LITERATURE REVIEW

Bionomics of medical importance of *Musca domestica*

Classification and geographical distribution

Musca domestica, commonly known as house fly, was classified as follow:

Phylum	Arthropoda
Class	Insecta
Order	Diptera
Suborder	Cyclorrhapha
Family	Muscidae
Genus	*Musca*
Species	*Musca domestica*

M. domestica probably the most widely distributed domestic insect species to be found (Greenberg, 1973; Smith, 1973). It occurs worldwide but is least abundant in Africa (Kettle, 1995). It was the most abundant fly specie collected (>90%) from several areas in Thailand (Sucharit *et al.*, 1976; Tumrasvin *et al.*, 1978; Sucharit and Tumrasvin, 1981), similar to the abundant capture (83.2%) in a poultry facility in Southeastern Brazil (Avancini and Silveira, 2000).

General morphology

Adult - The adult fly is medium sized non-metallic fly about 6-9 mm in length, depending on the conditions at which the larva grows up. The adult body, as in all insects, consists of three main units (head, thorax and abdomen). The color of thorax is grey with four dark longitudinal stripes on the dorsal side. The fourth longitudinal vein of the wing has a sharp upward bend, discerning house fly from other Muscidae. The abdomen is yellowish, with especially in males a dark posterior end, and contains the largest part of digestive and reproductive organs.

Egg - House fly egg is creamy-white long and narrow shape, measuring about 1.20× 0.25 mm.

Larva - The larva is smooth maggot. It has 12 visible segments (one head segment, three thoracic and eight abdominal segments). On the ventral side of the first segment there are two oral lobes transverse by parallel tubes, which converge on the mouth. The head is retracted into the thorax and its dark cephalopharyngeal skeleton can be seen through the translucent body of the larva. Respiration is amphipneustic with fan-shaped anterior spiracles on the second segment, undeveloped in first stage larvae, and dark, flat, plate-like spiracles on the posterior surface of the body. The posterior spiracle has one simple, reniform opening the first stage, and two nearly straight slits in the second stage. In the third larval stage, the fan-shaped anterior spiracles consist of 5-7 opening and the posterior spiracles like a letter D with three M-shaped sinuous slits and a button in the middle of the straight side of the D-shaped (Kettle, 1995).

Pupa – Immediately after moulting, the puparium is creamy white, but it steadily darkens through shades of redish brown until the mature puparium is almost black. Pupa case is barrel-like shaped, measuring 4-6×2-2.5 mm.

General biology

Life cycle

There are four distinct stages in the life of fly: egg, larva, or maggot, pupa and adult. Depending on the temperature, it takes from 6 to 42 days for the egg to develop into the adult fly. The length of life is usually 2-3 weeks but in cooler conditions it may be as long as three months (Rozendaal, 1997). Eggs are usually laid in masses on organic material such as manure and garbage. Hatching occurs within a few hours. The young larvae borrow into the breeding material. When the breeding medium is very wet they can live only on the surface, whereas in drier materials they may penetrate to a depth of several centimeters. The larvae develop rapidly, passing through three stages (instars). The first two stages last about 24 hr while the third for 3 or more days. After feeding stage is completed the larvae migrate to a drier place and burrow into the soil or hide under objects offering protection. The larvae form a capsule-like case, the puparium, within which the transformation from larva to adult takes place. This usually takes about 5 days, at the end of which the fly pushes open the top of the case and works its way out and up to the surface. Soon after emergence, the adult fly spreads its wings and the body dries and hardens. Adult become sexually mature 2-3 days, and four days after copulation, females deposit their first batch of eggs. Under natural conditions an adult female rarely lays eggs more than five times, and seldom lays more than 120-130 eggs on each occasion.

Food & feeding

Both male and female flies feed on all kinds of humans food, garbage and excreta, including sweat, and animal dung. Under natural condition flies seek a wide variety of food substances. Because of the structure of their mouthpart, food must be either in the liquid state or readily soluble in the salivary gland secretions or in the crop. Common source of food are milk, sugar, syrup, blood, meat broth and many other materials found in humans settlements. The flies evidently need to feed at least two or three times a day.

Breeding sites

House fly rarely breeds in meat or carrion but it prefers to breed in dung, garbage and waste from food processing, organic manure, sewage, and accumulated plant materials (Rozendaal, 1997).

Ecology of adult

Literature on *M. domestica* is vast. Being a classical eusynanthropic, endophilous and markedly communicative species, it has adapted to domestic living, feeding and breeding on humans food, organic wastes and feces (Kettle, 1995). Adult is diurnal, favor by high temperatures and low humidity, but, as the name house fly implies, it is more active in shade than in sunlight. At night it normally rests, although it adapts to some extent to artificial light (Rozendaal, 1997). Adult can fly up to 3.5 km (2 miles) from its site of emergence with ease, and has been found up to 8 km (5 miles) away (Smith, 1973). Its distribution is greatly influence by its reaction to light, temperature, humidity, and surface color and texture. The preferred temperature for resting is between 35°C and 40°C. Oviposition, mating, feeding, and flying all stop at temperatures below 15 °C (Rozendaal, 1997).

Medical importance

The most medical importance of *M. domestica* is being a mechanical carrier of several pathogens that causing illness in humans. They fulfill all the conditions required of a disease vector, namely: (1) eusynanthropy, i.e., close existence with man, sharing artificial biocoenosis (anthropobiocoenosis); (2) consumption of both contaminated and non-contaminated food; (3) great flight activity and dispersal and (4) constant alternation between feces and food (Greenberg, 1971). Numerous observations indicate that some diseases occur more frequently over the summer and when flies are most numerous (Brown and Adkins, 1972).

Because of its habitats, the adult is capable to transmit a large number of diseases to humans. The transmission takes place mechanically: pathogens may be ingested by the fly and deposited with the fecal spots, ingested and deposited with the vomit, or spread around by the labellar and legs. Larva feeding on infected material can produce infected adult (Smith, 1973). The house fly is thereby able to transmit virus diseases (poliomyelitis and Coxsackie virus, both via human feces); bacterial diseases (many diarrheal and enteric fevers, infantile summer dysentery, typhoid and paratyphoid fevers, bacillary dysentery, all via humans feces; conjunctivitis, tuberculosis, leprosy, plague; streptococci and staphylococci); protozoa parasites (cysts and trophozoites, trypanosomes, amoebic dysentery); tapeworms and nematodes (*Thelazia* spp., etc.) and other arthropods (egg of *Cordylobia* spp., nymph of

Pediculus spp.) (Greenberg, 1971; Smith, 1973; Monzon *et al.*, 1991; Sukontason *et al.*, 2003). Furthermore, house fly is able to transmit *Aeromonas caviae*, pathogen of diarrhea in livestock and humans (Nayduch *et al.*, 2001).

Moreover, *M. domestica* is also involved in mechanical transmission of multiple antibiotic-resistant bacteria in hospital environments (Graczyk *et al.*, 2001). The ultrastructural studies of the mouthpart of house fly revealed that the fly's prestomal teeth are able to damage host tissues via tearing and sucking up cell (Kovacs *et al.*, 1990). This type of damage presumably can allow microorganisms to invade as shown by the successful transfer of Aujeszky 's virus by house fly (Medveczky *et al.*, 1988). *M. domestica* has been implicated as disseminators of trachoma and epidemic conjunctivitis in eastern countries (Greenberg, 1973).

In addition to the ability of transmitting pathogens, adult has been reported as a nuisance in slaughterhouse and on meat, fish, sweets, fruits and other foodstuffs in market places (Greenberg, 1973). In large number of flies can be an important nuisance by disturbing people during work and at leisure. Flies soil the inside and outside of houses with its faeces. Furthermore, it can also have negative psychological impact because their presence is considered a sign of unhygienic condition (Rozendaal, 1997). Economic loss in poultry (e.g., decrease of chicken egg production, reduced value due to unhygienic appearance of eggs contaminated with fly's feces) and dairy farm (e.g, decrease of cattle milk production) has been recorded (Eldridge and Edman, 2000). Moreover, the swarming of large number of flies, emerged from livestock farms, to neighboring domestic settlements may result in considerable social and legal problems for farmers (Kelling, 2001).

Aside from being medically important agent of adult, house fly larva has also been accounted for myiasis-causing fly of vertebrates (Zumpt, 1965). Such cases as intestinal, urino-genital, traumatic, aural and nasopharyngeal myiasis are recorded (Smith, 1973; Eldridge and Edman, 2000).

Bionomics of medical importance of *Chrysomya megacephala*

Classification and geographical distribution

Chrysomya megacephala is a blow fly species, commonly known as Oriental latrine fly. It was classified as follow:

Phylum	Arthropoda
Class	Insecta
Order	Diptera
Suborder	Cyclorrhapha
Family	Calliphoridae
Genus	*Chrysomya*
Species	*Chrysomya megacephala*

C. megacephala is distribute throughout the Asian regions, Australasian, Pacific, South Africa, and South America (Zumpt, 1965; Well and Kurahashi, 1994; Kurahashi and Chowanadisai, 2001). In Thailand, it has been found the second most abundant fly species collected and predominant among blow fly species collected (Sucharit *et al.*, 1976; Tumrasvin *et al.*, 1978; Sucharit and Tumrasvin, 1981).

General morphology

Adult - *C. megacephala* adult is metallic greenish blue with purple reflections. It has short stout body with a noticeably large head. The total body length is 8-11 mm in length. The eyes are unusually large and a very prominent shade of red.

Egg - Egg is larger than that of house fly, measuring about 1.40×0.40 mm. Under natural conditions in Thailand, it gives rise to larva within one day.

Larva – The larva is creamy-muscoid shaped. It is similar to house fly larva, but larger in size. In the third-larval stage, the posterior spiracles have three straight slits that definitely difference from house fly larva, whereas the anterior spiracle shows 11-13 branches on the second segment or prothorax.

Pupa - Pupa case looks like house fly, but bigger in size. Mature puparium is mahogany brown, the anterior projecting spiracles are yellow fan-shaped.

General biology

Life cycle

Life cycle of *C. megacephala* is holometabolous, the same as of house fly. Well and Kurahashi (1994) reported the development of *C. megacephala* at 27°C that completed by all larvae by the following ages: egg hatch, 18 hr; first molt, 30 hr; second molt, 72 hr; pupariation, 144 hr; adult emergence, 234 hr. The adult longevity is shown to be dependent on temperature and humidity. At temperatures of 25-29°C and 75% relative humidity, flies live an average of 54 days (90 in maximum); at lower humidity flies appear to live longer (Greenberg, 1973). Females lay their eggs in batch of 150-300 eggs.

Food & Feeding

Food and feeding behavior of *C. megacephala* resemble of those *M. domestica*. Nevertheless, larva of this blow fly is primarily carrion feeder, and adult shows a preference of fresh remain of corpse and/or carrion (Bohart and Gressitt, 1951).

Breeding site

C. megacephala prefers to breed in meat, carrion, sweet food, urine or excrement; hence, the common name of the Oriental latrine fly. Females deposit their eggs on suitable oviposition sites such as in garbage, high humid areas, decay animals or corpses.

Ecology of adult

C. megacephala is a hemisynanthropic to eusynanthropic exophilous species. Adults have pronounced activity peak during the heat of the afternoon. *C. megacephala* is one of the first species to become active in the early morning hours and is one of the last species to depart carrion at nightfall (Byrd and Castner, 2000). This species seldom oviposits on isolated human feces but in accumulated large masses, and fresh rather than old carrion is preferred (Bohart and Gressitt, 1951).

Medical importance

The most medical importance of *C. megacephala* is mechanical carrier of several pathogens that causing illness in humans similar to *M. domestica*. However, the investigation in Chiang Mai, northern Thailand, indicated the greater mechanically agent than *M. domestica* (Sukontason *et al.*, 2003). The medically important aspects such as annoyance and/or myiasis-producing fly of *C. megacephala* resemble to those noticed in *M. domestica* (Zumpt, 1965).

Fly control strategy

<u>Physical methods</u> (WHO, 1986)

Physical control methods are easy to use and avoid the problem of insecticide resistance, but they are not very effective when fly densities are high. They are particularly suitable for small-scale use in hospitals, hotels, supermarkets and other shops selling meat, vegetables and fruits.

Fly traps: Large number of flies can be caught with fly traps. Picken *et al.* (1994) recommended standard inverted cones traps for captured *M. domestica* whereas *C. megacephala* can be captured in traps fitted with horizontal entry cones or in cone traps in which the bait was placed inside the cone chamber.

Sticky tapes: Commercially available sticky tapes, suspended from ceilings, attract flies because of their sugar content. Flies landing on the tapes are trapped in a glue.

Light trap with electrocutor: Flies attracted to the light are killed on contact with electrocuting grid that covers it. Blue and ultraviolet light attract blow flies but is not very effective against house flies (Rozendaal, 1997).

<u>Chemical methods</u> (WHO, 1986; Rozendaal, 1997)

Control with insecticides should be undertaken only for a short period when absolutely necessary because flies develop resistance very rapidly. The application of effective insecticides can temporarily lead to very quick control, which is essential during outbreaks of cholera, dysentery or trachoma.

Insecticide vaporizers: This method is effective only in places with little ventilation. Insecticide vaporizers such as strips of absorbent material impregnated with dichlorvos are commercially available.

Introduction of toxic materials to resting site: Materials that can be impregnated with insecticide (e.g., organophosphorus compounds, carbamates and pyrethroids) include bed nets, curtains, cotton cords, cloth or gauze bands and strong paper strips. This method is cheap and has a long residual effect. However, it does not work in rooms with an air draught under the ceiling.

Attraction of flies with toxic baits: Traditional toxic baits made use of sugar and water or other fly-attracting liquids containing strong poison such as sodium arsenite. Improvements became possible with the development of organophosphorus and carbamate compounds that are highly toxic to flies but relatively safe to humans and other mammals.

Treatment of resting sites with residual insecticides: Surfaces on which flies rest can be sprayed with a long-lasting insecticide. This method has both an immediate and a long-term effect. However, the risk of resistance developing in flies is greater with residual sprays than with other chemical treatments used against adult flies.

Space-spraying: The treatment is carried out by spraying with pressurized aerosol spray cans, hand-operated sprayers or small portable power-operated sprayers. The principal is to fill a space with a mist of droplets that are picked up by the insects when they fly.

Treatment of breeding sites with larvicides: Chemical substances that kill larvae are mainly use on dung on farm. An importance advantage is that control at this stage tackles the problem at its base. However, the use of larvicides may favour the development of resistance, therefore, the choice of compound should be made carefully.

The advantages of using chemical insecticides were previously mentioned. On the other hand, such drawbacks of insecticide resistance resulting from repeating usage have been prevalently noted (WHO, 1986). Other means of fly control without chemical insecticide usage have also been suggested (WHO, 1986; Rozendaal, 1997).

Biological control

Regarding biological control, an alternative selective approach to control flies is the deployment of natural enemies. The enemies of the adult flies are fungi, of which there are many species, most notably *Empusa muscae* and bacteria. Immature stages are parasitized by Hymenoptera, and preyed upon by ants, beetles, other fly maggots, and mites (Smith, 1973). Scott *et al.* (1991) have been reviewed the important biological control agents, pteromalid parasitoids, that limit populations of filth flies, especially house flies, *M. domestica*, and stable flies, *Stomxys calcitrans*. Under relatively stable conditions and in a suitable climate, fly predators, hymenoptera parasitoids, may limit the fly population to an acceptable level. However, active biological control by release of parasitoids or predators in the natural areas have not been very effective, and it is doubtful if it will become important except in certain isolated areas (Keiding, 1986).

In conclusion the most effective method of controlling flies that breed in domestic wastes is efficient garbage and sewage disposal systems. The improvement of environmental sanitation and hygiene is the most important fundamental step for the success of fly control due to provides longer-lasting results, is more cost-effective and usually has other benefits.

Toxicity and residual effects of synthetic insecticides

Organophosphorus compounds: The most commonly used for the control of house fly are malathion and fenitrothion. Mode of action of organophosphorus compounds is anticholinesterases. Symptoms in insects roughly follow the general pattern of nerve poisoning including, restlessness, hyperexcitability, tremors and convulsions, and paralysis (Matsumura, 1985). They have also been found to inhibit lipid synthesis and chitin synthase (Coats, 1982).

Carbamates: The most commonly used for the contro of house fly are propoxur and bendiocarb, which classified as moderately hazardous. Carbamates are very selective inhibitors of cholinesterase. The symptoms in insect are primarily poisoning of the central nervous system (Matsumura, 1985).

Pyrethroids: This group includes the most recently developed residual insecticides such as deltamethrin, permethrin, lambdacyhalothrin and cypermethrin. The pyrethroids are moderately hazardous and they are safe for spray personnel and house owners. The primary action of pyrethroids is on the insect nervous system, as shown by the fact that they produce such rapid paralysis (Matsumura, 1985).

Toxicity test methods against insects

There are several ways to administer insecticides to insect. The most commonly employed method for insects including flies is topical application, where the insecticide is dissolved in a relatively less toxic and volatile solvent, such as, acetone or absolute ethanol, and is then allowed to come in contact with particular location on the body surface. The results obtained with the topical application procedure can be a very reliable indication of relative contact toxicity of any insecticide to a certain insect (WHO, 1980; Matsumura, 1985; Saito *et al.*, 1992).

However, this topical application method cannot be used in dipterous larvae according to the topical application cannot deliver a sufficient quantity of insecticide. The dipping method is used for dipterous larvae (Matsumura, 1985).

Toxicity effect of crude plant extracts against flies

The impacts of synthetic insecticides on humans and environment safety are concerned. Several crude plant extracts have been studied regarding their toxicity effect against different species of insects including flies. The ethanolic extract of *Nerium oleander*

(Apocynaceae) leaves has been mentioned as having insecticidal effect against second-instar larvae of false stable fly *Muscina stabulans* (Diptera: Muscidae). The LC_{50} was 113.66 ppm, and this dose yielded the delay of larval and pupal developmental period, suppressed oviposition and adult longevity of the survivors (El-Shazly *et al.*, 1996). The ethanol extract of *Calotropis procera* has insecticidal activity against different stages of flesh fly *Sarcophaga haemorrhoidalis* (Moursy, 1997). Two volatile oils of *Matricaria chamomilla* and *Clerodendron inerme* have been investigated the toxicology on adult house fly *M. domestica* by using topical application, with the LC_{50} of the former and the latter being 76 and 84 μg/fly, respectively (Shourkry, 1997). The acetone extract of pomegranate, *Punica granatum,* has also been shown the larvicidal activity on third-instar larvae of *Chrysomyia albiceps,* with LC_{50} being 25 ppm (Morsy *et al.*, 1998).

Eucalyptol properties and toxicity reports

Eucalyptol, the major constituent of *Eucalyptus* spp. leave oil, is considered as interested compound for medical use. This due to several reports of *Eucalyptus* spp regarding the toxicity against insects and medicinal usefulness (Faouzia *et al.*, 1993; Inder Pal and Hideo, 1997; Lamiri *et al.*, 2001).

The medicinal value of Eucalyptus oil was recognized in Australia since 1788. Interest in the chemistry of this essential oils began with Cloez in 1870, who named the principal component of *Eucalyptus globulus* oil as eucalyptol, and it was later identified as cineole by Jahns in 1884 (Ghisaberti, 1996). Although eucalyptol or cineole is a natural constituent of a number of aromatic plants and their essential oil fraction (Table 1), however it is the major of constituent of *Eucalyptus* spp leave oil.

Table 1 Occurrence of eucalyptol in the essential oil from aromatic plants

Botanical name	Common name	Content (%)
Artemisia ponica	Roma mugwort	12-23
Artemisia abiritium	Wormwood	3.7
Artemisia herbo-alba	Not available	0.5-15
Eucalyptus globulus	Eucalyptus globulus	70-80
Ocimum basilicum	Sweet basil	8
Rosmarinus officinalis	Rosemary	12-47
Salvia officinalis	Sage	8-23
Salvia lavandulaefolia	Spanish sage	11.8-41.2
Elettaria cardamomum	Cardamon matou	13.1-51.3
Hedichium flavum	Garland flower	42.2
Menthe piperita	Peppermint	5-18
Menthe spicate	Spearmint	6

Chemical structure of eucalyptol

Eucalyptol ($C_{10}H_{18}O$); molecular weight = 154.24) CAS no. 470-82-6. Synonyms: 1,3,3-Tri-methyl-2-oxabicyclo[2.2.2]-octane; 1,8-epoxy-*p*-menthane; cineole; cajeputol. Structural formula of eucalyptol is shown in Figure 1. Eucalyptol is a colorless, optically active liquid possessing a camphoraceos odor with a spicy, cooling taste. It is insoluble in water but soluble in organic solvents such as alcohol, oil, chloroform, and ether (Tripathi *et al.*, 2001).

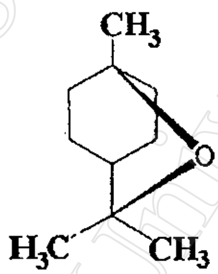

Figure 1 Structural formula of eucalyptol

Eucalyptol is given GRAS (Generally Recognized As Safe) status by the Flavor and Extract Manufacturer's Association (FEMA, 1965) and is approved by the Food and Drug Administration (FDA, USA) for food use [CFR (Code of Federal Regulation) 172.510] (De Vincenzi *et al.*, 2002). Eucalyptol has several uses in pharmaceutical and industrial preparations such as flavoring agent and expectorant (in chronic bronchitis). It has bactericidal properties and finds quasi-pharmaceutical applications such as disinfecting wounds and cleaning congested nasal passage (Acharya *et al.*, 2001)

Toxicity of eucalyptol to insects

Eucalyptol was assessed regarding the toxicity with several species of insects. The fourth-instar larvae of bug *Triatoma infestans* have been tested with eucalyptol using impregnated paper test, with the LD_{50} being 1.38 mg/cm^2 (Laurent *et al.*, 1997). It has also been investigated for the possible fumigant, contact, and ingestion activity against lesser grain borer *Rhyzopertha dominica*, and red flour beetle *Tribolium castaneum*. The results showed that the main action were contact and/or ingestion (Prates *et al.*, 1998). Eucalyptol extracted from *Artemisia annua,* which has purity 98%, was applied with *T. castaneum*. The LD_{50} value for larvae was 108.04 µg/mg weight using contact method, while LD_{50} value for adult was 1.52 mg/L air by fumigant toxicity method (Tripathi *et al.*, 2001). Sánchez-Ramos and Castañera (2001) reported the natural monoterpenes substances including eucalyptol, as having quite high acaricidal activity, by vapour action, against mobile stages of stored pest mite *Tyrophagus putrescentiae*. Eucalyptol at 50 µg/ml air caused 100% mortality in some important stored-product pest insects, including the rice weevil, *Sitophilus oryzae*, the red flour beetle, *T. castaneum*, the sawtoothed grain beetle, *Oryzaephilus surinamensis*, the house fly, *M. domestica*, and the German cockroach, *Blattella germanica* (Lee *et al.*, 2003).

<u>Toxicity of eucalyptol to mammals and humans</u>

The subacute toxicity of eucalyptol was studied in rats and mice. Mice were more susceptible to eucalyptol than rat. Toxicity was found in male rats at dose higher than 600 mg/kg while no toxic effect was seen in mice up to 1200 mg/kg. Following the accidental exposure of humans beings, death was reported only in two cases after ingestion of 3.5-5 ml of essential eucalyptus oil, but a number of recoveries have also been described for much higher amounts of oil (De Vincenzi *et al.*, 2002).

PURPOSE OF THE STUDY

To assess the toxicity of eucalyptol against adults and third-stage larvae of *M. domestica* and *C. megacephala* by using topical application method for adults and dipping method for third-stage larvae.