CHAPTER 2

LITERATURE REVIEW

1. <u>Biology of experimental honey bees</u>

There are five honey bees species in Thailand. Four of them, *Apis florea*, *Apis dorsata*, *Apis cerana*, and *Apis andreniformis* are native wild species of Thailand. *Apis mellifera* is an imported species (Wongsiri, 1989). For this research selection of honey bees for experiment, only two species of honey bees were used. They are *A. florea* and *A. cerana*, which are easily found nesting in feral colonies in Thailand.

A. florea , the dwarf honey bee, is a native wild species of honey bee in Thailand. Its ecological niche is usually the stratum of dense bushes and small trees of the tropics (Ackatanakul, 1983). The diameter of its nest is appoximately 20 centimetres. The body size of this honey bee is smaller than *A. cerana*, *A. mellifera*, and *A. dorsata*, but bigger than *A. andreniformis*.Geographically,it is widely distributed in Thailand, India, Pakistan, Sri lanka, Indonesia, China, and Malaysia. *A. florea* is a good pollinator and is easy to maintain in tropical fruit orchards. Their nests and honey products can help increase the income of villagers in Thailand (Lekprayoon and Wongsiri, 1989). (Figure 2.1)

A. cerana , the Eastern honey bee, is very similar to the European honey bee, *A. mellifera* . The natural nesting sites of *A. cerana* are usually found in hollow trees, under roofs, and inside houses in caves ; they are often hidden in cavities, consisting of about five or six vertical combs. This honey bee is widely distributed in Asia. The diameter of its nest is appoximately 30 centimeters. Its body is larger than that of *A. florea* but smaller than that of *A. mellifera* and *A. dorsata.* (Figure 2.2)

Taxonomically, *A. florea* and *A. cerana* can be classified as follows :

Kingdom	Metazoa
Phylum	Arthropoda
Class	Insecta
Order	Hymenoptera
Super-family	Apoidea
Family	Apidae
Subfamily	Apinae
Genus	*Apis*
Species	*A. florea, A. cerana*

Figure 2.1 : *Apis florea*

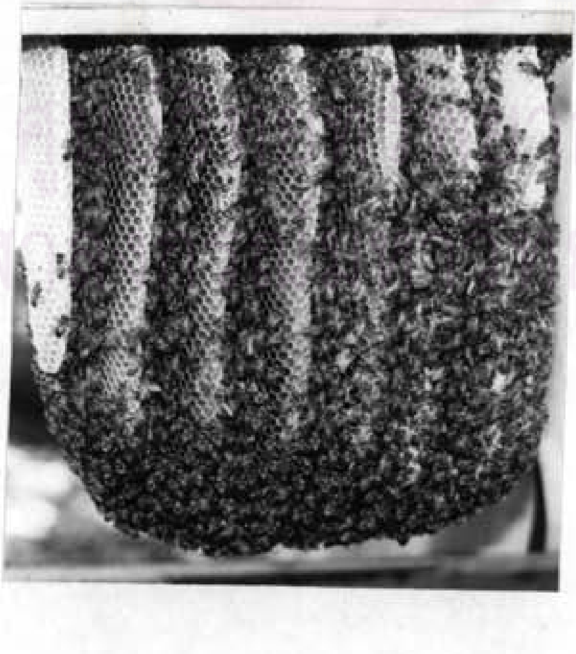

Figure 2.2 : *Apis cerana*

2. The importance of honey bees to man and environment

Man has had a special affinity for honey bees and both creatures have been closely associated with each other for a long time. Honey bees are very beneficial insect to man and environment.

2.1 Honey bees and man

Man has harvested the direct benefit of honey bee as producer of honey and other hive products since prehistorical period. Nowadays, honey, beeswax, propolis, pollen, royal jelly and bee venom are the bee products of economic importance and are used in apitherapy or as health foods (Vaissiere, 1992).

2.2 Honey bees and agriculture

Sustainable development of agriculture in the 21st century will necessitate a reorientation of the present crop production technologies. Instead of making extensive use of chemical fertilizers, biocides, irrigation facilities, and heavy machinery for yield enhancement, a shift towards biologically-based agriculture, such as increased photosynthetic efficiency, biological nitrogen fixation, efficient nutrient uptake and biological cross-pollination, is now needed to increase food productivity. In the future, the full utilization of these biological-based and more environmentally-friendly resources should be emphasised. For example, the yields of different cultivated crops could be increased through cross-pollination by honey bees. The vital role honey bees can play in enhancing the productivity levels of different crops, such as fruits, nuts, vegetables, pulses, oils and forge crops, has often been underestimated, especially in developing countries all over the world.

Honey bees are good pollinators. Pollination is the tranfer of pollen grains from the anther (male part of the flower) to the stigma (the female part) of the same or another flower of the same plant species. This is the first step towards fertilization which is the union of the male nucleus of germinated pollen grains with the female (oosphere) of the egg or ovule. The ovule, after fertilization, develops into the seed. A plant is considered to be self-pollinated/self-fertile when its anther is pollinated by its own pollen. In this case, pollen grains from the anther fall on the stigma of the same flower. Some self-fertilised species are automatically pollinated by pollens from their own flowers, but more often, the construction of the flowers is such that wind or insects are needed to tranfer pollen from the anthers of the flowers to their own stigmas.

However, in many other plants, the flower cannot be fertilized with pollen from the same plant (self-sterile) but needs pollen from another plant of the same species for fertilization. This phenomenon is known as cross-pollination. (Figure 2.3)

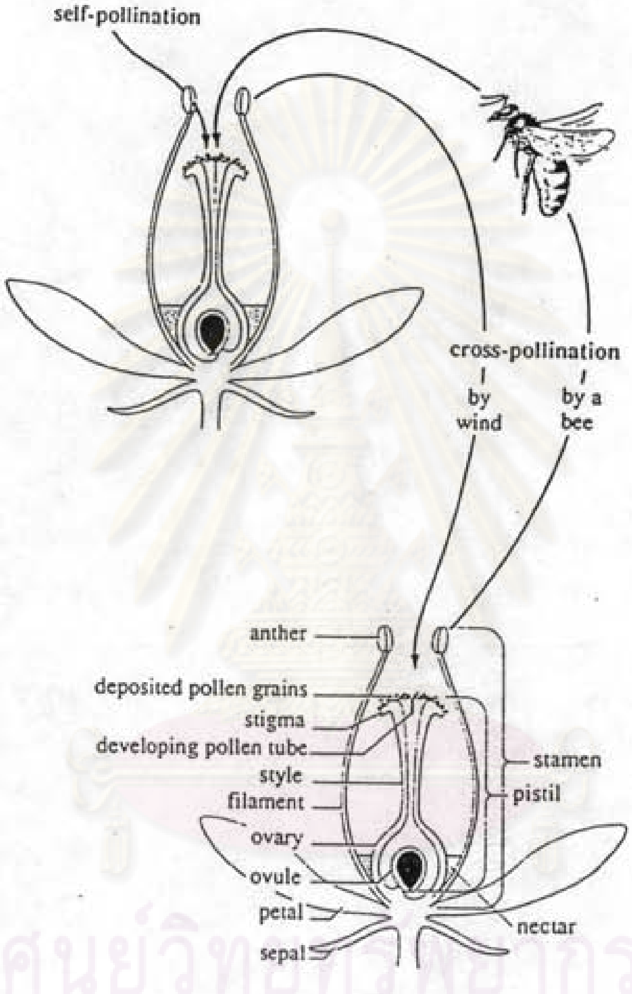

Figure 2.3 : The mechanism by which a honey bee pollinates a flower.

(Crane, 1990)

Many cultivated plants do not yield seeds or fruits without cross-pollination of their flowers. The degree of self-fertilisation varies in different cultivars/varieties of such plants.Cross-pollination of such crops by honey bees is the surest, most effective, and cheapest method of increasing their yields. Other agronomic practices like application of manure, fertilisers, irrigation, and pesticides are also important, but the desired results may not be obtained without the use of honey bees as pollinators. (Crane, 1990)

Honey bees enhance the productivity levels of different crops through cross-pollination. As a matter of fact, the main significance of honey bees and beekeeping is pollination, whereas hive products such as honey and beeswax are of secondary value. The income from agriculture is many times greater than their value as honey and beeswax producers. Moreover, it is not only the self-sterile varieties/cultivars which require cross-pollination, but self-fertilised plants would also produce more seeds of a better quality if pollinated by honey bees rather than by other insects. (Verma and Partap, 1993)

For many years, beekeepers and others have been interested in computing the monetary value of pollination of agricultural crops by honey bees. The first estimate was made by Metcalf et al. (1962). Based on the 1957 crop statistics, they valued the crops in the U.S. benefiting from bee pollination at $ 4.5 billon per year. By 1971 this estimate was increased to $ 7.6 billion (Ware, 1973). A similar review based on the 1981 statistics indicated an annual value of almost $ 19 billion for the crops and commodities to which bees contribute by their pollination activities (Levin and Waller, 1989).

Presently, the value of bee pollination in crop production is estimate estimated at US $ 20 billion per year (USDA-ARS, 1991). A recent FAO report indicated that the direct contribution of pollination in increasing the farm harvests in 20 Mediterranean countries and others in the region, is about US $ 2 billion annually. (Cadoret, 1992).

2.3 Honey bees and environment

The transfer of pollen from stamens to stigma is of prime importance for the reproduction and survival of most plant species. Honey bees play a very important role as pollinators of wild plant species and this is crucial to the maintanance of the genetic diversity of the latter.

Interest is again focused on honey bees as environmental indicators of chemical and electromagnetic pollution. Many contaminants are accumulated in/or on the plants (directy or from the soil) which are then transferred to honey bees via pollen and nectar. The chemical contaminants include industrial effluents such as fluorine, cyanide, sulphur dioxide, phenol compounds and hydrocarbons, metal such as lead and arsenic and various radioactive elements. A comprehensive study has been published on the effects of high-voltage transmission lines on honey bee colonies (Greenberg et al., 1978). In the USA, honey bees are being used to study effects of microwaves on insects such as would be generated in the NASA project to transmit solar energy to the earth via gaint satellites (Crane, 1990).

3. Toxicity of insecticides to honey bees

Ever since the pesticides have been applied to crops, bees have been found to be poisoned by some; almost all pesticides that kill bees are insecticides. Early insecticides were made of arsenic compounds and they appeared to be very harmful to bees. After the second World War, large-scale mechanisation was introduced in agriculture, and chemical pesticides were massively used for pest control in ways that killed large numbers of bees. Beekeepers in many countries were faced with a crisis , and some felt that beekeeping was doomed. (Crane, 1990)

In 1942 in Arizona, 7,231 colonies were lost through pesticide application to cotton. In 1967, Sevin (carbaryl) applied to cotton killed an estimated 70,000 colonies of bees in California, about 15% of all the colonies in the state. The destruction of bees have occured on just about every crop that blooms and from many flowering weeds. Here are some examples:

-In Washington, 33,000 colonies were killed by application Sevin (carbaryl) on corn in 1967.

-In Arizona, the number of honey bee colonies dropped from

116,000 to 60,000 between 1963 and 1977 due to the destruction of honey bee by pesticides (McGregor, 1978).

-In 1967, the estimated national loss from all pesticide poisoning was 500,000 colonies. Between 1976-1978, 69 % , 56 % , 16 % , and 6 % honey bee colonies in Washington, Arizona, California, and Winconsin respectively were lost or damaged by pesticides.

-In 1984, 24 occurrence of bee poisonings were reported to the Washington State Department of Agriculture. This represented a loss of over $ 1 million to the beekeepers.

A comprehensive 1967 economic survey of 30 commercial Washington beekeepers showed considerable economic loss due to insecticide damage. For instance, the operators suffered a loss of $ 204,000 from insecticide poisoning in 1967, sustaining a 3.2 % loss on the investment instead of an expected 11.2 % gain they would have accrued without the adverse effect of insecticides. A three-year study in Washington (1979 to 1981), showed that 66-79 % of all honey bee colonies received at least one kill from pesticides every year. Another well-documented series of heavy bee losses due to pesticide poisoning came from California, where beekeepers lost an average of 62,500 colonies a year from 1962 to 1973. For example, in 1970 , 89,000 out of a total 521,000 were lost from pesticides.

When Sevin (carbaryl) was first used in Pacific Northwest orchards, one beekeeper lost several thousand colonies in less than a month. As Sevin was registered for use on additional crops starting in the late 1950's, its impact upon bees became even more devastating. Currently, special new formulation of carbaryl such as Sevin XLR are not nearly as toxic to bees as the older formulations ; this has helped to reduce the problem.

Other bee species have also been killed. When diazinon was misused for aphid control on alfalfa hay fields while the plant was in partial bloom, many adult alkali bees were killed, causing a 95 % reduction in alkali bee larvae in three nearby soil nesting sites. The losses in potential seed production and pollinators totaled $ 287,000 and two years later the alkali bees had regained only 25 % of their initial population size. In 1987, the application of Metasystox-R on blooming alfalfa caused a 90 % reduction in alkali bees in four beds, a loss of $ 500,000. In 1988, alfalfa leaf-cutting bees in four fields were kille from insecticidal drift. The loss totaled $ 275,000 (Johansen, 1977).

The problem of pesticide toxicity to honey bees, the primary pollinators of crops which require cross-pollination, is an area of particular concern in Austalia where agricultural crop production in 1980 was valued at about $A 5,500 million (Melkshaam, 1985).

Apart from the problem of pesticide residues in honey bees and bee products, there is also a related adverse impact of pesticides on human health and welfare.In England,Needham and Stevenson(1966) reported that 20 of 31 samples of allegedly poisoned honey bees contained insecticides. On the other hand, Martin (1978)) demonsatrated that honey bees could carry sugar syrup containing insecticide at concentration well above the LD_{50} with no apparent ill effects. Smith and Wilcox (1990) reported that residues of oxytetracycline (< 0.254 ppm) were found in the broodnest honey in six of nine colonies of *Apis mellifera* treated in the spring with antibiotic extender patties or antibiotic dusting, and in all three colonies treated with patties in spring and summer. Spitter et al.(1986) reported that 50 of 70 bee and pollen samples were found to contain methyl parathion and/or carbaryl in USA.

In Thailand, Grasshopper Research Center, Department of Agricuture (1978) reported that after spraying with fenitrothion by air to kill grasshoppers in corn crops in Amphur Kantalak and Amphur Khun Han Changwat Srisagate, 24 colonies of *A. dorsata* nesting on big trees in that area were subsequently found to be killed.

Most of the beekeepers located in the northern part of the country revealed that they are confronted with serious insecticide problems (Wongsiri and Tangkanasing, 1986). Buranapawang and Boongird (1989) reported that insecticide applications were not only harmful to honey bee activities, but also to pollen viability. They found that tangerine pollen could not germinate the pollen tube after the application of some insecticides to dehisced anther. The formulation of insecticide to be used during tangerine blooms should be re-considered.

4. Effect of pesticides on honey bees

While collecting nectar and pollen from flowers, honey bees may come into contact with pesticides deposited upon the plants. Many pesticides are capable of killing larvae (brood) in all stages as well as adult bees. Most often, pesticides kill the field bees (foragers) without causing serious effects to the colony. In some instances the bees died in large numbers after returning to the hive. Many bees are lost in the field and between the treated field and the colony. The colony is weakend but not usually killed. In extreme instances pesticides are carried by the foragers to the hive where they may kill brood and young workers in the colony. As a consequence, the entire population of the colony may die (Crane, 1990).

Pesticides can be absorbed by the bees through one or more of the following routes: oral, respiratory, and dermal. Oral intake is likely to occur when nectar and/or pollen are contaminated. For a pesticide to be toxic via this route, it must be absorbed and the efficiency of absorption depends upon the characteristics of the pesticide. For example, bees could carry lethal doses of organophosphate dimethoate in their honey stomachs without showing signs of intoxication (Anderson and Wojtas, 1986).

Contamination of nectar occurs in a number of plants treated with systemic insecticides. However, there is usually little danger of contamination through nectar. On the other hand, several scientists have reported that dimethoate (applied at the rate of 11 kg/ha) killed the bees as a result of nectar contamination.

The major cause of bee poising is contamination of pollen by microencapsulated insecticides. For example, when Penncap-M ® capsules are applied to agricaltural crops, the capsules are carried by foraging bees to their hives and stored in the brood frames together with pollen. The hive bees then feed the contaminated pollen to the developing brood and this results in the loss of the entire colony. Foraging bees may be killed while collecting and transporting this pollen. The young hive bees are usually killed while storing and feeding the contaminated pollen, and the brood is killed by the poisoned food.

Insecticides such as DDVP, some organochlorine pesticides such as chlordane, and compounds such as nicotine can be present in suffient concentration in the air to be toxic to bees by way of their trachael system. Another possible problem in this regard is the absortion and subsequent release of volatile pesticides with fumigant properties. Beeswax has excellent absorptive properties. Combs exposed to DDVP for 48 hours absorb sufficient pesticide to kill the bees within two to six minutes.

Direct contact is probably the major way by which the pesticides are acquired by the bees. Interception of pesticide droplets in the air during spraying operations and contact with sprayed surfaces are the most likely sources of contamination. The toxicity of the air-borne droplets varies according to the method of application, and the amount of pesticide available to bees decreases with increasing absorption of pesticide by the surface.

Bees usually lose their sense of time when exposed to sub-lethal doses of pesticides like parathion. However, it is not clear whether this phenomenon is the result of changes in the "internal clock" of the poisoned bees or in the manner in which they communicate this "time" to other bees. Disruption in the communication of distance also occurs due to pesticide poisoning.

Outbreaks of European Foulbrood and Sacbrood Virus infections were observed to follow applications of carbaryl insecticide in the foraging area of the affected colonies. The first records of the occurrence of Chalkbrood disease came from colonies that were exposeed to fenetrothion spray. (Atkin, 1992)

Anderson and Atkins (1968) determined the relationship between pesticide toxicity and honey bees by laboratory and field tests, and they classified the toxicity into three groups :

1. Highly toxic group (LD_{50} 0.001-1.9 µg/bee) : Severe losses may be expected if these materials are used when bees are present during treatment or within a day thereafter.

2. Moderately toxic group (LD_{50} 2.0-10.99 µg/bee) : These can be used in the vicinity of bees if the dosage, timing and method of application are correct but should not be applied directly on bees in

the field or at the colonies.

3. Relatively nontoxic (LD_{50} above 11.00 µg/bee) : These can be used around bees with a minimum of injury.

5. Symptoms of bee poisoning

One of the obvious signs of poisoning is the presence of a large number of dead or dying bees at the hive entrance. These adult bees are foragers which have been exposed to pesticides sprayed on flowering plants (Verma and Pratab, 1993). The mortality figures in Table 2.1 are used as guidelines to assess the extent of bee poisoning by pesticides.

Table 2.1 : Extent of bee poisoning by pesticides

Number of dead bees per day at entrance	level of poisoning
100	Normal death rate
200-400	Low
500-1000	Medium
Over 1000	High

Source: FAO Bulletin 68/3 1988.

As a result of organophosphorous poisoning, dying bees extend their tongues through which nectar is regurgitated, and a moist and sticky mass of dead bees is often found at the hive entrance. Fast-acting insecticides kill foraging bees in the field itself, and only a small number of such bees manage to return to the hive. Sometimes, the whole bee colony may die instantly. Strong bee colonies suffer greater losses due to pesticide poisoning than weaker ones because the former have a large number of foraging bees.

Foraging bees often carry residual pesticides in their pollen loads while returning to the hive. As a result, the behaviour of bees in the hive changes abruptly. Honey bees in such colonies become more agitated or aggressive. When the hive with pesticide-affected forager bees is opened, they often fly off the top bars of the hive and sometimes straight into the face of the beekeeper handling them. Other symptoms of pesticide poisoning include stupefaction, paralysis, and abnormal, jerky, or spinning movements. Carbaryl poisoning causes bees to crawl around at the hive entrance. They lose their ability to fly and ultimately die within two to three days after poisoning.

Nurse bees in pesticide-affected colonies lose their ability to remove dead bees from the hive ; as a result the hive entrance is completely blocked. Pesticide poisoning also affects the colony strength because there is a break in the brood-rearing cycle and often dead or deserted colonies cease foraging ; consequently there is a sharp decline in food storage, and incoming foragers are attacked at the hive entrance by other bees.

6. Factors contributing to bee poisoning

Johansen and Mayer (1990) described factors contributing to bee poisoning :

6.1 Bloom

The amount of bloom in a crop often governs the number of bee visiting that crop. The number of foragers affects the magnitude of a bee kill. For example, a highly toxic insecticide sprayed on an alfalfa field at 10% bloom results in less bee kill than the same material applied when the field is at 100 % bloom. The more intensive the bloom is, the more bees are visiting the crops, and thus, the more are being killed.

6.2 Residue exposure

Residue is the amount of insecticide present on a plant after it had been sprayed. The amount of residue and its toxicity decreases with time as the chemical degrades. Residual action is of paramount concern because it largely determines whether an insecticide can be safely used on a blooming crop.

6.3 Air temperature

Temperature also affects the residul action of insecticides. In general, there is a dramatic increase in residual killing action of insecticides occurring under low temperatures. Chemicals applied during cool weather retain a longer residual hazard.

6.4 Timing

The time of day during which an insecticide is applied has an impact on the hazard to foraging bees. No chemical harmful to bees should ever be applied during the day when bees are foraging.

6.5 Formulation

Formulation also affects bee hazard of insecticides. Dust formulations are usually more hazardous to bees than sprays, and wettable powders often have a longer residual effect than emulsifiable concentrates.

6.6 Colony strength

Populous colonies always suffer greater losses than weak ones because greater numbers of foragers are exposed to the insecticidal residue.

6.7 Distance

The distance of colonies from treated fields is inversely proportional to the number of bees killed.

6.8 Forage

A lack of suitable alternative sources of pollen and nectar severely aggravates bee poisoning in many areas, since the bees are often forced to forage on the treated plants.

6.9 Body size

Body size appears to have a direct effect on the susceptibility of bees to insecticides. In general, smaller bees are more susceptible than larger ones.

6.10 Selectivity

The ideal selective pesticides usually do not harm the bees, but they do kill other insect pests.

7. The study of pesticide effects on honey bees

The study of pesticide effect on honeybees is vital because agriculture must have pesticides to control many agricultural pests. Agriculture also requires honey bees for pollinating over 50 of the 250 crops grown in the U.S.A. to produce high quality seeds and fruits at commercial quantities. The farmers must therefore be taught how to use proper pesticides so that pests are controlled and the honey bees and other benificial insects are allowed to survive.

An ideal pesticide is selectively nontoxic to honey bees, but it should be harmful and effective in controlling the target insect pests. Since it is seldom possible to develop a truely ideal pesticide,only the best possible compromises are available on the market. To determine whether a chemical is suitable for widespread use in agriculture, its relative toxicity on honey bees must be throughly investigated.

To determine the toxicity of pesticides on honey bees, laboratory and field investigations must be conducted seperately, and the results of both investigation must be correlated. To apply a pesticide to the best advantage, detailed qualitive and quantitative studies of its effects on sensitive indicators like honey bees should also be carried out. This is best done under controlled laboratory conditions. Large-scale field studies based on the preliminary data obtained in laboratory tests should then follow to determine the practical effects of its use in actual situation.

These chemicals to be tested can be applied in many different forms. The most important forms are as baits, dips, dusts, fumigants, granules, side dressings, and sprays. Comparisons are made in field tests to determine the differences in toxicity that occur under different conditions such as day and night applications, airplane and ground machine treatements, high volume, low volume and ultra-low volume sprays, treatments over or near covered colonies, dust and spray applications, and so on.

There has been a renewed interest recently in the priniples of *economic entomology* which is currently popularly referred to as the *plant-pest management* and/or *insect pest management strategy.* In the most recently revised list on the toxicity of pesticides to honey bees determined from laboratory tests, Atkins (1992) provided informations which allow to predict the expected hazard of a pesticide to honey bees in field applications.

Through experience and by observation of field applications and field tests, a useful rule of thumb method of determining the anticipated toxicity hazard of a pesticide to honey bees in the field is available by utilizing the laboratory data. In most instances the LD_{50} value of a pesticide in micrograms (μg) per bee can be directly converted to the equivalent number of pounds of chemical per acre when applied as a spray or dust to the aerial portions of plants [LD_{50}(in μg/bee) x 1.12 is equivalent to the amount in kg/hectre] (the LD_{50} value is the amount of pesticide which will kill 50 percent of the bees contacted). Since the LD_{50} of parathion is 0.175 μg/bee, we would expect that 0.175 lb/acre of parathion would kill 50 percent of the bees foraging in a treated area at the time of treatment or shortly afterwards.

The slope value (probits) of a pesticide may also be utilizied to determine the anticipated increase or decrease of honey bee hazard in relation to the LD_{50} value. In general, a pesticide with a slope value of 4 probits or higher can often be made safer to honey bees by lowering the dosage only slightly.Conversely, by increasing the dosage only slightly the pesticide can become highly hazardous to bees. This information is particularly useful when the LD_{50} in μg/bee is approximately equal to the normal dosage in lbs/acre needed in the field to control the pest populations. For example, consider a pesticide which is normally applied at dosages of 0.5 to 1.5 lbs/acre to control pest insects, and has a LD_{50} value of 1.0 μg/bee. Further, suppose that the slope value of this pesticide is 2.0 probits. Then, if this chemical is applied at 0.5 lb/acre, we would expect a 28 percent kill of bees in the field; at 1 lb/acre, we would expect a 50 percent kill; and, at 1.5 lbs/acre,we would expect a 64 percent kill.

Suppose the slope value of this pesticide is 16 probits. Under these conditions, if this chemical is applied at 0.5 lb/acre, we would expect no kill of bees in the field; at 1 lb/acre, we would expect a 50 percent kill; and, at 1.5 lbs/acre, we would expect 100 percent kill. These examples illustrate the basic principle that the toxicity or non-toxicity of a pesticide can be determined from its dosage. Pesticides normally considered toxic always have a threshold dosage below which they cause no harmful effect.

The expected bee mortalities at other selected slope value and dosages of a pesticide at an LD_{50} value of 1.0 µg/bee, are tabulated in Table 2.2.

Any pesticide with known LD_{50} and slope value can be similarly perused by substituting the LD_{50} value of the chemical into the centre (the LD_{50} or the 1.0 lb/acre) column of this table and multiplying the LD_{50} value by the other factors (0.1, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 3.0, or 10.0) to obtain the proper range of field dosages per acre. Then, using the slope value closest to the known slope value for the particular pesticide, the anticipated percent mortalities for this chemical can be read from the table (Atkin, 1992).

Table 2.2 : Examples of anticipated honey bee mortality when a pesticide with a LD_{50} value of 1.0 is applied at selected slope values and at increasing and decreasing dosages.

Slope value	Percent mortality at following dosages (lb/acre):									
	0.1	0.25	0.5	0.75	1.0	1.25	1.5	1.75	3.0	10.0
	Below LD_{50}*				LD_{50}*	Above LD_{50}*				
2	3	12	28	42	50	57	64	68	82	97
4	-	1	12	32	50	66	72	82	96	-
6	-	-	2	17	50	76	91	97	-	-
16	-	-	-	3	50	93	-	-	-	-

Most comparative toxicity studies of pesticides on honey bees are carried out on *A. mellifera* . Comparative toxicity studies on *A. cerana* have also been carried out, but less frequently. To date, few studies have been carried out on the comparative toxicity of pesticides on *A. florea* and *A. dorsata* (Amornsak, 1982). Several researchers have compared the relative toxicities of commonly used pesticides on *A. cerana* and these have been classified as highly toxic, moderately toxic, and non-toxic (Verma and Partap, 1993).

List of pesticides with high, moderate, and least toxicity to *A. cerana*

Group 1 : Highly toxic pesticides

Carbaryl 50 % WP, Carbophenothion 20 EC, Cypermethrin 10 EC, Decamethrin 20 EC, Dichlorvos 100 EC, Dimethoate 30 EC, DDVP 100 EC, Monocrotophos 36 WSC, Oxydemeton-methyl 25 EC, Parathion, Phosphamidon 100 EC, Phorate, Permethrin 25 EC, Quinalphos 25 EC, Sumithion 50 EC, Thiometon 25 EC

Group 2 : Moderately toxic pesticides

BHC 50 percent, Carbyl 50 WP, DDT 50 per cent, Dieldrin, Endrin, Hinosan 50 EC, Heptachlor 10 WP, Malathion 50 EC, Methyl demeton, Monocrotophos 40 EC, Trichlorfan 50 EC, Diazinon 20 EC, Ethyl parathion 46 %, Fenitrothion 100 EC, 50 EC, Fenthion 100 EC, Formothion 25 EC, Gramma BHC 20 %, Lindane, Metacide 50 EC, Matasystox 50 EC, 25 EC, Mevinphos, Methyl Parathion 50 EC, Dithane M-45 75 WP, Foltaf 80 WP, Difolitan 50 WP, Hexacap 50 WP, Bavistin 50 WP

Group 3 : Relatively non-toxic pesticides

Endosulfan 35 EC, Menazon 70 DP, Phosalone 35 EC

8. <u>General characteristics of neem</u>

In Thailand the neem tree is known by local names such as : "Quinin","Dao", "Sadao", "Salium", "Kadao" and "Jatang". The scientific name is : *Azadirachta indica* A. Juss, or : *Melica indica* Brand, or *Melia azadirachta* L. The neem tree is a large evergreen tree about 40 - 50 ft. tall, and is commonly found in Southeast Asia, East Africa, Sahara, and many parts of Central America (Boonyarit, 1983). At present *A. indica* also occurs in tropical, and subtropical areas of Africa, America and Australia. During the last 20 years neem has been introduced to many countries mainly for afforestation and fuel wood production in dry areas, and for other purposes, such as for use as an avenue or shade tree and as a producer of natural pesticides.

In Thailand, Bundit (1983) reported that the neem tree is found in Central and Eastern regions of Thailand.There are two varieties , *A. indica* and *A. indica* var. *siamensis*. Thai neem tree differs from the Indian variety in botanical and other characteristics. The fruits are the most important source of the ingredients of neem that affect to insects various ways. They are produced in drooping panicles usually once, or sometimes, twice a year. The oval fruits are 1.4-2.4 cm. long and when ripe have a yellowish sweet pulp that encloses a brown seed kernel embedded in a hard white shell. In Thailand *A. indica* var. *siamensis* blooms from December to January and ripens from March or April.

The tree starts to yield friuts after three to ten years,and the annual yield is about 10-12 kg/tree but this depends on a number of environmental factors such as rainfall and soil condition (Ermel et al., 1986).

Figure 2.4 : *Azadirachta indica* tree.

Figure 2.5 : *Azadirachta indica* var. *siamensis* tree.

8.1 Active ingredients

Due to its various effects on insects, azadirachtin is considered to be the most important active principle in the neem seed kernels. However, the quantity of this compound present in neem seed kernels may vary considerably because of environmental factors and possibly also because of genetic variability. The highest yield of azadirachtin obtained to date was about 10 g/kg of seed kernels. Azadirachtin has deterrent, anti-ovipositional, antifeedant, growth-disrupting (growth-regulating), fecundity-and fitness-reducing properties on insects.

Azadirachtin is a steroid-like tetranortriterpenoid(limonoid). The structural formula of this compound was first proposed in 1972 (Butterworth and Morgan, 1972), but the final elucidation of the complicated molecule was achieved only recently.

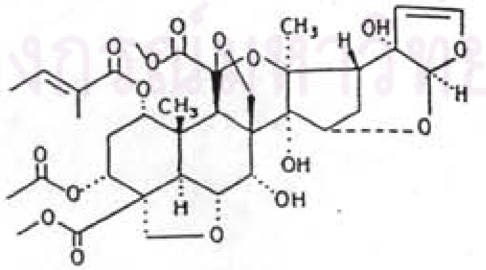

Figure 2.6 : Chemical structure of azadirachtin.

According to some authors, azadirachtin (AZ) is formed by a group of closely related isomers called AZ.A to AZ.G. AZ.A is the most important compound in terms of its quantity in neem seed kernel extracts, and AZ.E is regarded as the most effective insect growth regulator (Rembold, 1987). A considerable number of other active compounds were isolated from neem seed kernels such as salanin, salannol, salannol acetate, 3-deacetylsalannin, azadiradion, 14-epoxy azadiradion, gedunin , nimbinen and diacetylnimbenen (Jones et al., 1989). However, the quantity of these compounds present in neem seed kernels varies considerably because of environmental factors and possibly also for genetic reasons.

8.2 Influence of neem derivatives on insects

Neem extract was effective on many kinds of insects, mites, nematodes and some plant diseases. Its effectiveness on insects depends on the type and the developmental stage of the insects. It can be classified into following categories (National Research Council, 1992) :

(a) Disrupting or inhibiting the development of eggs , larvae, or pupae.

(b) Blocking moulting and metamorphosis.

(c) Disrupting mating and sexual communication.

(d) Repelling larvae and adult.

(e) Deterring females from laying eggs.

(f) Sterilizing adults.

(g) Poisoning larvae and adult.

(h) Deterring feeding and blocking the swallowing process (that is, reducing the motility of the gut).

(i) Sending metamorphosis wary at various stages.

(j) Inhibiting the formation of chitin.

Because of their relatively weak contact effect on insects and their special mode of action, neem-based pesticides in most cases are not harmful, or at most only slightly harmful, to important natural enemies of pests. Neem-based pesticides are remarkably benign to spiders , butterflies, and insects such as bees that pollinate crops and trees, ladybugs that consume aphids, and wasps that act as parasites on various crops pest. (Saxena, 1987)

Rembold et al.(1981) found that the neem extract did not inhibit feeding activities of honey bee larvae but higher concentrations resulted in higher mortality rate.

The addition of 1 % (wt/vol) of crude ethanolic extract of neem seed to a 50 % (vol/vol) sugar syrup resulted in a 50 % reduction in feeding by honey bees (*A. mellifera*) in laboratory cages (Jacobson et al., 1981).

Schmutterer and Holst (1987) found that worker bees were affected only after repeated spraying of highly concentrated neem products onto the flowers of plants. Under these extreme conditions, the workers carried contaminated pollen or nectar to the hives and fed it to the brood. However, only small hives then showed insect-growth regulating effects; medium-sized and large bee populations were unaffected.

Margosan-O ® proved to be nontoxic to honey bee workers in the United State after application as a direct contact product in concentrations up to 4418 ppm AZ/ha.

In Thailand, Malaipan et al. (1992) studied the comparative effects of spraying insecticides (Solone, Endosanfan) and neem extract to the thrips and honey bee pollinators of Pomelo. The result showed that the number of thrips in flowers sprayed with neem extract was not significantly different from those sprayed with the other insecticides. However, the fruit yield seemed to be higher in neem extract treated plants.

There are also other reports which indicate the efficiency of neem seed extracts as pest-control agents. Sombatsiri et al.(1987) reported that the methanol neem extracts at 3.3 % concentration could be highly effective in controlling American bollworm with no statistical difference in cotton production from those plants treated with cyhalothrin, but better than those treated with Bactospein ®.

9. General characteristics of pyrethrum

Pyrethrum (*Chrysanthemum cinerarifolium*)

Pyrethrum is perennial plant of temperate origin. It grows to about 60 cm tall. *Chrysanthemum* spp. originates in the Middle-East. The cultivation of *Chrysanthemum cinerarifolium* originates in Yugoslavia and is now spread throughout the world. After World War 1, Japan became the main producer and export country.The annual production of pyrethrum in various countries (Kenya, Tanzania, Rwanda, Ecuador, Japan and Yugoslavia) was increasing between 1967 and 1972. In developing countries, pyrethrum is cultivated mainly for export.

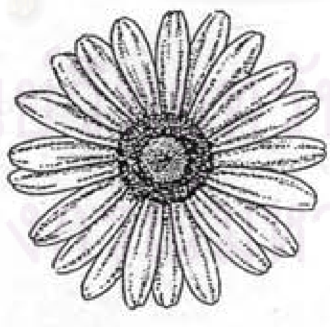

Figure 2.7 : Pyrethrum flower

Pyrethrum can grow at heights of 2000 metres above sea-level and can endure frost up to 12 degrees Celsius below zero. The flower of pyrethrum contains active ingredients called pyrethrins. Flowers should not be harvested before at least three-quarters of the disc florets have opened. Harvesting too early or too late will result in a poor quality of the active ingradient (Martin ,1983). Crop yields may be as high as 200-1000 kg/ha. The active ingradients of the flowers can be extracted by organic solvents (Reay, 1969). Bright sunlight quickly reduces the effectiveness of the pyrethrins, and pyrethrum flowers lost their insecticidal properties very quickly during storage. Pyrethrins can also be synthesized, and these are often known as pyrethroids. These so -called pyrethroids often show higher insecticidal activities than the natural compounds (Casida, 1973).

Extraction of the pyrethroids from natural plants is costly, but the elucidation of the structure of the natural pyrethroids made possible the synthesis of related compounds which possess similar or higher insecticidal activity than the natural compounds. The synthetic pyrethroids are also more stable in light and atmosphere and they could be cheaper to manufacture. They are often more specific in their action than the natural compounds. The first pyrethrin analoque (or pyrethroid) to be synthesized was allethrin (Schechter et al., 1949 cited in Mutsumura, 1975). The synthetic pyrethroids are preferred as substitutes to the natural ones.

9.1 Cyhalothrin

Cyhalothrin is a novel pyrethroid which was developed in 1977. It is principally used to combat a wide range of pests in public health as well as in veterinary medicine.

The molecular formula : $C_{23}H_{19}ClF_3NO_3$

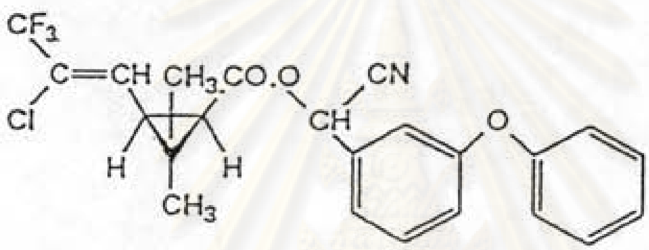

Figure 2.8 : Chemical structure of cyhalothrin

Chemical name : α - cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-difluoroprop-1-enyl)-2,2-dimetyl-cyclopropanecarboxylate

Cyhalothrin is a pyrethroid insecticide with a high level of activity (application rate up to 20 g/ha) against a wide range of *Lepidoptera, Hemiptera, Diptera,* and *Coleoptera* species. It also has some mitecidal activity. The compound is a stomach, contact, and residual insecticide. It shows adulticidal, ovicidal and, particularly larvicidal activities (Pruszynski and Mrowczynski, 1990).

Cyhalothrin has been shown to be toxic to honey bees (*A. mellifera*) in laboratory tests see Table 2.3 .

Table 2.3 : Toxicity of cyhalothrin for honey bees

(expressed as 24-h LD_{50} µg per bee)

Formulation	Topical application	Oral administration	Reference
Technical cyhalothrin	0.051	0.97	Gough et al.(1984)
Cyhalothrin 5% EC	0.057	0.57	Gough et al.(1984)

In common with other pyrethroids, the high laboratory toxicity of cyhalothrin is not translated into a significant field hazard to the bees. In two trials on flowering rape, cyhalothrin was applied at midday by helicopter at a concentration of 10 g/ha to fields where hives of honey bees were located. A toxic stardard and untreated control were used for comparison. Bees were actively foraging during spraying, and the hives were oversprayed. Mortality, foraging activity, activty at the hive, and brood development were monitored before and after treatment, and pollen, honey, and wax were analyzed for residues.

Apart from a suppression of foraging lasting up to 1.5 h, the cyhalothrin formulation had no effect on the bees, whereas the toxic standard killed large numbers. Only low levels of residues were detected (pollen, 0.44µg/g; honey, 0.01 µg/g ai/ha, wax, 0.01 µg/g). It was concluded that, at 10 g ai/ha, cyhalothrin formulation EC is non-hazardous to honey bees on flowering rape (Gough et al., 1985).

Lewis et al.(1990) designed to assess the effects of the residues of cyhalothrin on honey bees (*Apis mellifera*). A laboratory residual toxicity test and a semi-field "tunnel" trial were used in order to compare the results of the two tests. The laboratory residual toxicity test gave an extremely severe result, probably due in large part to the unnaturally prolonged and continuous exposure and stress that the bees experienced under the test conditions. The results were also highly variable, thus limiting the ability of the test to predict field hazard. The results of the laboratory test were not reflected in tunnel trial : there was no increase in mortality but some limited effects on foraging activity.