CHAPTER 5

DISCUSSION

1. Toxicity and residual effect of cyhalothrin on *A. florea* and *A. cerana*

Toxicity of Thai neem extract and cyhalothrin on *A. florea* and *A. cerana* was studied by using topical application and feeding methods . The results of LC_{50} were evaluated and analysed using probit analysis program (Finney, 1974).

The results showed that cyhalothrin was highly toxic to honey bees because the LD_{50} values (24 hours) of cyhalothrin at 95 % confidence interval on *A. florea* and *A. cerana* were found to be 0.0003 and 0.0034 µg/bee respectively, which were less than the relatively safe level of 2 µg/bee.

Cyhalothrin was also found to be more toxic by contact than by oral route to both *A. florea* and *A. cerana* . This is because cyhalothrin (synthetic pyrethroids) has a neurotoxic action. Cyhalothrin causes a long-lasting prolongation of the transient increase in sodium permeability of the nerve membrane during excitation. This results in long-lasting trains of repetitive impulses in the sense organ and a frequency-dependent depression of the nerve impulse in nerve fibres. In particular,an initial period of sensory hyperexcitability leads successively to loss of

coordination, prostration, convulsions and death (Matsumura, 1975). In this experiment, direct contact exposure to cyhalothrin caused erratic movement in the bees and they became unable to fly. Stupefaction and paralysis followed,and the treated bees became moribund and dead within a very short time. From Tables 4.1 and 4.2, % mortality among the treated bee (*A. florea* and *A. cerana*) could be observed within three hours of treatment with cyhalothrin. (Figure 5.1) Cyhalothrin has a rapid knock down effect on insects.

Cyhalothrin can control many pest species. The normal application rate of cyhalothrin 2.5 % EC is 8-16 ml per 20 l of water. This is roughly equal to a cyhalothrin concentration of 0.002 % which is less than the LC_{50} value of the contact toxicity of cyhalothrin on *A. cerana* but more than the LC_{50} value of the contact toxicity of cyhalothrin on *A. florea*. Thus, if cyhalothrin is used as pesticide, we must be aware of its extreme toxicity on *A. florea*.

Gough et al.(1984) reported that LD_{50} value of cyhalothrin on *A. mellifera* by topical application and feeding methods were 0.95 µg /bee and 0.57 µg/bee respectively. These values clearly indicated that cyhalothrin is less toxic on *A. mellifera* than on *A. florea* and *A. cerana*.

A. florea and *A. cerana* are more susceptible to cyhalothrin than *A. mellifera*, and *A. florea* is the most susceptible of the three bee species to this pesticide.(Figure 5.2). The main reason for this extreme

susceptibility of *A. florea* to this pesticide is its greater surface to volume ratio. Furthermore, *A. florea* is also a different type of bee with a host of physiological, biochemical, and behavioral patterns which differ from the other bee type (Danka et al., 1986). Admad and Johansen (1973) examined the difference in the enzyme systems and other possible sources of the variation in tolerance.

The results of residual effect of cyhalothrin on *Antigonon leptopus* on *A. florea* and *A. cerana* indicated that there is no residual toxicity on *A. florea* by topical application and feeding methods after application 24 hours and 6 hours respectively. No residual effect was noted from *A. cerana* treated by topical application and feeding methods 6 hours and 1 hours respectively after application.

Cyhalothrin showed a higher residual effect on *A. florea* than on *A. cerana* because the LD_{50} value of cyhalothrin to *A. florea* was less than its LD_{50} value to *A. cerana*.

Johansen and Mayer (1990) reported that the persistence of residual toxic effect of cyhalothrin on honey bees was more than 24 hours which was similar to the residual effect on *A. florea* observed in this experiment, but different to the residual effect on *A. cerana*. This would be due to difference as in the climate between Thailand and the USA. There is a longer period of sunshine in Thailand, and this probably accounts for why cyhalothrin degrades more rapidly in this country. The direct toxicity of cyhalothrin on honey bees is high but its residual

activity persists for only a few hours.

An interesting observation was made regarding the repellant effect of cyhalothrin on honey bees. In this research, the repellent effect of cyhalothrin on honey bees was found to be significantly higher than those of the neem extract and the control treatment ($p<0.05$; Table 4.44).

Atkins (1992) found that when a bee repellant was used on crops in combination with a toxic pesticide, it will repel bees significantly during the daylight hours of the first day of application. In combination with the following hours of darkness, it could reduce bee losses from pesticides by at least of 50 %. Such a bee repellant would be worthy of development, if its cost was economical. The residues of most pesticides remain toxic for two to five days, but since the number of bees killed is reduced by 50 percent each day following the application, only the loss of bees during the first day is considered to be critical.

Atkin and Kellum (1981) reported that demeton, disulfoton and permethrin are all effective as repellants for one to two days in protecting bees from toxic pesticides on cotton and sanflower as well as seed alfalfa. Johansen and Mayer (1990) found that pydrin application caused a reduction in the number of foraging bees. They attributed this reduction to the changes in flower odour and the changes in the behavior of scout bees brought about by sublethal poisoning of pydrin. The changes in the behaviour of the scout bees led to a reduction in the recruitment of other bees to the treated flowers.

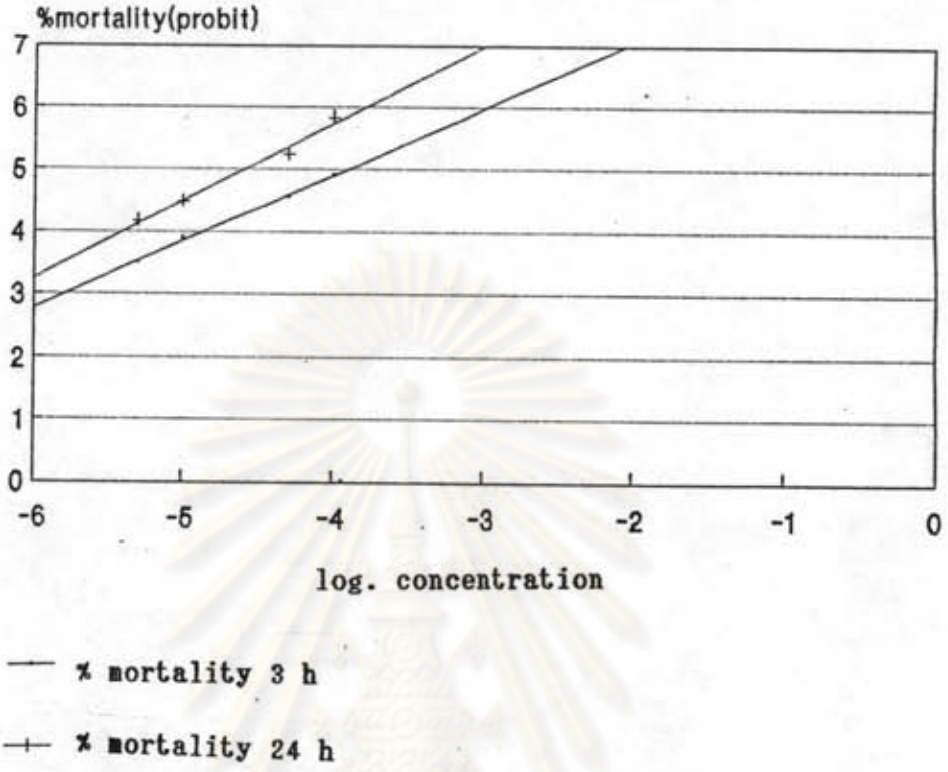

Figure 5.1 : Comparative toxicity of cyhalothrin on *A. florea* at 3 h and 24 h.

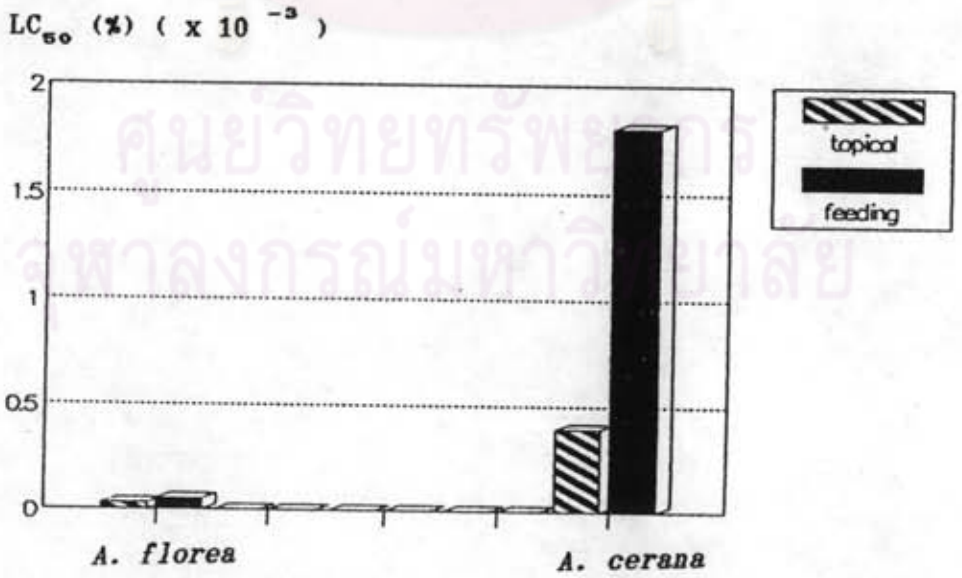

Figure 5.2: Comparative toxicity of cyhalothrin on *A. florea* and *A.cerana*

2. Toxicity and residual effect of Thai neem extract to *A. florea* and *A. cerana*.

The various neem extracts tested in this research were found to be relatively non-toxic to honey bees. The results were shown in Tables 4.36 and 4.37. The LD_{50} values of the various neem extracts on *A. florea* and *A. cerana* were more than 11 µg/bee (Figure 5.3). The quantities of azadirachtin in various neem extracts were also found to be different ; neem-seed crude extract > neem-seed extract > neem oil > Neemix® > Advantage® > Margosan-O® > neem-leaf extract (Figure 5.4).

The toxicity of each neem extract on *A. florea* was also found to be different ; neem-seed extract > neem oil > Margosan-O® > Neemix® > neem-seed crude extract > Advantage® > neem leaf extract (Figure 5.5). Similarly, the toxicity of each neem extract on *A. cerana* were also different; neem-seed crude extract > neem-seed extract > neem oil > Margosan-O® > Neemix® > Advantage® > Neem-leaf extract when using the LD_{50} value of azadirachtin in µg/bee as a criterion. (Figure 5.6). Neem leaf-extract was the least toxic on *A. florea* and *A. cerana*. The reason is that neem-leaf extract has no azadirachtin. *A. florea* was more susceptible to neem oil than *A. cerana* because of its greater surface to volume ratio. Neem oil effected the honey bees by plugging their respiratory holes (spiracles) (Mitra, 1963).

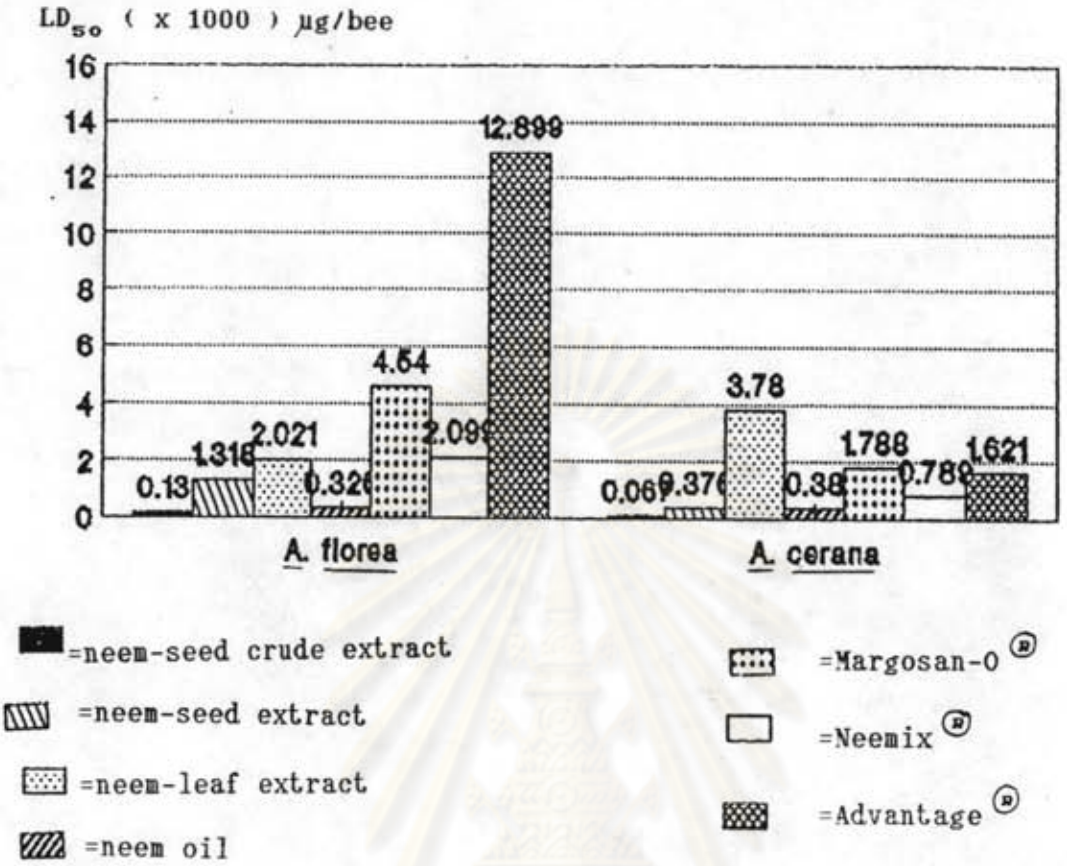

Figure 5.3 : Comparative toxicity of various neem extracts on *A. florea* and *A. cerana* (LD_{50} µg/bee).

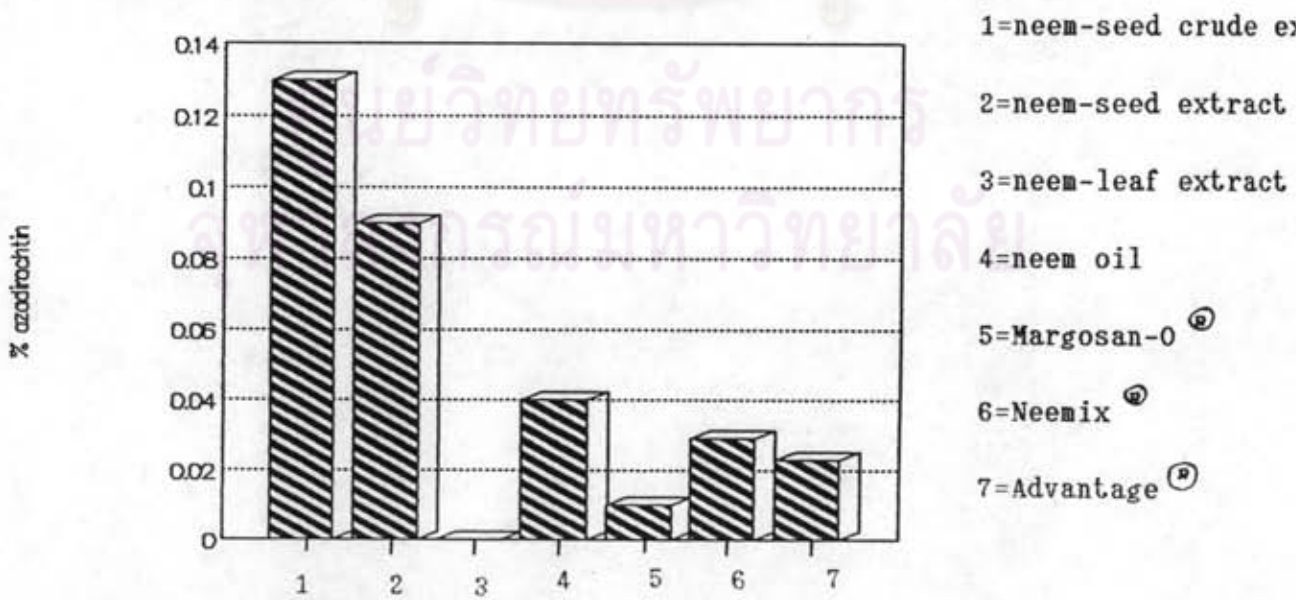

Figure 5.4 : Quantity of azadirachtin in various neem extracts.

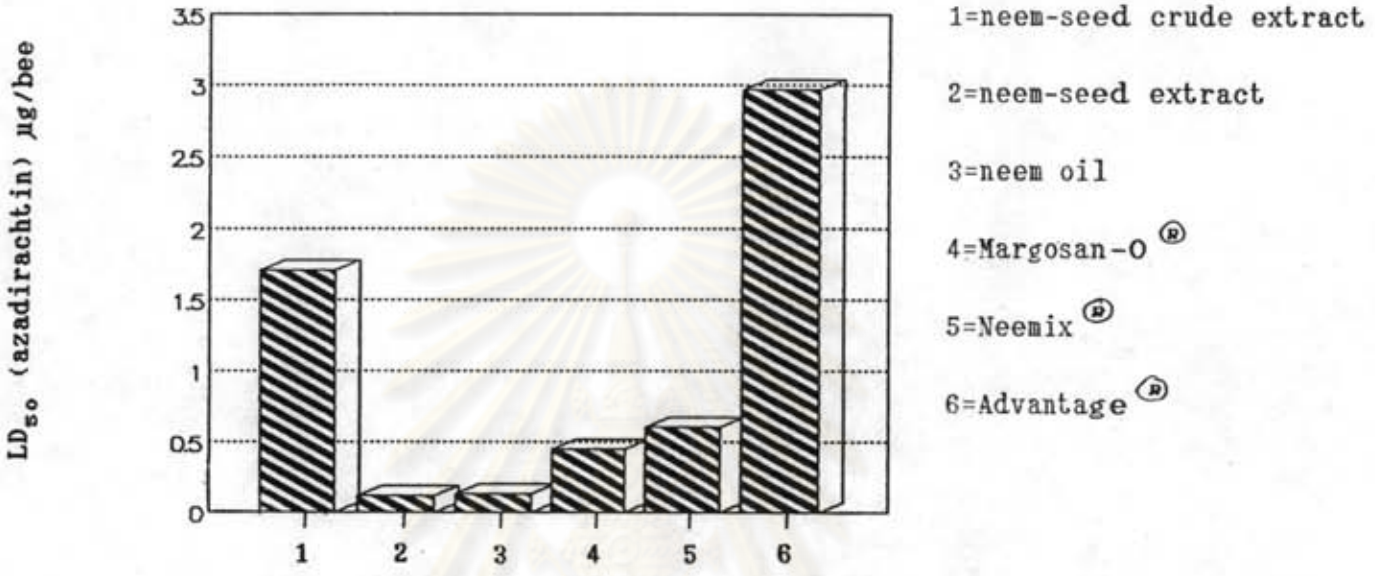

Figure 5.5 : Comparative toxicity of various neem extracts on *A. florea*

(LD_{50} azadirachtin value)

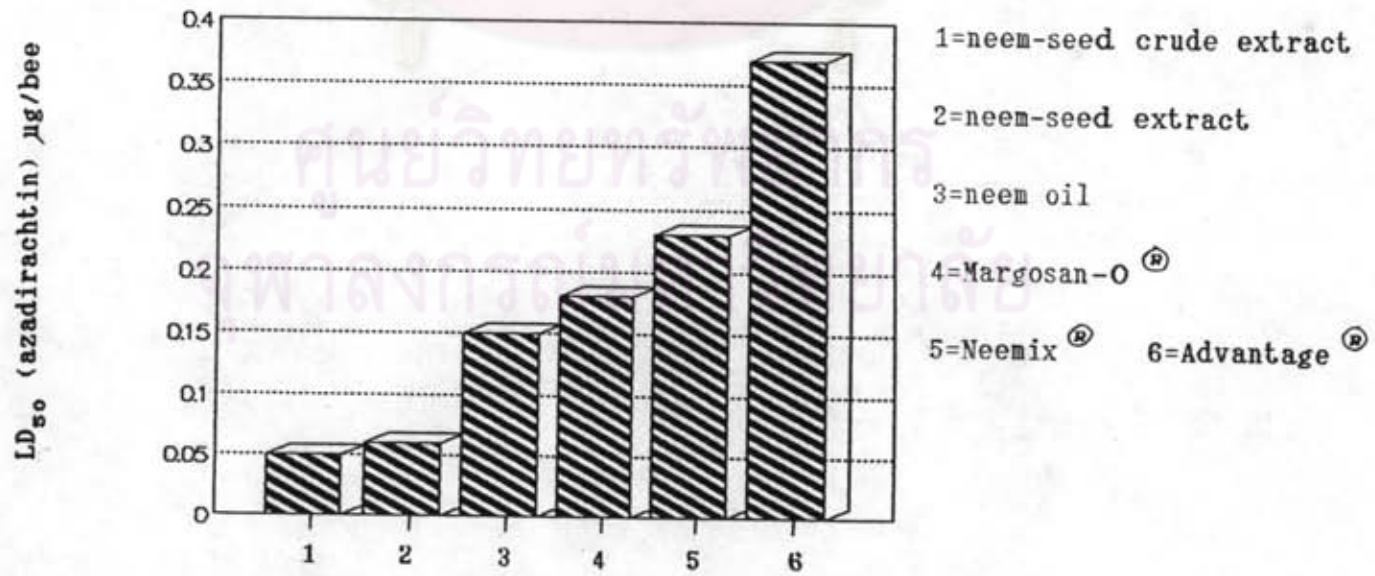

Figure 5.6 : Comparative toxicity of various neem extracts on *A. cerana*

(LD_{50} azadirachtin value)

The toxicity of various neem extracts differs from one type to another because different neem extracts contain different combinations of active compounds such as azadirachtin, meliantriol, salanin, nimbidin etc. Thai commercial neem extract has a mixture of citronella grass and galingale which contain citronellal and geraniol. At times, the elucidation of the precise mode of action is difficult to assess because of the complex ways and the concerted or synergistic effect of the neem compounds present in leaves, bark, seed, oil, cake or extractions. However, most of the effects have been identified and categorized. The neem extract affects the honey bees by poisoning and deterring feeding activities. The mode of action of azadirachtin on honey bees is thought to reside in its ability to interfere with the monooxygenase enzyme (MFO) system which functions by damaging foreign substances within the body. This quickly weakens and subsequently kills the affected honey bees.

Schulze and Schluter (1984) also demonstrated the inhibition of polypeptides in *Epilachna varivestis* by electrophoresis. These reports indicate some effect of neem products on proteins (such as enzymes), and the DNA and RNA which take part in proteins synthesis. However, much work still needs to be done on this respects before a definite conclusion can be drawn.

The oral toxicity of neem extract on honey bees was somewhat inconsistent, presumably because of the feeding-deterrent effect and the ability of the neem extract to block swallowing processes in the honey

bees. At high concentrations, the mortality of honey bees decreased because the neem extract deterred feeding. Thus, there were less honey bee poisoning in the case of neem extracts with high concentrations.

From the results of this research, *A. cerana* was found to be more susceptible to neem extract than *A. florea*. The main reason for the higher susceptibility in *A. cerana* was not its greater surface to volume ratio but rather due to the differences its physiological and behavioural characteristics when compared to *A. florea*. An *A. florea* nest was found on a neem tree at Changwat Samut-Songkhram, and this is shown in Figure 5.7 .

Figure 5.7 : *A. florea* nest on a neem tree at Changwat Samut-Songkhram.

It was shown that the neem extracts were relatively non-toxic to honey bees. In some parts of Asia, neem honey commands premium prices, and people promote apiculture by planting neem trees (Report of an Ad Hoc panel of the Board on Science and Technology for International Development National Research Council, 1992).

The relationship between honey bees and neem trees has been established for a long time. The evolution of the honey bee on adaptation to toxicity of neem extracts is interesting. Similarly, toxicity of cyhalothrin (synthetic pyrethroids from a pyrethrum flower) on *A. florea* and *A. cerana* were higher than *A. mellifera* because the pyrethrum tree is not a native plant. The adaptation of *A. mellifera* is better than that of *A. florea* and *A. cerana*.

Scientists have studied the effectiveness of neem extracts and found that neem extracts control many pest in low concentrations. Schmutterer (1990) employed 4 % neem seed extract to control the diamond back moth. Heyde (1984) reported that 3 % neem oil affected the adults of the brown rice planthopper. Field application rates of Neemix®, Advantage®, and Margosan-O® were less than the toxicity levels of neem extracts on honey bees.

The residual effects of neem extract on honey bees were minimal. The results obtained in this research indicated a lack of residual effect on honey bees two to three hours after spraying neem extracts. Neem extracts have short persistance and bio-degradability. Chirathamjaree

et al.(1993) reported that seven days after spraying of neem extract 500 ppm azadirachtin, only 0.08 ppm was found as residue in Chinese Kale plots. However, Barnby (1989) reported no azadirachtin residues after 16 days of irradiation with ultraviolet germicidal light.

3. The study of a field trial to assess the effect of neem extract on *A. cerana* .

From the study of a field trial to assess the effect of neem extract on *A. cerana*, it can be concluded that the conditions within the beehive (such as weigh, egg, brood, adult, nectar and pollen collection) were not significantly different between the control beehive and that treated with neem extract. But, there was a significant effect on the number of larvae in the ninth week. Swarming also occured in some neem extract test cages. This could be due to the poor condition within the beehives. The results were similar to those of Schmutterer and Holst (1987) who observed that the worker bees were affected after spraying neem extract. Under these extreme conditions, the workers carried contaminated pollen or nectar to the hives and fed it to brood. However, only small hives showed insect-growth regulating effects.

Poor conditions of beehives were also observed in the control group. However, this was comparatively better than in the treated cages. The conditions within the test cages were different from those of the natural setting.

The behaviour of the insect pollinators in cages was basically similar to the behavior of those found in the open plots, except for the following aspect. The foraging speed during the day of the honey bees in the cage were slower than the foraging speed in the open plot because of the effect from the shadow of the blue nylon cage, which resulted in reduced light and low temperature.

The effect of neem extract on larvae of *A. cerana* was also investigated to confirm the field tests. The results showed that the % of abnormal larvae in neem extract treatment was significantly higher than that of the control group ($p \leq 0.05$), especially on three-day old larvae. One- and two-day old larvae were affected by expelled larvae from beehives.

Rembold (1987) studied the mode of action of neem and activity of azadirachtin, which he isolated from the seed kernels of the neem. He concluded that azadirachtin is an insect growth inhibitor which interferes with the neuroendocrine regulation of juvenile and molting hormones. The main cellular targets appeared to be the malphighian tubules and the corpus cardicum of the insects. Azadirachtin reduces the turnover of neurosecretory material in the corpus cardicum. Consequently, levels of the morphogenetic juvenile and molting hormones are shifted and concomitantly decreased after azadirachtin injection.