CHAPTER 3

RESULTS

3.1 Yield of the essential oils

All spice seeds were ground into powder. 100 g of each spice powder was extracted for essential oils by hydrodistillation for approximately 4 h using a Clevenger-type apparatus. Essential oil of pale yellow color was extracted from *A. graveolens* L. with yield of 0.87 to 1.13 %w/w (1.00-1.30 %v/w). Whereas *Z. piperitum* provided light yellow essential oil of 0.35 - 0.44 %w/w (0.40-0.50 %v/w) yield. (Figure 3.1).

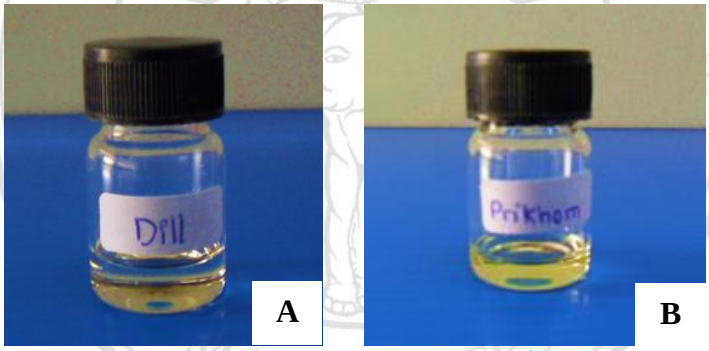

Figure 3.1 The essential oils from A) *A. graveolens* L. and B) *Z. piperitum*

3.2 Chemical composition of the essential oils

The chemical compositions of *A. graveolens* L. and *Z. piperitum* essential oils were analyzed by Gas Chromatography (GC) 6890 Agilent Technologies/ MSD 5973 5973 Hewlett Packard series GC-MS. The compositions were identified based on the comparison of their relative retention time and mass spectra with those of standards, W8N05ST (Wiley ver. 8.0) library data of the GC-MS system. *A. graveolens* L. and *Z. piperitum* oil mass spectral is shown in Figures 3.2 and 3.3, respectively. The amounts of components are also listed in Table 3.1 and Table 3.2

3.2.1 Chemical composition of *Anethum graveolens* L. essential oil

GC-MS analysis resulted in the identification of twenty-four components, accounting for 96.2% of the total oil shown in Table 3.1. Of these, the constituents could be grouped into three sub-classes with monoterpene hydrocarbon 21.8%, oxygenated monoterpene 40.5% and phenylpropene 33.9%. The major peaks of the essential oil constituents exhibited at 12.607, 20.892, 22.954 and 38.718 min (Figure 3.2), each with peak height of 19.2%, 12.3%, 26.0%, 33.1%, of which m/z values consistent with the mass spectral patterns of (-)-limonene (98% similarity), dihydrocarvone (98% similarity), (+)-carvone (96% similarity), *trans*-isodillapiole (90% similarity), respectively. The minor components (≥ 0.5%) were shown at 12.860 and 20.498 min (Figure 3.2) with 2.0% and 1.4% abundance, and their m/z value were consistent with the mass spectral patterns of β-ocimene (96% similarity) and (+)-isodihydrocarvone (99% similarity), respectively.

Table 3.1 Chemical composition of *A. graveolens* L. essential oil identified by GC-MS analysis

Peak no.	Compound	Retention time (min.)	Match Quality	Molecule Weight	%
1	α-(+)-Pinene	8.277	97	136	Tr
2	(+)-Sabinene	9.899	97	136	Tr
3	β-Pinene	9.999	94	136	Tr
4	β-Myrcene	10.715	91	133	0.1
5	1,5,8-*P*-Menthatriene	11.262	94	136	0.3
6	(-)-Limonene	12.607	98	136	19.2
7	β-Ocimene	12.860	96	136	2.0
8	γ -Terpinene	13.724	96	136	Tr
9	(-)-α-Thujone	15.034	95	152	Tr
10	α -4-Dimethylstyrene	15.157	96	132	0.1
11	*cis*-Limonene Oxide	17.167	97	152	Tr
12	*trans*-Limonene Oxide	17.390	95	152	0.1
13	Dill ether	19.599	95	152	0.1
14	(+)-Isodihydrocarvone	20.498	99	152	1.4
15	Dihydrocarvone	20.892	98	152	12.3
16	Dihydrocarveol	21.550	97	154	0.3
17	*cis*-Carveol	21.861	98	152	0.2
18	(+)-Carvone	22.954	96	150	26.0
19	*cis*-Dioxide carvone	23.976	90	166	Tr
20	(*E*)-Anethol	24.311	97	148	0.2
21	Thymol	24.940	93	150	Tr
22	Myristicin	34.182	98	192	0.4
23	Elemicin	35.586	98	208	0.2
24	*trans*-Isodillapiole	38.718	90	222	33.1

Tr.: trace amount <0.05%

Total 96.2%

3.2.2 Chemical composition of *Zanthoxylum piperitum* essential oil

Sixty three compounds have been identified in essential oil from *Z. piperitum*, accounting for 92.7% of the total oil composition as shown in Table 3.2. Of these, the components could be grouped into six sub-classes with monoterpene hydrocarbon 68.6%, oxygenated monoterpene 19.5%, sesquiterpene hydrocarbon 3.2%, oxygenated sesquiterpene 1.0%, phenylpropene 0.2%, and miscellaneous compounds 0.2%. The major compounds of the essential oil were exhibited at 10.097, 12.612 and 40.215 min (Figure 3.3) with 14.6%, 23.2%, and 11.9% abundance, of which their identity was consistent to the mass spectral pattern of sabinene (96% similarity) , β-phelladrene (94% similarity) and brevifolin (94% similarity). The minor compounds (≥ 0.5%) of the essential oil were exhibited at 8.053, 8.329, 10.144, 10.832, 11.267, 11.819, 12.929, 13.352, 13.799, 14.234, 15.115, 15.673, 15.926, 16.760, 17.600, 19.727, 20.062, 20.785, 22.994, 27.054, 28.523, 29.868, 32.424 and 34.146 min with 0.8%, 3.1%, 0.8%, 4.6%, 0.9%, 1.4%, 2.2%, 1.1%, 2.2%, 0.9%, 1.5%, 0.9%, 3.4%, 1.0%, 0.7%, 0.5%, 1.3%, 0.5%, 6.4%, 2.3%, 0.6%, 0.5%, 0.6% and 0.5% (Figure 3.3), of which their m/z rations were consistent to the mass spectral pattern *α*-thujene (94% similarity) , α-(+)-pinene (96% similarity) , β-*L*-pinene (94% similarity) , β-myrcene (95% similarity) , α-phellandrene (98% similarity) , α-terpinene (97% similarity) , *cis*-ocimene (98% similarity), β-ocimene (96% similarity), γ-terpinene (96% similarity), 4-thujanol (98% similarity) , α-terpinolen (97% similarity) , *cis*-β-terpineol (91% similarity) , linalool (97% similarity) , (*E*)-*p*-menth-2-en-1-ol (91% similarity) , 4-terpinenol (96% similarity), *L*-cryptone (95% similarity), β-fenchol (91% similarity), *cis*-piperitol (90% similarity) , (+)-piperitone (96% similarity) , terpinyl acetate (91% similarity) , geraniol acetate (91% similarity) , caryophyllene (99% similarity) , germacrene D (98% similarity), δ-cadinene (99% similarity).

Table 3.2 Chemical composition of *Z. piperitum* essential oil identified by GC-MS analysis

Peak no.	Compound	Retention time (min.)	Match Quality	Molecule Weight	%
1	Hexanal	4.187	95	100	0.1
2	α-Thujene	8.053	94	136	0.8
3	α-(+)-Pinene	8.329	96	136	3.1
4	Camphene	8.852	96	136	0.1
5	(+)-Sabinene	10.097	96	136	14.6
6	β-Pinene	10.144	94	136	0.8
7	β-Myrcene	10.832	95	136	4.6
8	α-Phellandrene	11.267	94	136	0.9
9	3-Carene	11.484	97	136	Tr
10	α-Terpinene	11.819	98	136	1.3
11	β-Phelladrene	12.612	96	136	23.2
12	*cis*-Ocimene	12.929	97	136	2.2
13	β-Ocimene	13.352	98	136	1.1
14	γ -Terpinene	13.799	96	136	2.2
15	4-Thujanol	14.234	96	154	0.9
16	α-Terpinolene	15.115	98	136	1.5
17	*p*-Cymenene	15.175	97	132	0.1
18	*cis*-β-Terpineol	15.673	91	154	0.9
19	Linalool	15.926	97	154	3.4
20	(*E*)-*p*-Menth-2-en-1-ol	16.760	91	154	1.0
21	α-Campholenal	16.878	94	152	Tr
22	Neo-Allo-Ocimene	17.047	97	136	Tr
23	4-terpinenol	17.600	96	154	0.7
24	4-Isopropyl cyclohexone	18.352	96	140	0.1
25	1,4-Dimethoxybenzene	18.687	96	138	Tr
26	(+)-*P*-Menth-1-en-4-ol	19.445	97	154	0.2
27	*L*-Cryptone	19.727	95	138	0.5

28	β-Fenchol	20.062	91	154	1.3
29	*cis*-Piperitol	20.785	90	154	0.5
30	(*E*)-Carveol	21.396	98	152	Tr
31	(*Z*)-Geraniol	21.807	97	154	0.1
32	2-Methyl-3-phenyl-propanal	22.142	98	148	0.2
33	(+)-Carvone	22.336	94	150	0.1
34	(+)-Piperitone	22.994	96	152	6.4
35	Phellandral	23.699	95	152	0.2
36	Bornyl acetic ether	24.169	99	196	0.3
37	(*E*)-Anethol	24.628	98	148	0.1
38	α-(+)-Pinene	24.827	93	136	Tr
39	Carvacrol	25.344	97	150	0.1
40	Terpinyl acetate	27.054	91	196	2.2
41	Chavibetol	27.430	98	164	Tr
42	Nerol acetate	27.683	91	196	0.2
43	Copaene	28.035	99	204	Tr
44	Geraniol acetate	28.523	91	196	0.6
45	β-Elemene	28.770	99	204	0.2
46	Methyl eugenol	29.445	98	178	0.1
47	Caryophyllene	29.868	99	204	0.5
48	α-Caryophyllene	31.267	98	204	0.1
49	Naphthalene	32.254	99	204	0.1
50	Germacrene D	32.424	98	204	0.6
51	Germacrene B	33.029	95	204	0.2
52	α-Amorphene	33.217	98	204	0.1
53	β-Elemene	33.376	94	204	0.1
54	α-Farnesene	33.582	98	204	0.1
55	δ-cadinene	34.146	99	204	0.5
56	*trans*-Nerolidol	35.797	91	222	0.1
57	(+)-Spathulenol	36.296	99	220	0.3

58	β-(-)-Caryophyllene epoxide	36.431	99	220	0.4
59	Cedren-8-ol	37.166	99	222	0.4
60	Isospathulenol	38.588	99	220	0.1
61	Brevifolin	40.215	94	196	11.9
62	Farnesol	41.690	97	222	0.1
63	Farnesol acetate	45.744	97	264	0.2

Tr.: trace amount <0.05%

Total 92.7 %

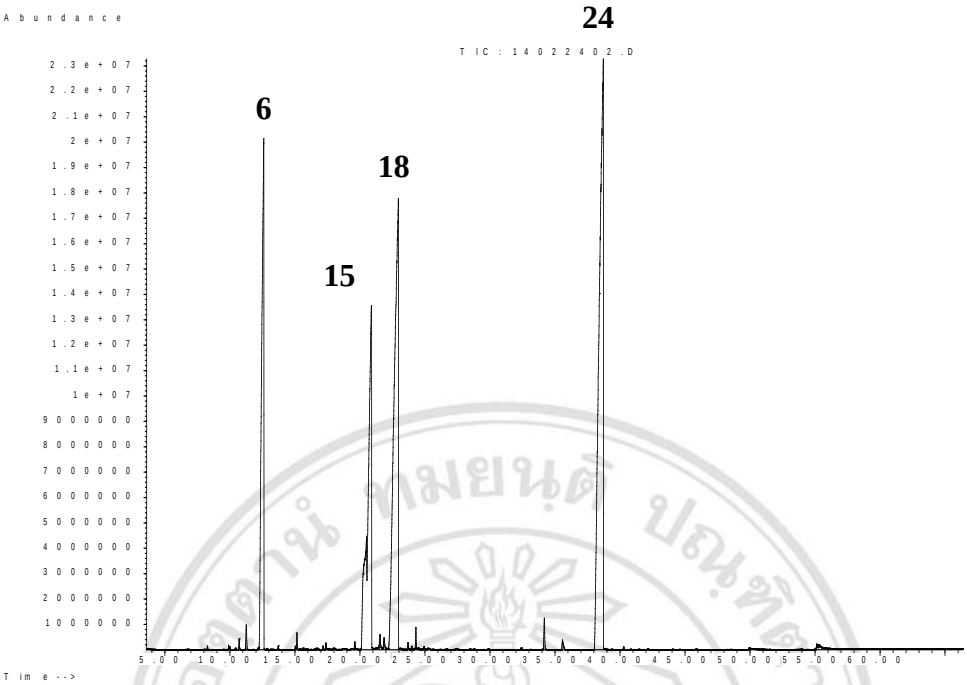

Figure 3.2 The total ion chromatogram of *A. graveolens* L. oil

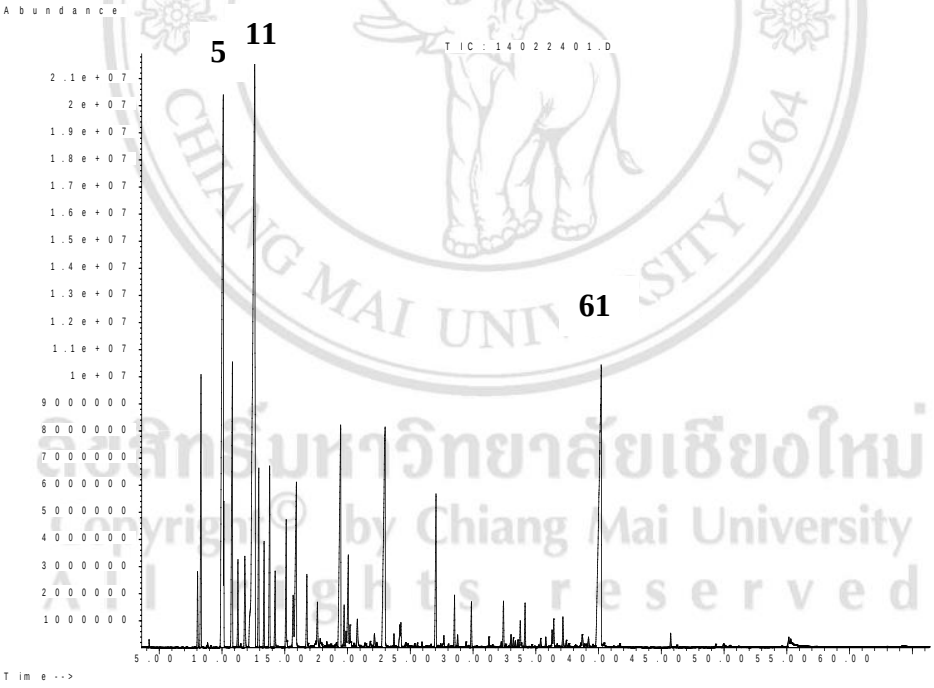

Figure 3.3 The total ion chromatogram of *Z. piperitum* oil

3.3 Antifungal activity of the essential oils on mycelial growth

The antifungal activity of essential oils from *A. graveolens* L. and *Z. piperitum* were determined by the poisoned food technique in potato dextrose agar plate against aflatoxigenic fungi (*A. flavus*) (2.6.1). The fungal growth at 28±2 ^{o}C was monitored by measuring colony diameters every day for 9 days. The results of *A. graveolens* L. oil are shown in Figures 3.4 and 3.5 and the results of *Z. piperitum* oil are shown in Figures 3.6 and 3.7.

3.3.1 Antifungal activity of *A. graveolens* L. oil on mycelial growth

Five concentrations (0.25, 0.5, 1.0, 1.5, 2.0 µL/mL) of *A. graveolens* L. essential oil were tested for their antifungal activities. The mycelial growth was retarded by 3 days for *A. flavus* at 1.0 µL/mL and 6 days at 1.5 µL/mL *A. graveolens* L. oil. The complete growth inhibition of *A. flavus* was observed at the MIC value of 2.0 µL/mL oil shown in Figure 3.4 and the fungal colony at 9th day of inoculation shown in Figure 3.5, the fungal growth colony diameter was shown in Table 3.3. The results indicated that fungal colony diameter increased with incubation time but gradually decreased when increasing concentration of *A. graveolens* L. oil. The oil significantly reduced fungal growth with inhibition percentage of 71.10%, 75.35%, 86.91% and 92.10% at 0.25, 0.5, 1.0, and 1.5 µL/mL concentrations, respectively, on the 9th day of incubation.

Table 3.3 Mean diameter of *A. flavus* colony grown in agar medium supplemented with five different concentrations of the essential oil from *A. graveolens* L.

Concentration of *A. graveolens* oil (µL/mL)	Diameter of fungal colony* (cm)
0	8.48±0.03[a]
0.25	2.45±0.08[b]
0.5	2.09±0.51[b]
1.0	1.11±0.09[c]
1.5	0.68±0.04[d]
2.0	0.40±0.00[d]

* Values are mean (n = 3) ± standard deviations. Values followed by the same letter in each column are not significantly different in ANOVA and Duncan Multiple Range Test ($p < 0.05$).

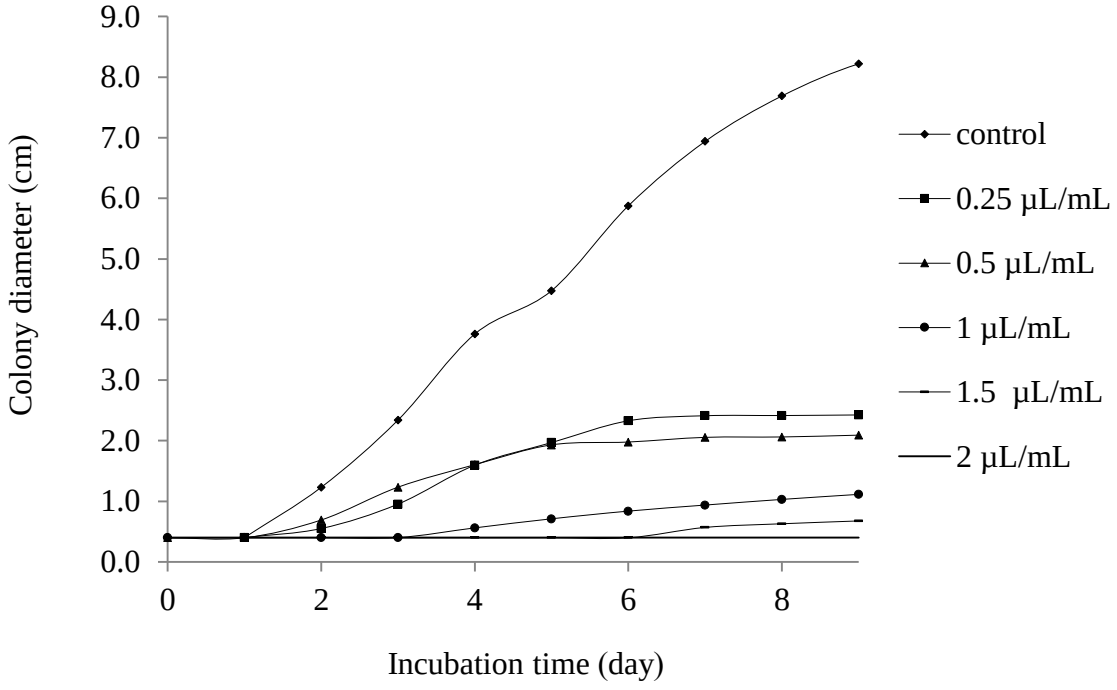

Figure 3.4 Effects of the different concentrations of *A. graveolens* oil on the colony growth of *A. flavus* in PDA medium

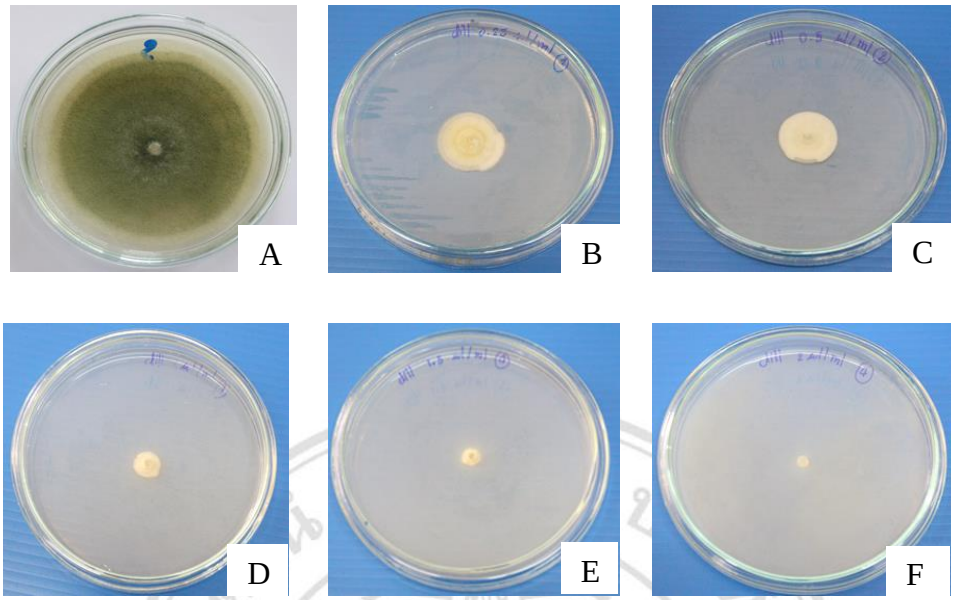

Figure 3.5 *A. flavus* colony grown on PDA control medium (A) and PDA with 0.25 µL/mL (B), 0.5 µL/mL (C), 1.0 µL/mL (D), 1.5 µL/mL (E) and 2.0 µL/mL (F) of *A. graveolens* oil after incubation at 28±2 ºC for 9 days

3.3.2 Antifungal activity of *Z. piperitum* oil on mycelial growth

Essential oil from *Z. piperitum* was tested for its antifungal activity at 8 different concentrations (1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 µL/mL) . The mycelial growth was retarded by 3 days for *A. flavus* at 2.5 µL/mL and 5 days at 3.0 µL/mL of oil. A complete growth inhibition of *A. flavus* by *Z. piperitum* oil was observed at the MIC value of 4.5 µL/mL shown in Figure 3.6 and the 9th days old fungal colonies shown in Figure 3.7, and their diameters were shown in Table 3.4. The results indicated that fungal colony diameter increased with increasing incubation time but gradually decreased when increasing concentration of *Z. piperitum* oil. The oil significantly reduced fungal growth with inhibition percentage of 46.34, 62.50, 77.83, 82.78, 86.43, 89.03 and 91.04 at 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 µL/mL concentrations, respectively.

Table 3.4 Mean diameter of *A. flavus* colony grown in agar medium supplemented with the essential oil from *Z. piperitum* at eight different concentrations

Concentration of *Z. piperitum* oil (µL/mL)	Diameter of fungal colony* (cm)
0	8.48±0.03[a]
1.0	4.55±0.01[b]
1.5	3.18±0.26[c]
2.0	1.88±0.14[d]
2.5	1.46±0.12[e]
3.0	1.15±0.06[f]
3.5	0.93±0.18[f, g]
4.0	0.76±0.07[g]
4.5	0.40±0.00[h]

* Values are mean (n = 3) ± standard deviations. Values followed by the same letter in each column are not significantly different in ANOVA and Duncan Multiple Range Test ($p < 0.05$).

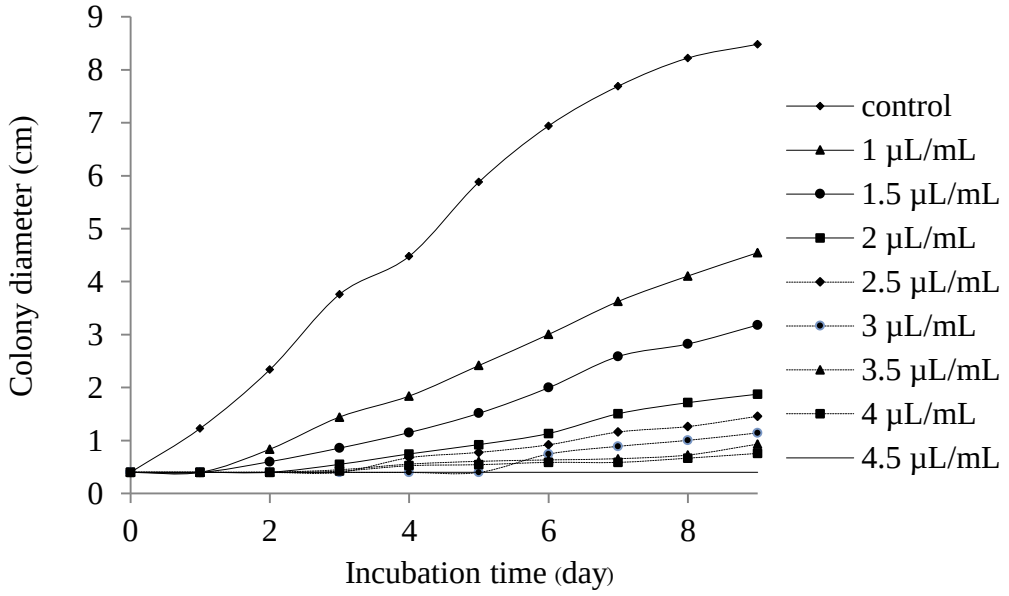

Figure 3.6 Effects of the different concentrations of *Z. piperitum* essential oil on the colony diameter growth of *A. flavus* in PDA medium

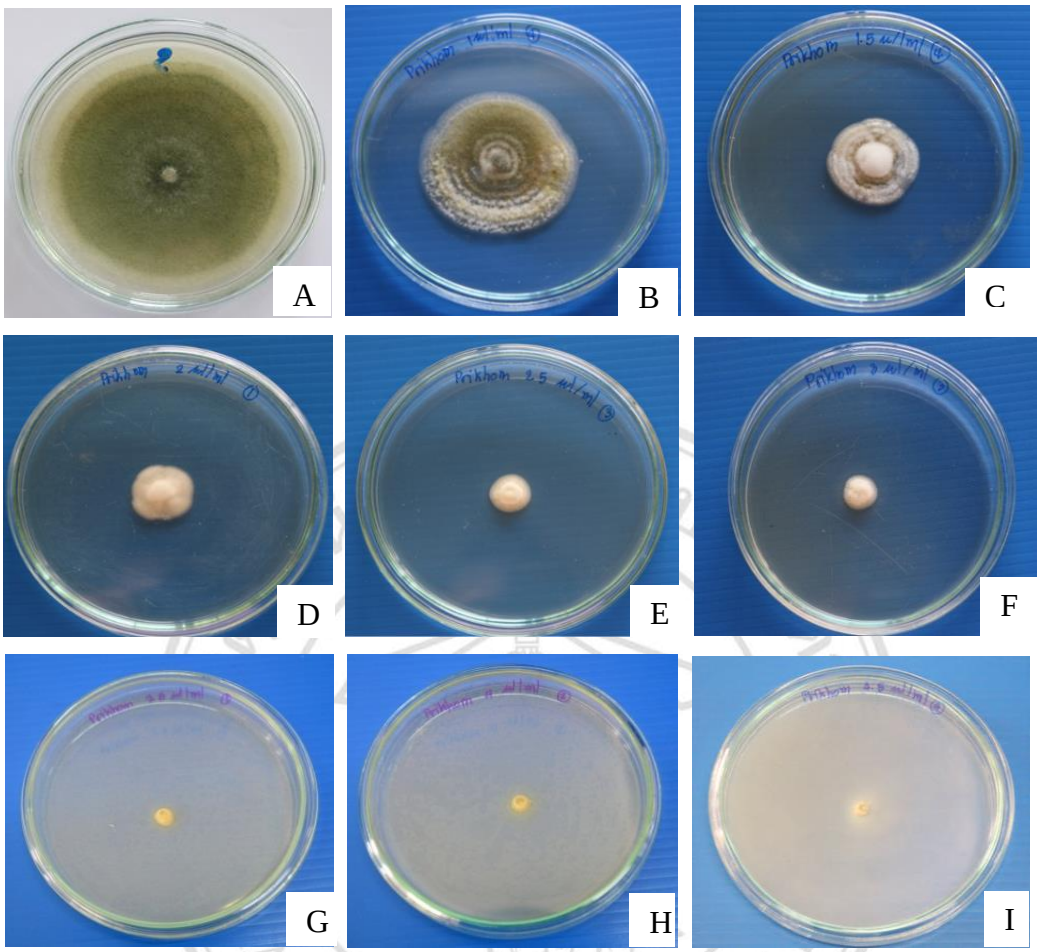

Figure 3.7 *A. flavus* colony grown on PDA control medium (A) and PDA with 1.0 µL/mL (B), 1.5 µL/mL (C), 2.0 µL/mL (D), 2.5 µL/mL (E), 3.0 µL/mL (F), 3.5 µL/mL (G), 4.0 µL/mL (H) and 4.5 µL/mL (I) of *Z. piperitum* oil after incubation at 28±2 ºC for 9 days

3.4 Antifungal activity of essential oils in liquid culture

The antifungal activity of *A. graveolens* and *Z. piperitum* oils against aflatoxigenic fungi was performed in broth medium (2.6.2). The cells were harvested on 9^{th} day and weighed in both wet and dry forms.

3.4.1 Antifungal activity of *A. graveolens* L. oil on mycelial mass

The antifungal activity of *A. graveolens* L. oil on mycelial wet and dry weight was determined in PDB medium (Table 3.5). Five different concentrations of the essential oil were observed to be effective in remarkably inhibiting the biomass production of the fungi (Figure 3.8) and mycelial sizes were smaller when the concentrations of essential oils were increased (Figure 3.9). Mycelial growth at 0.25 µL/mL was decreased to approximately half of that of the control. *A. graveolens* oil was found to be most effective (96.43% inhibition, no mycelial mass observed) at 2.0 µL/mL concentration. The results illustrate that mycelial fungal mass increased with increasing incubation time but gradually decreased when increasing concentration of *A. graveolens* L. oil.

3.4.2 Antifungal activity of *Z. piperitum* oil on mycelial mass

Inhibitory effect of *Z. piperitum* oil on mycelial dry weight was determined in PDB medium (Table 3.6). Eight different concentrations of the essential oil were observed to be gradually effective in inhibiting the biomass production of the fungi (Figure 3.10). High concentrations of essential oil resulted in small size of mycelial fungal mass (Figure 3.11). Mycelial growth in the presence of 2.0 µL/mL *Z. piperitum* oil was decreased to approximately half of that of the control while the most effective concentration (no observed mycelia) was 4.5 µL/mL.

Table 3.5 Mean wet and dry mycelial weight of 9-day old of *A. flavus* in PDB liquid medium supplemented with the essential oil from *A. graveolens* oil at five different concentrations

Concentration of *A. graveolens* oil (µL/mL)	Wet mycelial weight (g)	Dry mycelial weight (g)	% Inhibition*
0	7.1673±0.1604	0.1205±0.0041[a]	-
0.25	3.1120±0.1381	0.0581±0.0110[b]	51.78
0.5	2.5331±0.3644	0.0437±0.0011[c]	63.37
1.0	1.6164±0.0822	0.0350±0.0064[c]	70.95
1.5	1.4228±0.1298	0.0176±0.0065[d]	85.54
2.0	1.0421±0.0129	0.0043±0.0048[e]	96.43

* Calculated from dry weight

Values are mean (n = 3) ± standard deviations. Values followed by the same letter in each column are not significantly different in ANOVA and Duncan Multiple Range Test ($p < 0.05$).

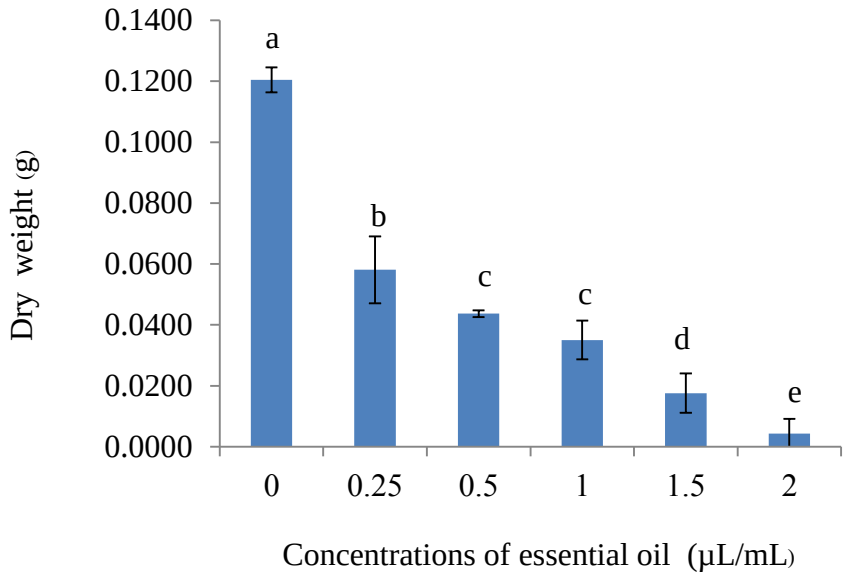

Figure 3.8 Dry weight of mycelial mass of *A. flavus* shaken for 9 days in liquid medium supplemented with the essential oil from *A. graveolens* L. at five different concentrations

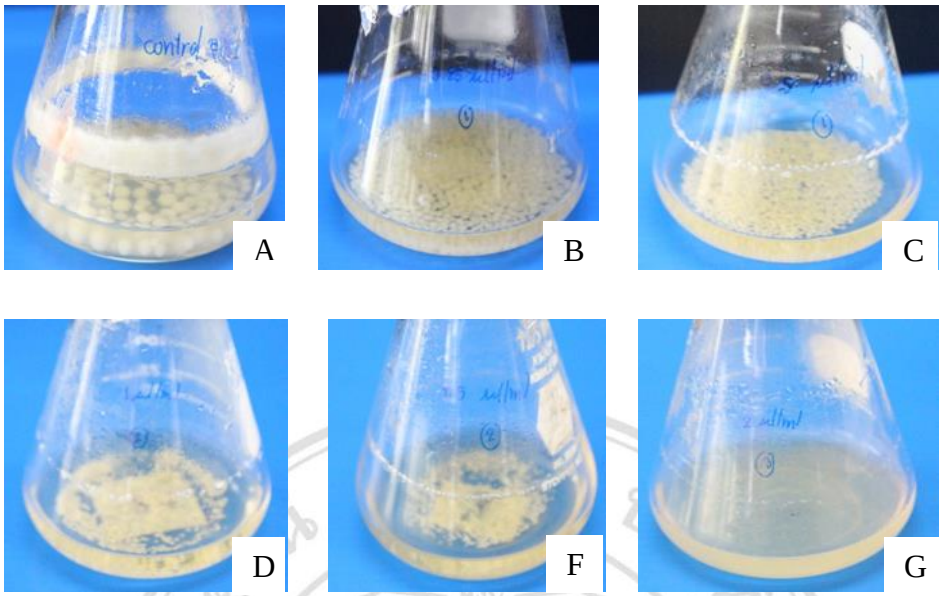

Figure 3.9 Mycelial material of *A. flavus* in control (A), 0.25 µL/mL (B), 0.5 µL/mL (C), 1.0 µL/mL (D), 1.5 µL/mL (E) and 2.0 µL/mL (F) of *A. graveolens* L. essential oil after incubation at 28±2 °C for 9 days

Table 3.6 Mean dry mycelium weight (g) of 9-day old *A. flavus* in liquid medium supplemented with the essential oil from *Z. piperitum* at eight different concentrations

Concentration of *Z. piperitum* oil (µL/mL)	Wet mycelium weight (g)	Dry mycelium weight (g)	% Inhibition*
0	7.6651±0.7791	0.1346±0.0027^{a}	-
1.0	5.9172±0.5735	0.1159±0.0056^{b}	13.89
1.5	4.2699±0.3560	0.0935±0.0117^{c}	30.53
2.0	2.8692±0.2753	0.0656±0.0076^{d}	51.26
2.5	1.7935±0.0481	0.0376±0.0023^{e}	72.06
3.0	1.6609±0.0237	0.0332±0.0011^{e}	75.33
3.5	1.5419±0.1032	0.0296±0.0006^{e,f}	78.00
4.0	1.3728±0.0282	0.0251±0.0026^{f}	81.35
4.5	1.1940±0.1376	0.0047±0.0047^{g}	96.51

* Calculated from dry weight. Values are mean (n = 3) ± standard deviations. Values followed by the same letter in each column are not significantly different in ANOVA and Duncan Multiple Range Test ($p < 0.05$).

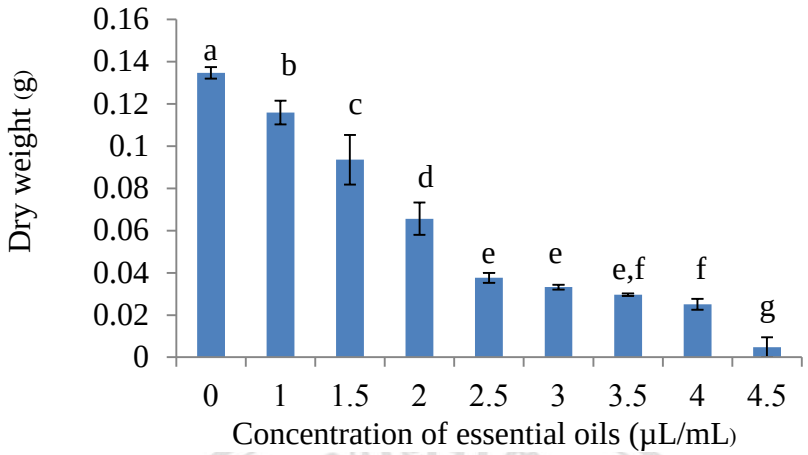

Figure 3.10 Dry weight of mycelial mass of *A. flavus* shaken for 9 days in liquid medium supplemented with the essential oil from *Z. piperitum* at eight different concentrations.

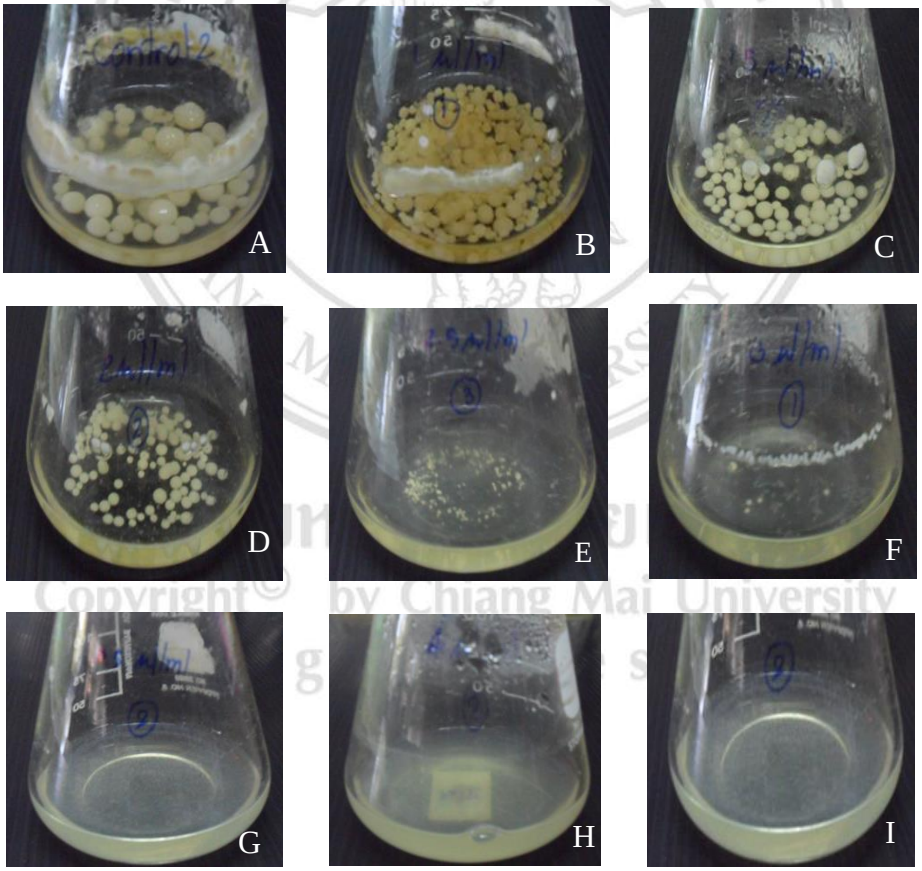

Figure 3.11 Mycelial material of *A. flavus* in control (A), 1.0 µL/mL (B), 1.5 µL/mL (C), 2.0 µL/mL (D), 2.5 µL/mL (E), 3.0 µL/mL (F), 3.5 µL/mL (G), 4.0 µL/mL (H) and 4.5 µL/mL (I) of *Z. piperitum* oil after incubation at 28±2 ^{o}C for 9 days.

3.5 Light microscope examination of the effect of the essential oils on *A. flavus* morphology

Fungal hypha morphology was observed by slide culture. The PDA plates with and without 1.0 µL/mL of *A. graveolens* L. and *Z. piperitum* essential oils, respectively (2.6.3) were examined under a light microscope at 20x and 40x magnification.

3.5.1 The effect of *A. graveolens* L. and *Z. piperitum* oils on morphology of *A. flavus*

The microscopic observation results of fungus treated with 1.0 µL/mL by *A. graveolens* L. and control without oil are shown in Figure 3.12 and 3.13 The control agar plate (Figure 3.12 A) had more conidia and budding conidia spores of *A. flavus* than the ones with 1.0 µL/mL of *A. graveolens* L. and *Z. piperitum* oils (Figure 3.12 B and C) , respectively. The result supported by Figure 3.13 showing morphology degenerative changes in the hyphal and conidial head without and with 1.0 µL/mL for *A. graveolens* L. and *Z. piperitum* oils. Mycelial filament structure without essential oil was regular cell structure homogenous with cylindrical principal axes (Figure 3.13 A), clearly visible conidia sterigmata bearing one a large and radiate conidial heads, and the conidiophore profuse conidiation on conidial heads (Figure 3.13 D). However, The hyphae structures of fungus exposure to oil, appeared spiral coil, thin flat and decreased hyphal diameters due to the lack of cytoplasm (Figure 3.13 B and C). Furthermore, the conidial heads treated with 1 µL/mL of both essential oils were distorted, languish conidial heads structures and flatten conidiophore (Figure 3.13 E and F).

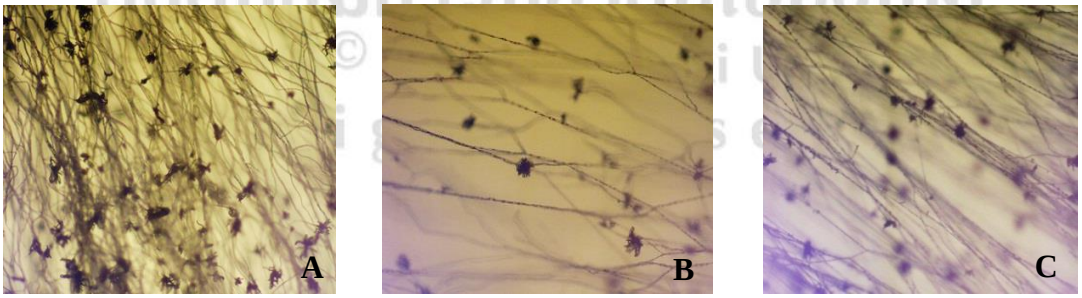

Figure 3.12 Morphology of *A. flavus* under 20X microscope on PDA without (A) and with the essential oils of *A. graveolens* L. (B) and *Z. piperitum* (C) at 1 µL/mL

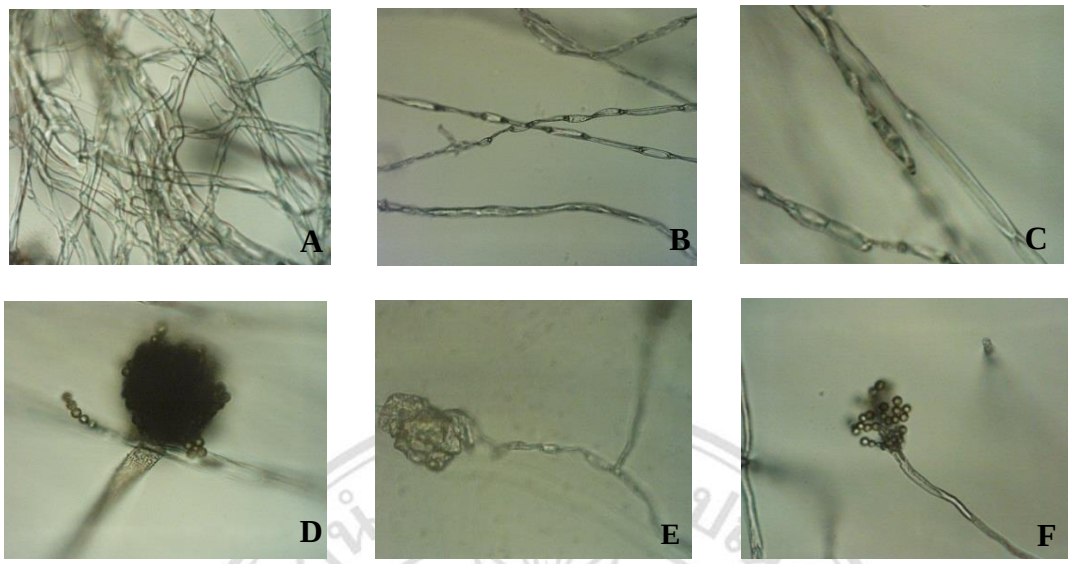

Figure 3.13 Morphology of *A. flavus* under 40X microscope on PDA without (A,D) and with the essential oils of *A. graveolens* L. (B,E) and *Z. piperitum* (C,F) at 1 µL/mL

3.6 Effect of essential oils on ergosterol content in plasma membrane of *A.flavus*

The effect of *A. graveolens* L. and *Z. piperitum* oils on the ergosterol content in the plasma membrane of *A. flavus* was studied. In our study, the ergosterol content was determined by previously described methods by Tian et al., (2012) . This sterol quantitation method is indicative of the ergosterol and 24(28) dehydroergosterol contents based on the exclusive spectral absorption at 230 and 280 nm of extracted sterols (2.6.4).

3.6.1 Effect of ergosterol content in plasma membrane from *A. graveolens* L. and *Z. piperitum* oils

The result indicated that the ergosterol content in the plasma membrane of *A. flavus* was reduced by 0.25 µL/mL of *A. graveolens* L. and 1.0 µL/mL of *Z. piperitum* oils. After 4-day inculation wet mycelial masses in the presence of added *A. graveolens* L. and *Z. piperitum* oils were 4.39 g and 3.72 g, respectively, compared to the control (9.56 g) . After incubated with 0.25 µL/mL for *A. graveolens* L. oil, a reduction of the ergosterol content in the plasma membrane of *A. flavus* was observed at 0.17±0.01 %w/w (32% reduction) of the control. Whereas *Z. piperitum* oil decreased the ergosterol content to 0.18±0.02 %w/w (28% reduction) compared to the control (0.25±0.03 %w/w).

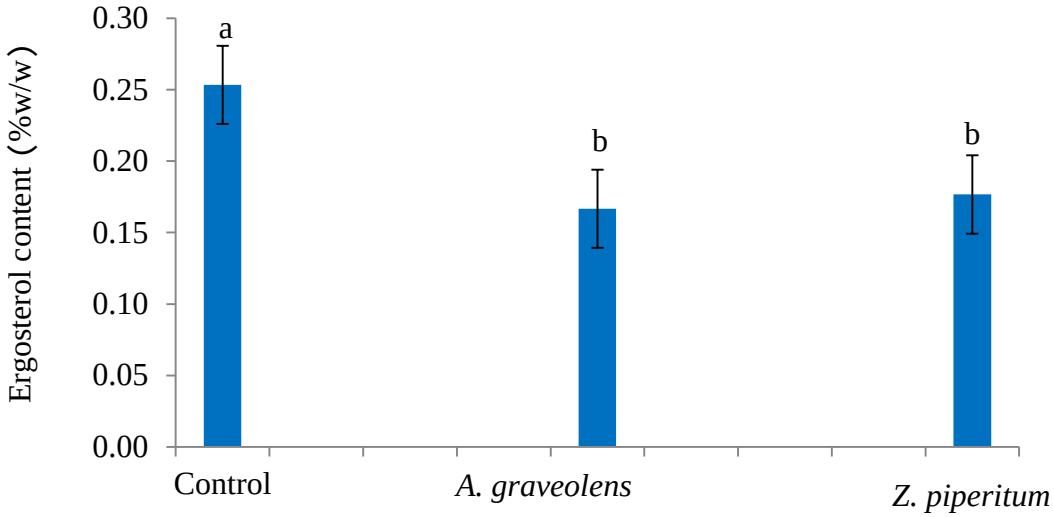

Figure 3.14 Effect of essential oils from *A. graveolens* L. and *Z. piperitum* on ergosterol content in plasma membrane of *A. flavus* in PDB for 4 days. Values are mean (n = 3) ± standard deviations. Values followed by the same letter in each column are not significantly different in ANOVA and Duncan Multiple Range Test ($p < 0.05$).

3.7 Efficacy of essential oils as antifungal coat in dry chili

Dried bird chili, a model product, coated with *A. graveolens* L. and *Z. piperitum* oil were tested for potential to protect against *A. flavus* infection. Pour plate culture at 10^{-5} dilution of infected chili is shown in Figure 3.12, after incubation at 28 ^{o}C for 9 days (2.6.5).

3.7.1 The effect of *A. graveolens* L. and *Z. piperitum* oils on dry chili model

Essential oils from *A. graveolens* L. and *Z. piperitum* were tested for their potential to protect against *A.flavus* infection on dry chili. The results indicated that the dry chili control had more fungal hypha than essential oil coated chili and number of colony decreased when concentration of the essential oil increased. The concentrations of essential oil at 2.0 and 4.0 µL/mL of *A. graveolens* L. oil resulted in reduction of fungal development by 84.00% and 93.32%, respectively (Figure 3.12 B and C) . While *Z. piperitum* oil reduced 45.20 % and 89.32 % (Figure 3.12 D and E) at 4.5 and 9.5 µL/mL respectively, compared with the control (Figure 3.12 A).

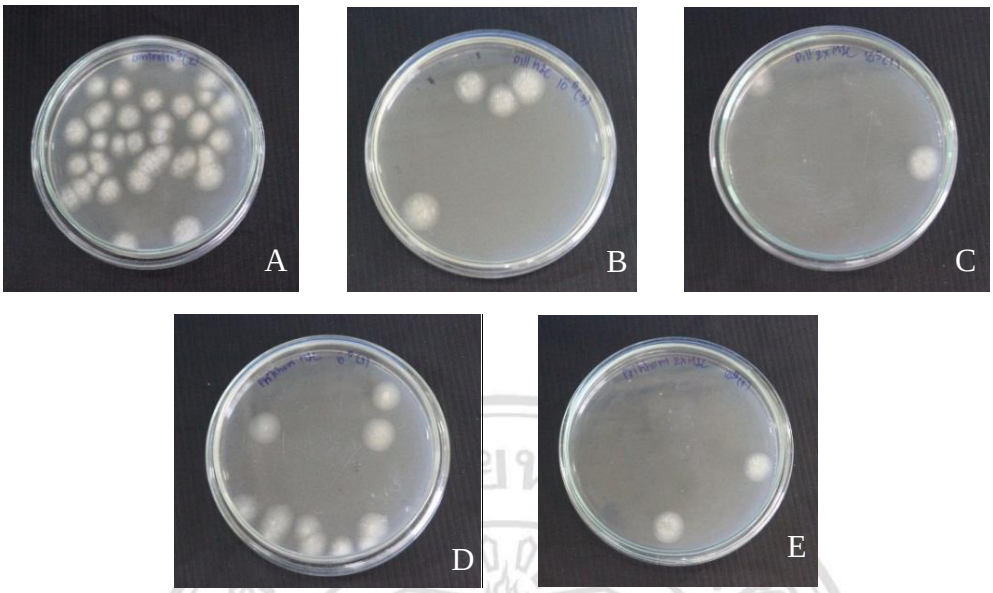

Figure 3.15 Pour plates culture after 9 days of incubation of dried bird chili non-coated (A), coated with 2.0 µL/mL (B), 4.0 µL/mL (C) *A. graveolens* L. oil and with 4.5 µL/mL (D) and 9.0 µL/mL (E) *Z. piperitum* oil.