Songklanakarin J. Sci. Technol.
30 (5), 667-672, Sep. - Oct. 2008

Songklanakarin Journal of Science and Technology
http://www.sjst.psu.ac.th

Original Article

Contact toxicity of the crude extract of Chinese star anise fruits to house fly larvae and their development

Guntharee Sripongpun*

Department of Environmental Science, Faculty of Science, Silpakorn University, Nakorn Pathom 73000, Thailand

Received 25 April 2008; Accepted 6 August 2008

Abstract

Contact toxicity of the ethanol crude extract of Chinese star anise fruits, *Illicium verum* Hook. F. (Illiciaceae) to house fly larvae, *Musca domestica* L. (Diptera: Muscidae) and their development were determined by a dipping method. Younger larvae were more susceptible than older. The median lethal concentration (LC_{50}) values of the crude extract to the 2nd instar larvae at 24, 48 and 72 hours were $7.4x10^4$, $4.1x10^4$ and $3.2x10^4$ mg/l, respectively. Furthermore, treated extracts affected house fly development in pupal and adult stages. The number of test larvae developed to pupae and adults was less than that of control group. The crude extract at concentration $8.5x10^4$ mg/l completely inhibited development from larvae to pupae. In addition, small pupae were found at every concentration between $2.5x10^3$ -$105x10^3$ mg/l. Small treated pupae were observed more at higher concentrations. Some of them could continually develop to adults but their developmental percentages were less than those of normal sized pupae in treatments and in control group. The crude extract of Chinese star anise fruits can be applied as an optional point source control of house fly.

Keywords: *Illicium verum*, *Musca domestica*, larvicide, pest management

1. Introduction

House fly, *Musca domestica* L. (Diptera: Muscidae) is an ideal vector for transmission of many pathogens in human and animal because of its biology and ecology. House flies can be found worldwide, except Antarctica. Therefore, they become very important in public health concern. Chemical insecticides are commonly used for their control. Nevertheless, the widespread and inappropriate application of chemical insecticides produce the risk of developing pest resistance, side effects to non-target species, and long lived residues in the environment (Kristensen and Jespersen, 2003; Nivsarkar *et al.*, 2001; Taşkin *et al.*, 2004).

Consequently, plant products become more interesting. Many plants have been reported about their potential insecticidal actions on larvae and/or adults of house flies via crude extracts or extracted active compounds (Russell *et al.*, 1976; Gbewonyo and Candy, 1992; Shalaby *et al.*, 1998; Liao, 1999; Morsy *et al.*, 2001; Singh, 2001; Sukontason *et al.*, 2004; Abdel Halim and Morsy, 2006). Some results also showed their effects on metamorphosis or emergence or fecundity or life span of house flies (Liao, 1999; Sukontason *et al.*, 2004; Abdel Halim and Morsy, 2005; Abdel Halim and Morsy, 2006).

Chinese star anise, *Illicium verum* Hook. F. (Illiciaceae) is a small evergreen tree. Its original is in the south of China and the north of Vietnam. The pericarp has aromatic odour and sweet taste. The star anise fruit is traditionally used in the symptomatic treatment of digestive disturbance such as epigastic bloating, impaired digestion, eructation, and flatulence. It is also traditionally used as an adjunctive therapy for the painful component of spasmodic colitis (Bruneton, 1995). The anise has a volatile content of 2.5-3.5% in fresh fruit and 8-9% in dried material. The main component in star anise essential oil is trans-anethole, a paramethoxyphenyl propene which accounts for about 85-90% (by weight). The rest includes terpene, pinene, β-phellandrene, α-diterpene,

*Corresponding author.
Email address: gunthare@su.ac.th

limonene, estragole, safrol and terpineol (Tuan and Ilangantileke, 1997). The non - polar extract at $9.6 x 10^5$ mg/l caused 100% mortality of *Tribolium castaneum* adults, but only caused 55% mortality in *Sitophilus zeamais* adults. In addition, the eggs of *T. castaneum* failed to develop to adults when they were treated with non-polar star anise extracts at $1.0 x 10^4$ mg/l. Moreover, 70% mortality of *T. castaneum* adults occurred in rice treated with the non-polar extract at 0.01 mg of extract/mg of rice after 21 days exposure. Non-polar extracts completely suppressed F_1 adult emergence in both species, while polar extracts only caused a significant reduction in F_1 adult emergence (Ho *et al.*, 1995). Later, Kim *et al.* (2003a) and Kim *et al.* (2003b) reported about contact activity of the methanol extract from *Illicium verum* fruits at $3.5 x 10^3$ mg/l by filter paper diffusion method caused 100% mortality 1 day after treatment against *Lasioderma serricorne* (F.) and *Callosobruchus chinensis* (L.) adults, while it caused 100% mortality 4 days after treatment against *Sitophilus oryzae* (L.) adults.

However, scientific studies of the effects of Chinese star anise fruits on house fly larvae and their development have not been reported. Therefore, contact toxicity tests based on the median lethal time (LT_{50} and LT_{95}) and the median lethal concentration (LC_{50} and LC_{95}) of the crude extract of Chinese star anise fruits to house fly larvae were investigated. In addition, the development of larvae exposed to that crude extract was examined to provide an optional control strategy of house fly.

2. Materials and Methods

2.1 Insects tested

House fly adults were collected from a canteen in Silpakorn University, Nakorn Pathom province, Thailand, and identified by the Department of Biology, Faculty of Science, Silpakorn University. Adult flies were kept and colonized in 0.3x0.3x0.3 m cages at room temperature 28-31°C. They were reared with sugar, water soaked into cotton wool, and a combination of fish feed and bird food (after being softened with water) at a weight ratio of 1:2 as a food source and an oviposition site. The food was changed daily, while the other items were available throughout the period of the study. To minimize insecticide residue that might be found in test insects and to allow house flies to be familiar with the laboratory condition, the 3rd generation larvae were used in these experiments.

2.2 Plants preparation

Dry fruits of Chinese star anise were washed, dried in the oven at 50°C for 6 hours and ground to fine powder. The result from a preliminary dipping method showed that the aqueous extract did not affect on the 2nd instar larvae of house fly. Then, the powder was extracted with 95% ethanol at room temperature for 48 hours in darkness and filtered. The filtrate was concentrated to dryness by a rotary evaporator at 40°C. Twelve percent yield (% w/w) was achieved by this procedure. The stock of extract was kept in a closed container at 4°C. The determined concentrations for toxicity tests were prepared by using 5% ethanol as a diluent.

2.3 Toxicity test of house fly larvae based on LT_{50} and LT_{95}

The crude extract of Chinese star anise fruits at $1.5 x 10^5$ mg/l was immediately prepared as previously described. Twenty house fly larvae were randomized for each replication of the 1st, 2nd, and 3rd instar larvae. Three replications were set up per treatment at room temperature (30°C). First, test larvae were placed into one side of a petri dish (0.1x 0.015 m) and 4 ml of the crude extract was added to another side. Next, this petri dish was leaned to let the larvae dip in the extract for 5 seconds. Then, the extract was leaned to another side; the larvae were transferred to the rearing container. The container containing food of larvae was covered with muslin cloth and tied with rubber ring. For the control, the larvae were dipped in 5% ethanol. Finally, the mortality of larvae was checked by softly touching each one with a small paint brush (No. 0) at 0, 4, 6, 8, 12, 18, 22, 24, 48, 72 and 96 hours, respectively; and those which showed no response were considered dead. Mortality was calculated using Abbot's formula.

$$\% \ Mortality = \frac{(\% \ test \ mortality) - (\% \ control \ mortality)}{100 - (\% \ control \ mortality)} \ x \ 100$$

The lethal time values (LT_{50} and LT_{95}) for the 1st, 2nd, 3rd larvae were determined by EPA Probit Analysis Program.

2.4 Toxicity test of house fly larvae based on LC_{50} and LC_{95}

According to the result of toxicity test of house fly larvae based on LT_{50} and LT_{95} (Topic 2.3), the 2nd instar larvae were chosen as the representative animals for further experiments because their susceptibilities were between those of the 1st and 3rd instar larvae. A pretest of the crude extract of Chinese star anise fruits was done against the 2nd instar larvae by dipping method as previously described to find the appropriate concentration range for contact toxicity of the test extract. The range was further divided into 11 concentration levels, viz. 0, $2.5 x 10^3$, $5.0 x 10^3$, $10 x 10^3$, $15 x 10^3$, $20 x 10^3$, $25 x 10^3$, $45 x 10^3$, $65 x 10^3$, $85 x 10^3$ and $105 x 10^3$ mg/l. These concentration levels were applied by dipping method to 20 larvae for each replication. Three replications were carried out per treatment (concentration). The lethal concentration values (LC_{50} and LC_{95}) at 24, 48, and 72 hours were determined by EPA Probit Analysis Program. Later, all living larvae were studied further for their development.

2.5 Effects of the crude extract of Chinese star anise fruits on house fly larvae development by dipping method

After dipping with the extract of Chinese star anise fruits, living larvae for each concentration from the toxicity test of house fly larvae based on LC_{50} and LC_{95} (Topic 2.4) were reared further with untreated food in a container which was covered with muslin cloth and tied with rubber ring. Percentages of the succeeded house fly larvae and pupae that could develop to next stages were expressed as mean $\pm$ S.D. (standard deviation). The morphological appearance of the resultant pupae and adults was also observed.

Data from each treatment of the 3rd instar larvae developed to pupae were statistically analyzed using one-way ANOVA and means were compared according to Duncan's multiple range test. Significant difference was considered at $p<0.05$. However, not a large number of living test pupae were left to develop to adults for each treatment, especially at high concentrations. Therefore, the total number of living pupae was pooled at each test concentration and the percentage of pupa developed to adult was reported as mean $\pm$ S.D.

3. Results and Discussion

As shown in Table 1, younger larvae were more susceptible than older. The lethal time values (LT_{50} and LT_{95}) of the crude extract of Chinese star anise fruits at $1.5\text{x}10^5$ mg/l applied to the 1st, 2nd, 3rd instar house fly larvae by dipping method were 7.7 and 18.0, 9.8 and 22.5, 18.7 and 60.9 hours, respectively.

Marcus and Lichtenstein (1979) reported that trans-anethole was the major insecticidal agent present in anise oil of *Pimpinella anisum* L. with an LD_{50} of 75 µg/fly when it was topically applied to house flies. Trans-anethole was also a major component found in volatile oil of *Illicium verum* Hook. F. (Duke 1985). Therefore, trans-anethole should act as a prominent effect on house fly larvae mortality in this experiment as well. Contact LC_{50} and LC_{95} by the dipping method of the crude extract of Chinese star anise fruits to the 2nd instar larvae of house flies after 24, 48 and 72 hours of exposure are shown in Table 2. At 95% confidence limits, the LC_{50} values were between $7.4\text{x}10^4$ - $3.2\text{x}10^4$ mg/l while the LC_{95} values were between $24.0\text{x}10^4$ - $11.0\text{x}10^4$ mg/l. At early treatment period, larval movement was always observed. This may be because of skin irritation by trans-anethole (Thermo Fisher Scientific Inc., 2008). Later, the motion of larvae decreased until died, which was in accordance with the pharmacologic effects of trans-anethole, viz. reduction in motor activity, lowering of body temperature and hypnotic effects (IPCS INCHEM, 2008). The body colour of dead larvae became more brownish especially the lower part of the body with prolonged treatments. It was in good agreement with digestive tract irritation and cell necrosis caused by continuous intake of high dose level of trans-anethole (IPCS INCHEM, 2008 and Newberne *et al.*, 1999).

The results in this study showed obviously that the tendency for the development of test larvae to pupae decreased with a rise of the crude extract concentration (Table 3). The larvae treated with the crude extract at $8.5\text{x}10^4$ mg/l could not develop to pupae completely. At lower concentration, some larvae could continually develop. Most of these larvae developed to pupae within 5 days. Morphological appearance of the pupae was not different from that of control. However, two sizes of treated pupae were

Table 1. Lethal time of the crude extract of Chinese star anise fruits at $1.5\text{x}10^5$ mg/l against house fly larvae at 95% confidence limits

	Stage of test larvae		
	1st instar	2nd instar	3rd instar
LT_{50} (95% CI) (hour)	7.7 (7.0-8.5)	9.8 (9.0-10.7)	18.7 (17.1-20.6)
LT_{95} (95% CI) (hour)	18.0 (15.7-21.6)	22.5 (19.9-26.4)	60.9 (51.4-75.6)
Slope $\pm$ SE	4.5$\pm$0.4	4.6$\pm$0.4	3.2$\pm$0.2
χ^2	7.2	6.8	3.9
N	60	60	60

Table 2. Lethal concentrations of the crude extract of Chinese star anise fruits against house fly larvae for various experimental periods at 95% confidence limits

Experimental period (hour)	LC_{50} (95% CI) $\text{x}10^4$ mg/l	LC_{95} (95% CI) $\text{x}10^4$ mg/l	Slope$\pm$SE	χ^2	N
24	7.4 (6.6-8.4)	24.0 (18.3-37.1)	3.2±0.4	3.5	60
48	4.1 (3.7-4.5)	13.3 (11.1-16.9)	3.2±0.3	9.5	60
72	3.2 (2.8-3.6)	11.0 (8.9-14.7)	3.1±0.3	8.1	60

Table 3. Percentage of house fly larvae* developing to pupae when were treated with various concentrations of the crude extract of Chinese star anise fruits at 30°C

Concentration of the crude extract x10^3 mg/l	Average percentage of test larvae developed to pupae	Percentage of normal sized pupae**	Percentage of small sized pupae***
0 (control)	100^{a}	100^{a}	-
2.5	91 ± 2^{ab}	91 ± 8^{ab}	9 ± 8^{ab}
5.0	91 ± 2^{ac}	89 ± 5^{ac}	11 ± 5^{ac}
10.0	87 ± 3^{ad}	85 ± 4^{ad}	15 ± 4^{ad}
15.0	85 ± 0.1^{bcd}	80 ± 11^{bcd}	20 ± 11^{bcd}
20.0	80 ± 10^{bcd}	76 ± 5^{bcde}	24 ± 5^{bcde}
25.0	63 ± 2^{e}	70 ± 11^{bcde}	30 ± 11^{bcde}
45.0	50 ± 6^{ef}	60 ± 12^{e}	40 ± 12^{e}
65.0	40 ± 15^{f}	60 ± 12^{e}	40 ± 12^{e}
85.0	0^{g}	0^{f}	0^{f}

* Values within each column followed by the same superscript are not significantly different at p = 0.05
** Normal sized pupae was about 2 mm wide x 5 mm long
*** Small sized pupae was about 1 mm wide x 3 mm long

Table 4. Comparative percentage of pupae developing to adults from total pupae, normal sized pupae, and small sized pupae at 30°C

Concentration of the crude extract x10^3 mg/l	Percentage of total pupae developed to adults*	Percentage of normal sized pupae developed to adults**	Percentage of small sized pupae developed to adults***
0 (control)	98.25(56/57)	98.25(56/57)	-
2.5	90.74(49/54)	91.84(45/49)	80.00(4/5)
5.0	88.68(47/53)	91.49(43/47)	66.67(4/6)
10.0	81.25(39/48)	85.37(35/41)	57.14(4/7)
15.0	77.29(34/44)	82.86(29/35)	55.56(5/9)
20.0	69.70(23/33)	76.00(19/25)	50.00(4/8)
25.0	63.16(12/19)	71.43(19/14)	40.00(2/5)
45.0	58.34(7/12)	71.43(5/7)	40.00(2/5)
65.0	40.00(2/5)	66.67(2/3)	0(0/2)

* Each value in parenthesis is the number of total pupae developed to adults/the number of total pupae
** Each value in parenthesis is the number of normal sized pupae developed to adults/ the number of total pupae
*** Each value in parenthesis is the number of small sized pupae developed to adults/ the number of total pupae

observed. The first group had the same size as that of control, which was about 2 mm wide x 5 mm long. The size of the second group was smaller, which was about 1 mm wide x 3 mm long. Majority of control pupae changed to adults within 8 days, while the treated normal and small pupae became adults within 10 and 11 days, respectively. The tendency of the treated pupae developed to adults decreased more when they were treated with higher concentrations of the extract (Table 4). In addition, the number of small pupae developed to adults was less than that of normal pupae in treatments and in control group.

The crude extract of Chinese star anise fruits can be applied in control of house fly at breeding sites. It can affect both on contact toxicity and the development of house fly larvae. However, the applied concentration should not be less than 8.5x10^4 mg/l. This concentration could completely inhibit the development of the 2nd instar larvae to pupae and could reduce the distribution of house flies. However, the laboratory results might be different from those in the field. Therefore, environmental factors such as light intensity,

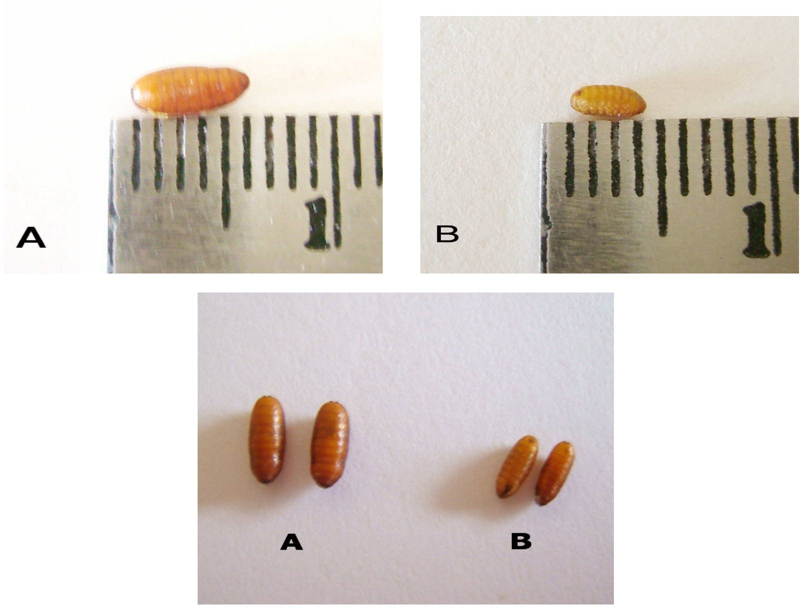

Figure 1. Comparative appearance of pupae developed from test larvae between
(A) normal sized of test pupae or control pupae and
(B) small sized of test pupae

Figure 2. Comparative appearance of adult developed from test pupae between
(A) normal sized of test pupae or control pupae and
(B) small sized of test pupae

temperature, and season should be further studied for application in the field. Different solvents should be also tried more in order to be able to get different results. Then, advantage and disadvantage of each solvent can be compared to choose the most optimum solvent for that situation. Furthermore, the strong smell of Chinese star anise fruits is of interest to be used in the form of house fly repellent sticks. It was also reported that anethole and anisaldehyde were both found to increase the toxicity to house flies when applied simultaneously with parathion, paraoxon, carbaryl, carbofuran, DDT, or pyrethrum (Marcus and Lichtenstein, 1979). In addition, trans-anethole synergized the toxicity of thymol in *Spooptera litura* (Passreiter *et al.*, 2004). Therefore, the crude extract of Chinese star anise becomes more affective. Other crude plant extracts or extracted active compounds have been reported to have effects on house fly toxicity and/or development as previous mentioned in introduction section. Consequently, the combination of these with the crude extract of Chinese star anise is also of interest in house fly control.

4. Conclusion

The results of this study provide more information about contact toxicity of the crude extract of Chinese star anise fruits to house fly larvae and their development. This crude extract can be applied as an optional control of house fly at breeding sites.

Acknowledgements

This research project was partially supported by research grants from Silpakorn University Income. The author appreciates the experimental assistance of Miss Chalalai Rungruang. Department of Environmental Science, Faculty of Science, Silpakorn University is also thanked for providing laboratory facilities and equipment for this study.

References

Abdel Halim, A.S. and Morsy, T.A. 2005. The insecticidal activity of *Eucalyptus globulus* oil on the development of *Musca domestica* third stage larvae. Journal of the Eqyptian Society of Parasitology. 35, 631-636.

Abdel Halim, A.S. and Morsy, T.A. 2006. Efficacy of *Trigonella foenum-graecum* (fenugreek) on third stage larvae and adult fecundity of *Musca domestica*. Journal of the Eqyptian Society of Parasitology. 36, 329-334.

Baki, M.A., Rahman, M.A.A., Khatune, N.A., Zahid, R.A., Khalequzzaman, M., Husain, M.M. and Sadik, G. 2002. Synergic effects of *Wedelia calendulacea* Less. plant extracts with lambda cyhalothrin on common housefly *Musca domestica* L. Online Journal of Biological Science. 2, 688-689.

Bruneton, J. 1995. Pharmacognosy, Phytochemistry, Medicinal Plants. Hatton CK., Translator. Intercept Ltd., Hampshire SP 101YG, UK. pp. 441-443.

Duke, J.A. 1985. CRC Handbook of Medicinal Herbs. CRC Press, Inc., Florida, U.S.A., p. 247.

Gbewonyo, W.S. and Candy, D.J. 1992. Separation of insecticidal components from an extract of the roots of male *Piper quineense* (west African black pepper) by gas chromatography. Toxicon. 30, 1037-1042.

Ho, S.H., Ma, Y., Goh, P.M.and Sime, K.Y. 1995. Star anise, *Illicium verum* Hoof f. as a potential grain protectant against *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Postharvest Biology and Technology. 6, 341-347.

Kim, S., Park, C., Ohh, M., Cho, H. and Ahn, Y. 2003a. Contact and fumigant activities of aromatic plant extracts and essential oils against *Lasioderma serricorne* (Coleoptera: Anobiidae). Journal of Stored Products Research. 39, 11-19.

Kim, S., Roh, J., Kim, D., Lee, H. and Ahn, Y. 2003b. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. Journal of Stored Products Research. 39, 293-303.

Kristensen, M. and Jespersen, J.B. 2003. Larvicide resistance in *Musca domestica* (Diptera: Muscidae) population in Denmark and establishment of resistant laboratory strains. Journal of Economic Entomology. 96, 1300-1306.

Liao, S.C. 1999. Mortality and repellency effects of essential oils from citrus against the housefly and German cockroach. Zhonghua Kunchong. 19, 153-160.

Marcus, C. and Lichtenstein, E.P. 1979. Biologically active components of anise: Toxicity and interactions with insecticides in insects. Journal of Agriculture and Food Chemistry. 27, 1217-1224.

Morsy, T.A., Rahem, M.M. and Allam, K.A. 2001. Control of *Musca domestica* third instar larvae by the latex of *Calotropis procera* (Family: Asclepiadaceae). Journal of the Eqyptian Society of Parasitology. 31, 107-110.

Newberne, P, Smith, R.L., Doull, J., Goodman, J.I., Munro, I.C., Portoghess, P.S., Wagner, B.M., Well, C.S., Woods, L.A., Adams, T.B., Lucas, C.D. and Ford, R.A. 1999. The FEMA GRAS assessment of trans-anethole used as a flavouring substance. Food and Chemical Toxicology. 37, 789-811.

Nivsarkar, M., Cherian, B. and Padh, H. 2001. Alpha-terthienyl: A plant-derived new generation insecticide. Current Science. 81, 667-672.

Passreiter, C.M, Wilson, J., Andersen, R. and Isman, M.B. 2004. Metabolism of thymol and trans-anethole in larvae of *Spodoptera litura* and *Tricholplusia ni* (Lepidoptera: Noctuidae). Journal of Agriculture and Food Chemistry. 52, 2549-2551.

Russell, G.B., Sigh, P. and Fenemore, P.G. 1976. Insect-control chemicals from plants. III. Toxic lignans from *Libocedrus bidwillii*. Australian Journal of Biological Sciences. 29, 99-103.

Shalaby, A.A., Allam, K.A., Mostafa, A.A. and Fahmy, S.M. 1998. Insecticidal properties of citrus oils against *Culex pipiens* and *Musca domestica*. Journal of the Eqyptian Society of Parasitology. 28, 595-606.

Singh, D. 2001. Correspondence: bioinsecticides from plants. Current Science. 78, 7-8.

Sukontason, K.L., Boonchu, N., Sukontason, K. and Choochote, W. 2004. Effects of eucalyptol on house fly (Diptera: Muscidae) and blow fly (Diptera: Calliphoridal). Revista do Instituto de Medicina Tropical de São Paulo. 46, 97-101.

Taşkin, V., Kence, M. and GöÇmen, B. 2004. Determination of malathion and diazinon resistance by sequencing the MdαE7 gene from Guatemala, Colombia, Manhattan, and Thailand housefly (*Musca domestica* L.). Russian Journal of Genetics. 40, 377-380.

The International Programme on Chemical Safety (IPCS) INCHEM. 466. 2008. Trans-anethole (WHO Food Additives Series 14). http://www.inchem.org/ducuments/jecfa/jecmono/v14je02.htm [April 3, 2008]

Thermo Fisher Scientific Inc. Material Safety Data Sheet. 2008. Trans-Anethole, 99% (GC). http://fscimage.fishersci.com/msds/240.46.htm [April 3, 2008]

Tuan, D.Q. and Ilangantileke, S.G. 1997. Liquid CO_2 extraction of essential oil from star anise fruits (*Illicium verum* H.). Journal of Food Engineering. 31, 47-57.

United States Environmental Protection Agency. 2001. EPA Probit Analysis Program Used for Calculating LC/EC Value Version 1.5. United States Environmental Protection Agency, Cincinnati, Ohio.