RESULTS

2.1 Assessment of eucalyptol toxicity against adults *M. domestica* and *C. megacephala* by using topical application method

***M. domestica*:**

Eucalyptol toxicity was determined at 24-hr after adult flies being topically tested. The eucalyptol toxicity against adult *M. domestica*, evaluated by LD_{50}, LD_{95} and LD_{99} values were presented in Table 4. All were dead after had been treated with concentrated eucalyptol (100%). In addition, only males dead all after being treated with 50% (v/v) eucalyptol. Males were more susceptible than females in all concentration used. The LD_{50}, LD_{95} and LD_{99} of males were 118, 460 and 987 μg/fly, respectively, whereas those of females were 177, 500 and 895 μg/fly, respectively. No significant difference was found between males and females based on the overlapping of 95% confidence interval at LD_{50} value. No mortality was observed in flies of the control group.

Table 4 Toxicity activity of eucalyptol against *M. domestica* adults using topical application method

Sex	*M. domestica*		
	LD_{50} (95% CI) μg/fly	LD_{95} (95% CI) μg/fly	LD_{99} (95% CI) μg/fly
Male	118 (91-154)	460 (354-598)	987 (759-1283)
Female	177 (153-202)	500 (392-754)	895 (623-1682)

***C. megacephala*:**

Eucalyptol toxicity was determined at 24-hr after adult flies being topically tested. The results shown *C. megacephala* adults of both sexes, after treated with concentrated eucalyptol (100%), were absolutely dead. The eucalyptol toxicity against adult *C. megacephala*, evaluated by LD_{50}, LD_{95} and LD_{99} were presented in Table 5. Concerning the LD_{50}, LD_{95} and LD_{99} value, the toxicity of eucalyptol against males was slightly lower than those females. The LD_{50}, LD_{95} and LD_{99} of males were 197, 380 and 549 μg/fly, respectively, while those of females were 221, 422 and 608 μg/fly, respectively. There was no significant difference between males and females mortality, based on the overlapping of 95% confidence interval at LD_{50} value. No mortality was observed in flies in the control group.

Table 5 Toxicity activity of eucalyptol against *C. megacephala* adults using topical application method

Sex	*C. megacephala*		
	LD_{50} (95% CI) μg/fly	LD_{95} (95% CI) μg/fly	LD_{99}(95% CI) μg/fly
Male	197 (177-218)	380 (323-495)	549 (435-816)
Female	221 (199-244)	422 (357-560)	608 (478-924)

2.1.1 Life span of alive flies after adults being tested with eucalyptol using topical application

Life span of all alive flies was determined after being tested with each concentration of eucalyptol, using topical application. Median value and range of adult life span for each fly group are shown in Table 6. No alive flies of *M. domestica* and *C. megacephala* were found after being topically tested with concentrated (100%) eucalyptol. For lessor concentrated of 50% (v/v) eucalyptol, only male *M. domestica* were deadly.

The life span of adult male and female *M. domestica* varied according to the concentration of eucalyptol application. In males, the median (range) life spans of *M. domestica* tested with 25%, 12.5%, 6.25% (v/v) eucalyptol, absolute ethanol and untreated flies were 5 (2-17), 8 (2-19), 13 (3-33), 15 (2-30) and 17 (2-31) days, respectively (Table 6, Figure 2). Non significant different of median life span were detected in males treated with 6.25% eucalyptol and control group (absolute ethanol) (Mann Whitney *U* test; $P = 0.791$). However, median life span of males treated with 25% and 12.5% differed significantly with the control flies (absolute ethanol) (Mann Whitney *U* test; $P = 0.003$ and $P = 0$, respectively).

In females *M. domestica*, the median (range) life spans flies tested with 50%, 25%, 12.5%, 6.25% (v/v) eucalyptol, absolute ethanol and untreated flies were 2 (2-3), 4 (2-18), 8 (2-20), 17 (5-29), 15.5 (2-33), and 19 (4-32) days, respectively (Table 6, Figure 2). There was no significant different of median life span in females treated with 6.25% eucalyptol and control group (absolute ethanol) (Mann Whitney *U* test; $P = 0.987$). However, median life span of females treated with 50%, 25% and 12.5% differed significantly with the control flies (absolute ethanol) (Mann Whitney *U* test; $P = 0.006$, $P = 0$ and $P = 0$, respectively).

As for *C. megacephala*, the life span of flies treated with the same concentration of eucalyptol was similar with those of M. *domestica*. In males, the median (range) life spans of flies tested with 50%, 25%, 12.5%, 6.25% (v/v) eucalyptol, absolute ethanol and untreated flies were 5 (2-8), 7 (2-16), 12 (2-31), 15 (4-48), 19.5 (3-49) and 21 (3-49) days, respectively (Table 6, Figure 3). Non significant different of median life span were detected in males of 6.25% eucalyptol group and control group (absolute ethanol) (Mann Whitney *U* test; $P =$

0.112). However, median life span of males treated with 50%, 25% and 12.5% differed significantly with the control flies (absolute ethanol) (Mann Whitney *U* test; $P = 0.015$, $P = 0$ and $P = 0$, respectively).

Regarding females *C. megacephala*, the median (range) life spans flies tested with 50%, 25%, 12.5%, 6.25% (v/v) eucalyptol, absolute ethanol and untreated flies were 6 (2-12), 12 (3-21), 13 (4-30), 20 (4-49), 19.5 (2-49) and 23 (2-50) days, respectively (Table 6, Figure 3). There was no significant different of median life span in females treated with 6.25% eucalyptol and control group (absolute ethanol) (Mann Whitney *U* test; $P = 0.548$). However, median life span of females treated with 50%, 25% and 12.5% differed significantly with the control flies (absolute ethanol) (Mann Whitney *U* test; $P = 0.032$, $P = 0.002$ and $P = 0$, respectively).

Table 6 Life span of alive flies [median (range)] after adults being topically tested with varying concentration of eucalyptol

Concentration of eucalyptol (%v/v)	*M. domestica* (Days)		*C. megacephala* (Days)	
	Male	Female	Male	Female
50	-	2 (2-3)*	5 (2-8)*	6 (2-12)*
25	5 (2-17)*	4 (2-18)*	7 (2-16)*	12 (3-21)*
12.5	8 (2-19)*	8 (2-20)*	12 (2-31)*	13 (4-30)*
6.25	13 (3-33)**	17 (5-29)**	15 (4-48)**	20 (4-49)**
Absolute ethanol	15 (2-30)***	15.5 (2-33)***	19.5 (3-49)***	19.5 (2-49)***
Natural control	17 (2-31)	19 (4-32)	21 (3-49)	23 (2-50)

* Significant difference from control group (absolute ethanol), Mann Whitney *U* test; $P < 0.05$.
** Not significantly different from control group (absolute ethanol), Mann Whitney *U* test; $P > 0.05$.
*** Not significantly different from natural control, Mann Whitney *U* test; $P > 0.05$.

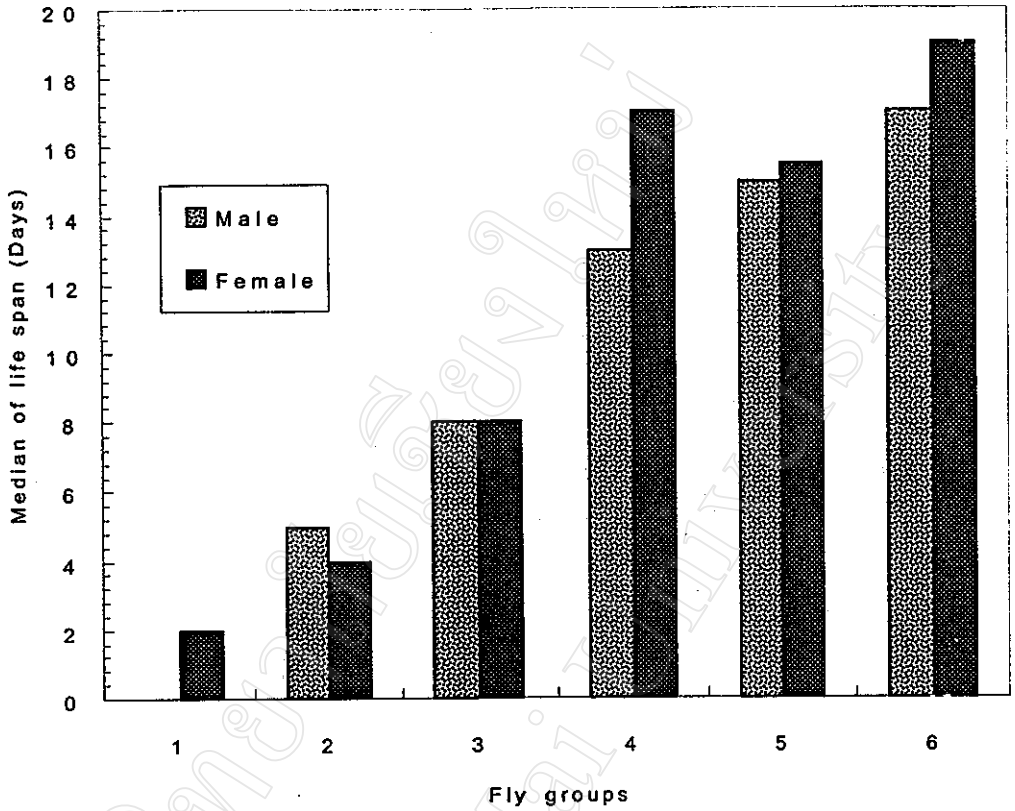

Figure 2 Median life span of adult *M. domestica* after adults being topically tested with eucalyptol at the concentration of 50% (group 1), 25% (group 2), 12.5%(group 3) and 6.25% (group 4), respectively. Control were group 5 (tested with absolute ethanol) and group 6 (natural control).

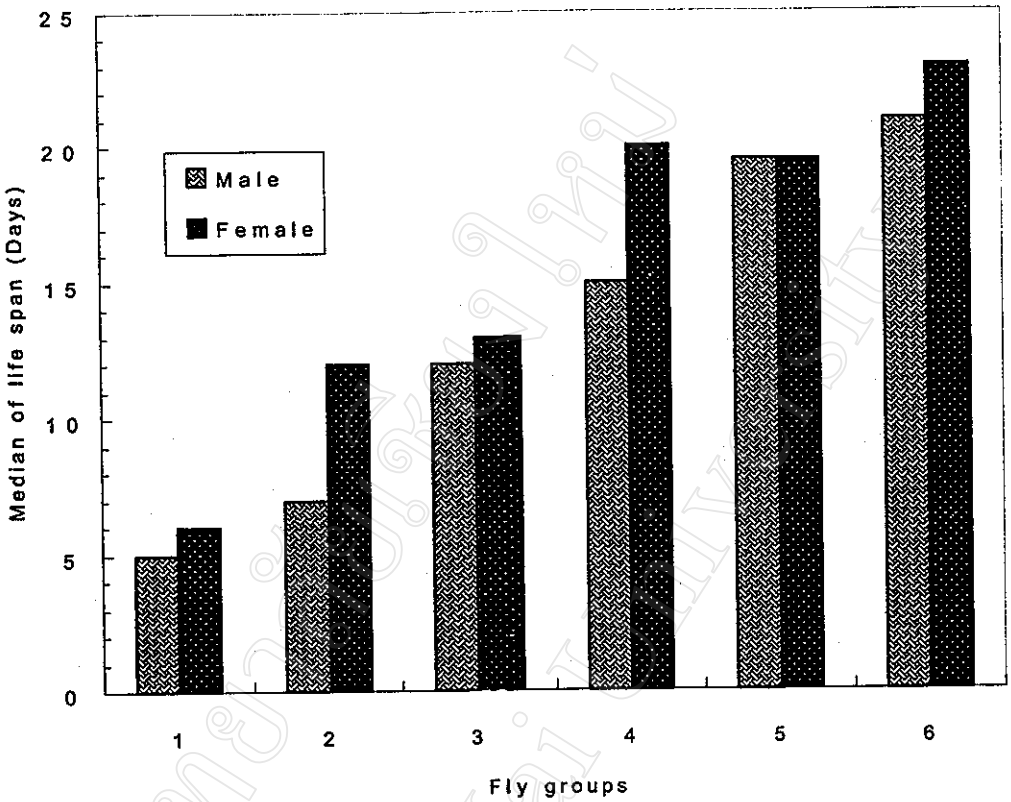

Figure 3 Median life span of adult *C. megacephala* after adults being topically tested with eucalyptol at the concentration of 50% (group 1), 25% (group 2), 12.5% (group 3) and 6.25% (group 4), respectively. Control were group 5 (tested with absolute ethanol) and group 6 (natural control).

2.2 Assessment of eucalyptol toxicity against third-stage larvae of *M. domestica* and *C. megacephala* by using dipping method

The eucalyptol toxicity against third-stage larvae of *M. domestica* and *C. megacephala* using dipping method, evaluated by LC_{50}, LC_{95} and LC_{95} value, were presented in Table 7. The LC_{50}, LC_{95} and LC_{99} of *M. domestica* were 101, 239 and 388 μg/μl, respectively. In contrast, the third-stage larvae of *C. megacephala* were more tolerance to eucalyptol than those *M. domestica*. The LC_{50}, LC_{95} and LC_{99} value of eucalyptol on *C. megacephala* were 642, 1,539 and 2,511 μg/μl, respectively. Significant difference between LC_{50} value of *M. domestica* lower than *C. megacephala* larvae was present. No mortality of larvae was observed of the control group.

Table 7 Toxicity activity of eucalyptol against third-stage larvae *M. domestica* and *C. megacephala* using dipping method

Species of flies	LC_{50} (95% CI) μg/μl	LC_{95} (95% CI) μg/μl	LC_{99} (95% CI) μg/μl
M. domestica	101 (92-111)	239 (203-305)	388 (305-558)
C. megacephala	642 (585-710)	1539 (1272-2044)	2511 (1915-3797)

2.2.1 Emergence of adult after the third-instar larvae being dipped with each concentration of eucalyptol

After third-instar larvae being dipped with concentration of eucalyptol, they were reared to determine the success of emergence. As shown in Table 8, all larvae of *M. domestica* died when being dipped using 100% eucalyptol, thus no emergence of flies was found. When larvae were dipped with 50%, 25%, 12.5% and 6.25% (v/v) eucalyptol, the emergence were 50%, 67%, 35% and 60%, respectively. In contrast, larvae in both control groups (after being dipped with absolute ethanol and larvae from same egg batch of the tested group) were well developed to be adults (92% and 93% of emergence rates, respectively).

Regarding *C. megacephala*, emergence after larvae being dipped with 100%, 50%, 25%, 12.5%, and 6.25% (v/v) eucalyptol were 100%, 93%, 90%, 93% and 73%, respectively. The success of fly emergence in all treated groups were not significant difference from both control groups (80% of those dipped with absolute ethanol and 68% of larvae from the same egg batch of tested group) [$P < 0.05$; Chi-square test].

Table 8 Emergence of *M. domestica* and *C. megacephala* after larvae being dipped with eucalyptol

Concentration (%v/v)	% emergence	
	M. domestica	*C. megacephala*
100	-	100 (15/15)
50	50 (1/2)	93 (42/45)
25	67 (2/3)	90 (53/59)
12.5	35 (9/26)*	93 (55/59)
6.25	60 (31/52)*	73 (44/60)**
Absolute ethanol	92 (55/60)	80 (48/60)
Natural control	93 (56/60)	68 (41/60)

* Significant difference from control groups ($P < 0.05$; Chi-square test [Epi Info 6])
** Significant difference from the tested groups ($P < 0.05$; Chi-square test [Epi Info 6])

2.2.2 Surface ultrastructure of third stage larvae of *M. domestica* and *C. megacephala* after being treated with 100% eucalyptol using scanning electron microscope (SEM)

***M. domestica*:**

Generally, the morphology of the third stage *M. domestica* larva is muscoid-shaped and more slender than calliphorid larvae. The body consists of 12 segments; a cephalic, 3 thoracic, and 8 abdominal segments. The pointed end of the larva is anterior, and the broadly truncate end is posterior. As for the structure of cephalic region, a pair of dorsal organs (antennae) and a pair of maxillary palp complex (terminal organ) appear distally. Beneath the terminal organ is the oral groove, an array of cuticular ridges associated with the mouth. The anterior spiracles are fan-shaped, placed on each dorsolaterally edge of prothorax, each having five to seven opening. The posterior end of larva smoothly rounded where the posterior spiracle located. The posterior spiracles are D-shaped with three M-shaped sinuous slits and a button in the middle of the straight side of the D-shaped.

Correspondingly, the larva that was tested with absolute ethanol, serving as control in this experiment, was observed surface changes by using SEM. The scanning electron micrographs (Figure 4) showed that this larva had normal morphology compared with the general housefly larva described above. The anterior end consists of normal cephalic region including antennae, maxillary palp complex, oral groove and, especially, the anterior spiracle (Figure 4A). The multiple-pointed of intersegmental spines between prothorax and mesothorax and the integument are normal appeared (Figure 4B). Posteriorly viewed of the caudal segment, the morphology of posterior spiracle is also typical appearance (Figure 4C).

In contrast, those larvae treated with concentrated eucalyptol showed some aberrant appearances. As seen in figure 5A, the whole body is abnormally wrinkly muscoid-shaped and inflated. Focusing with high magnification, some parts of integument are covered with several sized of blebs (Figure 5B). Furthermore, the posterior spiracles are difficult to observe their define the structures because they are coated with eucalyptol residuum (Figure 5C).

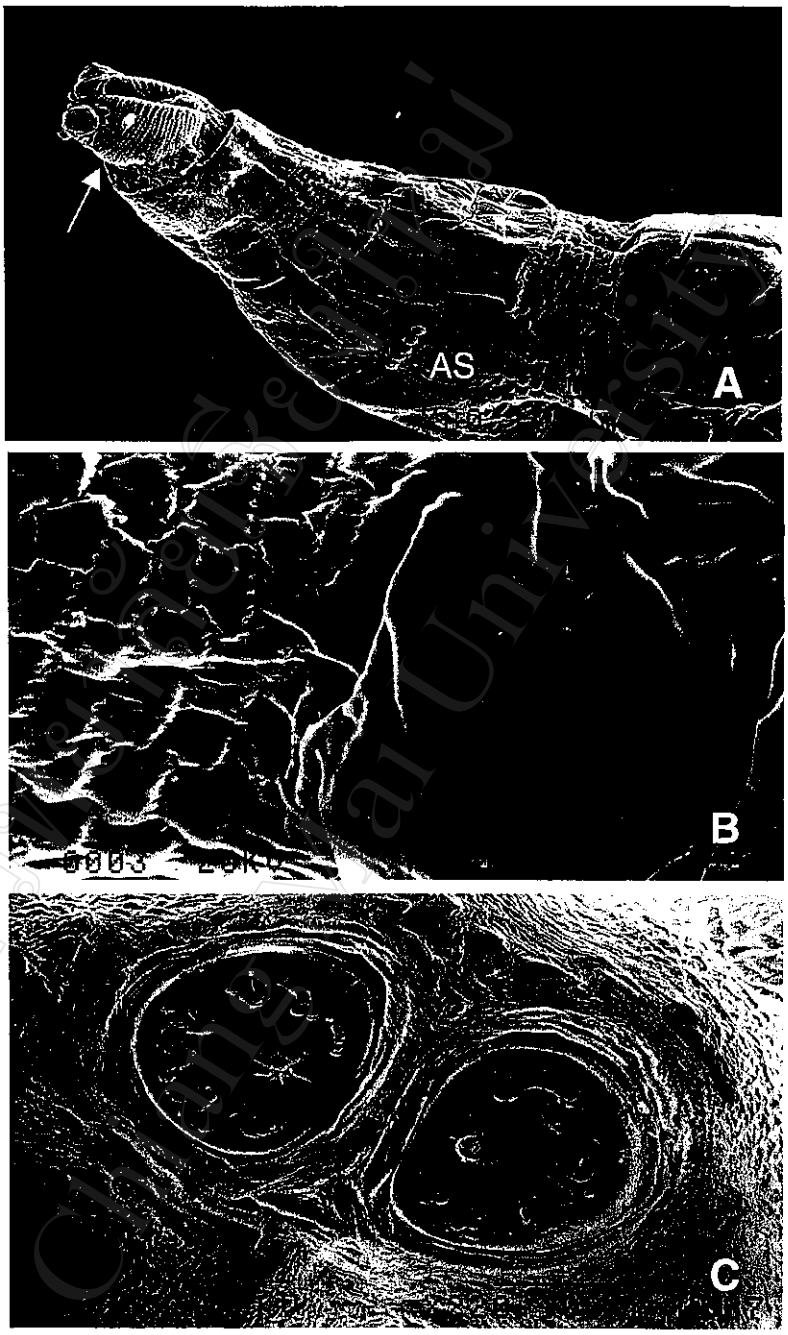

Figure 4 SEM micrographs of third-stage larva *M. domestica* after being dipped with absolute ethanol. (A) Cephalic region (arrow) and prothorax showing anterior spiracle (AS). (B) Multiple-pointed of intersegmental spines and integument.(C) A pair of posterior spiracle.

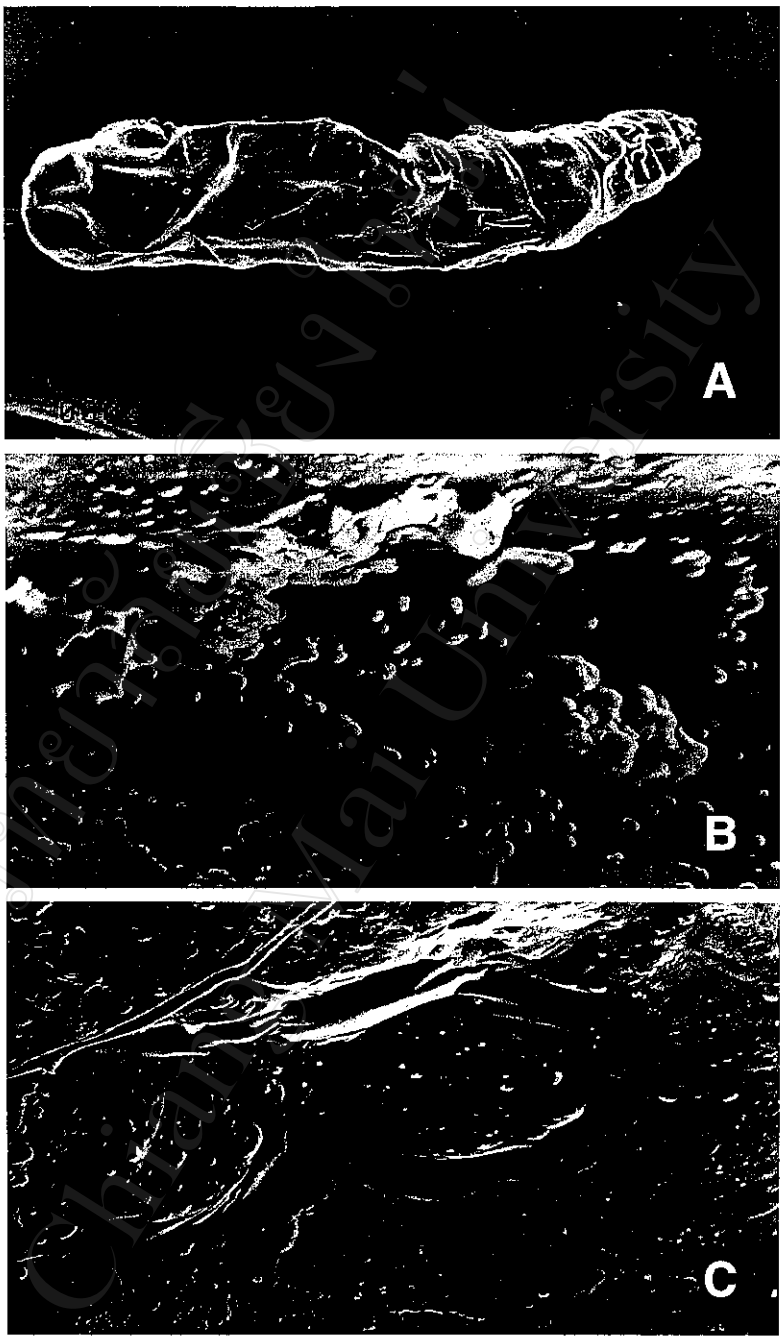

Figure 5 SEM micrographs of third-stage larva *M. domestica* after being dipped with concentrated eucalyptol. (A) Whole larva. Anterior end at right, posterior end at left. (B) Integument covered with residual grains. (C) Posterior spiracle covered with residuum.

***C. megacephala*:**

The muscoid-shape of third-stage larva *C. megacephala* is similar to those *M. domestica*, but larger size. Larva treated with absolute ethanol as control was observed the surface changes using SEM. The anterior region is normal and no sign of aberrant is found (Figure 6A). The intersegmental spines and integument are common (Figure 6B). The posterior end has complete structure (Figure 6C), while three spiracular slits are clear (Figure 6D).

Differently, larvae treated with the concentrated eucalyptol showed strange appearance. As seen in Figure 7A, the whole body is abnormally distorted muscoid shaped, whereas the posterior part was swelling. Focused on the cephalic region, distorted cephalic structures were observed (Figure 7B). The antenna and maxillary palp were departed (Figure 7C). The integument is covered with residuum and, in some parts, several deep pores with raised edge are seen (Figure 7D). The intersegmental spines are swelling, not in the form of regular appearance. Many breaches occur in some areas of the body (Figure 7E). At the posterior end, a pair of posterior spiracle is covered with thick material (Figure 7F).

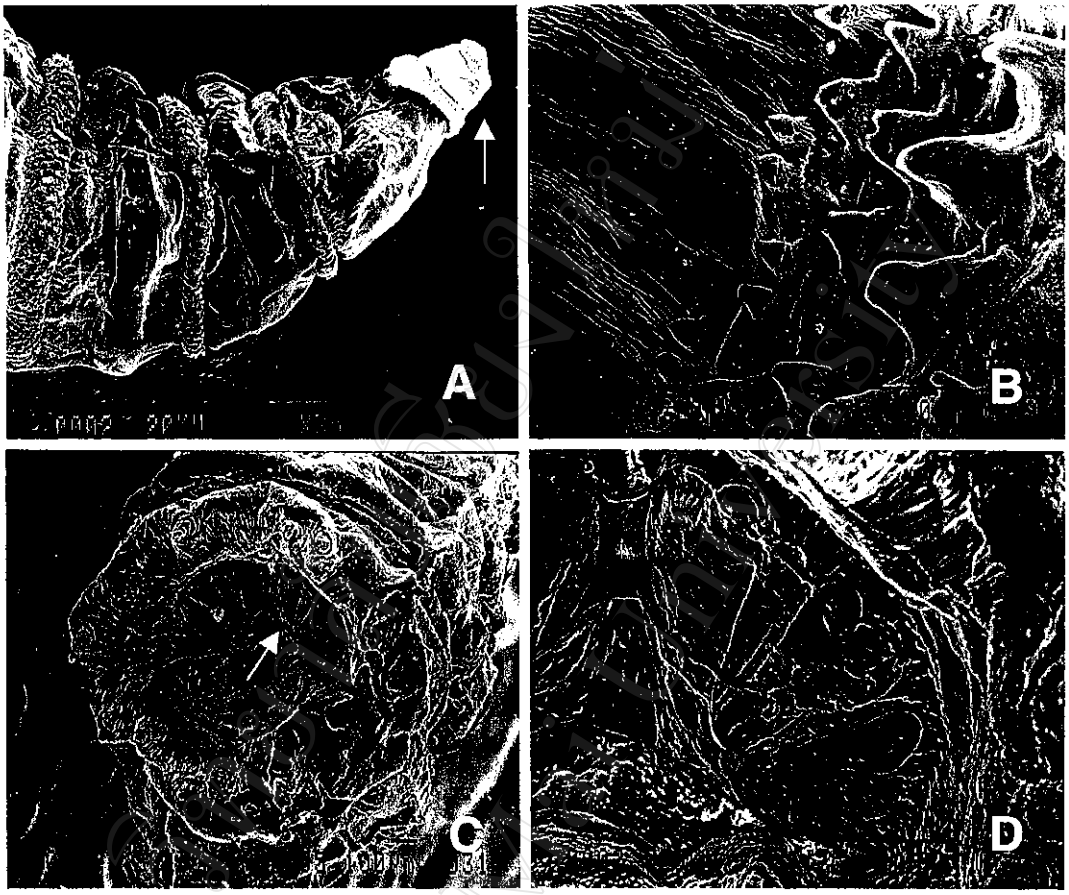

Figure 6 SEM micrographs of third-stage larva *C. megacephala* after being dipped with absolute ethanol. (A) Cephalic region (arrow) and thoracic segments (B) Intersegmental spines and smooth integument. (C) Posterior view showing a pair of posterior spiracle (arrow). (D) Higher magnification of posterior spiracle showing 3 normal spiracular slits.

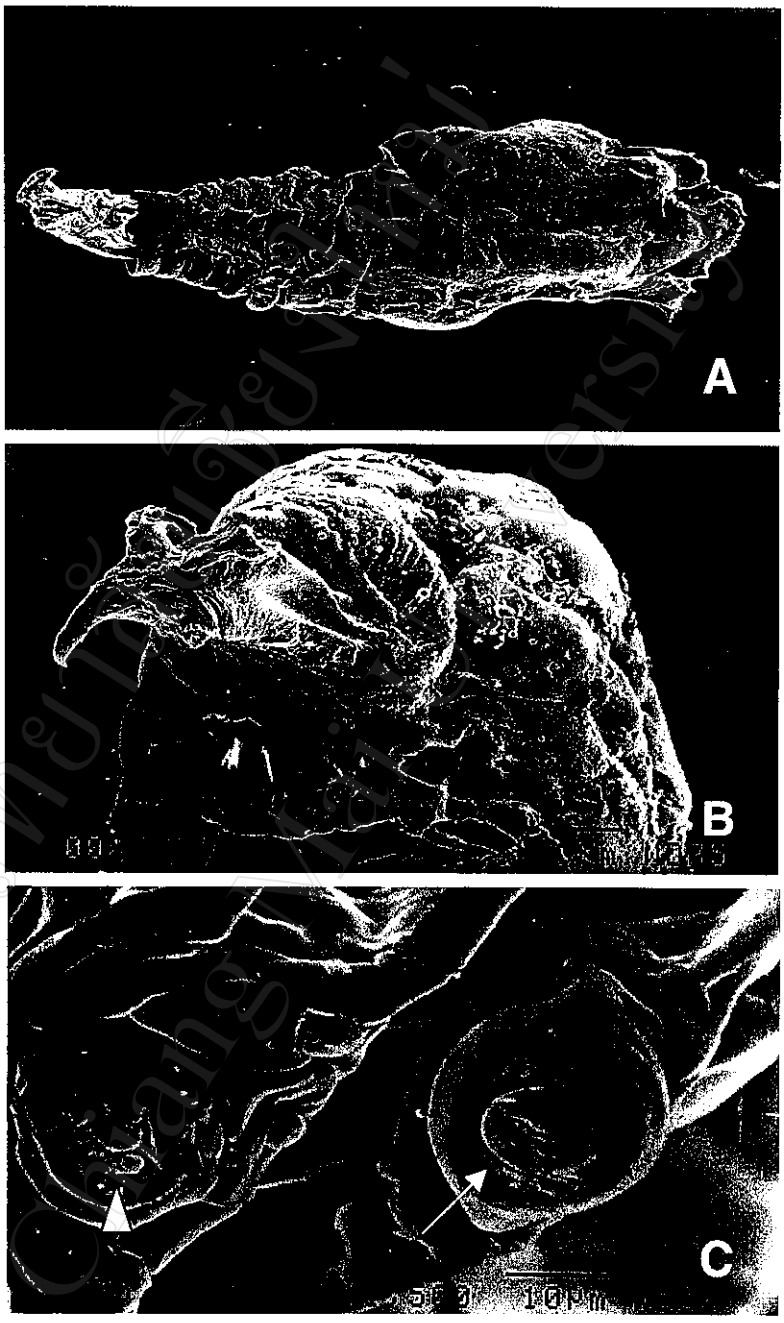

Figure 7 SEM micrographs of third-stage larva *C. megacephala* after being dipped with concentrated eucalyptol. (A) Whole larva showing swelling at the posterior half of body. Anterior end at left, posterior end at right. (B) Cephalic region showing distortion. (C) Departed of the antenna (arrow) and abnormal of maxillary palp (triangle).

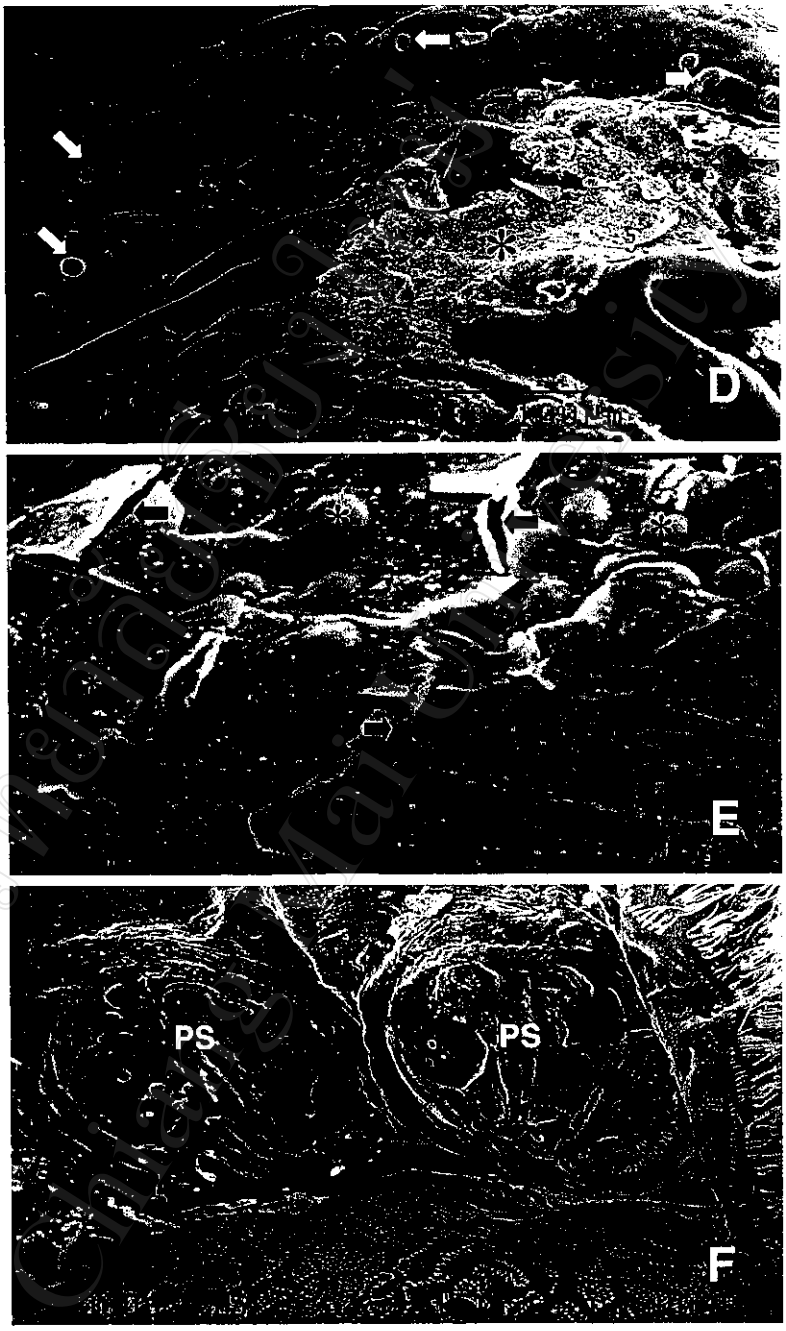

Figure 7 (cont.) SEM micrographs of third-stage larva *C. megacephala* after being dipped with concentrated eucalyptol. (D) Integument covered with residuum (asterisk). Several pores (arrows) also appeared. (E) Swelling intersegmental spines (asterisks) and several breaches in some parts (arrows). (F) Posterior spiracle (PS) covered with thick materials.