CHAPTER 4

RESULTS AND DISCUSSION

Preliminary study: Effect of five volatile compounds on inhibition of mycelium growth of four postharvest decay fungi of longan fruit *in vitro*

Among the four longan fruit decay fungi, *L. theobromae, Pestalotiopsis* sp., *Phomopsis* sp. and *Curvularia* sp. *L. theobromae* grew most rapidly *in vitro,* reaching the maximum 90 mm plate diameter within three days, while *Pestalotiopsis* sp., *Phomopsis* sp., and *Curvularia* sp. colonies required 7, 7, and 10 days respectively to reach the same diameter (Appendix Table 1-5, Appendix Figure 1-5). Both *trans*-hexenal and hexanal had antifungal effects against the fungi, with *trans*-2-hexenal effective against all of the fungi at 198 $\mu l \cdot l^{-1}$, while hexanal required 264 $\mu l \cdot l^{-1}$. Hexanal was inhibitory against the most serious postharvest pathogen *L. theobromae* at 198 $\mu l \cdot l^{-1}$. 2-Nonanone, methyl benzoate, and methyl salicylate did not completely inhibit all of the fungi at 264 $\mu l \cdot l^{-1}$, the highest level used. This study was similar to previous reports of the effects of hexanal and *trans*-2-hexenal on postharvest pathogenic fungi (Archbold *et al.,* 1997a; Fan, 2006; Arroyo, 2007; Neri *et al.,* 2006; Song *et al*., 2007; Spotts *et al*., 2007). Hexanal was chosen for subsequent study based on overall antifungal activity as well as because it is commercially available at a reasonable cost in Thailand. Hexanal is used as a food additive, and it has low mammalian toxicity (EAFUS, 2006). The most severe pathogenic microorganism, *L. theobromae*, was chosen to use in the subsequent studies4.1 Effect of hexanal on growth and morphology of mycelium, germination and morphology of spores, and activity of four cell wall degrading enzymes of *L. theobromae*

Experiment 4.1 Effect of hexanal on growth and morphology of mycelium, germination and morphology of spores and activity of four cell wall degrading enzymes of *L. theobromae*

4.1.1 Effect of fumigation time on mycelial growth, spore germination and morphology of *L. theobromae in vitro*

The colony of *L. theobromae* grew very fast on PDA media. The colony covered the 90 mm diameter plate surface within 48 h. The potential of hexanal for controlling *L. theobromae* colony growth depended on hexanal concentration more than fumigation time. Hexanal at 66 µl l^{-1}, fumigating for 1, 2, 24, and 48 h, had almost the same effect in inhibiting the colony growth of *L. theobromae in vitro* (Figure 4.1, Table 4.1). This should be caused by too low hexanal concentration to control the pathogen. Therefore, increasing fumigation time did not increase the inhibitory effect. At a hexanal concentration of 132 µl l^{-1}, the inhibitory effect increased when the fumigation time increased from 1 to 2 h. However, this effect did not happen when the fumigation time increased above 2 h. It is likely that hexanal at this concentration reached its equilibrium point in the headspace at 2 h. Therefore, increasing of fumigation time could not increase the hexanal concentration in the head space. Hexanal at concentrations of 198, 300, 600 and 900 µl l^{-1} completely inhibited *L. theobromae* colony growth. After the *L. theobromae* colonies that had been fumigated with 198, 300, 600 and 900 µl l^{-1} were transferred to culture on new PDA media in hexanal-free environments, none of the colonies grew, indicating that hexanal at these concentrations had fungicidal effects on *L. theobromae* (Table 4.1).

The effect of hexanal on *L. theobromae* spore germination was also tested. It was found that *L. theobromae* spores could not germinate after hexanal fumigation at 198, 300, 600 and 900 µl l^{-1} for 1, 2, 24 and 48 h, indicating that hexanal at these concentrations had fungicidal effects on *L. theobromae* spores (Table 4.2). However, hexanal at 132 µl l^{-1} fumigation showed only fungistatic effects on spore germination, delaying it to 36 h (24 h for fumigation plus 12 h for incubation in the hexanal-free environment). For hexanal at 66 µl l^{-1}, germinated spores could be observed at 30 h (24 h for fumigation with 6 h for incubation in the hexanal-free environment), 6 h earlier than at 132 µl l^{-1}. At 52 h (48 h for fumigation and 6 h for incubation in the

hexanal-free environment) or longer, the spores germinated and mycelia grew very fast until it was not possible to determine germination under a light microscope.

For microscopy studies, *L. theobromae* spores which were not fumigated germinated normally on PDA media under a hexanal-free atmosphere were shown in Figure 4.2A. For hexanal-fumigated spores, the spores could not germinate at 198, 300, 600 and 900 µl l^{-1} for all fumigation periods (Figure 4.2C-E). Under low hexanal concentrations (66 and 132 µl l^{-1}), some spores germinated at 30 and 46 h. However, they showed some abnormal mycelial characteristics such as excessive branching (Figure 4.2F-I), swelling (Figure 4.2J-K), and excessive vacuole formation inside the cells (Figure 4.2I).

The fungicidal or fungistatic effects of hexanal in this study might be depended on its solubility and capacity to interact with the cytoplasmic membrane (Knobloh *et al*., 1988). Kubo *et al.* (2003) suggested that *trans*-2-hexenal caused a disruption and disorganization of the plasma membrane and cell components with a cell lysis. The severe damage caused by *trans*-2-hexenal on *Botrytis cinerea* membranes and cell walls may have led to the observations of morphological deformation, cell collapse, and deterioration of the conidia (Fallik *et al*., 1998). The morphological deformations of *L. theobromae* mycelia, excessive branching, swelling of the cell wall, and an increase of large vacuoles were also found after hexanal fumigation of *Collectrichum acutatum* (Arroyo *et al.,* 2005) and *B. cinerea* (Arras *et al*., 1995). However, the site and primary mechanism of action of hexanal was not clear from this and prior studies as these responses could be secondary effect.

Many researchers had reported that fungal spores were more sensitive to volatile compound than mycelium. (Fallik *et al.*, 1998; Neri, 2007) but in this study the spore and mycelium of *L. theobromae* tended to have the same sensitivity with hexanal.

4.1.2 Effect of hexanal on mycelial morphology of *L. theobromae* on PDA

The normal growth characters of *L. theobromae* mycelia were a long tube with branched which it looked like a big branch from the trunk of a tree (Figure 4.3A-C). After fumigating of the mycelia with hexanal for 24 h, abnormal mycelia were found. This included development of many vacuoles inside the cells (Figure

4.3D-E) and breakage of cell walls (Figure 4.3G-H). It was also observed that the degree of hexanal toleration by *L. theobromae* mycelia depended on its age. Abnormal characteristics of the 3 and 7 days old mycelia were observed when fumigated with hexanal at 954 and 1,434 µl l^{-1}, respectively. For 14 days old mycelia, an abnormal characteristic was observed when fumigated at even the lowest concentrations (Table 4.3). This might be caused aging mycelia had thinner cell walls, or the ability to metabolize hexanal was reduced by the aging process. On another hand, hexanal might be metabolized to some toxic substances in aging mycelia at higher rates than the young mycelia.

4.1.3 Effect of hexanal on extracellular enzyme activities of *L. theobromae* on PDA

Hexanal fumigation did not affect the activities of polygalacturonase, pectin methyl esterase and cutinase of *L. theobromae* mycelium (Table 4.4). In this study, cellulase was the only enzyme of *L. theobromae* mycelium where activity was dramatically reduced by hexanal fumigation. Cellulase activities were reduced about 7-fold (from 11,046 to 1,617 µm $h^{-1}mg^{-1}$) after hexanal at 447 µl l^{-1} and about 10-fold after hexanal at 954 and 1,431 µl l^{-1}. For cell wall degrading enzymes activity, cellulase was the only enzyme with activity reduced by hexanal fumigation. This might be caused by the affinity between hexanal and cellulase.

The reduction in cellulase activity in the extracellular wash of *L. theobromae* caused by hexanal could be due to effects on protein production and/or secretion, or by direct interaction with proteins that could impair their function. Myung *et al*. (2007) reported that *Botrytis cinerea* exposed to *trans*-2-hexenal vapors exhibited changes in protein expression patterns and that *trans*-2-hexenal was recovered in the extracellular protein. Although it is not clear that a reduction in cellulase activity would reduce growth of *L. theobromae in vitro* or pathogenicity on longan fruit, this apparent specificity for cellulase may provide an opportunity to more clearly define the mode of action of hexanal.

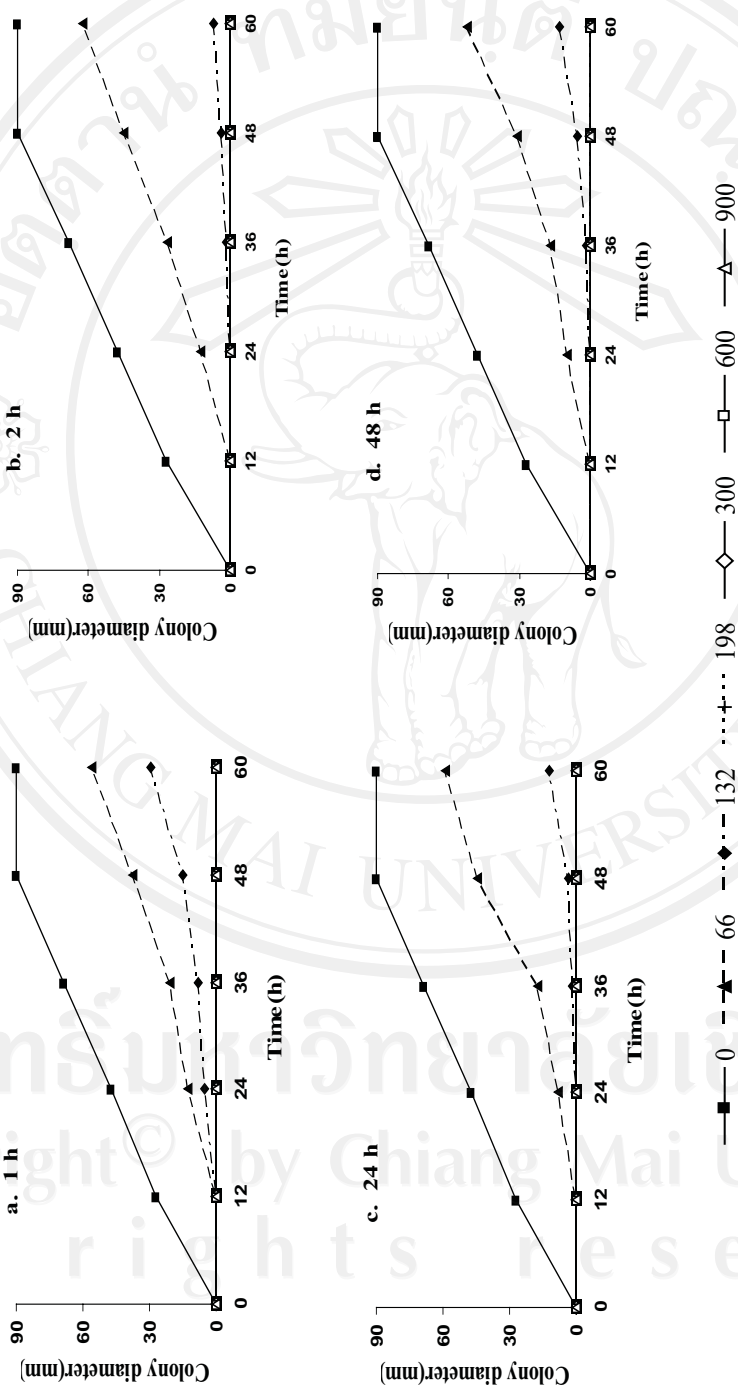

Figure 4.1 Colony diameter (mm) of *L. theobromae* fumigated with seven hexanal concentrations (µl l^{-1}) for 1, 2, 24 and 48 h *in vitro*.

Table 4.1 The percentage of mycelial inhibition versus controls of *L. thobromae* fumigated with hexanal at six concentrations for 1, 2, 24 and 48 h *in vitro*.

Hexanal conc.	Fumigation time(h)				
µl l^{-1}	1	2	24	48	mean
0	0 e	0 e	0 e	0 e	0 D
66	59 c	43 d	67 bc	66 bc	59 C
132	78 b	94 a	100 a	92 a	91 B
198	100 a	100 a	100 a	100 a	100 A
300	100 a	100 a	100 a	100 a	100 A
600	100 a	100 a	100 a	100 a	100 A
900	100 a	100 a	100 a	100 a	100 A
mean	77 A	77 A	81 A	80 A	79
%CV			9.41		

*= fungistatic or ** = fungicidal

Means with the same letter in columns did not significantly differ by LSD at P=0.05.

Table 4.2 Percent inhibition of spore germination versus controls at 6 and 12 h for *L. theobromae* fumigated with hexanal at 66, 132, 198, 300, 600 and 900 µl l^{-1} for 1, 2, 24 and 48 h *in vitro.*

Hexanal concentration µl l^{-1}	Fumigation time (h)							
	1		2		24		48	
	6 h	12 h	6 h	12 h	6 h	12 h	6 h	12 h
66	100*	100*	100*	100*	22	16	uc	uc
132	100*	100*	100*	100**	100*	81	94	88
198	100**	100**	100**	100**	100**	100**	100**	100**
300	100**	100**	100**	100**	100**	100**	100**	100**
600	100**	100**	100**	100**	100**	100**	100**	100**
900	100**	100**	100**	100**	100**	100**	100**	100**

*= fungistatic or ** = fungicidal.

uc = uncountable

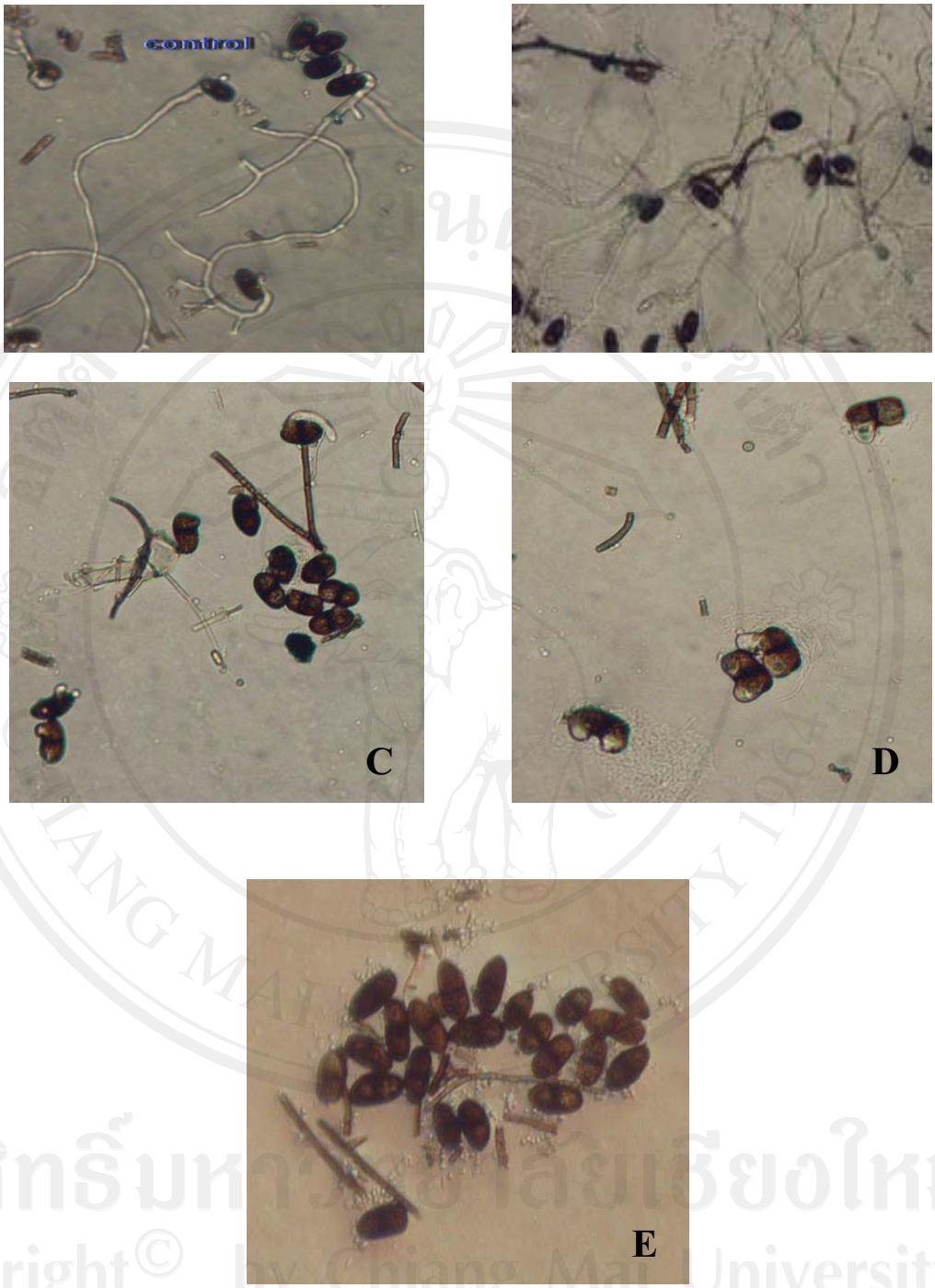

Figure 4.2 Effect of hexanal on spore germination and morphology of *L. theobromae* after hexanal fumigation for 24 h at 25° C (Magnification x400).

A: spore germination of control at 6 h. B: spore germination of control at 12 h.
C – E: ungerminated spore F – I: branch mycelium J – K: swollen mycelium

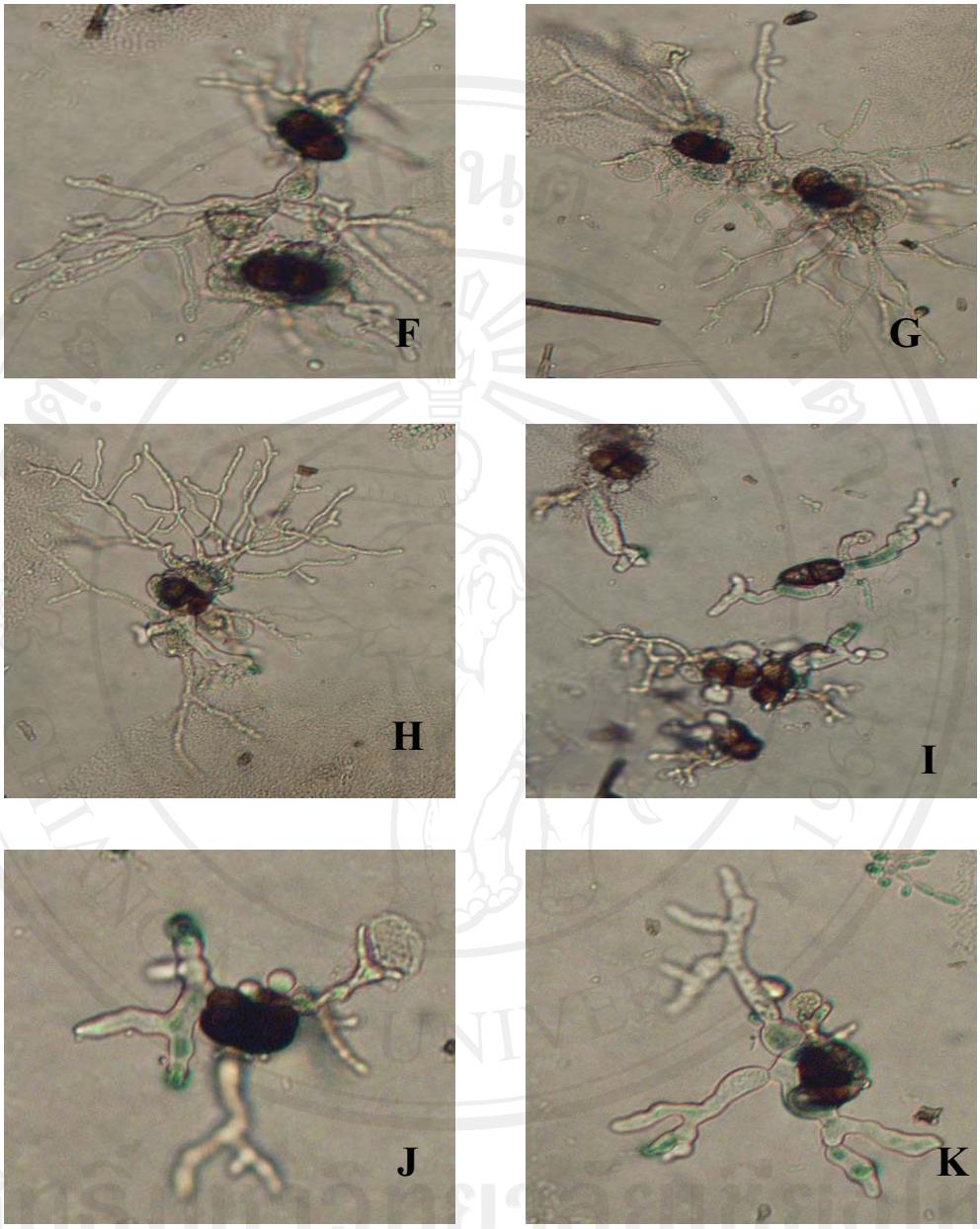

Figure 4.2(continued) Effect of hexanal on spore germination and morphology of *L.theobromae* after hexanal fumigation for 24 h at 25^{o} C (Magnification x400).

A: spore germination of control at 6 h. B: spore germination of control at 12 h.

C – E: ungerminated spore F – I: branch mycelium J – K: swollen mycelium

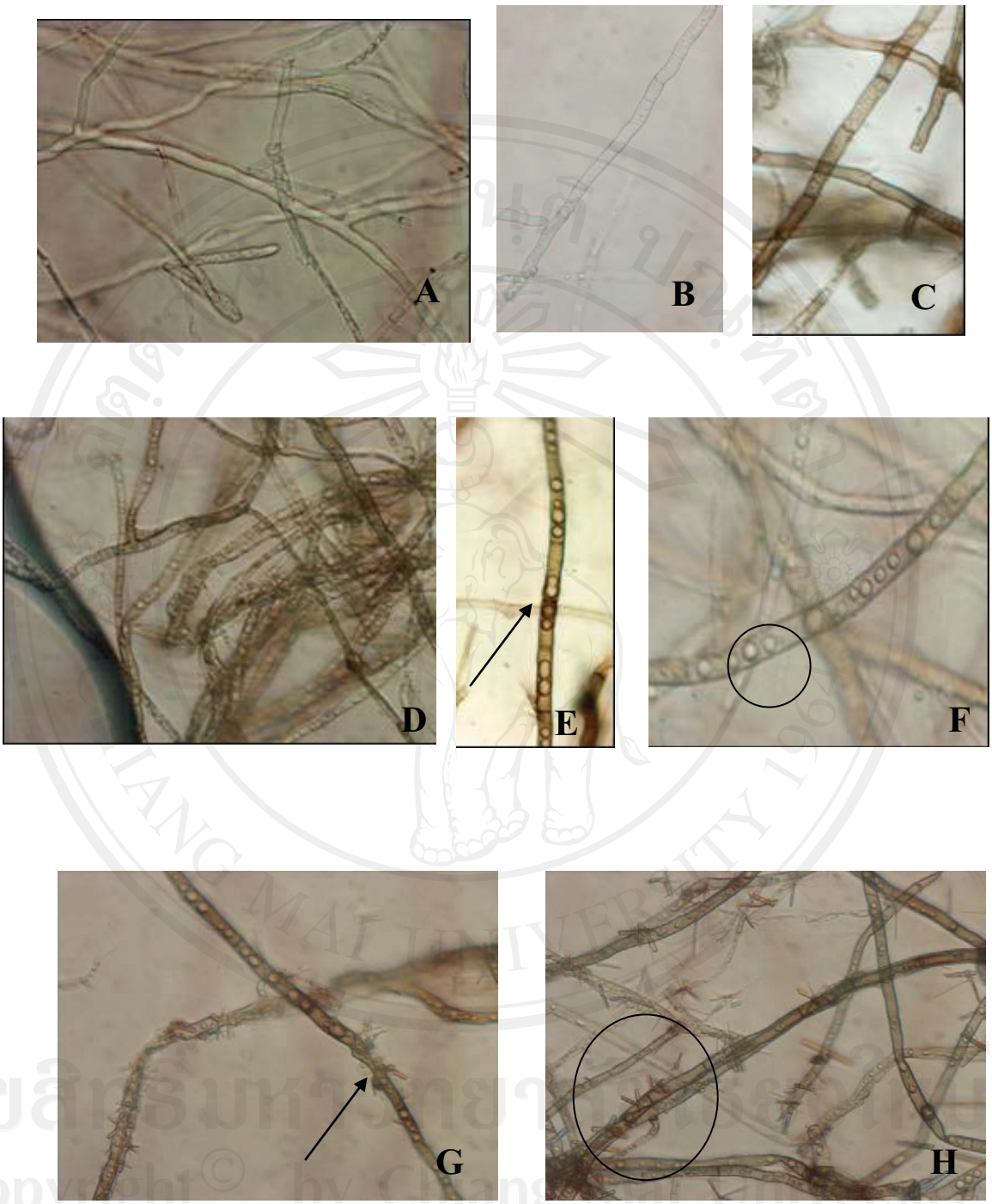

Figure 4.3 Effect of hexanal on mycelial morphology of *L. theobromae* aged 3, 7 and 14 days old after fumigation with hexanal at 478, 956 and 1,431 µl l^{-1} for 24 h at 25°C *in vitro* (Magnification x100).

A – C: normal mycelium D – F: vacuolization G –H: broken mycelium

Table 4.3 Morphological change of *L. theobromae* mycelia aged 3, 7 and 14 days old after fumigation with hexanal at 478, 956 and 1,431 µl l^{-1} for 24 h at 25°C *in vitro*.

Mycelium age (days)	Morphological change after hexanal fumigation		
	447 µl l^{-1}	956 µl l^{-1}	1,431 µl l^{-1}
3	-	+	+
7	-	+	+
14	+	+	+

- = no morphological change

+ = morphological change:

Table 4.4 Effect of hexanal on activities of four cell wall degrading enzymes (mean ± se) produced by *L. theobromae* mycelia aged 14 days after hexanal fumigation at four concentrations at 25° C for 24 h *in vitro.*

Hexanal Concentration µl l^{-1}	Cellulase (µ mol h^{-1} mg^{-1}protein)	Polygalacturonase (A575 $min^{-1}mg^{-1}$ protein)	Pectin methyl esterase (A620 $min^{-1}mg^{-1}$ protein)	Cutinase (A405 $min^{-1}mg^{-1}$ protein)
0	11,046±493 a	0.1590±0.008 a	-23.29±1.142 a	0.3534±0.082 a
477	1,617±556 b	0.1290±0.072 a	-37.06±2.625 a	0.3788±0.066 a
954	1,069±704 b	0.1598±0.027 a	-33.24±3.019a	0.4375±0.053 a
1,431	1,079±522 b	0.0810±0.035 a	-31.39±1.486 a	0.3596±0.061 a

Means with the same letter in columns did not significantly differ by LSD at P=0.05.

Experiment 4.2 Effect of hexanal on postharvest decay of longan fruit

4.2.1 Influence of hexanal concentration and fumigation time on longan fruit decay

The effect of hexanal concentration and period of fumigation for control of postharvest decay of longan fruit were determined by using 0, 300, 600 or 900 μl l^{-1} hexanal for periods of 0, 1, 2, 3, 4 or 6 h. Hexanal fumigation at all concentrations reduced fruit decay of *L. theobromae*-inoculated fruit if held at ambient temperature (Tables 4.5, 4.6) or at 5 °C for 30 d (Tables 4.8, 4.9). However, the percentage of fruit decay was over 30% by 2-3 days after fumigation for all fumigation periods and most hexanal concentrations (Tables 4.5-4.16). These results agreed with Rasrinaul (1996) who reported that acetaldehyde fumigation of longan fruit at 0.125% for 8 h was effective for control of longan postharvest decay. Its effectiveness depended on concentration, fumigation time and cultivar. It should be noted that the higher concentrations and longer fumigation periods were less fungistatic or fungicidal.

To assess the severity of fungal development, fungal incidence was scored by the percentage of fruit surface covered with the *L. theobromae* mycelia. At the ambient storage temperature, hexanal at all concentrations reduced the severity of fruit decay. Hexanal at 900 μl l^{-1} for a 2 h fumigation period was determined to be the most effective treatment (Tables 4.5-4.16). Therefore, this treatment combination was selected for use in studying the effect of fumigation temperature

4.2.2 Influence of hexanal fumigation temperature on longan fruit decay

For determining the proper fumigation temperature, *L. theobromae*-inoculated longan fruit were fumigated with 900 μl l^{-1} for 2 h at 5°C, 40°C and ambient temperature. The results revealed that fumigation temperature at 40°C could not be used to control *L. theobromae* fruit decay. The fruit decay percentage was over the maximum acceptable fruit decay (30%) within 5 days of storage (Figure. 4.5-4.10). For 5°C and ambient temperature fumigation, fruit decay was not significantly different; both temperatures held the fruit decay percentage under the maximum acceptable for 10 days. Fruit decay exceeded the maximum by the 15th day of storage.

For fruit infection severity scores, the most infection severity was found with the 40°C fumigation temperature. The severities at 5°C and ambient temperatures did not show significant differences. From these results, it could be concluded that hexanal at 900 µl l^{-1} for 2 h at ambient temperature was the most suitable for fumigating longan and controlling *L. theobromae* fruit decay

4.2.3 Hexanal metabolism by longan fruit

The analysis of hexanal and longan samples by GC-MS was showed that the hexanal resulted in the presence of several compounds. There were 13 aroma volatile compounds produced by longan fruit. After the longan fruit were fumigated with hexanal, there were 19 compounds found in the headspace of the samples (Table 4.17).

Table 4.5 Fruit decay (%) of *L. theobromae*-inoculated longan fruit stored at ambient temperature after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage		
	1	2	5
T1V0	50 b-d	85 a-c	100 a
T1V300	10 fg	25 gh	45 cd
T1V600	10 fg	55 d-f	65 bc
T1V900	5 fg	27 f-h	80 ab
T2V0	50 b-d	95 ab	100 a
T2V300	25 d-g	30 f-h	45 cd
T2V600	0 g	20 gh	45 cd
T2V900	0 g	5 h	15 e
T3V 0	40 c-e	80 a-d	100 a
T3V300	0 g	20 gh	60 b-d
T3V600	15 e-g	40 e-g	50 b-d
T3V900	5 fg	35 e-g	35 de
T4V0	70 b	100 a	100 a
T4V300	25 d-g	40 e-g	60 b-d
T4V600	40 c-e	70 b-d	75 ab
T4V900	67 bc	60 c-e	80 ab
T6V0	80 ab	95 ab	100 a
T6V300	30 d-f	40 e-g	65 bc
T6V600	65 bc	70 b-d	80 ab
T6V900	100 a	95 ab	98 a
%CV	58.84	37.67	28.86
Conc.	**	**	**
Time	**	**	**
Conc.x Time	**	**	**

[z]T = Fumigation time, V= Volume of hexanal

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.6 Fungal incidence score of *L. theobromae*-inoculated longan fruit stored at ambient temperature after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage		
	1[n]	2	5
T1V0		3.2 ab	3.6 a
T1V300		0.7 fg	1.0 c-f
T1V600		1.8 c-f	1.8 bc
T1V900		0.4 g	1.9 bc
T2V0		3.3 ab	3.9 a
T2V300		0.6 g	4.0 a
T2V600		0.4 g	0.7 d-f
T2V900		0.1 g	0.2 f
T3V 0		2.8 a-c	3.9 a
T3V300		0.4 g	0.1 c-e
T3V600		0.8 fg	1.0 c-f
T3V900		1.0 e-g	0.6 ef
T4V0		3.6 a	4.0 a
T4V300		1.2 d-g	1.4 b-e
T4V600		2.1 d-e	1.9 bc
T4V900		2.6 abc	1.0 c-f
T6V0		3.3 ab	3.9 a
T6V300		1.0 e-g	1.5 b-d
T6V600		2.3 b-d	2.0 b
T6V900		3.3 ab	3.3 a
%CV		31.74	20.11
Conc.		**	**
Time		**	**
Conc.x Time		*	**

[z]T = Fumigation time, V= Volume of hexanal [n]= no data observation

Scores are: 0 = no visual evidence of fungi, 1 = less than 10%, 2 = 10-30%, 3 = 31-70%, and 4 = more than 70% of the surface affected with fungus.

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.7 Phytotoxicity scores of *L. theobromae*-inoculated longan fruit stored at ambient temperature after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage		
	1[n]	2	5
T1V0		0	0 e
T1V300		0.2	0.2 e
T1V600		1.3	1.1 d
T1V900		1.6	1.9 b-d
T2V0		0	0 e
T2V300		2	4.0 a
T2V600		1.7	2.2 bc
T2V900		0.9	1.6 cd
T3V 0		0	0 e
T3V300		0.8	3.0 bc
T3V600		0.8	1.5 cd
T3V900		1.4	2.2 bc
T4V0		0	0 e
T4V300		1.3	2.1 bc
T4V600		1.6	2.5 b
T4V900		1	2.5 b
T6V0		0	0 e
T6V300		1.6	1.6 cd
T6V600		2.4	2.5 b
T6V900		1.5	1.5 cd
%CV		40.96	23.83
Conc.		**	**
Time		ns	**
Conc.x Time		ns	**

[z]T = Fumigation time, V= Volume of hexanal [n] = no data observation

Scores are: 0 = no deterioration, 1 = slight deterioration, 2 = moderate deterioration, 3 = severe deterioration and 4 = tissue completely collapsed. Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.8 Fruit decay (%) of *L. theobromae* inoculated longan fruit stored at 5°C after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage					
	5	10	15	20	25	30
T1V0	0 d	0 e	0 b	65 ab	70 ab	100 a
T1V300	0 d	0 e	0 b	10 ef	15 cd	60a-c
T1V600	0 d	0 e	0 b	30 c-f	30 b-d	55a-c
T1V900	0 d	0 e	0 b	27 c-f	20 b-d	30 c
T2V0	0 d	0 e	5 b	60 a-c	60 ab	75 a-c
T2V300	5 cd	0 e	5 b	10 ef	40 b-d	50 bc
T2V600	0 d	0 e	0 b	10 ef	27 b-d	45 bc
T2V900	0 d	5 de	5 b	10 ef	15 cd	35 bc
T3V 0	0 d	0 e	0 b	55 b-d	65 ab	75 a-c
T3V300	0 d	0 e	0 b	25 d-f	35 b-d	60a-c
T3V600	0 d	0 e	5 b	20 ef	20 b-d	55a-c
T3V900	0 d	20 cd	20 b	30 c-f	15 cd	50 bc
T4V0	5 cd	0 e	5 b	4 f	4 d	100 a
T4V300	0 d	5 de	5 b	3 f	3 d	50 bc
T4V600	20 bc	30 c	20 b	3 f	0 d	50 bc
T4V900	35 b	73 a	50 a	4 f	4 d	65 a-c
T6V0	0 d	0 e	10 b	90 a	95 a	100 a
T6V300	10 cd	10 de	15 b	40 b-e	50 bc	75 a-c
T6V600	80 a	55 b	50 a	40 b-e	60 ab	80 ab
T6V900	80 a	70 ab	60 a	55 b-d	65 ab	80 ab
%CV	110.40	86.61	112.37	75.61	83.88	51.73
Conc.	**	**	**	**	**	**
Time	**	**	**	**	**	**
Conc.x Time	**	**	**	ns	ns	ns

[z]T = Fumigation time, V= Volume of hexanal

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05 (*), 0.01 (**),* or not significant (ns).

Table 4.9 Fungal incidence score of *L. theobromae* inoculated longan fruit stored at 5°C after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage					
	5	10	15	20	25	30
T1V0	0 c	0 b	0.1 cd	1	1.4	1.7
T1V300	0 c	0 b	0.1 cd	0.2	0.3	0.4
T1V600	0 c	0 b	0.1 cd	0.5	0.5	0.3
T1V900	0 c	0 b	0 d	0.4	0.4	0.4
T2V0	0 c	0 b	0.1 cd	0.7	0.8	1.5
T2V300	0.1 c	0 b	0.1 cd	0.2	0.6	0.1
T2V600	0 c	0 b	0.1 cd	0.2	0.4	0.1
T2V900	0 c	0 b	0.1 cd	0.1	0.2	0.1
T3V 0	0 c	0 b	0.1 cd	0.7	0.9	1.3
T3V300	0 c	0 b	0.1 cd	0.3	0.6	0.5
T3V600	0 c	0 b	0.2 cd	0.4	0.4	0.4
T3V900	0 c	0.3 b	0.3 c	0.6	0.4	0.9
T4V0	0.1 c	0 b	0.1 cd	1	1.7	1.7
T4V300	0 c	0.1 b	0.1 cd	1.1	0.9	0.1
T4V600	0.6 c	0.6 b	0.3 c	0.2	0.7	0.3
T4V900	1.6 b	1.7 a	1.0 b	1.9	1.6	0.9
T6V0	0 c	0 b	0.2 cd	1.1	1.6	1.8
T6V300	0.2c	0.2 b	0.2 cd	0.6	0.8	0.5
T6V600	2.8 a	1.6 a	1.2 ab	0.9	1.3	1.3
T6V900	2.2 ab	2.0 a	1.6 a	1.7	1.8	1.4
%CV	127.50	147.47	154.02	72.43	74.98	72.44
Conc.	**	**	**	**	**	**
Time	**	**	**	**	**	**
Conc.x Time	**	**	**	**	ns	ns

[z]T = Fumigation time, V= Volume of hexanal

Scores are: 0 = no visual evidence of fungi, 1 = less than 10%, 2 = 10-30%, 3 = 31-70%, and 4 = more than 70% of the surface affected with fungus.

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.10 Phytotoxicity scores of *L. theobromae*-inoculated longan fruit stored at 5°C after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage					
	5	10	15[n]	20	25	30
T1V0	0 h	0 g		0	0 f	0 d
T1V300	1 f-h	1.9 c-f		2.9	2 b-e	0.7 cd
T1V600	0.4 gh	1.2 fg		2.4	1.9 c-e	1.6 bc
T1V900	0.2 h	0.2 g		2.7	1.7 de	1.2c
T2V0	0 h	0 g		0	0 f	0 d
T2V300	1.8 c-f	2.9 a-c		2.4	1.6 e	0.7 cd
T2V600	1.7 d-f	2.3 b-f		2.3	2 b-e	1.1 cd
T2V900	0.4 gh	1.5 ef		2.1	0.6 f	0.5 cd
T3V 0	0 h	0 g		0	0 f	0 d
T3V300	3.3 a	2.7 a-d		2.4	2.5 a-d	2.3 ab
T3V600	2.9 a-c	2.2 b-f		2.2	1.7 de	0.8 cd
T3V900	2.7 a-d	2.7 a-d		3.2	2.4 b-e	3 a
T4V0	0 h	0 g		0	0 f	0 d
T4V300	2.6 a-d	2.9 a-c		3	2.3 b-e	1.1 cd
T4V600	2.0 b-f	2.9 a-c		2.5	2.8 a-c	1.2c
T4V900	1.4 e-g	3.9 a		3.1	3.5 a	1.8 a-c
T6V0	0 h	0 g		0	0 f	0 d
T6V300	3.0 ab	3.3 ab		2.9	2.9 ab	1.8 bc
T6V600	2.5 a-e	2.8 a-c		2.3	2.7 a-c	1.2c
T6V900	1.9 b-f	1.6 d-f		2.1	2.2 b-e	1.3 bc
%CV	56.7	49.8		28.93	39.89	77.21
Conc.	**	**		**	**	*
Time	**	**		ns	**	**
Conc.x Time	**	**		ns	**	*

[z]T = Fumigation time, V= Volume of hexanal [n] = no data observation

Scores are: 0 = no deterioration, 1 = slight deterioration, 2 = moderate deterioration, 3 = severe deterioration and 4 = tissue completely collapsed.

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.11 Fruit decay (%) of non-inoculated longan fruit stored at ambient temperature after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage				
	1	2	5	8	14
T1V0	0	0	0	0	15 bc
T1V300	0	0	0	0	0 c
T1V600	0	0	0	0	0 c
T1V900	0	0	0	0	5 bc
T2V0	0	0	0	0	5 bc
T2V300	0	0	0	0	10 bc
T2V600	0	0	0	0	15 bc
T2V900	0	0	0	5	40 a
T3V 0	0	0	0	0	15 bc
T3V300	0	0	0	0	5 bc
T3V600	0	0	0	0	0 c
T3V900	0	0	0	0	0 c
T4V0	0	0	0	0	5 bc
T4V300	0	0	0	0	25 ab
T4V600	0	0	0	0	10 bc
T4V900	0	0	0	0	10 bc
T6V0	0	0	0	0	10 bc
T6V300	0	0	0	0	0 c
T6V600	0	0	0	0	0 c
T6V900	0	0	0	0	0 c
%CV	-	-	-	894.43	171.83
Conc.	-	-	-	ns	*
Time	-	-	-	ns	ns
Conc.x Time	-	-	-	ns	*

[z]T = Fumigation time, V= Volume of hexanal

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.12 Fungal incidence scores of non-inoculated longan fruit stored at ambient temperature after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage				
	1	2	5	8	14
T1V0	0	0	0	0	0.2 bc
T1V300	0	0	0	0	0 c
T1V600	0	0	0	0	0 c
T1V900	0	0	0	0	0 c
T2V0	0	0	0	0	0.1 c
T2V300	0	0	0	0	0.2 bc
T2V600	0	0	0	0	0.2 bc
T2V900	0	0	0	0	0.9 a
T3V 0	0	0	0	0	0.2 bc
T3V300	0	0	0	0	0 c
T3V600	0	0	0	0	0 c
T3V900	0	0	0	0	0 c
T4V0	0	0	0	0	0.1 bc
T4V300	0	0	0	0	0.5 ab
T4V600	0	0	0	0	0.2 bc
T4V900	0	0	0	0	0.2 bc
T6V0	0	0	0	0	0.2 bc
T6V300	0	0	0	0	0 c
T6V600	0	0	0	0	0 c
T6V900	0	0	0	0	0 c
%CV	-	-	-	2.23	28.12
Conc.	-	-	-	ns	*
Time	-	-	-	ns	ns
Conc.x Time	-	-	-	ns	*

[z]T = Fumigation time, V= Volume of hexanal

Scores are: 0 = no visual evidence of fungi, 1 = less than 10%, 2 = 10-30%, 3 = 31-70%, and 4 = more than 70% of the surface affected with fungus.

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.13 Phytotoxicity scores of non-inoculated longan fruit stored at ambient temperature after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage				
	1	2	5	8	14
T1V0	0 f	0 e	0 e	0 g	0
T1V300	0.1 f	0.9 c-e	2.1 a-c	1.5 d-f	0.6
T1V600	0.9 e	1.4 b-d	2.1 a-c	2.3 a-d	2.5
T1V900	1.2 e	0.9 c-e	1.8 a-c	2.2 a-d	2.5
T2V0	0 f	0 e	0 e	0 g	0
T2V300	1.4 de	0.2 e	1.6 bc	1.5 d-f	1.3
T2V600	1.5 de	1.5 a-c	2.0 a-c	2.1 a-d	2.8
T2V900	2.4 ab	1.5 a-c	1.8 a-c	1.6 c-f	2.2
T3V 0	0 f	0 e	0 e	0 g	0
T3V300	2.8 a	2.2 ab	2.6 ab	2.4 a-d	2.6
T3V600	1.6 c-e	2.4 a	2.0 a-c	0.7 fg	1.8
T3V900	2.5 ab	1.6 a-c	2.8 a	1.7 c-f	2.7
T4V0	0 f	0 e	0 e	0 g	0
T4V300	2.0 b-d	0.5 de	0.5 de	0.9 e-g	2
T4V600	2.9 a	2.1 ab	1.6 bc	2.0 b-e	2.7
T4V900	2.8 a	0.5 de	2.0 a-c	2.3 a-d	3.3
T6V0	0 f	0 e	0 e	0 g	0
T6V300	2.3 a-c	1.8 a-c	2.6 a	2.9 ab	2.9
T6V600	2.6 ab	1.6 a-c	1.1 cd	2.8 a-c	2.7
T6V900	4.0 a	2.2 ab	2.3 ab	3.2 a	2.8
%CV	20.99	31.74	30.28	32.79	31.23
Conc.	**	**	*	**	**
Time	**	**	**	**	ns
Conc.x Time	**	**	*	**	ns

[z]T = Fumigation time, V= Volume of hexanal

Scores are : 0 = no deterioration, 1 = slight deterioration, 2 = moderate deterioration, 3 = severe deterioration and 4 = tissue completely collapsed.

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05 (*), 0.01 (**),* or not significant (ns).

Table 4.14 Fruit decay (%) of non-inoculated longan fruit stored at 5°C after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage					
	5	10	15	20	25	30
T1V0	0	0	0	0	15 bc	25
	0	0	Days of storage	0	0 c	0
T1V600	0	0	0	0	0 c	5
T1V900	0	0	0	0	0 c	5
T2V0	0	0	0	0	30 b	5
T2V300	0	0	0	0	0 c	10
T2V600	0	0	0	0	0 c	0
T2V900	0	0	0	0	0 c	0
T3V 0	0	0	0	0	0 c	25
T3V300	0	0	0	0	0 c	10
T3V600	0	0	0	0	100 a	0
T3V900	0	0	0	0	0 c	0
T4V0	0	0	0	0	5 c	5
T4V300	0	0	0	0	0 c	0
T4V600	0	0	0	0	0 c	0
T4V900	0	0	20	0	0 c	0
T6V0	0	0	0	10	15 bc	20
T6V300	0	0	0	0	0 c	5
T6V600	0	0	0	0	0 c	10
T6V900	0	0	0	5	0 c	0
%CV	-	-	894	455	163.37	234.61
Conc.	-	-	ns	ns	**	**
Time	-	-	ns	**	**	ns
Conc.x Time	-	-	ns	ns	**	ns

[z]T = Fumigation time, V= Volume of hexanal

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.15 Fungal incidence scores of non-inoculated longan fruit stored at 5°C after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage					
	5	10	15	20	25	30
T1V0	0	0	0	0	0.2 bc	0.3
T1V300	0	0	0	0	0 c	0
T1V600	0	0	0	0	0 c	0
T1V900	0	0	0	0	0 c	0
T2V0	0	0	0	0	0.3 b	0
T2V300	0	0	0	0	0 c	0
T2V600	0	0	0	0	0 c	0
T2V900	0	0	0	0	0 c	0
T3V 0	0	0	0	0	0 c	0
T3V300	0	0	0	0	0 c	0
T3V600	0	0	0	0	4.0 a	0
T3V900	0	0	0	0	0 c	0
T4V0	0	0	0	0	0.1 c	0
T4V300	0	0	0	0	0 c	0
T4V600	0	0	0	0	0 c	0
T4V900	0	0	0	0	0 c	0
T6V0	0	0	0	0.1	0.2 bc	0.3
T6V300	0	0	0	0	0 c	0.1
T6V600	0	0	0	0	0 c	0
T6V900	0	0	0	0.2	0 c	0
%CV	-	-	-	13.29	10.94	10.63
Conc.	-	-	-	ns	**	**
Time	-	-	-	ns	**	ns
Conc.*Time	-	-	-	ns	**	ns

[z]T = Fumigation time, V= Volume of hexanal

Scores are: 0 = no visual evidence of fungi, 1 = less than 10%, 2 = 10-30%, 3 = 31-70%, and 4 = more than 70% of the surface affected with fungus.

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

Table 4.16 Phytotoxicity scores of non-inoculated longan fruit stored at 5°C after hexanal fumigation at 0, 300, 600 and 900 µl l^{-1} for 1, 2, 3, 4 and 6 h at ambient temperature.

Treatment[z]	Days of storage					
	5	10	15	20	25	30
T1V0	0	o g	0 c	0	0 f	0
T1V300	1.6	1.8 c-e	2.8 ab	1.6	2.9 a	2.5
T1V600	1.6	1.2 ef	2.0 b	2	2.7 ab	1.7
T1V900	2.4	1.8 c-e	3.5 a	0.8	1.8 b-d	1.2
T2V0	0	o g	0 c	0	0 f	0
T2V300	2	0.4 fg	2.0 c	1.6	0.5 ef	0.1
T2V600	2.5	1.4 d-f	2.4 ab	1.7	1.3 c-e	0.3
T2V900	2.7	3.2 a	3.1 a	1.8	1.9 b-d	1.5
T3V 0	0	o g	0 c	0	0 f	0
T3V300	2.8 -	1.9 b-e	3.2 a	1.3	2 a-c	1.2
T3V600	2.7	2.1 a-e	3.3 a	1.4	1.7 b-d	0.9
T3V900	3.1	2.3 a-d	3.3 a	1.6	1.6 c-e	1
T4V0	0	o g	0 c	0	0 f	0
T4V300	3.2	2.2 a-e	2.9 ab	1.8	1.6 c-e	1.5
T4V600	2.9	2.8 a-c	3.1 a	1.4	1.7 b-d	0.8
T4V900	3.6	3.2 a	3.5 a	2.4	2.9 a	2.2
T6V0	0	o g	0 c	0	0 f	0
T6V300	2.8	o g	3.3 a	2.6	2.7 ab	2.2
T6V600	3.8	2.7 a-c	3.2 a	2.8	2.7 ab	1.8
T6V900	3.1	4.0 ab	3.5 a	2.3	2.9 a	1.8
%CV	20.65	32.02	21.22	30.65	25.55	38.68
Conc.	**	**	**	**	**	**
Time	**	**	**	**	**	**
Conc.x Time	ns	*	*	ns	**	ns

[z]T = Fumigation time, V= Volume of hexanal

Scores are: 0 = no deterioration, 1 = slight deterioration, 2 = moderate deterioration, 3 = severe deterioration and 4 = tissue completely collapsed.

Means followed by different letters within columns are significantly different by Fisher's Least Significant Difference (*P*=0.05). For main effects, F values are significant at *P=0.05* (*), *0.01* (**), or not significant (ns).

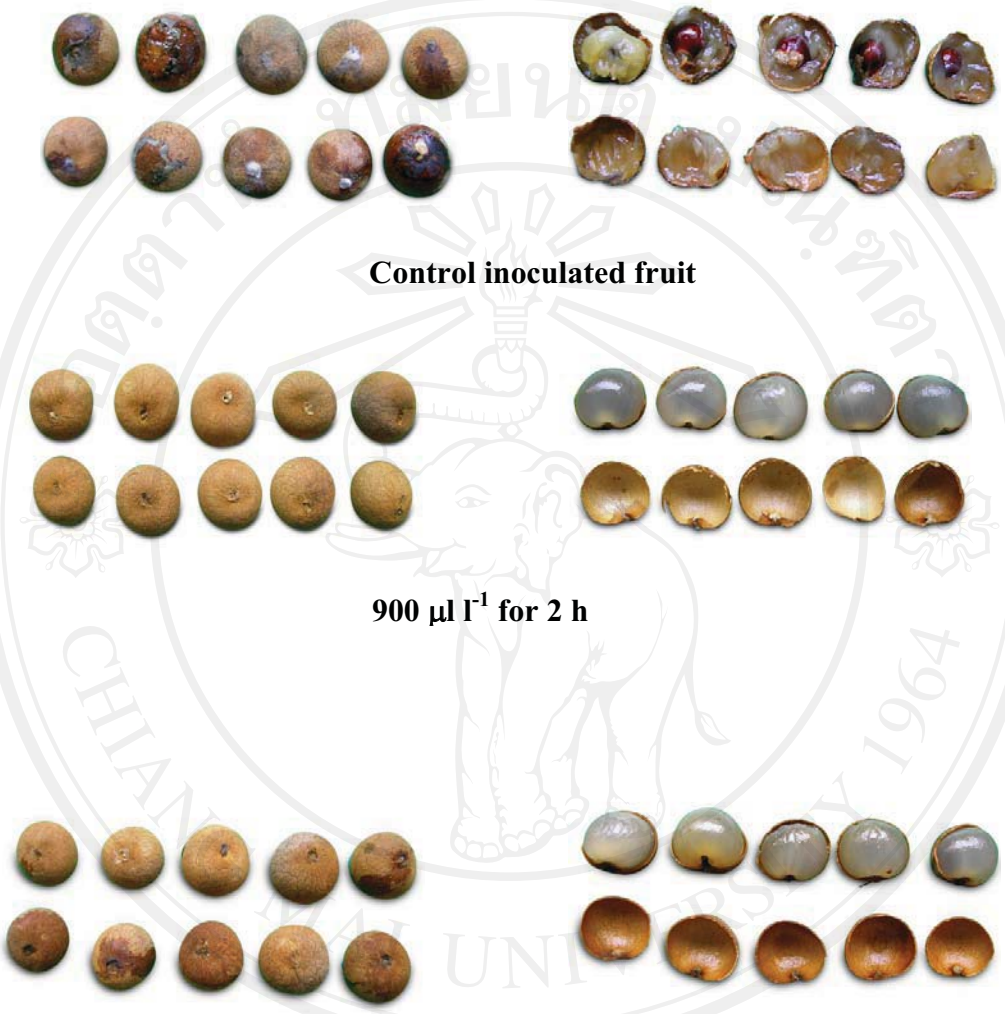

Figure 4.4 *Lasiodiplodia theobromae*-inoculated longan fruit stored at ambient temperature after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature versus the control (non-hexanal fumigation treatment).

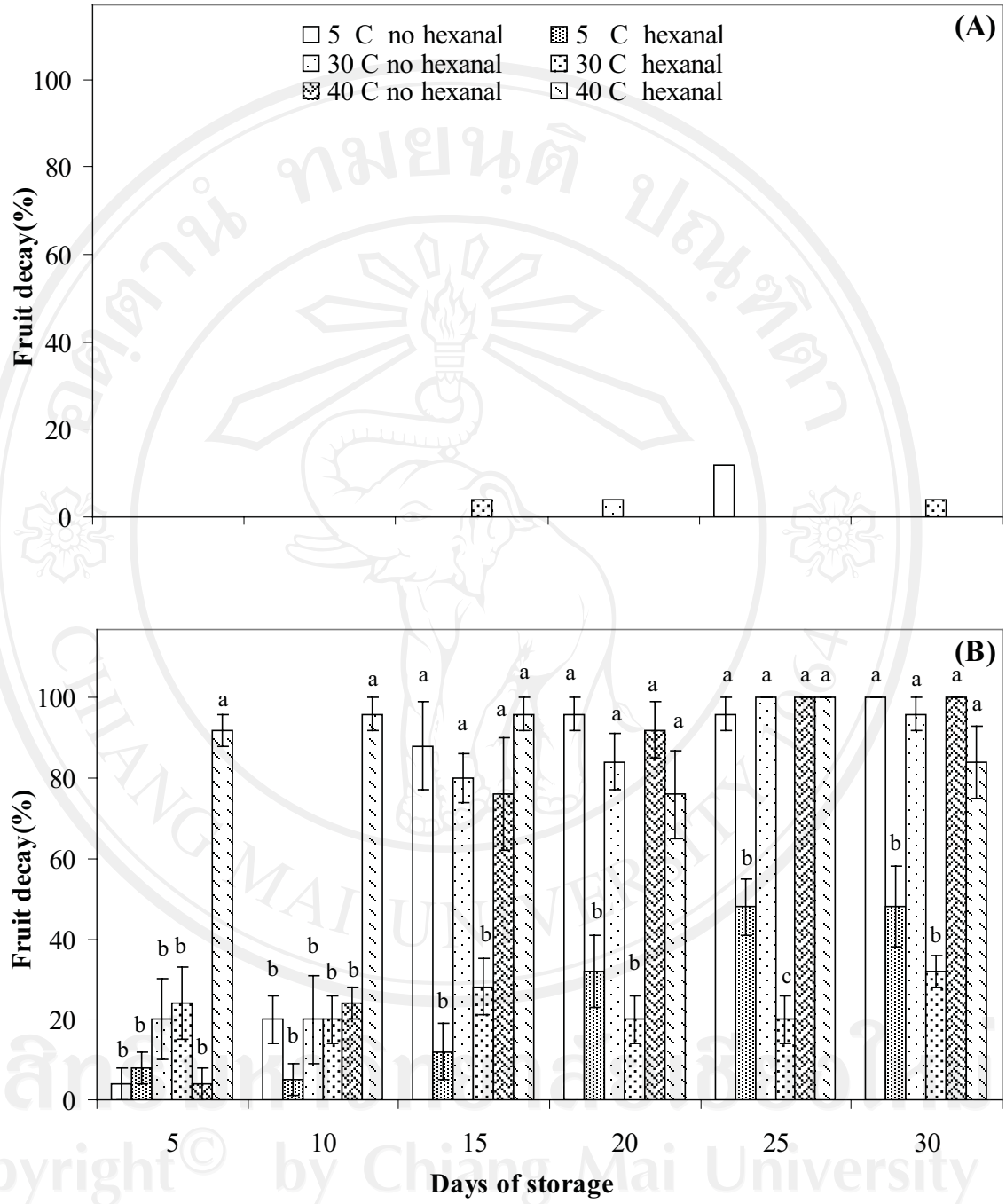

Figure 4.5 Percentage of fruit decay of non-inoculated (A) and *L. theobromae* inoculated (B) longan fruit stored at ambient temperature after hexanal fumigation at 900 µl l^{-1} for 2 h at 5°C, ambient temperature and 40°C. Means followed by different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05)

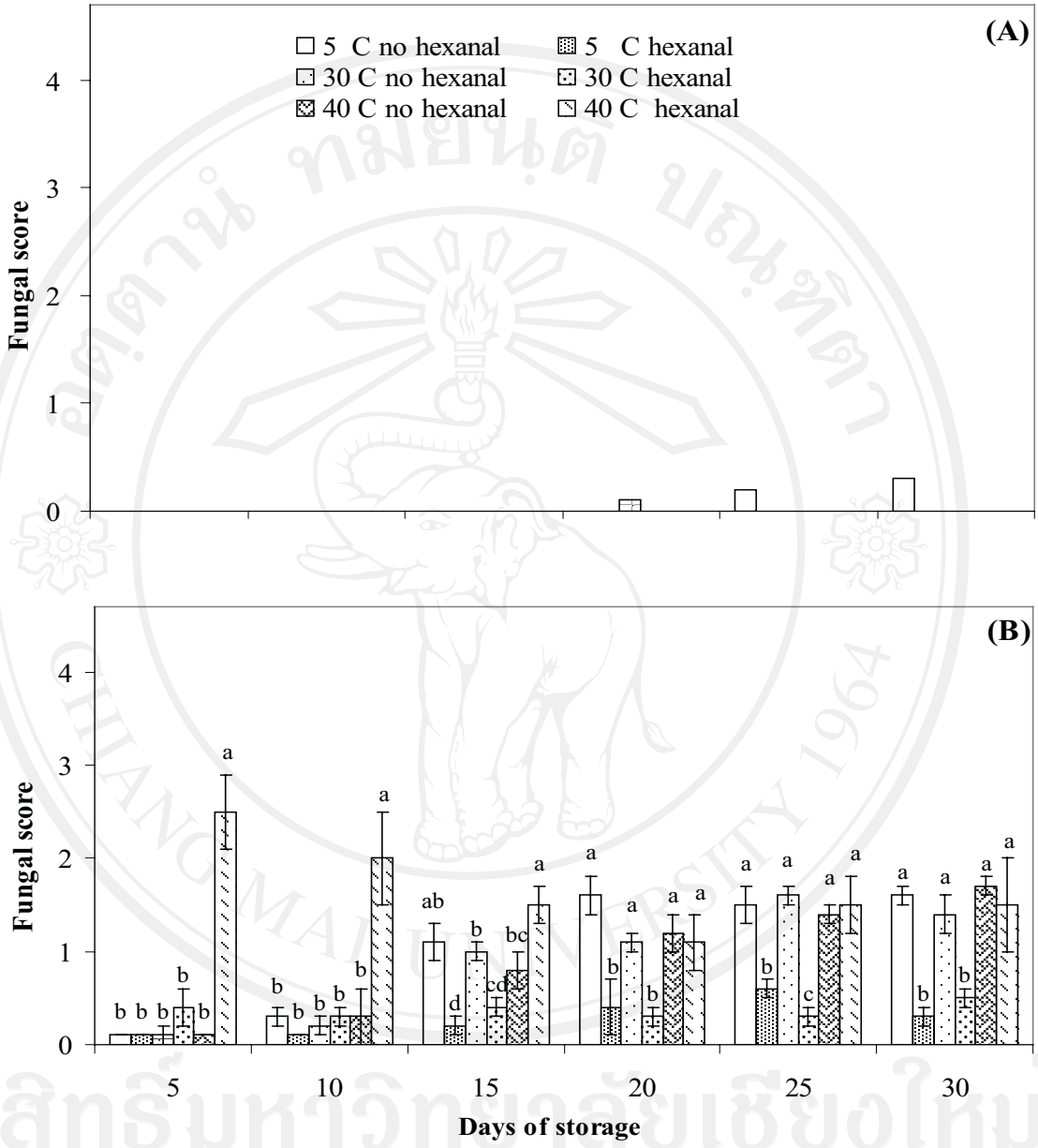

Figure 4.6 Fungal incidence score of non-inoculated (A) and *L. theobromae*. inoculated (B) longan fruit stored at ambient temperature after hexanal fumigation at 900 µl l^{-1} for 2 h at 5°C, ambient temperature and 40°C. Scores are: 0 = no visual evidence of fungi, 1 = less than 10%, 2 = 10-30%, 3 = 31-70%, and 4 = more than 70% of the surface affected with fungus. Means followed by different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05)

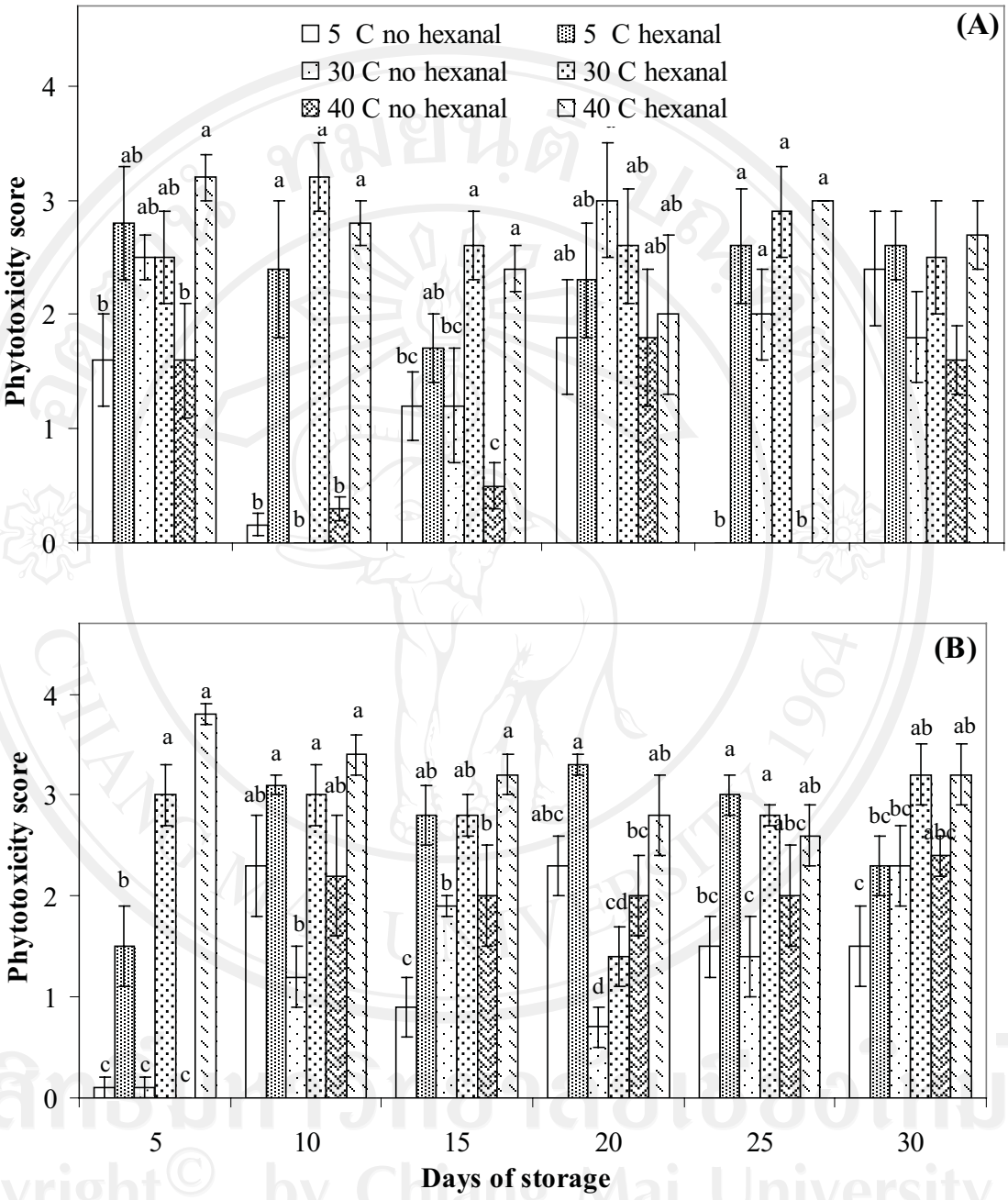

Figure 4.7 Phytotoxicity score of non-inoculated(A) and *L. theobromae* inoculated (B) longan fruit stored at ambient temperature after hexanal fumigation at 900 µl l^{-1} for 2 h at 5°C, ambient temperature and 40°C. Scores are: 0 = no deterioration, 1 = slight deterioration, 2 = moderate deterioration, 3 = severe deterioration and 4 = tissue completely collapsed. Means followed by different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

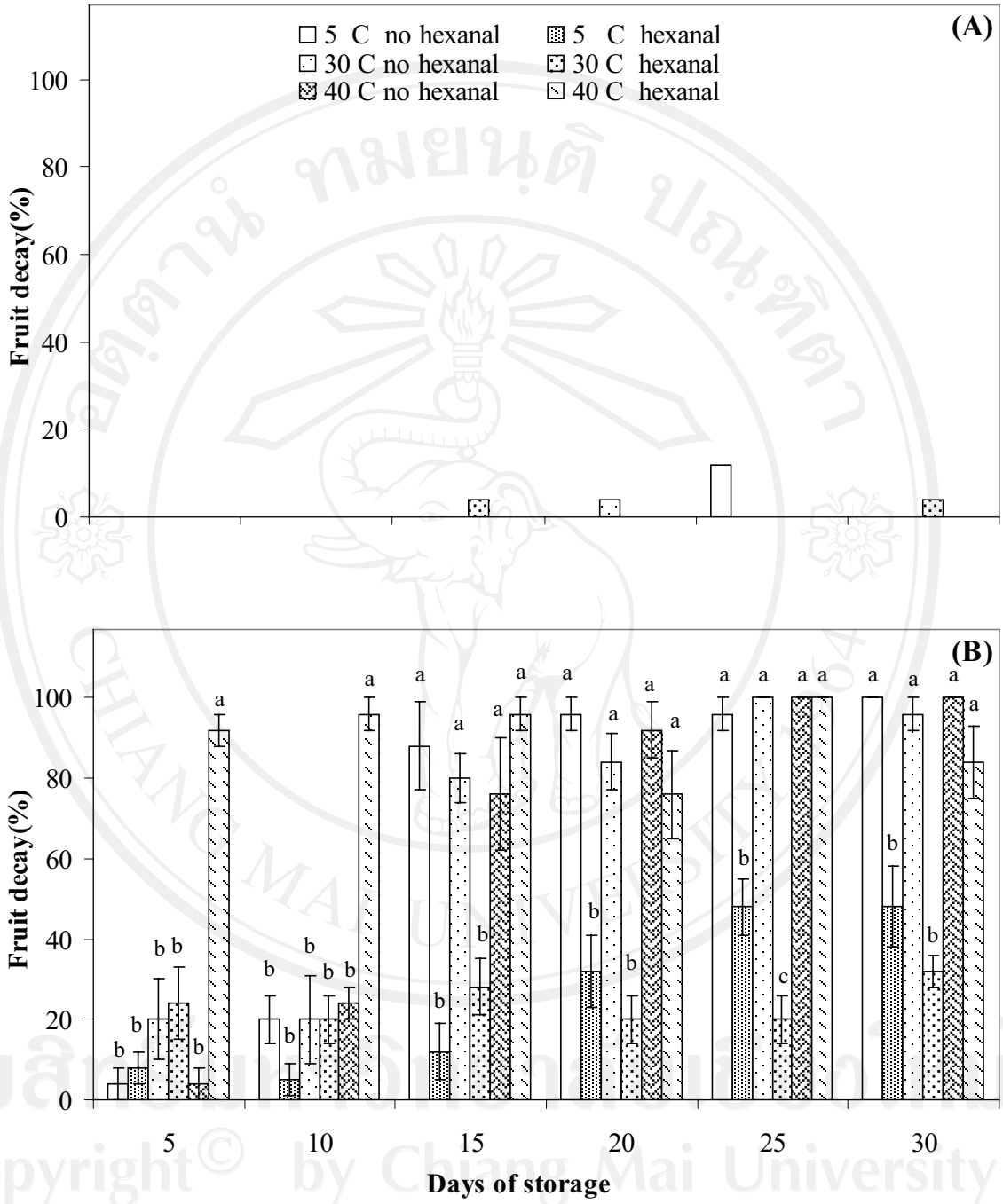

Figure 4.8 Percentage of fruit decay of non-inoculated (A) and *L. theobromae* inoculated (B) longan fruit stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at 5°C, ambient temperature and 40°C. Means followed by different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

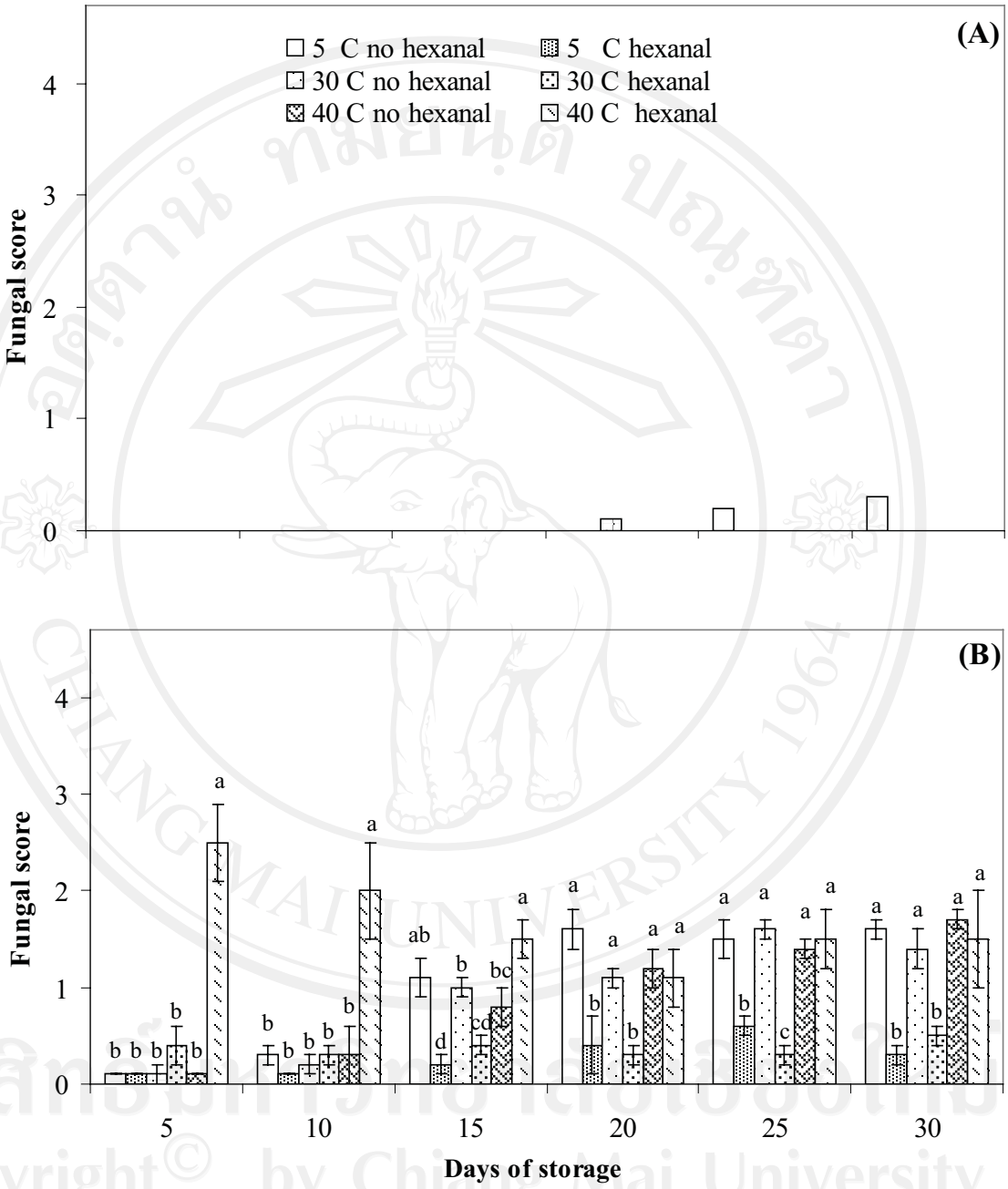

Figure 4.9 Fungal incidence scores of non-inoculated (A) and *L. theobromae* inoculated (B) longan fruit stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at 5°C, ambient temperature and 40°C. Scores are; 0 = no visual evidence of fungi, 1 = less than 10%, 2 = 10-30%, 3 = 31-70%, and 4 = more than 70% of the surface affected with fungus. Means followed by different letters within days are significantly different by Fisher's Least Significant Difference (P=0.05).

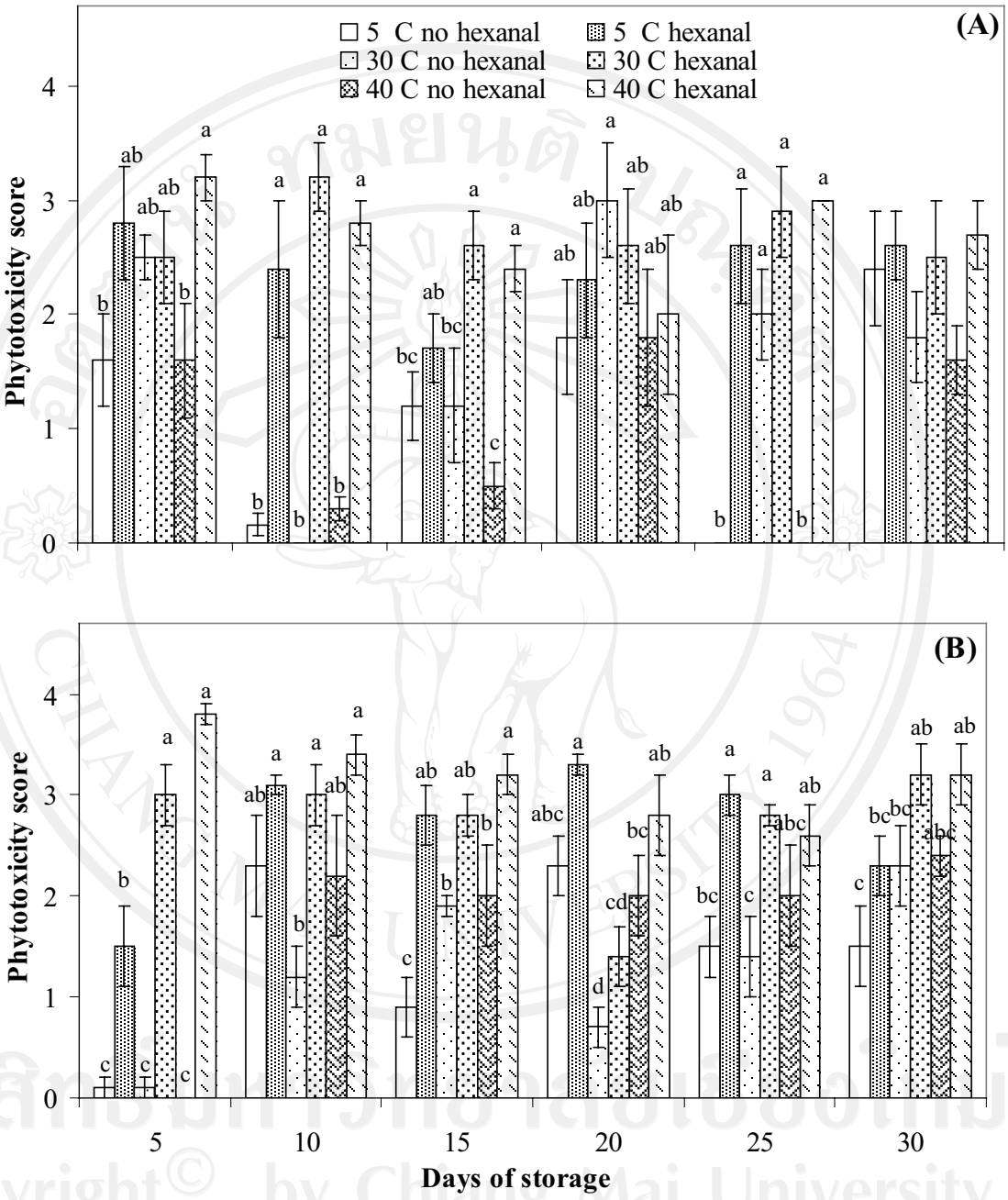

Figure 4.10 Phytotoxicity scores of non-inoculated (A) and *L. theobromae* inoculated (B) longan fruit stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at 5°C, ambient temperature and 40°C. Scores are: 0 = no deterioration, 1 = slight deterioration, 2 = moderate deterioration, 3 = severe deterioration and 4 = tissue completely collapsed. Means followed by different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

Table 4.17 Volatile compounds in the headspace at ambient temperature of hexanal, longan fruit cv. Daw, and longan fruit with hexanal at 900 µl l^{-1} for 24 h, analyzed by GC-MS.

Compound	Treatment		
	Hexanal	Longan	Longan+hexanal
1,2- benzenedicarboxylic acid, diethyl ester		/	/
1,2-heptanediol			/
1,3,5,7,9,11-hexaethylbicyclo[5,5,1]hexasiloxane		/	
1,3,5,7-tetraethyl-1-ethylbutoxysiloxycyclotetrasiloxane		/	/
1,3,6-octatriene, 3,7-dimethyl-		/	
1-hexanol		/	/
1-pentanol	/		
2-hexanone	/		
2-methylpentyl hexanoate			/
2-octenal , 2-butyl-		/	/
acetic acid, ethyl ester		/	
acetic acid, hexyl ester		/	/
benzeneacetic acid			/
butanoic acid, hexyl ester			/
carbon dioxide	/	/	/
cyclobutanecarboxylic acid,2-pentyl ester			/

Table 4.17 (continued) Volatile compounds in the headspace at ambient temperature of hexanal, longan fruit cv. Daw, and longan fruit with hexanal at 900 µl l^{-1} for 24 h, analyzed by GC-MS.

Compound	Treatment		
	Hexanal	Longan	Longan+hexanal
diethyl phthalate	/		
dithiocarbonic acid		/	/
estra-1,3,5(10)-trien-17-one			/
ethanol		/	/
ethyl acetate			/
hexanal	/		/
hexanoic acid	/		/
hexanoic acid, ethyl ester		/	/
hexanoic acid, hexyl ester		/	
hexanoic acid, methyl ester			/
pentanal	/		

Experiment 4.3 Effect of hexanal on longan fruit quality

4.3.1 Weight loss

Fresh weight of longan fruit declined continuously during cold storage (Figure 4.11, Appendix Table 6). The percent weight loss of the treatments showed few differences during the study period, indicating that hexanal and the fumigation technique did not affect this trait. Although in general wax coating has been effective in reducing weight loss of coated fruit, Sta-fresh coating did not reducing weight loss. This could be caused by the coating technique, i.e., dipping. The nature of the longan fruit, a rough pericarp covered with dust from its dead epidermal cells may have prevented the coating substance from covering the entire longan fruit surface continuously and resulted in cracks in the coating layer.

4.3.2 Total soluble solids content

The TSS of all treatments varied in a narrow range, 14.62-16.95 %, (Figure 4.12, Appendix Table 7) without a pattern of change during the study period. Thus, neither hexanal nor Sta-fresh affected the TSS of the fruit.

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4.3.3 pH

Hexanal fumigation and coating did not affect the juice pH of longan fruit. The pH of pericarp varied in a narrow range, 4.97-5.55 (Figure 4.13, Appendix Table 8), without a pattern during the study period. The pH of aril also varied in a narrow range, 6.75-7.2 (Figure 4.13, Appendix Table 9), without a pattern during the study period.

4.3.4 Electrolyte leakage

Electrolyte leakage of the pericarp tended to increase during the study period in all treatments. Sta-fresh coating did not affect the electrolyte leakage of the fruit. This would infer that the coating did not affect the membrane of the pericarp cells. Electrolyte leakage of the pericarp increased 2-3 folds after hexanal fumigation in both Sta-fresh coated and uncoated fruit (Figure 4.14, Appendix table 10). Hexanal

likely had some effects on the integrity of membrane of the pericarp cells which promoted the electrolyte leakage.

4.3.5 Firmness

Firmness of the pericarp varied in a narrow range, 0.89 – 1.24 N, during the study period without a pattern in any treatment (Figure 4.15 Appendix Table 11). Coating and fumigation did not affect the firmness of the pericarp. The variation which was found likely came from the lack of uniformity of the fruit samples.

4.3.6 Pericarp and aril color

The L* values and C-values of the outer pericarp of coated and uncoated fruits were not different (Figure 4.16, Appendix tables 12-13). Therefore, Sta-fresh coating did not affect the brightness and the chrome of the pericarp. L* values of both coated and uncoated fruit were reduced after fumigation. This meant that after fumigation the brightness of the fruit pericarp was reduced. The reduction of C values after fumigation meant that the outer pericarp color of fumigated fruit were more pale than the non-fumigated fruit. Hue angle of the outer pericarp was reduced during storage for both coated and uncoated fruit (Figure 4.16, Appendix Table 14). Fumigation further reduced the hue angle of fruit, making fruit more reddish-brown.

L* Values of the inner pericarp were reduced during storage in all treatments (Figure 4.17, Appendix Table 15). Coating did not affect L*values, while fumigation reduced the brightness of the inner pericarp. C Values of the inner pericarp increased during storage in all treatments (Figure 4.17, Appendix table 16). Therefore, the inner pericarp color turned darker during storage. Coating did not affect the C-value, while fumigation promoted a dark color of the inner pericarp. Hue angle of the inner pericarp was reduced during storage in all treatments (Figure 4.17, Appendix Table 17). Coating did not affect the hue angle while fumigation reduced it in the inner pericarp. Therefore, the inner pericarp color turned more yellowish-brown after fumigation and during the storage period.

For aril color, L* values, C values and hue angles changed in narrow ranges during the storage time. Coating and hexanal fumigation did not affect these

three characters of the arils (Figure 4.18, Appendix Tables 18- 20). L* Values of the arils were stable during the storage period. C Values showed some reduction but hue angle (Figure 4.18, Appendix Table 20) increased during storage. This made the aril pale-white instead of clear. This could have been due in part to water loss and deterioration during storage.

The changes of the inner pericarp color showed that hexanal could penetrate the inner pericarp during fumigation. The color changes of the inner and outer pericarp caused by hexanal were unwanted because they affected fruit quality. Therefore, pericarp color changes would be a concern during hexanal fumigation.

4.3.7 Hexanal residue in pericarp and aril

Hexanal was found in the pericarp of both fumigated and non-fumigated fruit. This confirmed that hexanal could be naturally produced by longan pericarp (0.04 – 1.04 μg in 1 g of pericarp) (Figure 4.19A, Appendix Table 21). The hexanal content of coated and uncoated fruit did not exhibit significant differences. Coating also did not affect the residue content of hexanal in the pericarp after fumigation. Hexanal in fumigated fruit was 5 – 11 times higher that for the non-fumigated fruit (8.20 – 10.56 μg in 1 g of pericarp). Hexanal in pericarp of all treatments declined continuously during storage. For non-fumigated fruit, dramatic reductions (1.62 to 0.51 and 0.92 to 0.28) occurred on day 6^{th} – 9^{th} of storage. This reduction of hexanal in pericarp was coincident with fruit rot symptoms which occurred during storage. Therefore, hexanal reduction could be one of the factors which reduced the degree of tolerance of longan fruit to fruit diseases. Hexanal content in pericarp of fumigated fruit dropped 2-3-fold during the 24 day-storage-period (8.20-10.56 to 3.63-3.68 μg in 1 g of pericarp). Thus, hexanal had a fungicidal effect and the levels of hexanal which were left in the pericarp may be high enough to inhibit the growth of fruit diseases. This may have resulted in the observation that none of the hexanal-fumigated fruit showed fruit rot symptom during the study period.

Hexanal content of aril tissue was very low. The hexanal residue in arils was 1.26 – 2.42 μg in 1g after the fumigation (Figure 4.19B, Appendix Table 22). These data showed that hexanal also penetrated through the pericarp to the aril. Hexanal content in the arils declined during the storage period. This decline might be

caused by metabolism of hexanal by the fruit cells, or hexanal diffusion back to the surrounding atmosphere.

4.3.8 Colony forming units (CFUs) of microorganisms isolated from the pericarp and aril

The numbers of colony forming units of microorganisms on longan fruit pericarp were reduced by hexanal fumigation and the reduction was increased by the combination of hexanal and fruit coating (Figure 4.20A, Appendix Table 23). After storing the fruit for 6 –18 days, the hexanal-fumigated fruit with Sta-fresh coating had a lower number of CFUs than the other treatments. However, the fungicidal effect of hexanal fumigation was greatest at the 24th day of storage when there did not have CFUs in any hexanal treatments. This result showed that coating did not affect the survival of microorganisms but coating had some synergistic effect with hexanal for controlling the microorganisms on the pericarp.

Hexanal fumigation showed its fungicidal effect on the number of CFUs on the longan fruit aril only on the 24th day after storage (Figure 4.20B, Appendix Table 24). In the earlier storage period, neither hexanal fumigation nor coating showed any control of CFUs on the aril. The results revealed that hexanal in pericarp was higher than in arils. Therefore, hexanal fumigation was more effective on CFUs on the pericarp than on the aril.

4.3.9 Fruit quality acceptance

The consumer acceptance scores for quality of the fruit revealed that coating and hexanal fumigation reduced the scores of the outer side of pericarp (Figure 4.21A, Appendix table 25). Only hexanal fumigation reduced the scores of the inner side of the pericarp (Figure 4.21B, Appendix Table 26). These results indicated that the color change and hexanal aroma of the pericarp were important factors affecting consumer acceptance of the fruit. For the aril, the edible part of the fruit, hexanal fumigation reduced the score (Figure 4.21C, Appendix Table 27). The aroma scores of the fruit were reduced by storage time and hexanal fumigation. The reduction in aril aroma scores was mainly caused by the unpleasant scent of hexanal residue inside the aril after the fumigation. The taste of longan aril was affected the

same as the aril aroma. Hexanal fumigated fruit had lower aril scores for taste than the unfumigated fruit (Figure 4.21E, Appendix Table 29). For the overall scores of the fruit, the control had the highest scores throughout the storage period, while hexanal-fumigated fruit had the lowest scores (Figure 4.21F, Appendix Table 30).

Even though hexanal controlled fruit rotted fungi, and it was known to be a GRAS (generally recognized as safe) substance, the use of hexanal for fumigation for longan will need to be improved before commercial acceptance due to adverse pericarp color changes and effects on aroma and taste. For use of hexanal fumigation of longan, the compromise between the fungicidal effect and the reduction of fruit quality should be solved. Hexanal fumigation might be combined with other treatments in controlling longan fruit rot such as ozone, chitosan coating, and precooling.

4.3.10 Fruit decay and fungal incidence

At day 21 of cold storage, decaying fruit were found in control, Sta-fresh coated and hexanal treatments were 7.5, 7.5 and 5.0 %, respectively (Figure 4.22A, Appendix Table 31). The decayed fruit in control and coating treatments were increased dramatically (57 and 37 %, respectively) over the limit for storability acceptance by day 24th. Therefore, the storage lives of the fruit in both treatments ended at the 21st day of storage. Decayed fruit in the hexanal treatment were only found on day 21st. These results were showed that hexanal could nearly completely control the fruit decay of longan fruit. Coating also reduced longan fruit decay, but it was not effective enough to give a significant extension of the storage life of the fruit. The combination of coating plus hexanal fumigation gave the best result. This treatment did not show any decayed fruit in the study period.

The incidence of fungal mycelia on the longan fruit surface was recorded. All of the decayed fruit exhibited 10-30% coverage of their surface with fungal mycelia. The mycelial growth did not show any patterns or preferred areas on the fruit (Figure 4.22B, Appendix Table 32).

Even though fungal infection was controlled by hexanal fumigation, phytotoxic symptoms (Figure 4.22C, Appendix table 33), including a brown color of the outer and inner pericarp occurred. For fruit quality, the aroma of fruit was

changed by hexanal and its metabolites and would be of concern. The synergistic interaction between hexanal and coating might be a good sign for developing a new treatment but avoiding the adverse effects of hexanal fumigation on longan fruit.

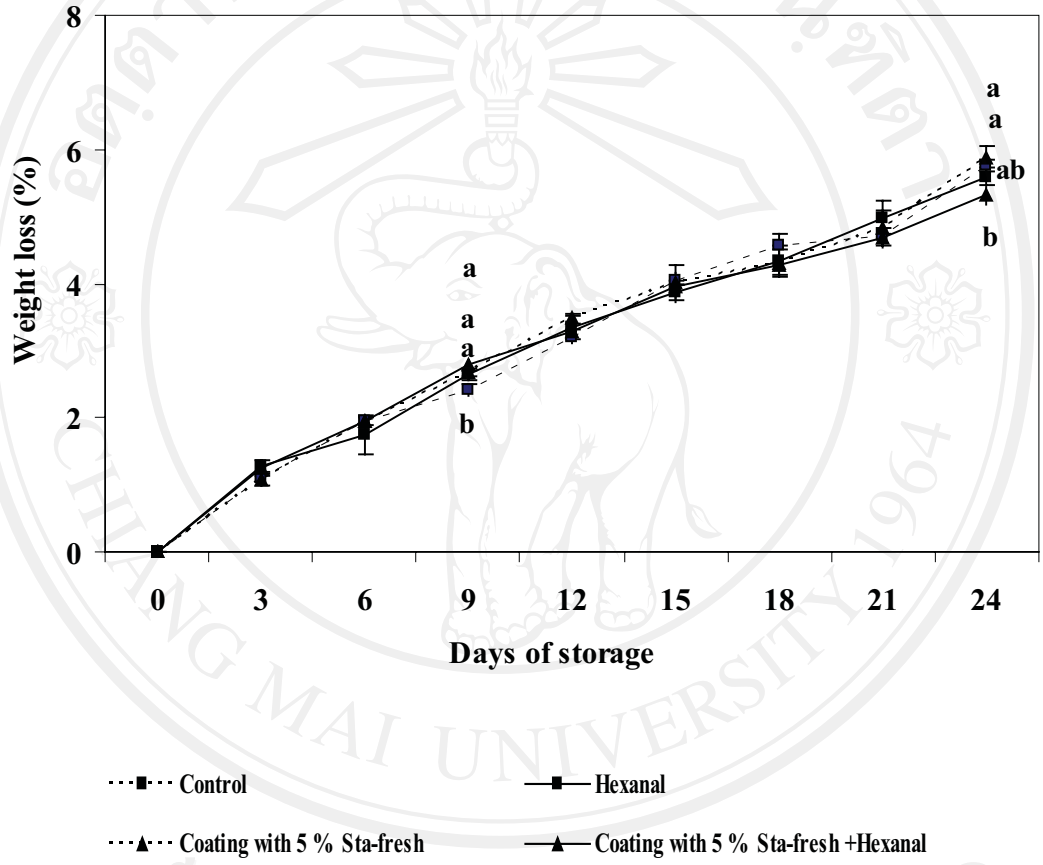

Figure 4.11 Percentage of weight loss of longan fruit cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

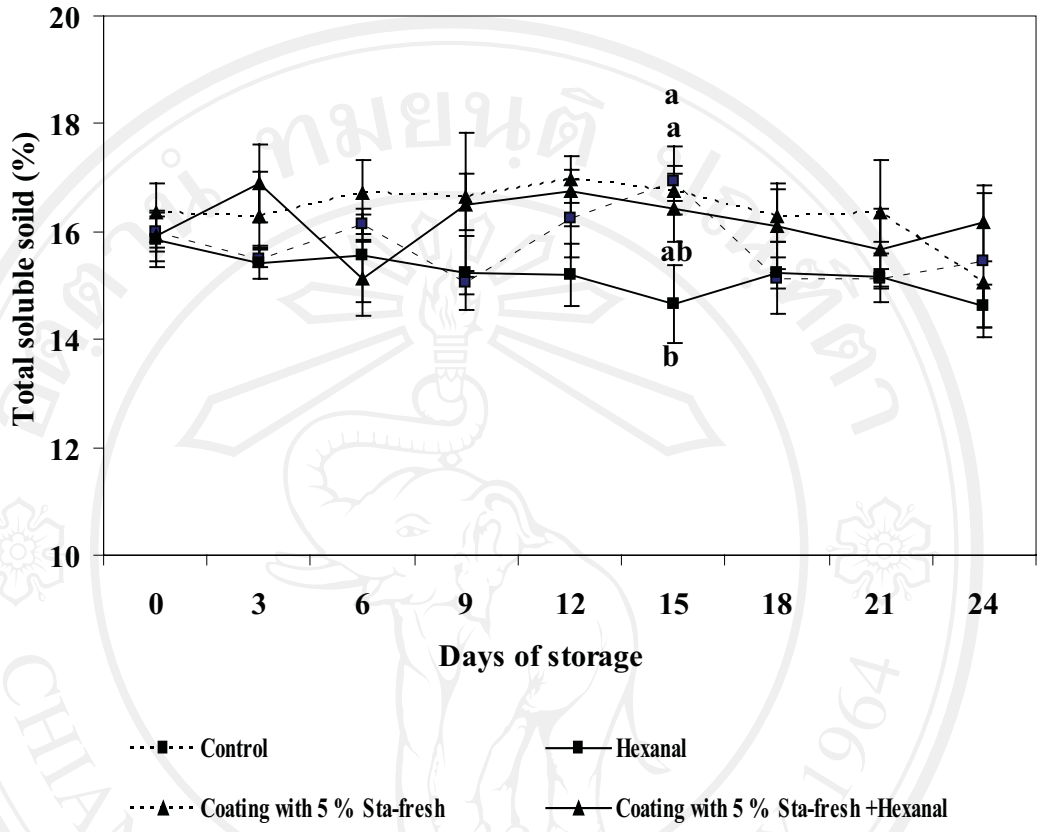

Figure 4.12 Total soluble solids (% brix) of longan fruit aril cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

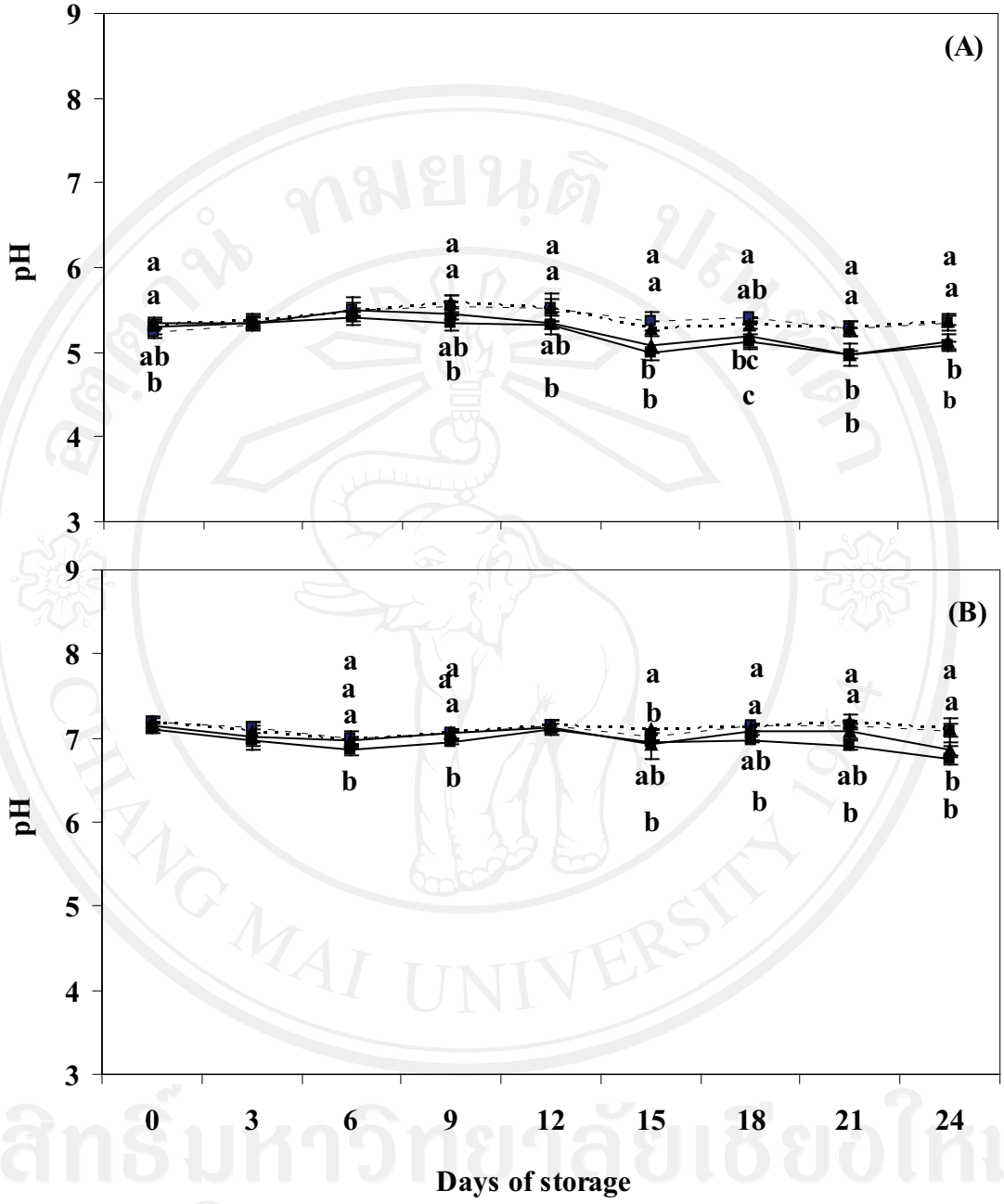

Figure 4.13 pH of longan fruit pericarp (A) and aril (B) cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

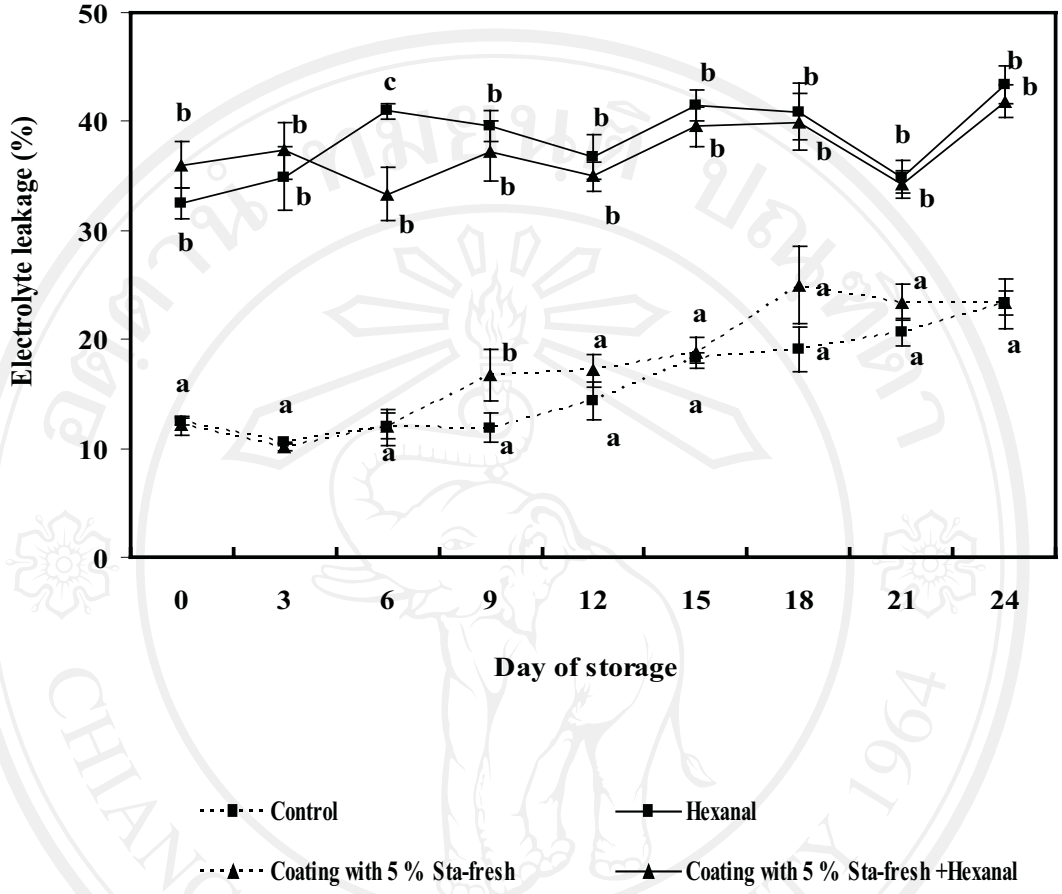

Figure 4.14 Electrolyte leakage of longan fruit pericarp cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature.

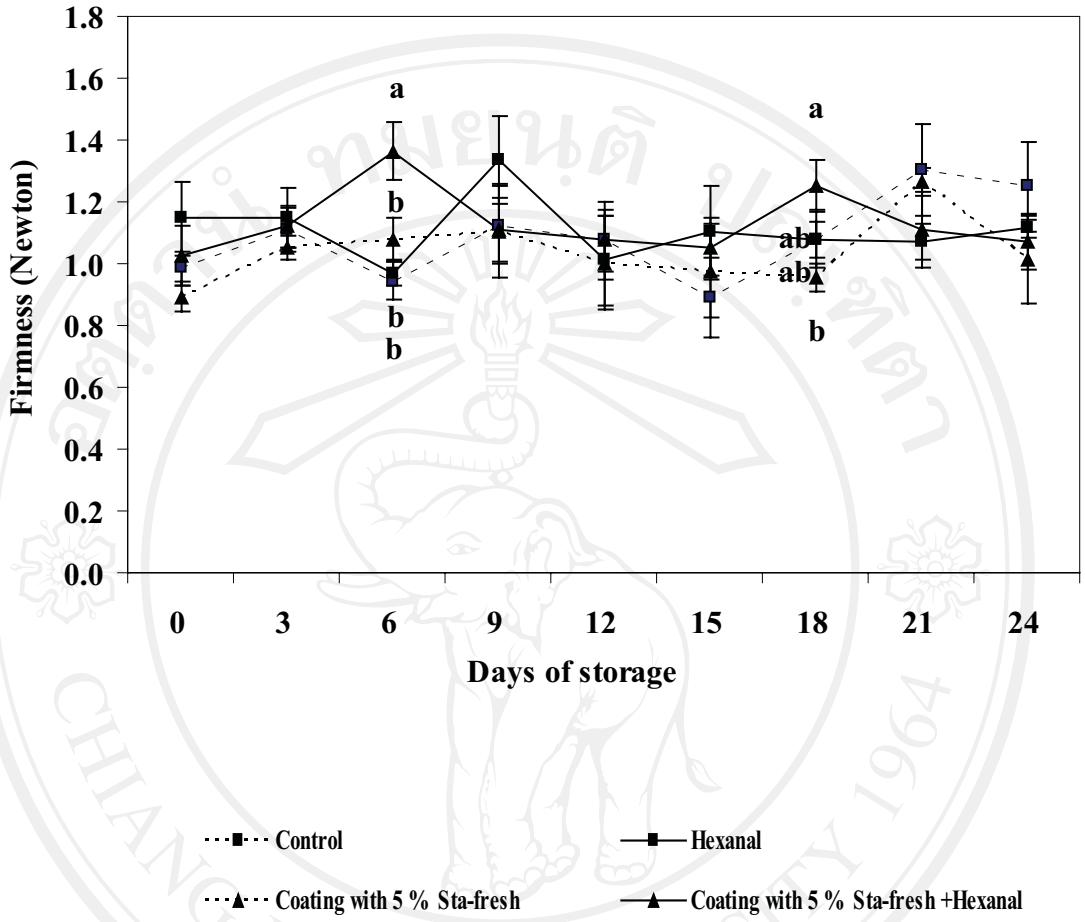

Figure 4.15 Firmness (Newton) of longan fruit pericarp cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature.

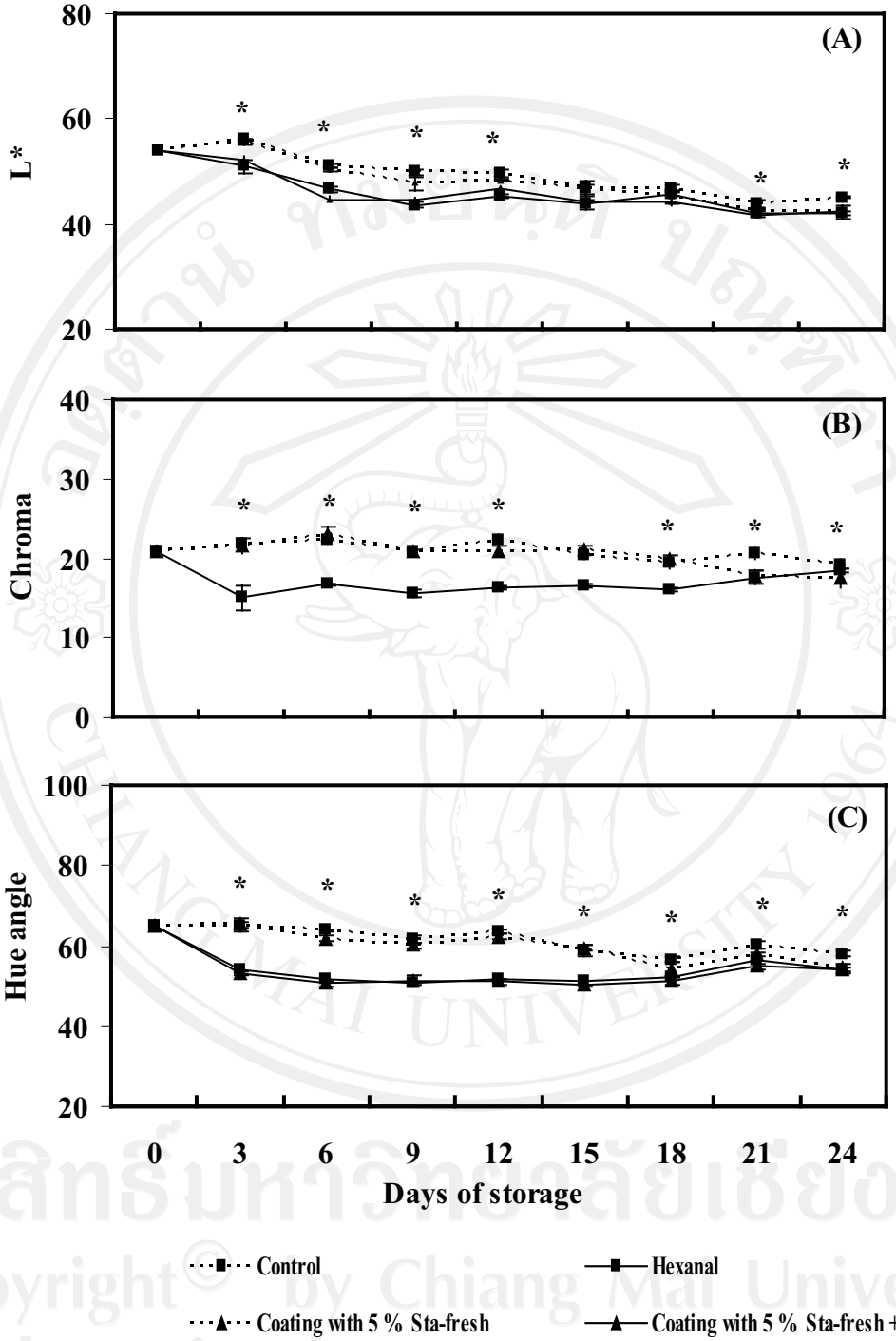

Figure 4.16 L* values (A), chroma values (B) and hue angles (C) of outer longan fruit pericarp cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Main effects of hexanal within days at P=0.05 are indicated by an asterisk.

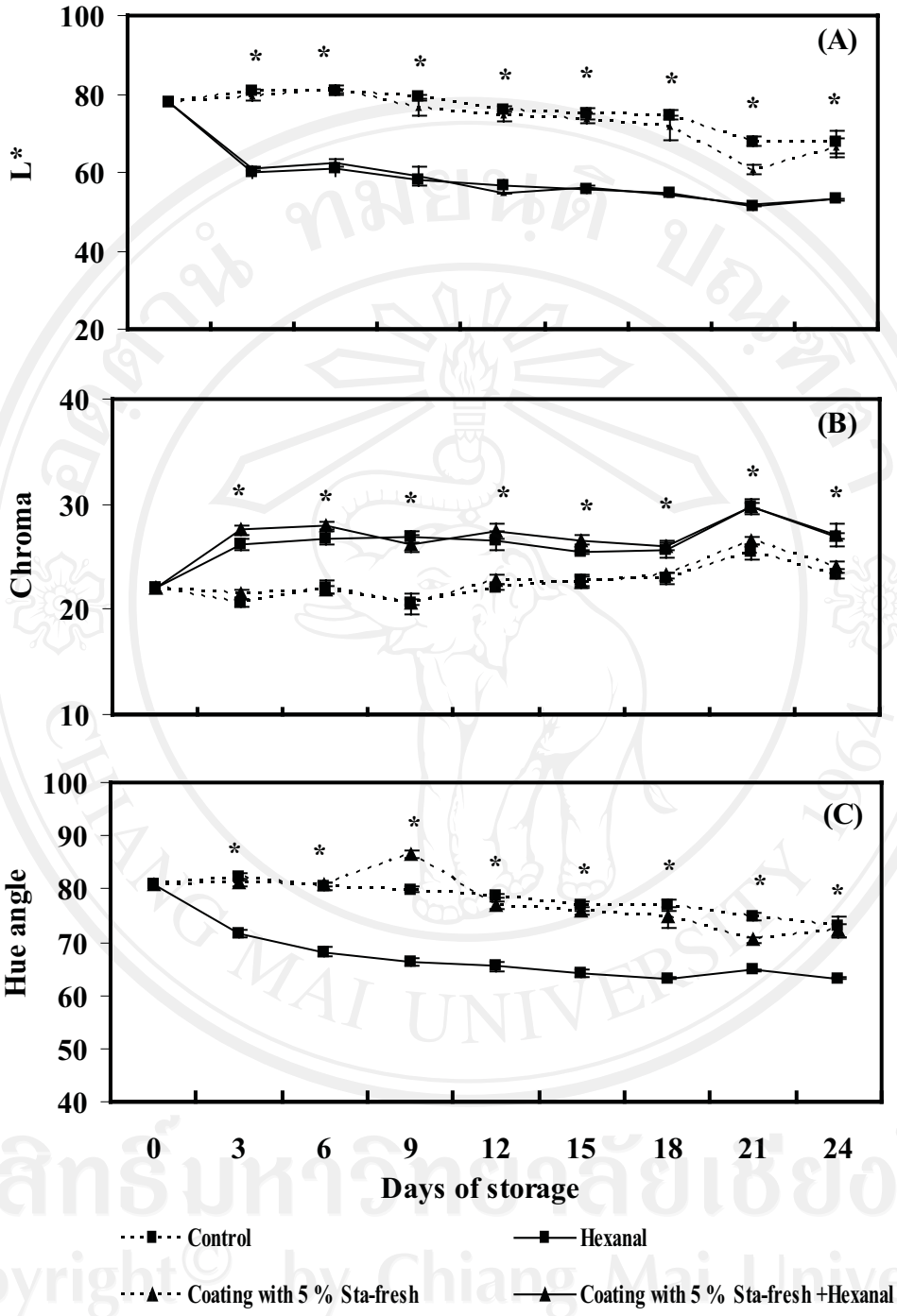

Figure 4.17 L* values (A), chroma values (B) and hue angles (C) of inner longan fruit pericarp cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Main effects of hexanal within days at P=0.05 are indicated by an asterisk.

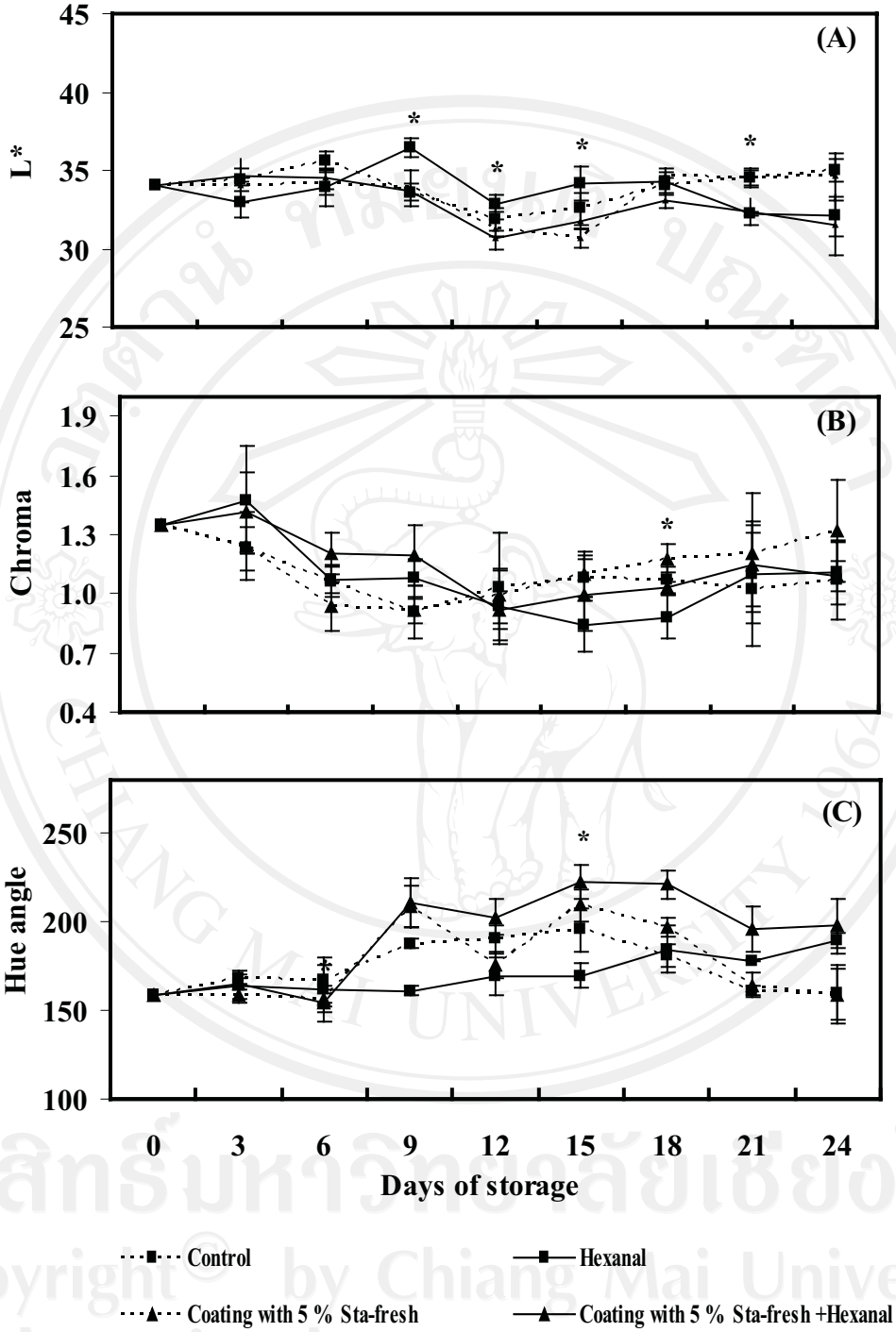

Figure 4.18 L* values (A), chroma values (B) and hue angles (C) of aril longan fruit pericarp cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Main effects of hexanal within days at P=0.05 are indicated by an asterisk.

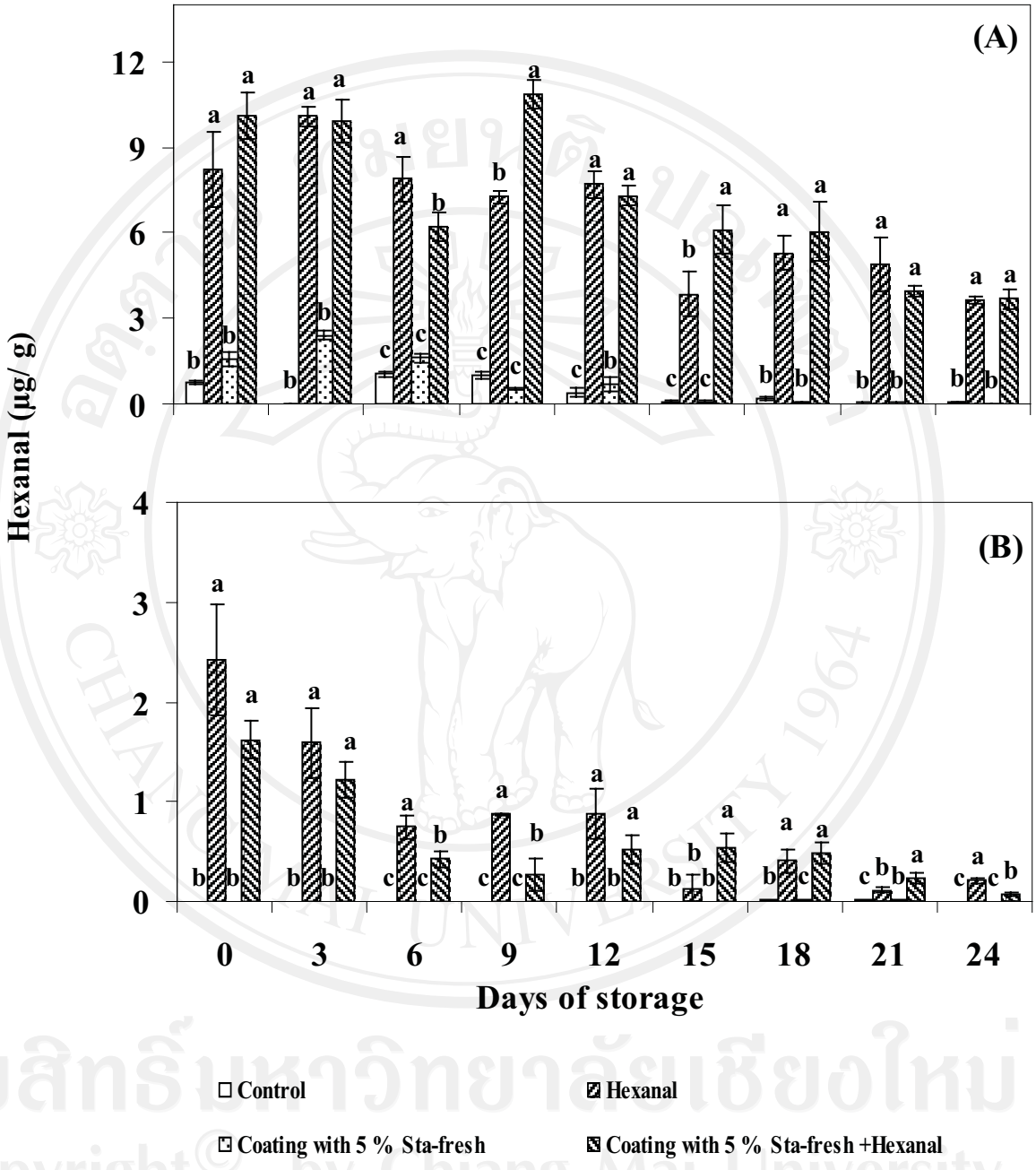

Figure 4.19 Hexanal residue (µg/g) of longan fruit cv. Daw in pericarp (A) and aril (B) stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Means with different letters within days are significantly different by Fisher's Least Significant Difference (P=0.05).

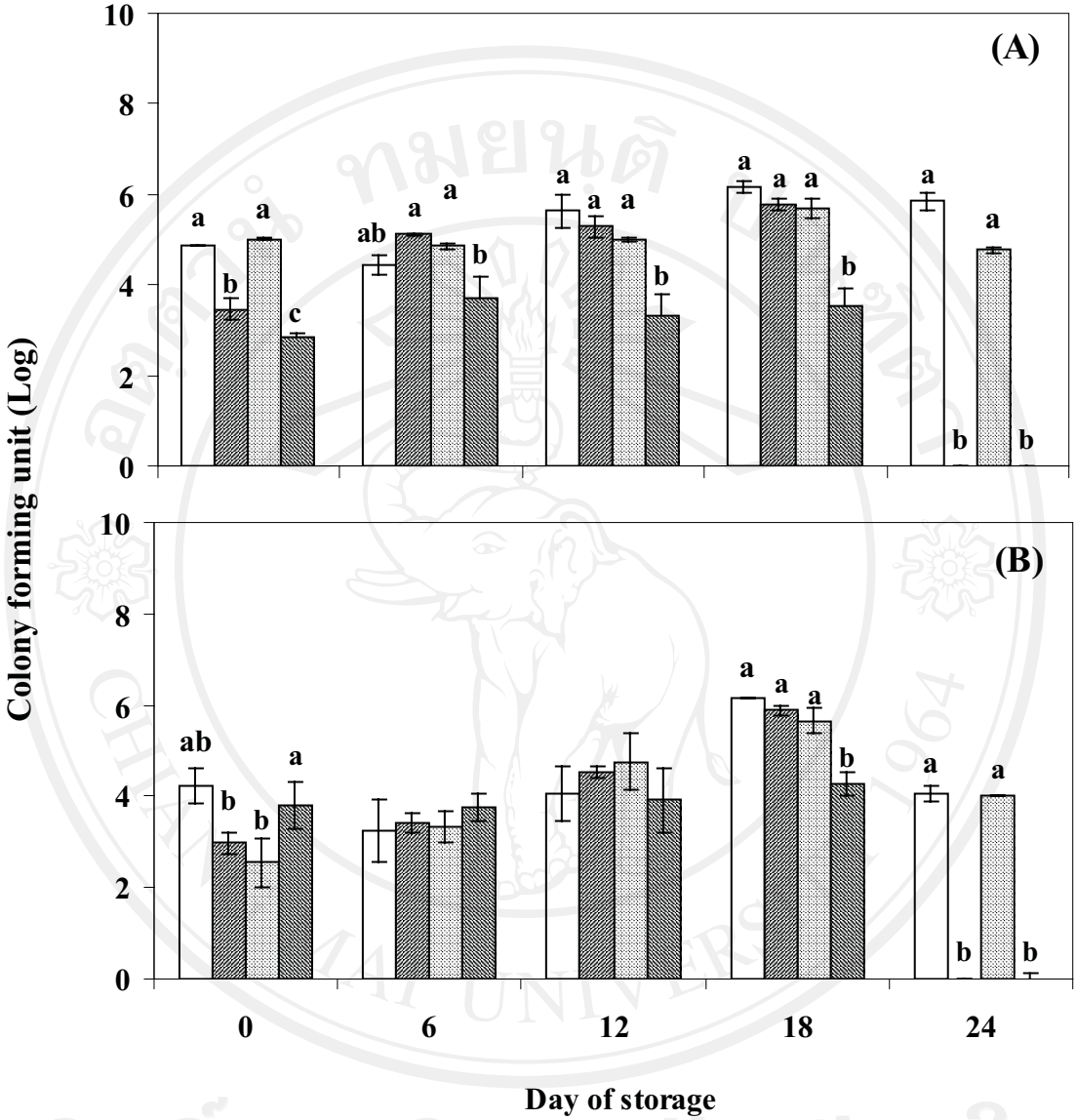

Figure 4.20 Colony forming unit (log g^{-1}) of longan fruit cv. Daw in pericarp (A) and aril (B) stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

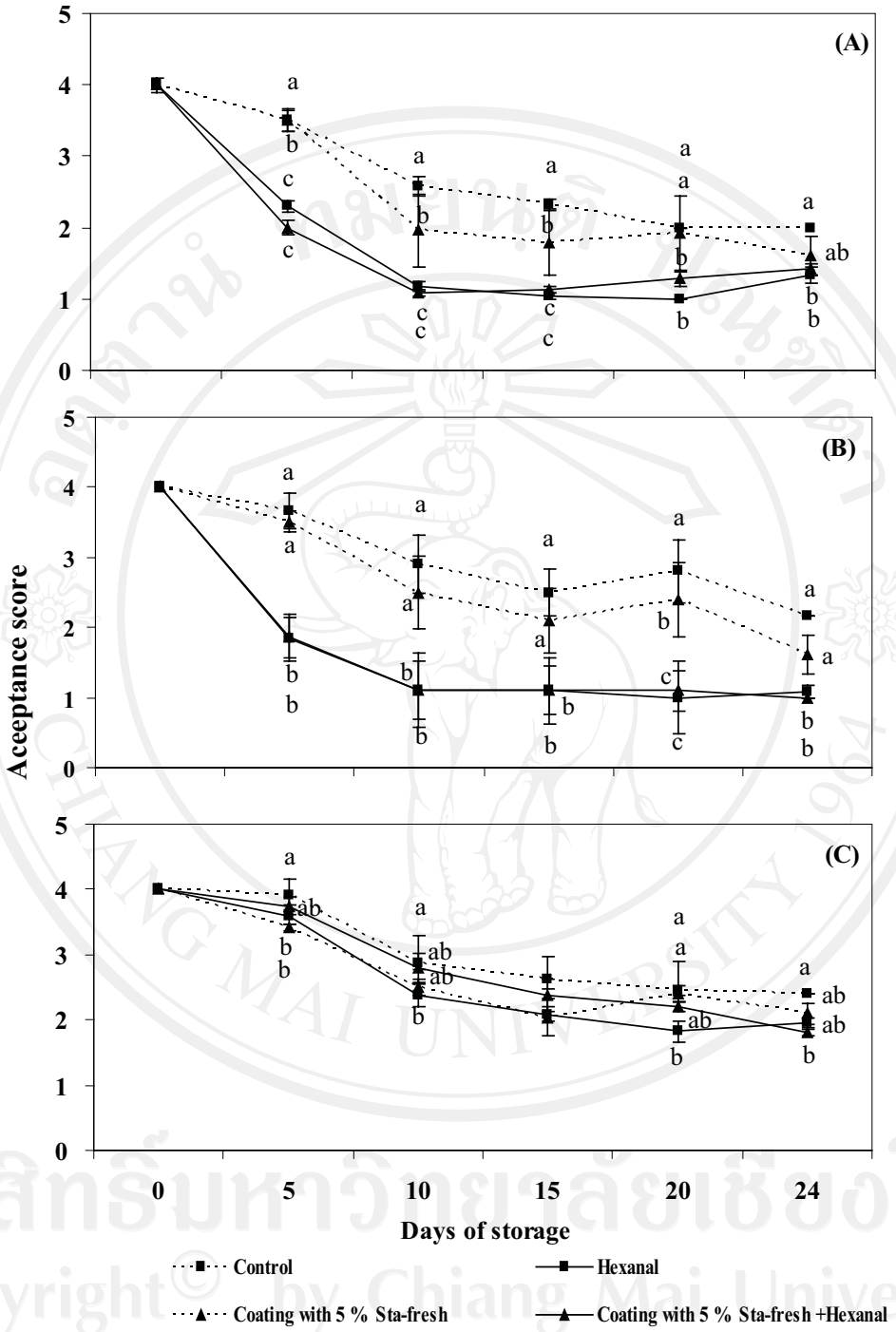

Figure 4.21 Outer peel (A), inner peel(B), aril (C), aroma (D), flavor(E) and overall (F) acceptance score of longan fruit cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Scores are: 1 = mostly dislike, 2 = moderately dislike, 3 = neither like nor dislike, 4 = moderately like, and 5 = mostly like). Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

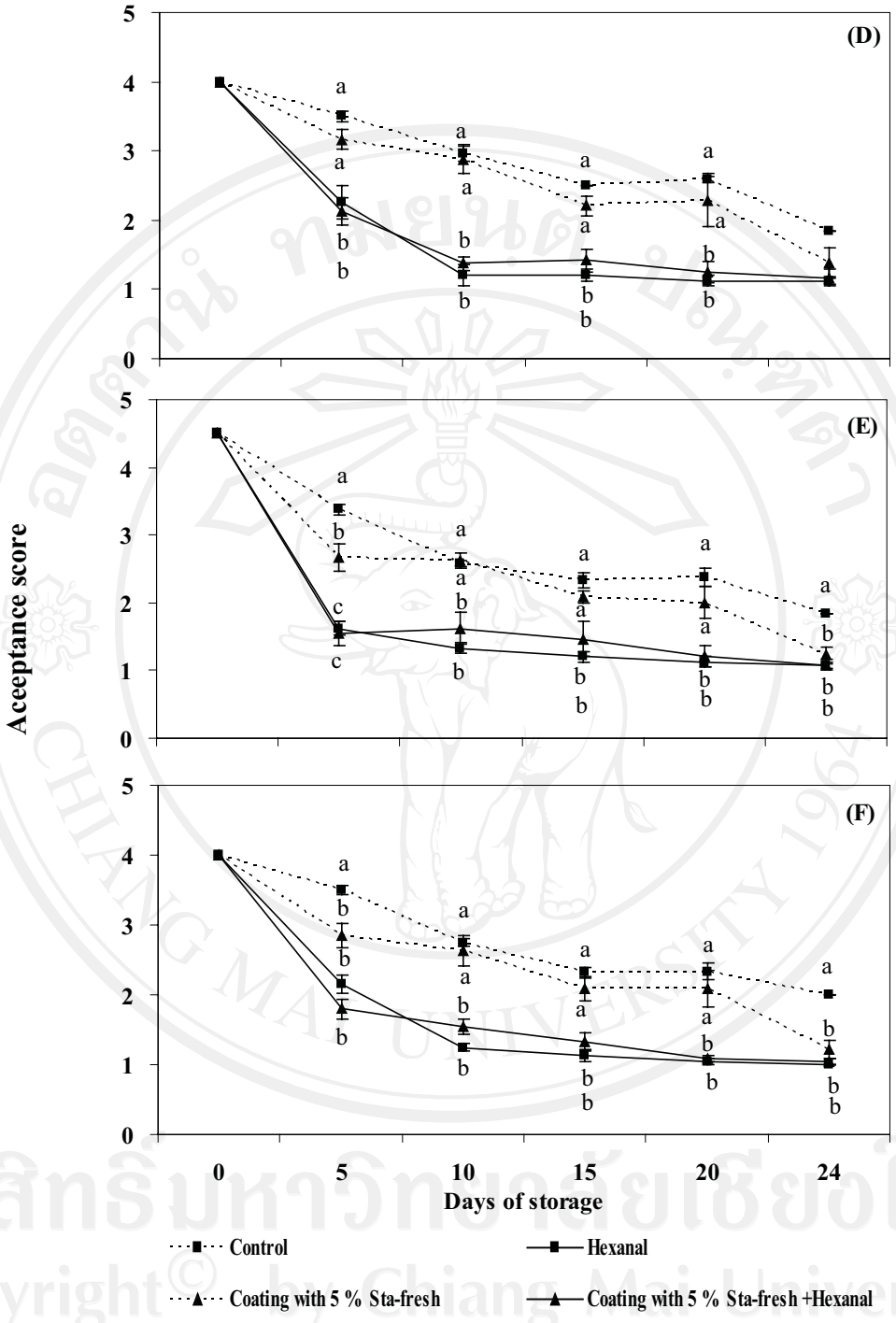

Figure 4.21 (continued) Outer peel (A), inner peel(B), aril (C), aroma(D), flavor(E) and overall (F) acceptance score of longan fruit cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Scores are: 1 = mostly dislike, 2 = moderately dislike, 3 = neither like nor dislike, 4 = moderately like, and 5 = mostly like. Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

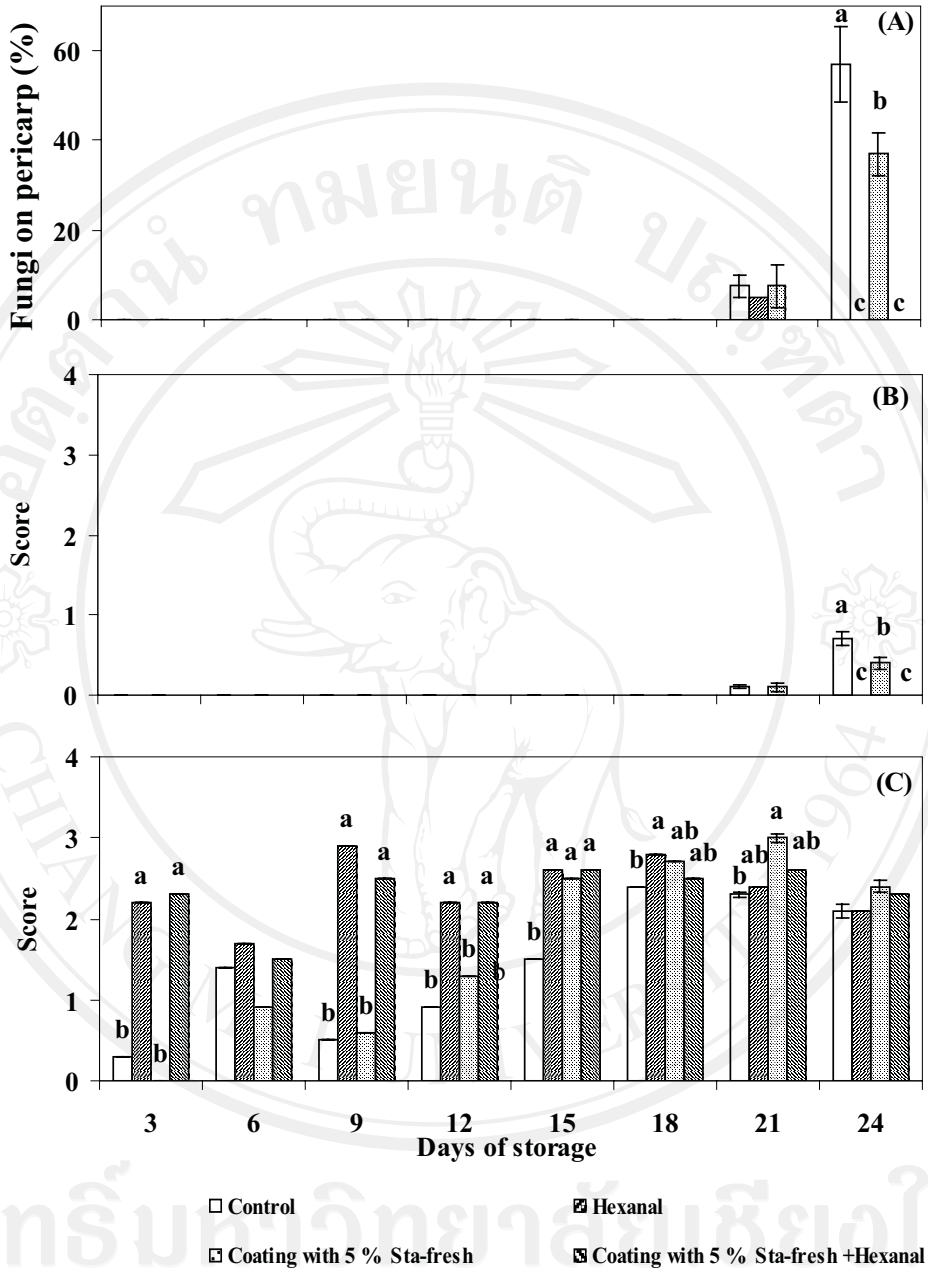

Figure 4.22 The percentage fungal incidence (A), fungi score (B) and phytotoxicity score (C) on longan fruit pericarp cv. Daw stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Scores are: 0 = no visual evidence, 1 = less than 10% of the surface, 2 = 10-30% of the surface, 3 = 30-70% of the surface and 4 = more than 70% of the surface. Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

Experiment 4.4 Effect of hexanal on some chemical components and some biochemical characteristics of longan fruit pericarp inoculated by *L. theobromae*

4.4.1 Phenolic compounds

The phenolic compound content in longan pericarp of the non-inoculated and inoculated fruit showed some reductions during the storage period (Figure 4.23A, Appendix Table 34). The hexanal fumigated fruit also showed reduced phenolic compound content of the pericarp. These might be caused by some chemical reactions which changed the phenolic compounds to new substances that could not be extracted or measured. Therefore, the measured phenolic compound contents were decreased during storage period and after hexanal fumigation.

4.4.2 Polyphenoloxidase activity (PPO)

The specific polyphenoloxidase activity of the longan pericarp declined during the storage period (Figure 4.23B, Appendix Table 35). Hexanal fumigation increased PPO activity of the cell. These results agreed with the dark brown color development on the pericarp after fumigation. *L. theobromae* inoculation did not increase the PPO activity of the longan pericarp, but the PPO activity of the pericarp of *L. theobromae* inoculated fruit after hexanal fumigation tended to increase during the storage period. This might be a synergistic effect between hexanal and *L. theobromae* infection

4.4.3 Peroxidase activity (POD)

The peroxidase activity of the pericarp tended to increase during the storage period (Figure 4.23C, Appendix Table 36). This phenomenon may have been caused by the autolysis of the pericarp cells after harvest and the infection of *L. theobromae*. Hexanal fumigation also increased the specific peroxidase activity of the cells. These results, along with increase in electrolyte leakage after fumigation, showed that the cells might be injured by the fumigation treatment.

4.4.4 Polygalacturonase

Polygalacturonase content in the pericarp could not be measured directly due to the lack of a polygalacturonase standard. Therefore, polygalacturonase content was measured by the absorbance at 575 nm per mg protein of the sample. This showed the relative levels of polygalacturonase in the samples instead of the absolute polygalaturonase activity (Figure 4.24A, Appendix Table 37). *L. theobromae* infection did not affect the polygalacturonase activity in the pericarp. However, polygalacturonase activity across all treatments tended to increase during storage. Hexanal fumigation also increased polygalacturonase activity in both inoculated and noninoculated fruit.

4.4.5 Cellulase

Cellulase activity in the pericarp responded in the same way as polygalacturonase activity. Cellulase activity also increased with storage time and hexanal fumigation (Figure 4.24B, Appendix Table 38).

Polygalacturonase is an enzyme that causes breakdown of the middle lamella between cell walls. Cellulase is an enzyme which causes breakdown of the cell wall. The increase of polygalacturonase and cellulase activities after hexanal fumigation may be signs of deterioration of pericarp cells.

Figure 4.23 Non-inoculated and *L. theobromae* inoculated longan fruit stored at 5° C for 30 days after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature versus no hexanal. A= non-inoculated fruit with hexanal. B= non-inoculated fruit without hexanal. C = Inoculated fruit with hexanal. D= Inoculated fruit without hexanal.

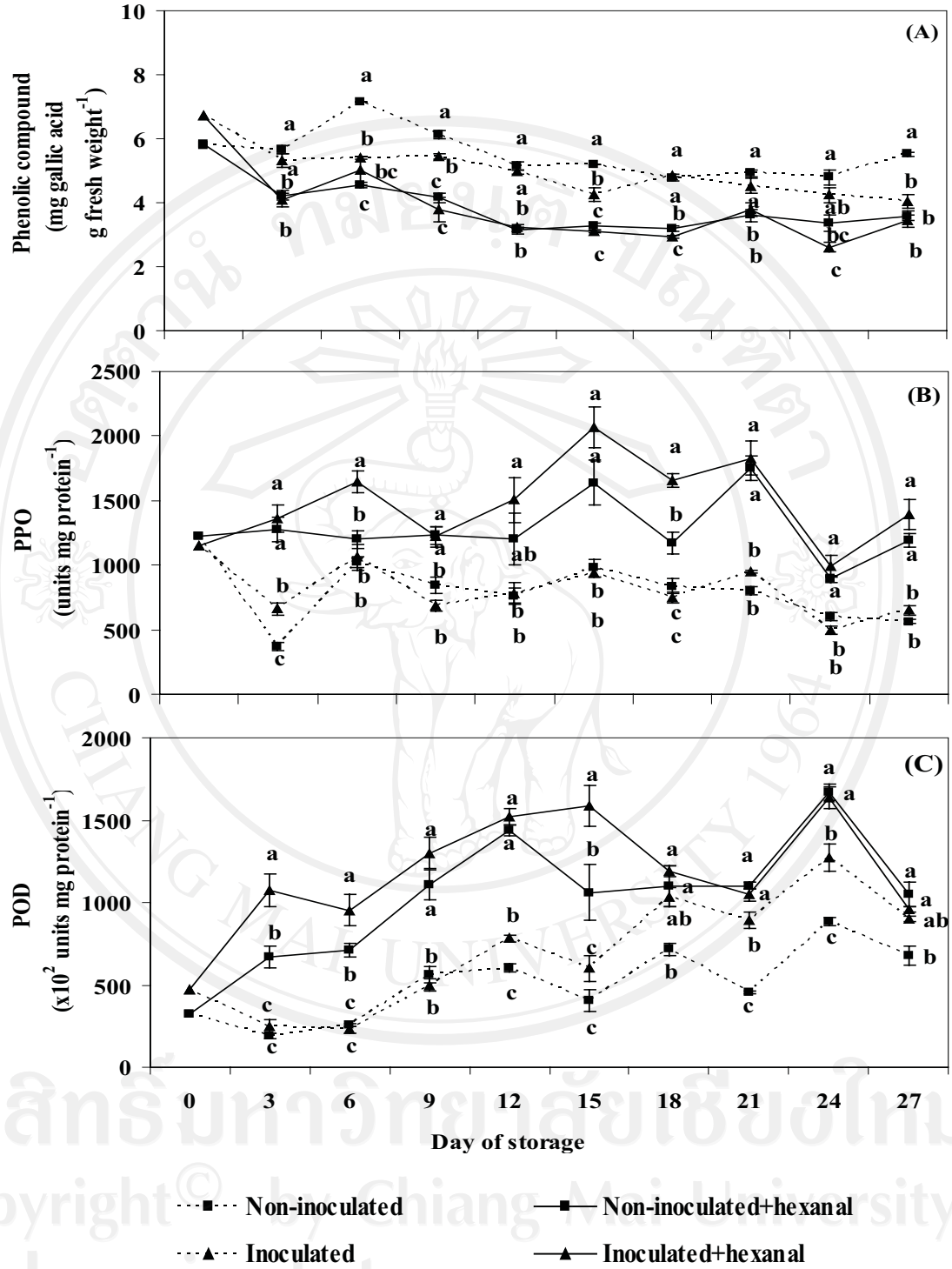

Figure 4.24 Phenolic compound (A), polyphenoloxidase activity (B) and peroxidase activity (C) of longan pericarp cv. Daw from non-inoculated and *L. theobromae*-inoculated fruit stored at 5°C after hexanal fumigation at 900 µl l^{-1} for 2 h at ambient temperature. Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).

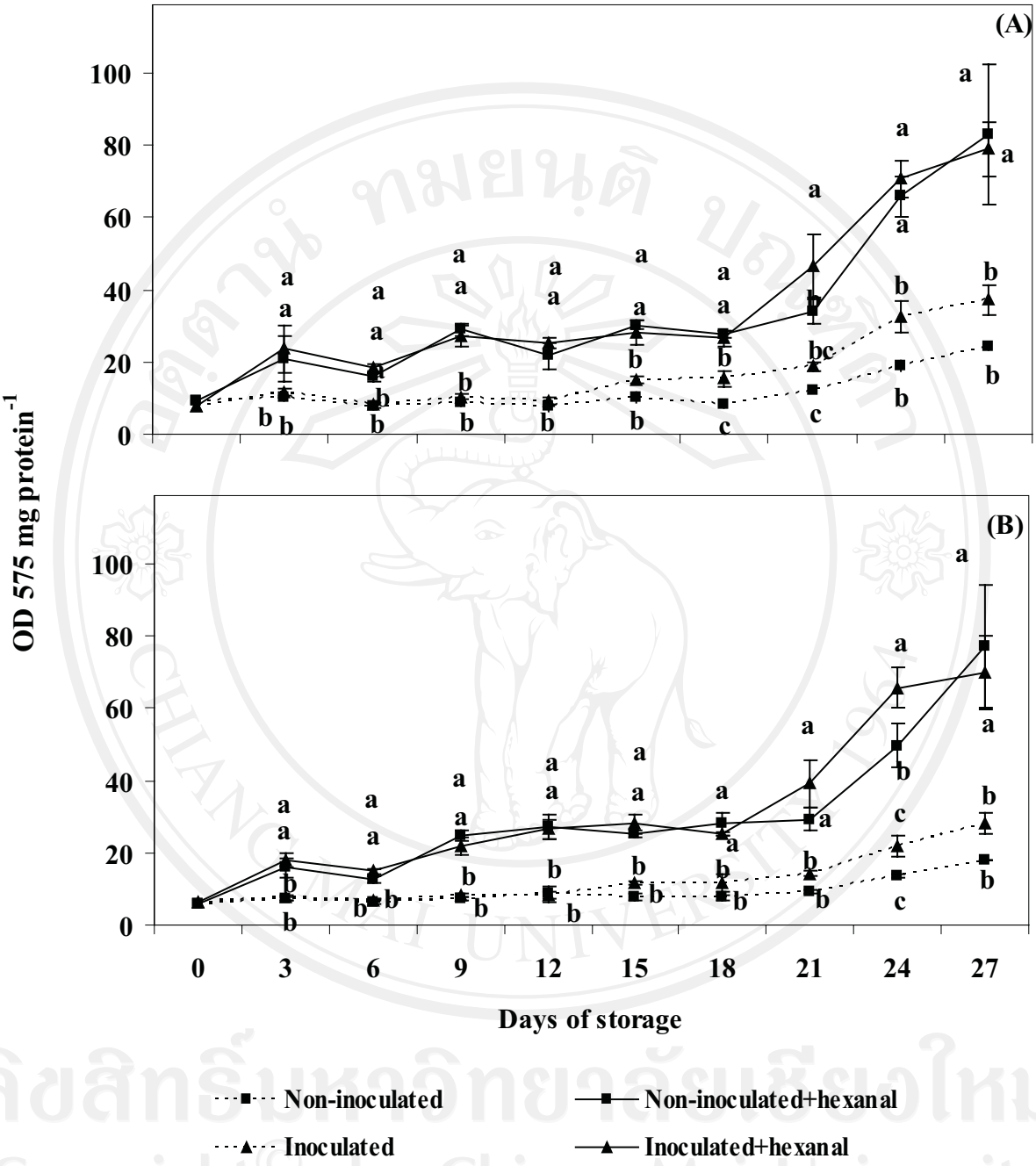

Figure 4.25 Activities of polygalacturonase (A) and cellulase (B) of longan pericarp cv. Daw from non-inoculated and *L. theobromae*-inoculated fruit stored at 5°C after fumigation with hexanal at 900 µl l^{-1} for 2 h at ambient temperature. Means with different letters within days are significantly different by Fisher's Least Significant Difference (*P*=0.05).