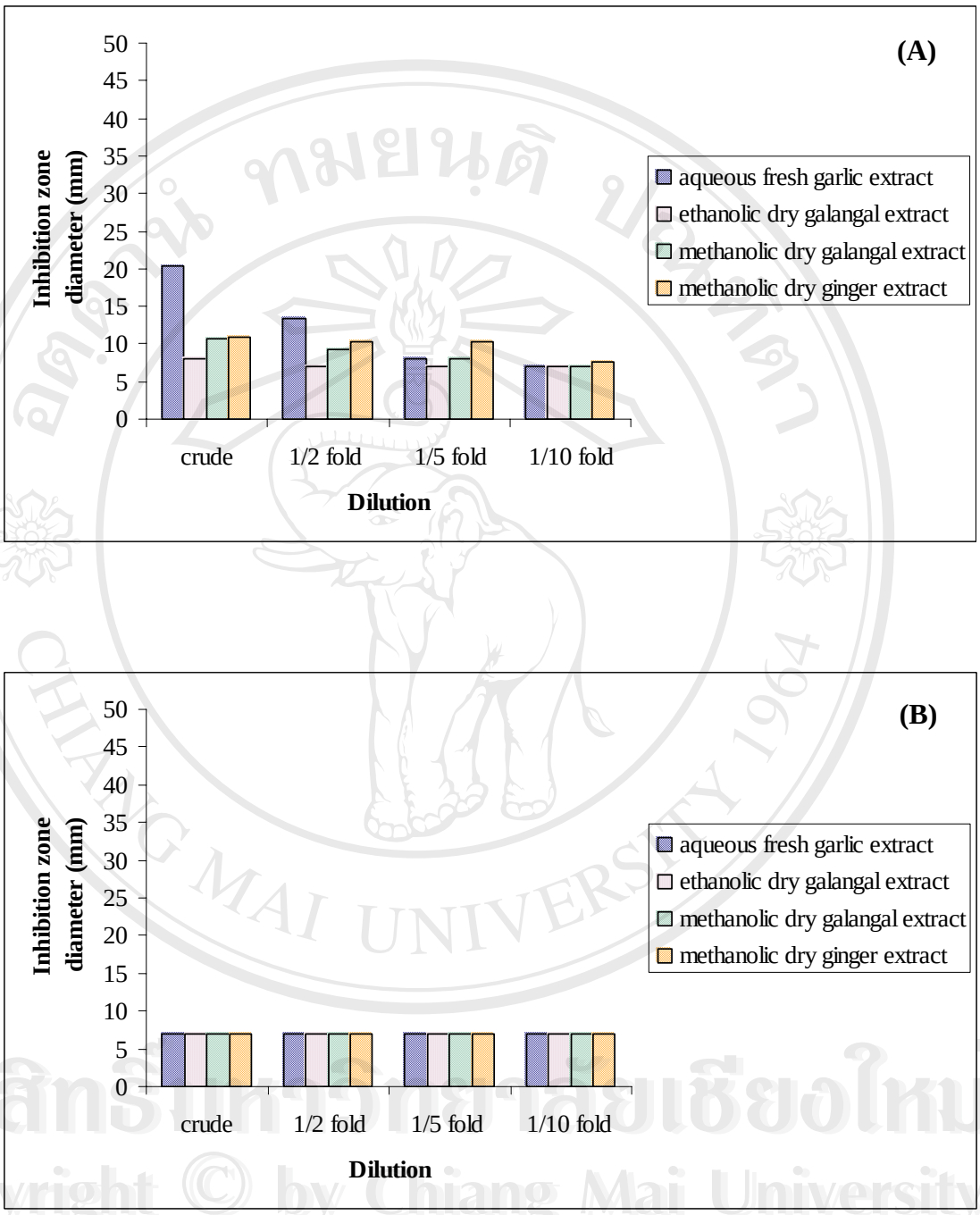


Fig. 4.25 The effect of four plant extracts on growth inhibition of *A. niger*1 (evaluated in term of inhibition zone diameter) in DAY 1 **(A)** and DAY 5 **(B)**

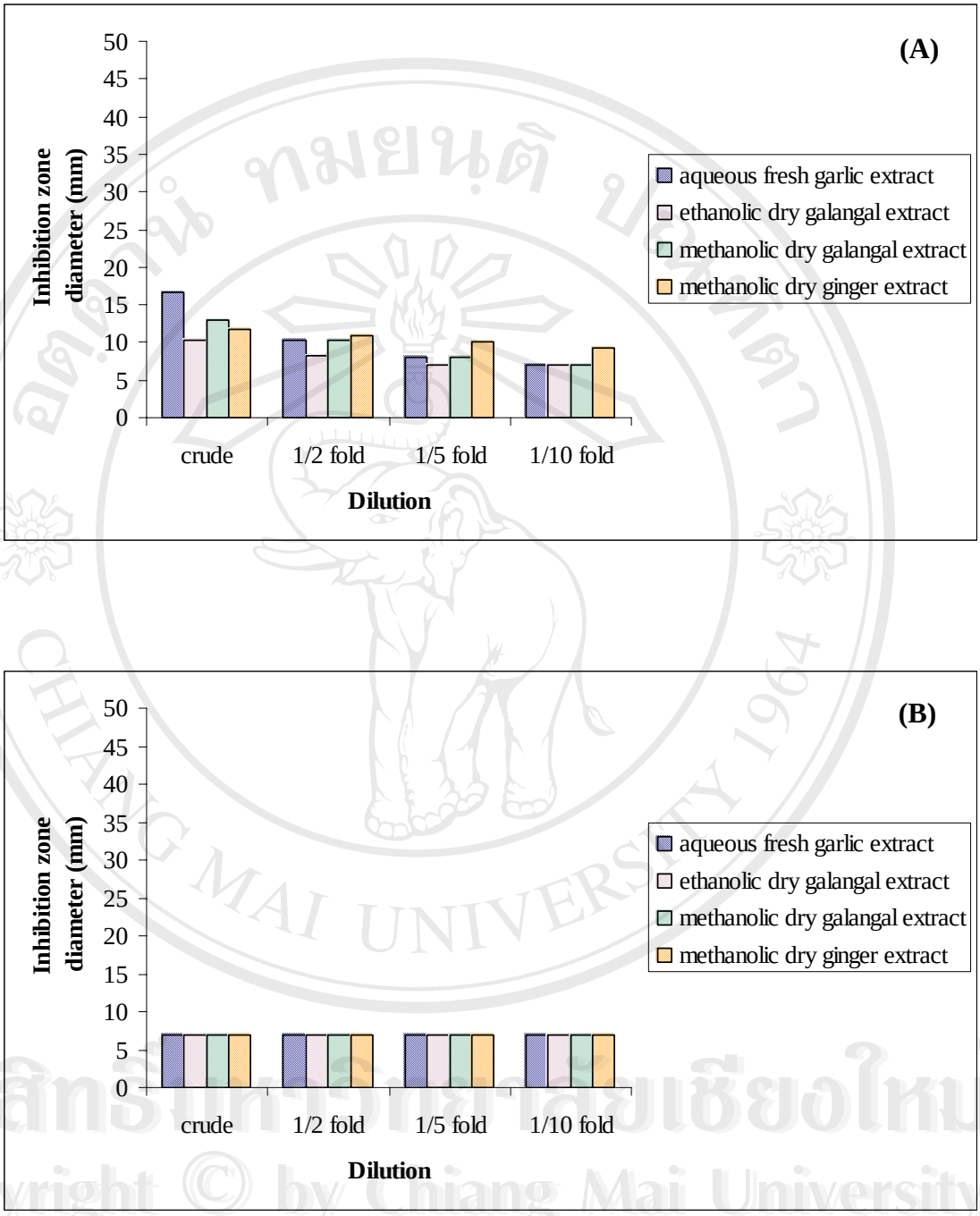


Fig. 4.26 The effect of four plant extracts on growth inhibition of *A. niger*2 (evaluated in term of inhibition zone diameter) in DAY 1 **(A)** and DAY 5 **(B)**

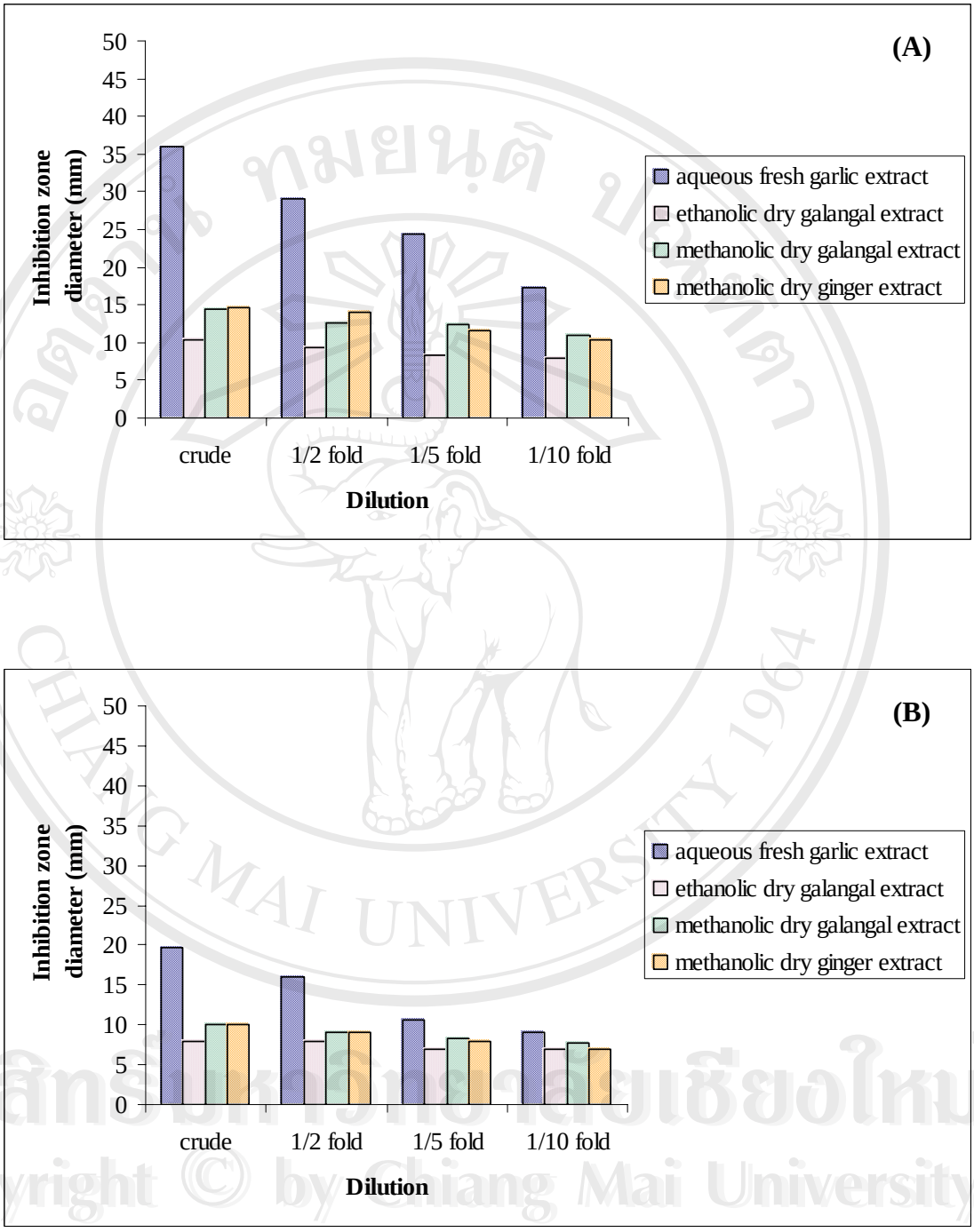


Fig. 4.27 The effect of four plant extracts on growth inhibition of *Penicillium* sp.1 (evaluated in term of inhibition zone diameter) in DAY 1 **(A)** and DAY 5 **(B)**

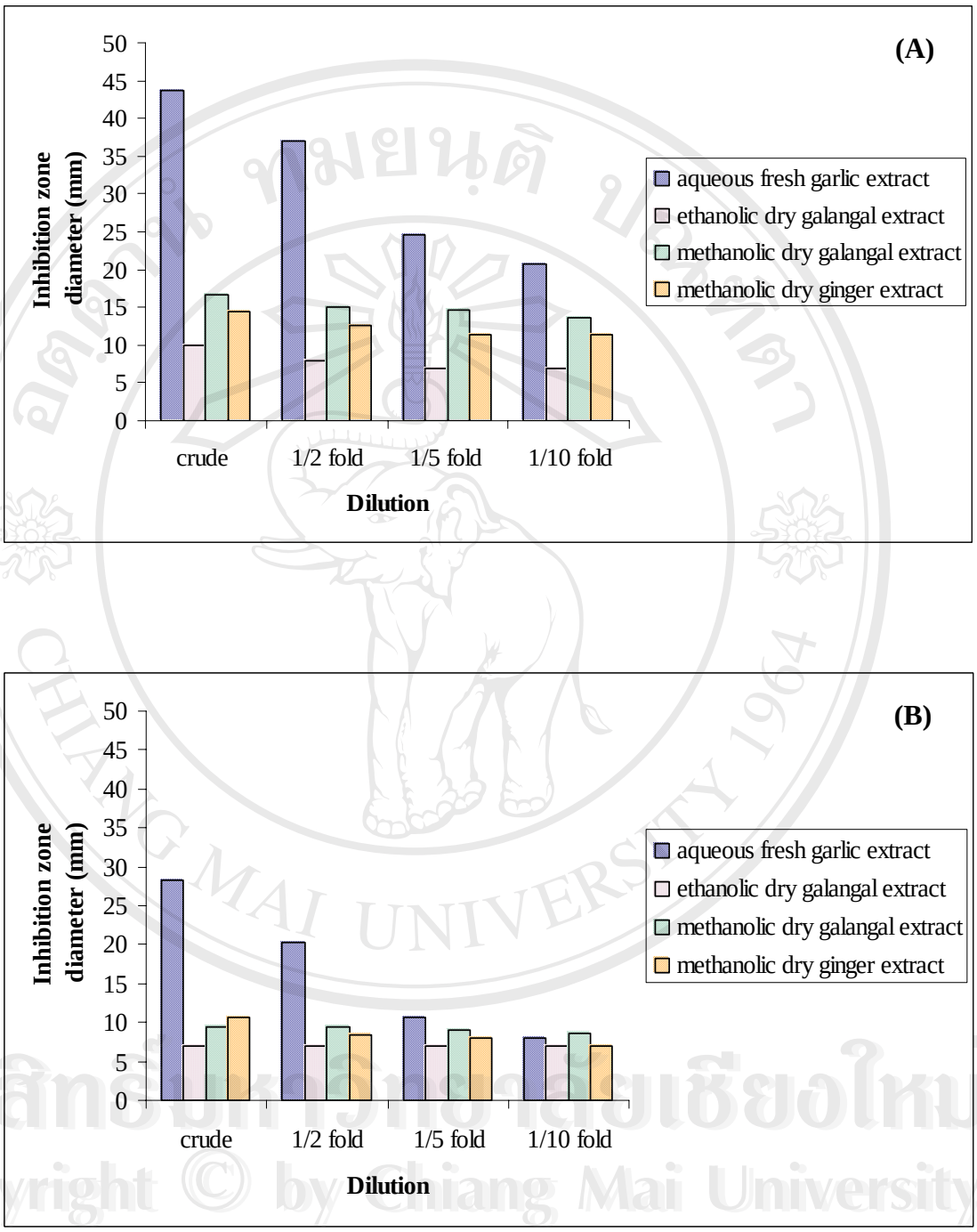


Fig. 4.28 The effect of four plant extracts on growth inhibition of *Penicillium* sp.2 (evaluated in term of inhibition zone diameter) in DAY 1 **(A)** and DAY 5 **(B)**

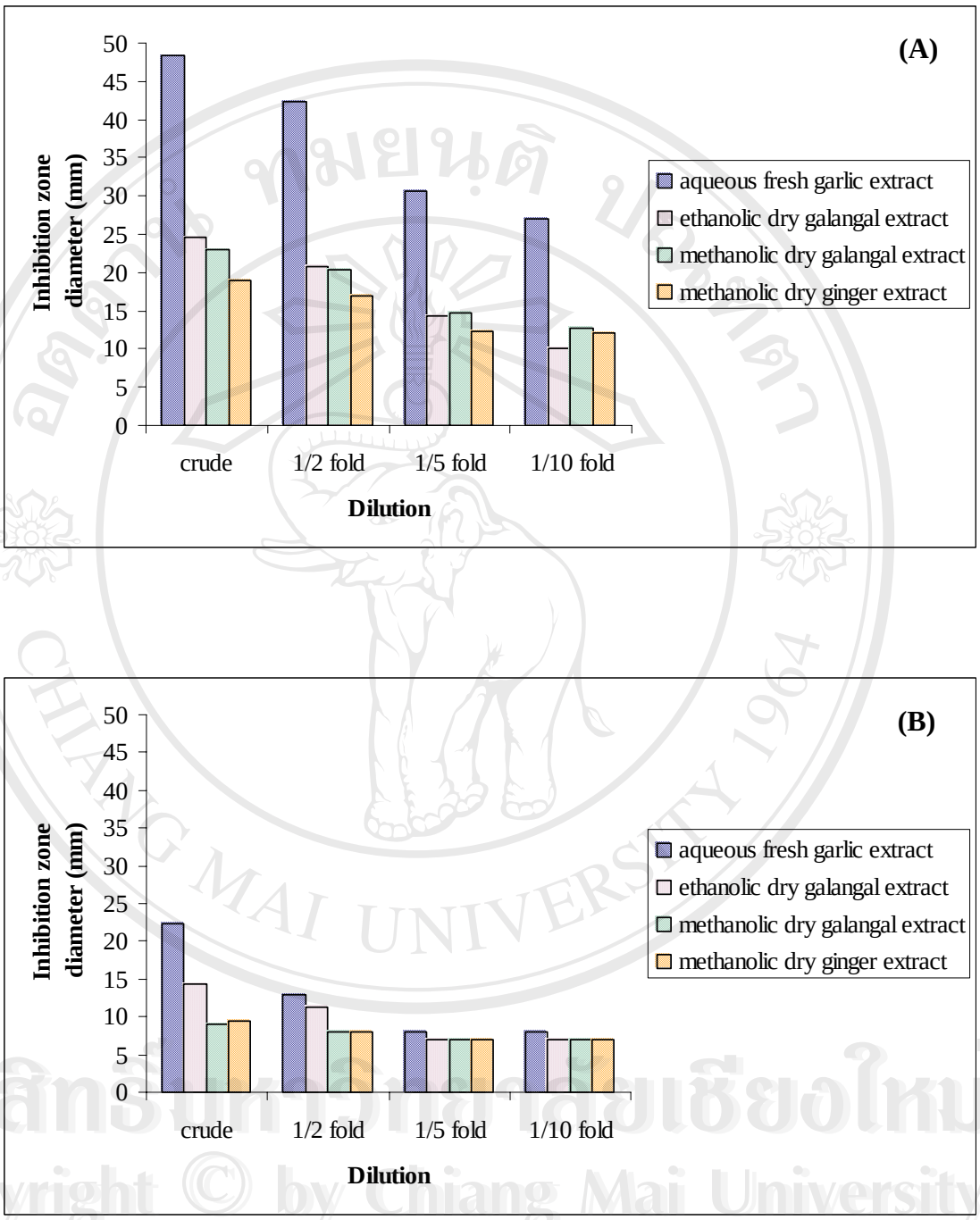


Fig. 4.29 The effect of four plant extracts on growth inhibition of *Penicillium* sp.3 (evaluated in term of inhibition zone diameter) in DAY 1 **(A)** and DAY 5 **(B)**

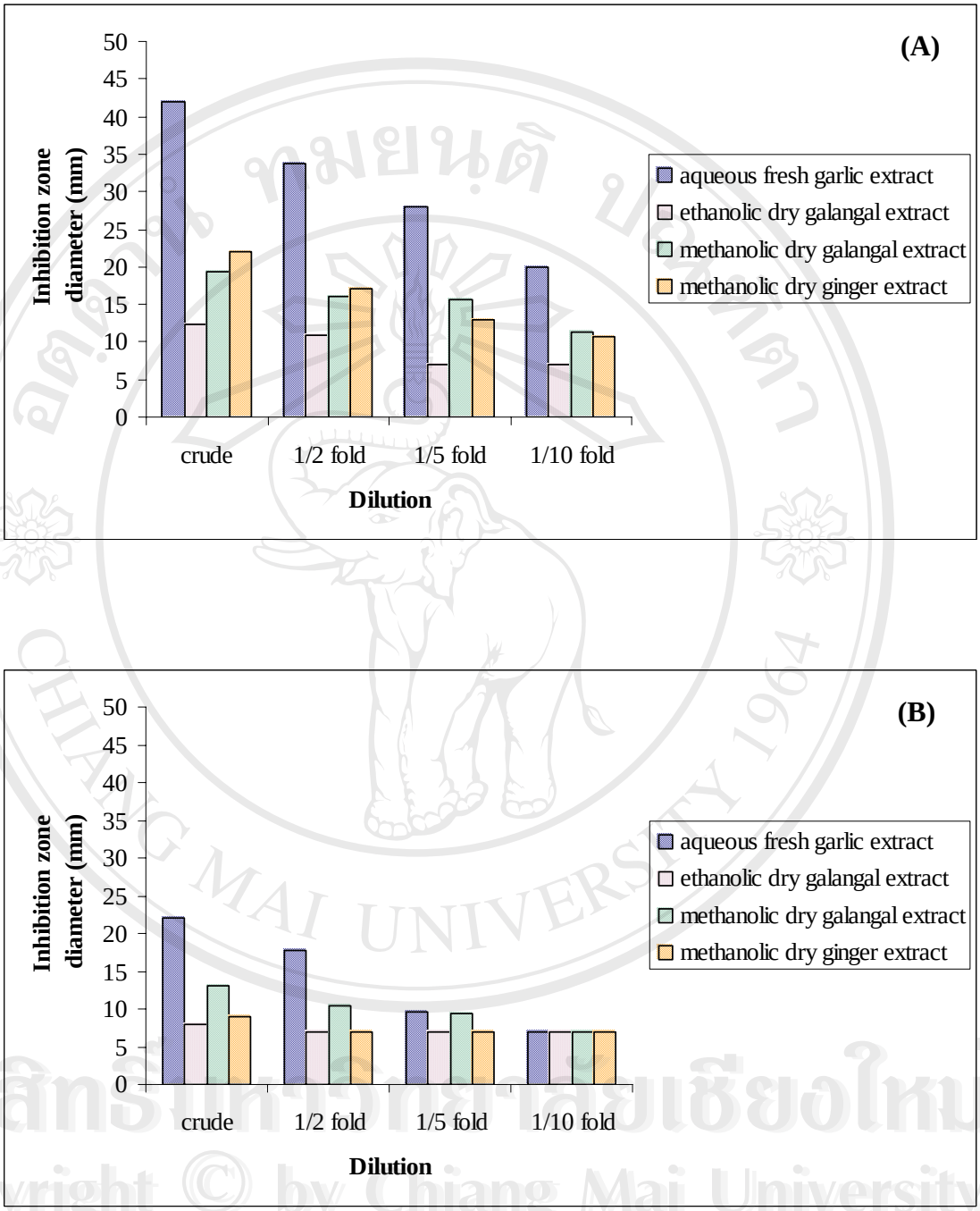


Fig. 4.30 The effect of four plant extracts on growth inhibition of *Penicillium* sp.4 (evaluated in term of inhibition zone diameter) in DAY 1 **(A)** and DAY 5 **(B)**

4.2.6 Effect of plant extracts on fungal inoculated shallot

In the in vivo study, shallot was treated with the plant extracts (aqueous fresh garlic extract, ethanolic dry galangal extract, methanolic dry galangal extract and methanolic dry ginger extract) in order to investigate the antifungal activity on shallot. The experiment was divided into two groups. The first set plant extracts were test the ability against the growth of contaminated and/or indigenous fungi on shallot. The second one was set to determine the anti three isolated fungi (*A. niger*2, *Penicillium* sp.3, *Penicillium* sp.4) inoculated on shallot. The result was described in the latter paragraph.

As the data in table 4.3, the growth of fungi was found in entire control group or no inhibition effect was observed. In contrast to the treatment used methanolic dry galangal and menthanolic dry ginger extract, these showed 100% inhibition similar to treated with ethanol and methanol. Aqueous fresh garlic extract and ethanolic dry galangal extract exhibited low inhibition ability (30% and 60%, respectively). Weight loss of shallot was assumed as the fungal assimilate water for their activity and growth. From the obtained outcome, there was no correlation between percent of inhibition and weight loss. This might be suggested that, it is the effect of high amount water evaporation. In the case of treatment used distilled water which higher percentage of inhibition more than aqueous fresh garlic extract and ethanolic dry galangal extract, it might due to the variation among bulb of shallot.

Table 4.3 Effect of plant extracts on shallot bulbs (no inoculation)

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
% inhibition	0.00^{e}	76.67^{b}	100.00^{a}	100.00^{a}	30.00^{d}	60.00^{c}	100.00^{a}	100.00^{a}
% weight loss	4.87^{a}	3.79^{c}	4.38^{ab}	4.31^{abc}	4.22^{bc}	4.53^{ab}	4.53^{ab}	4.85^{a}

(A) control (no treated extract); (B) distilled water; (C) ethanol; (D) methanol; (E) aqueous fresh garlic extract; (F) ethanolic dry galangal extract; (G) methanolic dry galangal extract; (H) methanolic dry ginger extract

^{a,b,c,d,e} Different letters indicates significant differences between the mean of column factors in each of row parameters. Significant level was set at $P<0.05$

The shallot bulbs inoculated with *A. niger*2 is presented in Table 4.4. The results showed that most extracts significantly (P<0.05) inhibited *A. niger*2 except the methanolic dry ginger extract. For the percentage of inhibition, the strongest inhibitory effect (93.33%) was obtained from ethanolic dry galangal extract and methanolic dry galangal extract, whereas, the percentage of inhibition of the aqueous fresh garlic extract and methanolic dry ginger extract were 90% and 80%, respectively. All treatment showed low percentage of weight loss compared to the control**.** However, there were no correlation between percentage of inhibition and percentage of weight loss. The best result was obtained from treatment used ethanolic dry galangal extract with 93% inhibition and 3.72% weight loss.

Table 4.4 Effect of plant extracts on shallot bulbs inoculated with *A. niger*2

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
% inhibition	76.67^{c}	60.00^{d}	96.67^{a}	93.33^{a}	90.00^{ab}	93.33^{a}	93.33^{a}	80.00^{bc}
% weight loss	5.12^{a}	3.63^{c}	4.78^{ab}	4.35^{abc}	4.18^{bc}	3.72^{c}	5.01^{ab}	4.44^{abc}

(A) control (no treated extract); (B) distilled water; (C) ethanol; (D) methanol; (E) aqueous fresh garlic extract; (F) ethanolic dry galangal extract; (G) methanolic dry galangal extract; (H) methanolic dry ginger extract

^{a,b,c,d} Different letters indicates significant differences between the mean of column factors in each of row parameters. Significant level was set at P<0.05.

The conclusion of the effect of plant extracts on shallot bulbs inoculated with *Penicillium* sp.3 is in Table 4.5. It was found that all extracts inhibited the growth of *Penicillium* sp.3, except the methanolic dry galangal extract (83.34%). The percentage of inhibition of aqueous garlic, ethanolic dry galangal and methanolic dry ginger extracts showed strong inhibitory effect (100%). Moreover, aqueous fresh garlic extract and methanolic dry ginger extract showed the equal percentage of inhibition (96.67%). All treatment showed lower percentage of weight loss than control, there is no correlation between percentage of inhibition and percentage of weight loss. The best result was obtained from ethanolic dry galangal extract which gave 100 % inhibition and 3.87% weight loss.

Table 4.5 Effect of plant extracts on shallot bulbs inoculated with *Penicillium* sp.3

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
% inhibition	86.67^{b}	76.67^{c}	100.00^{a}	96.67^{a}	96.67^{a}	100.00^{a}	83.34^{bc}	96.67^{a}
% weight loss	4.73^{a}	2.70^{d}	4.09^{bc}	4.37^{ab}	4.49^{ab}	3.87^{c}	4.14^{bc}	4.05^{bc}

(A) control (no treated extract); (B) distilled water; (C) ethanol; (D) methanol; (E) aqueous fresh garlic extract; (F) ethanolic dry galangal extract; (G) methanolic dry galangal extract; (H) methanolic dry ginger extract.

^{a,b,c,d} Different letters indicates significant differences between the mean of column factors in each of row parameters. Significant level was set at $P<0.05$.

Table 4.6 shows the effect of plant extracts on *Penicillium* sp.4 inoculated on shallot bulb. The results showed that the inhibitory effects of all plant extracts, were significantly different (P<0.05). The highest inhibition (100%) was obtained from ethanolic dry galangal and methanolic dry ginger extracts. the methanolic dry galangal extract also inhibited the growth of *Penicillium* sp.4. All treatment showed lower percentage of weight loss than control; there is no correlation between percentage of inhibition and percentage of weight loss. The best result was obtained from ethanolic dry galangal extract which gave 100 % inhibition and 3.75% weight loss.

Table 4.6 Effect of plant extracts on shallot bulbs inoculated with *Penicillium* sp.4

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
% inhibition	76.67^{c}	76.67^{c}	100.00^{a}	100.00^{a}	90.00^{b}	100.00^{a}	96.67^{ab}	100.00^{a}
% weight loss	4.75^{a}	3.23^{c}	4.19^{ab}	4.20^{ab}	3.62^{bc}	3.75^{bc}	4.14^{ab}	4.04^{b}

(A) control (no treated extract); (B) distilled water; (C) ethanol; (D) methanol; (E) aqueous fresh garlic extract; (F) ethanolic dry galangal extract; (G) methanolic dry galangal extract; (H) methanolic dry ginger extract.

^{a,b,c} Different letters indicates significant differences between the mean of column factors in each of row parameters. Significant level was set at P<0.05.

From the results, it can be concluded that all plant extracts could inhibit the inoculated fungal strains. Ethanolic dry galangal extract could more effectively inhibit the fungal growth than aqueous fresh garlic, methanolic dry galangal and ginger extracts because it could effectively inhibit the growth of *A. niger*2, *Penicillium* sp. 3 and *Penicillium* sp.4. Whereas, methanolic dry ginger extract could inhibit the growth of *Penicillium* sp.3 and *Penicillium* sp.4, and methanolic dry galangal extract could only effectively inhibit the growth of *A. niger*2. The ethanolic dry galangal extract had the highest inhibitory effect on the fungal growth due to possibly the efficiency of antimicrobial substance existed in each plant. Since, it involved with the solubility between the solvent extracts and a substance in

the plants. If the solvent extracts have a low polarity, it can be soluble with a low polar substance. To compare between the antimicrobial constituents (diterpene) of galangal extracted by ethanol and methanol, it can be suggested that diterpene extracted by ethanol may found in higher level due to ethanol has less polarity than methanol. Furthermore, the extracted antimicrobial constituents obtained from using different solvent extracts might contain different chemical composition as the efficiency of fungal growth inhibition of each extracted plants was different.

For the weight loss, the lowest weight loss was obtained from ethanolic dry galangal extract. The weight loss of shallot bulbs was the result of fungal metabolism. In addition, time and conditions of the storage were also the cause of weight loss.

The color of shallot bulb surface treated with methanolic dry galangal and ginger extracts, it changed from purple-red to purple-blue, whereas, the color of shallot bulb surface treated with ethanolic dry galangal extract did not change. It may cause from the reaction between plant extracts and bulb surface. The solvent extracts (ethanol and methanol) also influenced on the sheath of bulbs observed by using ethanol and methanol as a control.

4.2.7 Effect of plant extracts on fungal inoculated onion

The inoculated onion bulbs by fungi were incubated at room temperature. After 15 days, inoculated fungi were not observed but some bulbs were infected by other microorganisms as the soft and rot presented. Therefore, this experiment could not observe the efficiency of plant extracts on the inoculated onion bulbs. From the characteristic of symptom, it was probably soft rot disease (Delahaut and Stevenson, 2004) caused by *Erwinia carotovora* subsp. *carotovora* (Perombelon and Kelman, 1980). The soft rot bacterium can enter the neck tissue at early maturity phase. As the rot progress, invaded scale become soft and foul-smelling was detected (Brewster, 1994).