ADULTICIDAL EFFECT OF NERIUM INDICUM MILL. AZADIRACHTA INDICA A. JUSS AND ANNONA SQUAMOSA LINN. LEAF EXTRACTS ON MUSCA DOMESTICA LINN.

SUJITRA WANPUT

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Thesis

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was submitted to the Faculty of Graduate Studies, Mahidol University for the degree of Master of Science (Public Health) major in the Infectious Diseases and Epidemiology

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ABSTRACT

This study was designed for herbal insecticidal treatment so as to replace the use of chemical insecticides. The *Nerium indicum* Mill.,*Azadiracta indica* A. JUSS and *Annona squamosa* Linn. leaves were extracted with water. The solution was fed to the female adult house flies . The mortality rate of them was observed and recorded at 12, 24, 36 and 48 hours. Different herbal leaf extracts with different dilutions of herbal leaf extracts on female adult house fly at 24 and 48 hours were compared. Analysis of data was undertaken by using 2 way ANOVA and heterogenity data were recorded. The nonparametric data was analyzed by using Kruskal-wallis. The results found that at 24 hours, type and dilution of herbal leaf extracts did not have significant effect on female adult house flies. The dilution of herbal leaf extracts did not have significant effect on the mortality rate of female adult house flies. However, type of herbal leaf extracts had a significant effect on the mortality rate of female adult house flies at 48 hours. Female adult house flies killed by *Nerium indicum* Mill.leaf extracts were more than *Azadiracta indica* A. JUSS. and *Annona squamosa*.Linn. leaf extracts. *Azadiracta indica* A. JUSS. was more effective in terms of mortality rate of female adult house flies than *Annona squamosa* Linn. leaf extracts.

KEY WORDS : MUSCA DOMESTICA LINN. / NERIUM INDICUM MILL. AZADIRACTA INDICA A. JUSS/ ANNONA SQUAMOSA LINN.

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การฆ่าแมลงวันบ้านระยะตัวเต็มวัยโดยการใช้น้ำสกัดจากใบยี่โถ ใบสะเดาและใบน้อยหน่า (ADULTICIDAL EFFECT OF NERIUM INDICUM MILL. AZADIRACTA INDICA A. JUSS AND ANNONA SQUAMOSA LINN. LEAF EXTRACTS ON MUSCA DOMESTICA LINN.

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คณะกรรมการควบคุมวิทยานิพนธ์ : วงเดือน ปั้นดี, Dr.P.H., สุเทพ ศิลปานันทกุล, Ph.D.

บทคัดย่อ

ในการศึกษาครั้งนี้ได้ทำการศึกษาเกี่ยวกับการใช้สมุนไพรแทนที่การใช้ยาฆ่าแมลง โดนการใช้ใบยี่โถ ใบสะเดา ใบน้อยหน่า คั้นสดกับน้ำหลังจากนั้นนำไปให้แมลงวันบ้านเพศเมียระยะตัวเต็มวัยกินและสังเกตและจดบันทึกจำนวนการตายของแมลงวันบ้านเพศเมียระยะตัวเต็มวัยที่เวลา 12, 24, 36, และ 48 ชั่วโมง เปรียบเทียบการใช้ชนิดของในสมุนไพรที่แตกต่างกันและความเข้มข้นของน้ำสมุนไพรที่แตกต่างกันต่อจำนวนการตายของแมลงวันบ้านเพศเมียระยะตัวเต็มวัยที่เวลา 24 และ 48 ชั่วโมง ข้อมูลที่ได้วิเคราะห์ด้วย 2 ways ANOVA พบว่าข้อมูลที่ได้มีการกระจายไม่เหมือนกัน จึงวิเคราะห์ด้วย Kruskal- wallis ผลการศึกษาที่ได้พบว่าที่เวลา 24 และ 48 ชั่วโมงความเข้มข้นของน้ำสกัดจากใบสมุนไพรไม่มีผลต่อการตายของแมลงวันบ้านเพศเมียระยะตัวเต็มวัย และที่เวลา 24 ชั่วโมง ชนิดของใบสมุนไพรไม่มีผลต่อการตายของแมลงวันบ้านเพศเมียระยะตัวเต็มวัยที่ 48 ชั่วโมง ชนิดของใบสมุนไพรมีผลต่อการตายของแมลงวันบ้านเพศเมียระยะตัวเต็มวัยโดยน้ำสกัดจากใบยี่โถมีผลต่อการตายของแมลงวันบ้านเพศเมียระยะตัวเต็มวัยมากกว่าน้ำสกัดจากใบสะเดา และใบน้อยหน่า น้ำสกัดจากใบสะเดามีผลต่อการตายของแมลงวันบ้านเพศเมียระยะตัวเต็มวัยมากกว่าน้ำสกัดจากใบน้อยหน่า

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LIST OF CONTENTS

		Page
ACKNOWLEDGEMENT……...		iii
ABSTRACT……...		iv
LIST OF TABLES……...		viii
LIST OF FIGURE……...		x
CHAPTER		
I	INTRODUCTION……...	
	Rationale……..	1
	Research Objectives …….	3
	Scope of Study ……	3
	Limitation of the study ……...	4
	Definition of Terms……	4
II	LITERATURE REVIEW…….	
	1. House fly	7
	2. *Nerium indicum* Mill……..	15
	3. *Azadiracta indica* A. Juss……...	17
	4. *Annona squamosa* Linn.	18
	5. Related Researches……	21
III	RESEARCH METHODOLOGY……	
	Experimental Design	23
	Material and Equipment	23
	Method of examinations……..	29
	Data Analysis……..	30

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LIST OF CONTENTS (CONT.)

CHAPTER		Page
IV	RESULT……	31
V	DISCUSSION……	39
VI	CONCLUSION AND RECOMMENDATION…..	42
REFERENCES……		44
APPENDIX A……		52
APPENDIX B……		54
BIOGRAPHY……		56

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LIST OF TABLES

Table		Page
1	Mean and standard deviation of mortality of female adult flies From different type of herbal leaves,dilution of leaves extract, times	33
2	Result of varience analysis(2 way) of the mortality of female adult flies different type of herbal leaves,dilution at 24 hours	34
3	Result of varience analysis(2 way) of the mortality of female adult flies different type of herbal leaves,dilution at 48 hours	35
4	Mean comparison of mortality of female adult flies among *Nerium indicum* Mill. *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts	36
5	Percentage of mortality of female adult flies by time and types of *Nerium indicum* Mill. *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts	37

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LIST OF FIGURES

Figure		Page
1.	Scope of the study	6
2.	Life cycle of house fly	9
3.	Adult of house flies	25
4.	*Nerium indicum* Mill. leaves	26
5.	*Azadiracta indica* A. Juss. leaves	27
6.	*Annona squamosa* Linn. leaves	27
7.	Methods of examinations	29
8.	Graph of mortality rate of female adult house flies	38

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CHAPTER 1
INTRODUCTION

A. Introduction and Rationale

The house fly, *Musca domestica Linnaeus*, is the well known cosmopolitan pest being always found in municipality, solid waste disposal, cattle and poultry farms, slaughterhouses and food processing factories. They are not only nuisance but also being vectors of various disease – causing organisms for human and animals that cause serious public health problem (1). The *Musca domestica Linnaeus* has been regarded as a potential and important vectors for mechanical transmission of several enteric,skin and eye diseases, and helminths and viral infection (i.e.poliomyelitis or ECHO virus) Shigellosis, amoebic dysentery and other diarrhoeal diseases may be transmitted under unsanitary condition by house fly. As few as 100 Shigella bacteria may cause infection in man (2). House fly can carry eggs and cysts of many intestinal worms e.g. pinworm (*Enterobius spp.*), roundworm (*Ascaris spp.*), whipworm (*Trichiuris spp.*) hookworm (*Ancylostoma spp*. and *Necator spp*.), Strongyloides, tapeworm (*Taenia spp.*) *Dipylidium spp.*, etc., and may in some cases contribute to the spread of helminthic diseases to man and animals (3). Mechanical transmission of diseases by house fly had tremendous impact to socio-economic system. Moreover, the socio economic loss from psychological effects, reduce of product, tourism and Thai ‘s villager are immemse value so that we can estimate. Therefore, house fly must be continuously controlled

According to this problem, the method of controlling house fly have developed. There are four methods in controlling population of house fly which consist of environment sanitation, mechanical, chemical and biological controls (4).

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House fly can be managed to some extent by sanitation measures that reduce the accumulation of waste materials serve as the breedind sites.

Using chemical insecticides is an easy and effective method of controlling adult flies. On the other hand , house fly has developed resistance to insecticides which require higer concentration and more frequent treatment. Resistance is a major problem of flies control . House fly resistance has been an world widely increasing problem in the past decades . Effect to manage this dilemma become difficult because of the development of resistance to nearly all insecticides used in flies control (5) . Many regions of the world have reported that house fly developed resistance to organochorine, organophosphate, carbamate and synthetic pyrethroids at different levels (6).

For organophosphorus compounds, melathion resistance is high and almost universal while other organophosphorus compounds develop moderately to highly resistance in some part of the world. However multi- resistance to most of this group has reported from almost part of the world (7). Resistance to organochlorine compounds including DDT, HCT and cyclodienes is still present practically everywhere (8). Carbamate resistance is often found in house fly population which has already developed organocholine or organophosphorus group. Pyrethroid resistance had developed repidly and at high level after photostable pyrethroids spraying in Germany,Switzerland,United Kingdom and spread to other areas such as Chaina and Japan (9). However, multi-resistance occurred in some areas such as house fly has developed resistance to all insecticides (10).

According to the problems of insecticides resistance,environmental and health hazard, The control of house fly can not be maintained without the development of new compounds such as herbal insecticides which will afford new prospects for the future of flies control (11).

Nerium indicum Mill. *Azadiracta indica* A. Juss *and Annona squamosa* Linn. are trees that can general growth in Thailand and they have reported that can

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killed house fly (12). In this study was conducted by using *Nerium indicum* Mill. *Azadiracta indica* A. Juss *and Annona squamosa* Linn leaves extract to kill adult of house flies that they can repay chemical insecticides.

B. Research Objectives

1. General Objective

To determine adulticidal effect of *Nerium indicum* Mill. , *Azadiracta indica* A. Juss *and Annona squamosa* Linn. leaf extracts on *Musca domestica Linn.*

2. Specific Objective

To compare adulticidal effect of *Nerium indicum* Mill. , *Azadiracta indica* A. Juss *and Annona squamosa* Linn. leaves extract on *Musca domestica Linn.* at dilutioon of *Nerium indicum* Mill.,*Azadiracta indica* A. Juss *and Annona squamosa* Linn. leaf extracts are 1,2,3 and 4 fold for 24 and 48 hours in each test

C. Research Hypothesis

1. Adulticidal effect *of Nerium indicum* Mill. , *Azadiracta indica* A. Juss *and Annona squamosa* Linn. leaf extracts on *Musca domestica Linn.*

2. Different leaf herbal extracts had different adulticidal effect in the same time

3. Different dilution from leaf herbal extracts had different adulticidal effect in the same time

D. Scope of the study

In this research, three herbal leaves was collected from same tree that growth in Raknumpoo district Muang amphur Nakhonsawan along in this study

This study was studied in Laboratory room

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Adulticidal effect *of Nerium indicum* Mill*., Azadiracta indica* A. Juss *and Annona squamosa* Linn. leaf extracts were determined on eating of *Musca domestica Linn*

E. Limitation of the study

The research was studied only *Musca domestica Linn*. Because they are the predominant species (83% of flies) (13)

House fly was rared in laboratory room by ourself in order to avoid contaminates from insecticides that might accumulate in house fly before using in this study and house fly in the fourth generation was used in this study (14)

In this study, do not starvation of house fly before used in the test

F. Definition of Terms

House fly : flies in family Muscidae and scientific name were *Musca domestica Linn*., collected from poultry farms from Nakhonpathom, colonizing in laboratory room and the fourth generation of *Musca domestica Linn*. was used in this study

***Nerium indicum* Mill** : overripe leaves of *Nerium indicum Mill.* that have dark green leaves

***Azadiracta indica* A. Juss** : overripe leaves of *Azadiracta indica* that have dark green leaves

***Annona squamosa* Linn.** : overripe leaves of *Annona squamosa Linn* that have dark green leaves

Adulticidal: Musca domestica Linn in cages died from ate *Nerium indicum* Mill. *, Azadiracta indica* A. Juss *and Annona squamosa* Linn. leaves extract

1 fold dilution : Ratio of *Nerium indicum* Mill*., Azadiracta indica* A. Juss *and Annona squamosa* Linn. leaves 100 grams crushed with water 100 cc.and squeezed only water

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2 fold dilution : The using of 1 fold dilution 10 cc. To mixed with water 10 cc.

3 fold dilution : The using of 2 fold dilution 10 cc. To mixed with water 10 cc.

4 fold dilution : The using of 3 fold dilution 10 cc. To mixed with water 10 cc.

House fly died : *Musca domestica Linn* can not walked and when using probe to touched them, they can not moved (15)

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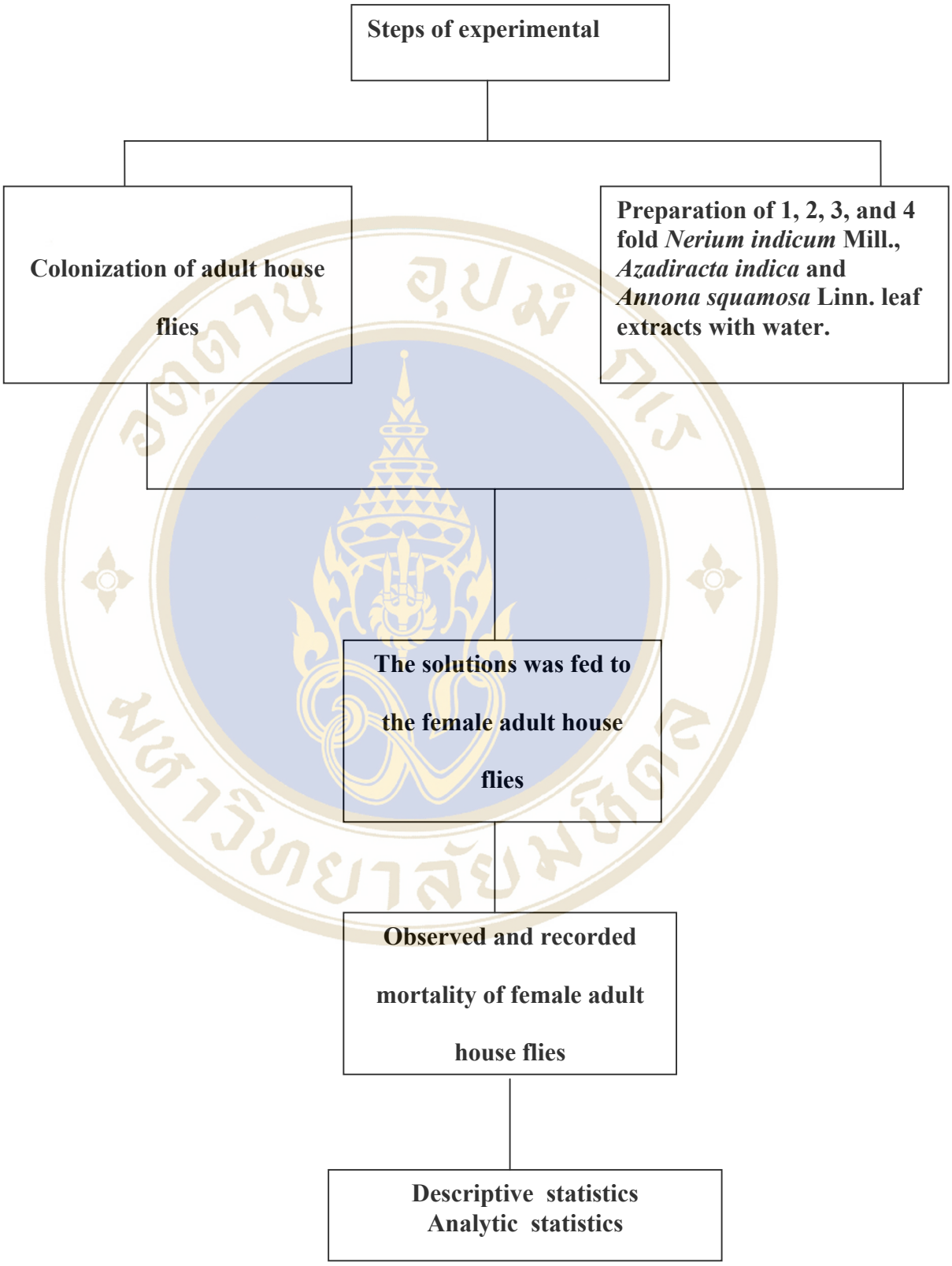

Fig 1 Scope of the study

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CHAPTER 2
LITERATURE REVIEW

2.1 House fly

2.1.1 Taxonomy and characteristics of house fly

Common name	House fly
Scientific name	*Musca domestica , Linnaeus*.
Phylum	Arthropoda
Class	Hexapoda
Order	Diptera
Family	Muscidae
Sub Family	Muscinae
Genus	Musca
Species	M. domestica

In *Musca domestica* , house fly is the most common and widespread species. House fly is scavenger and the connection between the house fly and man is manifold. House fly breeds in accumulation of waste , manure, etc.,feed on food and waste . By this habit , house fly become one of public health problems as annoyance and as a potential carrier of disease that cause many serious diseases such as typhoid fever, cholera, dysentery,tuberculosis, antrax,ophthalmic and infentile diarrhea.

2.1.2 General discription

Musca domestica Linn. is a medium-sized, mouse-gray insect,usually 6 to7 mm. In length , with a wing span approximately twice this distance. When at rest,however, the wings are directed somewhat posteriorly,so as to give to the fly a

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triangular appearance when viewed from above. The wings, otherwise clear and transparent,are somewhat yellowish at the base.

2.1.3 The head

In anterior view , the large compound eyes,purplish brown in color , occupy either side of the head and appear very slightly bean – shaped,due to the faintly sinuous outline of their inner margins. The surface of each compound eye is divided into approximately 4,000 facets, each irregularly hexagonal in outline. Each facet functions as a lens , permitting light to stimulate the visual unit (ommatidium) immediately beneath.In the house fly the surface of the compound eye is free from hair. At the very top of the head (vertex), situated in a small triangle with the point directed forward, may be seen the three ocelli,or simple eyes,the surface of which is not divided into facets.Extending from the vertex forward to the base of the antennae is a dull-black stripe ,the frontal vitta. In male specimens the vitta practically fills the space between the eyes. In females the front is much wider , and though the vitta is much wider also, particularly above, there remains on either side a definite area of the light golden color , separating the vitta from the eyes.

2.1.4 The wings

The wing membrane itself averages 6.2 mm. Long by 2.48 mm. wide , and except for the supporting veins is transparent throughout. Viewed beneath the binocular dissecting scope under reflected light, the wing surface shows many iridescent areas, with delicate shades of green and pink predominating. This is due to the refraction of light by many wrinkles and corrugations, microscopic in size.

2.1.5 The legs

The six legs of a fly are all essentially similar.Each consists of a basal coxa,a small, triangular trochanter, an elongated and comparatively robust femur, a tibia of about the same length as the femur but more slender throughout, the distal extremity being of greater diameter than the proximal, and the five segmented tarsus . The first tarsal segment (basitarsus) is much longer than the others.This condition is best developed on the third pair of legs.Between the bases of the two pulvilli is a

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tapering , bristlelike structure, the empodium. It remains to be shown whether the empodium exercises any special sensory function. All segments of the legs bear a considerable number of hairs, bristles, or spines .

2.1.6 The abdomen

The first abdominal segment is considerably reduced, being visible only ventral side, but the second , third, fourth and fifth are well developed.The basal half of the abdomen, especially on the lateral surface, is frequently yellowish or transparent buff in color.This feature is usually more pronounced in males than females . The median dorsal region of the anterior segments is marked by a brown or blackish longitudinal band. The rest of the abdominal surface is brownish gray with a yellowish pollinose sheen (16).

2.1.7 Life cycle of house fly

The house fly has a complete metamorphosis with distinct egg, larva, maggot or pupa and adult stages. The overwinters house fly were in either larval or pupal stage under manure piles or in other protected locations. In warm summer conditions are generally optimum for the development of the house fly and it can complete its life cycle in as little 7-10 days, and as many as 10 to 12 generations may occur in one summer (17,18)

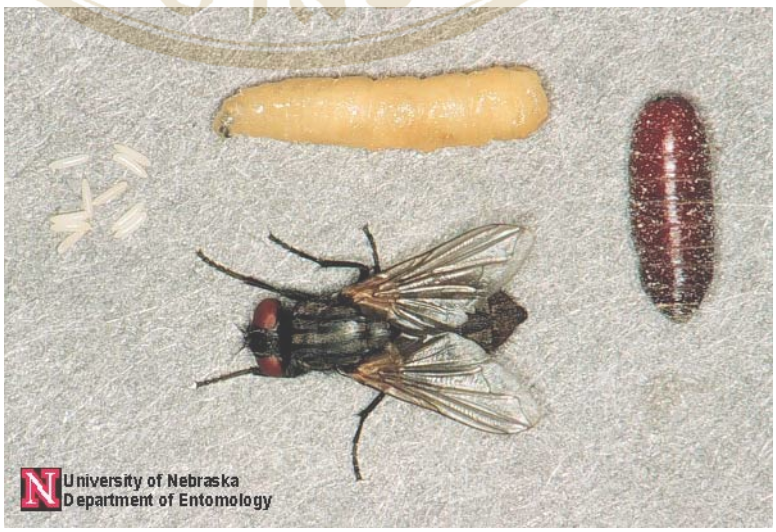

Figure 2 Life cycle of the house fly *Musca domestica Linn.* (clockwise)

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1. Egg

The eggs are pearly white in color and measure approximately one millimeter in length. The greatest diameter, which is near the posterior end, measures a little more than one-fourth of the length. Both ends are bluntly rounded, but the anterior is always more tapering (16) Each female fly can lay up 500 eggs in batches (about 75 to 150 eggs in each batch) in 3-4 days.The number of eggs produced is a function of female size, which is principally a result of larval nutrition. The egg stage requires 8 to 24 hrs for develop to larva stage. Eggs can not withstand desiccation and die if they die out and can not tolerate on temperature below 15 ^{o}c and above 40 ^{o}c. (19)

2. Larva

The larva, or maggot, passes through three stages or instars. All stages have twelve easily recognized segments, of which the second, or postoral, segment is really double, giving a total of thirteen (16). There are no eyes,legs, antennae, or other appendages. In the first- stage larva, only the two posterior spiracles are present . These are situated on the obliquely truncate posterior end. Each consists of a pair of small, sllitlike apertures on a slight prominence. After the first molt, the slits become larger and more conspicuous. The posterior spiracles of the third stage each have three strongly sinuous slits surrounded by a heavily chitinized ring with a conspicuous, perforated button that extends inward from the mesial side. Second- and third – stage larvae have anterior spiracles as well.

First- stage larvae have been known to molt anywhere from twenty hours to four days after hatching, depending on environmental conditions. The second instar requires from twenty-four hours to several days to complete development, while the third stage feeds from three to nine days before undergoing pupation

3. Pupa

The process of pupation consists of a general contraction of the larva within its own integument so what the latter comes to from a cylindrical puparium, in normal specimens about 6.3 mm. in length. The puparium shows a slight

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gradual increase in a diameter from front to rear, except that both ends are bluntly rounded (16). The puparium stage lasts about three to five days in warm weather, but may be prolonged to 7-14 days during cooler period (19). The puparium stage definitive color is a rich, dark brownto dark black. The emerging fly escape from the pupa case through the use of an alternatively swelling and shrinking sac, called the ptilinum, on the front of its head which it uses like a pneumatic hammer.

4. Adult

The adult house fly is 6 to 7 mm. in length, which is the female usually larger than in male. The eyes are reddish and the mouthparts are spnging. The thorax bears four narrow black stripes and there is a sharp upward bend in the fourth longitudinal wing vein. The space between the eyes can separate the sexes, which in females is almost twice as broad as in male.

Adult flies are omnivorous, feeding on a variety of fresh or putrid animal or vegetable substances. Adults of *Musca domestica Linn*. generally avoid direct sunlight, preferring to seek shelter in buildings inhabited by man or animals . House fly are often called domestic or synanthropic fllies because of their close association with man and his household. *Musca domestica Linn* adults are almost active during the cooler part of the day, but in the temperature below 10 ^{o} c they become inactive(19).

2.1.8 Mating of adults

House fly copulate in the superimposed position with the male above the female and facing in the same direction. In nature, the sexes are usually about equal, but occasionally there is considerable departure from normal expectancy. When the size of the average adult, in random sampling, runs small, the males will be more numerous,while if the average fly captured is of superior weight and size, such is never the case.This would appear to be in part an expression of the amount of food available during the larval state, especially so when nourishment is insufficient.

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2.1.9 Oviposition

Insofar as temperature affects the ovipositional activity of flies, it is the temperature of the air rather than of the substratum which proves the deciding factor. The above- mentioned workers state that all oviposition ceased at an atmospheric temperature of 10°even though the upper layers of dung remained at 15°c- 20°c

The female fly is rather delibrate in depositing her eggs. She walks over the materials that is to serve as food for her larvae,seeking crevices and cracks in which the ova may a measure of protection. By action of certain muscles, she compress her abdomen and the oviposition shoots out. She then pushes or 'worms'the oviposition a far into the crevice as possible. One egg, or several, may be laid in a single spot, but all will be more or less hidden if opportunity permits.

2.1.10 The food of adult flies

The food of the adult flies is important in two ways: first, to maintain the body processes- that is, to sustain the life of the individual ; and, secondly, to provide for the maturation of the sex organs and thus make possible reproduction. For house fly some of sugar or assimilable starch proved necessary for normal longevity. In addition, a protein ingredient was required for the production and deposition of eggs.

2.1.11 Longevity

In the height of summer, it is doubtful if flies ordinarily live longer than two or three weeks. At lower temperatures, however, this may probably be extended to as much as three months. If there is true hibernation of adults, such individuals must, presumably, live even longer (16).

2.1.12 Breeding places

The house fly deposited the eggs on decaying, fermenting or rotting organic matter both animal and vegetable origin. It really bred in meat or carrion (20), but preferred to breed in pig manure since its antennae were attracted by pig manure volatiles such as butanoic acid, dimethyl disulphate, dimethyl trisulphide, 3-methyl

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butanoic acid, phenol, benzene, ethanol, indole and 3 methyl indole (21). Moreover, garbage provided the main madium for breeding. The garbage in connection with canning of vegetables and fruits, breweries, distillers, sugar plants, various wastes from food processing and industry was also important breeding sources (22).

2.1.13 Population growth of house fly

On the average a female will mature 120 eggs in a batch (range 100-150) and deposit four to six batches of ebbs during a lifetime of 2 to 4 weeks in a summer. With a life cycle from egg to egg of 3 weeks, ten to twelve generations can be produced a year in the warmer temperature regions of the world. In colder regions breeding will be restricted to the warmer months, and the winter passed as slowly growing larvae and pupae, some of which will survive to emerge, when warmer conditions return (23).

2.1.14 Disease from house fly

The pathogens recovered from flies range from viruses to helminthes, and include the viruses of poliomyelitis and infectious hepatitis, the bacteria associated with cholera *(Vibrio)*, enteric infections caused by species of *Salmonella* and *Shigella*, pathogenic *Escherichia coli*, haemolytic streptococci, *Stretococcus aureus*, agent of trachoma, bacteria conjunctivitis, antrax, diphtheria, tuberculosis, leprosy, and yaws. In addition flies can carry the cycles of protozoa, including those of *Entamoeba histolytica* (24).

2.1.15 House fly control

1. Sanitation

House fly develop in moist manure and wet, decaying organic matter of all kinds found around swine area. The sanitation program is essential to keep down for population in and around swine facilities. All manure, spilled feed, wet straw and decaying plant materials should be removed twice a week to break the breeding cycle of the flies. Which can complete a life cycle from egg to adult in 7 to 10 days. This can be done by spreading manure and other waste material and dry or by placing it in pits or lagoons to liquefy. If a liquid manure pit is use it should not be

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allowed to accumulate the manure above the waterline , either floating or sticking to the sides, since this is an ideal site for egg-laying(25).

2. Mechanical control

The proper use of screens and flytraps are two methods of mechanical fly control. First, in enclosed pig housing. Second, doors and windows should be screened to prevent entry of flies. Many kinds of flytraps are available, such as electric, and employ a black light with an electrically charged grill to kill the insects, or they may be baited with a fly- attractant material. Traps do appear to be helpful in tight, enclosed areas where good sanitation practices are followed. However, in area of heavy fly population, traps are not effective in reducing the number of flies to satisfactory levels. They are best used as a supplement to other fly-control activity (26).

3. Chemical control

WHO (1984) recommended that there are six main methods for the application for chemical fly control : 1) Treating- breeding place with larvicides. 2)Applying residual treatment , mainly sprays, to resting and other surfaces frequently visited by flies. 3) Introducing toxic-resting site mainly impregnated strips and cords etc. 4) Toxic baits. 5) Space sprays and directs spraying of fly aggregation indoors, and 6) Fumigation (27).

4. Biological control

Despite their long association with humans, house fly still remain among the most difficult livestock pests to control. The increase resistance of flies to chemical insecticides has been long emphasized the urgency to seek alternative means to chemical control. Filth-breeding flies have been targets for biological control efforts in various parts of the world. Numerous surveys and ecological studies have been conducted on several species of parasitic Hymenoptera (e.g. *Spalangia* and *Muscidifurax*) and predator such as Staphylinid beetles (*Carcinop pumilo*) that contribute to the reduction of the filth-breeding fly population and their breeding sites. Various pathogens such as fungi (e.g. *Enthomopthera*), bacteria (e.g. *Bacillus*

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thuringiensis) have also been used with some successful result in the control of manure-breeding flies (28).

2.2 The use of plants extracts in controlling insect pests

2.2.1 *Nerium indicum* Mill.

Latin names : Nerium odorum / N. indicum Mill.

English names : Sweet-Scented Oleander, Indian Oleander

Indian names : Karavira

Family : Apocynaceae (29)

Description : These are evergreen flowering shrubs that are found wild in the Orient. They can grow up to 20 feet tall. All parts of this plant are poisonous if eaten. They are great for cultivating outdoors in far South. The leaves are dark green, leathery and lance-shaped ; they average 6 inches in length and 1 inch wide. The flower bloom in clusters at the ends of the shoots in the summer. They have a tubular base, but open out at the tips.They may be single, semi-double or double, and come in red, apricot, pink or white. They usually begin to bloom from March to May and continue until autumn. The Oleander, as this plant is commonly called, requires a minimum winter temperature 45 degrees. All parts of their plant contain a watery latex.

Potting : Oleanders need full sun and soil that has adequate drainage. They flourish in sandy areas. Pruning consists of cutting out dead, crowded, and unwanted growths in late winter, before new growth begins. At that time, it is wise to give them a dressing of fertilizer. Fertilizing their roots with manure or compost would be beneficial. When they are grown indoors in pots, the best soil mixture consists of two parts sandy loam and one part of equal portions of well-decayed manure and leaf mold or peat moss, with sand added freely. Repotting of small plants should be done in March and April. Older plants that are already growing in large pots

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or tubs are not repotted annually, instead, they are given a yearly top-dressing with fresh compost. After repotting, they should be shaded from direct light and sprayed with water twice a day until they are established. When the weather is warm and settled all danger of frost has passed, the plants may be set outside. Throughout the summer, these plants need a large amount of water. Setting them in saucers of water is a good way to accomplish this. When the flower buds are developing, the young shoots that develop at the bases of the flower trusses must be removed. Failure to do this is a common cause of the plant s failing to bloom. As soon as the flowers have faded, the shoots of the previous year's growth should be pruned by two-thirds or more. Less water is required after they are pruned, but as they start into growth the water supply should gradually be increased. In early fall, the Oleanders should be returned to the greenhouse or other light place where they are to spend the winter. Only enough water is needed throughout the winter to keep the leaves from shriveling (30)

Toxicology : All parts of the plant and even the smoke is poisonous. The dried plant in hay or fodder can also cause poisoning . Animals may even die if they drink water in which leaves or flowers of the plant have fallen. A few leaves are sufficient to cause the death of a sheep. A number of active heart glycosides are present in the plant. The symtoms produced are similar to thos of tulp and slangkop poisoning(34). The symtoms from eating were headache, nausea, vomitting, dazzled, dropped blood pressure and die from ventricular fibrillation.In child the symtoms from eating cardiac arrhythmia and severe CNS depression and in adult have psychological symtoms (31)

Principal constituents : The roots, bark and seeds contain cardio-active glycosides, formerly designated as neriin, neriodorein and karabin; the bark also contains scopoletin and scopolin. The alcoholic root bark showed the presence of a-amyrin, b-sitosterol; the ether fraction showed kae chloroform fraction showed odoroside (29).

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2.2.2 *Azadiracta indica* A. Juss

Latin name : *Azadiracta indica*

Local name : Neem, Nim (32)

Description : Neem is an evergreen tree which widely distributes in Thailand and also occurs in Southern Laos and Western Cambodia (33). In Thailand , it grows well in all regions of the country. The most common varieties are *Azadiracta indica* var Siamesis and *Azadiracta indica* var India. In Thai traditional medicine Neem is used for various purposes. Flowers have been used as element tonic, for the treatment of fever and nasal polyposis. Petiole is used as antipyretic, while stem bark is used for the treatment of diarrhea and amoebic dysentery (34). Fruits are used as anthelmintic, for the treatment of haemorrhoids and abnormal urination(34,35,36). Root bark is used as antipyretic, emetic and for skin disease (34,37). Leaves are used as element tonic, gastric secretion stimulation and insecticide (34,35,36)

Some chemicals isolated from neem.

The chemical constituents reported can be divided into several classes

1. Limonoids

Neem is bitter in taste. The bitterness is due to an array of complex compounds called “triterpenes” or more specifically “limonoids”. They have been isolated rom various parts of the neem tree. So far, at least nine neem limonoids have demonstraated an ability to block insect growth, affecting a range of species that includes some of the most deadly pests of agriculture and human health. New limonoids are still being discovered in neem, but azadirachtin, salannin, meliantriol, and nimbolide are the best known and, for now at least, seem to be the most significant (38).

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2. (Poly) phenolics

The phenolics compounds have been isolated from flowers, leaves, bark, and fruits of the neem tree, for example flavonoids, flavonolglycosides, dihydrochalcones, coumarins, and tannins

3. Carbohydrates and proteins

The gum exuded from the stem of neem is a very complex combination of proteins and heteropolysaccharides. The proteins are linked very tightly to the polysaccharides, which constitute the major components. The nature of these complexes hampers the total elucidation of the srructure of the gum.

4. Sulphurous compounds

Some part of neem, such as the seeds, posses a characteristic odorous smell.

5. Alkaloids

Alkaloids are present in the bark, fruits and in the leaves of neem

6. Others

Nimbocetin, a substitued aromatic ester from the fruits.

Acid: oxalic acid from the leaves and indole acetic acid, indole pyruvic acid, tiglic acid, and fatty acid from the seeds.

5-hydroxymethylfurfural from the fruits. This compounds may represent a degradation product of saccharides (40).

2.2.3 *Annona squamosa* Linn.

Annona squamosa Linn. or custard apple belongs to the family Annonaceae. Other English names of this plant are sugar apple and sweetsop. In Thailand, *Annona squamosa* has many local names such as Noinaa (central) , Noinae (Southern), Manonae (Northern), Mak khiap (Northeastern) (41,42). *Annona squamosa* is native to tropical America. It is now widely grown throughout the tropics. They are cultivated in north eastern and central parts of Thailand.

Description : The sugar apple tree ranges from 3-6 m in height with open crown of irregular branches, and some – what zigzag twigs. Deciduous leaves,

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alternately arranged on short, hairy petioles, are lanceolate or oblong, blunt tipped, to 5-15 cms long and 2-5 cms wide; dull-green on the upperside, pale, with a bloom, below; slightly hairly when young; aromatic when crushed. Along the branch tips, opposite the leaves, the fragrant flowers are bome singly or in groups of 2 to 4. They are oblong, 2.5-3.8 cms long, never fully open; with 2.5 cms long,dropping stalks, and 3 fleshy outer petals, yellow-green on the outside and pale-yellow inside with a purple or dark- red spot at the base. The 3 inner petals are merely tiny scales. The compound fruit is nearly round, ovoid, or conical; 6-10 cms long; its thick rind composed of knobby segments, pale-green,bluish-green, or in one form, dull, deep-pink externally (nearly always with a bloom); separating when the fruit is ripe and revealing the mass of conically segments, creamly-white, glistening, delightfully fragrant, juicy, sweet, delicious flesh . Many of the segments enclose a single obloncylindric, black or dark-brown seed about 1.25 cms long. There may be a total of 20 to 38, or perhaps more, seeds in the average fruit. Some trees, however, bear seedless fruits.

Origin and Distribution : The origin home of the sugar apple is unknown. It is commonly cultivated in tropical South America, not often in Central America, very frequently in southern Mexico, the West Indies, Bahamas and Bermuda, and occasionally in southern Florida. In Jamaica, Puerto Rico, Barbados, and in dry regions of North Queenland, Australia, it has escaped from cultivation and is found wild in pastures, forests and along roadsides(43).

Ethnomedical information : Various parts of *Annona squamosa* Linn. have long been in Thai folk medicine. For example *Annona squamosa* roots can be used as laxative, emetic and anti-snake venom, and detoxication. The stem bark can be used for the treatment of wound, periodontitis, diarrhoea, snake bite and as astringent. Leaves can be used as anthelmintic, pediculicide, scabicide, and anti-inflammation. Fruit can be used for the treatment of neck and ear abcesses, ring worm, Taenia vesicolor, herpes virus, snakebite and parasites. Seeds can be used as an anti- edema and anti-head lice (44,44,45).

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Phamacological actions : The various pharmacological action of *Annona squamosa* Linn. have been studied as follows

1. Uterine stimulant action : The water extract of different parts of *Annona squamosa* were tested in isolated uterus of estrogen treated animals. Seed (46),leaves (47), and stem (48) extract all stimulated the contraction of uterus.

2. Fungicidal action : The antifungal activity of water extract were determined by the disk agar diffusion method against the two dermatophytes : *Trichophyton mentagrophytes* and *Microsporum gypseum.* The distrilled water extract of leaves at dilution 1:10,1:50 and 1:500 inhibited *T. mentagrophytes* 50,16 and 10% respectively and at dilution 1:50, 1:500 inhibited *M. gypseum* 33 and 13 % respectively (49)

3. Insecticidal action : It was found that a 95% ethanol extract of roots and leaves of custard apple at 5% concentration showed insecticidal activity against female *Dacus cucurbitae* and *Macrosiphoniella sanborni* (50). The dry ether extract (0.062g) of the leaves diluted with 0.2 ml acetone killed 91% of *Aulacophora foveicollis* (red pumkin beetle) (51)

4. Parasiticidal action : The *in vitro* parasiticiddal activities of various organic solvent extracts from the leaf and the seed of *Annona squamosa* in coconut oil were studied against human head lice and male ticks from dogs in comparison with a preparation used in folk medicine which employed crude extracts of *Annona squamosa* seed in coconut oil. The petroleum ether extracts in coconut oil of the leaf and the seed at the ratio of 1:1 were found to have the most potent actioon against head lice *in vitro* which killed 90% of the lice (LT_{90}) in 53 and 26 minutes, respectively. The second potent extract were choroform extract in coconut oil (1:1) from the leaf and ethanolic extract in coconut oil (1:2) from the seed with the same LT_{90} of 67 minutes. A preliminary phytochemical screening of the petroleum ether extracts of the leaf and the seed indicated the presence of alkaloids and steroid-type compounds(52).

Toxicity : The seeds are acrid and poisonous . Bark, leaves and seeds contain the alkaloid, anonaine. Six other aporphine alkaloids have been isolated from the leaves and stem: corydine, roemerine. Norcorydine, norisocarydine, isocorydine

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and glaucine.Aporphine, norlaureline and dienone may be present also. Powdered seeds, also pounded dried fruits serve as fish poison and insecticides in India. In maxico, the leaves are rubbed on floors and put in hen's nests to repel lice (43).

2.3 Related researches

Studies on effect of *Nerium indicum* Mill, *Azadiracta indica*, *Annona squamosa* Linn. leaves extract.

Rao (53) reported the toxicity of petal of *Nerium indicum* Mill with Rice weevil (*Sittophilus oryzae* L.) and the chemical extract from bark and leaves of *Nerium indicum* Mill are insecticides from reported by Gupta and Thorsteinson (54)

Khalsa et.al., (55) the chemical extract from bark , leaves, root, flower of *Nerium indicum* Mill have toxicity with *Musca nebulo* Wiedermann .

Sookvanichsilp et. al.,(56) tested various organic solvent extracts from seeds and leaves of *Annona squamosa* Linn. at 1:10 (w/v) dilution in propylene glycol and tested dried extract on toxicity of rabbit eye and skin.The result showed that the organic solvent extract in propylene glycol produced rugged cornea, edema and erythema of conjunctiva and ear skin. The different organic solvent extracts produced adverse effects on rabbit eyes in the following order of severity : ether > petroleum > chloroform > ethanol >, and those on rabbit ear skin were petroleum ether > ether > chloroform > ethanol

Jaswanth A et. al.,(57) suggested the potential mosquitocidal effect of methanolic extract of leaves of *Annona squamosa* Linn. on *Culex quinquefasciatus*

Aahmed El Tahir et. al., (58) reported the methanolic extract of *Annona squamosa* Linn leaves showed high antiplasmodial activity with IC_{50} values of 2 and 30 µl/ml on 3D7 and Dd2, respectively

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Watt and Breyer – Brandwijk (59) reported many parts of *Annona squamosa* Linn. used to be insecticides.

Aliero,B.L.(60) reported exposure of the larvae of *Anopheles* mosquito to undiluted extracts of seed oil, leaf, and bark of *Azadiracta indica* for 12 hours led to 100, 98, and 48% mortality, respectively.

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CHAPTER 3
MATERIALS AND METHODS

Experimental Design

This study is an experimental research with factorial design type 3 x 4. Each experiment was done in 3 replication, which were the number of hours after house flies were dies from eating 3 types of leaf extracts, such as *Nerium indicum* Mill., *Azadiracta indica* A. Juss. and *Annona squamosa* Linn. leaf extracts and length of testing time in each replication, which had 4 level as 12,24,36 and 48 hours respectly, and dilution of leaf extracts solution had 4 levels as 1,2,3 and 4 fold.

Preparation of house fly

Female house flies used in this experiment were obtained from poultry farms in Nakhonpathom.

Materials and Equipment

Nerium indicum Mill leaves(overripe,dark green)	1 kg
Azadiracta indica A. Juss leaves(overripe,dark green)	1 kg
Annona squamosa Linn. leaves(overripe,dark green)	1 kg
Cage (15 x15 x 15 cm)	13
Cage (50x 50 x50 cm)	2
Seed of pounded meat	1 kg
Rice bran mixed fish meal	1 kg
Zinc cup (12.5 x 6 cm)	6
Ice cream cup (7.5 x 10 cm)	13

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Plastic jar (14 x 14 x 17 cm)	2
Thin cloth (1 x 1 m)	1
Stone mortar (17.5 x 11.5 cm)	1
Scale “camry brand” sized 1 kg	1
Beaker 200 ml	1
Granulated sugar	1 kg
Cotton wool	1 roll
Ether 99.5%	
Long tube	1
Short tube	1
Sawdust	2 kg
Sieve (35 x 20 cm)	1
Water (left for 7 day ,for eradicate chlorine)	

1. Colonization of house fly

Adults of the house fly (Musca domestica) employed in this study were maintained in the laboratory. House fly colony was maintained under laboratory condition at room temperature and relative humidity 80 – 85 %. Adult flies were reared in cage (50 x 50 x 50 cm.) Adult flies were fed with granulated sugar in zinc cup (12.5 x 6 cm.) and water soaked in cotton wool in zinc cup (12.5 x 6 cm) in separation cup. 1 day later, brought the mixture seed of pounded meat , rice bran mixed fish meal and water (150gm:50gm:100ml), put in the cage(50 x 50 x 50 cm.) for the stock females oviposition. Eggs were laid approximately 2 days after fertilization and hatched to give rise to larva about 8-10 hours later. About 500 newly hatched larvae were transferred to a plastic jar (14 x 14 x 17 cm.) containing about the mixture seed of pounded meat, rice bran mixed fish meal and water (225gm:75gm:200ml), then covered with the sawdust 3 cm in depth. The nursing material was covered with thin cloth to protect undesirable insect infestation. The duration of larva was developed about 4-5 days. The late third stage instar larvae creeped up to pupate in the layer of sawdust. When almost all larvae became pupae, they were collected from the sawdust by sieving and transferred to the zinc cup (12.5 x 6 cm). This pupa was placed in the cage and it took 4-5 days for adult

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emergence. This colonization of house fly was repeated, then the fourth generation was used in the experiment later.

Fig 3 Adult of house flies

2. Preparation of *Nerium indicum* Mill, *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts with water

Before used *Nerium indicum* Mill, *Azadiracta indica* and *Annona squamosa* Linn. leaves. They were cut their central core of leaf ,very sliced their leaves

1.1 fold dilution was, collected *Nerium indicum* Mill or *Azadiracta indica* or *Annona squamosa* Linn. leaf 100 gm (from Raknumpoo district Muang amphur Nakhonsawan) put into stone mortar (17.5 x 11.5 cm) then added water into stone mortar 100 ml, fine crushed about 10 minutes. Then filtered with thin cloth (50 x 50 cm) , squeezed only water for used in experiment later.

2.2 fold dilution was, brought 1 fold dilution 10 ml mixed with water 10 ml, then shaked well about 5 minutes and used in experiment later.

3.3 fold dilution was, brought 2 fold dilution 10 ml mixed with water10 ml, then shaked well about 5 minutes and used in experiment later.

4.4 fold dilution was, brought 3 fold dilution 10 ml mixed with water10 ml, then shaked well about 5 minutes and used in experiment later.

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Water from *Nerium indicum* Mill , *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts. in each test kept in the refrigerator.Temperature of the refrigerator was 5 – 8°c and before used must shaked well. Water from *Nerium indicum* Mill, *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts used 2 days with each test (1 replication)

Fig 4 *Nerium indicum* Mill. leaves

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Fig 5 ***Azadiracta indica*** **A. Juss. leaves**

Fig 6 ***Annona squamosa*** **Linn. leaves**

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3. Methods of examinations

1. Colonization of adult house flies, only adult female house fly were selected

2. Carried 25 female adult house flies into cage (15 x 15 x 15 cm). Every cage had ice cream cup (7.5 x 10 cm) contained granulated sugar 10 gm. Granulated sugar was food of adult house fly.

3. Carried 1, 2, 3 and 4 fold dilution of *Nerium indicum* Mill leaf extract 5 ml soaked in cotton wool in ice cream cup, put into each cage that had granulated sugar in ice cream cup too.

4. Carried 1, 2, 3 and 4 fold dilution of *Azadiracta indica* leaf extracts 5 ml soaked in cotton wool in ice cream cup, put into each cage that had granulated sugar in ice cream cup too.

5. Carried 1, 2, 3 and 4 fold dilution of and *Annona squamosa* Linn. leaf extracts 5 ml soaked in cotton wool in ice cream cup, put into each cage that had granulated sugar in ice cream cup too.

6. Observed and recorded mortality of female adult house flies at 12, 24, 36 and 48 hour.

7. Every 12 hour, added 1, 2,3 and 4 fold dilution of *Nerium indicum* Mill, *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts 5 ml into the same ice cream cup to protect drying.

4. Control Group

Control group consisted of water 5 ml soaked in cotton wool in ice cream cup and granulated sugar10 gm in ice cream , put into cage (15 x 15 x 15 cm). Then carried 25 female adult house flies into cage. Every 12 hour, added water 5 ml into the same cup. Observed and recorded mortality of female adult house flies at 12, 24, 36 and 48 hour.

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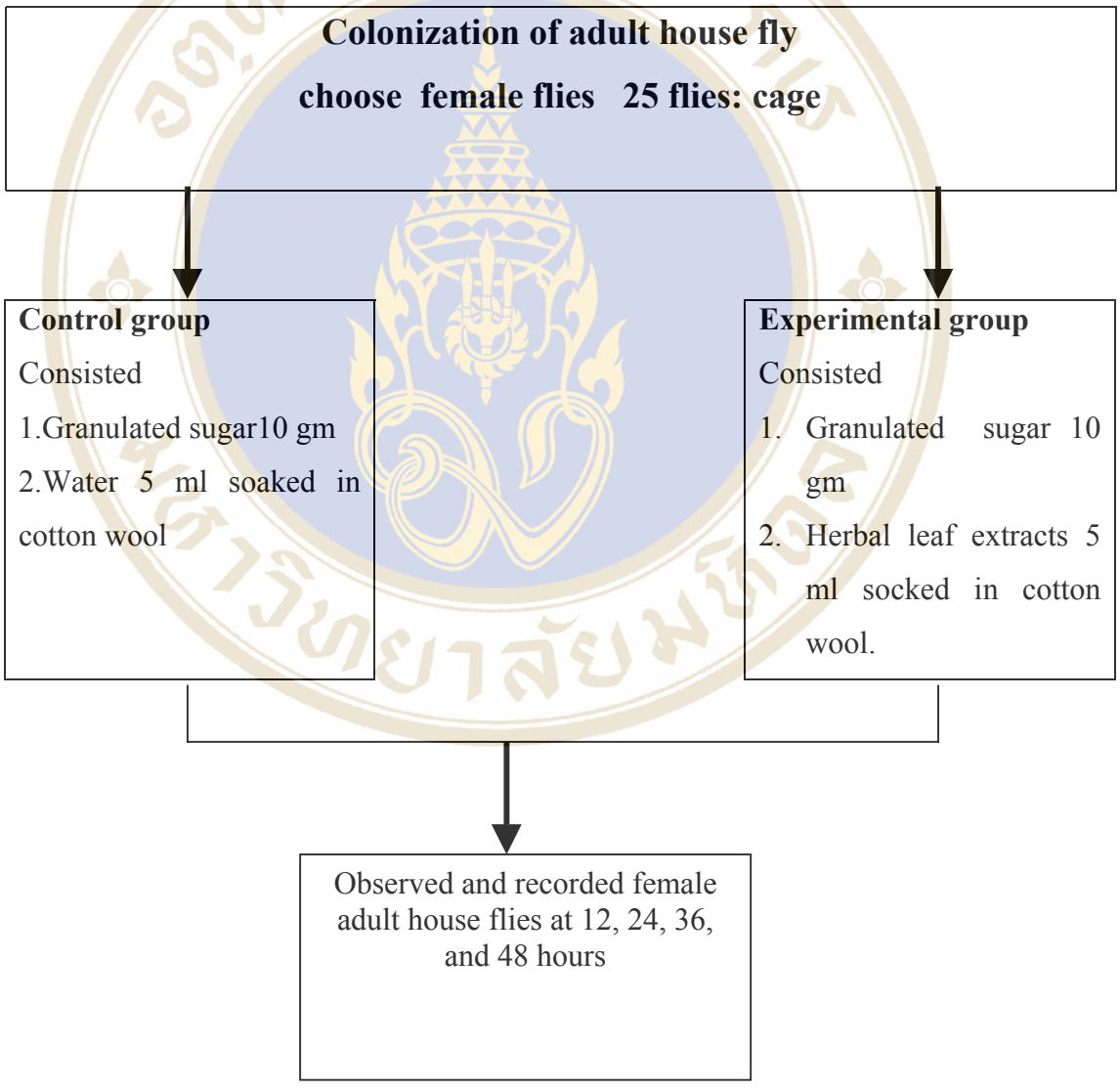

Fig 7 Methods of examinations

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Qualities Controlling

- Only one person performed all of the experiments in the whole research work.

- The experimenter was trained in house fly colonization and studied about characteristic of house fly.

Research Variables

Independent variables

- Types of herbal leaves (*Nerium indicum* Mill, *Azadiracta indica* and *Annona squamosa* Linn .)

- Dilution of *Nerium indicum* Mill, *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts.

Dependent variable

- Mortality of female adult house flies.

Data Analysis

1. Descriptive statistics : mean and standard deviation of the mortality of female adult house flies from ate *Nerium indicum* Mill, *Azadiracta indica* and *Annona squamosa* Linn . leaves extract in each experiment.

2. Statistical analysis : 2-way ANOVA and find difference by using lsd method

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CHAPTER 4
RESULTS

In this study, those female adult house flies were reared in cage during November 2003 to January 2004 and testing was conducted on January 2004 .

Experimental results was observed and recorded of mortality of female adult house flies from eating different type of herbal leaves and different dilution of herbal leaf extracts at 12, 24, 36, and 48 hours.

Comparison of different type herbal leaves and different dilution of herbal leaf extracts on female adult house flies at 24 and 48 hours.

Nerium indicum Mill.

The study result found that female adult house flies ate each dilution of *Nerium indicum* Mill. leaf extracts, They were died increase if the time was prolong. The experiment was design that the house flies ate 1 fold dilution of *Nerium indicum* Mill. leaf extracts, and were observed and recorded their mortality at 12, 24, 36, and 48 hour. The average numbers of death from 1 fold dilution of *Nerium indicum* Mill.leaf extracts were 1.3 +1.5, 3.7 +1.2, 14.0 + 6.1 and 21.7 + 1.5, respectively. The average numbers of death from 2 fold dilution of *Nerium indicum* Mill. leaf extracts were 2.7 + 3.8, 8.3 + 11.0, 13.7 + 8.6 and 18.3 + 6.4, respectively. The average numbers of death from 3 fold dilution of *Nerium indicum* Mill. leaf extracts were 1.0 + 1.7, 5.3 + 5.5, 12.7 + 9.0 and 21.3 + 3.1, respectively. The average numbers of death from 4 fold dilution of *Nerium indicum* Mill. leaf extracts were 1.3 + 2.3, 8.7 + 10.7, 14.7 + 9.0 and 23.3 + 1.5, respectively. (Table 1)

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Azadiracta indica A.Juss.

The study result found that when female adult house flies ate each dilution of *Azadiracta indica* leaf extracts, They were died increase if the time was prolong. The experiment was design that the house flies ate 1 fold dilution of *Azadiracta indica*. leaf extracts, and were observed and recorded their mortality at 12, 24, 36, and 48 hours. The average numbers of death from 1 fold dilution of *Azadiracta indica.* leaf extracts were 1.0 + 1.7, 4.3 + 6.7, 8.7 + 11.5 and 12.7 + 10.0, respectively. The average numbers of death from 2 fold dilution of *Azadiracta indica*. leaf extracts were 1.3 + 2.3, 4.7 + 6.4,12.0 + 11.3 and 15.3 + 9.5, respectively. The average numbers of death from 3 fold dilution of *Azadiracta indica*. leaf extracts were 1.0 + 1.0, 2.3 + 3.2, 4.0 + 4.4 and 5.0 + 5.3, respectively. The average numbers of death from 4 fold dilution of *Azadiracta indica*. leaf extracts were 0.7 + 0.6 , 0.7 + 0.6, 1.3 + 0.6 and 1.7 + 1.2, respectively. (Table 1)

Annona squamosa Linn.

The study result found that female adult house flies ate each dilution of *Annona squamosa* Linn. leaf extracts, They were died increase if the time was prolong., The experiment was design that the house flies ate 1 fold dilution *Annona squamosa* Linn. leaf extracts , and were observed and recorded their mortality at 12, 24, 36, and 48 hours. The average numbers of death from 1 fold dilution of *Annona squamosa* Linn. leaf extracts were 0.7 + 0.6, 1.7 +1.5, 3.3 + 2.1 and 4.3 + 2.1 , respectively. The average numbers of death from 2 fold dilution of *Annona squamosa* Linn. leaf extracts were 0.7 + 3.8, 1.0 + 1.0, 1.0 + 1.0 and 1.0 + 1.0, respectively. The average numbers of death from 3 fold dilution of *Annona squamosa* Linn. leaf extracts were 0.3 + 0.6, 0.3 + 0.6, 0.7 + 0.6 and 0.7 + 0.6, respectively. The average numbers of death from 4 fold dilution of *Annona squamosa* Linn. leaf extracts were 0.3 + 0.6 , 0.3 + 0.6 , 1.7 + 1.5 and 2.0 + 2.0, respectively. (Table 1)

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Table 1 Mean and standard deviation of mortality of female adult house fly from different type of herbal leaves, dilution of leaves extract, times (3 replications)

Type	Dilution (fold)	Lengths of experimental time (hours)			
		12	24	36	48
		Mean+S.D.	Mean+S.D.	Mean+S.D.	Mean+S.D.
Nerium indicum Mill.	1 fold	1.3+1.5	3.7+1.2	14.0+6.1	21.7+1.5
	2 fold	2.7+3.8	8.3+11.0	13.7+8.6	18.3+6.4
	3 fold	1.0+1.7	5.3+5.5	12.7+9.1	21.3+1.5
	4 fold	1.3+2.3	8.7+10.7	14.7+9.1	23.3+1.5
Azadiracta indica	1 fold	1.0+1.7	4.3+6.7	8.7+1.5	12.7+10.0
	2 fold	1.3+2.3	4.7+6.4	12.0+11.3	15.3+9.5
	3 fold	1.0+1.0	2.3+3.2	4.0+4.4	5.0+5.3
	4 fold	0.7+0.6	0.7+0.6	1.3+0.6	1.7+1.2
Annona squamosa Linn.	1 fold	0.7+0.6	1.7+1.5	3.3+2.1	4.3+2.1
	2 fold	0.6+1.2	1.0+1.0	1.0+1.0	1.0+1.0
	3 fold	0.3+0.6	0.3+0.6	0.7+0.6	0.7+0.6
	4 fold	0.3+0.6	0.3+0.6	1.7+1.5	2.0+2.0

The adulticidal effects were collected by separated to 2 factors such as types and dilution of the herbal leaves.Three types of the herbal leaves were *Nerium indicum* Mill. *Azadiracta indica* .and *Annona squamosa* Linn. and each herbal had dilution as 1, 2, 3 and 4 fold. Data was analyzed by using 2 factors Factorial experiment , and 2 ways interaction testing result were as follows

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The 2 ways interaction testing at 24 hour result found that there were no interaction between the 2 factors ($p>0.01$) (Table 2). It meanted that each independently factor affected to mortality of female adult house flies at 24 hours , the analysis of main effect at 24 hour result found p-value > 0.01 .It meanted that at 24 hours, different types of herbal leaves and different dilution of herbal leaves were not affected mortality of female adult house flies (Table 2).

Table2 Result of variance analysis (2 ways) of the mortality of female adult house fly, different types of herbal leaves , dilution at 24 hour

Source of variation	Sum of squares	df	Mean squares	F	p-value
Main effect	216	5	43.2	1.414	0.255
Type	196.222	2	98.111	3.211	0.058
Dilution	19.778	3	6.593	0.216	0.884
2-Way interaction	67.556	6	11.259	0.968	0.892
Type x Dilution	67.556	6	11.259	0.368	0.892
Explained	283.556	11	25.778	0.844	0.602
Residual	733.333	24	30.556		
Total	1016.889	35	29.054		

The 2 ways interaction testing at 48 hours result found that there were no interaction between the 2 factors ($p>0.01$) (Table 3). It meant that each independently factor affected to mortality of female adult house flies at 48 hours, the analysis of main effect at 48 hour result found that p-value < 0.01 .It meanted that at 48 hour, different types of herbal leaves or different dilution of herbal leaves were affected to mortality of female adult house flies (Table 3). Factor 1 was type herbal leaves and factor 2 was dilution of herbal leaves. The result found that, the dilution of herbal leaves had p-value > 0.01. It meanted that at 48 hour, different dilution of herbal leaves were not affect mortality of female adult house flies. But different types of herbal leaves had p-value < 0.01 . It meant that at 48 hour, different types herbal leaves were affect mortality of female adult house flies. (Table 3)

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Table 3 Result of variance analysis (2 ways) of the mortality of female adult house fly from different types of herbal leaves , dilution at 48 hour

Source of variation	Sum of squares	df	Mean squares	F	p-value
Main effect	2373.667	5	474.733	19.989	<0.001
Type	2272.222	2	1136.111	47.836	<0.001
Dilution	101.444	3	33.815	1.424	0.26
2-Way interaction	330.889	6	55.148	2.322	0.066
Type x Dilution	330.889	6	55.148	2.322	0.066
Explained	2704.556	11	245.869	10.352	<0.001
Residual	570	24	23.75		
Total	3274.556	35	93.559		

The data analysis at 48 hour, showed that different types of herbal leaves were affect mortality of female adult house flies. The analysis found variances of data by using Levene's test were statistically different ($p < 0.01$).The variances of types of herbal leaves was heterogenity. The data was translated to 10 base logarithm and test found variance of data again by using Levene's test. The variances were statistically different ($p < 0.01$), and the data was analyzed by using Kruskal – wallis (Free distribution analysis) (Table 4) (see Appendix B)

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Table 4 Mean comparison of mortality of female adult flies among *Nerium indicum* Mill. *Azadiracta indica*. and *Annona squamosa* Linn. leaves extract

Type	n	$\bar{x}$	S.D.	x^2	df	p-value
Nerium indicum Mill.	12	21.2 [a]	3.7	20.76	2	< 0.001
Azadiracta indica	12	8.7 [b]	8.6			
Annona squamosa Linn	12	2.0 [c]	2.0			

After the data was analyzed by using Kruskal – wallis. The result expressed that each herbal leaves had affected to mortality female adult house fly ($p<0.01$) at 48 hours and *Nerium indicum* Mill.had affected to mortality of female adult house flies more than the *Azadiracta indica* and *Annona squamosa* Linn. and the *Azadiracta indica*. had affected to mortality of female adult house flies more than the *Annona squamosa* Linn.

In this study, Dilution of herbal leaf extracts were not affected mortality of female adult house flies. It meanted that different dilutions of herbal leaves extract did not effects to different mortality of female adult house flies.

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Table 5 Percentage of mortality of female adult house fly by time and types of *Nerium indicum* Mill. *Azadiracta indica* A. JUSS. *Annona squamosa* Linn. leaf extracts (Total number start 300 house flies)

Type	Time	$\bar{x}$	S.D.	Minimum	Maximum	Percentage of dead
Nerium indicum Mill	12	1.583	2.234	0.0	7.0	6.33
	24	6.500	7.305	0.0	21.0	26.0
	36	13.750	7.124	6.0	25.0	55.0
	48	21.167	3.689	11.0	25.0	84.7
Azadiracta indica A. JUSS.	12	1.000	1.348	0.0	4.0	4
	24	3.000	4.492	0.0	12.0	12
	24	3.000	4.492	0.0	12.0	12
	36	6.500	8.328	1.0	25.0	26
	48	8.667	8.574	1.0	25.0	34.7
Annona squamosa Linn	12	0.500	0.674	0.0	2.0	2
	24	0.833	1.030	0.0	3.0	3.3
	36	1.667	1.614	0.0	5.0	6.7
	48	2.000	2.000	0.0	6.0	8

From table 5, it showed that in the same time , percentages of dead of female adult house flies from *Nerium indicum* Mill leaf extracts were more than *Azadiracta indica* leaf extracts and *Annona squamosa* Linn. leaf extracts. Percentage of death from *Azadiracta indica* A. JUSS leaf extracts more than *Annona squamosa* Linn. leaf extracts. If time increased , The death of house flies were increased, too.

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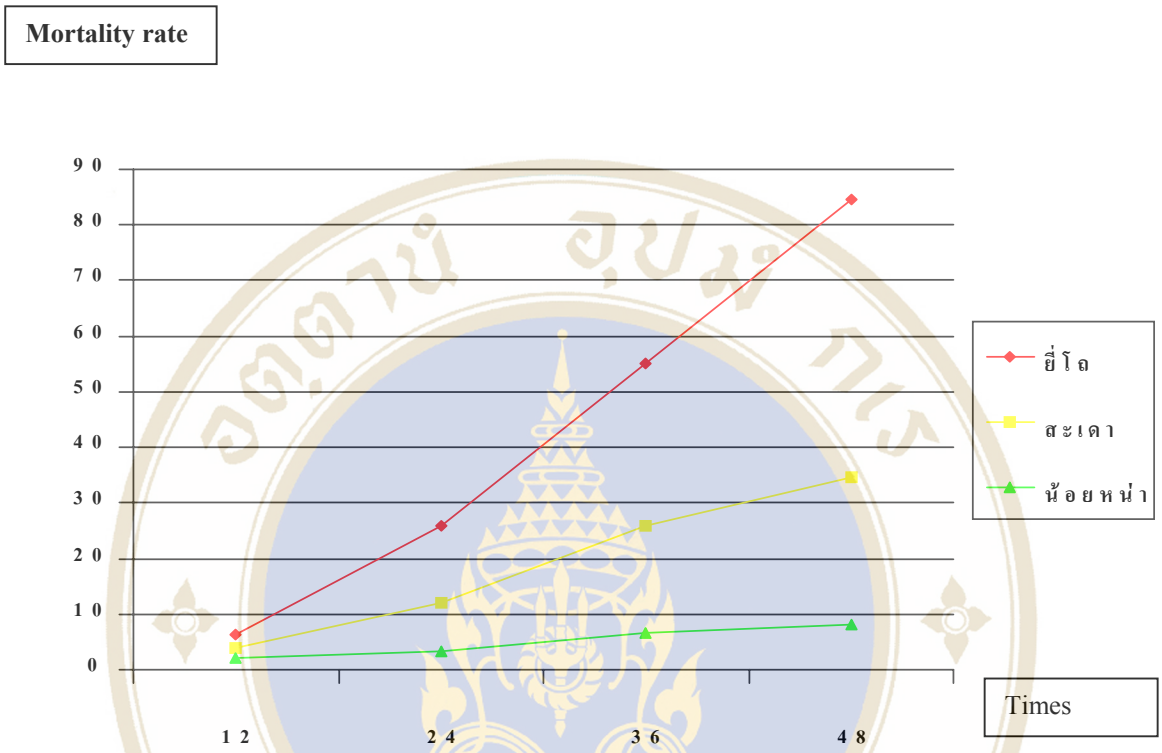

Fig 8 Graph of mortality rate of female adult house flies at 12, 24, 36, and hours

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CHAPTER 5
DISCUSSION

Nerium indicum Mill., *Azadiracta indica* and *Annona squamosa* Linn. leaf were extracted by water.The solutions were kept in the refrigerator with temperature at 5-8 ^{o} c and used for 2 days in each experiment. Testing method was adulticidal effects of *Nerium indicum* Mill. , *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts on female adult house flies. Mortality of female adult house flies was observed and recorded at 12, 24, 36, and 48 hours, and compared at 24 and 48 hours in each type and dilution of herbal leaf extracts. It found that female adult house flies ate *Nerium indicum* Mill. , *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts, and if time go on , they increasingly died.The flies were killed by *Nerium indicum* Mill. leaf extracts more than *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts. *Azadiracta indica* leaf extracts killed the house flies more than *Annona squamosa* Linn. leaf extracts, did

The toxicity of *Nerium indicum* Mill come from latex of stem, root , bark, leaves and seed that its chemical substances and pharmacologicity looked like cardiac glycosides.leaf of *Nerium indicum* Mill. has much cardiac glycosides e.g. neriin (nerioside), 1-strophanthin rosagenin, cornevin, oleandrin-6, oleandrin-4, desacetyloleandrin, pseudo-curanine, neriin D, neriin F, neriin E, 16-anhydrogitoxigenin,16-diacetylanhydro-oleandrin, adynerin (adyneroside), nerigenin, oleandrigenin, acetylgitoxigenin (61). The important toxicity of *Nerium indicum* Mill. leaves is oleandrin (Forlinerin) that has toxicity like digitalis. The poison contact of human is rash and dermatitis. The gastric poison is headache, nausea, vomit, to be dazzled, arrhythmia, drop blood pressure, ventricular fibrillation and dead. In low dose , it stimulates circulating system and in high dose it contracture. The female adult house flies were died by *Nerium indicum* Mill. leaf extracts if they ate high dose

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of *Nerium indicum* Mill. leaf extracts. Some flies were survived, because they ate low dose of *Nerium indicum* Mill. leaf extracts. The result from studied conducted by Gupta and Thorsteinson (54)found that the chemical extract from bark and leaves of *Nerium indicum* Mill. were insecticides. The study conducted by Khalsa et.al., (55) found that the chemical extract from bark, leaves, root, flower of *Nerium indicum* Mill. have toxicity with *Musca nebulo* Wiedermann . The study conducted by Jongkol and Veena (62) studied property of cardiovascular action and active constituent of *Nerium oleander*, L.: comparison with digitalis, found that cardiac glycosides in leaves and bark of *Nerium oleander*, L like digitalis.

Azadiracta indica consistd of azadiractain (63), azadirachotolide (64), azadirolide,iso (65), hyperoside (66), meldenin (67), nimbaflavone (68), nimbinal, 6-dwacetyl (69), nimbocinolide (70), nimbocinolide,iso (71), nimbolide (72,73), nimocinol (74), nimbolide, 28-deoxo (69), nimbocinone (75), nimocinolide, iso (76), nimonol (74), nimonol,14,15-epoxy (77), quercetin (78), rhamnetin, iso (79), rutin (78), salannol,2'3'-dehydro (80), stigmasterol (75). Azadirachtin, the most well known component, is found in *Azadiracta indica* , possessed several biological activities including antibacterial (81), antiparasitic (82), insecticide (83,84), larval growth inhibition (85), insect development inhibition (86,87,88), and larvacidal activities (89,90,91). *Azadiracta indica* leaf extracts had effect female adult house flies less than *Nerium indicum* Mill. because *Azadiracta indica* is bitter in taste and Azadirachtin, component found in *Azadiracta indica* possessed several biological activities including larval growth inhibition, insect development inhibition and larvacidal activities that it has toxicity in larval stage but in this experiment used female adult house flies . The sunny *Azadiracta indica* component is decompose, and the experiment was test in January 2004 that during morning to afternoon was sunny weather. Suthep *et.al.* (14) found that azadirachtin of *Azadiracta indica* seed extracts was larval growth inhibition and was toxicity larval stage.

Annona squamosa Linn. consisted of anonaine, bomeol, camphene, camphone, carvone, eugenol, geraniol, thymol, menthone and pinene that it has acrid smelling and water from *Annona squamosa* Linn. leaf extracts was sticky. *Annona squamosa* Linn. leaf extracts has effect female adult flies less than *Azadiracta indica*

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and *Nerium indicum* Mill. leaf extracts because of acrid smelling and sticky of *Annona squamosa* Linn. leaf extracts. From observed, female adult house flies always fly on the top of the cage.

Dilution of herbal leaf extracts did not significantly affect on female adult house flies at 24 and 48 hours. Because the dilution intervals of herbal leaf extracts were too closed to discriminate them, statistic analysis was not significant effect.

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CHAPTER 6
CONCLUSION AND RECOMMENDATIONS

This research is an experiment laboratory research studying results of adulticidal effect of *Nerium indicum* Mill., *Azadiracta indica* A.Juss. and *Annona squamosa* Linn. leaves extract on *Musca domestica* Linn.Then observed and recorded mortality of female adult house fly at 12, 24, 36, and 48 hour. Data of mortality of female adult house fly was analyzed , comparison mortality of female adult house fly at 24 and 48 hour. Then data was analyzed to find mean, standard deviation. Comparison variance of mortality of female adult house fly by using Levene's test and compared mortality of female adult house fly in each type of herbal leaves extract and dilution of herbal leaves extract by using Kruskal- Wallis test. It was summarized that

1. Results of the study

In this study, when female adult house fly ate each dilution of *Nerium indicum* Mill.or *Azadiracta indica* or *Annona squamosa* Linn. leaves extract., female adult house fly were died increase when time increase too. At 24 hour different type of herbal leaves extract were not affect different mortality of female adult house fly and at 48 hour different type of herbal leaves extract were affect mortality of female adult house flies.At 24 and 48 hour different dilution of herbal leaves extract were not affect different mortality of female adult house fly.

2. Result of the hypothesis

2.1 Female adult house flies were killed from *Nerium indicum* Mill., *Azadiracta indica* and *Annona squamosa* Linn. leaf extracts.When time increase , female adult house were increase died from ate *Nerium indicum* Mill.,*Azadiracta indica* and *Annona squamosa* Linn. leaf extracts.

2.2 At 48 hour different type of herbal leaves extract were killed different mortality of female adult house fly. *Nerium indicum* Mill.had affect

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mortality of female adult house fly more than *Azadiracta indica*. and *Annona squamosa* Linn. leaves extract and *Azadiracta indica*. had affect mortality of female adult house fly more than *Annona squamosa* Linn. leaves extract.

2.3 At 24 and 48 hour different dilution of herbal leaves extract were not affect mortality of female adult house fly.

Recommendation from application

To choose 4 fold dilution of *Nerium indicum* Mill. leaves extract to used in adulticidal of adult house fly and wear protecting clothes e.g. mask, gloves when contact parts of *Nerium indicum* Mill.

Suggestion for future researches

1. In this study was elementary study, Then in the next study must change extract methods for pure chemical substance from herbal leaves.
2. Next experiments will change from herbal leaves to seeds, bark.
3. Next experiments will try to bring herbal leaves extract mix with food that adult house fly like e.g. fish meal
4. Next experiments will test herbal leaves extract with other stage of house fly e.g. larva

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APPENDIX A

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Recorded Table

Replication 1

ชั่วโมงที่	ควบคุม	สะเดา 1เท่า	สะเดา 2เท่า	สะเดา 3เท่า	สะเดา 4เท่า	ยี่โถ 1เท่า	ยี่โถ 2เท่า	ยี่โถ 3เท่า	ยี่โถ 4เท่า	น้อยหน่า 1เท่า	น้อยหน่า 2เท่า	น้อยหน่า 3เท่า	น้อยหน่า 4เท่า
12													
24													
36													
48													

Replication 2

ชั่วโมงที่	ควบคุม	สะเดา 1เท่า	สะเดา 2เท่า	สะเดา 3เท่า	สะเดา 4เท่า	ยี่โถ 1เท่า	ยี่โถ 2เท่า	ยี่โถ 3เท่า	ยี่โถ 4เท่า	น้อยหน่า 1เท่า	น้อยหน่า 2เท่า	น้อยหน่า 3เท่า	น้อยหน่า 4เท่า
12													
24													
36													
48													

Replication 3

ชั่วโมงที่	ควบคุม	สะเดา 1เท่า	สะเดา 2เท่า	สะเดา 3เท่า	สะเดา 4เท่า	ยี่โถ 1เท่า	ยี่โถ 2เท่า	ยี่โถ 3เท่า	ยี่โถ 4เท่า	น้อยหน่า 1เท่า	น้อยหน่า 2เท่า	น้อยหน่า 3เท่า	น้อยหน่า 4เท่า
12													
24													
36													
48													

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APPENDIX B

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The Kruskal-wallis one way analysis of variance by ranks

$$H = \frac{12}{N(N+1)} \sum_{j=1}^{k} \frac{R_j^{\,2}}{n_j} - 3(N+1)$$

where k = number of samples

n = number of cases in $j$yh sample

N= $\sum n$, the number of case in all sample combined

R = sum of ranks in jth sample (column)

$\sum$(j,k) directs one to sum over the K samples (column)

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