DISCUSSION

M. domestica and *C. megacephala* are the two most medically important fly species in Thailand, with the prevalence of combined species being >90% of adult fly populations collected (Sucharit *et al.*, 1976; Sucharit and Tumrasvin, 1981). Both play major role as the mechanical carriers of numerous bacterial species in the market places in urban areas of the country (Sukontason *et al.*, 2003). In this regard, control of these fly populations, particularly during epidemic diseases caused by them, is needed. In this present study, the toxicity of pure compound eucalyptol was assessed against both species. Pure compound of eucalyptol was chosen because of the previous documents as having some toxicity degrees to many insects (e.g., triatomine bug *Triatoma infestans*, red flour beetle *Tribolium castaneum*, lesser grain borer *Rhyzopertha dominica*, rice weevil *Sitophilus oryzae*, sawtoothed grain beetle *Oryzaephilus surinamensis*, house fly *M. domestica*, Hessian fly *Mayetiola destructor*, German cockroach *Blattella germanica*, stored food mite *Tyrophagus putrescentiae*) (Laurent *et al.*, 1997; Prates *et al.*, 1998; Lamiri *et al.*, 2001; Sánchez-Ramos and Castañera, 2001; Tripathi *et al.*, 2001; Macchioni *et al.*, 2002; Lee *et al.*, 2003). The non-toxic of eucalyptol to humans and other mammals, based on the use in pharmaceuticals and a variety of cosmetic preparations (Laurent *et al.*, 1997), was also taken into account.

As for the standard test method of insecticide toxicity against adult insects, it depends on the vectorial capacity of particular species. In mosquito, as females only play major role as vector of pathogens, therefore almost females have been employed. About *M. domestica*, Brown and Pal (1971) denoted that more female house fly has been tested than males, however only males was used in some assessments. Both sexes have been tested in some cases. In this experiment, both male and female flies were investigated due to the non significant difference between them as the mechanical carrier of pathogens (Sukontason *et al.*, 2003). Correspondingly, males and females of Hessian fly *M. destructor* have been tested of insecticidal efficacy with essential oils (Lamiri *et al.*, 2001).

The toxicity of pure compound eucalyptol to adult *M. domestica* using topical application indicated that males were more susceptible than females. This was accordance with several insecticide bioassay tests of house fly (Brown and Pal, 1971). The susceptibility difference is partly due to their smaller size of males (Brown and Pal, 1971). As for the mortality, the LC_{50} values of house fly in this present result (118 µg/fly in males and 177 µg/fly in females) were higher than those applied with volatile oils of *Matricaria chamomilla* and *Clerodendron inerme* on adult *M. domestica*, with the LC_{50} of the former and the latter being 76 and 84 µg/fly, respectively (Shourkry, 1997). On the other hand, the LC_{50} value of this study was lower than several monoterpenoids against *M. domestica* using topical application — LD_{50} ~200 or >500 µg/fly (Rice and Coats, 1994). The screening test of eucalyptol performed using fumigation method indicated the concentration at 50 µg/ml air caused 100% mortality of adult *M. domestica*. It may be that fumigation method was more appropriate for bioassay test of eucalyptol against *M. domestica* or other flies, and this subject needed further investigations.

Concerning the comparable of susceptibility of both fly species examined, *M. domestica* was more significantly susceptible than *C. megacephala* (Tables 4 and 5). The reason for such difference between species was unknown. It may be the due to the morphological difference, as suggested by Brown and Pal (1971). Adult *C. megacephala* is larger than *M. domestica*; 8-11 mm and 6-9 mm, respectively (Zumpt, 1965). On the other hand, biological information indicated that *C. megacephala* is more promiscuous in some behavioral aspects such as feeding, habitats, breeding places, etc (Greenberg, 1973). More exposure to various environments may produce stronger and/or healthy than the less one. Some intrinsic factors within the body might involve in detoxification of insecticide. These presumptions needed more evidence.

Regarding the life span of adult flies, it was demonstrated that flies had been topically subjected to eucalyptol yielded shorter alive duration, when using the criteria of median and range. Both sexes of *M. domestica* and *C. megacephala* showed resemble circumstance. Increased concentration of eucalyptol is resulted in shorter longevity of flies. It was clearly demonstrated that males were more susceptible to eucalyptol than females. Adults subjected with eucalyptol at 12.5% (v/v) or 115 μg/fly produced ~2-fold shorter life span. Although this was the evidence in the laboratory, however shorter longevity would decrease the vectorial capacity of both sexes, as far as the capacity of being mechanically carrier is concerned (Sukontason *et al*, 2003). Likewise, the shorter life span of flies in this experiment agreed with El-Shazly *et al.* (1996). The dipping of second-stage larvae of false stable fly *Muscina stabulans* in an extract of *Nerium oleander* decreased approximately 2-fold life span of adult survivors. Moreover, this could prolong the larval and pupal developmental rates as well as decreased adult emergence. Tripathi *et al.* (2001) contributed the reduction of growth rate and food consumption in adult *T. castaneum* subjected to 1,8- Cineole. Thus, these phenomena could produce later effects of adult flies treated with this substance via feeding deterrence and postingestive toxicity. As to the reproductive potential, even though it has not been performed in this study, eucalyptol has been documented as suppressing the hatching of *T. castaneum* and reducing the subsequent survival rate of larvae (Tripathi *et al.*, 2001). Regarding this, such reproductive potential aspects are therefore merit further investigation in fly of both species.

The assessment of pure compound eucalyptol on third-stage larvae *M. domestica* indicated moderate larvicide effect (LD_{50} = 101 μg/μl) while low effect against *C. megacephala* (LD_{50} = 642 μg/μl). Quite high lethal dosages were although resulted from dipping method, the resembling methodology of topical application for fly larvae (Kawada *et al.*, 1987). Tripathi *et al.* (2001) documented the LD_{50} of ~150 μg/mg wt of 1-8, Cineole on *T. castaneum* larva. The low toxicity in this study were similar to report of Laurent *et al.* (1997) that commercial pure compound of eucalyptol (1-8, Cineole) had weak larvicidal action on the fourth instar nymph of *T. infestans*, using topical application. It was suggested by Laurent *et al.* (1997) that as the essential oils are made up of volatile compounds, then it is possible that when they are applied directly on the insects, their potency is quickly lost due to the evaporation of the active components. This discussion may be corrected in some circumstances. However, this unlikely to be the case in this experiment, since the diluted eucalyptol in a bottle had been closed immediately after dilution to prevent evaporation, and it was utilized as soon as possible. Accordingly, the low larvicidal action of eucalyptol on third-stage larvae *M. domestica* and *C.*

megacephala should be from another factors. One would be due to the thickness of larval integument — the outer covering of the living tissues of an insect, and acts as a barrier between tissues and the environment. Based on the section, those of *M. domestica* and *C. megacephala* were ~17 μm (n=9) and ~29 μm (n=5), respectively (data not shown), thus efficiently preventing from the penetration of intrude substance. The other explanation would be the bionomic of both larval species. Larvae of *M. domestica* and *C. megacephala* are terrestrial, and to be found in quite diverse habitats (e.g., dung, garbage, excrement, decay materials, slaughterhouse, foodstuff, damp places, carrion), thus they could tolerate to various conditions.

Although eucalyptol produced low to moderate toxicity against larvae of *M. domestica* and *C. megacephala*, the mode of action of this substance should be taken into account. It has shown that the insecticidal activity of eucalyptol (cineole) was mainly contact and/or ingestion on *R. dominica* and *T. castaneum*, which are important pests of stored grain (Prates *et al.*, 1998). The contact response would be the case of *M. domestica* and *C. megacephala*, since morphological changes of treated larvae were confirmed using microscopy. Determined under dissecting microscope, only swelling of the larvae was observed. From results of larvae treated with concentrated eucalyptol [100% (v/v) or 0.902 g/ml)] under high magnification of SEM, the whole body surface was remarkably morphological alterations induced by this substance. Bleb formation, partial fractures and deformation of integumental spines were the striking phenomena, and these were similarly observed in larvae of both species. These indicated the contact action of eucalyptol towards the body surface or integument. None of the above findings were found in larval integument of control group (treated with absoluted ethanol). These destructive effects on *M. domestica* and *C. megacephala* larvae treated with eucalyptol were similar to the intestinal parasites administrated by antiparasitic drugs. In helminths, surface damages of adult liver fluke *Opisthorchis viverrini* after *in vitro* incubation in praziquentel include blebbing, swelling, erosion and disruption of the integument (Apinhasmit and Sobhon, 1996). Increase of such damages depended upon concentration of drug administered and duration of incubation. The treated of *Taenia taeniaeformis* with mebendazole demonstrated the degeneration of tegument with grooves, holes, and craterlike structures (Verheyen *et al.*, 1978). Topographic changes of *Capillaria hepatica* worms were in the form of disorganized cuticle and absence of surface uniformity (El Gebaly *et al.*, 1996).

Having the volatile property, eucalyptol applied as topical would produce lesser toxicity than fumigation or the impregnated paper test. However, these two latter methods require a rather longer observation period (Laurent *et al.*, 1997). Accordingly, such other efficacy of eucalyptol as repellent or attractiveness of flies should be examined. Some documents as its repellent to cockroaches (Maugh, 1982) or attractiveness toward banana weevil *Cosmopolites sordidus* (Ndieqie *et al.*, 1996), grasshopper *Hypochlora alba* (Blust and Hopkins, 1987), Mexican fruit fly *Anastrepha ludens* (Robacker, 1991) have been found.

In this current study, toxicity of eucaplytol against adult or larva of *M. domestica* and *C. megacephala* varied from low to moderate susceptibility. However, some modes were documented that could enhance the insecticidal toxicity. The small addition of compounds, called synergist, enormously increased the toxicity (Matsumura, 1985). D-limonene, the component of citrus peel oil extract, was synergized by piperonyl butoxide; when combined, these compounds produced a synergistic ratio of 3.2 and more rapid mortality of adult fleas *Ctenocephalides felis* (Hink and Fee, 1986). Hence, the combination of eucalyptol with synergist is of interested subject for fly control, since the nontoxic to humans, other mammals, natural predators, as well as the environmental safety have to be highly concerned.

Although eucalyptol was not satisfactory promising bio-insecticide compound toward adult and larvae of *M. domestica* and *C. megacephala* through topical or dipping methods, it may produce better efficacy such as repellent or attractiveness. The other application of fumigation or impregnated paper test was suggested in this regard.