# Chapter 4

## EFFECT OF 1′-ACETOXYCHAVICOL ACETATE AND β-ASARONE ON RIPENING: PHYSIOLOGY AND MODE OF ACTION

### 4.1 Introduction

In Chapter 3, the active ingredient inhibiting growth of *Colletotrichum gloeosporioides* (Penz.) Sacc. in sweetflag is β-asarone , and in galanga is 1′-acetoxychavicol acetate. The effective dosage of β-asarone and 1′-acetoxychavicol acetate was 200 ppm and 370 ppm, respectively. The information are however still the results achieved from the laboratory scale. More information is required for application of these active substances under the field conditions, especially directly on mango fruit. Effects of environmental conditions, fungal infection, fruit maturity should also be taken into account. The optimum conditions for the effective application of crude extract must therefore be closely studied.

It was reported here and there about the side - effect of natural product on physiology and development of fruit especially in the ripening process (Wing, 1993). It might also be the case when apply β-asarone and 1′-acetoxychavicol acetate. These need to be observed, since the ripening process or respiration rate of the fruit will affect the market life of the fruit as well.

In this study the effect of β-asarone and 1′-acetoxychavicol acetate to control anthracnose disease on mango fruit as well as effect on fruit physiology and the mode of action of active substance on fungal development will be reported.

### 4.2 Material and Methods

#### 4.2.1 Study on optimum dosage and minimum fungicidal concentration (MFC)

The experiment was divided into two parts: for poison food technique and for fruit dipping method according to the suggest by Zehr *et al.* (1978) and Griffin(1988). $ED_{50}$ dosage achieved from previous studies (Chapter 3, 3.35, 2) for β-asarone (200 ppm) and 1′-acetoxychavicol acetate (370 ppm) were used as the basic value (X-value) . The concentration of

active substances were varied from 0, 0.25X, 0.75X, X, 1.25X, 1.5X, 1.75X and 2X, respectively and mixed into poison food PDA. The solution of active ingredients were mixed with adjuvant (25 % acetone: tween 80 = 10:1) to increase the dissolvability of active substance in PDA. Colony of *Colletotrichum gloeosporioides* (Penz.) Sacc. was transfered to the poison food PDA and percentage inhibition was measured (Dhingra and Sinclair, 1986).

The optimum dosage was calculated based on the Trend Comparison, orthogonal polynomial ($Y= a + bx + cx^2$) and the Optimum Dosage (OD) calculated from the following formula:

$$\mathbf{OD = MFC = -b / 2c} \quad \text{..... (Uppraditsakul, 1980)}$$

On the fruit dipping experiment, the optimum dosage was used as the basic concentration (X value) the dosage was then varied to become 0, 0.25X, 0.75X, X, 1.25X, 1.75X and 2X, respectively. The concentrations of active substance in the dipping solution varied as shown in Table 4.1.

**Table 4.1** Dosage of β-asarone and 1′-acetoxychavicol acetate in different dipping solutions according to the concentrations.

| Concentrations of dipping solution | Dosage (ppm) | |
|---|---|---|
| | β-asarone | 1′-acetoxychavicol acetate |
| 0.25X | 92.5 | 160 |
| 0.5X | 185.0 | 320 |
| 0.75X | 277.5 | 480 |
| X | 370.0 | 640 |
| 1.25X | 462.5 | 800 |
| 1.5X | 555.0 | 960 |
| 1.75X | 647.5 | 1,120 |
| 2X | 740.0 | 1,280 |

**Figure 4.1** Incubation of mango fruit in transparent plastic bag keeping at room temperature for 7 days.

The experiment was conducted based on factorial in completely randomized design (2x8) where type of the active substance as the first factor and concentration as the second.

Full mature mango fruits cultivar Nam – Dok – Mai, were used for the studied. Fruits were firstly thoughroughly washed and shade-dried for 3 hr before dipped in spore suspension of *Colletotrichum gloeosporioides* (Penz.) Sacc. (spore concentration $6x10^6$ spores/ml) for 5 minutes and dry at room temperature for another 3 hr then dipped in the dipping solution of active substance. Treated mango fruits were incubated under moisture condition in transparent plastic bag kept at room temperature (25 $^{\circ}$C) for 7 days (Figure 4.1).

Data collection:

Areas of disease symptom on the surface peel of mango were detected. Area infected were categorized into 6 groups (disease rating) according to method used by Korpraditsakul, *et al.* (1991).

| | |
|---|---|
| Level 0 | no disease symptom |
| Level 1 | symptom 1 – 5% of overall peel area |
| Level 2 | symptom 6 – 15% of overall peel area |
| Level 3 | symptom 16 – 30% of overall peel area |
| Level 4 | symptom 31 – 50% of overall peel area |
| Level 5 | symptom > 50% of overall peel area |

Uppraditsakul (1980)'s formula was again used to calculated the optimum dosage and minimum fungicidal concentration (MFC) to confirm the same parameter studied in the previous experiment (Chapter 3).

**4.2.2 Effect of active substance on fruit ripening physiology.**

The confirmed optimum dosage was used as basic concentration of the dipping solution and compared with benomyl at 225 ppm (suggested concentration from the Company). The control treatment used sterile distilled water mixed with adjuvant (25%acetone: triton X – 100 10:1). The experiment was conducted based on completely randomized design (CRD), used Nam – Dok – Mai mango 300 fruits. Dipping in spore suspension and in active substance solution and incubation of mango fruits were follow the same procedure explained in 4.2.1.

Data collection:

**1) Weight change (loss)**

Fruit were weighed every 2 days. Percent weight loss were calculated:

$$\frac{W_1 - W_2}{W_1} \times 100$$

$W_1$ = weight at the first day of experiment

$W_2$ = weight at the day measured.

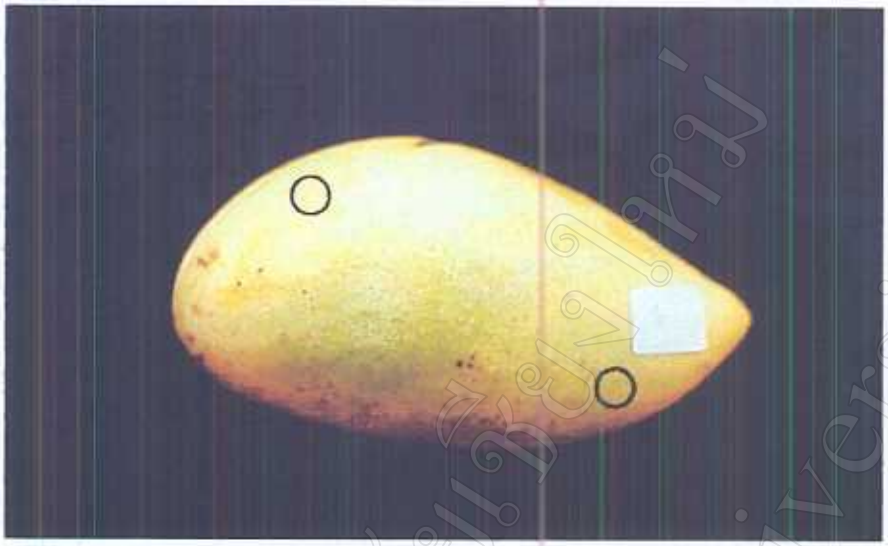

**Figure 4.2** Positions on mango fruit used to detect the fruit firmness and changes in peel and flesh colour. ( O = detected position)

**2) Fruit firmness**

Using fruit firmness testor (Model SHIMPO FGV – 50 A ) pressed both directly on the peel and on the flesh (mesocarp) at two positions on each fruit as shown on Figure 4.2. Detection took place every 2 days.

**3) Change in peel and flesh colour**

Effects of 1′-acetoxychavicol acetate, $\beta$ – asarone and Benomyl on disease appearance, peel and flesh colour were compared on fruits at 25 days of storage.

**4) Rate of Respiration**

Treated fruits were put in the plastic bag as shown in Figure 4.3. Fruits were kept under room temperature condition (25°C). $CO_2$ concentration excreted from the fruit through respiration were sucked from the plastic chamber and measure by Kanomax – Portable $CO_2$ Indicator Model 2312 every two days for 32 days/(Static method, Smith, 1995) and calculated as follow :

Calculated formula of $CO_2$ static system mg $CO_2$ $kg^{-1}$ $hr^{-1}$

$$= \frac{\text{free volume (m/s) x \% } CO_2\text{, diff x 321.75}}{\text{sample wt (kg) x mins scaled x (273+store temp)}}$$

$$\text{and \% } CO_2 \text{ diff.} = \frac{(\%\ CO_2 1 - \%\ CO_2 2)}{\text{Exp. Box volume}} \text{ X 100}$$

**5) Chemical change of the fruit**

Fruit juice was collected from the treated fruits every 5 days and used for detection of pH tritable acid (TA) assessment using reaction of critic acid and colour changing through phenophthalene (Green field *et al.*, 1993). Total soluble solid (TSS) of the fruit juice was measured using Digital Refractometer (Atago PR – 101).


**Figure 4.3** Chamber for measure $CO_2$ concentration by studying of respiration rate.

**6) Consummer's Perception (Sensory evaluation)**

Edible quality of flesh were tested by 5 panels every 5 days for 25 days (5 times). Testing parameters were mesocarp colour (Chaiyavanna, 1993), flesh aroma (Kanatham, 1994), taste (Kanatham, 1994), texture feeling when eat (Liumnak, 1998), and general acceptability (Chaiyavanna, 1993).

### 4.2.3 Mode of action studies

Results from previous studies showed unfavorable of β-asarone on fruit ripening. The substance could inhibit the fungal growth relatively good, but percentage weight loss and $CO_2$ emission were enhanced. Fruit ripening process was accelerated. β-asarone was therefore no more interesting and excluded from the experiment, only 1′-acetoxychavicol acetate remained the most potential substance for the further studying.

According to Hippe (1988) fungicide usually inhibits fungal growth and development by affecting the ultrastructure and physiological and biochemical activities at the cellular level. The comprehensive understanding of the Mode of Action is therefore necessary, by studies with sophisticated equipment such as electron microscope, GCMS, HPLC, etc. In this study change in ultrastructure of the fungal cell as affected by 1′- acetoxychavicol acetate will be emphasized using light microscope and transmission electron microscope (TEM).

**1) Preparation of studied materials**

Poison food technique was used by mixing 1′-acetoxychavicol acetate at different concentration; 0, 0.5X, X, 1.5X and 2X (X = 670 ppm.), respectively, into PDA. Colony of *Colletotrichum gloeosporioides* (Penz.) Sacc. was transferred to the PDA and incubated at room temperature for 15 days and used as studied material for microscope studies.

**2) Microscope studies**

For light microscope study, outer ridge of *Celletotrichum. gloeosporioides* (Penz.) Sacc. colony was dug out and embedded in paraffin before fine cut with ultramicrotome followed the method of Brown (1977). Sliced rectangular plates were then dyed with methylene blue before elucidated under light microscope (400X). Effect of antifungal substance on hypha

development can be observed from dye absorbance and darkening of the cell wall and organelles (Brown, 1977).

Effect of 1′- acetoxychavicol acetate on cell ultrastructure was further elucidated by transmission electron microscope (TEM). According to the method of O' Connell *et al.* (1985) outer ridge of the *Colletotrichum. gloeosporioides* (Penz.) Sacc. colony was firstly fixed for 12 hr in 3% glutaraldehyde solution. Sample was then transferred into 2% osmiumtetoxide in buffer solution (phosphate buffer) and soaked for 6 hrs. Sample was taken out and dipped in set of small beakers filled with acetone gradually increase in the concentration. Dipping was taking place in each beaker for 30 min long to allow acetone to replace the osmiumtetoxide. The dipping temperature by was kept at 4°C constantly. Acetone saturated samples were then fixed in Spur's resin before section with ultramicrotome and dyed with uranyl acetate and lead acetate before elucidated under transmission electron microscope (Jeol Electron Microscope Model 501 –2) in Central Laboratory of Research and Development Institute, Kasetsart University.

### 4.3 Result and Discussion

#### 4.3.1 Optimum dosage (OD) and minimum fungicidal concentration (MFC)

**1) Poison food studies**

The studies were divided into 2 experiments; PDA poison food and fruit dipping method. The PDA poison food technique showed a relative stronger effect of β-asarone than 1′-acetoxychavicol acetate (ACA) in control colonial growth of *Colletotrichum gloeosporioides* (Penz.) Sacc. A smaller colony diameter was observed on β-asarone mixed PDA than on 1′- acetoxychavicol acetate (ACA) (Figure 4.4 and 4.5).

By comparing the percentage of inhibition between β-asarone and 1′- acetoxychavicol acetate (ACA) at the same concentration (Table 4.3), it was found that β-asarone had a significant higher inhibition percentage than 1′- acetoxychavicol acetate (ACA), but only in the range of upto the concentration fro 0.25X to X. At the higher concentration of 1.25X to 2X both substances promoted similarly high inhibition percentage, but not significantly difference. (Table 4.2).

**Table 4.2** Percentage inhibition of β-asarone and 1′- acetoxychavicol acetate at different concentration on *Colletotrichum gloeosporioides* (Penz.) Sacc.

| Active ingredient | Percent inhibition at each concentration [1/] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.25X | 0.5X | 0.75X | X | 1.25X | 1.5X | 1.75X | 2X |
| β-asarone | 52.78[a 2/] | 62.59[a] | 65.55[a] | 71.11[a] | 71.67[NS] | 71.85[NS] | 75.73[NS] | 87.04[NS] |
| 1′- acetoxychavicol acetate | 39.87[b] | 44.43[b] | 52.10[b] | 57.33[b] | 68.14 | 72.24 | 79.83 | 92.06 |
| CV (%) | 15.20 | 14.96 | 13.34 | 12.06 | 13.46 | 16.15 | 14.90 | 10.87 |

[1/] Percent inhibition average from 6 replications

[2/] means in the same column followed by different subscript differs significantly at P≤0.05


**Figure 4.4** Growth of *Colletotrichum gloeosporioides* (Penz.) Sacc. on poison food PDA mixed with 1′- acetoxychavicol acetate (ACA) at different concentration compared to control (CT).


**Figure 4.5** Growth of *Colletotrichum gloeosporioides* (Penz.) Sacc. on poison food PDA mixed with β-asarone at different concentration compared to control (CT).

According to the correlation equation for optimum dosage (OD) and poison food technique, minimum fungicidal concentration (MFC) of the effective substances were:

1) β-asarone : $Y = 19.20 + 7.4x - 0.01x^2$

$$OD = MFC = \frac{-b}{2c} = 370 \text{ ppm}$$

2) 1′-acetoxychavicol acetate : $Y = 40.96 + 51.2x + 0.04x^2$

$$OD = MFC = \frac{-b}{2c} = 640 \text{ ppm}$$

which could be demonstrated in the curve from of the correlation equation as showed in Figure 4.6.

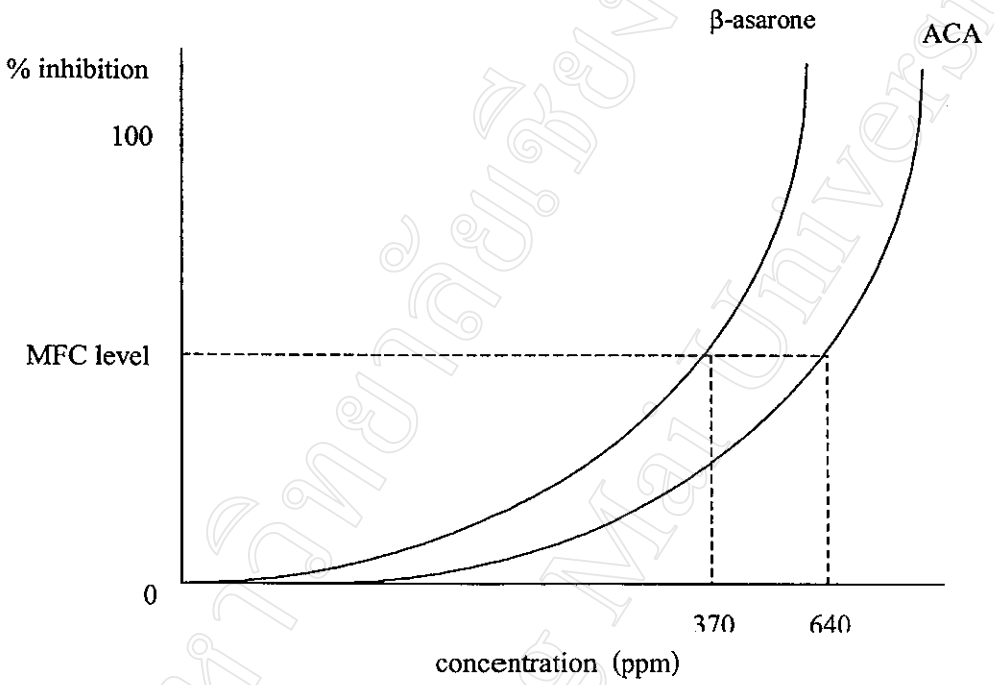


**Figure 4.6** Correlation equation showed optimum dosage (OD) from poison food technique and minimum fungicidal concentration (MFC).

**2) Fruit dipping method**

In this experiment Nam –Dok – Mai mango fruits were dipped in different concentrations either of β-asarone or 1′-acetoxychavicol acetate (ACA) and incubated for 18 days. Area of disease symptom on the surface peel were categorized as the so call " disease rating " or "disease incidence" as shown in table 4.3.

**Table 4.3** Disease incidence on mango fruits at 18 days after dipped in active substance solution.

| Active ingredient | Concentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 01 | 02 | 0.25X | 0.5X | 0.75X | X | 1.25X | 1.5X | 1.75X | 2X |
| β-asarone | 5 | 4.9 | 4.2 | 3.2 | 3.1 | 0.8 | 0.7 | 0.4 | 0.3 | 0.3 |
| 1′-acetoxychavicol acetate | 5 | 4.9 | 4.7 | 3.5 | 3.5 | 1.3 | 1.2 | 1.2 | 1.1 | 1.0 |
| LSD | NS | | | | | | | | | |

Remarks: 01 = control ($H_2O$)

02 = control ($H_2O$ + adjuvant)

Disease incidence (Disease rating, Korproditsakul *et al.*, 1991)

Level 0 = No symptom

Level 1 = symptom 1 – 5 % of overall peel area

Level 2 = symptom 6 – 15 % of overall peel area

Level 3 = symptom 16 – 30 % of overall peel area

Level 4 = symptom 31 – 50% of overall peel area

Level 5 = symptom > 50 % of overall peel area

β-asarone and 1′-acetoxychavicol acetate (ACA) had the similar disease rate, which mean the similar efficiency to control mango fruit-rot disease. Comparing the effect of different concentration used, the higher concentration the lower disease incidence (Table 4.3) was observed. The effects are clearly seen in Figure 4.7.

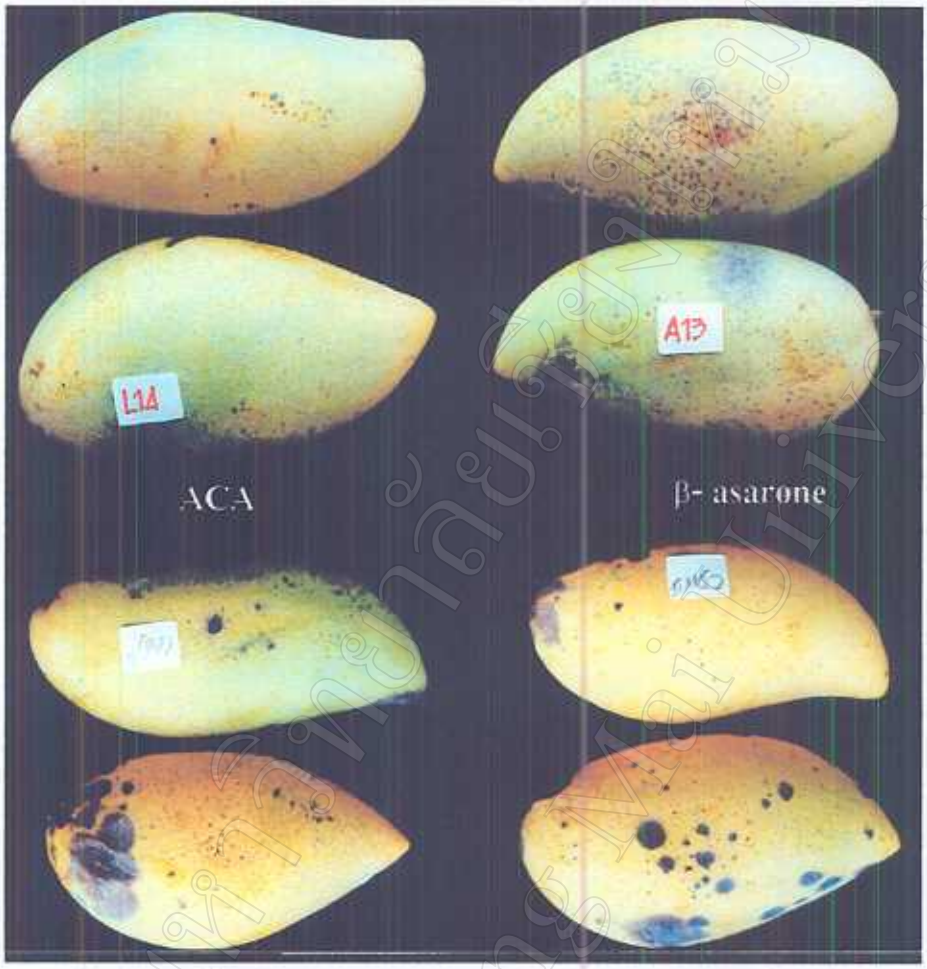


**Figure 4.7** Effect of β-asarone or 1′-acetoxychavicol acetate (ACA) (at the concentration of 1.5x) on control of anthracnose disease compared to control (water treatment or water plus adjuvant).

The optimum dosage for β-asarone or 1′-acetoxychavicol acetate (ACA) were calculated based on the data on Table 4.3 using Uppraditsakul' s formular (1980 ). The result revealed that optimum dosage or minimum fungicidal concentration (MFC) for each substance as followed:

1) $\beta$-asarone : $Y = 24.1 + 7.5x - 0.01x^2$

$$OD = MFC = \frac{-b}{2c} = 375 \text{ ppm}$$

2) $1'$-acetoxy chavicol acetate: $Y = 48.8 + 53.6x - 0.04x^2$

$$OD = MFC = \frac{-b}{2c} = 670 \text{ ppm}$$

The optimum dosage (or MFC) achieved from the fruit dipping method was slightly higher than those achieved by the poison food technique; 375 compared to 370 ppm for $\beta$-asarone, and 670 compared to 640 ppm for $1'$-acetoxychavicol acetate. The results confirmed the accuracy of the effective concentration of active substance to be used to control *Colletotrichum gloeosporioides* (Penz) Sacc. Since such kind of experiments were never conducted and reported before, these concentrations of $\beta$-asarone and $1'$-acetoxychavicol acetate were accepted for further studying.

### 4.3.2 Effect of $\beta$ - asarone and $1'$ – acetoxychavicol acetate (ACA) on disease rating compare with benomyl.

According to the method recommended by Korproditsakul, *et al.* (1991) which suggest to categorized the disease rating into 6 levels from no symptom (1), symptom 1-5% of peel area (2), symptom 6-15% of peel area (3), symptom 16-30 of peel area (4), symptom 31-50% of peel area (5), and over 50% of peel area (6), respectively.

In this studies mango fruit (Nam-Dok-Mai) treated with $\beta$ - asarone, ACA, adjuvant, benomyl and water (control) were evaluated the symptom area at day 21 after storage. The result in Table 4.4 revealed that ACA was the most effective substance to control mango fruit rot followed by benomyl and $\beta$ - asarone respectively. Fruits treated with ACA showed the symptom area of only 6-15%, where as those treated with benomyl and $\beta$ - asarone showed the same symptom area 16-30% of the peel area. Control fruit showed 3 1 - 50% symptom area on the peel.

**Table 4.5** Disease rate of mango fruits treated with β - asarone and 1′ – acetoxychavicol acetate on day 21 after storage

| Treatment | Replication | | | | | | | | | | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| β - asarone | 2 | 3 | 4 | 3 | 2 | 3 | 4 | 3 | 4 | 3 | 3.1 [b] |
| 1′ – acetoxy chavicol acetate (ACA) | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 2.6 [c] |
| Adjuvant | 4 | 5 | 5 | 5 | 4 | 5 | 5 | 5 | 5 | 5 | 4.8 [a] |
| benomyl | 2 | 3 | 4 | 3 | 3 | 3 | 2 | 3 | 4 | 3 | 3.0 [b] |
| Water (control) | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 4.9 [d] |

Means in the same column followed by different superscript differs significantly at $p<0.05$

### 4.3.3 Effect of β-asarone and 1′-acetoxychavicol acetate on fruit ripening physiology

In the Table 4.5 was the percent loss of fruit weight after dipping in β-asarone and ACA compared with the effect of benomyl, adjuvant and water (control). Fruit treated with water and adjuvant demonstrated the highest percent weight loss when keeping from 5 to 15 days. β-asarone and as well as ACA gave the similar lowest percent weight loss like Benomyl. These parameters suggested the positive effect of natural substance (β-asarone and ACA). Since Benomyl is the synthetic chemical product. Therefore β-asarone and ACA might be the natural substance to replace benomyl for future postharvest mango treatment.

**Table 4.5** Percent loss of fruit weight after treated with β-asarone and 1′-acetoxychavicol acetate (ACA )

| Dipping solution | Percent weight loss after the treatment (days) | | | | | |
|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30[1/] |
| β-asarone | 0.88ab | 1.01ab | 1.64b | 2.43b | 3.01ab | 4.33a 2/ |
| 1′-acetoxychavicol acetate (ACA ) | 0.83b | 0.95b | 1.63b | 2.51b | 2.81b | 3.65ab |
| Adjuvant | 0.89ab | 1.12a | 2.34a | 3.15a | 3.54a | 3.92a |
| benomyl | 0.90a | 1.05ab | 1.57b | 2.63b | 2.95ab | 3.34b |
| Water (control) | 0.91a | 1.10a | 2.25a | 3.8a | 3.32b | 3.8a |
| CV (%) | 18.54 | 16.33 | 21.81 | 19.35 | 14.49 | 15.87 |

[1/] days after dipping in studied solution

[2/] means in the same column followed by different subscript differs significantly at P≤ 0.05

**4.3.4 Firmness of the fruit**

Peel and flesh firmness were measured using firmness tester (Model SHIMPO FGV-50A). The results showed in Table 4.6 In general peel of mango fruits were very hard when unripe. After 5 days of storage peel firmness decreased around 15% and decrease drastically after 10 and 15 days of storage to become very soft at 25 days of storage. Fruit treated with ACA showed significantly higher peel firmness than those treated with β - asarone and benomyl, adjuvant and water (Table 4.6)

**Table 4.6** Peel firmness ($kg/cm^2$) of mango fruit treated with β- asarone and 1′-acetoxychavicol acetate.

| Dipping solution | Day of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| β - asarone | 223.35 | 163.75b [1/] | 79.84 b | 56.58 c | 40.67 bc | 31.13 b | 16.69 c |
| 1′ – acetoxy chavicol acetate (ACA) | 223.35 | 177.73 a | 96.37 a | 69.15 a | 49.88 a | 39.17 a | 21.15 a |
| Adjuvant | 223.35 | 151.17 c | 71.37 c | 48.97 ab | 36.11 bc | 26.67 c | 18.39 c |
| benomyl | 223.352 | 161.33 b | 80.44 b | 65.37 b | 45.75 b | 32.13 b | 19.67 b |
| Water (control) | 223.35 | 142.67 d | 64.44 d | 47.38 d | 31.56 | 25.56 c | 13.75 d |
| CV (%) | - | 18.37 | 15.04 | 17.89 | 21.44 | 13.50 | 14.07 |

[1/] means in the same column followed by different subscript differs significantly at $P \leq 0.05$

Similar to the peel firmness, the flesh (mesocarp) firmness also drastically decreased only on 5 days of storage and decrease gradually until fruit decay on day 25. Also similar to the effect on peel firmness ACA could prolong the flesh firmness, which were higher than those from fruit treated with β - asarone. In the case of flesh firmness ACA gave the same firmness value as benomyl (Table 4.7).

The positive effect of ACA on peel firmness and flesh firmness, which better similar to benomyl suggested the high potential of this substance for the postharvest treatment.

The better the fruit firmness is the higher tolerance to the transport can be expected and also the longer shelflife could be achieved. From these reasons it might suggest the best possibility of ACA, if the other parameters like effect on chemical quality still very positive.

**Table 4.7** change in flesh firmness in different storage day as affceted by β - asarone and 1′ – acetoxychavicol acetate .

| Dipping solution | Day of storage | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | 20 | 25 |
| β - asarone | 124.85NS [1/] | 0.63 NS | 0.28 b [3/] | 0.22 ab | 0.16 b | 0.59 |
| 1′ – acetoxy chavicol acetate | 124.85 | 0.62 | 0.33 a | 0.24 a | 0.25 a | 0.09 |
| Adjuvant | 124.85 | 0.64 | 0.24 c | 0.22 ab | 0.18 b | ND 2/ |
| benomyl | 124.85 | 0.64 | 0.30 c | 0.23 ab | 0.21 a | 0.06 |
| Water | 124.85 | 0.63 | 0.24 a | 0.21 b | 0.13 c | ND |
| **CV (%)** | - | 25.34 | 19.84 | 13.45 | 18.37 | |

1/ fruit still unripe

2/ no determination due to fruit rot condition

3/ means in the same column follwed by different subscript differs significantly at P≤ 0.05

**4.3.5 Change in peel colour and flesh colour**

In figure 4.8 and 4.9 are the fruit characteristics at 25 days after treated with 1′ – acetoxychavicol acetate, β-asarone, benomyl, adjuvant and control (water). Mango fruits treated with β-asarone and control were seriously infected by anthracnose disease, when compared to these treated with 1′ – acetoxychavicol acetate and benomyl. Peel colour and flesh colours were similar by all the treatments.

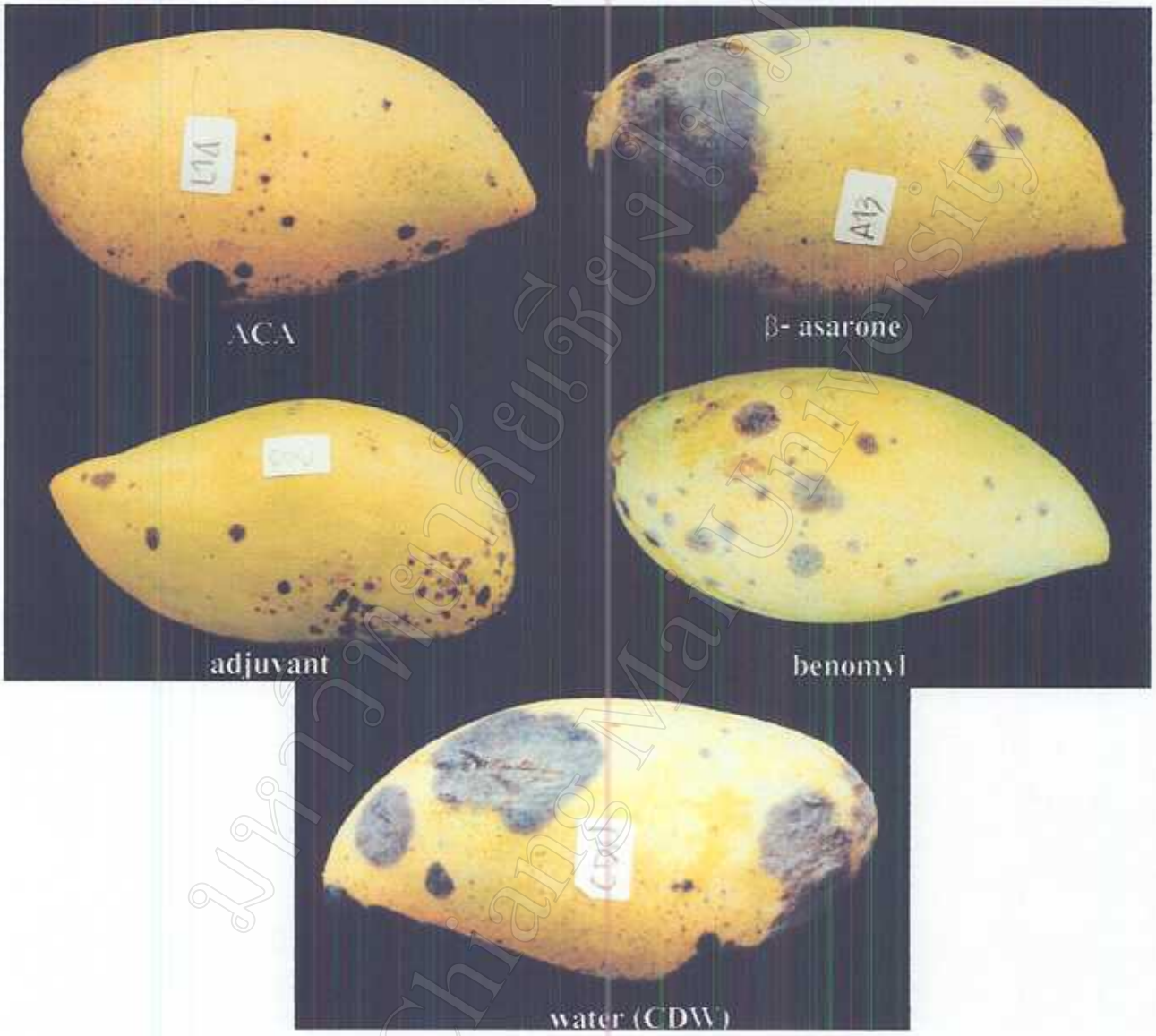


**Figure 4.8** Disease infested and peel colour of Nam-Dok-Mai mango fruit treated with 1′-acetoxychavicol acetate (L14), β- asarone (A13), benomyl, adjuvant (Cadj) and water (CDW)

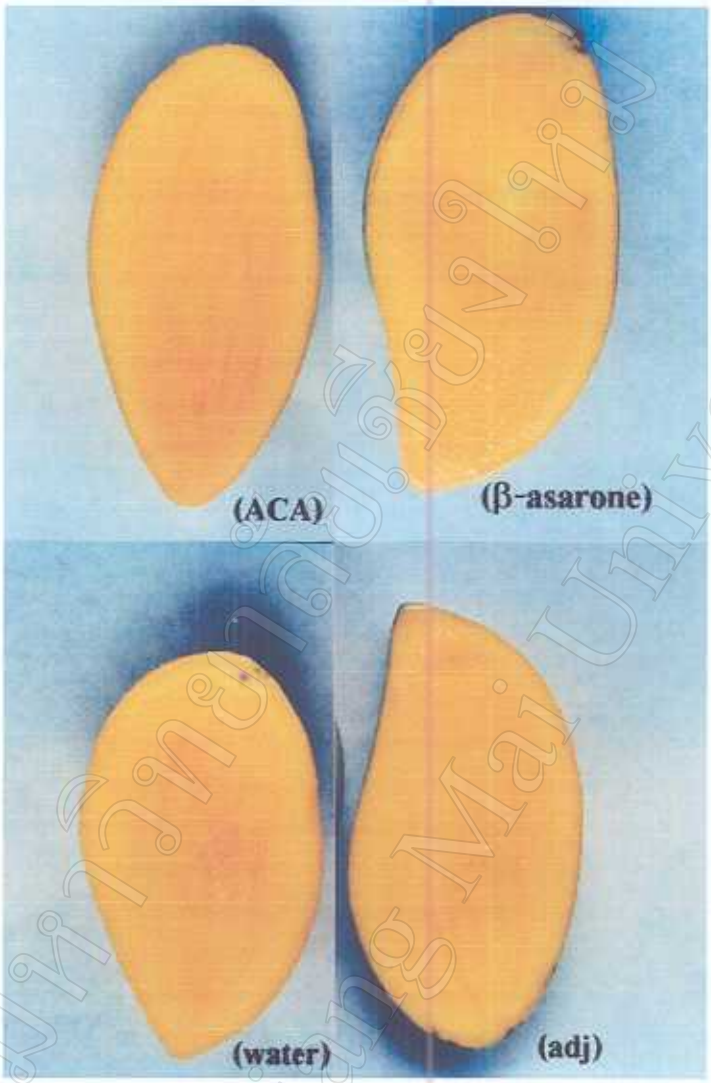


**Figure 4.9** Flesh colour and juiciness of mango fruit at 25 days (storage at room temperature 25 °C) after treated with 1′ – acetoxychavicol acetate (upper left) , β-asarone (upper right), adjuvant (lower right) and water (lower left).

**4.3.6 Rate of respiration**

As shown in Figure 4.10 the respiration rate of mango fruit generally increased gradually from the first days of storage and reached the peak at around 14 days to 18 days of storage. The peak of the respiration rate was around 180 mg. $CO_2$. $kg^{-1}$,$hr^{-1}$. and decreased gradually afterward.

Mango fruits treated with $\beta$ - asarone showed higher respiration rate than ACA treated and control, especially when the fruit arrived their highest respiration activity. Comparing between fruits treated with ACA and bemomyl, it was found a lower respiration rate in fruit treated with ACA, at least by the first 18 days of storage.

These results suggested the most beneficial activity of ACA in delaying the fruit ripening when compared to benomyl and $\beta$ - asarone respectively. A lower respiration rate could prolong the shelflife of fruit in the market. In the opposite way the higher respiration rate could shorten the shelflife of mango. In this case ACA trended to be a better substance for postharvest treatment of mango compared to the $\beta$ - asarone or even benomyl.

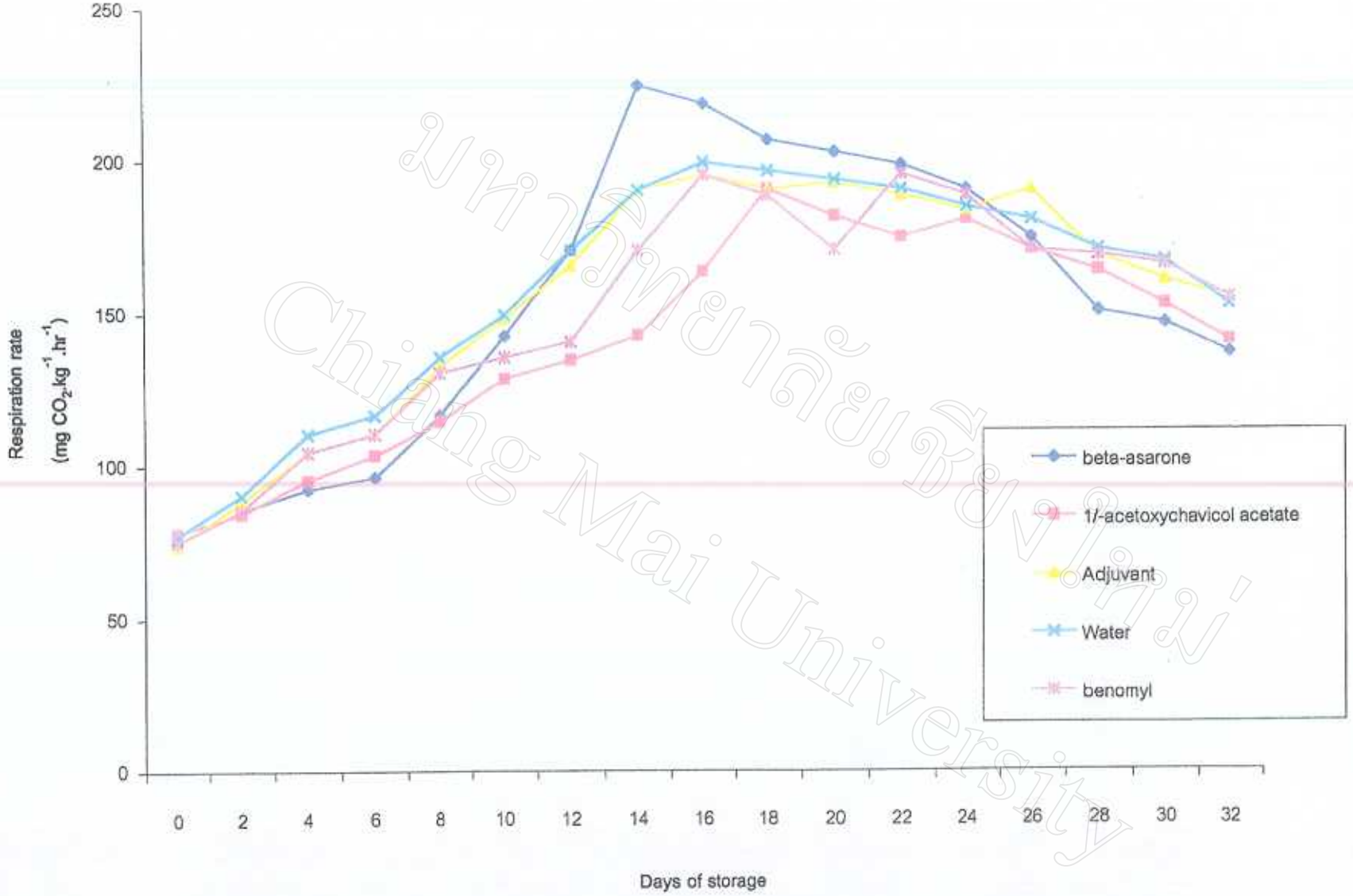


**Figure 4.10** Change in respiration rate of mango fruits as affected by β-asarone and 1′ - acetoxychavicol acetate.

**4.3.7 Chemical change of the fruit**

In the Figure 4.11 are pH value, tritable acid and total soluble solids of mango fruit during the storage for at 25 days. It was found that pH value of fruit juice increased form 3.7-4.46 from the first day of storage to be the highest peak at around 6 on day 15 after storage and remained almost constance afterward. β - asarone and ACA had no effect on pH changing pattern (Statistical analysis showed no significant difference)

Tritable acid firstly increased on the first 5 days of storage, decreased however after 10 days of storage and remained relative constance afterward. Tritable Acid also was not affected by β - asarone and ACA. Pattern of tritable acid changing was similar by all treatments in the whole storage study.

Change in total soluble solids during the storage time measured by hand refractometer showed slightly increase in the degree brix from around 15° by the beginning of storage to be the highest peak at around 18-20 °brix at 10 days after storage, after that the total soluble solid gradually dropped down (Figure 4.11).

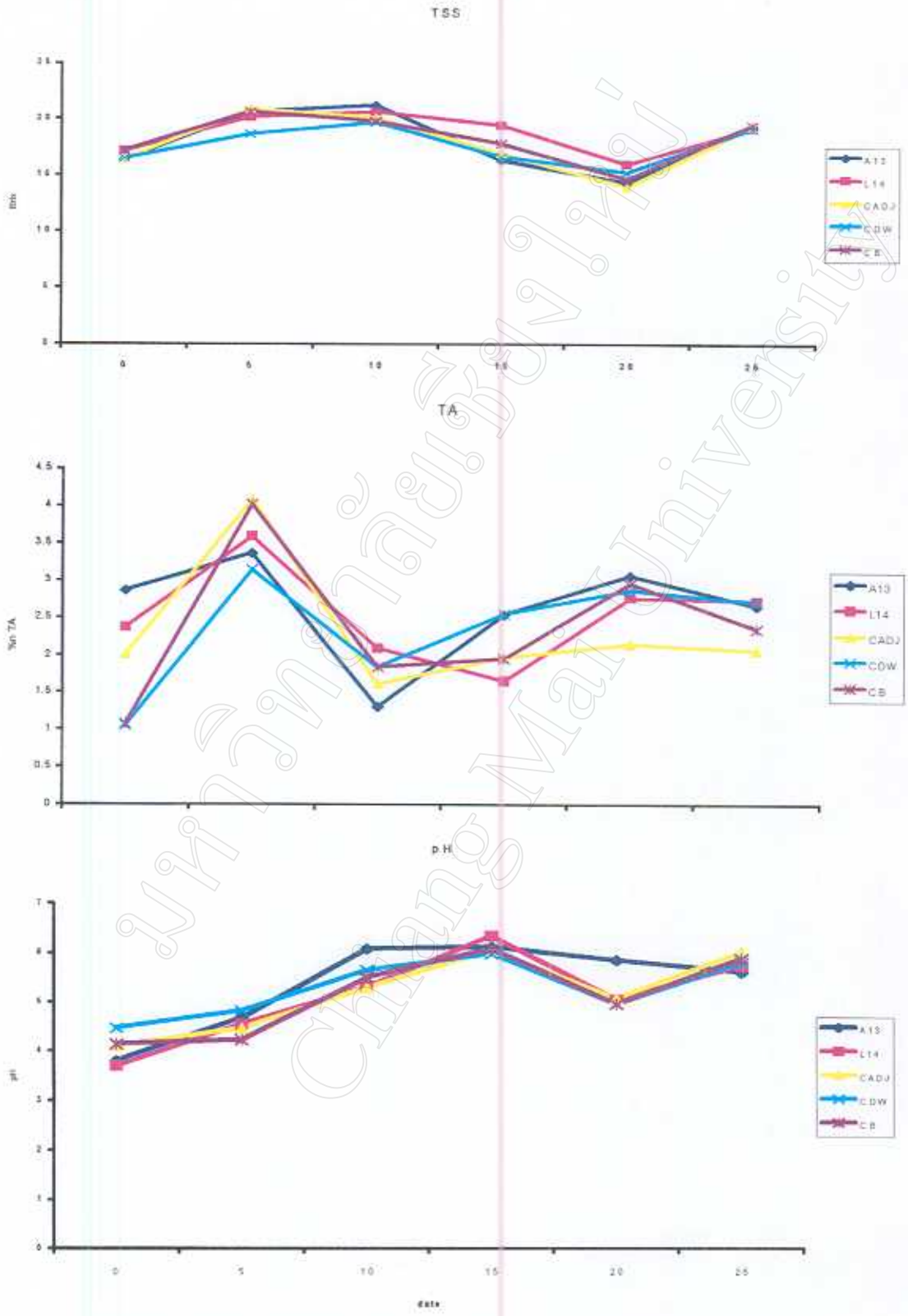


**Figure 4.11** Change in pH, percent titrable acid, and total soluble solid of mango fruits treated with β - asarone, 1′ – acetoxychavicol acetate and benomyl.
A13 = β - asarone, L14=ACA, CADJ=adjuvant, CDW= water(control) CB=benomyl

**4.3.8 Consumer perception ( Sensory evaluation)**

**1) Fruit characteristics**

Fruit characteristics were stuied by sensory evaluation method, for which 5 panels wre used (Table 4.8)

During ripening, Nam-Dok-Mai mango fruit changed the flesh colour from pale to dark yellow within 10 days of storage (ripening stage). After that flesh colour remained yellow even until 25 days of storage. Flesh aroma also remained relatively constant even up to 25 days of storage. The taste was however change from sweet and sour in the early stage of ripening to become very sweet at 25 days of storage. All the treated substances; $\beta$ - asarone, $1'$ – acetoxychavicol acetate and benomyl showed no significant effect on flesh colour, flesh aroma and taste, this means no effect of the treatments on fruit ripening (Table 4.8).

**2) Consumer's perception**

For the consumer's perception (Table 4.9) on the aspect of texture perception and general acceptability, $\beta$ - asarone, $1'$ – acetoxychavicol acetate and benomyl had no effcet on the two parameter of the fruit. In general fruit texture on Nam-Dok-Mai fruit were relative soft even at 10 days of storage it remained up to15 days, however on day $25^{th}$ of storage the teste panel gave the score of unacceptable. This was because of the change in peel colour to become dark brown and very soft texture up to juicy texture in some fruits.

**Table 4.8** Effect of β - asarone, 1′ – acetoxychavicol acetate on changes in flesh colour, flesh aroma and taste of mango fruit during of storage (Consumer's Perception).

| Treatment | Flesh colour | | | | Flesh aroma | | | | Taste | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1[1/] | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| β - asarone | 3 | 4 | 3.8 | 3.6 | 3.6 | 3.8 | 3.6 | 3.2 | 3 | 5.8 | 2.8 | 6 |
| 1′- acetoxy chavicol acetate | 3 | 3.2 | 3.5 | 3.2 | 3.9 | 3.6 | 3.8 | 3.2 | 3 | 5.8 | 5.8 | 6 |
| Adjuvant | 4 | 4.4 | 3.9 | 3.6 | 4 | 3.0 | 3 | 3.2 | 4.8 | 5.8 | 5.8 | 6 |
| Benomyl | 3.8 | 4.6 | 4 | 3.4 | 3.8 | 3.6 | 3.6 | 4 | 4 | 5 | 5 | 6 |
| Water | 3.5 | 3.6 | 4.2 | 3.4 | 3.6 | 3.6 | 3.4 | 3.2 | 3.8 | 5.4 | 5.6 | 6 |

[1/] Days of storage

Score of flesh colour : 1= white, 2=whitish-yellow, 3=pale yellow, 4=dark yellow, 5=yellowish orange, 6=orange, 7=orangish-red

Score of flesh aroma : 0=fermented, 1=raw smell, 2=no raw smell, 3=slightly ripe, 4=overripe smell

Score of flesh taste : 0=obnormal, 1=no taste, 2=sour, 3=sweet and sour, 4=slightly sweet, 5=medium sweet, 6=very sweet

**Table 4.9** Consumer's perception of the fruits treated with β - asarone, 1′ – acetoxychavicol acetate

| Treatment | Texture (when eat) | | | | General acceptability | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| β - asarone | 2.6 | 2.4 | 2.4 | 2.5 | 6.4 | 8 | 8 | 1.8 |
| 1′ - acetosychavicol acetate | 3.2 | 2.4 | 2.4 | 1.8 | 4.6 | 7.4 | 7.4 | 1.8 |
| Adjuvant | 3.2 | 2 | 2 | 2 | 6.6 | 6.4 | 6.4 | 1.4 |
| Benomyl | 3.8 | 2 | 2 | 2 | 6.4 | 6 | 6 | 1.6 |
| water | 3.2 | 2.2 | 2.2 | 1.8 | 5.8 | 7.4 | 7 | 2 |

Score of texture : 1 – 6 (Very hard to hard)

Score of acceptability : 1= un-acceptable, 2=very dislike, 3=dislike, 4=slightly-dislitec, 5=acceptable, 6=slightly like, 7=like, 8=more like, 9=very like

### 4.3.9 Mode of action

It can be concluded from many previous studies comparing the effect of β - asarone, 1′ – acetoxychavicol acetate (ACA), that β - asarone was unfavorable to be used for mango postharvest treatment. β - asarone could inhibit the fungal growth relative good but percentage weight loss (Table 4.5 ), $CO_2$ emission were enhanced (Figure 4.11), fruit ripening process was accelerated, fruit firmness decreased faster than fruit treated with ACA (Table 4.7). β - asarone was therefore excluded form the experiment. Only ACA remained the most interesting substance for the further studying.

**1) Light microscope studied**

As shown in Figure 4.12 hypha of *Colletotrichum gloeosporioides* (Penz.) Sacc. Absorbed more methylene blue when cultivated on higher concentration of ACA (2X > X >control). In control treatment hyphae looked transparent whereas hyphae grown in PDA mixed with ACA at the concentration X only the cell wall absorbed methylene blue. Hyphae grown on to X poison food PDA cell wall and most of cell organelle absorbed methylene blue and became dark blue in colour (Figure 4.12).

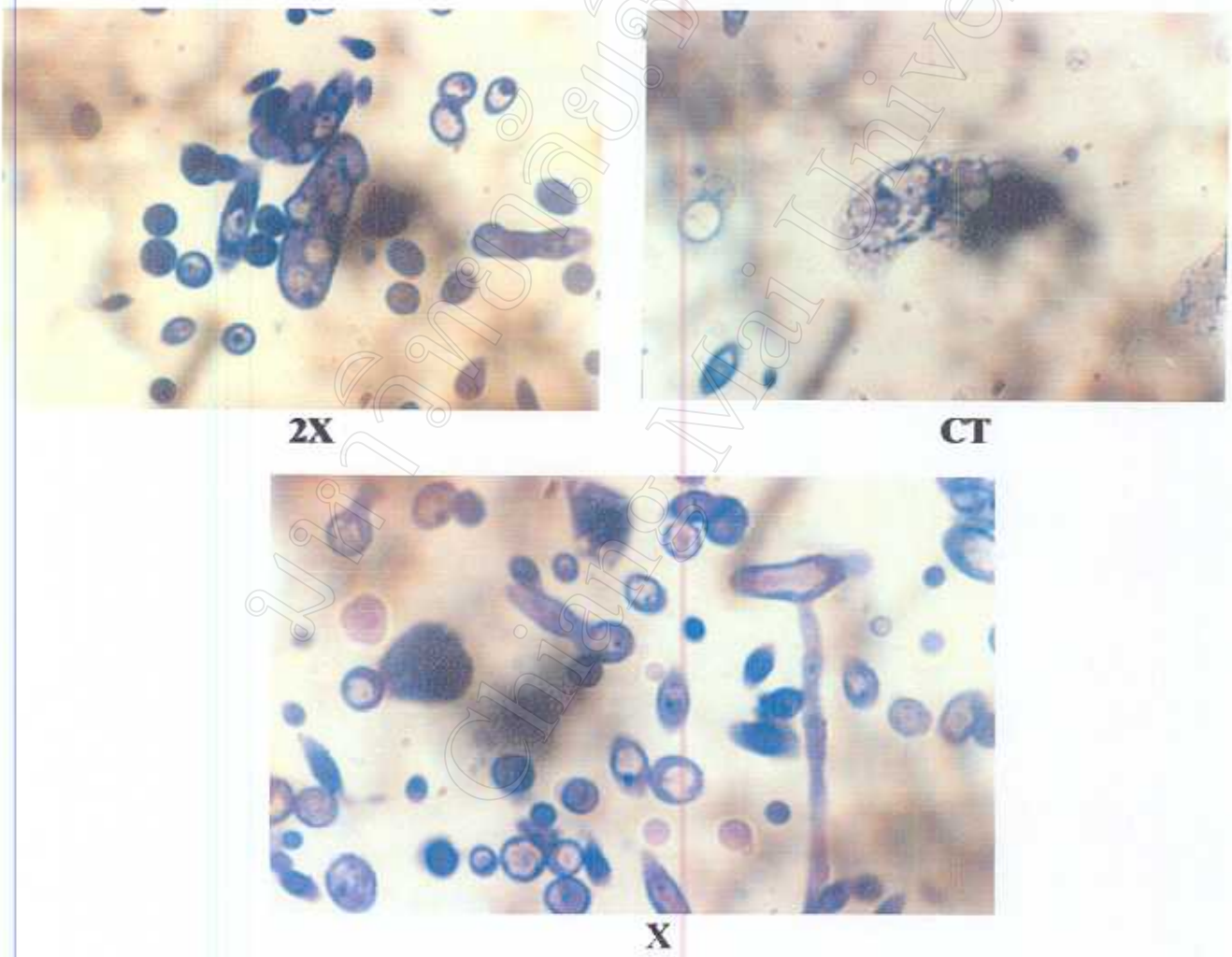


**Figure 4.12** Methylene blue absorbed hyphae of *Colletotrichum gloeosporioides* Penz. (Sacc.) after grown in different concentration of 1′ – acetoxy chavicol acetate. (CT= normal, X= Poisonfood ACA=670 ppm., 2X=poisonfood ACA=1340 ppm)

**2) Transmission Electron Microscope study**

According to Hippe' (1988) antifungal agent affected growth and development of pathogen both in biochemical and cellular aspects. To achieve the comprehensive understanding of the mode of action of fungicidal substance, electron microscopy study should be found out to provide importance data on ultrastructural change and biochemical as well as biophysical effects on fungi. Hippe' (1988) reviewed the effect of fungicidal on fungal growth and development and reported possible activities of fungicide in many aspects; e.g. inhibition of respiration process, lipid peroxidation, chitin synthesis inhibition, sterol-biosyhthsis inhibition. In the case of Benzimidazoles fungicide (benomyl, carbendazim, nocodazole), the fungicide activity occurred by binding to $\beta$ -tubulin and thus disrupting microtubule function, which consequently disturbed cell structure and influence cell development in especially spitzenkörper region.

In this study there was no facility to study the effect of 1'-actoxychavicol acetate on biological and biochemical change in the cell of *Colletotrichum gloeosporioides* (Penz.) Sacc., but the ultrastructural changes was observed by using by Transimission Electron Microscope (TEM). From the previous results (Light Microscope studied), *Colletotrichum gloeosporioides* (Penz.)Sacc. cell treated with ACA also showed a better absorbance of uranyl acetate and lead actate, which gave a darker colour of cell organelles (Figure 4.13). The figure also showed abnormal development of septate on the ACA treated hypha. Cell of *Colletotrichum gloeosporioides* (Penz.) Sacc. with normal growth usually consisted of a big vacuole, which took more than 90% of the cell volume (Figure 4.13), whereas in no vacuole was clearly observed the ACA treated cell. Cell organelle distributed throughout the cell.

These are very few information concerning the effect of ACA on fungal growth and development. Mann (1987) reported that ACA was the product of pentose phosphat pathnway in the group of phenyl propanoid with containing alkyl group as the active site. Dahmen (1988) also reported that the fungicide containing alkyl group usually effected the fungal growth and development by effecting site chain methylation which conseguently inhibit the sterol formation of the fungus. The active position for site chain methylation usually occurred on the C24. Enzyme for slerol methyltransferase was interfered. This finding was also confirmed by Köller (1992). There were no possibility to study the same effect of ACA on fungal growth and development of

tonoplast and cellmembrane i.e. the septum development, may confirm the previous explaination of Mann (1987), Dahmen (1988) and Köller (1992).

Darker colour of the cells both studied under light microscope and transmission electron microscope also suggested the possibility of the abnormal development of the membrane. According to Smtthanan (1998) normal cell or tissue has no ability or incomplete efficiency to exude the strange substance out of the cell. This however required more detail information.

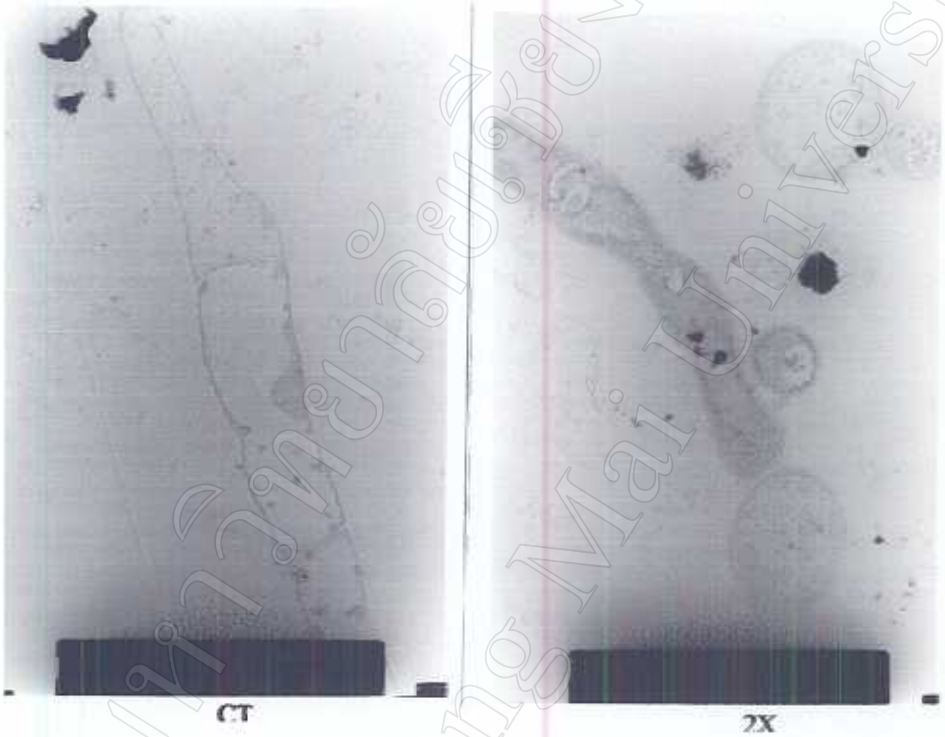


**Figure 4.13** Ultrastructure of *Colletotrichum gloeosporioides* (Penz.) Sacc. Hypha without (control, left) and with (right) the treatment with 1′-acetoxychavicol acetate (ACA).

The cross section of the hypha cell also confirmed the disappearance of vacuole in the cell treated with ACA (Figure 4.14). In some case cell dimension also changes from round cross section to be irregular shape. All these pictures from TEM confirmed unclearly effect of ACA on cell wall development and cell membrane . These however required more precious studies, in especially effect of ACA on tonoplast (membrane of the vacuole). The effects of ACA on abnormal development of septate and the disappearance of vacuole reminded the result of fungicide on sterol-biosynthesis with consequently effect membrane formation as reviewed by Hippe′ (1998). Hippe′ also explained that sterol-biosynthesis-inhibiting (SBI) fungicides, contained functionally related

compounds belonging to different chemical group (e.g. azoles, pyrimidines, morpholines and allylanmines). Their selective fungitoxic action occurs at various

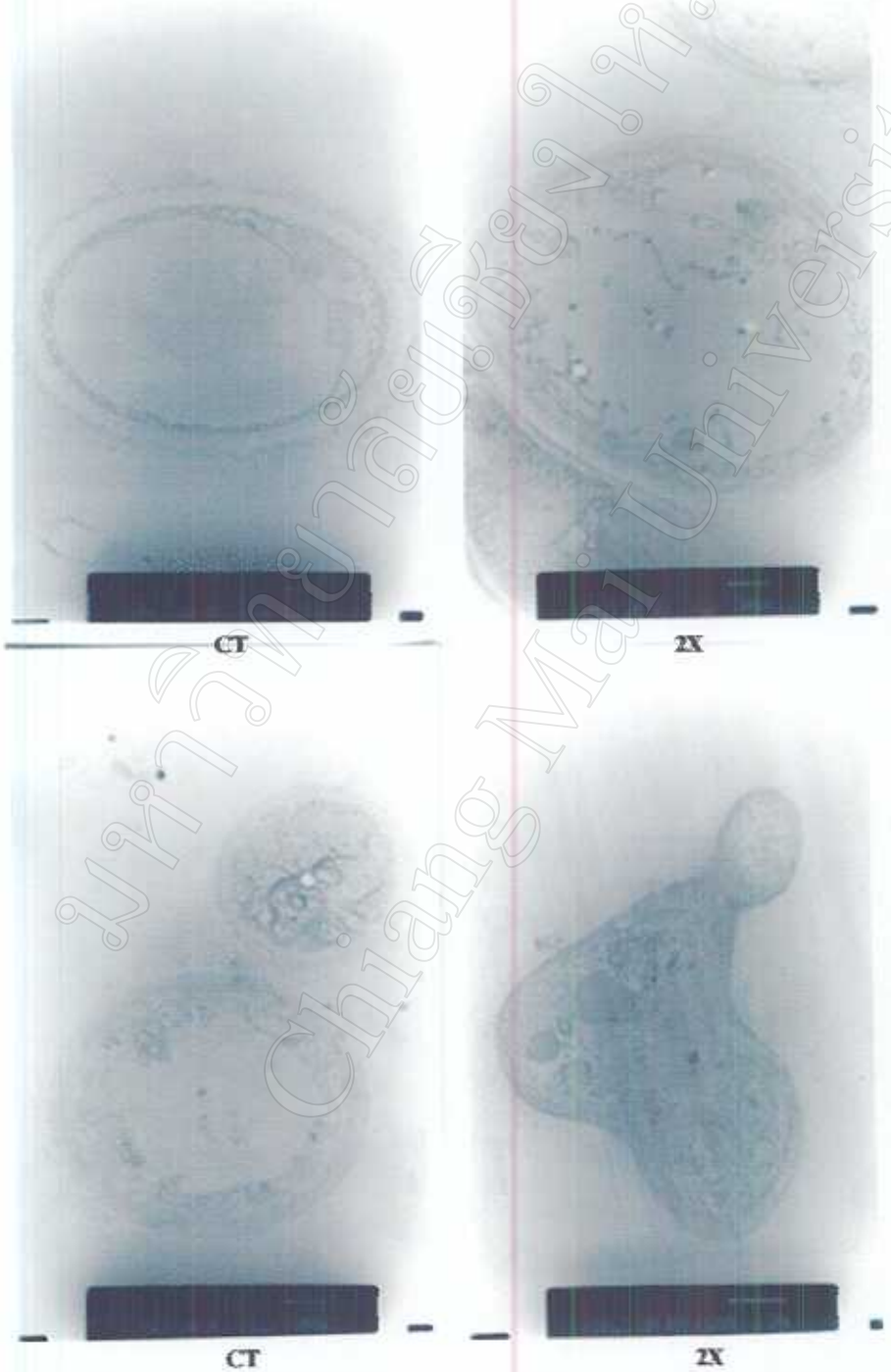


**Figure 4.14** Ultrastructure of *Colletotrichum gloeosporioides* (Penz.) Sacc. Crosssection of hypha cell organelles (control upper left) and cell treated with 1′-acetoxychavicol acetate (upper right, lower left and right).

steps in the sterol pathway. Varying the SBI-fungicide concentration at the time of application, both *in vitro* and *in vivo* experiments result in a common sequence of ultrastructural changes leading to cellular degeneration and finally cell death. The sequence included undulations of the plasmalemma, increases and destabilization of the ER, disturbances of the mitochondria, accumulation of lipid-bodies, vacuolization and exocytosis, considerable thickening of cell walls, and incomplete septa formation.

How ACA affected growth and development of *Colletotrichum gloeosporioides* (Penz.) Sacc. hyphae, or does ACA affected growth and development of *Colletotrichum gloeosporioides* (Penz.) Sacc. through the same mode of action as suggested by Hippe' (1998), is not yet clear from these research results. Results shown in Figure 4.15, however demonstrated Spitzenkörper of the hyphae to be the major point response to the active substance, due to it high metabolic activity and also the biosynthesis of membrane, cell wall, and many important organelles of the cell. Jipipob (2002) studied the chemical activity of ACA on growth and development of *Colletotrichum gloeosporioides* (Penz.) Sacc. and suggested that the demethylation activity of alkyl group (-CH = $CH_2$ ) in the chemical structure of ACA (Figure 4.16).

**Figure 4.15** Spitzenköper of *colletotrichum gloeosporioides* (Penz.) Sacc. showing active site of membrane and wall formation.

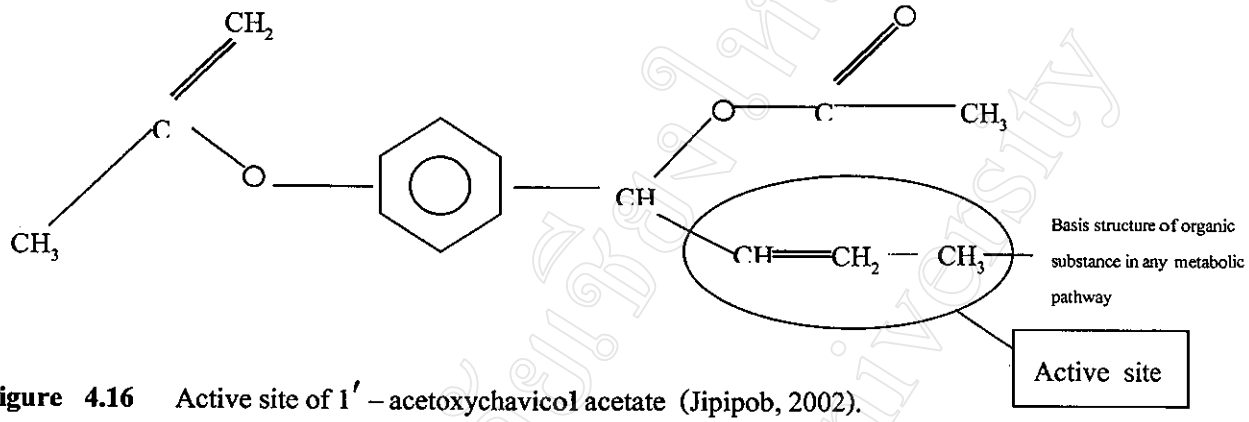


**Figure 4.16** Active site of 1′ – acetoxychavicol acetate (Jipipob, 2002).

### 4.3.10 Conclusion

Effect of β-asarone and 1′ – acetoxychavicol acetate on ripening physiology and effect on ultrastructure of *Colletotrichum.gloeosporioides* (Penz.)Sacc. (Mode of Action) were studied. The results can be concluded that:

1) The optimum dosage (or minimum fungicidal concentration, MFC) to inhibit growth and development of *Colletotrichum gloeosporioides* (Penz.) Sacc. of β-asarone and ACA were 375 ppm and 670 ppm, receptively. Both substances gave the similar best result as benomyl. Therefore a high potential to replace benomyl in postharvest mango treatment.

2) β-asarone showed relatively better result to inhibit *colletotrichum gloeosporioides* (Penz.) Sacc. but a disadvantage in enhancing respiration rate of mango fruit, which consequently caused a lower fruit firmness and percent weight loss. Together with a risk to be banded of β-asarone due to its carcinogenic activity in animal, 1′ – acetoxychavicol acetate has more potential.

3) For mode of action studies, ACA affect the fungus growth and development by interfere with membrane development in, especially, tonoplast and septate formation. Cytoplasm of the fungus cell are more condensed and darker in colour when studied under light microscope and transmission electron microscope.