

**TOXICITY OF SOME NEW PESTICIDES TO
HONEY BEES AND THEIR RESIDUES IN
HONEY AND POLLEN**

THESIS

By

AMIT CHOUDHARY

Submitted to



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PALAMPUR-176 062 (H.P.) INDIA**

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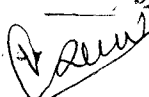
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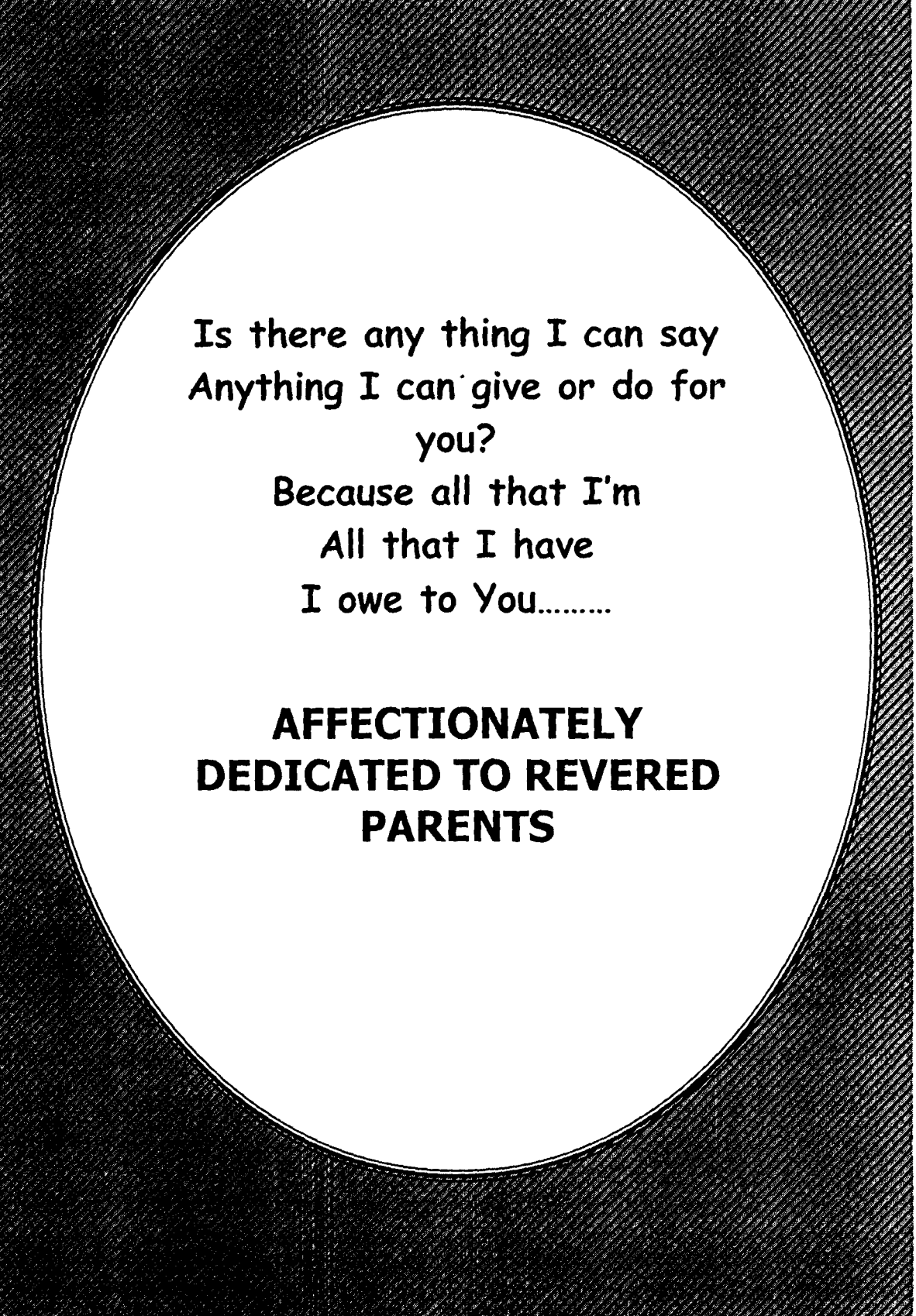
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Is there any thing I can say
Anything I can give or do for
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Because all that I'm
All that I have
I owe to You.....

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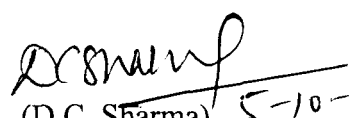
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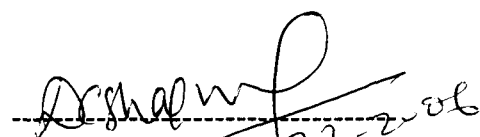
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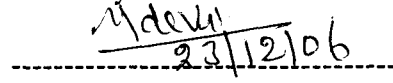

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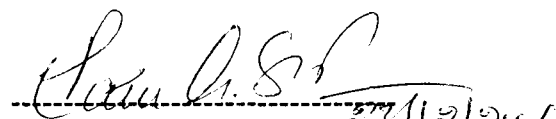
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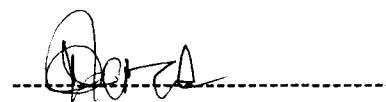
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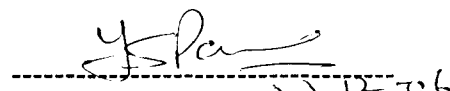
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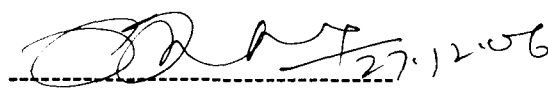
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Needless to say, errors and omissions are mine.

Place : Palampur

Date : 5th Oct 2024


(Amit Choudhary)

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INTRODUCTION

In modern agriculture the use of agrochemicals have become imperative for the protection of plants to enhance and sustain the productivity and yield in order to meet the growing demands for food (Anonymous, 2006). At present, the world pesticide market is around 0.6 billion \$, which is highest among other agricultural inputs. Though the per hectare pesticide consumption in India is very low yet the scenario is quite unhealthy, because we are still using and manufacturing a number of hazardous pesticides which are banned elsewhere. Hill farmers use only a small quantity of pesticide compared to other states but the chances of pollution are very high due to fragility of hill ecosystem. Besides this farmers use newly introduced chemicals without knowing their hazards to consumer and safety to non target organisms and environmental pollution they cause. Such injudicious use of plant protection chemicals cause poisoning and other side effects to beneficial insects including honey bees (Kevan and Baker, 1983; Sihag, 1988).

The exposure of honey bees to pesticides may occur through contaminated pollen/nectar or by their direct contact on the unevenly sprayed field crops (Jaycox, 1964; King, 1964). The presence of pesticide residues even at the low amount in the honey bee body systems may prove detrimental to their important metabolic functions (Shivrana and Jain, 1994). It is also known that the contaminated pollen fed to the brood may poison and kill larvae in the early stages of development. Some times the effects may be delayed so that the death occurs in pre- pupae, pupae and newly emerged bees. Contamination of colony's beewax and stored food with pesticides may also cause long term effects due to persistent physiological changes or delayed exposure and are responsible for weakened and lost colonies from killed brood and adult bees (Atkins and Kellum, 1986).

The problem of pesticide toxicity to honey bees- the primary pollinator of crops requiring cross pollination is an area of prime concern as pesticide use in such crops result in high bee mortality. Some times it exceeds 90 per cent in some apiaries (Wedberg and Erickson, 1986). In India, only about 10 lakh bee colonies are available at present as against an estimated need of 737 lakh owing to extensive agriculture, deforestation and use of pesticides (Ramarethinum, 1998).

Rapeseed and mustard comprise of very important and attractive bee foraging crops. These provide a vital source of nectar and pollen in the period of scarcity of food during winters. In India, these crops are grown over an area of 4419 thousand ha with low average productivity of 869 kg ha⁻¹ (Anonymous, 2004). To manage the major insect pests of these crops 0.2 per cent of the total pesticides were applied. The toxic effects on foragers due to indiscriminate use of pesticides on mustard crop have been well documented by Attri and Sharma (1969) and Thakur *et al.* (1981). Besides this, the extensive use of pesticides in agricultural crops has also resulted in the presence of residues in food (Gunther, 1969; Thrasyvoulou and Pappas, 1988), nectar, pollen and honey (Anderson and Atkins, 1968; Waller and Barker, 1979; Loper and Ross, 1982; Hameed and Singh, 1997).

Indian honey has good place in international market but with the growing demand of organic food, liberalization of food trade (GATT), developments in food science and technology, expanding world economy, improvements in transport and communication and growing awareness towards increased safety to environment and public health it becomes necessary to produce and supply quality product. The quality parameters (including pesticide residues) were underlined by World Trade Organization (WTO) and International Plant Protection Convention (IPPC)- Sanitary and Phytosanitary (SPS)

guidelines of the Government of India. China is facing the problem of exporting honey to the European countries due to the presence of antibiotic residues, which are not permissible as per Codex Alimentarius Commission (Warner, 2002). Such problem may arise with the export of honey produced in Himachal Pradesh (India) because it was also found contaminated with pesticide residues (Yadav, 1995; Sharma and Kashyap, 2002).

Though the safety of different pesticides to honey bees was worked out but there is need to evaluate the toxicity of some newer pesticides entered recently in market along with their residues in nectar and pollen. The pesticide residue monitoring in honey produced in different localities of Himachal Pradesh is also very important to protect the consumer health. Keeping these facts in view the present investigations have been carried out with the following objectives:

- i) To evaluate residual/ persistent toxicity of new pesticides against *Apis cerana cerana* and *Apis mellifera*,
- ii) to evaluate the extent of pesticidal residues in nectar and pollen,
- iii) to monitor the level of pesticides in honey produced in different localities of Himachal Pradesh. and
- iv) to study the effect of residues on physico- chemical properties of honey.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The widespread use of pesticides in modern agriculture throughout the world have become necessary for the protection of the plants against insect pests and diseases to obtain higher yields to meet out the food requirement of increasing population but the injudicious use of pesticides has resulted in contamination of agroecosystem and agriculture produce including nectar and pollen and caused heavy losses to the pollinators (*Apis cerana cerana* and *Apis mellifera*). Such contaminated nectar and pollen when brought to hive may cause damage to brood besides the contamination of the stored honey. Pesticides viz. *Bacillus thuringiensis* subsp. *kurstaki*, cypermethrin, endosulfan, lambda cyhalothrin and neem formulations are recommended in the state whereas, imidacloprid, spiromesifen and thiacloprid are being recommended in India and elsewhere due to their higher efficacy against the pests of oilseed crops without knowing their toxic effects to honey bees, residue problems and other side effects. Several workers made attempts to study the efficacy of these pesticides against pests of *Brassica* crops but so far very little information is available on the toxicity to honey bees (residual/ persistent), residues in nectar and pollen and honey under different agroecological regions of Himachal Pradesh. The literature pertaining to honey bee toxicity, persistent toxicity, residue estimation in nectar, pollen and honey and their effects on physico- chemical properties of honey is reviewed under the following heads:

2.1 TOXICITY OF PESTICIDES TO HONEY BEES

2.1.1 Contact Toxicity

2.1.1.1 Synthetic Pesticides

Cypermethrin was recorded to be highly toxic compared to other synthetic pyrethroids tested *viz.* decamethrin, fenvalerate and permethrin to honey bees (Gerig, 1979). Similarly, Gromiszowa (1982) also reported the higher contact toxicities of cypermethrin and permethrin to honey bees. Kasamatsu and Kawachi (1986) reported LD₅₀ value of cypermethrin to the tune of 0.036 µg bee⁻¹.

The LD₅₀ (contact toxicity) values (µg bee⁻¹) of various insecticides to honey bee foragers were deltamethrin (0.0043) > cypermethrin (0.008) > fenpropathrin (0.014) > methomyl (0.023) > triazophos (0.10) > profenofos (0.26) > sulprofos (0.61). In terms of safety index (LD₅₀ value divided by recommended dose), the order was triazophos (0.08) > methomyl (0.04) = fenpropathrin (0.04) > cypermethrin (0.1) > deltamethrin (0.11) > profenofos (0.12) > sulprofos (0.46) (Saleh *et al.*, 1989). Three pyrethroid insecticides *viz.* Danitol (fenpropathrin), Fastac (alpha-cypermethrin) and Sumicidin (fenvalerate) were found highly toxic when contact toxicity was tested under laboratory conditions (Wael and Laere, 1989).

Significantly higher LD₅₀ value of technical grade cypermethrin (0.16 ± 0.21 µg bee⁻¹) and fenitrothion (0.27 ± 0.031 µg bee⁻¹) compared to commercial formulations *viz.* Cymbush (0.26 ± 0.07 µg bee⁻¹) and Folithion (0.38 ± 0.2 µg bee⁻¹) were observed by Bendahou *et al.* (1997). It was also reported that newly emerged bees (age < 1 day) were more susceptible (LD₅₀ values 0.6 ± 0.03 and 0.27 ± 0.08 µg bee⁻¹ for cypermethrin and fenitrothion, respectively) compared to adult bees (LD₅₀ values 0.21 ± 0.01 and 0.42 ± 0.10 µg bee⁻¹ for cypermethrin and fenitrothion, respectively).

Celli (1974) advocated that endosulfan should not be used in agriculture, as it caused toxic effects to honey bees. In contrary to this, endosulfan was found comparatively safer against *A. cerana* by Kapil and Lamba (1974). The LC₅₀ value of

endosulfan to the tune of 0.4503 per cent to *A. c. indica* was recorded by Singh *et al.* (1974). Arzone (1975a) also reported the toxic nature of endosulfan to honey bees. The LD₅₀ values of different pesticides against *A. mellifera ligustica* x *A. mellifera adansonii* hybrids were determined by Batista *et al.* (1975) and it was reported that endosulfan was comparatively non-toxic with LD₅₀ value of 14.87 µg bee⁻¹ to this hybrid. Stevenson and Walker (1975) also found that endosulfan had significantly lower contact toxicity value compared to azinphosmethyl, fenitrothion and malathion. Thakur *et al.* (1981) recorded LC₅₀ value of 1178 mg kg⁻¹ for endosulfan, which make it comparatively safer to honey bees than fenitrothion, fenthion and trichlorfon. However, Wightman and Whitford (1982) concluded that none of the pesticide including endosulfan was entirely safe for use with Hymenoptera pollinators.

Toxicity studies of endosulfan and its breakdown products conducted in the laboratory revealed that technical endosulfan, alpha endosulfan, endosulfan sulphate and beta endosulfan were moderately toxic (LD₅₀ 2.0-3.6 µg bee⁻¹), photo beta endosulfan and photo alpha endosulfan had intermediate toxicity (LD₅₀ 12.0-24.0 µg bee⁻¹), however others viz. endosulfan ether, diol and hydroxy ether were practically non toxic to *A. c. indica* (Nath *et al.*, 1984).

Brasse (1985) and Svendsen (1985) observed that endosulfan containing pesticides were slightly harmful to hymenopteran pollinators including *A. mellifera*, in contrary to this, Lingappa *et al.* (1985) reported it to be entirely safe to *A. c. indica*.

A high contact toxicity value of endosulfan (comparable to phosphamidon and phosalon) to honey bees was reported by Panda *et al.* (1989). The order of toxicity of some chemicals against *A. mellifera* was worked out to be monocrotophos> dimethoate>

phosphamidon> malathion> carbaryl> chlorpyrifos> isoproturon> quinalphos> mancozeb> endosulfan> neem oil (Abrol and Andotra, 1997).

Imidacloprid was toxic to honey bees but its toxicity was significantly reduced by adding a surfactant 'Sylgard' (Mayer and Lunden, 1994). The influence of imidacloprid (Gaucho® used as a seed dressing for sunflower) on honey bees was examined by Ambolet *et al.* (1999) in 3 tunnel and 8 field trials in France. In none of the tests, imidacloprid had any adverse effect on the vitality, the foraging activity or the behaviour of the honey bees. Contact LD₅₀ values of imidacloprid to the tune of 0.005 and 0.024 µg bee⁻¹ were reported by Suchail *et al.* (2000) after 24 and 48 hours of exposure of honey bees, respectively.

Lambin *et al.* (2001) reported that topical application of imidacloprid at the doses of 0.005, 0.010, and 0.020 µg bee⁻¹ did not produce any lethal effect. It was also reported that lowest dose of 0.0012 µg bee⁻¹ had no effect on the gustatory function of *A. mellifera*. Nauen *et al.* (2001) obtained the contact LD₅₀ value of imidacloprid ranging between 0.049 and 0.102 µg bee⁻¹ against *A. mellifera* whereas, values were 0.070 and 0.050 µg bee⁻¹ after 72 and 96 hours of exposure, respectively (Suchail *et al.*, 2001).

It was observed by Maus *et al.* (2003) that imidacloprid seed dressing posed only negligible risk to honey bees because ten days after exposure of honey bee colonies to the treated sunflower the residues of imidacloprid and its main secondary metabolites *viz.* olefin imidacloprid and hydroxy imidacloprid were detected in traces (< 1.5 µg kg⁻¹). Schmuck *et al.* (2003a) reported contact LD₅₀ values of imidacloprid to *A. mellifera* between 0.040 and 0.104 µg bee⁻¹. While determining the LD₅₀ values (contact toxicity) of commercial neonicotinoid insecticides against honey bees, Iwasa *et al.* (2004) observed that nitro substituted compounds were more toxic to the honey bees with LD₅₀ values of

0.018 $\mu\text{g bee}^{-1}$ for imidacloprid, 0.022 $\mu\text{g bee}^{-1}$ for clothianidin, 0.030 $\mu\text{g bee}^{-1}$ for thiamethoxam and 0.138 $\mu\text{g bee}^{-1}$ for nitenpyram compared to cyano substituted neonicotinoids (LD_{50} values of 7.1 $\mu\text{g bee}^{-1}$ for acetamiprid and 14.6 $\mu\text{g bee}^{-1}$ for thiacloprid).

Imidacloprid can be determined in various body parts of *A. mellifera* viz. head, thorax, abdomen, haemolymph, midgut and rectum within 24 hours of treatment (@ 100 $\mu\text{g kg}^{-1}\text{bee}$). It was observed that haemolymph had the lowest and rectum had the highest level of total imidacloprid. The studies revealed that imidacloprid was readily distributed and metabolized into five metabolites viz. 4,5-hydroxy-imidacloprid, 4,5-dihydroxy-imidacloprid, 6-chloronicotinic acid, and olefin and urea derivatives in honey bee bodies (Suchail *et al.*, 2004).

Cyhalothrin was reported to have high contact toxicity to honey bees (Smart and Stevenson, 1982; Gough and Wilkinson, 1984). Similarly, Lewis *et al.* (1990) reported a low LD_{50} value of lambda cyhalothrin to the tune of 0.051 $\mu\text{g bee}^{-1}$ to *A. mellifera*.

Pilling (1992) reported the synergistic effect of mixing of piperonyl butoxide with lambda cyhalothrin. Similar action was noticed after mixing of lambda cyhalothrin with fungicides, which reduced the LD_{50} value of lambda cyhalothrin from 0.068 to 0.004 $\mu\text{g bee}^{-1}$ (Pilling and Jepson, 1993). The contact toxicity of cyhalothrin was found to be least (LD_{50} 0.036 $\mu\text{g bee}^{-1}$) to alkali bee, *Nomia melanderi*, intermediate (LD_{50} 0.022 $\mu\text{g bee}^{-1}$) to the honey bees, *A. mellifera* and highest (0.002 $\mu\text{g bee}^{-1}$) to alfalfa leafcutter bee, *Megachile rotundata* (Mayer *et al.*, 1998).

Spiromesifen (Oberon[®] 240SC) a good insecticide/ acaricide belongs to new chemical class called tetrone acids. It has a unique mode of action and classified as a

lipid biosynthesis inhibitor. It was reported to be harmless against honey bees with LD₅₀ values of more than 200 µg bee⁻¹ (Anonymous, 2005).

Thiacloprid (Calypso® 240SC) is a new insecticide, having a systemic ingredient chemically related to imidacloprid. Thiacloprid is an acute contact and stomach poison with systemic properties. It has broad spectrum of activity particularly against sucking and biting pests and is safe against honey bees (Horvat, 2001).

Elbert *et al.* (2000) confirmed the safety of thiacloprid to honey bees, which allowed its application during the blossom period of bee-attractive crops. Jeschke *et al.* (2001) reported that contact LD₅₀ value to foragers of *A. mellifera* varied with in the range of 10.00 to 40.00 µg bee⁻¹ after 48 hours of exposure. Similarly, Soloveva (2002) reported the low contact toxicity of thiacloprid to honey bees.

2.1.1.2 Chitin Synthesis Inhibitor (CSI)

Novaluron: a benzoylphenyl urea derivative is a potential chitin synthesis inhibitor. The application of diflubenzuron did not increase the mortality among the adults of honey bees. However, some damage to brood, larvae, pre-pupae and pupae were noticed by Egger (1977) after 11 days of spraying. It was found that mortality occurred due to decrease in tensile strength and cuticular rigidity (Ker, 1978). Wilkaniec and Wojtowski (1979) documented diflubenzuron to be non toxic to honey bees in laboratory testing.

The use of chitin synthesis inhibitor BAYSIR 8514 in bee colonies was proposed by Herbert *et al.* (1986) to protect honey bees from ectoparasitic mites; *Tropilaelaps clareae* and *Varroa jacobsoni*. Usha and Kandasamy (1986) categorized diflubenzuron as the safest pesticide because no mortality was observed when adults of *A. cerana* were exposed to treated surface (@10000 mg L⁻¹) for 90 minutes. The insect growth regulators

caused little or no damage to adult honey bees and the typical effects were noticed only in the stage just after moulting (Engels, 1990). Likewise, Gupta and Chandel (1995) found that topical application of $100 \mu\text{g bee}^{-1}$ was non toxic to adult bees.

Tasei (2001) reviewed the effects of insect growth regulators on honey bees and concluded that the toxic effects of the insect growth regulators (IGR's) or ecdysone or juvenile hormone mimics or chitin synthesis inhibitor were noticed in larval stages but these compounds were safe to adults of honey bees.

2.1.1.3 Biopesticides

Rembold and Czoppelt (1981) applied purified azadirachtin topically to the third instar larvae of honey bees, which caused detrimental effects at a lowest dose of $0.25 \mu\text{g larvae}^{-1}$. A dose of $196 \mu\text{g a.i.}$ of azadirachtin caused neither acute nor delayed toxic action in any of the test insect viz. *A. mellifera* and *Osmia lignaria* (Ladurner *et al.*, 2005).

Davidson *et al.* (1977) and Vandenberg (1990) reported that *Bacillus sphaericus* was safe to honey bees but, in the latter case, lifespan was found to be reduced by technical powder and formulated product of another bacterium *B. thuringiensis* var. *tenebrionis* at the highest tested concentration of 10^8 spores mL^{-1} sucrose syrup.

Spinosad is a natural insecticide derived from an actinomycete bacterium species, *Saccharopolyspora spinosa*, which displays the efficacy of a synthetic insecticide. It consists of the two most active metabolites, designated as spinosyn A and D. Spinosad has a high level of efficacy for lepidopteran larvae, as well as some Diptera, Coleoptera, Thysanoptera, and Hymenoptera, but has limited or no activity to other insects and exhibits low toxicity to mammals and other wildlife (Mayes *et al.*, 2003).

Mayer *et al.* (2001) worked out the contact toxicity (LD_{50}) of spinosad against adult *A. mellifera* bees to the tune of $0.078 \mu\text{g bee}^{-1}$. On the basis of LD_{50} , the honey bee

(LD₅₀ 0.612 µg gbee⁻¹) was the most susceptible followed by the alkali bee (0.773 µg gbee⁻¹) and the leafcutter bee (1.908 µg gbee⁻¹).

2.1.2 Stomach Toxicity

2.1.2.1 Synthetic Pesticides

Gromiszowa (1982) reported that cypermethrin had the high level of oral toxicity to honey bees which was at par with permethrin. The LD₅₀ (oral toxicity) values of three pyrethroid pesticides viz. Danitol (fenopropathrin), Fastac (alpha-cypermethrin) and Sumicidin (fenvalerate) were reported to the tune of 0.423, 0.056 and 0.046 µg bee⁻¹, respectively (Wael and Laere, 1989).

Oral toxicity of endosulfan was markedly high (Arzone, 1975b). Stevenson and Walker (1975) found that endosulfan had significantly lower oral toxicity compared to azinphosmethyl, fenitrothion and malathion. In contrary to this, Giordani *et al.* (1978) reported endosulfan and its sulphate isomer were highly toxic to honey bees. Endosulfan had LC₅₀ value of 1178 mg kg⁻¹, which was less compared to fenitrothion, fenthion and trichlorfon (Thakur *et al.*, 1981). Akwatanakul and Amornsak (1983) found that all the tested pesticides including endosulfan belonging to three different groups viz. organochlorine, organophosphorus and carbamate were highly toxic to *A. mellifera* when administered orally. Similar observations against *A. cerana* and *A. florea* were recorded by Amornsak and Akwatanakul (1983).

Nath *et al.* (1984) and Nath and Sharma (1985) observed that two endosulfan isomers (endosulfan I and II) were interconvertible inside honey bee (*A. c. indica*) body and were moderately toxic. Jain and Guman (1992) reported non toxic nature of endosulfan to *A. florea* and *A. dorsata* in oral feeding tests.

No adverse effects were observed on bees fed with imidacloprid spiked sugar solution up to $20 \mu\text{g L}^{-1}$ (Ambolet *et al.*, 1999). LD_{50} value of imidacloprid to the tune of $0.005 \mu\text{g bee}^{-1}$ was reported after 48 hours of exposure by Suchail *et al.* (2000) and between 0.048 to $0.081 \mu\text{g bee}^{-1}$ by Nauen *et al.* (2001). It was also reported that sugar syrup spiked with imidacloprid up to $20 \mu\text{g L}^{-1}$ did not produce any adverse effect on honey bees. In contrary to this, Schmuck *et al.* (2001) reported a different range of LD_{50} (oral toxicity) value of imidacloprid to honey bees (0.004 - $0.041 \mu\text{g bee}^{-1}$). Decourtye *et al.* (2003) worked out the oral LD_{50} value against *A. mellifera* as $0.153 \mu\text{g bee}^{-1}$. The lowest observed effect concentration was less to summer bees ($12 \mu\text{g kg}^{-1}$) compared to winter bees ($48 \mu\text{g kg}^{-1}$). Oral LD_{50} values of imidacloprid to *A. mellifera* varied from 0.040 to $0.104 \mu\text{g bee}^{-1}$ (Schmuck *et al.*, 2003a) and 0.004 to $0.021 \mu\text{g bee}^{-1}$ (Gregore and Bozi, 2004).

High oral toxicity of lambda cyhalothrin against honey bees was reported by Prakash and Kumaraswami (1984), Arzone and Patetta (1986), Rieth and Kevin (1987), Shivrana and Jain (1994) and Mayer *et al.* (1998).

Oral LD_{50} value of spiromesifen was reported to be $200 \mu\text{g bee}^{-1}$ (Anonymous, 2005). Liu *et al.* (2004) also documented spiromesifen to be non toxic to honey bees.

Schmuck *et al.* (2003b) reported that the chloronicotinyl insecticide, thiacloprid, posed little hazard to *A. mellifera* when it was applied at the rate of $480 \text{ g a.i. ha}^{-1}$ (Calypso® 240SC). Similarly, Garzia and Rapisarda (2004) observed no significant impairment of pollination activity of a hymenopteran pollinator (*Bombus terrestris*). It was suggested that thiacloprid could be preferred over other compounds for use during flowering.

2.1.2.2 Chitin Synthesis Inhibitor

Benzoylphenyl urea derivative; diflubenzuron was found safe for brood production when 5.9 and 0.59 mg L⁻¹ concentrations were fed to bees in sugar syrup. However, a higher dose of 59 mg L⁻¹ significantly reduced the brood production (Barker and Taber, 1977).

Barker and Waller (1978) observed that food consumption, brood area and emergence of new workers decreased in the colonies fed with 100 mg L⁻¹ of diflubenzuron. In contrary to this, the spray concentrations of 0.11, 0.20 or 0.40 kg a.i.ha⁻¹ were found safe to the foraging honey bees by Emmett and Archer (1980). Diflubenzuron fed at the rates of 1.0 or 10 mg kg⁻¹ in sugar cake, significantly reduced sealed brood but reduction in the adult population was recorded only at the 10 mg kg⁻¹ rate (Stoner and Wilson, 1982). Tasei (2001) concluded that feeding a small quantity of CSI may cause damage to brood.

2.1.2.3 Biopesticides

Naumann and Isman (1996) fed the formulated product of azadirachtin to the first and third instar larvae of honey bees and reported the acute LD₅₀ values to the tune of 37 and 61 µg g⁻¹ body weights for first and third instar larvae, respectively. Ladurner *et al.* (2005) found azadirachtin dose as high as 196 µg a.i. did not produce any toxic effects to the adults of *A. mellifera* and *O. lignaria*.

Mortalities were not observed in the honey bees when workers were infected in the laboratory with 2 x 10⁷, 2 x 10⁸ or 4 x 10⁸ spores of *B. thuringiensis*, or the corresponding amounts of exotoxin was fed in sugar solution or sugar candy. The multiplication of spores of *Bacillus* was inhibited by acidity and the presence of honey inhibine in the gut (Marletto *et al.*, 1972). Similarly, no adverse effects to caged honey

bees were observed when *B. thuringiensis* subsp. *alesti* (0.0018 g cm^{-3}) was fed to the bees (Celli, 1974). It was also reported that honey bees could safely ingest 9×10^5 spores day^{-1} of *B. thuringiensis* subsp. *kurstaki* from Dipel.

Krieg *et al.* (1980) reported that adult honey bees fed for seven days with *B. thuringiensis* subsp. *israelensis* at a concentration of 5×10^8 spores + crystals per ml of 50 per cent sucrose solution did not produce any toxic effects. Similarly, Vandenberg and Shimanuki (1986) tested two commercial formulations ABG-6162 (ABG) and SAN410SC72 (SAN) of *B. thuringiensis* for their effects on honey bees by feeding the honey bees with diluted solution of toxin throughout their life. It was concluded that only the most dilute preparation of ABG had no significant effect on the lifespan of caged bees compared to untreated controls, however all other dilutions of ABG and SAN significantly reduced the LT_{50} .

Spinosad had oral LD_{50} value of $0.063 \text{ } \mu\text{g bee}^{-1}$ (Mayer *et al.*, 2001).

2.2 PERSISTENT TOXICITY OF PESTICIDES TO HONEY BEES

2.2.1 Synthetic Pesticides

El-Banby and Kansouh (1981) released honey bees on leaves, petals, nectar and pollen collected from cypermethrin treated cotton plants at different time intervals i.e. 0, 1, 2, 3 and 4 days after spray. Honey bee mortality was recorded only on one day after spray. Shires and Debray (1982) conducted trials for the evaluation of field weathered toxicity of Ripcord (cypermethrin) applied at the rate of $25 \text{ g a.i. ha}^{-1}$ on flowering oilseed rape. It was observed that colonies placed next to the sprayed fields showed slightly increased adult bee mortality on the day of application, which returned to normal after some time. A repellent action of Ripcord was also noticed soon after the spray that reduced the foraging activity in the field and activity at the hive. Similar studies were also

conducted by Benedek (1983) on flowering winter rape and lucerne. It was found that the direct contact toxicity of all compounds was high. Ambush (permethrin) and Chinetrin (permethrin + tetramethrin) showed high persistent toxicity compared to Decis (deltamethrin), Ripcord (cypermethrin) and Sumicidin (fenvalerate).

Benedek (1985) reported repellent action in alphamethrin (the [1alpha(S*), 3alpha]-(±)-isomer of cypermethrin) and deltamethrin treatments for 3-4 hours of spray. Similar effects of cypermethrin were observed upto 2 days of spray on oilseed rape but the number of bees observed in dead-bee traps in the first three days after spraying were three times more than the control treatment (Svendsen, 1985). When EC formulations of cypermethrin (15 g a.i. ha⁻¹), dimethoate (500 g a.i. ha⁻¹) and phosalone (1200 g a.i. ha⁻¹) were applied to the flowering *Phacelia campanularia* plants, low honey bee mortalities to the order of 15 and 7 per cent for cypermethrin and phosalone, respectively were observed compared to more than 97 per cent in dimethoate treatment (Murray, 1985).

The repellent action was initiated by the exposure of tarsi and ventral of bee's abdomen to pesticide (Rieth, 1986). Rathore *et al.* (1987) found that cypermethrin (0.01%) sprays affected the honey bee (*A. c. indica*) activity up to 9 days.

Order of field weathered toxicity of various pesticides to *A. mellifera* after 6 hours exposure was: Nurelle > Cybolt > Sevin > Methrin > Decis. However, after 24 hours of exposure the order became Nurelle = Meothrin = Sevin > Cybolt > Decis. In a different trial pesticides were applied to field plots in which hives were placed. It was observed that foraging activity of honey bee foragers decreased in the following order: Sevin > Meothrin > Nurelle > Decis > Cybolt (Atallah *et al.*, 1989)

In field trials in Italy, trichlorfon (2 g a.i. L⁻¹) and parathion (200 g a.i. L⁻¹) spraying on flowering *Phacelia tanacetifolia* caused high mortality of *A. mellifera* upto 2

days of application. It was found that alphasmethrin (5 g a.i. L⁻¹) was safe to honey bee colonies (Inglesfield, 1990; Lapietra and Allegro, 1990).

Ramakrishnan *et al.* (1974) reported that endosulfan application @ 0.07 per cent on sunflower caused 33.2 per cent honey bee mortality after 1 hour of spray, which reduced to zero per cent after 24 hours. Benedek *et al.* (1976) observed lower field weathered toxicities of cartap, endosulfan, kelevan and pirmicarb to honey bees compared to fenthion, fenitrothion, methyl parathion, phenthoate and parathion. Strong repellent action of pesticides including endosulfan was recorded against honey bees even after 24 hours of spray by Praagh (1980). Sorthia and Chari (1985) observed hundred per cent mortality of honey bees due to direct spray drift for all the insecticides tested (endosulfan, etrimfos, fenvalerate and quinalphos) within 24 hours, however mortality was lower when cages were introduced into the fields after spraying. Rathore *et al.* (1987) reported that honey bee visits to endosulfan treated (0.025 %) blooms declined just after spray upto six days, thereafter, normal bee activity was restored. The residual toxicity of endosulfan against honey bees, on sunflower, began to decrease six hours after spraying. Comparatively lesser number of dead bees (59) was reported in endosulfan than dimethoate and DDVP treatment (each 113). The residual toxicity of endosulfan persisted for five days (Singh *et al.*, 1989). Vaidya *et al.* (1995) and Sontakke and Dash (1996) also observed repellent action of endosulfan to honey bees. Endosulfan was found to be safe against hymenopteran pollinators in rapeseed by Singh *et al.* (1999). Endosulfan applied thrice (@1000 ml ha⁻¹) caused 66.64 per cent mortality to *A. mellifera* (Suchail *et al.*, 2001).

Imidacloprid used as seed treatment (@0.7 mg a.i. seed⁻¹) was found safe to *A. mellifera caucasia* by Ambolet *et al.* (1997). Mortality of bees caged with imidacloprid

treated alfalfa foliage was significantly lower after 8 hours compared to 2 hours of treatment (Mayer and Lunden, 1997). A dose of $20 \mu\text{g kg}^{-1}$ imidacloprid was found not to cause any objectionable adverse effects under field relevant exposure conditions (Schmuck, 1999). Similarly, Confidor® application (50 ml ha^{-1}) on apple and seed dressing of sunflower did not cause any adverse effect to bees (Cantoni *et al.*, 2001). However, daily consumption of imidacloprid $0.1 \mu\text{l L}^{-1}$ for ten days proved toxic to honey bees (Suchail *et al.*, 2001).

Cure *et al.* (2001), Tasei *et al.* (2001), Wallner (2001) and Stadler *et al.* (2003) reported that imidacloprid applied on *P. tanacetifolia* and seed treatment on sunflower did not produce any adverse effects to bees.

Imidacloprid granules and sprays were applied on flowering white clover followed by irrigation. Both the treatments were not found to have any effect on colony vitality or worker's behaviour. In contrary to this, exposure to dry non irrigated residues had severe impact on colony vitality (Gels *et al.*, 2002). Singh and Singh (2004) found low honey bee mortality in beehives kept in the mustard fields sprayed with imidacloprid at 140, 168 and 196 ml ha^{-1} .

Repellent action of lambda cyhalothrin to honey bees was observed by Fries and Wibran (1987). Lewis *et al.* (1990) carried out the studies to assess the effect of lambda cyhalothrin residues (field weathered toxicity) on honey bees by two methods i.e. laboratory estimation by releasing honey bees on oilseed rape (*Brassica napus*) and lucerne foliage collected 3, 8, 24, 48 and 96 hours after spray and placing honey bee colonies in the fields sprayed and covered with nylon net "semi field tunnel trial". It was found that first experiment showed high level of toxicity whereas, latter showed non significant mortality with respect to untreated check.

Karate[®] (lambda cyhalothrin) spray on crops had little effect on the honey bee foragers (Rotrekl, 1994), but Thompson and Folkard (2001) found Hallmark[®] (lambda cyhalothrin) to be highly toxic and caused heavy losses if applied in combination with fungicide.

Spiromesifen sprays on the crops did not produce toxic effects. In the plants it got metabolized readily, thereby did not cause mortality in foragers of honey bees (Anonymous, 2005).

2.2.2 Biopesticides

Schmutterer and Holst (1987) tested the effects of enriched and formulated neem seed kernel extract AZT-VR-K on honey bees in cage and field experiments. It was observed that such sprays did not produce harmful effects to adult foragers. Similarly, field applications of $> 150 \text{ mg a.i. L}^{-1}$ did not repel foragers from the blossoming *B. campestris* because individual bee acquired only small quantity $< 30 \text{ pg}$ of azadirachtin (Naumann *et al.*, 1994). Schmutterer (1990), Mordue and Blackwell (1993) and Thapa *et al.* (1997) did not observe any toxic effect of azadirachtin to adult honey bees.

In two different experiments, bees were released on caged cotton (*Gossypium* spp.) plants and on sprayed leaf samples. It was reported that neemazal at the higher tested dosage was safe to honey bees with only 0.74 per cent mortality in the former and 17.19 per cent mortality in the latter experiments (Mann and Dhaliwal, 2001).

2.3 PESTICIDE RESIDUES

2.3.1 Nectar

Jaycox (1964) and King (1964) reported that nectar and pollen got contaminated due to pesticide application on crops. Similarly, spraying of aldicarb, dimethoate and metasystox on crops poisoned the nectar (Mizuta and Johanson, 1972).

Waller and Barker (1979) reported that nectar from onion (*Allium cepa*) plants sprayed with 300 mg L⁻¹ a.i. dimethoate, contained residues up to 7 mg kg⁻¹ at 4 days after treatment, which dropped to 0.2 mg kg⁻¹ after 14 days. It was also documented that a dose of 0.2 mg kg⁻¹ produced harmful effects in honey bees.

Belanger and Rivard (1980) and Barker *et al.* (1980) reported residues of dimethoate get translocated as such into nectar. Kelthane (dicofol) and methyl parathion too were found to contaminate the nectar with their residues by Petukhov (1981) and Loper and Ross (1982). Fielder and Drescher (1984) reported that nectar of flower got contaminated with insecticides *viz.* acephate, dimethoate, methomyl, phosalone and propoxur after pre- blossom treatment and caused toxic effects in *A. mellifera*. The residues of aldicarb and its sulfoxide and sulfone (aldoxycarb) metabolites were present in nectar of lucerne plants after a spray of 3.4 kg ai ha⁻¹ (Johansen *et al.*, 1984). The nectar samples collected from flowers and comb had dimethoate residues greater than 0.1 mg kg⁻¹ (each) after 8 days of treatment (Waller *et al.*, 1984). Rincker and George (1985) recorded the presence of aldicarb, carbofuran, dimethoate, methyl demeton and trichlorfon residues in nectar and reported that these were harmful to *A. mellifera*. Fieller (1987) determined acephate residues after pre-blossom treatments. It was reported that all the nectar samples collected during flowering (up to 16 days after start of flowering) had acephate and methamidophos residues, which were dangerous to honey bee broods.

Celli and Porrini (1988) reported that after spraying apple and dandelion, nectar was contaminated with deltamethrin and mancozeb. Similarly, cabbage, onion, carrot, clover and *Convolvulus arvensis* nectar had residues of methyl parathion. It was documented that floral morphology and placement of nectar contributed a lot in the level of contamination. While amount of nectar produced per flower, amount of pesticide used

and frequency of bee foraging were comparatively less important. According to Davis *et al.* (1988) residues of pesticides viz. carbofuran and dimethoate were persistent in nectar of *Vicia faba* flowers.

Thakur and Kashyap (1989) reported that highly systemic insecticides (demeton-S-methyl and phosphamidon) get translocated into nectar of flowers and caused honey bee mortality even after 24 hours of spray. Field studies were carried out by Tasei *et al.* (1994) on oilseed rape (*B. napus* cv. *oleifera*) to study the effects of sublethal doses of deltamethrin (12.5 g ai ha⁻¹) (Decis) on *B. terrestris*. It was reported that residues to the tune of 0.012 – 0.019 mg kg⁻¹ were present in nectar, which did not have lethal effects on *B. terrestris*.

Nectar of sunflower was analyzed for the presence of imidacloprid and its relevant metabolites and residues were found below detection limit of 10 mg kg⁻¹ (Ambolet *et al.*, 1999). Cure *et al.* (2001) and Schmuck *et al.* (2001) observed that imidacloprid residues in nectar of seed treated sunflower did not exceed LOQ (Limit of Quantifications) of 10 µg kg⁻¹. The content of organophosphorus insecticides residues in pollen on the day of application exceeded the minimum lethal concentrations, whereas, residue levels of pyrethroid compounds were close to the mid lethal concentration for *A. m. caucasia* (Illarionov, 2001). It was reported that traces of imidacloprid residues (3-10 µg kg⁻¹) were secreted in nectar of *P. tanacetifolia* flowers (Wallner, 2001).

Nectar samples of sunflower, rape and maize plants had imidacloprid residues below 5 µg kg⁻¹ (Maus *et al.*, 2003). Olivero *et al.* (2003) reported that fenitrothion residues were released in nectar of peach flowers thereby contaminating it. Gregore and Bozi (2004) recovered residues of imidacloprid to the tune of 1.9 µg kg⁻¹ in nectar of sunflower after seed treatment. Romaniuk *et al.* (2004) analyzed the occurrence of HCH

and DDT in trophic chain of bee families and concluded that starting from pesticide content in flowers (nectar and pollen) every component of the chain had residues of these persistent pesticides.

2.3.2 Pollen

The initial report by Morse *et al.* (1963) stated that bees could collect the contaminated pollen from the fields sprayed with pesticides and stored them in the hives, raised concerns about the aerial application of insecticides. It was reported that after field application, monocrotophos residues were observed in trapped and stored pollens (Stranger and Winterlin, 1975).

Residues of organophosphorus pesticide above tolerance limits in pollen were reported by Danielyan *et al.* (1976). Several claims have been made by the bee keepers that many colonies of *A. mellifera* were killed because they feed on contaminated stored food (pollen and honey) in winter periods when foraging was halted. Heavy loss of newly emerged bees and brood under similar conditions was reported by Johansen and Kious (1978).

Rhodes *et al.* (1979) conducted bioassay studies using *A. mellifera* as test organism by feeding pollen diet containing the residues of methyl parathion. Average adult mortality was found to be 29- 72 times higher than normal. Honey bees were collected from birdsfoot trefoil (*Lotus corniculatus*) treated fields and their pollen loads were examined for the residues. Penncap-M residues were found even after 9 days of treatment (Burgett and Fisher, 1980).

Barker *et al.* (1980) reported that dimethoate residues contaminate the pollen of lucerne. A spray treatment containing 304 mg L⁻¹dimethoate gave residues to the tune of 0.5 mg kg⁻¹ after one day of treatment.

Residues of aldicarb, carbofuran, demeton, dimethoate and trichlorfon were reported in the pollens of lucerne by George and Rincker (1982). Hand collected pollens from *Cupressus arizonica*, *Franseria confertiflora* and *Helianthus annuus* and bee collected pollen from *Taraxacum officinale* had methyl parathion residues, but their toxicity to pollinators differ due to difference in lipid content (Loper and Ross, 1982).

Residues of methyl parathion to the tune of 0-81 mg kg⁻¹ were present in the pollen samples collected from the damaged honey bee colonies (Atkins and Kellum, 1984).

In the filed trials, Fries (1985) reported that cypermethrin application (@ 44 g ai ha⁻¹) on blooming rapeseed repelled honey bees from the sprayed area. Residues to the tune of 0.22 µg g⁻¹ in pollen were also recovered after 2 hours of application, which decreased to 0.05 µg g⁻¹ after 34 hours. However, in case of 7.5 g a.i. ha⁻¹ deltamethrin treatment, residues to the tune of 0.30 and 0.16 µg g⁻¹ were recovered after 12 and 86 hours of application, respectively. Gayger and Dustmann (1985) reported very low level of γ-HCH residue (0.001-0.07 mg kg⁻¹) in the pollen samples.

Spittler *et al.* (1986) analyzed the 70 pollen samples associated with honey bee mortality and reported that fifty samples were found to contain more than 0.1 mg kg⁻¹ methyl parathion and fifteen contained more than 0.1 mg kg⁻¹ carbaryl, whereas, five samples contained the residues of both the insecticides, out of those only two samples had azinphos-methyl residues. The residue levels of cypermethrin (44 g ai ha⁻¹) and PP 321 (5 g ai ha⁻¹) in *B. campestris* pollen on the day of treatment were 1.9 µg g⁻¹ and 0.2 µg g⁻¹, respectively (Fries and Wibran, 1987).

Ruijter and Steen (1987) observed that forager bees brought contaminated pollen back to the hive due to that poisoning symptoms appeared in larvae. Affected larvae died

in pupal stage with in five days, all brood developed to second half of the larval phase died, adult bees those appeared after 10 days of spraying were malformed. Methyl parathion residues were reported in pollen of sunflower after two sprays (Wilson *et al.*, 1988a).

Wilson *et al.* (1988b) applied two aerial applications of parathion on flowering sunflower (*H. annuus*) and observed that pollens contained parathion residues upto 3.7 mg kg⁻¹, with an average of 0.5 and 0.9 mg kg⁻¹ after 7 and 10 days after treatment, respectively. Residues of parathion were several times greater on leaves than on the floral heads due to difference in the tissue surface area to mass ratio.

When fluvalinate (144 g ai ha⁻¹) was applied to flowering apple trees, residues to the tune of 0.26 and 0.13 µg g⁻¹ were recorded 1 and 6 days after spray in pollen, respectively (Haouar *et al.*, 1990). Fungicides viz. dichlofluanid and procymidone too contaminate the pollen of strawberry (Kubik *et al.*, 1992).

Malathion residues in canola pollens were recovered after 12 hours of application as ULV (Pankiw and Jay, 1992). Deltamethrin application (12.5 g a.i. ha⁻¹) had residues to the tune of 0.047 to 0.605 mg kg⁻¹ in anthers (Tasei *et al.*, 1994). The presence of fenoxycarb residues in pollen were documented by Uoglauh and Neumann (1994) using the *Aedes* larvae and flour beetle as test organism. It was also reported that flour beetle was more suitable test insect. Residues of organochlorines (DDT + DDE + DDD and HCH) amounted to 0.76, 0.34 and 0.19 µg kg⁻¹ in bees, pollen and honey, respectively (Wilde *et al.*, 1995).

Kubik *et al.* (2000) analyzed the pesticide residues of Captan, a contact and difenoconazole, a systemic fungicides applied on flowering apple trees in bee products and reported that contamination of pollen was higher (0.043 and 2.99 mg kg⁻¹ of

difenoconazole and captan, respectively) compared to honey. In pollen 0.0072-0.0130 $\mu\text{g g}^{-1}$ HCH was reported by Witkiewicz *et al.* (2000).

Tasei *et al.* (2000), Cure *et al.* (2001) and Schmuck *et al.* (2001) reported that residues of imidacloprid in honey or pollen collected by honey bees from treated sunflower did not exceed 10 $\mu\text{g kg}^{-1}$. Whereas, Maus *et al.* (2003) found imidacloprid residues below 5 $\mu\text{g kg}^{-1}$ in the pollens of maize, rape and sunflower. Gregore and Bozi (2004) reported the residues of imidacloprid to the tune of 3.9 $\mu\text{g kg}^{-1}$ in the pollens of sunflower. Highest residue levels of clothianidin in field studies on pollen were observed to be 6 $\mu\text{g kg}^{-1}$, which was safe for *Bombus impatiens* (Franklin *et al.*, 2004).

2.3.3 Honey

Gruch (1957) reported that after continuous use of inorganic insecticides for 75 years, arsenical pesticide residues to the tune of traces to 0.2 mg kg^{-1} were present in few samples of honey. Pourtellier and Telliew (1967) reported that French honey was contaminated with endosulfan and toxaphene residues.

Woodwell *et al.* (1971) also reported contamination of honey with pesticide residues. Barragan and Maria (1973) reported a very alarming situation in certain samples of Colombian honey, in which chlordane and dieldrin residues were detected. Ogota and Bevenue (1973) analyzed 291 honey samples and reported that chlorinated hydrocarbons were the major contaminant followed by organophosphorus group of pesticides. Maximum residues to the tune of 215 $\mu\text{g kg}^{-1}$ and 358 $\mu\text{g kg}^{-1}$ of DDT and its analogues and HCH, respectively were recorded.

Morton *et al.* (1974) observed that honey from colonies using treated water contained residues upto 50 mg kg^{-1} of 2,4,5- T. The concentration dropped to about 5 mg kg^{-1} within a week after the bees began using untreated water. Sundaram (1974)

determined fenitrothion residues in honey by gas liquid chromatography having flame photometric detector.

Grandi (1975) reported Italian honey to be contaminated with DDT, HCH and parathion residues ($0.1-4.2 \mu\text{g kg}^{-1}$). Dzilinski and Szymymrowska (1975) reported honey samples contamination with gamma- HCH residues. Similarly, Stranger and Winterlin (1975) observed that all the 56 samples collected from the state (Warrow) farms and other apiaries had residues to the tune of 0.01mg kg^{-1} of DDT and its analogues. Campas and Rampel (1976) reported that honey samples contained alpha- hexachlorocyclohexane, heptachlor and lindane residues (0.054 and 0.132 mg kg^{-1}). Residues of aldrin, DDT, DDD, dieldrin and lindane ranging from traces to $40 \mu\text{g kg}^{-1}$ were reported from Italy (Sabatini and Savigini, 1976). In contrary to this, samples from Italy were found free from any residues of organochlorine insecticides (Cerruti and Mannino, 1977; Sabatini and Savigini, 1977). Estep *et al.* (1977) reported that 56 per cent honey samples were contaminated with chlorinated hydrocarbon insecticides ($0.01-0.38 \mu\text{g kg}^{-1}$). Renvall (1977) reported Sweedish honey samples free from pesticide residues.

Cerruti and Mannino (1979) found fourteen samples of Italian and imported honey free from organochlorine pesticides. Emmett and Archer (1980) found small quantities (0.11 and $0.39 \mu\text{g g}^{-1}$) of diflubenzuron in samples of honey from hives placed in apple plots treated with this insecticide. Ruttner *et al.* (1980) reported that honey from K-79 treated honey bee colonies had residues (traces – 0.01mg kg^{-1}). Ritter (1980) reported kelthane residues to the tune of $0.2-0.7 \text{ mg kg}^{-1}$ in the honey samples collected from three times treated honey bee colonies.

Bentler and Frese (1981) analyzed 83 honey samples and reported that most of the samples were contaminated with organochlorine pesticide residues up to 1 ppb. Malinin *et*

al. (1981) analyzed honey samples for any probable residues through GC. It was also observed that residues to the tune of 0.07-0.52 mg kg⁻¹ were recovered in extracted and comb honey after 3-8 applications of pesticide, which reduced to the level of 0.028-0.035 mg kg⁻¹ after 6-7 months of storage after collection. It was also concluded that degradation of residues was independent of temperature. Tsvetkova *et al.* (1981) reported that besides traces of organophosphorus pesticides residues, organochlorine pesticide residues to the tune of 0.0002- 0.0060 mg kg⁻¹ were also recovered in Bulgarian honey.

Beck (1983) concluded that local Danish honeys present no risk to consumers but imported honeys had organophosphorus pesticides to the levels above 0.005 mg kg⁻¹. However, organochlorine pesticide residues posed the greatest risk. Each of twelve honey samples collected and analyzed by Grasso and Capei (1983) from different parts of north Italy contained p'-p' DDT (1.02- 39.47 µg kg⁻¹) and heptachlor epoxide (traces to 19.22 µg kg⁻¹). Heptachlor (0.34- 9.13 µg kg⁻¹) was present in 11, α- endosulfan (traces- 42.64 µg kg⁻¹) in 11, β- endosulfan (traces- 7.87 µg kg⁻¹) in 11, lindane (traces- 35.81 µg kg⁻¹) in 10 and o-p'- DDT (traces- 6.42 µg kg⁻¹) in 10 honey samples.

Liakos (1983) reported that treatment of beehive with malathion (0.05-5 g) at five days interval resulted in 0.003-0.008 mg kg⁻¹ and 0.009-0.054 mg kg⁻¹ residues in honey extracted by centrifugal and squeeze method, respectively. Twin *et al.* (1984) reported that low residue levels (0.-0.2 mg kg⁻¹) of both iprodione and phosalone were found in fresh honey samples two days after spraying the blooming winter oilseed rape. Subsequently, no residues were recovered in matured honey. Gayger and Dustmann (1985) found very small amounts of residues of chlorinated hydrocarbon (γ- HCH, 0.001- 0.004 mg kg⁻¹) insecticides in 21 German and foreign honey samples. Thrasyvoulou *et al.* (1985) reported that 54 honey samples out of total 61 collected from various parts of

Greece were found free from any type of pesticide contamination. Rest of samples contained ($1-5 \mu\text{g kg}^{-1}$) malathion residues.

Moilenen *et al.* (1986) reported that during 1978-84 no organochlorine pesticide was recovered from honey samples collected from Finland except PCB ($0.1-0.2 \text{ mg kg}^{-1}$). However, the levels were below the previously reported levels in honey that caused poisoning ($30-90$ and $70-170 \text{ mg kg}^{-1}$, respectively). Rexillius (1986) reported that chemicals used to control oilseed rape pests, contaminated the honey.

Twenty four samples from different apiaries in the Punjab were collected in 1980 and were analyzed for insecticidal residues. The results revealed that all the samples of honey were contaminated with both DDT and HCH at levels ranging from traces to $175 \mu\text{g kg}^{-1}$ and $5-85 \mu\text{g kg}^{-1}$, respectively (Chawla and Goyal, 1988).

Al- Rifai and Akeel (1997) reported the residues of 50 pesticides belonging to organochlorine, organophosphorus, pyrethroid and nitrogen containing compounds in honey samples collected from Jordan. The residues of amitraz, bromopropylate and tetradifon were not detected in any of the sample. Rathi *et al.* (1997) analyzed 27 samples of honey collected from Haryana, India. It was reported that all samples were contaminated with synthetic pesticide residues whereas, per cent honey contamination with residues of organophosphorus pesticides was highest.

Honey samples from Hoogly, West Bengal (India) were found to contaminate with α and β endosulfan to the tune of $0.001-0.100 \text{ mg kg}^{-1}$, whereas, γ - HCH was found in few samples (Karak *et al.*, 1999). Bogdanov *et al.* (2004) reported that 30 per cent of Swiss and 7 per cent of imported honey samples contained para- dichlorobenzene residues. Portuguese honeys were more contaminated with synthetic pesticide residues compared to

Spanish honeys. Most of the contaminants were found to be organochlorine pesticides Blasco *et al.* (2004).

Khan *et al.* (2004) reported that honeys were contaminated with all the major pesticides used to protect the crops. The most common contaminants were aldrin, BHC, endosulfan, HCH and quinalphos.

Besides insecticides, used to manage crop pests, honey samples were also found to contain the residues of acaricides *viz.* amitraz, bromopropylate, coumaphos, formic acid, fluvalinate, menthol, para dichoro benzene (Borneck and Merle, 1990; Al- Rifai and Akeel, 1997); fungicides difenoconazole (Kubik *et al.*, 2000) and antibiotics (Bogdanov, 2003).

2.4 RESIDUE ESTIMATION

2.4.1 Extraction and Clean Up of Pesticides in Nectar, Pollen and Honey

The methods and solvents used for extraction and clean up of insecticides in foraged pollen and nectar by honey bee (*A. mellifera*) on mustard crop are rare but an attempt has been made to review the literature for stored honey and pollen from colonies.

Stranger and Winterlin (1975) reported that due to the solubility of monocrotophos in water the extraction and clean up steps were combined with liquid partition. Hexane phase was used for the lipids present in pollens, whereas, honey samples were shaken with water and hexane in a reagent bottle. The hexane was aspirated off and aqueous phase was quantitatively transferred to separating funnel. The monocrotophos was extracted three times with chloroform along with two hexane washings to remove oils and waxes from the pollen.

To determinate the carbaryl residues in honey bees Belliardo *et al.* (1977) blended the sample with chloroform for 10 minutes, then filtering through anhydrous sodium

sulphate. Extracts were evaporated to dryness and redissolved in mixture of acetone, toluene and hexane (1:1:5). Lopper and Ross (1982) extracted the samples of hand collected pollens exposed to fumes of methyl parathion with acetone: xylene (19:1) mixture and then with hexane in a high speed blender. The extracts were filtered and concentrated at 40°C, transferred to volumetric flask and diluted with xylene.

Thrasyvoulou and Pappas (1988) reported that for the estimation of malathion and coumaphos in honey and wax, the samples were diluted with 4 per cent aqueous sodium chloride and 0.1 per cent triton x-100 and extracted three times with acetone: petroleum ether (1:1 v/v) in a blender for 5 minutes. The extracts were combined and filtered over anhydrous sodium sulphate. The samples were concentrated by placing them in water bath at 40°C under steam of dry air. Partitioning was done with hexane: acetonitrile (1:1 v/v). A novel clean up technique for the organochlorine was developed by Pederson and Higgins (1988). Sample was extracted with hexane and applied to a pipette containing 0.35 g activated florisil topped with layer of anhydrous sodium sulphate. The sample was allowed to filter slowly through the column, which was washed with hexane: butyl methyl ketone (47:3).

Atienz *et al.* (1993) used supercritical fluid extraction of fluvalinate residues in honey with carbon dioxide. Driss *et al.* (1994) described a simplified method for extraction and analysis of organochlorine insecticide residues in honey. Samples of honey were dissolved in water and extracted with petroleum ether. The extract was purified on florisil micro column.

Garcia *et al.* (1995) extracted the honey samples with acetonitrile: water (2:1) mixture. Partitioning was done with hexane and florisil or Sep pack cartridges were used for the purification of samples. The samples were concentrated under nitrogen and

recovered with hexane. Yadav (1995) dissolved the honey samples with distilled water and methanol. Extract was transferred to a separating funnel with few milliliters of concentrated sulphuric acid. It was washed twice with distilled water. Fraction containing n-hexane was dried over a plug of anhydrous sodium sulphate and concentrated under vacuum. Clean up was made with silica gel column covered with layer of anhydrous sodium sulphate. Mixture of benzene and n- hexane was used as eluant. Al- Rifai and Akeel (1997) analysed organochlorine, organophosphorus and pyrethroid pesticides from honey according to the S-8 method of Beaker (1987). Honey samples were mixed with a mixture of acetone: water (1:1) and blended for three minutes on a mechanical blender. Extraction was done with 10 ml of dichloromethane, 3 ml of saturated NaCl solution and 50 ml of distilled water. The organic layer was filtered through anhydrous sodium sulphate. The extraction was repeated twice with 10 ml dichloromethane. After complete filtration the sodium sulphate layer was rinsed three times with 20 ml dichloromethane. The extract was combined and concentrated over rotary vacuum evaporator at 38⁰C. This extract was cleaned by passing it through the glass column consisted of 0.5 g silica gel, 5 g mixture of activated carbon and silica gel (1: 15) and 1 g of anhydrous sodium sulphate. The residues of pesticides were eluted from the column with 250 ml mixture of dichloromethane, toluene and acetone (100:2:2). The eluant was collected and evaporated to dryness and recovered in hexane for GC analysis.

Karak *et al.* (1999) analyzed the residues of pesticides belonging to different groups from honey through multiresidue technique. Organochlorine pesticides were extracted by mixing honey samples with mixture of methanol: water (1:1) and partitioned three times in a separatory funnel by n- hexane. The organic layer was collected over anhydrous sodium sulphate and concentrated in rotary vacuum evaporator. Clean up was

done over a glass column filled with florisil silica gel mixture (1:1) saturated with hexane. Sample was eluted with hexane. Whereas, organophosphorus were extracted similarly, but the chloroform was used for liquid- liquid partitioning. Clean up was done on florisil packed column saturated with hexane and eluted with ethyl acetate. The emulsion was broken down by centrifuging the extract for 5 minutes at 5000 rpm. The extract was dried over the layer of anhydrous sodium sulphate and concentrated to dryness. The residues were recovered in hexane.

Lodevico and Li (2002) determined imidacloprid residues in coffee by GCMS (gas chromatography-mass spectrometry). Coffee beans were extracted with a mixture of methanol and 1 per cent H_2SO_4 (1:3, v:v). The cleanup procedure involved hexane partitioning and XAD-4 resin cleanup. Elution was done with methanol. Schoning and Schmuck (2003) analyzed the samples of pollen, flowers, leaves, nectar, honey, wax and bees for imidacloprid and its metabolites. The extraction was made in methanol: water. The extracts were cleaned on a column filled with diatomaceous earth followed by subsequent solid phase extraction on a silica gel column for the determination on LCMS. Khan *et al.* (2004) extracted the honey samples with ethyl acetate 40, 30 and 30 ml after dilution with 100 ml of 4 per cent aqueous solution of sodium sulphate. The extract was centrifuged at 3000 rpm for five minutes. The organic phase was filtered through a 5 cm layer of anhydrous sodium sulphate and concentrated *in vacuo*. Clean up step using column chromatography was not performed.

A HPLC technique was proposed by HongXia *et al.* (2005) for the analysis of imidacloprid residues in baked tobacco leaves. Imidacloprid was extracted with ethyl acetate and clean up was done by liquid liquid partition with 50 g L^{-1} aqueous sodium chloride followed by dichloromethane.

2.4.2 METHODS OF RESIDUE ESTIMATION

2.4.2.1 Bioassay

The available literature on the determination of microquantities of insecticide's deposits/ residues by bioassay was reviewed and used by various workers (Gunther and Blinn, 1956; Sun, 1957; Hoskins and Craig, 1962; Lal and Hameed, 1971; Attri, 1977; Thakur, 1978; Kashyap, 1979; Jacob and Verma, 1984). The methods used in bioassay have been grouped in to two broad categories:

- i) exposing test organism to insecticidal contaminated media directly and
- ii) exposing test organisms to residual extracts

Various techniques were suggested for exposing test organisms to residual extracts viz. dry film, aqueous suspension method, photomigration method, feeding method and topical application method. Out of these, dry film technique is the most commonly used method in which the insecticidal film is made on the filter paper/ glass plate/ plywood. Insecticidal films were made by pouring and drying 1 ml of insecticidal solution in petridishes (7.0 x 1.5 cm) and used against *Calendra granaria* L. as described by Krisjman and Berger (1949). This dry film technique of bioassay has been used for the estimation of insecticidal residues by various workers (Singh and Lal, 1966; Kavadia and Lal, 1967; Duhra and Hameed, 1990; Kashyap, 1979; Sharma, 1993; Sharma and Singh, 1993; Vir, 1996).

Usman (1963) used larvae of *Aedes aegypti* (L.) to study the residues and toxicity of some insecticides by bioassay method. Singh and Lal (1966) estimated malathion residues by bioassay using *Bracon brevicornis* Wesm. adults.

A significant correlation between the bioassay and chemical assay was developed and reported by Kavadia and Lal (1967) who used *Drosophila melanogaster* Meig. as test

insect. Lal and Hameed (1971) conducted bioassay studies by using *Bagrada cruciferarum* Kirkaldy as test insect. *D. melanogaster* was used as test insect for the estimation of insecticide residue by several workers (Srivastava and Nath, 1983; Jacob and Verma, 1984; Duhra and Hameed, 1990; Sharma and Singh, 1993). Uoglah and Neumann (1994) reported the bioassay method for the presence of fenoxycarb residues in pollen. It was documented that the use of *Aedes* sp. larva as a test organism did not give the satisfactory results, whereas, use of flour beetle, *Tenebrio molitor* gave the desired results.

2.4.2.2 Chromatographic Methods

Zweig and Sherma (1972) had given a general method for determination of endosulfan on GC fitted with ECD (6 feet x ¼ inch o.d.) aluminium tubing column. Thidemann (1979) recommended the use of Zeiss ERI 65-m spectrometer and integrator for residues estimation in honey by TLC (thin layer chromatography).

Loper and Ross (1982) analyzed the pollens of different plants through GC for the presence of methyl parathion residues and reported that concentration of different pesticides in pollens belonging to different plant origin depend upon the lipid content. Spittler *et al.* (1986) worked out the residues in dead bees and pollen through multi residue analysis scheme. HPLC determination of carbaryl on a reversed- phase column with detection by UV and fluorescence was also described. The residues of endosulfan were determined by various workers using GLC equipped with ECD, from vegetables and other commodities (Duhra and Hameed, 1990; Naik *et al.*, 1993; Vir, 1996; Gupta *et al.*, 1998).

Driss *et al.* (1994) described a GC method for analysis of organochlorine insecticides in which the recoveries of the pesticides (DDE, DDT, dieldrin, eldrin, HCH,

heptachlor epoxide and lindane) were more than 90 per cent. Al- Rifai and Akeel (1997) analyzed organochlorine pesticides on GC, Hewlett Packard model 5890 series II system, equipped with splitless inlet, an ECD and ultra 2 column (25 x 0.5 mm id x 0.1 μ m film thickness). Other GLC parameters kept were: carrier gas helium (4.8 psi, 1.2 min. purge time, 4 ml min⁻¹), make up gas argon: methane (95: 5) with total flow 57 ml min⁻¹. Oven temperature (80 to 235⁰C), injector temperature (270 ⁰C) and detector temperature (280 ⁰C). The determination of organophosphorus pesticides was also done on the same equipment using NPD with BP5X column (25m x 0.32 mm x 0.5 μ m). Other GLC parameters were carrier gas (nitrogen) flow rate 1.3 ml min⁻¹, make up gas (nitrogen) flow rate 30 ml min⁻¹, H₂ 3.3 ml min⁻¹ and air 110 ml min⁻¹. Oven temperature was programmed to vary from 70 to 268⁰C, injector temperature 250⁰C and detector temperature 280⁰C. The average recoveries following this method were reported to be 87-105 per cent for organochlorine, 79-85 per cent for pyrethroids and 75-90 per cent for organophosphorus pesticides.

Rathi *et al.* (1997) and Khan *et al.* (2004) analyzed residues of organochlorine and organophosphorus on GLC (Hewlett Packard 5890 A) using multiresidue technique. ECD Ni ⁶³ detector was used for the analysis of organochlorines with SPB- 5 column (30 m x 0.342 mm id x 0.25 μ m film thickness). Column temperature was programmed to vary from 150 to 280⁰C, injection port and detector temperatures were 280 and 300⁰C. Nitrogen gas flow rate was 60 ml min⁻¹. The GLC analysis for organophosphorus was done on NPD. The following parameters were employed: a methyl silicon mega bore column, 10m x 0.53 mm id x 2.65 μ m film thickness, column temperature was programmed to vary from 100 to 260⁰C. Temperature of injection port was 250⁰C, whereas detector temperature was 275⁰C. Flow rates of different gases were maintained as 18 ml min⁻¹ for

N₂, 1.5 ml min.⁻¹ for H₂ and 135 ml min.⁻¹ for zero gas. It was reported that limit of detection for organochlorines was 0.005- 0.01 µg kg⁻¹ and for organophosphorus it was 15-50 µg kg⁻¹.

Karak *et al.* (1999) carried out pesticide residue analysis in honey through GLC (Hewlett Packard 5890A) equipped with Ni ⁶³ (ECD) and coupled with 3392 A integrator for the analysis of organochlorine and synthetic pyrethroid pesticides. For the determination of organophosphorus pesticides GLC (Hewlett Packard 5890 Series-II) was used with FPD. The column used was DB-5 (megabore, J&W Scientific USA) with id 0.53 mm, length 30 mm and film thickness of 1.5µm. For organochlorine pesticides, oven, injector and detector temperatures were 200, 200 and 300⁰C, respectively with the flow rate of 30 ml min.⁻¹ of the carrier gas. For organophosphorus pesticides, oven, injector and detector temperatures were 220, 200 and 280⁰C. The flow rates of nitrogen gas, hydrogen gas and airflow were 30 ml min.⁻¹, 1.5 ml min.⁻¹ and 3 ml min.⁻¹, respectively. The recoveries of all the pesticides were found to vary from 81-90 per cent.

Tsipi *et al.* (1999), developed multiresidue method for the determination of organochlorine residues in honey. The determination of residues was performed by capillary GC equipped with ECD. The detection limits were found to be between 0.05 and 0.20 µg kg⁻¹. Antonescu and Mateescu (2001) analyzed chlorinated pesticide residues in honey by GC. Lodevico and Li (2002) determined imidacloprid residues in coffee beans by GCMS. Bogdanov (2003), enlisted recent methods used to detect pesticide residue in honey and concluded that both GLC and HPLC methods were effective in pesticide assay. However, GC methods with MS was considered most promising.

Two approaches based on sorptive extraction, solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE), in combination with LC- atmospheric pressure

chemical ionization mass spectrometry (MS) were assayed for analyzing six organophosphorus insecticides in honey. SBSE showed higher concentration capability and greater accuracy (between 5 and 20 times) and sensitivity (between 10 and 50 times) than SPME (Blasco *et al.*, 2004).

HongXia *et al.* (2005) determined imidacloprid residues in tobacco leaves by HPLC. The separation was performed on a Supelco LC-18 column (250 mm x 4.6 mm ID) with a mobile phase of acetonitrile+5 mM ammonium acetate (20+80 by volume) at a flow rate of 1 ml min⁻¹. The baseline separation between imidacloprid and the tobacco blank was achieved within 10 min. Detection wavelength was 270 nm. The limit of quantification was 0.04 mg kg⁻¹ and the recovery ranged from 89.8 to 95.4 per cent.

2.5 HALF LIFE AND WAITING PERIODS

2.5.1 Endosulfan

Wills and Mc Dowell (1987) reported 2.6 and 4.5 days half life for endosulfan in lettuce and turnip leaves, respectively. Greighsmith (1994) reported a field half life of 50 days.

2.5.2 Imidacloprid

Yang *et al.* (1999) reported that fifty per cent imidacloprid residues degraded with in 3-4 days when applied on tobacco leaves. Imidacloprid residues dissipated exponentially with time following foliar application on okra with a half life of 2-4 days (Indumathi *et al.*, 2001). Imidacloprid was applied as seed treatment (Gaucho 70 WS[®] 5 and 10 g a.i. kg⁻¹ seed) and foliar spray (Confidor[®] 200 SL[®] 20 and 40 g a.i. ha⁻¹) on *Brassica*, the half lives of 14.40 and 13.07 and 4.09 and 3.86 days were recorded, respectively. It was also concluded that the dissipation was independent of initial doses and followed a first order rate kinetics (Kumar and Dikshit, 2001).

Gopal *et al.* (2002) studied the persistence of imidacloprid (20 and 40 g a.i. ha⁻¹) in Indian mustard (cv. Pusa Bold) leaves and seeds. A half life of 4.2-5.0 days was proposed. Half life of 40.96 days for imidacloprid was proposed in soil after seed treatment of groundnut by Singh and Singh (2004). Suchail *et al.* (2004) reported that imidacloprid, which was rapidly metabolized into 5-hydroxyimidacloprid and olefin in *A. mellifera*, had a half life ranging between 4.5 and 5 hours.

2.5.3 Lambda cyhalothrin

Hill and Inaba (1991) reported that cyhalothrin residues dissipated biphasically with DT₅₀ of 1.3 weeks and a DT₉₀ of 14.5 weeks. Two different models were given for describing the dissipation of cyhalothrin residues in apple. According to these models, cyhalothrin residues reduced to half of their initial concentration after 10 days of treatment (Bostanian *et al.*, 1993). The residue half lives (RL₅₀) of lambda cyhalothrin in cauliflower were 2.0-2.2 days for the 2.5 EC formulation and 2.2-2.4 days for the 5.0 EC formulations (Sharma and Awasthi, 2002).

2.5.4 Spiromesifen

Spiromesifen had half life of 25 days in neutral conditions which shorten to 4 days in alkaline conditions. It was reported to be comparatively stable in acidic conditions (half life 53 days). In soil, initial deposits were found to dissipate to half in 2-6 days (Anonymous, 2005).

2.6 PHYSICO- CHEMICAL PROPERTIES OF HONEY AND PESTICIDES RESIDUE'S EFFECT

The Bureau of Indian Standards outlined the following quality characteristics for the grading of honey as Special, A or standard class vide its publication no. IS 4941: 1974 (Anonymous, 1974).

Table 2.1 Quality standards proposed by Bureau of Indian Standards for honey

Characteristics	Grade		
	Special	A	Standard
Specific gravity at 27 ⁰ C, min.	1.41	1.39	1.37
Moisture per cent by mass, max.	20.0	22.00	25.00
Total reducing sugars, per cent by mass, min.	70.00	65.00	65.00
Sucrose, per cent by mass, max.	5.00	5.00	5.00
Fructose: glucose ratio, per cent by mass, min.	1.00	1.00	1.00
Ash, per cent by mass, max.	0.50	0.50	0.50
Acidity expressed as formic acid, per cent by mass, max.	0.20	0.20	0.20
Fiehe's Test	-ve	-ve	-ve

The average composition of fresh honey from Himachal Pradesh were found to contain 81.50, 80.70, 68.33, 12.37, 32.43, 35.90, 4.1, 0.35 and 0.26 per cent total soluble solids (TSS), total sugars, total reducing sugars (TRS), non reducing sugars, glucose, fructose, ph, optical density (O.D.) and total colloidal content, respectively by Kaushik (1988).

The average optical density (O.D.), viscosity, moisture, refractive index (RI), density, specific gravity and pollen density of honey ~~were~~ found to be 0.138, 9.020 minutes, 16.92 per cent, 1.495, 1.624 gcm⁻³, 1.397 and 60210 pollen grains per 10g of honey, respectively in the *A. mellifera* honeys. The average chemical properties; total soluble solids (TSS), total reducing sugars (TRS), sucrose, fructose, glucose, fructose: glucose ratio, ash content and acidity as formic acid ~~were~~ found to be 83.08, 63.50, 5.75, 31.70, 33.10, 1.087, 0.241 and 0.114 per cent, respectively for *A. mellifera* honeys (Yadav, 1995). Relevant literature pertaining to the effects of pesticide residues on physico- chemical properties could not be traced.

MATERIALS AND METHODS

MATERIALS AND METHODS

Field trials for the present investigation, “Toxicity of some new pesticides to honey bees and their residues in honey and pollen”, were laid out during *rabi* seasons of 2003-04 and 2004-05 at the Experimental Farm of Department of Entomology, CSK HPKV, Palampur, located at 32.8⁰N latitude, 76.3⁰E longitude and at an elevation of 1260.80 meters a.m.s.l.

The laboratory experiments were conducted in the Toxicology Laboratory of Department of Entomology, CSK HPKV, Palampur. The details of materials and methods used during the course of present investigations are given as under:

3.1 MATERIALS

3.1.1 Biological

3.1.1.1 Honey bees

Colonies of *Apis cerana cerana* Fab. (Indian honey bee) and *Apis mellifera* L. (Italian honey bee) were maintained at the Department of Entomology apiary, following recommended package of practices. The forager bees were used for conducting the relative toxicity tests because both either too younger or too older bees showed variability in the results (Smirle *et al.*, 1984).

3.1.1.2 Culture of Vinegar Fly

Vinegar fly (*Drosophila melanogaster* Meig.) was used for bioassay of pesticides because of its sensitivity to most of the pesticides (Kavadia and Lal, 1967; Jacob and Verma, 1984). The artificial diet for raising the culture of flies was prepared as per the method given by Singh (1984). For handling and sexing, the adult flies were taken in a glass tube through a funnel and to make them docile, they were anaesthetized with the

help of chilling treatment. Males were separated by the presence of blackish spot on the abdomen. The one day old male flies were used for assaying the deposits/ residues of the pesticides.

3.1.1.3 Mustard Crop

Mustard crop (*Brassica juncea* (L.) Czern.) cv. *Varuna* was selected for conducting the experiments to estimate the persistence toxicity and residues in nectar and pollen. Crop was raised as per Package of Practices for *rabi* crops during the two years 2003-04 and 2004-05 at Experimental Farms of Department of Entomology, CSKHPKV, Palampur following randomized block design. No pesticides except the proposed treatments were applied.

3.1.2 Chemicals

3.1.2.1 Pesticides

Details of the formulations, technical grade purity and source of availability of pesticides used are presented in Table 3.1.

3.1.2.2 Solvents

Acetic acid, acetone (A.R.), acetonitrile (HPLC grade), dichloromethane (HPLC grade), ethyl acetate, n- hexane (A.R.) and toluene: ethyl acetate (85: 15) were used for extracting the residues from pollen, nectar and honey. All the solvents used were of analytic grade even though they were redistilled prior to use. Whereas, for the analysis of physico- chemical properties of honey, acetic acid, concentrated sulphuric acid, copper sulphate, diethyl ether, hydrochloric acid (specific gravity 1.18 at 20⁰C), methylene blue indicator, potassium ferrocynite, potassium iodide, potassium sodium tartrate, resublimed iodine, resublimed resorcinol, sodium acetate, sodium chloride, sodium bisulphate,

Table 3.1 Details of pesticides used in the present investigation

Sr. No.	Common name	Commercial formulation	Technical grade (% purity)	Chemical name (IUPAC / CAS)	Source of supply
a. Synthetic pesticides					
1.	Cypermethrin	Bilcyp 10EC	94.0	Cyano (3-phenoxyphenyl) methyl 3(2,2 – dimethyl cyclopropane carboxylate	Bayer Crop Science (India) Ltd., New Delhi, India
2.	Endosulfan	Thiodan 35EC	92.0	6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3oxide	Bayer Crop Science (India) Ltd., New Delhi, India
3.	Imidacloprid	Gaucha 70WS	95.0	1-(6- chloro-3-pyridylmethyl)- N-nitroimidazolidin-2- ylideneamine	Bayer Crop Science (India) Ltd., New Delhi, India
4.	Lambda cyhalothrin	Karate 5EC	95.0	(*) ¹ -α-cyano-3-phenoxybenzyl-(Z)- (*) ² -3-(2-chloro-3,3,3-trifluoropropenyl)-2,2-dimethylcyclopropanecarboxylate	Syngenta , India Limited
5.	Spiromesifen	Oberon 240SC	98.9	Butanoic acid, 3,3-dimethyl,2-oxo-3-(2,4,6-trimethyl phenyl)-1-oxaspiro(4,4)non-3-en-4yl ester	Bayer Crop Science (India) Ltd., New Delhi, India
6.	Thiacloprid	Calypso 240SC	---	N- (3 – (6- chloro – 3pyridin- 3 – ylmethyl) – thiazolidin - 2 – ylidene) - cyanamide	Bayer Crop Science (India) Ltd., New Delhi, India
b. Chitin synthesis inhibitor					
7.	Benzoylphenyl urea	Novaluron 10EC	---	1-(3-chloro- 4 – (1,1,2 – trifluoro- 2 – trifluoromethoxy – ethoxy) phenyl) – 3 – (2, 6 – difluorobenzoyl) urea	Indofil Chemical Company, Mumbai, India
c. Biopesticides					
8.	Azadirachtin	Achook 1500 ppm	---	Azadirachtin	Godrej Agrovet Limited, Gujarat, India
9.	<i>Bacillus</i>	Dipel 8L	---	Delta endotoxin and spores	Cheminova India Ltd

<i>thuringiensis</i> var. <i>kurstaki</i>		(17600 IU mg ⁻¹ / 3.5 %)	Mumbai, India	
10.	Spinosad	Success 2.5SC	75.0	(2 ¹ ,3aR,5a ¹ ,5bS,9S,13S,14R,16aS,16b ¹)-2-(6-deoxy-2,3,4-tri-O-methyl-alpha-L-mannopyranosyloxy)-13-(4-dimethylamino-2,3,4,6-tetraeoxy-beta-D-erythropyranosyloxy)-9-ethyl-2,3,3a,5a,5b,6,7,9,10,11,12,13,14,15,16a,16b-hexadecahydro-14-methyl-1H-8-oxacyclododeca[b]as-iNDacene-7,15-dione

^{*1} S and ^{*2} 1R, 3R or ^{*1} R and ^{*2} 1S, 3S

¹ R for Spinosyn A and S for Spinosyn D in spinosad which is a mixture of Spinosyn A and Spinosyn D

sodium carbonate, sodium hydroxide, sodium thiosulphate, sucrose standard and zinc acetate were used.

3.1.2.3 Other Materials

One day old vinegar flies were collected in a plastic vial and were anaesthetized by keeping the vial in the deep freezer (-4⁰C) for one minute. Sodium bicarbonate was reacted with sulphuric acid for producing CO₂ which was used for anaesthetizing the honeybee foragers. Artificial feeding of honey bees was done with 70: 30 sucrose solution. Other materials are given in Table 3.2 and 3.3.

Table 3.2 Chemicals used for extraction and cleanup of residues in nectar and pollen

Pesticides	Chemicals used for extraction	Chemicals used for clean up
Endosulfan	Sodium sulphate (anhydrous) Acetone: n-hexane (9:1)	Magnesium oxide, Celite, Activated charcoal, n- hexane
Imidacloprid	Methanol: Water {3: 1}(HPLC grade)	Dichloromethane (HPLC grade), Acetonitrile (HPLC grade), Toluene: Ethyl acetate (85: 15), Silica gel cartridges, Acetic Acid
Lambda cyhalothrin	Sodium sulphate (anhydrous) Acetone: n- hexane (9:1)	Magnesium oxide, Celite, Activated charcoal, n- hexane
Spiromesifen	Sodium chloride Acetone: Distilled water (2:1) Ethyl acetate: cyclohexane (1:1)	Silica gel: celite: florisil: activated charcoal (4:4:1:1), Acetone, n- hexane: toluene (65: 35)

Table 3.3 Chemicals used for extraction and cleanup of residues in honey samples

Pesticides	Chemicals used for extraction	Chemicals used for clean up
Organochlorine, cyclodiene and synthetic pyrethroids	Distilled water Methanol (A.R.) n- hexane (A.R.) Anhydrous sodium sulphate	Florisil: silica gel mixture (1:1) n- hexane (A.R.)
Organophosphorus	Anhydrous sodium sulphate n- hexane (A.R.)	Florisil: silica gel mixture (1:1) n- hexane (A.R.)

3.1.3 Instruments Used

3.1.3.1 Gas Liquid Chromatograph (GLC)

The GLC (Auto GC), PERKIN ELMER, USA make, equipped with electron capture detector (ECD), nitrogen phosphorus detector (NPD), flexible capillary columns (BPX10, BPX5, BPX225) and autosampler was used. It was connected with terminal integrator model PE 1022 and printer model OKIDATA-320.

3.1.3.2 High Performance Liquid Chromatograph (HPLC)

The HPLC, Waters USA make equipped with dual absorbance detector, C₁₈ column and binary pump system was used.

3.1.3.3 Microapplicator

Arnold Type CV 65 Buckard, Power unit microapplicator was used for treating the bees with known amount of pesticide volumes.

3.1.3.4 Other Equipments

Abbey's refractometer, B.O.D. incubator, deep freezer, electronic balance (Model-Anamed), muffle furnace, oven, UV-VIS spectrophotometer, remi's centrifuge, rotary vacuum evaporator, solvent distillation set, stereo microscope with haemocytometer and water distillation set.

3.2 METHODS

The methods followed for conducting the present investigations are given below:

3.2.1 Cleaning of Glassware

Contaminated glassware was cleaned first with water followed by two hour dip in a concentrated solution of chromic acid. The acid was washed away with running tap water. The glassware were further cleaned with teepol (soap solution neutral pH) and again washed with running tap water. Thereafter, these were washed with distilled water. This glassware were then dried up by pouring some amount of acetone and kept at 60⁰C in oven for one hour before use.

3.2.2 Toxicity Tests of Pesticides to Honey bees

3.2.2.1 Honey bees Collection

The foragers of both the honey bee species (*A. c. cerana* and *A. mellifera*) were collected with the cone type muslin hand net with a diameter of 30 cm. Honey bees were put in the plastic jars and placed in BOD incubator maintained at $25\pm 2^{\circ}\text{C}$ and 50% RH till the pesticide treatment. Honey bees were anaesthetized in a conical 250 ml flask with CO_2 . After treatment, honey bees were fed with 30 per cent (w/v) sucrose water solution. Solution was put in 5 cm diameter petridishes and in order to avoid direct contact of bees with the solution, clean sticks were put in the dishes covered with 0.31 cm plastic wire mesh (Sharma, 1987).

3.2.2.2 Contact Toxicity

To evaluate the contact toxicity, various concentrations of pesticides were prepared using respective solvents. To avoid any change in concentrations, the containers of solutions were properly capped and kept in deep freezer maintained at -4°C . One microlitre ($1.0\ \mu\text{l}$) of the pesticide solution was applied on the dorsal part of the thorax of each honey bee with the help of microapplicator. A minimum of twenty bees were taken for each observation and each concentration was replicated thrice. A control treatment was also maintained using application of only $1.0\ \mu\text{l}$ of respective solvent on the thorax of the honeybees. The bees were fed on sucrose solution *ad libitum* in glass chimmenies and kept in BOD incubator maintained at $25\pm 2^{\circ}\text{C}$ and 50% RH. The mortality was observed after 24/ 48 hours of exposure. Moribund bees were considered as dead.

3.2.2.3 Stomach Toxicity

Estimation of stomach toxicity was done by following the Mass Feeding Method of Kumar (1989). After uniform acclimatization, the honey bees were starved for three

hours. The insecticidal solutions were prepared in 30 per cent sucrose - water solution. Five milliliter (5ml) of this pesticide- sucrose solution was soaked in cotton and kept in petridish which was then covered by a wire mesh to avoid direct body contact of the bees with pesticide and allow only the probosis to reach the solution. Pesticide – sucrose solution was given only for one hour. After locating the solution the bees readily started feeding on pesticide- sucrose solution.

In these tests a group of twenty bees per dose per replication (4-6 doses) were fed with sucrose solution, which contained known concentration of the technical/ formulated pesticide. Due to their trophallactic behaviour, individual bees share the food in these tests and so received comparable doses. After consumption of the pesticide containing sucrose solution, bees were provided with untreated 30 per cent (w/v) sucrose solution. In control treatment honeybees were fed only with sucrose solution. Bees were then kept in BOD incubator maintained at $25 \pm 2^{\circ}\text{C}$ and 50% RH. The mortality data was recorded after 24 / 48 hours.

3.2.2.3.1 Estimation of Total Amount of Sucrose Solution Ingested by the Honey bee Foragers in One Feeding Load

This was to determine the actual amount of pesticide entering the body of the insect. The bees were starved for three hours. Thereafter, the bees were fed on 30 per cent sucrose solution soaked in cotton placed in petridish and covered with wire mesh for one hour. Then the individual bee was held in between the thumb and the forefinger and the abdomen was pressed in order to ooze out the contents of the crop. As soon as the material oozed out, it was immediately sucked into a graduated microcapillary (Plate 3). From this, the volume of the pesticide: sucrose solution ingested by the honey bee was worked out and from this the quantity of pesticide taken up by bees was calculated which

was later on used for the determination of LD₅₀ / LD₉₀ values. It was found that foragers of *A. c. cerana* consumed 8.0 µl and *A. mellifera* consumed 12.0 µl of 30 per cent (w/v) sucrose water solution.

3.2.3 Field Experiments for the Determination of the Persistence Toxicity and Periodic Residues Level in Nectar and Pollen

3.2.3.1 Sprays

Mustard crop (cv. *Varuna*), raised as per section 3.1.1.3, was sprayed with recommended concentrations of the pesticide (Table 3.4) at fifty per cent flowering of mustard crop during *rabi* seasons of 2003-04 and 2004-05 in the Experimental Farm of Department of Entomology, CSK HPKV, Palampur (Plate 1&2) .

The spray applications were made on 8th February, 2004 and 10th February, 2005 during Ist and IInd seasons, respectively.

Location of the experiment	: Experimental Farm, Department of Entomology, CSK HPKV, Palampur
Treatments	: Pesticides at the given rates (as per table 3.4)
Spraying dates	: 8 th February, 2004 and 10 th February, 2005
Sampling intervals	: 1, 4, 8, 24, 48, 72, 120, 240 and 360 hours of spray.
Stage of crop at spraying	: 50 per cent flowering
Name of the sprayer	: Knap Sack Sprayer
Weather conditions	: Figures 1 & 2 and Appendices 1&2

3.2.3.2 Sampling

3.2.3.2.1 Mustard Flower Stalks

Field weathered toxicity of pesticides was evaluated against the foragers of *A. c. cerana* and *A. mellifera*. Fifteen tender floral stalks (approx. 15 cm) were cut with the

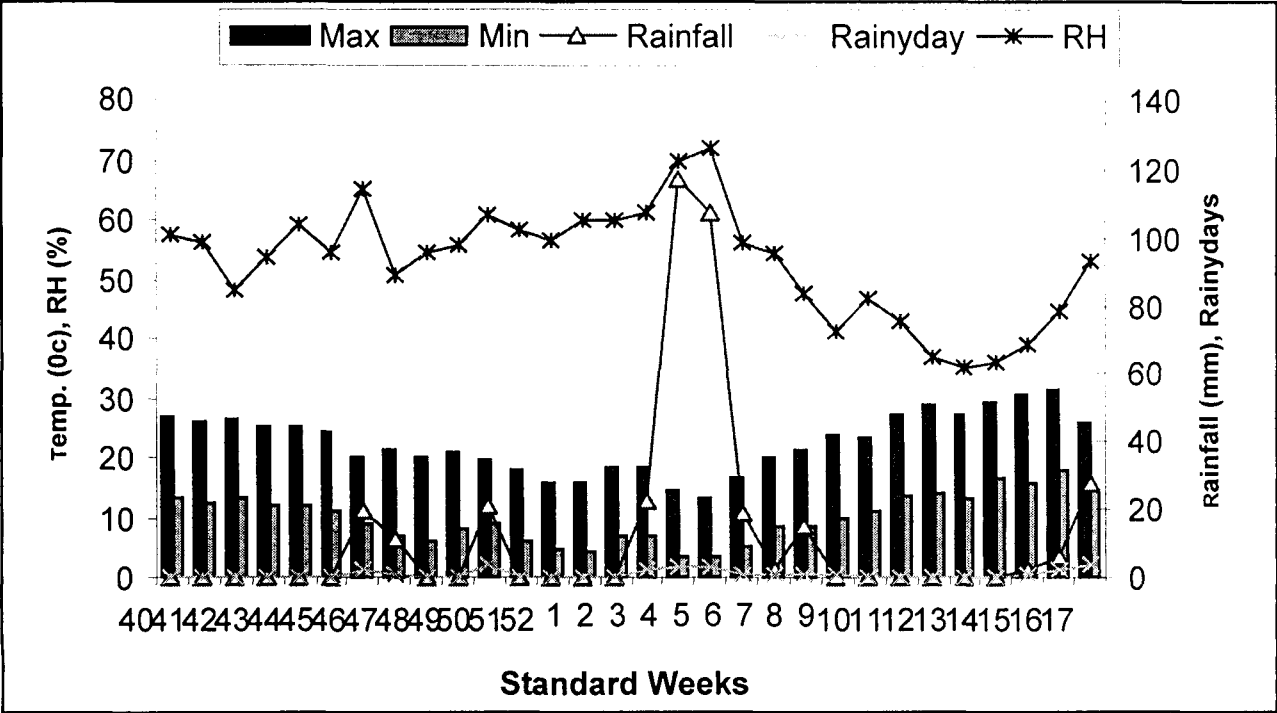


Fig. 3.1 Meteorological data during experimental period 2003- 04

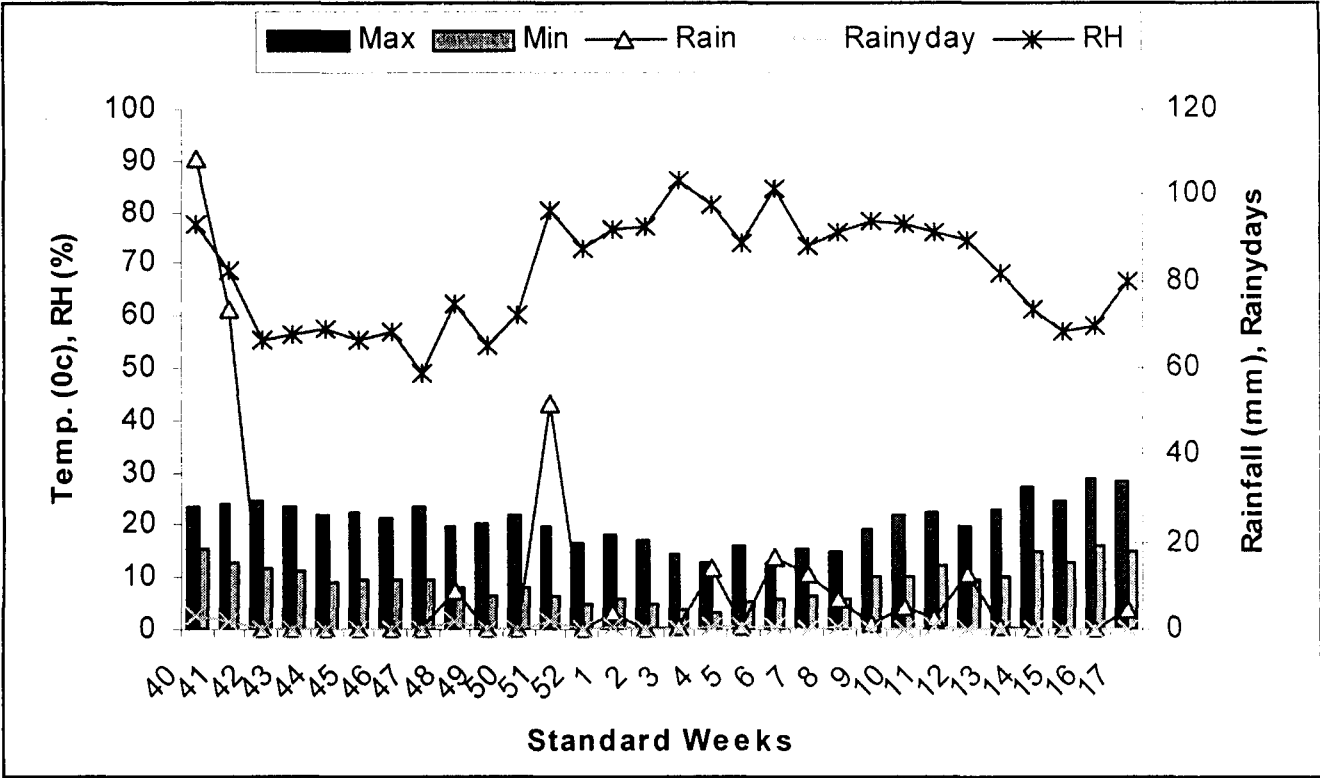


Fig. 3.2 Meteorological data during experimental period 2004-05

Table 3.4 Preparation of concentrations for field sprays

Sr. No.	Common name	Formulation	g a.i. ha ⁻¹	Conc. of the spray material (%)
T1	<i>B. thuriengiensis</i> var. <i>kurstaki</i>	Dipel 8L (3.5 % a.i.)	78.75	10.5 x10 ⁻²
T2	Cypermethrin	Ripcord 10EC	75.00	0.01
T3	Cypermethrin+ <i>B. thuriengiensis</i> var. <i>kurstaki</i>	Ripcord 10EC + Dipel 8L	37.5 + 39.37	0.005 + 5.25x10 ⁻²
T4	Endosulfan	Thiodan 35EC	525.00	0.07
T5	Imidacloprid (Foliar Treatment)	Confidor 17.8EC	25.00	0.003
T6	Imidacloprid (Seed Treatment)	Gaucha 70WS	21.00	5.00*
T7	Lambda cyhalothrin	Karate 5EC	75.00	0.01
T8	Lambda cyhalothrin+ Achook	Karate 5 EC + Achook 1500 ppm	37.5 + 1.687	0.005 + 0.15 [#]
T9	Spiromesifen	Oberon 240SC	225.00	0.03

* g a.i. kg⁻¹ seed; # on the basis of formulated product

help of a pair of scissors at different time intervals (0, 1, 4, 8, 24, 48, 72, 120, 240 and 360 hours after spray) from each replication without disturbing the deposits during handling.

These stalks were taken in polythene bags and kept in refrigerator.

Further, in order to maintain the freshness these stalks were transferred into the reagent bottles having standard solution of 10 per cent sucrose and 0.2 per cent mercuric chloride (HgCl₂). These stalks were then placed inside glass chimmenies and sixty anesthetized bees were released. Following this chimmenies were placed inside BOD incubator maintained at 25±2⁰C and 50% RH. The mortality counts were recorded after 24 hours of release. The bees were supplied with additional sucrose solution as dipped cotton wick after 8 hours of exposure to the pesticide treated surfaces. The moribund bees were considered dead when recording the mortality. The experiment was replicated thrice.



Plate 1



Plate 2

Plate 1 & 2 Field trials at Experiment Farm of Department of Entomology, CSK HPKV, Palampur during 2003-04 (Plate 1) and 2004-05 (Plate 2)



Plate 3 Nectar collection from the honey bee



Plate 4 Pollen collection from the honey bee

The mortality counts were converted to corrected per cent kill. The data were angular transformed for further analysis.

3.2.3.2.2 Nectar

Each *A. mellifera* forager was observed in the treated area. The foragers were collected with the insect collecting net and taken out of the net carefully, avoiding any pressure on its abdomen. The bees were caught carefully from the wings and a gentle pressure was applied on the abdomen upwards. The oozed out nectar around mouth parts was sucked up in lambda capillary tube and discharged into the vial (Plate 3). On an average a nectar load of 0.15 ± 0.01 mg per 20 bees was observed.

3.2.3.2.3 Pollen

The bees were collected as per section 3.2.3.2.2. The bees were caught carefully from the head and portion of the thorax in between two fingers. The pollens foraged were then removed with the help of a camel hair brush (No. 1) in vial (Plate 4). In each sample 20 foragers were taken. An average pollen load of 0.05 ± 0.002 mg per 20 bees was recorded.

Nectar and pollen samples were kept in marked and weighed vials. The nectar was then weighed along with vial and added solvent (4 ml). Similarly, samples were collected for other intervals. The samples were then stored in deep freezer at -4°C temperature till the samples were extracted, cleaned up and estimation of residues was done with bioassay and chromatographic techniques.

3.2.3.2.4 Honey

The honey samples (500 g) were collected from the commercial bee keepers of Himachal Pradesh during 2003, 2004 and 2005. The sampling was done from various

localities of the state along with some basic information (Table 4.56). These samples were stored at room temperature till residue extraction and estimation.

3.2.4 Preparation of Various Stock Solution and Working Concentrations

The following quantities (Table 3.5) of the technical materials of pesticides were accurately weighed on an aluminum foil with the help of electronic balance. Weighed quantities of technical materials of each pesticide were dissolved in respective solvents in 25 ml volumetric flasks. One ml of each of these pesticides contained 1000 ppm (solution A). From solution A, 5 ml was withdrawn and taken in 50 ml volumetric flask and volume made up to the mark with respective solvent (Solution B). This represented 100 ppm. Similarly, solution C and D of strengths 10 and 1 ppm, respectively were prepared. From these solutions, respective concentrations of each of the four pesticides were prepared for testing the mortality of the vinegar flies.

Further concentrations were prepared from the solution D by taking equal quantities of pesticide solution with respective solvent in descending order and tested with the test insect so as to arrive at approximately five concentrations of each pesticide giving range of mortality from 10-90 per cent. This range was later confirmed for each pesticide and also at various intervals before estimating the deposits in the sample extract. The respective concentrations of each pesticide were fixed and are given in Table 3.6.

Table 3.5 Amount of pesticides dissolved in 25 ml of respective solvents to get stock solution (solution A)

Sr. No.	Pesticides	Weight (mg)	Solvents used
1.	Endosulfan	27.17	Acetone (A.R.)
2.	Imidacloprid	26.32	Toluene (A.R.)
3.	Lambda cyhalothrin	26.32	Acetone (A.R.)
4.	Spiromesifen	25.28	Acetone (A.R.)

Table 3.6 Pesticide concentrations prepared to determine standard dosage mortality response curve against *D. melanogaster*

Sr. No.	Pesticide concentrations (ppm)			
	Endosulfan	Imidacloprid	Lambda cyhalothrin	Spiromesifen
1.	6.25 x10 ⁻²	2.00 x10 ⁻²	2.50 x10 ⁻²	1.50
2.	3.12 x10 ⁻²	1.00 x10 ⁻²	1.25 x10 ⁻²	0.75
3.	1.56 x10 ⁻²	0.50 x10 ⁻²	0.63 x10 ⁻²	0.38
4.	0.78 x10 ⁻²	0.25 x10 ⁻²	0.31 x10 ⁻²	0.19
5.	0.39 x10 ⁻²	0.13 x10 ⁻²	0.16 x10 ⁻²	0.09

3.2.4.1 Standard Dosage Mortality Response Curves for the Bioassay of Pesticides

Deposits/ Residues

During the present investigations, “dry film” method using *D. melanogaster* as test insect developed by Kavadia and Lal (1967), was used for the estimation of pesticide residues. Two millilitre of the test solution of the pesticides prepared vide Table 3.6 was taken in a pair of petridish (1 ml on each side) and the film was made by gentle swirling. After dryness, 20 anesthetized male test flies were released. A small amount of artificial diet was placed on the side of the lower petridish without disturbing the film. Each pair of petridish was tied with rubber bands to prevent flies to escape. Petridish with test insects were later placed in a desiccator at 80±5 per cent relative humidity. The desiccator was kept in an incubator at 26±1⁰C temperature and 80±5% RH. Mortality of flies was recorded after 24 hours of release taking moribund insects as dead. In the preliminary experiments, it was observed that neither anaesthetization through chilling nor the solvents of the pesticides had any effect on the mortality of the vinegar fly.

The concentrations giving mortality of adult flies in the range of 10-90 per cent were determined. When the range of mortality for each pesticide was known, the concentrations (Table 3.6) were finally replicated three times. Thus, five to six dosages of a pesticide giving mortality of flies between 10-90 per cent were determined and the mortality data were corrected by Abbot's (1925) formula. These data were subjected to Probit analysis (Finney, 1977). The regression equations thus worked out were used for working out the standard dosage mortality response curves for each pesticide.

3.2.4.2 Standard Calibration Curves for Chemical Assay

The chemical estimations of endosulfan, lambda cyhalothrin and spiromesifen were carried out by using Perkin Elmer Auto GC fitted with autosampler and detectors (ECD and NPD). The detector signals were supplied to PE 1022 terminal integrator with a computer to detect area (in PE units) and retention times (in minutes) for each peak (Table 3.7). The comparison was made with the standards obtained from the analytical grades. The injection volume was 1.0 μ l with acetone/ n- hexane. Imidacloprid residues were estimated using HPLC. The residues of various pesticides (organochlorine, cyclodiene, synthetic pyrethroids and organophosphorus) in honey were estimated by GLC (Table 3.8). The detection of endosulfan, lambda cyhalothrin and spiromesifen residues was carried out by GLC on ECD, whereas estimation of imidacloprid residues was carried on HPLC equipped with binary pump system and C₁₈ column.

The simultaneous estimation of residues of organochlorine and synthetic pesticides was carried on GLC equipped with ECD, whereas organophosphorus pesticides residues were analyzed on GLC equipped with NPD. The operational parameters for the above estimations on GLC and HPLC are listed in section 3.2.4.2.1.

3.2.4.2.1 Operational Parameters of Chromatograph

3.2.4.2.1.1 Nectar and pollen samples

Endosulfan, Lambda cyhalothrin and Spiromesifen (GLC)

Instrument	Auto GC
Column	BP 225
Carrier gas	N ₂
Detector	ECD
Calculating Type	Per cent
Peak data	Area
Start time	0.00 minutes
End Time (min.)	Endosulfan 15.0 Lambda cyhalothrin 30.0 Spiromesifen 25.0
Narrowest peak	1.4-3.2 s
Peak width	Fixed
Area sensitivity	50
Base sensitivity	8
Peak area reject	4.00000e+5
Report device	Epson#1
Plot made	Continuous
Chart speed	10mm min ⁻¹
Plot scale	Full
RT/Components	RT
Oven	Maximum temperature 450 ⁰ C Equil time 0.00 min.
Oven temperature programme	
	Temp. Time Rate
Step I	160 ⁰ C 0.00 3.00
Step II	240 ⁰ C 32.00 0.00

Inlets	Temp.	Pressure
Cap I	110 ⁰ C	4.0 psi
Cap II	240 ⁰ C	10.6 psi
Detector	ECD	Temperature 300 ⁰ C
Split valve		on
Carrier gas	N ₂ 30.0 ml min ⁻¹	

3.2.4.2.1.2 Honey Samples (GLC)

Organochlorine and synthetic pyrethroids

Instrument	Auto GC
Column	BP 225
Carrier gas	N ₂
Detector	ECD
Calculating Type	Per cent
Peak data	Area

Organophosphorus

Instrument	Auto GC
Column	BPX5
Carrier gas	N ₂
Detector	NPD
Calculating Type	Per cent
Peak data	Area

Start time	0.00 minutes	0.00 minutes
End Time	58 minutes	66 minutes
Narrowest peak	1.4-3.2 s	1.4-3.2 s
Peak width	Fixed	Fixed
Area sensitivity	50	100
Base sensitivity	8	16
Peak area reject	4.00000e+5	4.00000e+5
Report device	Epson#1	Epson#1
Plot made	Continuous	Continuous
Chart speed	10mm min ⁻¹	10mm min ⁻¹
Plot scale	Full	Full
RT/Components	RT	RT
Oven	Maximum temperature 450 ⁰ C	450 ⁰ C
	Equil time	0.00 min.
Oven temperature programme		
	Temp. Time Rate	Temp. Time Rate
Step I	160 ⁰ C 0.00 3.00	160 ⁰ C 0.00 3.00
Step II	240 ⁰ C 32.00 0.00	220 ⁰ C 10.00 3.00
		240 ⁰ C 10.00 0.00
Inlets		
	Temp. Pressure	Temp. Pressure
Cap I	110 ⁰ C 4.0 psi	250 ⁰ C 20.0 psi
Cap II	240 ⁰ C 10.6 psi	130 ⁰ C 5.0 psi
Detector	ECD Temperature 300 ⁰ C	NPD Temperature 300 ⁰ C
Split valve	on	on
Carrier gas	N ₂ 30.0 ml min ⁻¹	N ₂ 30.0 ml min ⁻¹
		H ₂ 5.0 ml min ⁻¹
		Zero gas 80.0 ml min ⁻¹

3.2.4.2.1.3 Imidacloprid (HPLC)

Instrument	Waters Auto HPLC
Column	C ₁₈
Solvent system	Acetonitrile: Water 40:60
Low Pressure	0 bar
High Pressure	275.8 bars
Programmed flow	Isocratic (1 ml min ⁻¹)
Detector	Dual absorbance detector
Wave length	270 nm
Filter type	Hamming
Ratio minimum	AU 0.10000
Calculating type	Per cent
Peak data	Area
Start time	0.00 minutes
End time	10.00 minutes
Narrowest peak	1.4- 3.2 S
Peak width	Fixed

3.2.5 Recovery and Analysis of Pesticides

3.2.5.1 Nectar and Pollen Samples

Recovery of pesticides was carried out to know the efficiency of analytical method used in the studies. Experiments were conducted by taking nectar and pollen samples from untreated plots and fortifying the samples with pesticides at levels viz. 2, 4, 8 and 16 ppm for endosulfan, imidacloprid, lambda cyhalothrin and spiromesifen. They were extracted and cleaned up following the methods described under section 3.2.5.3. The blank samples were also processed in the same way to find out the differences, if any, due to difference in substrate. Per cent recoveries of bioassay were also tested along with chemical assay.

3.2.5.2 Honey Samples

Recovery experiments were conducted by taking honey samples known to be collected by bees from pesticide free source of flora (*Plectranthus* sp.). These honey samples were fortified with different quantities of various pesticides belonging to one group. The samples were extracted and cleaned up following methods described in section 3.2.5.4. The blank samples were also processed in the same way to find out the differences, if any, due to difference in substrate. For the estimation of recovery 1.0 µl of the standard solutions was injected into the chromatograph and the peak area of the known standard sample was recorded. The procedure was repeated 3-4 times and the average was calculated. Per cent recovery was calculated as per the following formula:

$$\text{Per cent recovery} = \frac{\text{Average peak area of 2ppm equivalent sample}}{\text{Average peak area of 2ppm standard solution}} \times 100$$

Table 3.7 Retention time, area, detectability and sensitivity for different pesticides on GLC*

Pesticide	Qty. of pesticide (µg ml ⁻¹)	Pesticide volume (µl)	Retention time (min.)	Area (PE units)	Detection limit (ppb)	Sensitivity (pg)
Endosulfan I	10	1.0	6.890	215524800 ± 6.2249	0.10	5.00
Endosulfan II	10	1.0	13.173	256610272 ± 7.0059	0.10	5.00
Imidacloprid**	10	10.0	2.052	5065 ±2.005	10.0	5.00
Lambda cyhalothrin	10	1.0	24.153	111100928 ± 4.4463	0.50	2.00
Spiromesifen	10	1.0	19.552	27230590 ± 9.2244	0.10	5.00

*Detector ECD; Column BP225; Figures following ± standard deviations; ** detected through HPLC column C₁₈

Table 3.8 Retention time, area, detectability and sensitivity for different pesticides (organochlorine, cyclodiene and synthetic pyrethroids) on GLC*

Pesticide	Qty. of pesticide (µg ml ⁻¹)	Pesticide volume (µl)	Retention time (min.)	Area (PE units)	Detection limit (ppb)	Sensitivity (pg)
α HCH	0.02	1.0	3.938	53906108 ±5.0008	0.05	2.00
β HCH	0.02	1.0	5.360	59670368 ±4.6787	0.05	2.00
p, p' DDT	0.02	1.0	8.478	81887416 ±8.9987	0.10	5.00
Endosulfan I	0.05	1.0	9.073	246173600 ±7.7245	0.10	5.00
γ HCH	0.02	1.0	10.093	68743528 ±5.5599	0.05	2.00
p, p' DDD	0.02	1.0	10.763	30704548 ±6.3369	0.15	7.00
o, p' DDT	0.02	1.0	12.302	72677648 ±4.5699	0.10	5.00
Endosulfan II	0.05	1.0	16.077	351845920 ±5.4469	0.10	5.00
Dicofol	0.05	1.0	19.618	60885776 ±2.3364	0.50	2.00
Lambda cyhalothrin I	0.005	1.0	23.263	24765372 ±8.8801	0.50	2.00
Lambda cyhalothrin II	0.005	1.0	24.222	111100928 ±3.2564	0.50	2.00
Cypermethrin I	0.05	1.0	29.890	51031784 ±4.5684	1.00	5.00

Cypermethrin II	0.05	1.0	31.115	33712044 ±2.3057	1.00	5.00
Fenvalerate I	0.11	1.0	35.020	38830688 ±3.5421	0.50	2.00
Fenvalerate II	0.11	1.0	36.990	29046404 ±6.5241	0.50	2.00
Deltamethrin	0.586	1.0	42.678	32525964 ±2.1111	2.00	10.00

*Detector ECD; Column BP225; Figures following ± standard deviations

Table 3.9 Retention time, area, detectability and sensitivity for different pesticides (organophosphorus) on GLC*

Pesticide	Qty. of pesticide µg (ml ⁻¹)	Pesticide volume (µl)	Retention time (min.)	Area (PE units)	Minimum detection limit (ppb)	Sensitivity (pg)
Acephate	0.10	1.0	11.773	42114572 ± 4.0552	5.00	50.00
Monocrotophos	0.10	1.0	19.520	31399846 ± 3.5562	5.00	50.00
Dimethoate	0.05	1.0	21.590	60489560 ± 5.2214	2.00	20.00
Malathion	0.05	1.0	30.300	12672248 ± 4.5687	5.00	50.00
Chlorpyriphos	0.05	1.0	31.320	16748263 ± 6.2136	5.00	50.00
Quinalphos	0.05	1.0	39.275	20688406 ± 3.2210	5.00	50.00
Ethion	0.05	1.0	55.423	36645144 ± 2.1142	2.00	20.00

*Detector NPD; Column BPX5; Figures following ± standard deviations

3.2.5.3 Residue Analysis in Nectar and Pollen

3.2.5.3.1 Extraction and Clean Up

Residues of pesticides viz. endosulfan, imidacloprid, lambda cyhalothrin and spiromesifen were estimated from nectar and pollen. Quantities of the samples were taken by subtracting the weight of the empty vial from the weight of the vial containing sample. Thus known amount of the nectar and pollen sample was subjected to analysis.

For the extraction of residues from the nectar, samples were decanted as such along with 5 ml of respective solvent (Table 3.2). Whereas, pollens were grounded well in

pestle and mortar in 5 ml of respective solvent and 1 g of anhydrous sodium sulphate. The extract was filtered through Whatman filter paper No 1.

The extracted samples were concentrated to 3 ml in a rotary vacuum evaporator at 35°C. The concentrated extract was transferred to chromatographic column packed with specific adsorbent pre-washed with specific eluant (Table 3.2). The eluant was collected in glass flasks. The final volumes for nectar and pollen samples were made to 10 and 15 ml, respectively.

3.2.5.3.2 Storage

Residues were estimated through GLC/HPLC, if the estimation is delayed the extracted and cleaned samples were stored in stoppered reagent bottles in deep freezer maintained at -4°C until estimation.

3.2.5.3.3 Residue Analysis

Cleaned and stored sample bottles were taken out of the deep freezer and allowed to stand for some time under laboratory conditions so as to obtain the original level. The decreased level due to evaporation was made with the help of addition of respective solvent. The samples were analyzed for the presence of pesticides with bioassay method using *D. melanogaster* as a test insect and then by chemical assay.

3.2.5.3.3.1 Bioassay

The samples were processed for bioassay as per method mentioned earlier. If the residual film gave 100 per cent mortality, the extract was further diluted by stripping the solution. Consequently, the dilution giving 20- 80 per cent mortality was relied upon by confirming repeatedly.

3.2.5.3.3.2 Chemical Assay

A minimum quantity of 1.0 μ l equivalent sample was injected in GLC, whereas 10.0 μ l was injected in HPLC, and these were made ideal with the parameters as per section 3.2.3. Standard and recovery samples were also injected.

3.2.5.4 Honey Analysis

3.2.5.4.1 Physico- Chemical Properties

3.2.5.4.1.1 Physical Properties

The methodology used for the analysis of different physical parameters of the representative sample is described as follows:

3.2.5.4.1.1.1 Colour

Colour of honey samples was determined by recording the optical density of honey without dilution and presented in USDA colour specifications (Townsend, 1969). Sample of around 20 g honey was taken in a test tube and was placed in a water bath at 77 $^{\circ}$ C till the removal of air bubbles. It was allowed to cool down to room temperature. The absorbance of honey was read in a UV-VIS Spectrophotometer at 560 nm. Distilled water was taken as blank.

3.2.5.4.1.1.2 Moisture

Moisture (per cent by weight) of different honey samples was determined by the refractometer method. Refractometer reading was taken at 20 $^{\circ}$ C and moisture was calculated (Anonymous, 1974).

3.2.5.4.1.1.3 Density

Density of the honey was calculated by using specific gravity bottle (Anonymous, 1974). The bottle was cleaned, dried and weighed. It was filled with honey maintained at temperature $27 \pm 1^{\circ}$ C on thermostatically controlled water bath. The density was calculated as follows:

$$\text{Density} = \frac{B - A}{\text{Volume}}$$

Where ,

A= Weight (g) of the empty specific gravity bottle,

B= Weight (g) of the specific gravity bottle with the honey samples.

3.2.5.4.1.1.4 Pollen Counts

Determination of total count of pollen was made with the centrifuge machine at 3000 rpm for 5 minutes (Yadav, 1995). Honey samples (10 g) were weighed and dissolved in distilled water (50 ml) in a small clean beaker. This was transferred carefully to a 100 ml measuring cylinder and the cylinder was filled with distilled water up to 100 ml mark. This stock solution (10 ml) was centrifuged in 15 ml centrifuge tube at 3000 rpm for 5 minutes. The supernatant liquid was decanted cautiously without disturbing the sediment taking care to leave 1ml of the liquid with the sediment in the tube. The sediment was shaken well and completely transferred to a collecting tube. Centrifugation was repeated with supernatant and the sediments were pooled in a collection tube. The pooled sediments were stirred only with a drop of 0.5 per cent alcoholic basic fuchsin solution. It was centrifuged and supernatant was drawn and the sediment was shaken well and a drop of this solution was placed on the 1 mm squares on the haemocytometer and a coverslip was placed. Pollen grains were counted in 1 mm square at a magnification of 100x. This counting was repeated ten times and 10 different counts were taken with the dispersed sediment. The pollen density was calculated as follow:

The average number of the pollen grains counted over the haemocytometer were for the volume 0.1 mm (1mm X 0.1 mm depth). From this, the pollen grains present in 1

ml, (equivalent to the number present in 10 g of honey) of solution was calculated. The results were expressed as the number of pollen grains in 1 g of honey sample.

3.2.5.4.1.2 Chemical Properties

3.2.5.4.1.2.1 Total Soluble Solids (TSS)

TSS was determined with the help of refractometer. Honey was put on water bath maintained at 70°C for 15 minutes to avoid any differences due to granulation and temperature difference. A small drop was applied to the prismatic surface of the refractometer and TSS percentage was read on the scale.

3.2.5.4.1.2.2 Sugars

Sugars contribute the major portion of the honey. Total reducing sugars, sucrose, glucose, fructose and fructose- glucose ratio were determined as per Anonymous (1974).

3.2.5.4.1.2.2.1 Total Reducing Sugars (TRS)

Reagents used to find out the total reducing sugar were as follows:

- i) Soxhlet modification of Fehling's solution: This solution was prepared by mixing equal volumes of solution A and solution B immediately before using.
 - a) Solution A (copper sulphate solution): 34.639 g of copper sulphate crystals ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) were dissolved in water, diluted to 500ml. This solution was then filtered through glass wool or filter paper.
 - b) Solution B (Potassium sodium tartrate): 173 g of potassium sodium tartrate (Rochelle salt) and 50 g of sodium hydroxide were dissolved in water, diluted to 500 ml. This solution was allowed to stand for a day and filtered.
- ii) Methylene blue indicator: 0.2 per cent in water.

For the standardization of copper sulphate solution five millilitre each of solution A and solution B were pipetted out in to a porcelain dish of 100 ml capacity. This mixture

was heated to boiling on a asbestos gauze and standard invert sugar solution was added to it through a burette, about 1 ml less than the expected volume, which reduced the Fehling's solution completely (about 48 ml). 1 ml of methylene blue indicator was added while keeping the solution boiling. The titration was completed with in three minutes, end point being indicated by the change of colour from blue to red. From the volume of invert sugar solution used, the strength of copper sulphate solution was calculated by multiplying the titer volume by 0.001 (mg/ml of the standard invert sugar solution). This gave the quantity of invert sugar required to reduce the copper in 5 ml of copper sulphate solution.

Sample of honey (10 g) was placed in a 250 ml volumetric flask and diluted with 150 ml of water for the estimation of TRS. The contents of the flask were mixed thoroughly and the volume was made to 250 ml with water. Using separate pipettes, 5 ml each of solution A and solution B was taken in a porcelain dish. About 12 ml of honey solution was added from a burette and heated to boiling over an asbestos gauge. Then 1 ml of methylene blue indicator was added, while keeping the solution boiling, titration was completed with in three minutes. The end point was indicated by change of colour from blue to red. The volume (H) was noted in ml of honey solution required for the titration. Total reducing sugar was calculated by the following formula:

$$\text{TRS (Per cent by weight)} = \frac{250 \times 100 \times S}{H \times W}$$

Where,

T.R.S. = Total reducing sugars

S= Strength of the copper sulphate solution

H= Volume (ml) of honey solution required for titration

W= Weight (g) of honey

3.2.5.4.1.2.2.2 Sucrose

Reagents used to find out the total reducing sugar were as follows:

Solutions A and B were prepared as explained in section 3.2.5.4.1.2.2.1.

- i) Hydrochloric acid: Specific gravity 1.18 at 20⁰C (approximately 12N).
- ii) Standard invert sugar solution: 0.95 g sucrose solution was dissolved in 500 ml of water, 2 ml of concentrated hydrochloric acid was added to it boiled gently for 30 minutes and kept aside for 24 hours. It was neutralized with sodium carbonate and the final volume was made to 100 ml. 50 ml of this solution contained 0.05 g invert sugar.

To 100 ml of honey solution, 1 ml of concentrated hydrochloride acid was added and heated to near boiling. The solution was kept aside overnight. This inverted honey solution was neutralized with sodium carbonate and per cent reducing sugars were calculated (Anonymous, 1974).

Sucrose (per cent / weight) = Reducing sugars after inversion (per cent / wt.)- Reducing sugars before inversion (per cent / wt.) x 0.095

3.2.5.4.1.2.2.3 Glucose

Reagents required for glucose analysis were as follows:

- i) Iodine solution: 0.05 N
- ii) Sodium hydroxide solution: 0.1 N
- iii) Concentrated and standard sodium thiosulphate solution: 0.05 N

Fifty milliliters of honey solution was pipetted in to a 250 ml stoppered flask. To this, 40 ml of iodine solution and 25 ml of sodium hydroxide solution was added and the flask was stoppered and kept in dark for 20 minutes. It was acidified with 5 ml of

sulphuric acid and the excess of iodine was titrated quickly against standard sodium thiosulphate solution. A blank test was conducted by using 50 ml of water. The glucose (per cent /wt.) was calculated as follows:

$$\text{Glucose (per cent / wt.)} = B - S \times 0.004502$$

Where,

B= Volume of sodium thiosulphate solution required for blank

S= Volume of sodium thiosulphate solution required for the sample

3.2.5.4.1.2.2.4 Fructose:

Calculations required for the determination of fructose in honey

$$\text{Fructose (Per cent / wt.)} = \text{TRS (per cent / wt.)} - \text{Glucose (per cent / wt.)}$$

3.2.5.4.1.2.2.5 Fructose- Glucose Ratio

Fructose- glucose ratio was determined by the following calculation

$$\text{Fructose- glucose ratio} = \frac{\text{Fructose (per cent / wt.)}}{\text{Glucose (per cent / wt.)}}$$

3.2.5.4.1.2.3 Ash

The ash content of the honey samples were determined by igniting the honey in a muffle furnace at $600 \pm 20^{\circ}\text{C}$. Honey sample of 10 g was taken in porcelain dish with a few drops of olive oil to prevent spattering, heat carefully over a low flame until swelling ceased. Ash content was determined by using the following formula:

$$\text{Ash (per cent / wt.)} = \frac{100 (W_2 - W)}{W_1 - W}$$

Where,

W = Weight (g) of the empty dish

W₁ = Weight (g) of the dish with the material (honey) taken for the test

W₂ = Weight (g) of the dish with the ash

3.2.5.4.1.2.4 Acidity

Reagents required for estimating acidity were as follows:

Standard sodium hydroxide solution (0.05N) and phenolphthalein indicator solution (0.5 g dissolved in 100 ml of 50 % ethyl alcohol (v/v)).

Honey sample (10g) was taken in a suitable titration flask and dissolved it in 75 ml of distilled water. It was mixed thoroughly and titrated against standard sodium hydroxide solution using 6 drops of neutralized phenolphthalein solution (pink colour) indicator persisted for at least 10 seconds. A blank test was conducted on water to correct, the volume of standard sodium hydroxide solution used. Acidity was calculated as:

$$\text{Acidity (as formic acid) \% by wt.} = \frac{0.23 \times V}{W}$$

Where

V= Corrected volume of 0.05 N sodium hydroxide solution required for titration

W= Weight (g) of the sample taken for the test

3.2.5.4.1.2.5 Hydroxyl Methyl Furfural (HMF)

HMF was measured by spectrophotometric method as per Anonymous (2000). Reagents required for the test were as follows:

- i) Carrez Solution I: 15 g of potassium ferrocynite was diluted with 100 ml of distilled water.
- ii) Carrez solution II: 30 g of zinc acetate was diluted with 100 ml distilled water.
- iii) Sodium bisulphite solution: 0.20 g of the sodium bisulphite was dissolved in 100 ml of distilled water. The solution was prepared daily.

Five grams of honey sample was weighed in a small beaker and dissolved with around 25 ml of distilled water in a 50 ml volumetric flask. Simultaneously, 0.50 ml

(each) of carrez I and carrez II were added, mixed well and volume was made to 50 ml with distilled water. This solution was filtered through whatman No. 1 filter paper. First 10 ml was discarded. From the filtrate 5.0 ml of solution was added in to separate test tubes those already contained 5.0 ml of distilled water and 5.0 ml of sodium bisulphite solution. Contents were mixed well and absorbance was recorded at 284 and 336 nm using a spectrophotometer. HMF was calculated as follows

$$\text{HMF (ppm)} = \frac{(A_{284} - A_{336}) \times 149.7 \times 5}{\text{weight of honey taken (g)}}$$

Where

A_{284} and A_{336} are absorbance at 284 and 386 nm

3.2.5.4.2 Pesticide Residues

3.2.5.4.2.1 Extraction and Clean Up for Residue analysis

3.2.5.4.2.1.1 Organochlorine, Cyclodiene and Synthetic Pyrethroid Pesticides

Method of Ogota and Bevenue (1973) with slight modifications made by Chawla and Goyal (1988) and Karak *et al.* (1999) was used. Samples of honey (50 g) were taken in 250 ml conical flasks and mixed with 100 ml each of distilled water and methanol. This mixture was shaken for 3 hours on a mechanical shaker and filtered through Whatman No. 1 filter paper. For clean up of chlorinated hydrocarbon and synthetic pyrethroid residues, the above extract was transferred to a separatory funnel of 500 ml capacity. 100 ml of n-hexane was added to the extract. After shaking the funnel for 15 seconds, it was kept for separation of layers. The lower layer was drained out in to a conical flask and the organic layer was collected over anhydrous sodium sulphate. The process was repeated twice. This solution was concentrated to 1-2 ml through vacuum rotary evaporator and transferred to glass column pre packed with activated Florisil: Silical gel mixture (1:1).

Distilled n-hexane was used as eluant and final volume was made up to 30 ml in each sample.

3.2.5.4.2.1.2 Organophosphate Pesticides

Method of Rathi *et al.* (1997) and Khan *et al.* (2004) with slight modifications was used. A representative 10 g honey sample was diluted with 100 ml of 4 per cent aqueous solution of sodium sulphate in 250 ml conical flask. This mixture was shaken for 1 hour in a mechanical shaker and filtered through Whatman No. 1 filter paper.

Each sample of extracted honey with distilled water (50 ml) was transferred to a separatory funnel (250 ml) and extracted four times with n- hexane (50 ml). Each time the hexane phase was transferred to a tube and centrifuged for five minutes at 5,000 rpm in order to break up the emulsion that appeared in water and hexane inter-phase. Afterwards, the extract was dried over anhydrous sodium sulphate (15 g). This solution was concentrated to 1-2 ml through vacuum rotary evaporator at 35⁰C. Concentrate was transferred to glass column pre packed with activated florisil: silica gel mixture (1:1). Distilled n-hexane was used as eluant. The eluant was collected in flask and evaporated to dryness in a vacuum rotary pump (at 35⁰C). The dried residue was diluted with hexane to 5 ml and stored in clean reagent bottles. If necessary samples were stored as per section 3.2.5.3.2.

3.2.5.4.3 Sample Analysis

Cleaned and stored sample bottles were taken out of the deep freezer and allowed to stand for some time under laboratory conditions so as to obtain the original level. The decreased level due to evaporation was made with the help of addition of respective solvent. A minimum quantity of 1µl equivalent samples were injected in chromatograph

(GLC) which was made ideal with the parameters as per section 3.2.4.2.1.2. Standard and recovery samples were also injected.

3.2.6 Effect of Pesticides on Physico-Chemical Properties

Quantities of pesticides viz. HCH (5 ppm), DDT (5 ppm), endosulfan (5 ppm), dicofol (5 ppm), lambda cyhalothrin (2 ppm), fenvalerate (2 ppm), cypermethrin (15 ppm), deltamethrin (10 ppm), acephate, chlorpyrifos, dimethoate, ethion, malathion, monocrotophos and quinalphos (each 15 ppm), more than that recorded in the honey samples were dissolved in 25 ml of water. These pesticides were added in 500 g of honey sample found free of pesticide residues and were mixed thoroughly. Samples were kept at room temperature and after 30 days various physico- chemical properties were determined as per section 3.2.5.4.1. In control experiments only water was added in honey samples to assess the effect of storage. Similarly, differences between contaminated and non contaminated samples were analyzed in the collected samples.

3.3 CALCULATION

3.3.1 Determination of LC₅₀ Values for Contact and Stomach Toxicity Tests

The data obtained on the observed mortality were converted into per cent mortality. The corrected per cent mortalities were calculated by using Abbot’s formula (Abbot, 1925). This data was further subjected to probit analysis (Finney, 1977) to calculate LC₅₀, LC₉₀, heterogeneity and fiducial limits were calculated. The regression lines were plotted and slopes of the regression were determined. The relative toxicity values of different pesticides were also determined.

Corrected mortality

per cent

=

Per cent mortality in treatment

100

-

Per cent mortality in control

Per cent mortality in control

X 100

3.3.2 Determination of LD₅₀ Values for Contact and Stomach Toxicity Tests

For the determination of contact and stomach toxicity, LD₅₀ values were worked out using the following relationship given by Sihag and Kumar (1990).

$$LD_{50} = 10 V \times LC_{50}$$

Where

V = Volume of insecticidal solution applied to/ consumed by the honeybee.

In topical application tests

$$V = 1.0 \mu l$$

In mass feeding tests

V= The volume consumed by the test bees.

3.3.3 Determination of LT₅₀

The per cent mortalities (20-90 %) obtained from the persistent toxicity at different time intervals were subjected to probit analysis by Finney (1977) to get LT₅₀.

3.3.4 Bioassay

The corrected per cent mortality data of the vinegar flies were transformed to probits (Finney, 1977). The formula of pesticide residues in ppm:

	Amount of residues in µg for known ml of extract(exposed to insect)	X	Total volume of extract prepared in ml	X	Dilution, if any
=					
	Volume of extract (ml) exposed to insect	X	Weight of sample (g)		

3.3.5 Chromatography

The formula for pesticide residue estimation in unknown sample by GLC is as under:

$$C_2 = \frac{A_2 C_1 \times D \times V \times V_1}{A_1 \times V_2 \times W}$$

Where

A_1 = Peak area of standard sample in PE units

A_2 = Peak area of unknown sample in PE units

C_1 = Concentration of standard sample in ppm

C_2 = Concentration of pesticide in unknown sample in ppm

D = Dilution ratio

V = Total volume of extract (ml)

V_1 = Volume of standard sample injected

V_2 = Volume of unknown sample injected

W = Weight of sample taken in g

3.3.6 Sensitivity of Bioassay Method

Sensitive of “Dry film” method of bioassay was determined by using the equation given by Sun and Sun (1952).

$$\text{Sensitivity (ppm)} = D \times F / R \times V$$

Where

D = LC_{50} of pesticide (ppm)

F = Factor of accuracy and reliability of data, worked out as ratio of LC_{20} over LC_{80} .

V = Volume of pesticide extract taken in exposure chamber

R = Extraction ratio (quantity of plant material per ml of the solvent)

3.3.7 Half Life or RL_{50} Value

The half life of RL_{50} values of the four pesticides were calculated on the basis of formula given by Hoskins (1961).

$$t_{1/2} = \log 2 / k_1$$

Where

$t_{1/2}$ = Half life value of residues

k_1 = Slope of regression co- efficient (b) of the log residues in ppm (y) on the number of elapsed days (x).

3.3.8 Dissipation of Residues

Per cent dissipation = $100 - \frac{\text{Residue (ppm)}}{\text{Initial residue (ppm)}} \times 100$

RESULTS

RESULTS

Results on the present investigation, the toxicity of some new pesticides to honey bees and their residues in honey and pollen, are presented in this chapter. The findings of the experiments on the contact, stomach and persistent toxicities to honey bees (*Apis cerana cerana* and *Apis mellifera*), residues in nectar, pollen and honey and effects of pesticide residues on physico-chemical properties of honey are described in this chapter.

4.1 TOXICITY OF PESTICIDES TO HONEY BEES

Ten pesticides (Table 3.1) belonging to different groups were tested for contact and stomach toxicities against foragers of *A. c. cerana* and *A. mellifera*.

4.1.1 Contact Toxicity

4.1.1.1 *A. c. cerana*

The dose mortality response of *A. c. cerana* foragers to topically applied synthetic pesticides are given in tables 4.1-4.6, whereas, for the chitin synthesis inhibitor and biopesticides data are given in tables 4.7 and 4.8.

The mortality data of cypermethrin are presented in table 4.1 and log dose probit (LDP) line in fig. 4.1a. A dose of $0.005 \mu\text{g bee}^{-1}$ applied on the thorax gave 22.03 per cent mortality and $0.080 \mu\text{g bee}^{-1}$ dose caused 88.14 per cent corrected mortality. In control treatment 1.67 per cent mortality was recorded. The LD_{50} and LD_{90} values with fiducial limits were computed to be $0.0171 (0.0135 - 0.0215) \mu\text{g bee}^{-1}$ and $0.1155 (0.0695 - 0.1919) \mu\text{g bee}^{-1}$, respectively.

The data were significantly homogenous as indicated by $\chi^2_{\text{cal.}}$ value of 0.9576 against 7.80, the tabulated value of χ^2 at $p=0.05$ and 3df (degree of freedom). The slope of the LDP line was calculated to be 1.5427 ± 0.1964 .

The mortality data of *A. c. cerana* forager on 24 hours exposure to endosulfan are given in the Table 4.2 and the LDP line is presented in Fig. 4.1b. From the table it is seen that corrected mortality of 17.95 per cent was observed at dose of $2.00 \mu\text{g bee}^{-1}$ whereas, 82.91 per cent corrected mortality was recorded at $32.00 \mu\text{g bee}^{-1}$. The mortality recorded in control treatment was 2.50 per cent.

The LD_{50} and LD_{90} values computed from the data were 7.9871 and $52.1868 \mu\text{g bee}^{-1}$, respectively. The fiducial limits corresponding to LD_{50} and LD_{90} were $3.9442 - 16.1740 \mu\text{g bee}^{-1}$ and $31.1417 - 87.4538 \mu\text{g bee}^{-1}$, respectively. The data were homogenous as indicated by $\chi^2_{\text{cal.}}$ value of 0.0959, which was significantly less than tabulated value of 7.80. The regression line $Y = 3.5813 + 1.5722X$ gave the best fit and the slope was calculated as 1.5722 ± 0.2084 .

The mortality data and LDP line of imidacloprid are given in Table 4.3 and Fig. 4.1c. From the data, it is observed that $0.00625 \mu\text{g bee}^{-1}$ imidacloprid gave 13.79 per cent and $0.10 \mu\text{g bee}^{-1}$ gave 75.86 per cent corrected bee mortalities. Per cent mortality of 3.33 was recorded in control treatment. The LD_{50} and LD_{90} values with fiducial limits were computed to be $0.0317 (0.0252 - 0.0399) \mu\text{g bee}^{-1}$ and $0.2122 (0.1225 - 0.3677) \mu\text{g bee}^{-1}$, respectively. The data were significantly homogenous as indicated by $\chi^2_{\text{cal.}}$ value of 0.6172, which was less than the tabulated value of 7.80. The slope of the LDP line was 1.5529 ± 0.1918 .

Lambda cyhalothrin gave 15.00 per cent mortality at dose of $0.0025 \mu\text{g bee}^{-1}$ whereas, $0.040 \mu\text{g bee}^{-1}$ dose gave 81.67 per cent mortality against the 0.00 per cent in control treatment. The LD_{50} and LD_{90} values were computed to be $0.0124 \mu\text{g bee}^{-1}$ and $0.0783 \mu\text{g bee}^{-1}$, respectively. The corresponding fiducial limits were $0.0099 - 0.0156 \mu\text{g bee}^{-1}$ and $0.0461 - 0.1327 \mu\text{g bee}^{-1}$.

Table 4.1 Dosage mortality response of *A. c. cerana* foragers to cypermethrin after 24 hours of topical application

Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
80.00	1.9031	88.14	6.1821	6.06
40.00	1.6021	67.80	5.4621	5.59
20.00	1.3010	52.54	5.0637	5.11
10.00	1.0000	35.59	4.6308	4.64
5.00	0.6990	22.03	4.2288	4.17
χ ² (3df)	= 0.9576	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.5427	S.E. (b)	= 0.1964	
Y= 3.0995 + 1.5427X				
LD ₅₀ = 0.0171 (0.0135 – 0.0215) µg bee ⁻¹				
LD ₉₀ = 0.1155 (0.0695 – 0.1919) µg bee ⁻¹				

Table 4.2 Dosage mortality response of *A. c. cerana* foragers to endosulfan after 24 hours of topical application

Dosage (µg bee ⁻¹ x 1)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
32.00	1.5051	82.91	5.9502	6.00
16.00	1.2041	69.23	5.5015	5.50
8.00	0.9031	48.72	4.9674	5.00
4.00	0.6021	31.62	4.5211	4.52
2.00	0.3010	17.95	4.0808	4.02
χ ² (3df)	= 0.0959	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 1.5722	S.E. (b)	= 0.2084	
Y= 3.5813 + 1.5722X				
LD ₅₀ = 7.9871 (3.9442 –16.1740) µg bee ⁻¹				
LD ₉₀ = 52.1868 (31.1417 – 87.4538) µg bee ⁻¹				

Table 4.3 Dosage mortality response of *A. c. cerana* foragers to imidacloprid after 24 hours of topical application

Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
100.00	2.0000	75.86	5.7000	5.70
50.00	1.6990	65.52	5.4000	5.30
25.00	1.3979	44.83	4.8700	4.87
12.50	1.0969	24.14	4.3000	4.30
6.25	0.7959	13.79	3.9100	4.00
χ ² (3df)	= 0.6172	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.5529	S.E. (b)	= 0.1918	
Y= 2.6684 + 1.5529X				
LD ₅₀ = 0.0317 (0.0252 – 0.0399) µg bee ⁻¹				
LD ₉₀ = 0.2122 (0.1225 – 0.3677) µg bee ⁻¹				

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

The $\chi^2_{\text{cal.}}$ (1.7053) was significantly less than $\chi^2_{\text{tab.}}$ (7.8), which showed the homogeneity of data (Table 4.4). The slope of LDP line was calculated to be 1.6041 ± 0.1964 (Fig. 4.1d).

The mortality data recorded after 24 hours of exposure on topical application of spiromesifen to *A. c. cerana* are given in Table 4.5 and LDP line in Fig. 4.1e. From the table it is seen that spiromesifen doses ranged from 15.00 to 540.00 $\mu\text{g bee}^{-1}$ gave per cent corrected mortality ranging from 11.03 to 80.34. Untreated control recorded 3.33 per cent mortality. The LD_{50} and LD_{90} values computed from the data were 141.5198 and 1543.7161 $\mu\text{g bee}^{-1}$, respectively. The fiducial limits corresponding to LD_{50} and LD_{90} were $105.9417 - 227.6567 \mu\text{g bee}^{-1}$ and $780.2852 - 3054.0876 \mu\text{g bee}^{-1}$, respectively. The $\chi^2_{\text{cal.}}$ value 1.7007 was significantly less in comparison to tabulated value of 7.80 at $p=0.05$ and 3df. The slope of the LDP line was calculated to be 1.2350 ± 0.1536 .

A dose of 6.25 $\mu\text{g thiacloprid bee}^{-1}$ gave 15.25 per cent corrected mortality whereas, the dose of 100.00 $\mu\text{g bee}^{-1}$ gave 89.83 per cent corrected mortality. Mortality in control treatment was 1.67 per cent (Table 4.6).

The LD_{50} and LD_{90} values with fiducial limits were computed to be 22.6433 (18.6746 – 27.4554) and 107.3767 (73.3978 – 157.0858) $\mu\text{g bee}^{-1}$, respectively. The data were significantly homogenous as indicated by low $\chi^2_{\text{cal.}}$ value of 0.1377 against tabulated value of 7.80. The slope of LDP line (Fig. 4.1f) was 1.8959 ± 0.2089 .

As high as, 20.00, 0.75 and 10.00 $\mu\text{g bee}^{-1}$ doses of benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki*, respectively were tested against *A. c. cerana*. The per cent mortalities were recorded after 24 and 48 hours after application. It was found that after 24 hours 5.42, 6.64 and 2.00 per cent mortality were recorded for benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki*, respectively.

Table 4.4 Dosage mortality response of *A. c. cerana* foragers to lambda cyhalothrin after 24 hours of topical application

Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	% corrected mortality*	Empirical probit	Expected probit
40.00	1.6021	81.67	5.9040	5.84
20.00	1.3010	63.33	5.3406	5.36
10.00	1.000	36.67	4.6602	4.89
5.00	0.6990	28.33	4.4269	4.41
2.50	0.3979	15.00	3.9636	3.93
χ ² (3df)	= 1.7053	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 1.6041	S.E. (b)	= 0.1964	
Y= 3.2440 + 1.6041X				
LD ₅₀ = 0.0124 (0.0099 – 0.0156) µg bee ⁻¹				
LD ₉₀ = 0.0783 (0.0461 – 0.1327) µg bee ⁻¹				

Table 4.5 Dosage mortality response of *A. c. cerana* foragers to spiromesifen after 24 hours of topical application

Dosage (µg bee ⁻¹ x 1)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
540.00	2.7324	80.34	5.8538	5.74
270.00	2.4314	60.69	5.2715	5.36
135.00	2.1303	44.14	4.8526	4.98
45.00	1.6532	31.03	4.5049	4.39
15.00	1.1761	11.03	3.7801	3.79
χ ² (3df)	= 1.7007	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.2350	S.E. (b)	= 0.1536	
Y= 2.3438 + 1.2350X				
LD ₅₀ = 141.5198 (105.9417 – 227.6567) µg bee ⁻¹				
LD ₉₀ = 1543.7161 (780.2852 – 3054.0876) µg bee ⁻¹				

Table 4.6 Dosage mortality response of *A. c. cerana* foragers to thiacloprid after 24 hours of topical application

Dosage (µg bee ⁻¹ x 1)	Log dosage	% corrected mortality*	Empirical probit	Expected probit
100.00	2.0000	89.83	6.2718	6.26
50.00	1.6990	72.88	5.6098	5.60
25.00	1.3979	52.54	5.0637	5.06
12.50	1.0969	30.51	4.4902	4.50
6.25	0.7959	15.25	3.9742	3.96
χ ² (3df)	= 0.1377	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 1.8959	S.E. (b)	= 0.2089	
Y= 2.4311 + 1.8959X				
LD ₅₀ = 22.6433 (18.6746 – 27.4554) µg bee ⁻¹				
LD ₉₀ = 107.3767 (73.3978 – 157.0858) µg bee ⁻¹				

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

Fig 4.1a

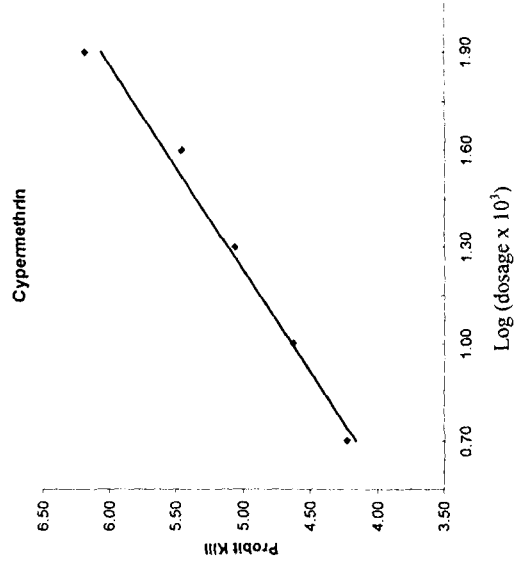


Fig 4.1b

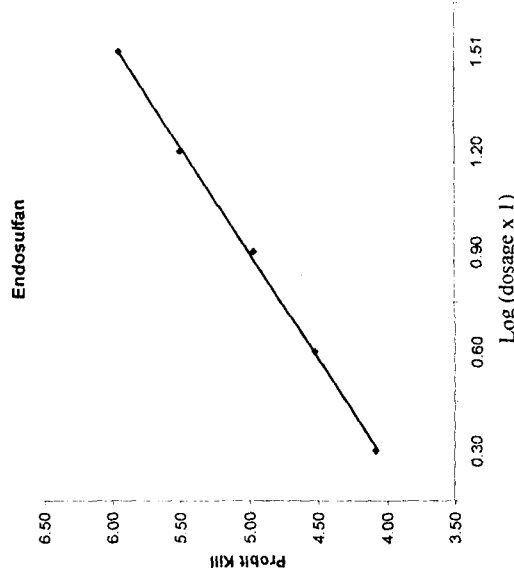


Fig 4.1c

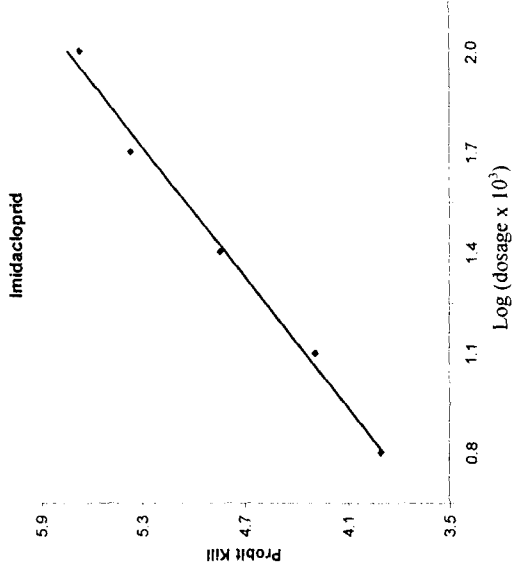


Fig 4.1d

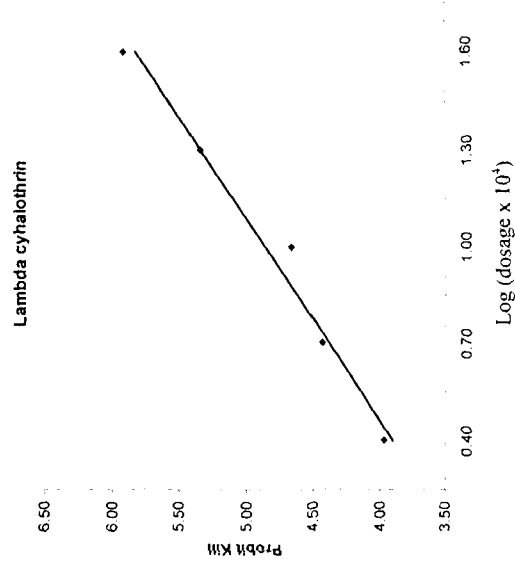


Fig 4.1e

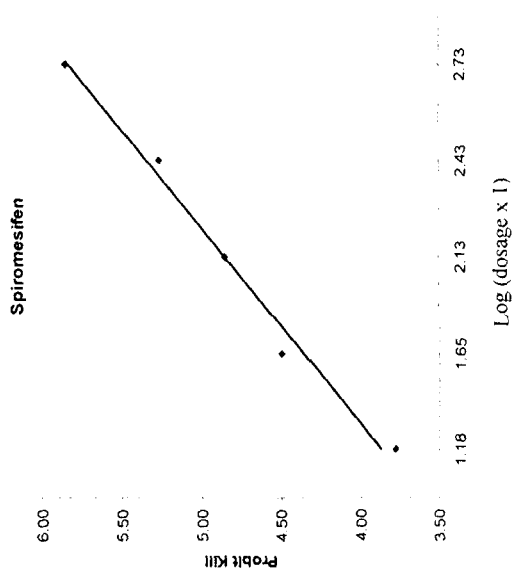


Fig 4.1f

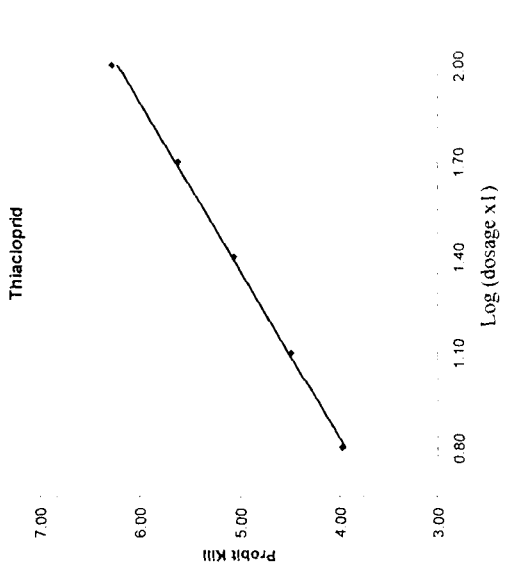


Fig. 4.1 Log dosage probit mortality response lines of *A. c. cerana* foragers to pesticides after 24 hours of topical application

Whereas after 48 hours, per cent mortalities were recorded to be 7.12, 6.87 and 2.33 per cent, respectively (Table 4.7). No mortality was recorded in untreated control.

Spinosad proved toxic even after 24 hours of exposure. Corrected mortality range of 11.86 to 81.36 per cent was observed at doses ranging from 0.0125 to 0.2000 $\mu\text{g bee}^{-1}$. The mortality recorded in control was 1.67 per cent (Table 4.8). The LD_{50} and LD_{90} values computed from the data were 0.0590 and 0.3525 $\mu\text{g bee}^{-1}$, respectively. The fiducial limits corresponding to LD_{50} and LD_{90} were 0.0474 – 0.0735 and 0.2133 – 0.5826 $\mu\text{g bee}^{-1}$, respectively. The mortality data were homogenous as it is clear from the low $\chi^2_{\text{cal.}}$ value of 1.0695 against the tabulated value of 7.80. The slope of the LDP line (Fig. 4.2 a) was 1.6514 ± 0.2021 . Whereas, after 48 hours of exposure LD_{50} and LD_{90} values were computed to be 0.0549 and 0.3101 $\mu\text{g bee}^{-1}$, respectively with corresponding fiducial limits of 0.0445 – 0.0677 and 0.1429 – 0.6728 $\mu\text{g bee}^{-1}$. The slope of the LDP line (Fig. 4.2b) was 1.7045 ± 0.1988 .

4.1.1.2 *A. mellifera*

Mortality data (Table 4.9) recorded after 24 hours of exposure of *A. mellifera* foragers to cypermethrin showed that 18.97 and 91.38 per cent corrected bee mortalities were observed at 0.0125 and 0.2000 $\mu\text{g bee}^{-1}$ doses, respectively. The mortality recorded in control treatment was 3.33 per cent. The LD_{50} and LD_{90} values computed from the data were 0.0438 and 0.2269 $\mu\text{g bee}^{-1}$, respectively. The fiducial limits corresponding to LD_{50} and LD_{90} were 0.0358 – 0.0535 $\mu\text{g bee}^{-1}$ and 0.1518 – 0.3391 $\mu\text{g bee}^{-1}$, respectively. Low $\chi^2_{\text{cal.}}$ value of 1.9928 against 7.80 the tabulated value, showed the homogeneity of mortality data. The slope of LDP line (Fig. 4.3a) was 1.7934 ± 0.2021 .

Table 4.7 Dosage mortality response of *A. c. cerana* foragers to pesticides (benzoylphenyl urea, azadirachtin and *B. thuringiensis*) after 24 and 48 hours of topical administration

Benzoylphenyl urea				Azadirachtin				<i>B. thuringiensis</i> subsp. <i>kurstaki</i>			
Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*	
	24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure
20.00	5.42	7.12	0.75	6.64	6.87	10.00	2.00	2.33	10.00	2.00	2.33
10.00	4.64	5.34	0.375	4.17	4.25	5.00	---	1.33	5.00	---	1.33
5.00	2.95	3.64	0.1875	1.09	1.17	2.50	---	---	2.50	---	---
2.50	---	---	0.0937	---	---	1.25	---	---	1.25	---	---
1.25	---	---	0.04687	---	---	0.625	---	---	0.625	---	---

LD₅₀ > 20.00 µg bee⁻¹

LD₅₀ > 0.75µg bee⁻¹

LD₅₀ > 10.00 µg bee⁻¹

Table 4.8 Dosage mortality response of *A. c. cerana* foragers to spinosad after 24 and 48 hours of topical application

Dosage (µg bee ⁻¹ x 10 ²)	Log dosage	24 hrs. exposure				48 hrs. exposure			
		%corrected mortality*	Empirical probit	Expected probit	% corrected mortality*	Empirical probit	Expected probit	% corrected mortality*	Empirical probit
20.00	1.3010	81.36	5.8927	5.90	84.48	5.9945	5.94	84.48	5.9945
10.00	1.0000	66.10	5.4152	5.38	67.24	5.4399	5.44	67.24	5.4399
5.00	0.6990	40.68	4.7647	4.88	44.83	4.8743	4.92	44.83	4.8743
2.50	0.3979	30.51	4.4962	4.36	29.31	4.4466	4.44	29.31	4.4466
1.25	0.0969	11.86	3.8200	3.84	13.79	3.9197	3.92	13.79	3.9197
χ ² (3df)	= 1.0695	χ ² (tab.) at 0.05 and 3df	= 7.80	χ ² (3df)	= 0.1886	χ ² (tab.) at 0.05 and 3df	= 7.80	χ ² (tab.) at 0.05 and 3df	= 7.80
b	= 1.6514	S.E. (b)	= 0.2021	b	= 1.7045	S.E. (b)	= 0.1988	S.E. (b)	= 0.1988
Y= 3.7265 + 1.6514X				Y= 3.7393 + 1.7045X					

LD₅₀= 0.0590 (0.0474 – 0.0735) µg bee⁻¹

LD₉₀= 0.3525 (0.2133 – 0.5826) µg bee⁻¹

LD₅₀= 0.0549 (0.0445 – 0.0677) µg bee⁻¹

LD₉₀= 0.3101 (0.1429 – 0.6728) µg bee⁻¹

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperial probit; X= Log dosage; Figures in parenthesis are fiducial limits

Fig 4.2a

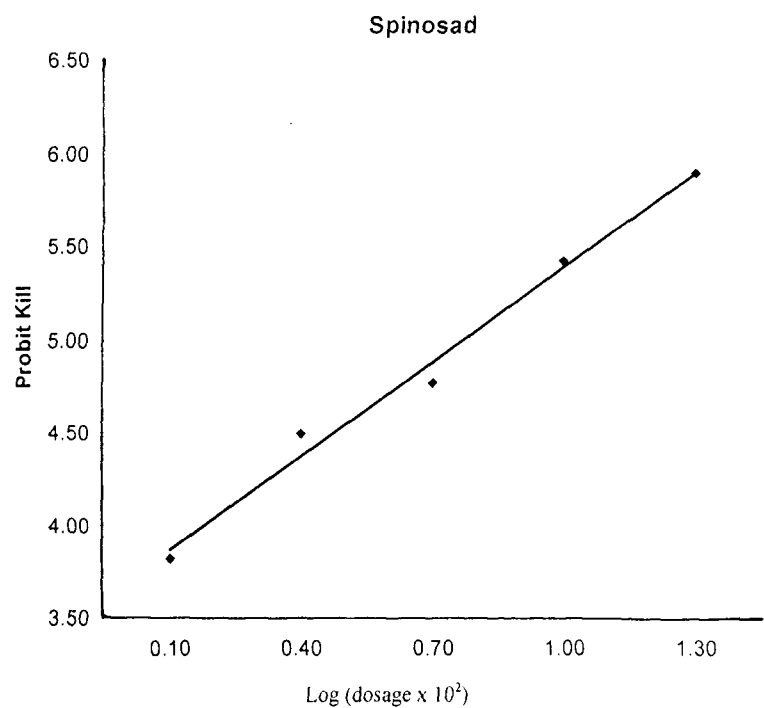


Fig 4.2b

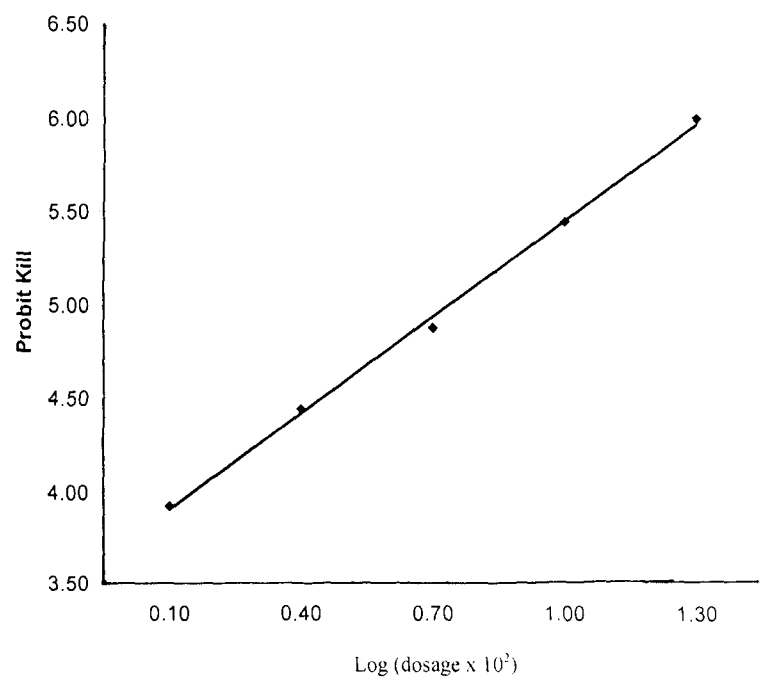


Fig. 4.2 Log dosage probit mortality response lines of *A. c. cerana* foragers to spinosad after 24 and 48 hours of topical application

Data showed that a dose of 2.00 μg endosulfan bee^{-1} gave 13.79 per cent whereas, 32.00 μg bee^{-1} dose caused 86.21 per cent corrected mortality of *A. mellifera* foragers. Mortality in control was 3.33 per cent (Table 4.10). The LD_{50} and LD_{90} values with fiducial limits were computed to be 9.0614 (7.4239 – 11.0601) μg bee^{-1} and 44.8833 (29.5289 – 68.2216) μg bee^{-1} , respectively. The data were significantly homogenous as $\chi^2_{\text{cal.}}$ (1.3928) value is significantly less than tabulated value of 7.80. The slope of the LDP line (Fig. 4.3b) was 1.8443 ± 0.2096 .

The mortality data of imidacloprid (Table 4.11) showed that 0.0300 μg bee^{-1} imidacloprid applied topically on the thorax gave 20.69 per cent whereas, 0.4800 μg bee^{-1} dose gave 79.31 per cent corrected mortality. Control treatment recorded 3.33 per cent bee mortality. The LD_{50} and LD_{90} values with fiducial limits were computed to be 0.1157 (0.0899 – 0.1487) μg bee^{-1} and 0.9864 (0.5321 – 1.8268) μg bee^{-1} , respectively.

The low $\chi^2_{\text{cal.}}$ value (1.3481) against the tabulated 7.80 showed the homogeneity in the mortality data. The LDP line (Fig. 4.3c) had the slope of 1.3769 ± 0.1877 .

From the Table 4.12 it is seen that mortalities to the tune of 21.08, 31.37, 51.96, 69.12 and 79.14 per cent were observed at corresponding lambda cyhalothrin doses of 0.00625, 0.0125, 0.025, 0.05 and 0.10 μg bee^{-1} , respectively. The LD_{50} and LD_{90} values along with their fiducial limits were computed to be 0.0241 (0.0188 – 0.0308) μg bee^{-1} and 0.1926 (0.1069 – 0.3467) μg bee^{-1} , respectively. The data were homogenous with low value of $\chi^2_{\text{cal.}}$ 0.7445 against the tabulated value of 7.80. The slope of LDP line (Fig. 4.3d) was 1.4208 ± 0.1926 .

Spiromesifen was applied topically to forager bees and mortalities after 24 hours of exposure were recorded (Table 4.13). It was observed that 31.50 μg bee^{-1} dose caused

Table 4.9 Dosage mortality response of *A. mellifera* foragers to cypermethrin after 24 hours of topical application

Dosage (µg bee ⁻¹ x 10 ²)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
20.00	1.3010	91.38	6.3668	6.18
10.00	1.0000	74.14	5.6476	5.65
5.00	0.6990	46.55	4.9135	5.12
2.50	0.3979	34.48	4.6011	4.60
1.25	0.0969	18.97	4.1221	4.10
χ ² (3df)	= 1.9928	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.7934	S.E. (b)	= 0.2021	
Y= 3.8501 + 1.7934X				
LD ₅₀ = 0.0438 (0.0358 – 0.0535) µg bee ⁻¹				
LD ₉₀ = 0.2269 (0.1518 – 0.3391) µg bee ⁻¹				

Table 4.10 Dosage mortality response of *A. mellifera* foragers to endosulfan after 24 hours of topical application

Dosage (µg bee ⁻¹ x 1)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
32.00	1.5051	86.21	6.0893	6.00
16.00	1.2041	68.97	5.4959	5.50
8.00	0.9031	41.38	4.7827	4.90
4.00	0.6021	24.14	4.2969	4.30
2.00	0.3010	13.79	3.9107	3.74
χ ² (3df)	= 1.3928	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 1.8443	S.E. (b)	= 0.2096	
Y= 3.2346 + 1.8443X				
LD ₅₀ = 9.0614 (7.4239 – 11.0601) µg bee ⁻¹				
LD ₉₀ = 44.8833 (29.5289 – 68.2216) µg bee ⁻¹				

Table 4.11 Dosage mortality response of *A. mellifera* foragers to imidacloprid after 24 hours of topical application

Dosage (µg bee ⁻¹ x 10 ²)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
48.00	1.6812	79.31	5.8169	5.82
24.00	1.3802	65.52	5.3989	5.41
12.00	1.0792	56.67	5.1687	4.99
6.00	0.7782	31.03	4.5041	4.57
3.00	0.4771	20.69	4.18.1	4.16
χ ² (3df)	= 1.3481	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.3769	S.E. (b)	= 0.1877	
Y= 3.5359 + 1.3769X				
LD ₅₀ = 0.1157 (0.0899 – 0.1487) µg bee ⁻¹				
LD ₉₀ = 0.9864 (0.5321 – 1.8268) µg bee ⁻¹				

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

20.69 per cent whereas, 500.00 $\mu\text{g bee}^{-1}$ dose caused 72.41 per cent corrected mortality. Mortality recorded in the untreated control was 3.33 per cent. The LD_{50} and LD_{90} values with fiducial limits were computed to be 159.7212 (118.2858 – 215.6713) $\mu\text{g bee}^{-1}$ and 2007.9101 (818.9081 – 4923.2664) $\mu\text{g bee}^{-1}$, respectively.

The data were significantly homogenous as indicated by χ^2_{cal} value of 0.0187 against 7.80 the χ^2_{tab} . The LDP line $Y = 2.4314 + 1.1657X$ had the slope of 1.1657 ± 0.1840 (Fig. 4.3e).

From the Table 4.14 and Fig. 4.3f it is seen that corrected mortality of 13.79 per cent was observed at the dose of 6.25 $\mu\text{g thiacloprid bee}^{-1}$ whereas, 86.21 per cent corrected mortality was recorded at 100.00 $\mu\text{g bee}^{-1}$. The mortality recorded in control treatment was 3.33 per cent. The LD_{50} and LD_{90} values computed from the data were 26.6903 and 144.7548 $\mu\text{g bee}^{-1}$, respectively. The fiducial limits corresponding to LD_{50} and LD_{90} values were 21.7089 – 32.8148 and 92.8803 – 225.6017 $\mu\text{g bee}^{-1}$, respectively. χ^2_{cal} value (0.3484) was significantly less in comparison to χ^2_{tab} value of 7.80 at $p = 0.05$ and 3df, which showed the homogeneity in mortality data. The slope of the LDP line was calculated to be 1.7454 ± 0.2020 .

From the Table 4.15 it is seen that benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki* containing pesticides were non toxic causing a maximum of 6.58, 8.74 and 4.67 per cent bee mortality at the tested doses of as high as 20.00, 0.75 and 10.00 $\mu\text{g bee}^{-1}$ after 48 hours of exposure, respectively. Mortalities in control treatment were 0.00 per cent for each pesticide.

It was recorded that per cent corrected mortality of *A. mellifera* foragers to the range of 18.64 to 79.66 was given by doses of 0.020 to 0.400 $\mu\text{g spinosad bee}^{-1}$, respectively after 24 hours of exposure (Table 4.16). However, at the same doses after 48

**Table 4.12 Dosage mortality response of *A. mellifera* foragers to lambda cyhalothrin
24 hours of topical application**

Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	% corrected mortality*	Empirical probit	Expected probit
100.00	2.0000	79.41	5.8208	5.82
50.00	1.6990	69.12	5.4993	5.42
25.00	1.3979	51.96	5.0502	5.05
12.50	1.0969	31.37	4.5155	4.60
6.25	0.7959	21.08	4.1970	4.20
χ ² (3df)	= 0.7445	χ ² (tab.) at 0.05 and 3df		7.80
b	= 1.4208	S.E. (b)	= 0.1926	
Y= 3.0356 + 1.4208X				
LD ₅₀ = 0.0241 (0.0188 – 0.0308) µg bee ⁻¹				
LD ₉₀ = 0.1926 (0.1069 – 0.3467) µg bee ⁻¹				

**Table 4.13 Dosage mortality response of *A. mellifera* foragers to spiromesifen after
24 hours of topical application**

Dosage (µg bee ⁻¹ x1)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
500.00	2.6990	72.41	5.5951	5.58
250.00	2.3979	58.62	5.2178	5.23
125.00	2.0969	44.83	4.8701	4.87
62.00	1.7924	31.03	4.5049	4.52
31.50	1.4983	20.69	4.1831	4.17
χ ² (3df)	= 0.0187	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.1657	S.E. (b)	= 0.1840	
Y= 2.4314 + 1.1657X				
LD ₅₀ =159.7212 (118.2858– 215.6713) µg bee ⁻¹				
LD ₉₀ = 2007.9101 (818.9081– 4923.2664) µg bee ⁻¹				

**Table 4.14 Dosage mortality response of *A. mellifera* foragers to thiacloprid after 24
hours of topical application**

Dosage (µg bee ⁻¹ x 1)	Log dosage	% corrected mortality*	Empirical probit	Expected probit
100.00	2.0000	86.21	6.0898	6.00
50.00	1.6990	65.52	5.3994	5.46
25.00	1.3979	46.55	4.9135	4.92
12.50	1.0969	29.31	4.4557	4.40
6.25	0.7959	13.79	3.9107	3.90
χ ² (3df)	= 0.3484	χ ² (tab.) at 0.05 and 3df		7.80
b	= 1.7454	S.E. (b)	= 0.2020	
Y= 2.5105 + 1.7454X				
LD ₅₀ = 26.6903 (21.7089 – 32.8148) µg bee ⁻¹				
LD ₉₀ = 144.7548 (92.8803 – 225.6017) µg bee ⁻¹				

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

Fig 4.3a

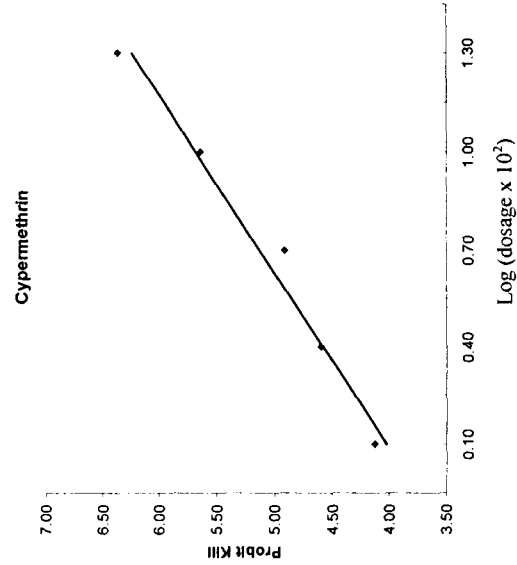


Fig 4.3b

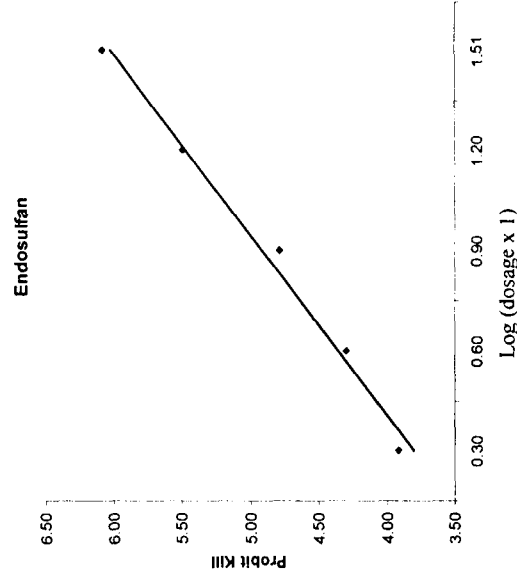


Fig 4.3c

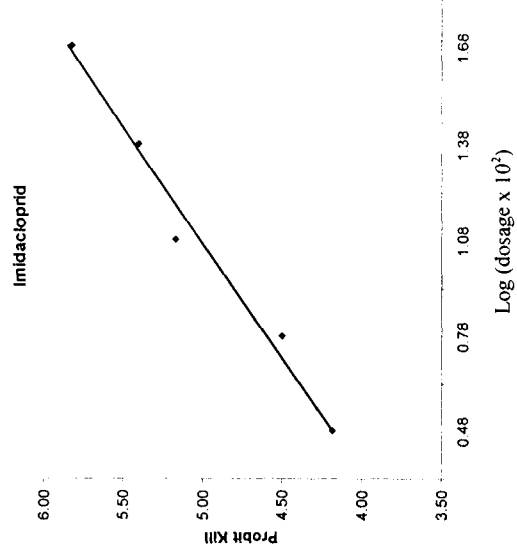


Fig 4.3d

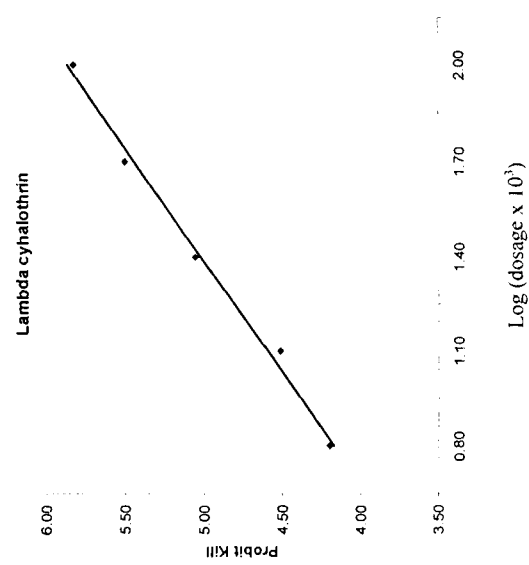


Fig 4.3e

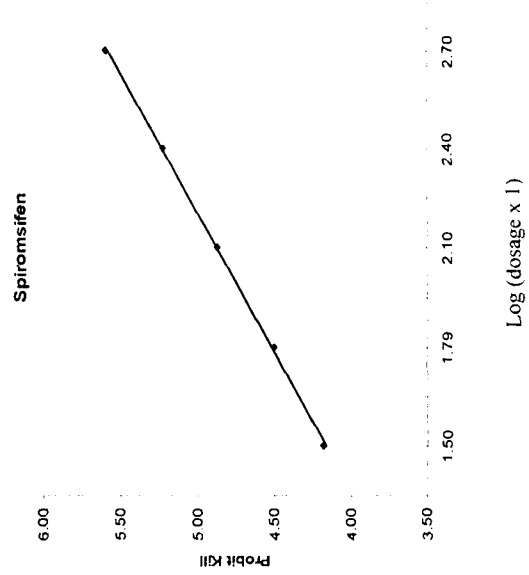


Fig 4.3f

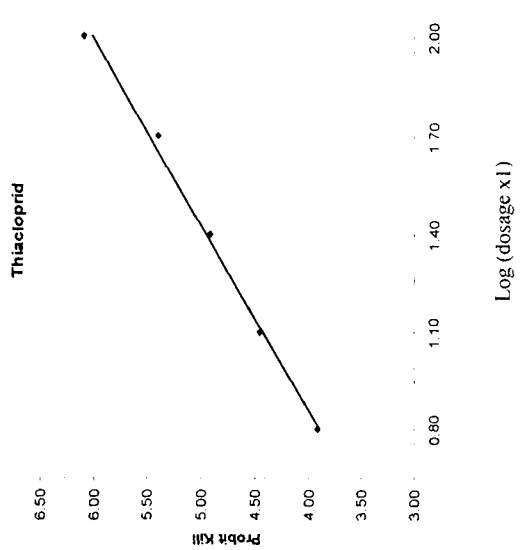


Fig. 4.3 Log dosage probit mortality response lines of *A. mellifera* foragers to pesticides after 24 hours of topical application

Table 4.15 Dosage mortality response of *A. mellifera* foragers to pesticides (benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki*) after 24 and 48 hours of topical application

Benzoylphenyl urea				Azadirachtin				<i>B. thuringiensis</i> subsp. <i>kurstaki</i>			
Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		24 hrs. exposure	24 hrs. exposure	48 hrs. exposure
	24 hrs.	48 hrs.		24 hrs.	48 hrs.		24 hrs.	48 hrs.			
20.00	5.52	6.58	0.75	8.48	8.74	10.00	4.26	4.67	4.26	4.26	4.67
10.00	4.41	4.75	0.375	6.78	7.10	5.00	1.20	2.10	1.20	1.20	2.10
5.00	1.02	1.42	0.1875	---	---	2.50	---	---	---	---	---
2.50	---	---	0.0937	---	---	1.25	---	---	---	---	---
1.25	---	---	0.0469	---	---	0.625	---	---	---	---	---

LD₅₀ > 20.00 µg bee⁻¹

LD₅₀ > 0.75 µg bee⁻¹

LD₅₀ > 10.00 µg bee⁻¹

Table 4.16 Dosage mortality response of *A. mellifera* foragers to spinosad after 24 and 48 hours of topical application

Dosage (µg bee ⁻¹ x 10 ²)	24 hrs. exposure				48 hrs. exposure			
	Log dosage	%corrected mortality*	Empirical probit	Expected probit	% corrected mortality	Empirical probit	Expected probit	
40.00	1.6021	79.66	5.8310	5.91	84.75	6.0258	6.04	
20.00	1.3010	67.80	5.4621	5.49	69.49	5.5101	5.61	
10.00	1.0000	59.32	5.2358	5.08	61.02	5.2798	5.18	
5.00	0.6990	40.68	4.7647	4.67	45.76	4.8945	4.74	
2.00	0.3979	18.64	4.1088	4.25	20.34	4.1704	4.31	
χ ² (3df)	= 1.8987	χ ² (tab.) at 0.05 and 3df	= 7.80	χ ² (3df)	= 2.4593	χ ² (tab.) at 0.05 and 3df	= 7.80	
b	= 1.3681	S.E. (b)	= 0.1881	b	= 1.4289	S.E. (b)	= 0.1988	
Y= 3.7281 + 1.3681X								

LD₅₀= 0.0851 (0.0670 – 0.1080) µg bee⁻¹

LD₉₀= 0.7352 (0.4022 – 1.3438) µg bee⁻¹

LD₅₀= 0.0745 (0.0579– 0.0959) µg bee⁻¹

LD₉₀= 0.5873 (0.3426 – 1.0069) µg bee⁻¹

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperial probit; X= Log dosage; Figures in parenthesis are fiducial limits

Fig 4.4a

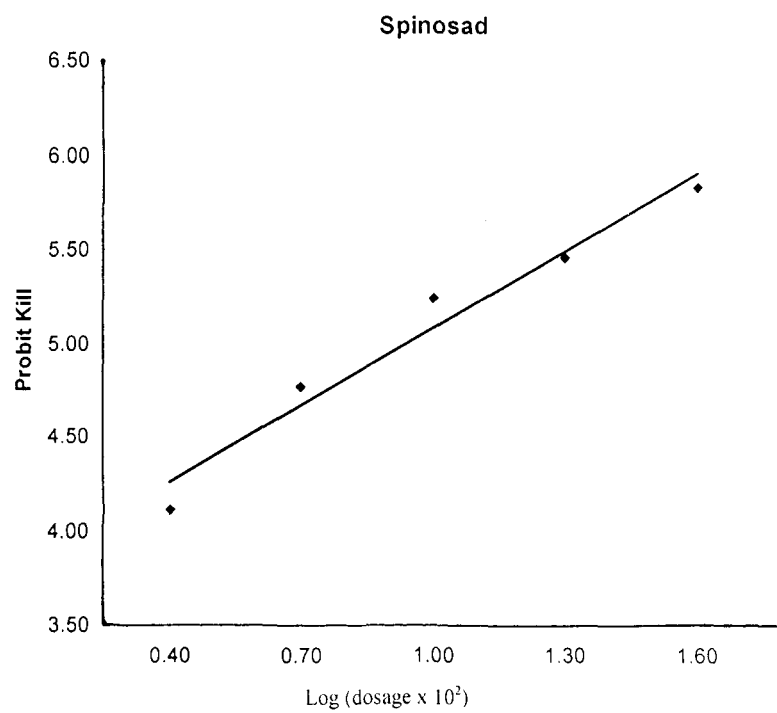


Fig 4.4b

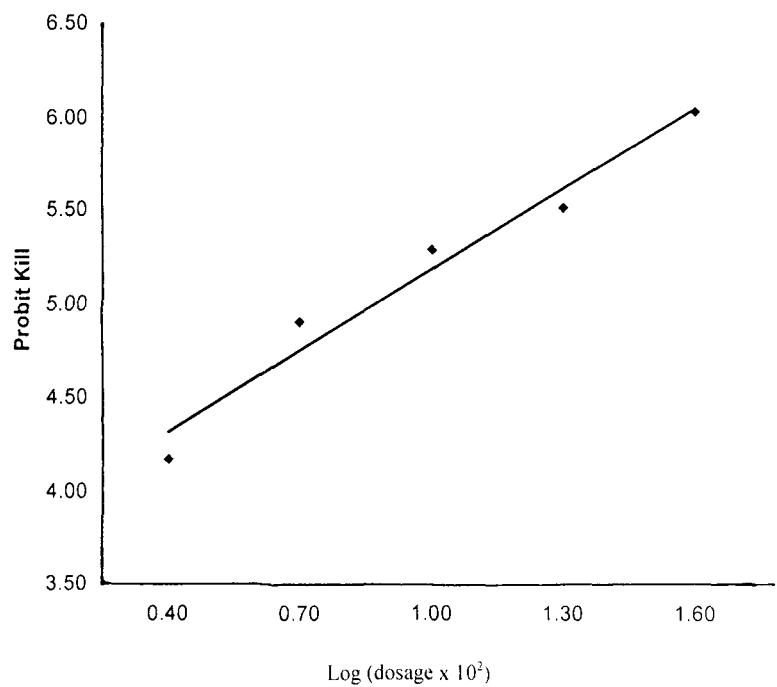


Fig. 4.4 Log dosage probit mortality response lines of *A. mellifera* foragers to spinosad after 24 and 48 hours of topical application

hours of exposure, per cent corrected mortality to the range of 20.34 to 84.75 was recorded. The LD₅₀ and LD₉₀ values with fiducial limits after 24 hours of exposure were recorded to be 0.0851 (0.0670 – 0.1080) and 0.7352 (0.4022 – 1.3438) $\mu\text{g bee}^{-1}$, respectively. The LD₅₀ values recorded after 48 hours of exposure were 0.0745 (0.0579 – 0.0959) and 0.5873 (0.3426 – 1.0069) $\mu\text{g bee}^{-1}$.

The slopes of LDP lines computed for 24 and 48 hours of exposure were 1.3681 ± 0.1881 and 1.4289 ± 0.1988 , respectively (Fig. 4.4a and 4.4b).

4.1.2 Stomach Toxicity

4.1.2.1 *A. c. cerana*

The data for the doses ($\mu\text{g bee}^{-1}$) of cypermethrin and corresponding bee mortalities are given in Table 4.17 and presented graphically in Fig. 4.5a. It was observed that cypermethrin doses of 0.00125 $\mu\text{g bee}^{-1}$ gave 18.97 per cent and 0.0200 $\mu\text{g bee}^{-1}$ gave 84.48 per cent corrected mortality. Control treatment gave 3.33 per cent mortality. The χ^2 test showed the homogeneity of test population as indicated by the low value of χ^2_{cal} . 0.1271 against the table value of 7.80. The slope of LDP line was 1.5327 ± 0.1953 . The LD₅₀ value worked out to be 0.0047 (0.0037 – 0.0059) $\mu\text{g bee}^{-1}$. The LD₉₀ value was 0.0322 (0.0190 – 0.0543) $\mu\text{g bee}^{-1}$.

The per cent corrected mortalities of 13.79 and 93.10 were recorded at doses of 2.00 to 24.00 $\mu\text{g bee}^{-1}$ of endosulfan, respectively (Table 4.18). The LD₅₀ value was computed to be 7.0041 (6.0062 – 8.1677) $\mu\text{g bee}^{-1}$. The LD₉₀ value was recorded to be 23.3278 (17.2187 – 31.6609) $\mu\text{g bee}^{-1}$. The data were homogenous as χ^2_{cal} value 3.2949 was significantly less than tabulated value. The LDP line (Fig. 4.5b) gave the best fit and slope of the regression line was calculated to be 2.4527 ± 0.2575 .

Table 4.17 Dosage mortality response of *A. c. cerana* foragers to cypermethrin after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
20.00	1.3010	84.48	6.0152	5.98
10.00	1.0000	68.97	5.4930	5.52
5.00	0.6990	50.00	5.0000	5.06
2.50	0.3979	36.21	4.6472	4.59
1.25	0.0969	18.97	4.1221	4.13
χ ² (3df)	= 0.1271	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.5327	S.E. (b)	= 0.1953	
Y= 3.9715 + 1.5327X				
LD ₅₀ = 0.0047 (0.0037 - 0.0059) µg bee ⁻¹				
LD ₉₀ = 0.0322 (0.0190 – 0.0543) µg bee ⁻¹				

Table 4.18 Dosage mortality response of *A. c. cerana* foragers to endosulfan after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 1)	Log dosage	%corrected mortality	Empirical probit	Expected probit
24.00	1.3802	93.10	6.4833	6.35
12.00	1.0792	68.97	5.4959	5.62
6.00	0.7782	48.28	4.9574	4.88
4.00	0.6021	17.24	4.1831	4.46
2.00	0.3010	13.79	3.9107	3.72
χ ² (3df)	= 3.2949	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 2.4527	S.E. (b)	= 0.2575	
Y= 2.9266 + 2.4577X				
LD ₅₀ = 7.0041 (6.0062 – 8.1677) µg bee ⁻¹				
LD ₉₀ = 23.3278 (17.2187 – 31.6609) µg bee ⁻¹				

Table 4.19 Dosage mortality response of *A. c. cerana* foragers to imidacloprid after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 10 ⁴)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
400.00	2.6021	86.21	6.0893	6.04
200.00	2.3010	65.52	5.3989	5.49
100.0	2.0000	51.72	5.0426	4.95
50.00	1.6990	24.14	4.2969	4.40
25.00	1.3979	13.79	3.9107	3.86
χ ² (3df)	= 1.1474	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.8071	S.E. (b)	= 0.2020	
Y= 1.3381 + 1.8071X				
LD ₅₀ = 0.0107 (0.0087 – 0.0130) µg bee ⁻¹				
LD ₉₀ = 0.0547 (0.0359 – 0.0830) µg bee ⁻¹				

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

Dosage mortality response data for imidacloprid are given in Table 4.19 and presented graphically in Fig. 4.5c. It was observed that per cent corrected mortality range of 13.79 to 86.21 was given by doses ranging from 0.0025 to 0.0400 $\mu\text{g bee}^{-1}$. Control treatment gave 3.33 per cent mortality. The χ^2 test showed the homogeneity of test population with low value of $\chi^2_{\text{cal.}}$ (1.1474) compared to table value of 7.80. The slope of LDP line was 1.8071 ± 0.2020 . The LD_{50} value worked out was $0.0107 \mu\text{g bee}^{-1}$ with fiducial limits of $0.0087 - 0.0130 \mu\text{g bee}^{-1}$. The LD_{90} value was $0.0547 \mu\text{g bee}^{-1}$ with fiducial limits of $0.0359 - 0.0830 \mu\text{g bee}^{-1}$.

Per cent corrected mortality range of 8.77 to 82.46 was observed at lambda cyhalothrin doses ranging from 0.00075 to 0.0120 $\mu\text{g bee}^{-1}$ (Table 4.20). The per cent mortality in control treatment was 5.00. The χ^2 test showed the homogeneity of the test population, as the calculated value of χ^2 (2.5446) was lower than the tabulated value (7.80). The LDP equation (4.5d) had a slope of 1.9298 ± 0.2092 . The LD_{50} and LD_{90} values worked out to be 0.0035 ($0.0029 - 0.0043$) $\mu\text{g bee}^{-1}$ and 0.0163 ($0.0110 - 0.0241$) $\mu\text{g bee}^{-1}$, respectively.

The mortality data of *A. c. cerana* foragers for spiromesifen administered orally are given in Table 4.21 and the LDP line is given in Fig 4.5e. From the table it is seen that corrected mortality of 20.69 per cent was recorded at a dose of 25.00 $\mu\text{g bee}^{-1}$, whereas 86.21 per cent corrected mortality was recorded at dose 600.00 $\mu\text{g bee}^{-1}$. Untreated control recorded 3.33 per cent mortality.

The LD_{50} and LD_{90} values were computed to be 111.7038 ($86.3969 - 144.4234$) and 925.5374 ($557.0416 - 1537.8015$) $\mu\text{g bee}^{-1}$, respectively. The data were homogenous as $\chi^2_{\text{cal.}}$ value (1.6071) was significantly less than table value (7.80). The slope of LDP line was calculated to be 1.3956 ± 0.1747 .

Table 4.20 Dosage mortality response of *A. c. cerana* foragers to lambda cyhalothrin after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 10 ⁴)	Log dosage	% corrected mortality*	Empirical probit	Expected probit
120.00	2.0792	82.46	5.9348	6.03
60.00	1.7782	73.68	5.6341	5.44
30.00	1.4771	38.60	4.7102	4.86
15.00	1.1761	26.32	4.3665	4.27
7.50	0.8751	8.77	3.6468	3.69
χ ² (3df)	= 2.5446	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 1.9298	S.E. (b)	= 0.2092	
Y= 2.0124 + 1.9298X				
LD ₅₀ = 0.0035 (0.0029 – 0.0043) µg bee ⁻¹				
LD ₉₀ = 0.0163 (0.0110 – 0.0241) µg bee ⁻¹				

Table 4.21 Dosage mortality response of *A. c. cerana* foragers to spiromesifen after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 1)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
600.00	2.7782	86.21	6.0898	6.04
300.00	2.4771	72.41	5.5951	5.61
150.00	2.1761	58.62	5.2178	5.19
75.00	1.8751	34.48	4.6011	4.76
25.00	1.3979	20.69	4.1831	4.09
χ ² (3df)	= 1.6071	χ ² (tab.) at 0.05 and 3df		= 7.80
b	=1.3956	S.E. (b)	= 0.1747	
Y= 2.1418 + 1.3956X				
LD ₅₀ = 111.7038 (86.3969 – 144.4234) µg bee ⁻¹				
LD ₉₀ = 925.5374 (557.0416 – 1537.8015) µg bee ⁻¹				

Table 4.22 Dosage mortality response of *A. c. cerana* foragers to thiacloprid after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 10)	Log dosage	% corrected mortality*	Empirical probit	Expected probit
120.00	2.0792	86.44	6.1003	5.87
60.00	1.7782	61.02	5.2798	5.36
30.00	1.4771	32.20	4.5379	4.85
15.00	1.1761	23.73	4.2850	4.34
7.500	0.8751	16.95	4.0439	3.83
χ ² (3df)	= 6.1648	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 1.6796	S.E. (b)	= 0.2028	
Y= 2.3386 + 1.6796X				
LD ₅₀ = 3.8416 (3.0867 – 4.7810) µg bee ⁻¹				
LD ₉₀ = 22.2607 (13.4310 – 36.8949) µg bee ⁻¹				

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

Fig 4.5a

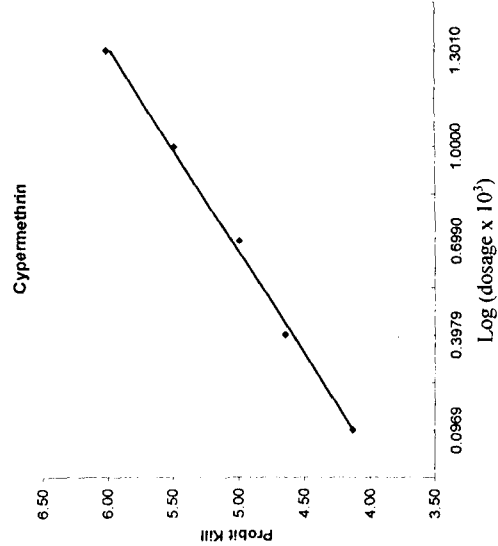


Fig 4.5b

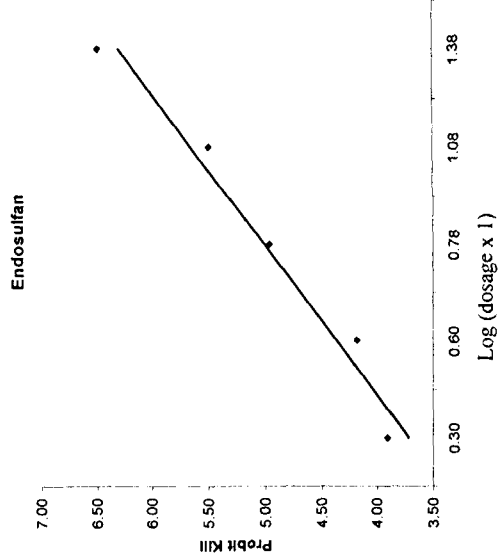


Fig 4.5c

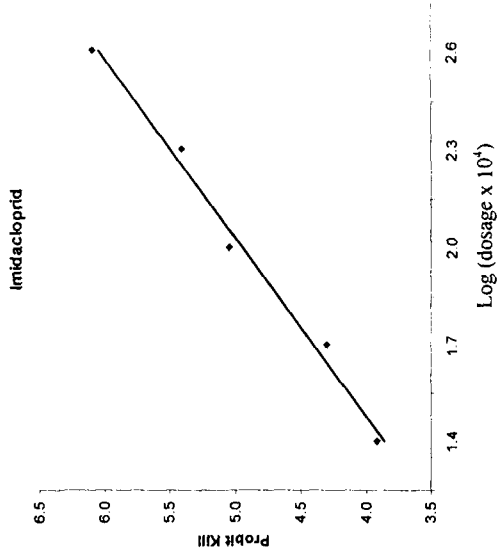


Fig 4.5d

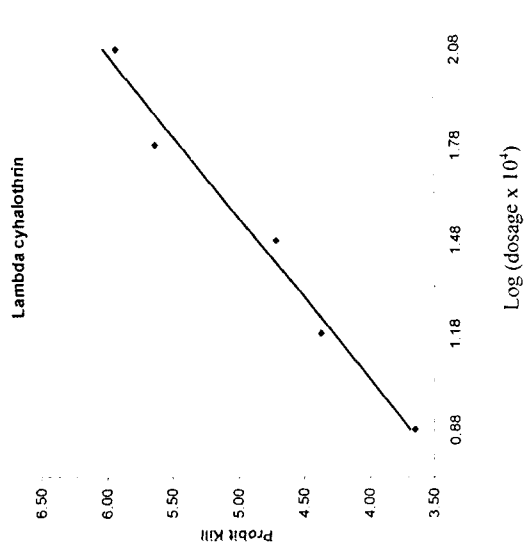


Fig 4.5e

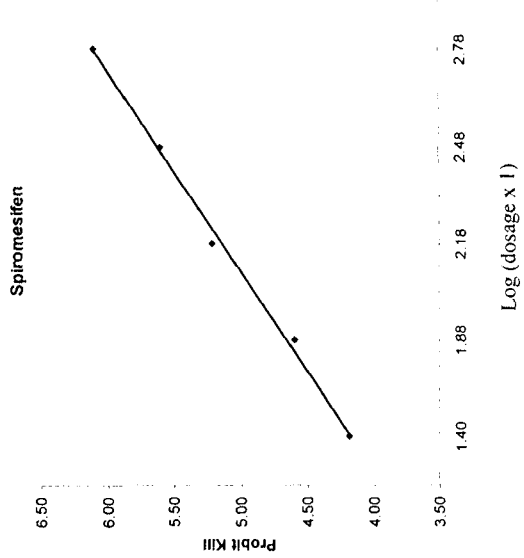


Fig 4.5f

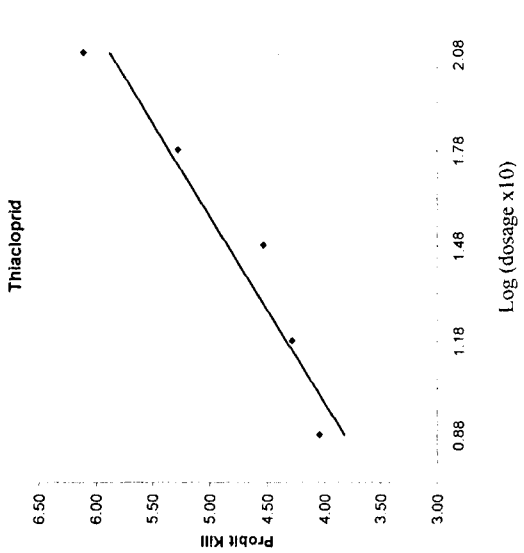


Fig. 4.5 Log dosage probit mortality response lines of *A. c. cerana* foragers to pesticides after 24 hours of topical application

It is observed from the Table 4.22 that a dose of $0.75 \mu\text{g bee}^{-1}$ of thiacloprid gave 16.95 per cent and $12.00 \mu\text{g bee}^{-1}$ gave 86.44 per cent corrected mortalities of *A. c. cerana* foragers. Control treatment gave 1.67 per cent mortality. The χ^2 test showed the homogeneity of test population with low value of $\chi^2_{\text{cal.}}$ (6.1648) compared to tabulated value of 7.80. The slope of LDP line was 1.6796 ± 0.2028 (Fig. 4.5f). The LD_{50} value was $3.8416 \mu\text{g bee}^{-1}$ with fiducial limits of $3.0867 - 4.7810 \mu\text{g bee}^{-1}$ whereas, the LD_{90} value was $22.2607 \mu\text{g bee}^{-1}$ with fiducial limits of $13.4310 - 36.8949 \mu\text{g bee}^{-1}$.

It is seen from the Table 4.23 that benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki* containing pesticides when administered orally to the foragers of *A. c. cerana* gave a maximum of 5.64, 5.86 and 4.74 per cent mortalities at the corresponding doses of 160.00, 6.00 and $80.00 \mu\text{g bee}^{-1}$, respectively after 48 hours of exposure. The untreated control did not record any mortality.

From the Table 4.24 and Fig. 4.6a and 4.6b it is seen that after 24 hours, per cent corrected mortalities of 17.24 and 82.76 were observed at doses of 0.0100 and $0.200 \mu\text{g bee}^{-1}$, respectively. Mortality in untreated control treatment was 3.33 per cent. At the same doses per cent corrected mortalities increased to 18.97 and 84.00, respectively after 48 hours of exposure. The LD_{50} and LD_{90} after 24 hours were 0.0402 (0.0309 – 0.0522) and 0.3611 (0.2009 – 0.6488) $\mu\text{g bee}^{-1}$, respectively those changed to 0.0359 (0.0274 – 0.0469) and 0.3281 (0.1832– 0.5880) $\mu\text{g bee}^{-1}$, respectively after 48 hours of exposure. The LDP lines framed for 24 and 48 hours of exposure gave the best fit with slopes of 1.3466 ± 0.1728 and 1.3330 ± 0.1755 , respectively.

Table 4.23 Dose mortality response of *A. c. cerana* foragers to pesticides (benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki*) after 24 and 48 hours of oral administration

Benzoylphenyl urea				Azadirachtin				<i>B. thuringiensis</i> subsp. <i>kurstaki</i>			
Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*	
	24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure
160.00	5.20	5.64	6.00	5.25	5.86	80.00	4.66	4.74	80.00	4.66	4.74
80.00	4.42	5.12	3.00	3.56	3.96	40.00	1.66	2.00	40.00	1.66	2.00
40.00	1.04	1.72	1.50	---	---	20.00	---	---	20.00	---	---
20.00	---	---	0.75	---	---	10.00	---	---	10.00	---	---
10.00	---	---	0.375	---	---	5.00	---	---	5.00	---	---

LD₅₀ > 160.00 µg bee⁻¹

LD₅₀ > 6.00 µg bee⁻¹

LD₅₀ > 80.00 µg bee⁻¹

Table 4.24 Dose mortality response of *A. c. cerana* foragers to spinosad after 24 and 48 hours of oral administration

24 hrs. exposure				48 hrs. exposure			
Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	%corrected mortality*	Empirical probit	Expected probit	% corrected mortality*	Empirical probit	Expected probit
200.00	2.3010	82.76	5.9463	5.94	84.00	5.9945	4.24
100.00	2.000	68.97	5.4959	5.53	70.69	5.5446	4.65
50.00	1.6990	53.45	5.0866	5.13	56.90	5.1764	5.18
20.00	1.3010	41.38	4.7827	4.59	43.10	4.8262	5.59
10.00	1.0000	17.24	4.0553	4.18	18.97	4.1221	6.00
χ ² (3df)	= 1.9215	χ ² (tab.) at 0.05 and 3df	= 7.80	χ ² (3df)	= 1.6626	χ ² (tab.) at 0.05 and 3df	= 7.80
b	= 1.3446	S.E. (b)	= 0.1728	b	= 1.3330	S.E. (b)	= 0.1755
Y= 2.8424 + 1.3446X				Y= 2.9279 + 1.3330X			

LD₅₀= 0.0402 (0.0309 – 0.0522) µg bee⁻¹

LD₉₀= 0.3611 (0.2009 – 0.6488) µg bee⁻¹

LD₅₀= 0.0359 (0.0274– 0.0469) µg bee⁻¹

LD₉₀= 0.3281 (0.1832– 0.5880) µg bee⁻¹

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Empirical probit; X= Log dosage; Figures in parenthesis are fiducial limits

Fig 4.6a

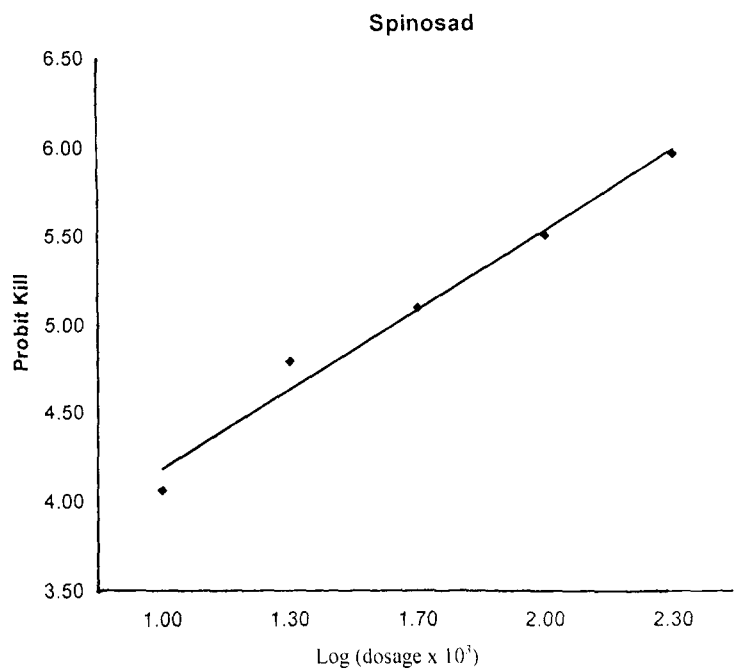


Fig 4.6b

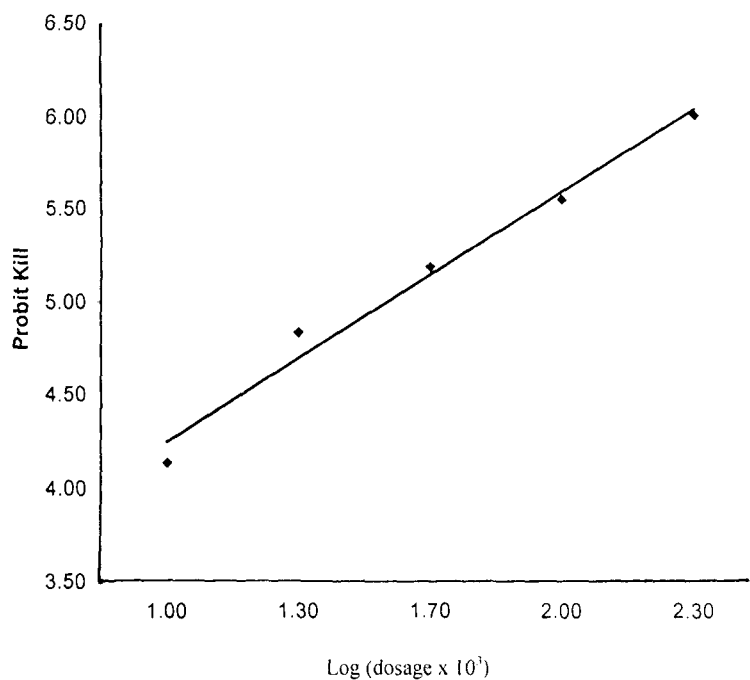


Fig. 4.6 Log dosage probit mortality response lines of *A. c. cerana* foragers to spinosad after 24 and 48 hours of topical application

4.1.2.2 *A. mellifera*

The mortality data of *A. mellifera* to the cypermethrin exposure are given in Table 4.25 and the LDP line is given in Fig. 4.7a. It is seen from the table that per cent corrected mortality of 20.00 occurred at dose of 0.0024 $\mu\text{g bee}^{-1}$ whereas, 88.33 per cent corrected mortality was recorded at dose of 0.0368 $\mu\text{g bee}^{-1}$ against zero per cent mortality recorded in control treatment.

The LD_{50} value was 0.0095 (0.0076 – 0.0119) $\mu\text{g bee}^{-1}$. The LD_{90} value was 0.0632 (0.0371 – 0.1077) $\mu\text{g bee}^{-1}$. The data were homogenous as $\chi^2_{\text{cal.}}$ value was low (3.4355) compared to table value of 7.80. The slope of LDP line was 1.5549 ± 0.1991 .

The mortality data of endosulfan are given in Table 4.26 and presented graphically in Fig. 4.7b. From the data it is observed that 2.00 $\mu\text{g bee}^{-1}$ dose of endosulfan gave 13.79 per cent corrected mortality after 24 hours of exposure. However, 24.00 $\mu\text{g bee}^{-1}$ dose gave 89.66 per cent corrected mortality. In control treatment mortality was 3.33 per cent. The LD_{50} and LD_{90} values were 7.1657 and 26.8021 $\mu\text{g bee}^{-1}$, respectively. The corresponding fiducial limits were 6.0757 – 8.4528 and 19.1426 – 37.5578 $\mu\text{g bee}^{-1}$, respectively. The $\chi^2_{\text{cal.}}$ value of 3.4484 was low compared to 7.80, the tabulated value. The slope of LDP line was 2.2370 ± 0.2429 .

It is observed from the Table 4.27 and Fig. 4.7c that imidacloprid doses of 0.00375 and 0.0600 $\mu\text{g bee}^{-1}$ recorded 16.67 and 86.67 per cent mortalities, respectively. Control treatment gave zero per cent mortality. The χ^2 test showed the homogeneity of test population as the calculated value of χ^2 was 1.0288, which was significantly lower than the table value of 7.80. The slope of LDP line was 1.7453 ± 0.1984 . The LD_{50} value was 0.0161 (0.0131 – 0.0197) $\mu\text{g bee}^{-1}$. The LD_{90} value was 0.0874 (0.0566 – 0.1349) $\mu\text{g bee}^{-1}$.

Table 4.25 Dosage mortality response of *A. mellifera* foragers to cypermethrin after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
36.80	1.5658	88.33	6.1916	5.98
18.40	1.2648	60.00	5.2533	5.49
9.60	0.9823	48.00	4.9582	5.04
4.80	0.6812	33.33	4.5692	4.55
2.40	0.3802	20.00	4.1584	4.07
χ ² (3df)	= 3.4355	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.5549	S.E. (b)	= 0.1991	
Y= 3.4820 + 1.5549X				
LD ₅₀ = 0.0095 (0.0076 – 0.0119) µg bee ⁻¹				
LD ₉₀ = 0.0632 (0.0371 – 0.1077) µg bee ⁻¹				

Table 4.26 Dosage mortality response of *A. mellifera* foragers to endosulfan after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 1)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
24.00	1.3802	89.66	6.2646	6.17
12.00	1.0792	65.52	5.3989	5.50
6.00	0.7742	48.28	4.9574	4.83
4.00	0.6021	20.69	4.1831	4.44
2.00	0.3010	13.79	3.9107	3.77
χ ² (3df)	= 3.4484	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 2.2370	S.E. (b)	= 0.2429	
Y= 3.0868 + 2.2370X				
LD ₅₀ = 7.1657 (6.0757 – 8.4528) µg bee ⁻¹				
LD ₉₀ = 26.8021 (19.1426 – 37.5578) µg bee ⁻¹				

Table 4.27 Dosage mortality response of *A. mellifera* foragers to imidacloprid after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
60.00	1.7782	86.67	6.1123	5.94
30.00	1.4771	66.67	5.4317	5.44
15.00	1.1761	46.67	4.9172	4.94
7.50	0.8751	26.67	4.3781	4.44
3.75	0.5705	16.67	4.0339	3.90
χ ² (3df)	= 1.0288	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.7453	S.E. (b)	= 0.1984	
Y= 2.8932 + 1.7453X				
LD ₅₀ = 0.0161 (0.0131 – 0.0197) µg bee ⁻¹				
LD ₉₀ = 0.0874 (0.0566 – 0.1349) µg bee ⁻¹				

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

The mortality data of lambda cyhalothrin recorded after 24 hours of exposure are tabulated in Table 4.28 and presented graphically in Fig. 4.7d. It was observed that 14.29 and 85.71 per cent corrected mortalities occurred at corresponding lambda cyhalothrin doses of 0.0020 and 0.0240 $\mu\text{g bee}^{-1}$, respectively. The χ^2 test showed the homogeneity of the test population as $\chi^2_{\text{cal.}}$ (2.3766) was significantly less than the $\chi^2_{\text{tab.}}$ (7.80). The LD_{50} and LD_{90} values were found to be 0.0061 and 0.0290 $\mu\text{g bee}^{-1}$, respectively. The fiducial limits of 0.0051- 0.0074 and 0.0193 – 0.0435 $\mu\text{g bee}^{-1}$ were recorded corresponding to LD_{50} and LD_{90} , respectively. The slope of LDP line was 1.8999 ± 0.2260 .

A dose of 25.00 $\mu\text{g bee}^{-1}$ of spiromesifen gave 20.69 per cent and 600.00 $\mu\text{g bee}^{-1}$ gave 75.86 per cent corrected mortality (Table 4.29). Control treatment gave 3.33 per cent mortality. The data were homogenous which was shown by χ^2 tests. The calculated value of χ^2 was 0.0923, which was significantly lower than the table value of 7.80. The LDP line had the slope of 1.0914 ± 0.1659 (fig. 4.7e). The LD_{50} value was 130.3387 $\mu\text{g bee}^{-1}$ with fiducial limits of 95.1036 – 178.6282 $\mu\text{g bee}^{-1}$. The LD_{90} value was 1946.6857 $\mu\text{g bee}^{-1}$ with fiducial limits of 838.1639 – 4521.2934 $\mu\text{g bee}^{-1}$.

The mortality data of *A. mellifera* foragers for thiacloprid are given in Table 4.30. From the table it is seen that mortality of 6.90 per cent was observed at dose of 1.1250 $\mu\text{g bee}^{-1}$ whereas, 84.48 per cent corrected mortality was recorded at dose of 18.00 $\mu\text{g bee}^{-1}$ against 3.33 per cent mortality recorded in control treatment. The LD_{50} value was computed to be 6.2934 $\mu\text{g bee}^{-1}$ with fiducial limits of 5.1591 – 7.6771 $\mu\text{g bee}^{-1}$. The LD_{90} value was recorded to be 29.3974 $\mu\text{g bee}^{-1}$ with fiducial limits of 19.7499 – 43.7576 $\mu\text{g bee}^{-1}$. The data were homogenous as indicated by $\chi^2_{\text{cal.}}$ value of 1.8325, which was significantly less than 7.80 of χ^2_{tab} at 5 per cent level of significance and 3df. The LDP line gave the best fit with slope of 1.9145 ± 0.2140 .

Table 4.28 Dosage mortality response of *A. mellifera* foragers to lambda cyhalothrin after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 10 ³)	Log dosage	% corrected mortality	Empirical probit	Expected probit
24.00	1.3802	85.71	6.0669	6.24
12.00	1.0792	75.00	5.6745	5.54
6.00	0.7782	46.43	4.9096	4.96
3.00	0.4771	32.14	4.5351	4.28
2.00	0.3010	14.29	3.9331	3.93
χ ² (3df)	= 2.3766	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 1.8999	S.E. (b)	= 0.2260	
Y= 3.5026 + 1.8999X				
LD ₅₀ = 0.0061 (0.0051 – 0.0074) µg bee ⁻¹				
LD ₉₀ = 0.0290 (0.0193 – 0.0435) µg bee ⁻¹				

Table 4.29 Dosage mortality response of *A. mellifera* foragers to spiromesifen after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 1)	Log dosage	%corrected mortality*	Empirical probit	Expected probit
600.00	2.7782	75.86	5.7031	5.72
300.00	2.4771	65.52	5.3994	5.39
150.00	2.1761	51.72	5.0431	5.06
75.00	1.8751	41.38	4.7827	4.73
25.00	1.3979	20.69	4.1831	4.21
χ ² (3df)	= 0.0923	χ ² (tab.) at 0.05 and 3df		= 7.80
b	= 1.0914	S.E. (b)	= 0.1659	
Y= 2.6915 + 1.9014X				
LD ₅₀ = 130.3387 (95.1036 – 178.6282) µg bee ⁻¹				
LD ₉₀ = 1946.6857 (838.1639 – 4521.2934) µg bee ⁻¹				

Table 4.30 Dosage mortality response of *A. mellifera* foragers to thiacloprid after 24 hours of oral administration

Dosage (µg bee ⁻¹ x 10)	Log dosage	% corrected mortality	Empirical probit	Expected probit
180.00	2.2553	84.48	6.0152	5.04
90.00	1.9542	63.79	5.3531	4.64
45.00	1.6532	31.03	4.5049	4.24
22.50	1.3522	20.69	4.1813	3.84
11.25	1.0512	6.90	3.5167	3.44
χ ² (3df)	= 1.8325	χ ² (tab.) at 0.05 and 3df		=7.80
b	= 1.9145	S.E. (b)	= 0.2140	
Y= 1.5561 + 1.9145X				
LD ₅₀ = 6.2934 (5.1591 – 7.6771) µg bee ⁻¹				
LD ₉₀ = 29.3974 (19.7499 – 43.7576) µg bee ⁻¹				

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

Fig 4.7a

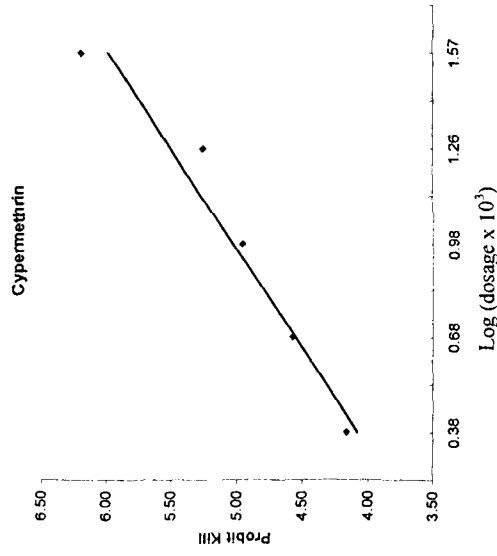


Fig 4.7b

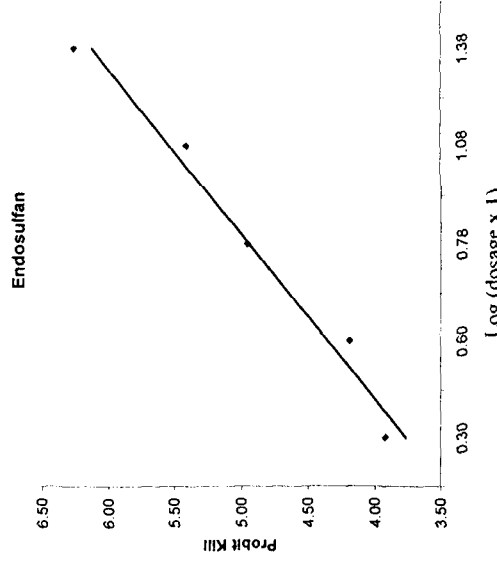


Fig 4.7c

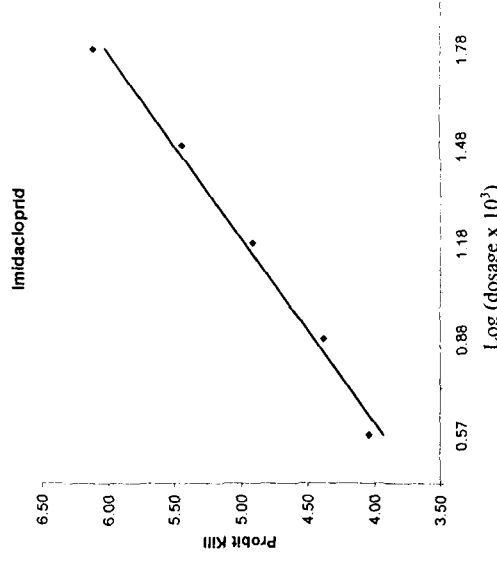


Fig 4.7d

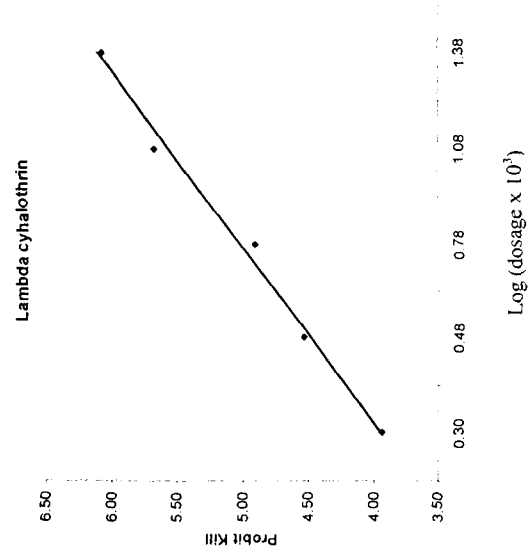


Fig 4.7e

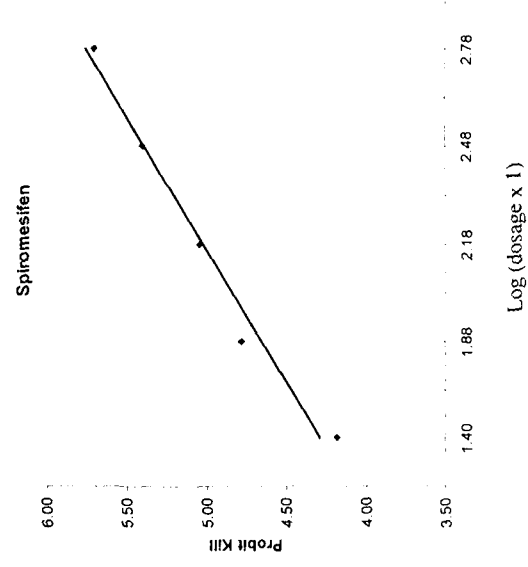


Fig 4.7f

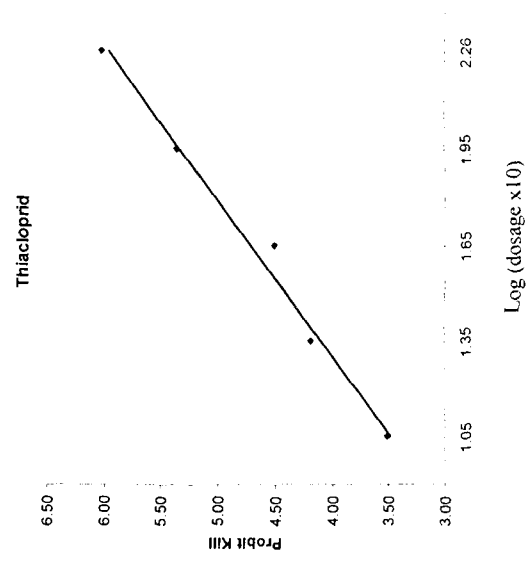


Fig. 4.7 Log dosage probit mortality response lines of *A. mellifera* foragers to pesticides after 24 hours of topical application

Benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki* containing pesticides when administered orally to the foragers of *A. mellifera* gave a maximum of 5.87, 3.56 and 4.68 per cent mortalities at the tested doses of 240.00, 9.00 and 120.00 $\mu\text{g bee}^{-1}$, respectively after 48 hours of exposure (Table 4.31), which showed that these pesticides were safe to honey bees. Per cent mortalities in corresponding untreated control treatments were 0.00 per cent.

Mortalities of *A. mellifera* foragers were recorded after 24 and 48 hours of exposure to spinosad. The data are presented in Table 4.32 and presented graphically in fig. 4.8a and 4.8b. It was recorded that after 24 hours of exposure, 16.67 to 85.00 per cent corrected mortalities were recorded at doses ranging from 0.0150 to 0.2400 $\mu\text{g bee}^{-1}$, respectively. At the same doses mortality values changed to 17.67 and 85.96 per cent after 48 hours of exposure. At both the exposure intervals, χ^2_{cal} values of 0.2993 and 0.4185 were significantly less than χ^2_{tab} of 7.80. The LD_{50} values of 0.0604 and 0.0577 $\mu\text{g bee}^{-1}$ were recorded after 24 and 48 hours, respectively. Whereas, LD_{90} values changed from 0.3752 to 0.3634 $\mu\text{g bee}^{-1}$. The slope of LDP lines were 1.6153 ± 0.1984 and 1.6037 ± 0.1980 for 24 and 48 hours of exposure, respectively.

On the basis of relative LD_{50} (relative to endosulfan), it was found that lambda cyhalothrin (0.0016) showed highest contact toxicity to the foragers of *A. c. cerana* followed by cypermethrin (0.0021), imidacloprid (0.0039), spinosad (0.0074), endosulfan (1.0000), thiacloprid (2.8349) and spiromesifen (17.7185). However, azadirachtin (>0.0939), *B. thuringiensis* subsp. *kurstaki* (>1.2520) and benzoylphenyl urea (>2.5040) were harmless (Table 4.33).

Table 4.31 Dosage mortality response of *A. mellifera* foragers to pesticides (benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki*) after 24 and 48 hours of oral administration

Benzoylphenyl urea				Azadirachtin				<i>B. thuringiensis</i> subsp. <i>kurstaki</i>			
Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*		Dosage (µg bee ⁻¹ x 1)	%corrected mortality*	
	24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure		24 hrs. exposure	48 hrs. exposure
240.00	5.85	5.87	9.00	3.41	3.56	120.00	4.65	4.68	120.00	4.65	4.68
120.00	4.75	4.75	4.50	1.17	1.24	60.00	1.05	1.51	60.00	1.05	1.51
60.00	---	---	2.25	---	---	30.00	---	---	30.00	---	---
30.00	---	---	1.12	---	---	15.00	---	---	15.00	---	---
15.00	---	---	0.56	---	---	7.50	---	---	7.50	---	---

LD₅₀ > 240.00 µg bee⁻¹

LD₅₀ > 9.00 µg bee⁻¹

LD₅₀ > 120.00 µg bee⁻¹

Table 4.32 Dosage mortality response of *A. mellifera* foragers to spinosad after 24 and 48 hours of oral administration

Dosage (µg bee ⁻¹ x 10 ²)	24 hrs. exposure				48 hrs. exposure			
	Log dosage	%corrected mortality*	Empirical probit	Expected probit	% corrected mortality*	Empirical probit	Expected probit	
24.00	1.3802	85.00	6.0364	5.98	85.96	6.0803	6.01	
12.00	1.0792	66.67	5.4316	5.49	66.67	5.4316	5.51	
6.00	0.7782	48.33	4.9582	5.00	50.27	5.0075	5.02	
3.00	0.4771	31.67	4.5239	4.51	33.00	4.5001	4.52	
1.50	0.1761	16.67	4.0339	4.01	17.67	4.0731	4.03	
χ ² (3df)	= 0.2993	χ ² (tab.) at 0.05 and 3df	= 7.80	χ ² (3df)	= 0.4185	χ ² (tab.) at 0.05 and 3df	= 7.80	
b	= 1.6153	S.E. (b)	= 0.1984	b	= 1.6037	S.E. (b)	= 0.1988	
Y=3.7387 + 1.6153X				Y= 3.7791 + 1.6037X				

LD₅₀= 0.0604 (0.0484 – 0.0753) µg bee⁻¹
LD90=0.3752 (0.2291 – 0.6146) µg bee⁻¹

LD₅₀= 0.0577 (0.0462 – 0.0721) µg bee⁻¹
LD90= 0.3634 (0.2228 – 0.5933) µg bee⁻¹

*Total number of insects treated 60; b= Slope of log dose probit line; S.E. = Standard error; Y= Imperical probit; X= Log dosage; Figures in parenthesis are fiducial limits

Fig 4.8a

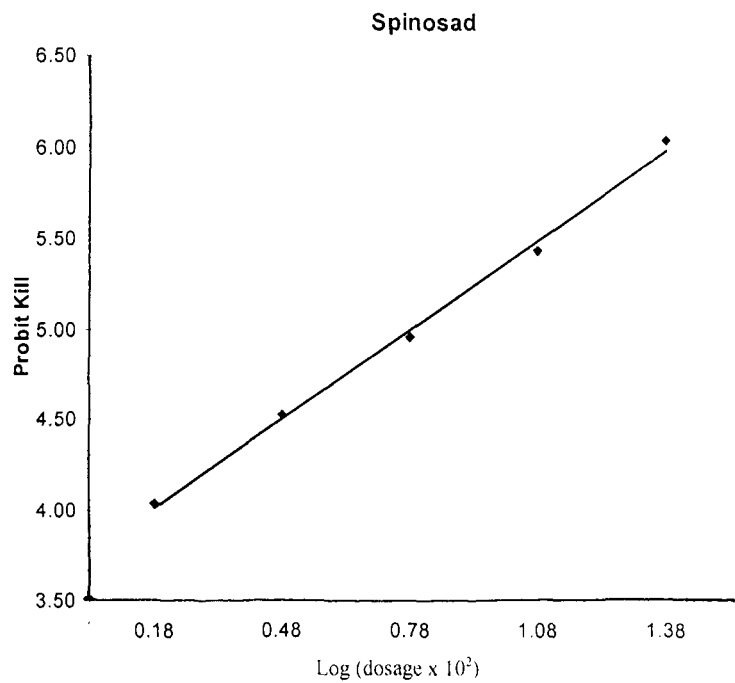


Fig 4.8b

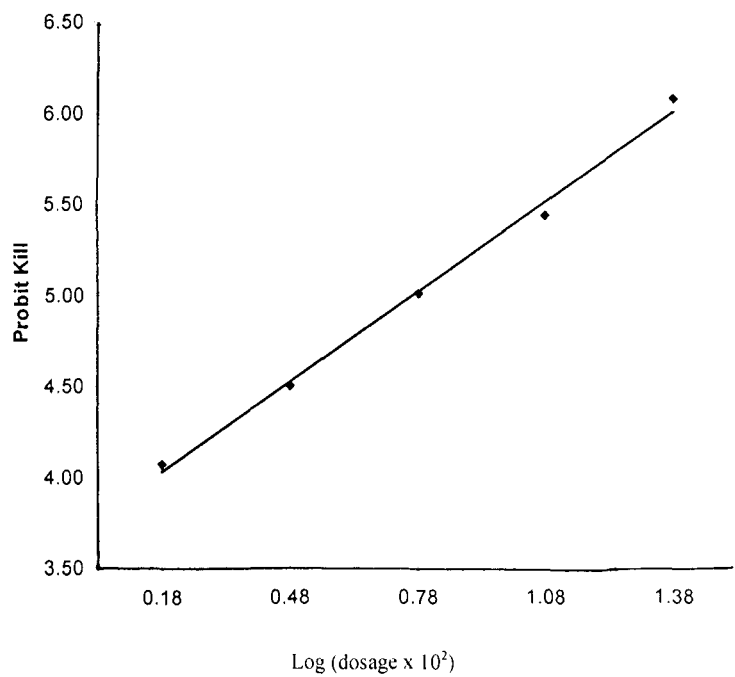


Fig. 4.8 Log dosage probit mortality response lines of *A. mellifera* foragers to spinosad after 24 and 48 hours of topical application

Almost similar trend was observed for the *A. mellifera* (Table 4.34). Lambda cyhalothrin (0.0027) was highly toxic followed by cypermethrin (0.0048), spinosad (0.0094), imidacloprid (0.0128), endosulfan (1.0000), thiacloprid (2.9455) and spiromesifen (17.6205). However rest of the pesticides, azadirachtin (>0.0828), *B. thuringiensis* subsp. *kurstaki* (>1.1036) and benzoylphenyl urea (>2.2072) were found safe.

Lambda cyhalothrin (0.0005) showed relatively high stomach toxicity followed by cypermethrin (0.0007), imidacloprid (0.0015), spinosad (0.0057), thiacloprid (0.5485), endosulfan (1.0000) and spiromesifen (15.9483). Whereas, azadirachtin (>0.8566), *B. thuringiensis* subsp. *kurstaki* (>11.4212) and benzoylphenyl urea (>22.8437) showed low toxicity to *A. c. cerana* foragers (Table 4.33). Similarly, oral toxicity of lambda cyhalothrin with respect to endosulfan was found highest (0.0008) to the *A. mellifera* followed by cypermethrin (0.0013), imidacloprid (0.0022), spinosad (0.0084), thiacloprid (0.8783), endosulfan (1.0000) and spiromesifen (18.1892). Azadirachtin (>1.2559), *B. thuringiensis* subsp. *kurstaki* (>16.7464) and benzoylphenyl urea (>33.4928) were harmless (Table 4.34).

4.2 FIELD WEATHERED TOXICITY OF PESTICIDES AGAINST HONEY BEES

The results of the experiments conducted at Palampur during *rabi* season 2003-04 and 2004-05 for determining the field weathered toxicity of pesticides on mustard bloom against *A. c. cerana* and *A. mellifera* foragers are presented in Tables 4.35 - 4.36 and 4.37 - 4.38. The mortality data were subjected to angular transformation and were statistically analyzed. The results of relative persistence toxicity of different pesticides against *A. c.*

Table 4.33 Relative safety of pesticides to *A. c. cerana* after 24 hours of topical and oral administration

Sr. no.	Pesticides	Toxicity after topical application		Relative* LD ₅₀	Calculated LD ₅₀ (µg gbee ⁻¹)		Toxicity after oral administration		Relative* LD ₅₀	Calculated LD ₅₀ (µg gbee ⁻¹)	
		LD ₅₀ (µg bee ⁻¹)	LD ₉₀ (µg bee ⁻¹)		LD ₅₀ (µg gbee ⁻¹)		LD ₅₀ (µg bee ⁻¹)	LD ₉₀ (µg bee ⁻¹)			
1.	Cypermethrin	0.0171	0.1155	0.0021	0.2192		0.0047	0.0322	0.0007		0.0603
2.	Endosulfan	7.9871	52.1868	1.0000	102.3987		7.0041	23.3278	1.0000		89.7962
3.	Imidacloprid	0.0317	0.2122	0.0039	0.4064		0.0107	0.0547	0.0015		0.1372
4.	Lambda cyhalothrin	0.0124	0.0783	0.0016	0.1589		0.0035	0.0163	0.0005		0.0449
5.	Spiromesifen	141.5198	1543.7161	17.7185	1814.3564		111.7038	925.5374	15.9483		1432.1000
6.	Thiacloprid	22.6433	107.3767	2.8349	290.2987		3.8416	22.2607	0.5485		49.2513
7.	Benzoylphenyl urea	>20.00	---	>2.5040	>2.5040		>160.00	---	>22.8437		>22.8438
8.	Azadirachtin	>0.75	---	>0.0939	>0.0939		>6.0000	---	>0.8566		>0.8566
9.	<i>B. thuringiensis</i> subsp. <i>kurstaki</i>	>10.00	---	>1.2520	>1.2520		>80.00	---	>11.4212		>11.4212
10.	Spinosad	0.0590	0.3525	0.0074	0.7564		0.0402	0.3611	0.0057		0.5154

Average weight of *A. c. cerana* foragers= 78 ± 2.1 mg

* Relative to endosulfan

Table 4.34 Relative safety of pesticides to *A. mellifera* after 24 hours of topical and oral administration

Sr. no.	Pesticides	Toxicity after topical application		Relative* LD ₅₀	Calculated LD ₅₀ (µg gbee ⁻¹)	Toxicity after oral administration		Relative* LD ₅₀	Calculated LD ₅₀ (µg gbee ⁻¹)
		LD ₅₀ (µg bee ⁻¹)	LD ₉₀ (µg bee ⁻¹)			LD ₅₀ (µg bee ⁻¹)	LD ₉₀ (µg bee ⁻¹)		
1.	Cypermethrin	0.0438	0.2269	0.0048	0.3650	0.0095	0.0632	0.0013	0.0792
2.	Endosulfan	9.0614	44.8833	1.0000	75.5117	7.1657	26.8021	1.0000	59.7142
3.	Imidacloprid	0.1157	0.9864	0.0128	0.9642	0.0161	0.0874	0.0022	0.1342
4.	Lambda cyhalothrin	0.0241	0.1926	0.0027	0.2008	0.0061	0.0290	0.0008	0.0508
5.	Spiromesifen	159.7212	2007.9101	17.6265	1331.0100	130.3387	1946.6857	18.1892	1086.1558
6.	Thiacloprid	26.6903	144.7548	2.9455	222.4191	6.2934	29.3974	0.8783	52.4450
7.	Benzoylphenyl urea	>20.00	---	>2.2072	>2.2072	>240.00	---	>33.4928	>33.4929
8.	Azadirachtin	>0.75	---	>0.0828	>0.0828	>9.00	---	>1.2559	>1.2559
9.	<i>B. thuringiensis</i> subsp. <i>kurstaki</i>	>10.00	---	>1.1036	>1.1036	>120.00	---	>16.7464	>16.7464
10.	Spinosad	0.0851	0.7352	0.0094	0.7092	0.0604	0.3752	0.0084	0.5033

Average weight of *A. mellifera* foragers = 120 ± 2.3 mg
* Relative to endosulfan

cerana and *A. mellifera* are also given. The data on the prevailing weather conditions are given in Appendix 1 and 2.

4.2.1 *A. c. cerana*

The field weathered toxicity of various pesticides to foragers of *A. c. cerana* were determined during 2003-04 and 2004-05. The mortality data are given in Tables 4.35 and 4.36. Results for the first year 2003-04 (Table 4.35) revealed that initial deposits of cypermethrin, cypermethrin+ *B. thuringiensis* subsp. *kurstaki*, imidacloprid (foliar spray), lambda cyhalothrin and lambda cyhalothrin + azadirachtin were highly toxic, caused 100.00 per cent mortality of *A. c. cerana* foragers, whereas endosulfan (32.33 % mortality) was intermediate. The foliar applications of *B. thuringiensis* subsp. *kurstaki* (6.37% mortality), spiromesifen (7.65 % mortality) and seed treatment of imidacloprid were non toxic. At 120 hours of spray, it was found that lambda cyhalothrin caused significantly high mortality (33.67%), which was followed by cypermethrin (29.67%), lambda cyhalothrin+ azadirachtin (18.98%), imidacloprid foliar treatment (11.83%) and cypermethrin + *B. thuringiensis* subsp. *kurstaki* (9.67%). The toxicity of these pesticides decreased with time and it reduced to 0.00 at 240 hours of spray. It is seen that during first year cypermethrin had slightly higher PT value (9407.97) than lambda cyhalothrin (9355.99), which was followed by lambda cyhalothrin + azadirachtin (8243.07), cypermethrin + *B. thuringiensis* subsp. *kurstaki* (8076.00), imidacloprid foliar treatment (6796.80), endosulfan (140.41), *B. thuringiensis* subsp. *kurstaki* (17.37), spiromesifen (6.16) and imidacloprid seed treatment (0.00). It was also found that with respect to endosulfan, cypermethrin and lambda cyhalothrin were respectively, 67.00 and 66.63 times more toxic.

Table 4.35 Field weathered toxicity of pesticides against *A. c. cerana* foragers during *rabi*, 2003-04

Pesticides (g a.i. ha ⁻¹)	Mean corrected per cent mortality (hrs.)*										Persistent Toxicity		LT ₅₀
	0	1	4	8	24	48	72	120	PT (hrs.)	(X)			
<i>B. thuringiensis</i> subsp. <i>kurstaki</i> (78.75)	6.37 (4.60)	4.33 (12.00)	2.33 (8.71)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	17.37	0.12			---
Cypermethrin (75.00)	100.00 (89.96)	100.00 (89.96)	96.32 (78.95)	91.86 (73.41)	82.67 (65.37)	70.33 (56.97)	56.33 (48.61)	29.67 (32.99)	9407.97	67.00			138.06
Cypermethrin + <i>B. thuringiensis</i> subsp. <i>kurstaki</i> (37.50+39.37)	100.00 (89.96)	100.00 (89.96)	92.32 (73.95)	81.01 (64.16)	73.00 (58.68)	52.67 (46.51)	29.73 (33.02)	9.67 (18.10)	8076.00	57.52			46.20
Endosulfan (525.00)	32.33 (34.63)	19.67 (26.30)	11.87 (20.13)	6.33 (14.53)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	140.41	1.00			---
Imidacloprid (foliar application) (25.00)	100.00 (89.96)	92.95 (74.57)	81.67 (64.63)	70.33 (56.97)	45.00 (42.17)	32.67 (34.83)	18.67 (25.58)	11.83 (20.10)	6796.80	48.41			21.02
Imidacloprid (seed treatment)(21.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	---	---			---
Lambda cyhalothrin (75.00)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	92.62 (74.21)	84.33 (66.65)	66.78 (54.78)	46.33 (51.92)	33.67 (35.45)	9355.99	66.63			72.81
Lambda cyhalothrin + Azadirachtin (37.50+1.687)	100.00 (89.96)	100.00 (89.96)	91.67 (73.19)	81.70 (66.64)	68.14 (55.61)	57.72 (49.42)	31.67 (34.23)	18.98 (25.81)	8243.07	58.70			54.90
Spiromesifen (225.00)	7.65 (16.04)	4.67 (12.47)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	6.16	0.04			---
CD (p=0.05)	0.73	0.70	1.94	1.83	1.33	1.36	1.30	0.32	---	---			---

* Average of three replications; Values in parentheses are arc sine transformed values; LT₅₀ = Median lethal time; PT = Persistent toxicity; X= Relative PT with respect to endosulfan

The LT_{50} values were found to be 138.06, 46.20, 21.02, 72.81 and 54.90 hours for cypermethrin, cypermethrin + *B. thuringiensis* subsp. *kurstaki*, imidacloprid, lambda cyhalothrin and lambda cyhalothrin + azadirachtin, respectively.

During 2004-05 (Table 4.36), it is seen that initial deposits of cypermethrin, cypermethrin+ *B. thuringiensis* subsp. *kurstaki*, imidacloprid (foliar application), lambda cyhalothrin and lambda cyhalothrin + azadirachtin were highly toxic causing 97.33 to 100.00 per cent mortality of *A. c. cerana* foragers. Endosulfan (28.48 % mortality) was intermediate. The foliar applications of *B. thuringiensis* subsp. *kurstaki* (3.62 % mortality), spiromesifen (4.67 % mortality) and seed treatment of imidacloprid were non toxic. It was found that toxicity of all the pesticides decreased with time up to 120 hours of spray, thereafter, it became harmless to the foragers of *A. c. cerana*. Lambda cyhalothrin treatment (25.00 % mortality) showed significantly higher toxicity than cypermethrin (22.67 % mortality) at 120 hours of spray. Toxicity posed by foliar treatment of imidacloprid (9.67 % mortality) was statistically at par with lambda cyhalothrin + azadirachtin (10.00 % mortality). Cypermethrin + *B. thuringiensis* subsp. *kurstaki* caused 3.35 per cent mortality.

Cypermethrin had slightly higher PT value (8904.55) than lambda cyhalothrin (8821.48), which was followed by lambda cyhalothrin + azadirachtin (7564.65), cypermethrin + *B. thuringiensis* subsp. *kurstaki* (7479.45), imidacloprid foliar treatment (6275.85), endosulfan (121.62), spiromesifen (3.50), *B. thuringiensis* subsp. *kurstaki* (2.97) and imidacloprid seed treatment (0.00) during 2004-05. It was also found that with respect to endosulfan, cypermethrin and lambda cyhalothrin were 73.22 and 72.53 times more toxic to *A. c. cerana*.

Table 4.36 Field weathered toxicity of pesticides against *A. c. cerana* foragers during *rabi*, 2004-05

Pesticides (g a.i. ha ⁻¹)	Mean corrected per cent mortality (hrs.)*										Persistent Toxicity		LT ₅₀
	0	1	4	8	24	48	72	120	PT (hrs.)	(X)			
<i>B. thuringiensis</i> subsp. <i>kurstaki</i> (78.75)	3.62 (10.96)	2.33 (8.77)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	2.97	0.02			---
Cypermethrin (75.00)	100.00 (89.96)	97.63 (81.12)	90.67 (72.18)	88.81 (70.44)	78.86 (62.60)	65.33 (53.90)	49.67 (44.79)	22.67 (28.42)	8904.55	73.22			98.46
Cypermethrin+ <i>B. thuringiensis</i> subsp. <i>kurstaki</i> (37.50+39.37)	100.00 (89.96)	92.44 (74.02)	84.12 (66.49)	78.80 (62.00)	69.49 (56.44)	50.00 (44.98)	21.00 (27.24)	3.35 (10.39)	7479.45	61.49			33.20
Endosulfan (525.00)	28.48 (32.24)	19.67 (26.31)	12.33 (20.54)	4.33 (12.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	121.62	1.00			---
Imidacloprid (foliar application) (25.00)	97.33 (80.88)	90.32 (71.85)	77.97 (61.98)	62.21 (52.04)	40.00 (39.21)	25.22 (30.13)	15.67 (23.30)	9.67 (18.08)	6275.85	57.60			14.07
Imidacloprid (seed treatment)(21.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	---	---			---
Lambda cyhalothrin (75.00)	100.00 (89.96)	100.00 (89.96)	94.92 (76.94)	84.32 (66.65)	76.00 (60.64)	62.08 (51.97)	45.77 (42.55)	25.00 (29.97)	8821.48	72.53			88.79
Lambda cyhalothrin + Azadirachtin (37.50+1.687)	100.00 (89.96)	95.94 (78.35)	86.62 (68.61)	79.00 (62.71)	62.00 (51.92)	45.09 (42.16)	25.33 (30.20)	10.00 (18.70)	7564.65	62.19			33.97
Spiromesifen (225.00)	4.67 (12.46)	2.33 (4.71)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.50	0.03			---
CD (p=0.05)	1.85	0.89	2.03	1.88	1.61	1.10	1.51	1.34	---	---			---

* Average of three replications; Values in parentheses are arc sine transformed values; LT₅₀ = Median lethal time; PT = Persistent toxicity; X= Relative PT with respect to endosulfan

The LT_{50} values for cypermethrin, cypermethrin + *B. thuringiensis* subsp. *kurstaki*, imidacloprid, lambda cyhalothrin and lambda cyhalothrin + azadirachtin were estimated to be 98.46, 33.20, 14.07, 88.79 and 33.97 hours, respectively.

Data for both the years (2003-04 and 2004-05) were pooled. It is evident from the Table 4.37 and fig. 4.9 that initial deposits of cypermethrin, cypermethrin+ *B. thuringiensis* subsp. *kurstaki*, imidacloprid (foliar application), lambda cyhalothrin and lambda cyhalothrin + azadirachtin were highly toxic causing 98.66 to 100.00 per cent mortality of *A. c. cerana* foragers. Endosulfan (30.40 % mortality) was intermediate. The foliar applications of *B. thuringiensis* subsp. *kurstaki* (4.99 % mortality), spiromesifen (6.16 % mortality) and seed treatment of imidacloprid were non toxic. It was found that toxicity of all the pesticides decreased with time up to 120 hours of spray, thereafter, it became non toxic to foragers of *A. c. cerana*. At 120 hours of spray the lambda cyhalothrin treatment (29.33 % mortality) showed significantly higher toxicity than cypermethrin (26.17 % mortality), which was followed by lambda cyhalothrin + azadirachtin (14.65 % mortality) and imidacloprid (10.75 % mortality). Cypermethrin + *B. thuringiensis* subsp. *kurstaki* treatment caused 6.51 per cent mortality.

Lambda cyhalothrin (9280.95) had the highest PT value followed by cypermethrin (9156.00), lambda cyhalothrin + azadirachtin (7906.20), cypermethrin + *B. thuringiensis* subsp. *kurstaki* (7775.70), imidacloprid foliar treatment (6536.10), endosulfan (135.00), *B. thuringiensis* subsp. *kurstaki* (12.64), spiromesifen (4.83) and imidacloprid seed treatment (0.00). It was also observed that lambda cyhalothrin and cypermethrin were respectively, 68.75 and 67.82 times more toxic than endosulfan.

Table 4.37 Field weathered toxicity of pesticides against *A. c. cerana* foragers during *rabi*, 2003-04 and 2004-05[#]

Pesticides g a.i. ha ⁻¹	Mean corrected per cent mortality (hrs.)*								Persistent Toxicity		LT ₅₀
	0	1	4	8	24	48	72	120	PT (hrs.)	(X)	
<i>B. thuringiensis</i> subsp. <i>kurstaki</i> (78.75)	4.99 (12.78)	3.33 (10.38)	1.16 (4.35)	---	---	---	---	---	12.64	0.09	---
Cypermethrin (75.00)	100.00 (89.96)	98.81 (85.54)	93.50 (75.57)	90.33 (71.93)	80.76 (63.98)	67.83 (55.43)	53.00 (46.70)	26.17 (30.70)	9156.00	67.82	115.36
Cypermethrin+ <i>B. thuringiensis</i> subsp. <i>kurstaki</i> (37.50+39.37)	100.00 (89.96)	96.22 (81.99)	88.22 (70.22)	79.50 (63.08)	71.24 (57.56)	51.33 (45.74)	25.36 (30.13)	6.51 (14.25)	7775.70	57.59	39.41
Endosulfan (525.00)	30.40 (33.43)	19.67 (26.31)	12.10 (20.33)	5.33 (13.27)	---	---	---	---	135.00	1.00	---
Imidacloprid (foliar application) (25.00)	98.66 (85.42)	91.63 (73.21)	79.82 (63.30)	66.27 (54.51)	42.50 (40.66)	28.94 (32.48)	17.17 (24.44)	10.75 (19.09)	6536.10	48.42	17.79
Imidacloprid (seed treatment)(21.00)	---	---	---	---	---	---	---	---	---	---	---
Lambda cyhalothrin (75.00)	100.00 (89.96)	100.00 (89.96)	97.46 (83.45)	88.47 (70.43)	80.16 (63.65)	64.43 (53.37)	58.88 (47.23)	29.33 (32.71)	9280.95	68.75	119.89
Lambda cyhalothrin + Azadirachtin (37.50+1.687)	100.00 (89.96)	97.97 (84.15)	89.14 (70.90)	80.35 (63.68)	65.07 (53.77)	51.40 (45.79)	28.50 (32.21)	14.65 (22.26)	7906.20	58.56	43.78
Spiromesifen (225.00)	6.16 (14.25)	3.50 (10.59)	---	---	---	---	---	---	4.83	0.04	---
CD (p=0.05) With in Environment	NS	NS	NS	NS	NS	NS	NS	NS	---	---	---
Environment	0.47	0.27	0.68	0.70	0.60	0.50	0.58	0.40	---	---	---
Treatment	0.95	0.54	1.28	1.22	0.96	0.80	0.91	0.63	---	---	---
Environment x Treatment	1.34	0.76	1.81	1.73	1.36	1.13	1.29	0.89	---	---	---

* Average of three replications; Values in parentheses are arc sine transformed values; LT₅₀ = Median lethal time; PT = Persistent toxicity; X= Relative PT with respect to endosulfan; [#] Pooled data

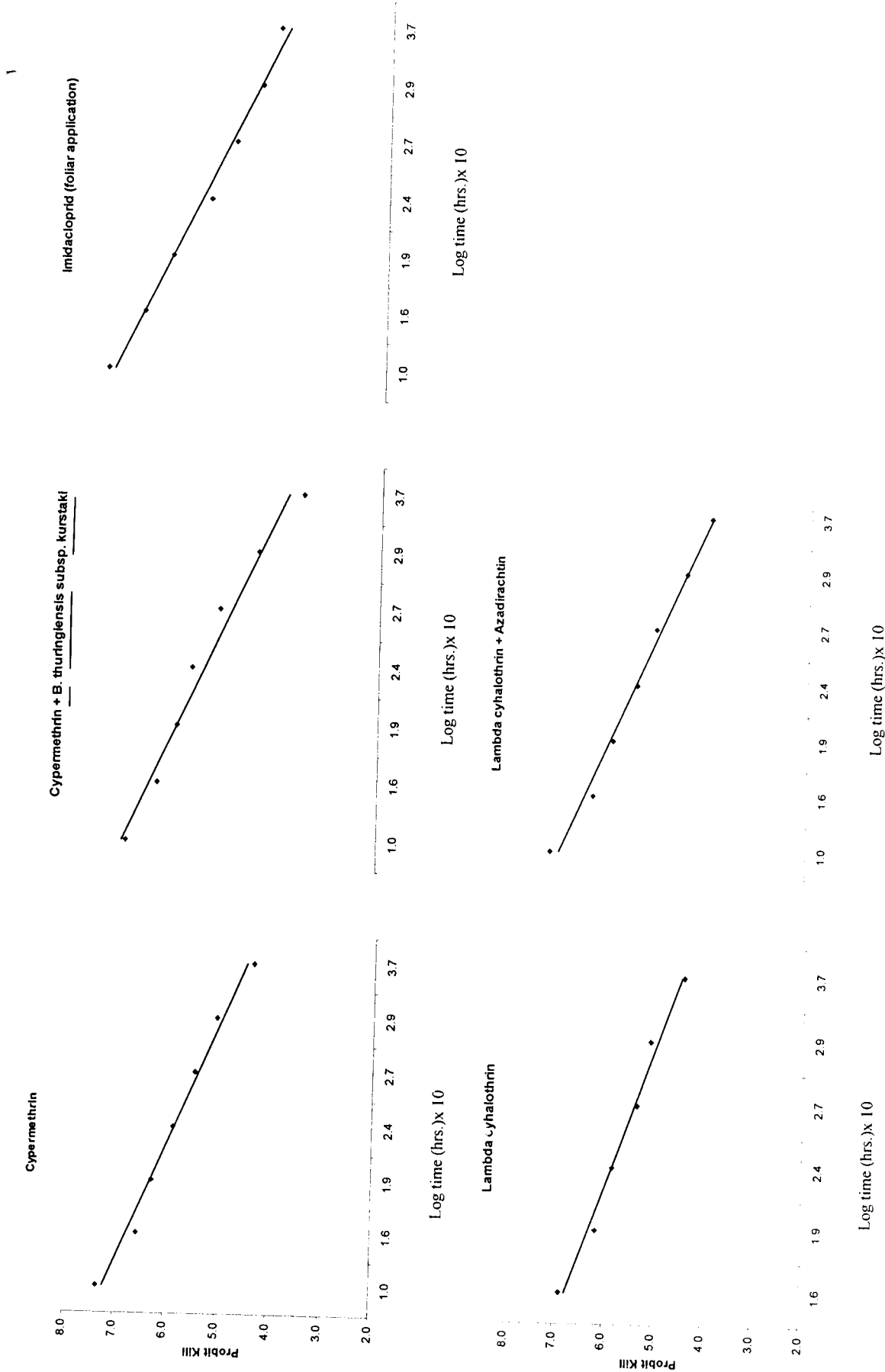


Fig. 4.9 Log time probit mortality response lines of *A. c. cerana* foragers to pesticide treatments during *rabi*, 2003-04 and 2004-05 (pooled)

The LT_{50} values for cypermethrin, cypermethrin + *B. thuringiensis* subsp. *kurstaki*, imidacloprid, lambda cyhalothrin and lambda cyhalothrin + azadirachtin were estimated to be 115.36, 39.41, 17.79, 119.89 and 43.78 hours, respectively.

4.2.2 *A. mellifera*

From the Table 4.38, it is seen that initial deposits of cypermethrin, cypermethrin + *B. thuringiensis* subsp. *kurstaki*, imidacloprid foliar treatment, lambda cyhalothrin and lambda cyhalothrin + azadirachtin were highly toxic showing cent per cent mortality of *A. mellifera* foragers in laboratory bioassay. The toxicity decreased thereafter, and at 240 hours of treatment no toxicity was recorded. At 120 hours of spray, it was found that lambda cyhalothrin (29.33 % mortality) treatment was highly toxic followed by cypermethrin (20.01 % mortality), lambda cyhalothrin + azadirachtin (12.00 % mortality) imidacloprid foliar treatment (10.33 % mortality) and cypermethrin + *B. thuringiensis* subsp. *kurstaki* (4.06 % mortality). Endosulfan treatment (31.33 % mortality) was intermediate in showing toxicity towards *A. mellifera* foragers, which decreased to zero at 24 hours of treatment. *B. thuringiensis* subsp. *kurstaki* (5.15 % mortality), spiromesifen (7.10 % mortality) and imidacloprid seed treatment were non toxic.

The highest PT value (8950.18) was observed for cypermethrin, which was followed by lambda cyhalothrin (8843.35), cypermethrin + *B. thuringiensis* subsp. *kurstaki* (7479.45), lambda cyhalothrin + azadirachtin (7108.35), imidacloprid foliar treatment (6419.70), endosulfan (127.74), spiromesifen (5.23), *B. thuringiensis* subsp. *kurstaki* (3.57) and imidacloprid seed treatment (0.00). It was also found that with respect to endosulfan, cypermethrin and lambda cyhalothrin were 70.07 and 69.23 times more toxic.

Table 4.38 Field weathered toxicity of pesticides against *A. mellifera* foragers during rabi, 2003-04

Pesticides g a.i. ha ⁻¹	Mean corrected per cent mortality (hrs.)*								Persistent Toxicity		LT ₅₀
	0	1	4	8	24	48	72	120	PT (hrs.)	(X)	
<i>B. thuringiensis</i> subsp. <i>kurstaki</i> (78.75)	5.15 (13.11)	2.00 (8.11)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.57	0.03	---
Cypermethrin (75.00)	100.00 (89.96)	100.00 (89.96)	94.26 (76.12)	88.00 (69.71)	81.21 (64.28)	63.88 (53.03)	49.34 (44.60)	20.01 (26.54)	8950.18	70.07	90.08
Cypermethrin+ <i>B. thuringiensis</i> subsp. <i>kurstaki</i> (37.50+39.37)	100.00 (89.96)	95.00 (77.16)	81.67 (64.63)	74.24 (59.47)	66.11 (54.37)	44.07 (41.57)	21.00 (27.24)	4.06 (11.56)	7479.45	61.49	28.86
Endosulfan (525.00)	31.33 (34.01)	18.33 (25.33)	10.81 (19.18)	3.40 (10.57)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	127.74	1.00	---
Imidacloprid (foliar application) (25.00)	100.00 (89.96)	89.67 (71.24)	76.33 (60.86)	66.32 (54.51)	44.33 (41.72)	24.67 (29.76)	16.33 (23.82)	10.33 (18.73)	6419.70	50.26	11.82
Imidacloprid (seed treatment)(21.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	---	---	---
Lambda cyhalothrin (75.00)	100.00 (89.96)	100.00 (89.96)	98.31 (82.60)	88.00 (69.71)	67.67 (55.32)	54.92 (47.80)	41.33 (39.99)	29.33 (32.74)	8843.35	69.23	75.84
Lambda cyhalothrin + Azadirachtin (37.50+1.687)	100.00 (89.96)	100.00 (89.96)	91.86 (73.39)	74.66 (59.76)	55.04 (47.87)	24.33 (29.53)	16.00 (23.56)	12.00 (20.24)	7108.35	55.65	24.92
Spiromesifen (225.00)	7.10 (15.44)	3.36 (10.55)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.23	0.04	---
CD (p=0.05)	0.68	1.62	2.05	1.86	0.97	1.13	1.81	1.72	---	---	---

* Average of three replications; Values in parentheses are arc sine transformed values; LT₅₀ = Median lethal time; PT = Persistent toxicity; X= Relative PT with respect to endosulfan

The LT_{50} values were found to be 90.80, 28.86, 11.82, 75.84 and 24.92 hours for cypermethrin, cypermethrin + *B. thuringiensis* subsp. *kurstaki*, imidacloprid (foliar application), lambda cyhalothrin and lambda cyhalothrin + azadirachtin, respectively.

During 2004-05 experiment similar trend was followed in which cypermethrin, cypermethrin + *B. thuringiensis* subsp. *kurstaki*, imidacloprid foliar treatment, lambda cyhalothrin and lambda cyhalothrin + azadirachtin treatments were highly toxic to the foragers of *A. mellifera* (Table 4.39). The mortality was not observed at 240 hours of treatment, however at 120 hours of treatment, lambda cyhalothrin was highly toxic causing 28.33 per cent mortality, which was followed by cypermethrin (18.67 %), lambda cyhalothrin + azadirachtin (5.67 %), imidacloprid foliar treatment (4.67 %) and cypermethrin + *B. thuringiensis* subsp. *kurstaki* (3.68 %). The endosulfan treatment caused 28.67 per cent mortality of *A. mellifera* foragers, which decreased to zero at 24 hours of spray. Low mortalities 4.06 and 6.36 were recorded for *B. thuringiensis* subsp. *kurstaki* and spiromesifen treatments. Imidacloprid seed treatment was found to be non toxic.

Lambda cyhalothrin (8556.49) and cypermethrin (8286.75) had highest PT values followed by cypermethrin + *B. thuringiensis* subsp. *kurstaki* (6844.80), lambda cyhalothrin + azadirachtin (6742.05), imidacloprid foliar application (6062.70), endosulfan (119.49), spiromesifen (5.38), *B. thuringiensis* subsp. *kurstaki* (3.04) and imidacloprid seed treatment (0.00). Cypermethrin and lambda cyhalothrin had 69.40 and 71.66 times more toxicity than endosulfan, respectively. The LT_{50} values were found to be 60.06, 22.88, 12.67, 76.51 and 19.97 hours for cypermethrin, cypermethrin + *B. thuringiensis* subsp. *kurstaki*, imidacloprid, lambda cyhalothrin and lambda cyhalothrin + azadirachtin, respectively.

Table 4.39 Field weathered toxicity of pesticides against *A. mellifera* foragers during *rabi*, 2004-05

Pesticides g a.i. ha ⁻¹	Mean corrected per cent mortality (hrs.)*										Persistent Toxicity		LT ₅₀
	0	1	4	8	24	48	72	120	PT (hrs.)	(X)			
<i>B. thuringiensis</i> subsp. <i>kurstaki</i> (78.75)	4.06 (11.61)	2.02 (8.16)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	3.04	0.03			---
Cypermethrin (75.00)	100.00 (89.96)	98.32 (82.62)	89.32 (20.91)	78.33 (62.33)	63.00 (52.51)	59.46 (50.43)	45.33 (42.30)	18.67 (27.24)	8286.75	69.40			60.06
Cypermethrin + <i>B. thuringiensis</i> subsp. <i>kurstaki</i> (37.50+39.37)	100.00 (89.96)	95.00 (77.19)	77.70 (61.80)	69.00 (56.15)	59.80 (50.63)	40.14 (39.29)	11.00 (19.34)	3.68 (11.04)	6844.80	57.33			22.88
Endosulfan (525.00)	28.67 (32.36)	18.33 (25.30)	8.33 (16.76)	4.37 (12.04)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	119.40	1.00			---
Imidacloprid (foliar application) (25.00)	100.00 (89.96)	89.67 (71.29)	73.00 (58.68)	62.16 (52.01)	39.94 (39.17)	20.74 (27.08)	14.00 (21.93)	4.67 (12.46)	6062.70	50.78			12.67
Imidacloprid (seed treatment)(21.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	---	---			---
Lambda cyhalothrin (75.00)	100.00 (89.96)	100.00 (89.96)	95.93 (78.39)	85.00 (67.19)	74.83 (59.86)	52.66 (46.51)	38.33 (38.23)	28.33 (32.14)	8556.49	71.66			76.51
Lambda cyhalothrin + Azadirachtin (37.50+1.687)	100.00 (89.96)	98.32 (82.64)	88.67 (70.30)	73.67 (59.10)	52.67 (46.51)	21.07 (27.30)	9.40 (17.84)	5.67 (13.76)	6742.05	56.47			19.97
Spiromesifen (225.00)	6.36 (14.60)	4.40 (12.10)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.38	0.05			---
CD (p=0.05)	0.25	2.57	1.93	1.56	1.46	2.41	2.14	1.44	---	---			---

* Average of three replications; Values in parentheses are arc sine transformed values; LT₅₀ = Median lethal time; PT = Persistent toxicity; X= Relative PT with respect to endosulfan

Data for both the years (2003-04 and 2004-05) were pooled. It is evident from the Table 4.40 and Fig. 4.10 that initial deposits of cypermethrin, cypermethrin+ *B. thuringiensis* subsp. *kurstaki*, imidacloprid (foliar application), lambda cyhalothrin and lambda cyhalothrin + azadirachtin were highly toxic causing cent per cent mortality of *A. mellifera* foragers. Endosulfan (30.00 % mortality) was intermediate. The foliar applications of *B. thuringiensis* subsp. *kurstaki* (4.60 % mortality), spiromesifen (6.73 % mortality) and seed treatment of imidacloprid were non toxic. It was found that toxicity of all the pesticides decreased with time up to 120 hours of spray, thereafter, it became non toxic to foragers of *A. mellifera*. At 120 hours of spray, the lambda cyhalothrin treatment (28.83 % mortality) showed significantly higher toxicity and was followed by cypermethrin (19.50 % mortality), lambda cyhalothrin + azadirachtin (8.83 % mortality) and imidacloprid foliar application (7.50 % mortality). The toxicity value for cypermethrin + *B. thuringiensis* subsp. *kurstaki* was 3.87 per cent.

Lambda cyhalothrin (8659.98) had the highest PT value followed by cypermethrin (8620.65), cypermethrin + *B. thuringiensis* subsp. *kurstaki* (7068.30), lambda cyhalothrin + azadirachtin (6925.65), imidacloprid foliar treatment (6240.90), endosulfan (123.56), spiromesifen (5.26), *B. thuringiensis* subsp. *kurstaki* (3.30) and imidacloprid seed treatment (0.00). It was also found that with respect to endosulfan, lambda cyhalothrin was 70.09 times and cypermethrin was 69.77 times more toxic. The LT_{50} values for cypermethrin, cypermethrin + *B. thuringiensis* subsp. *kurstaki*, imidacloprid, lambda cyhalothrin and lambda cyhalothrin + azadirachtin were found to be 76.25, 24.84, 14.39, 79.40 and 23.37 hours, respectively.

4.3 DISSIPATION OF PESTICIDE RESIDUES

4.3.1 Standard Log Dosage Probit Mortality Response Curves for the Bioassay

Table 4.40 Field weathered toxicity of pesticides against *A. mellifera* foragers during *rabi*, 2003-04 and 2004-05[#]

Pesticides g a.i. ha ⁻¹	Mean corrected per cent mortality (hrs.)*								Persistent Toxicity		LT ₅₀
	0	1	4	8	24	48	72	120	PT (hrs.)	(X)	
<i>B. thuringiensis</i> subsp. <i>kurstaki</i> (78.75)	4.60 (12.36)	2.01 (8.14)	---	---	---	---	---	---	3.30	0.03	---
Cypermethrin (75.00)	100.00 (89.96)	99.16 (89.29)	91.79 (73.52)	83.16 (65.97)	72.10 (58.40)	61.67 (51.73)	47.33 (43.45)	19.50 (26.87)	8620.65	69.77	76.25
Cypermethrin+ <i>B. thuringiensis</i> subsp. <i>kurstaki</i> (37.50+39.37)	100.00 (89.96)	95.00 (77.18)	79.68 (63.22)	71.62 (57.81)	62.95 (52.50)	42.10 (40.43)	16.00 (23.29)	3.87 (11.30)	7068.30	57.21	24.84
Endosulfan (525.00)	30.00 (33.18)	18.33 (25.32)	9.57 (17.97)	3.88 (11.30)	---	---	---	---	123.56	1.00	---
Imidacloprid (foliar application) (25.00)	100.00 (89.96)	89.67 (71.27)	74.65 (59.77)	64.24 (53.26)	42.13 (40.45)	22.70 (28.42)	15.17 (22.87)	7.50 (15.59)	6240.90	50.51	14.39
Imidacloprid (seed treatment)(21.00)	---	---	---	---	---	---	---	---	---	---	---
Lambda cyhalothrin (75.00)	100.00 (89.96)	100.00 (89.96)	97.12 (80.55)	86.50 (68.45)	71.25 (57.59)	53.79 (47.16)	39.83 (39.11)	28.83 (32.44)	8659.98	70.09	79.40
Lambda cyhalothrin + Azadirachtin (37.50+1.687)	100.00 (89.96)	99.16 (86.30)	90.26 (71.84)	74.14 (59.43)	53.85 (47.19)	22.70 (28.42)	12.70 (20.70)	8.83 (17.00)	6925.05	56.05	23.37
Spiromesifen (225.00)	6.73 (15.02)	3.80 (11.33)	---	---	---	---	---	---	5.26	0.04	---
CD (p=0.05) With in Environment	NS	NS	NS	NS	NS	NS	NS	NS	---	---	---
Environment	0.17	0.73	0.76	0.66	0.51	0.77	0.81	0.65	---	---	---
Treatment	0.35	1.45	1.32	1.14	0.81	1.22	1.29	1.03	---	---	---
Environment x Treatment	0.49	2.06	1.87	1.61	1.14	1.73	1.82	1.46	---	---	---

* Average of three replications; Values in parentheses are arc sine transformed values; LT₅₀ = Median lethal time; PT = Persistent toxicity; X= Relative PT with respect to endosulfan; [#]Pooled data

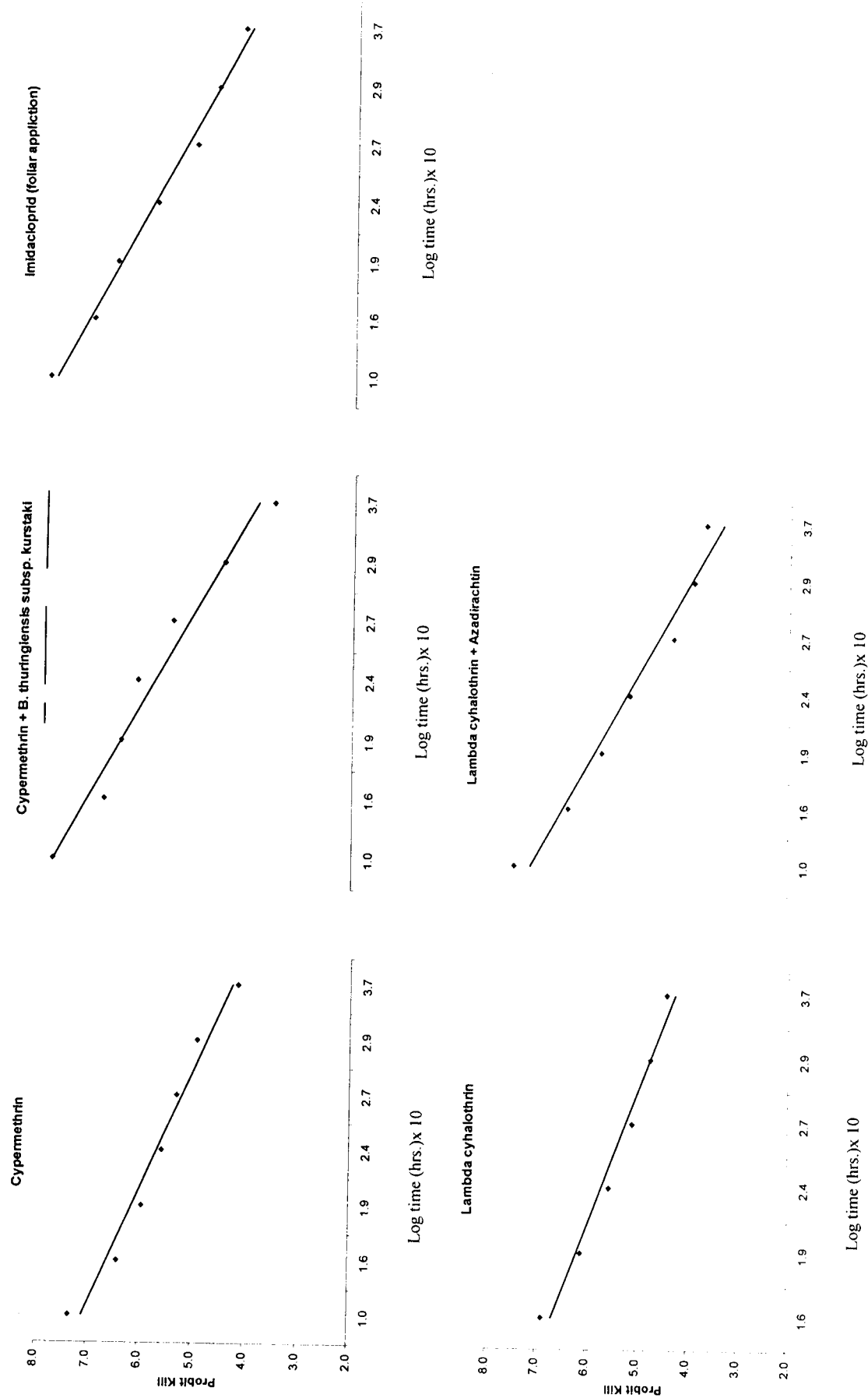


Fig. 4.10 Log time probit mortality response lines of *A. mellifera* foragers to pesticide treatments during *rabi*, 2003-04 and 2004-05 (pooled)

The toxicity data for the test insect (*D. melanogaster*) based on 100 insects following 24 hours exposure to various concentrations of test solutions of the four pesticides are given in Tables 4.41 to 4.44. The data were subjected to probit analysis (Finney, 1977). From the regression equations, expected probit values with respect to log concentrations were calculated and the log dosage probit mortality response curves were drawn (Fig. 4.11).

The deposits/ residues in extracts of field samples of nectar and pollen were estimated by substituting the mortality data in pesticide standard mortality regression equations.

4.3.1.1 Endosulfan

It is seen from the Table 4.41 that 0.0039 and 0.0625 ppm concentrations of endosulfan recorded 18.64 and 81.36 per cent corrected mortalities of *D. melanogaster*, respectively. Mortality in control treatment was 1.67 per cent. The LC_{50} and LC_{90} were computed to be 0.0183 and 0.1440 ppm, respectively after 24 hours of exposure.

4.3.1.2 Imidacloprid

It was observed that 22.03 and 84.75 per cent corrected mortalities were given by 0.0013 and 0.0200 ppm of imidacloprid concentrations, respectively. The per cent mortality in control treatment was 1.67. The LC_{50} and LC_{90} values were computed to be 0.0038 and 0.0258 ppm, respectively (Table 4.42).

4.3.1.3 Lambda cyhalothrin

From the Table 4.43 it was concluded that 15.00 per cent mortality of *D. melanogaster* occurred at 0.0156 ppm whereas, 86.67 per cent corrected mortality occurred at 0.250 ppm. Mortality in control was zero per cent. The LC_{50} and LC_{90} were computed to be 0.0660 and 0.3349 ppm, respectively after 24 hours of exposure.

Table 4.41 Dosage mortality response of *D. melanogaster* adults* exposed to the residue film of endosulfan technical grade on 24 hours exposure

Conc. (ppm)	Log (dosage x 10 ³)	%corrected mortality	Empirical probit	Expected probit
0.0625	1.7959	81.36	5.8927	5.80
0.0312	1.4942	61.02	5.2798	5.34
0.0156	1.1931	42.37	4.8083	4.90
0.0078	0.8921	30.00	4.4756	4.48
0.0039	0.5911	18.64	4.1088	4.06
Control	---	---		---
χ^2 (3df)	= 1.0674 (N.S.)	χ^2 (tab.) at 0.05 and 3df		= 7.80
b	= 1.4300	S.E. (b)	= 0.1900	

$Y = 3.1953 + 1.4300 X$

$LC_{50} = 0.0183$ (0.0143 – 0.0234) ppm
 $LC_{90} = 0.1440$ (0.0773 – 0.2682) ppm

Where
NS = Non significant; b = Slope; Y = Imperical probit; X = Log (dosage x 10³); *No. of insect treated= 100

Table 4.42 Dosage mortality response of *D. melanogaster* adults* exposed to the residue film of imidacloprid technical grade on 24 hours exposure

Conc. (ppm)	Log (dosage x 10 ³)	%corrected mortality	Empirical probit	Expected probit
0.0200	1.3010	84.75	6.0258	6.16
0.0100	1.0000	74.58	5.6620	5.64
0.0050	0.6990	61.02	5.2798	5.18
0.0025	0.3979	36.67	4.6602	4.68
0.0013	0.0969	22.03	4.2288	4.18
Control	---	---	---	---
χ^2 (3df)	= 0.6710 (N.S.)	χ^2 (tab.) at 0.05 and 3df		= 7.80
b	= 1.5451	S.E. (b)	= 0.1994	

$Y = 4.1017 + 1.5451 X$

$LC_{50} = 0.0038$ (0.0030 – 0.0048) ppm
 $LC_{90} = 0.0258$ (0.0157 – 0.0422) ppm

Where
NS = Non significant; b = Slope; Y = Imperical probit; X = Log (dosage x 10³); *No. of adults treated= 100

Table 4.43 Dosage mortality response of *D. melanogaster* adults* exposed to the residue film of lambda cyhalothrin technical grade on 24 hours exposure

Conc. (ppm)	Log (dosage x 10 ³)	%corrected mortality	Empirical probit	Expected probit
0.2500	1.3979	86.67	6.0160	6.02
0.1250	1.0969	68.33	5.4740	5.48
0.0625	0.7959	48.33	4.9330	4.96
0.0313	0.4949	25.00	4.3255	4.38
0.0156	0.1938	15.00	3.9636	3.86
Control	---	---	---	---
χ^2 (3df)	= 0.6559 (N.S.)	χ^2 (tab.) at 0.05 and 3df		= 7.80
b	= 1.8173	S.E. (b)	= 0.2020	

$Y = 3.5102 + 1.8173 X$

$LC_{50} = 0.0660$ (0.0542 – 0.0805) ppm

$LC_{90} = 0.3349$ (0.0424 – 0.0966) ppm

Where

NS = Non significant; b = Slope; Y = Imperical probit; X = Log (dosage x 10³); *No. of insect treated= 100

Table 4.44 Dosage mortality response of *D. melanogaster* adults* exposed to the residue film of spiromesifen technical grade on 24 hours exposure

Conc. (ppm)	Log (dosage x 10 ³)	%corrected mortality	Empirical probit	Expected probit
1.5000	2.1761	87.93	6.1715	6.14
0.7500	1.8751	67.24	5.4462	5.44
0.3750	1.5740	48.28	4.9574	4.88
0.1875	1.2730	25.86	4.3536	4.30
0.0937	0.9720	8.62	3.6355	3.74
Control	---	---	---	---
χ^2 (3df)	= 0.4444 (N.S.)	χ^2 (tab.) at 0.05 and 3df		= 7.80
b	= 2.0052	S.E. (b)	= 0.2124	

$Y = 1.7603 + 2.0052 X$

$LC_{50} = 0.4127$ (0.3434 – 0.4959) ppm

$LC_{90} = 1.7979$ (1.2503 – 2.5856) ppm

Where

NS = Non significant; b = Slope; Y = Imperical probit; X = Log (dosage x 10³); *No. of adults treated= 100

4.3.1.4 Spiromesifen

From the Table 4.44 it is seen that per cent corrected mortality range of 8.62 to 87.93 per cent were given by 0.0937 to 1.500 ppm of spiromesifen concentration, respectively against the 3.33 per cent in control treatment. The LC_{50} and LC_{90} values were calculated to be 0.4127 and 1.7979 ppm, respectively.

4.3.2 Recovery of Pesticides from Mustard Nectar and Pollen

4.3.2.1 Nectar

The results of the recovery of four pesticides from mustard nectar are given in table 4.45 and 4.46. The highest recovery was obtained with imidacloprid 88.90 ± 0.54 and 93.55 ± 0.53 per cent by bioassay and chromatographic methods of residue estimation, respectively. The recovery of endosulfan was 86.54 ± 0.73 and 93.01 ± 0.81 per cent by bioassay and chromatographic methods of residue estimation, respectively. Whereas, for lambda cyhalothrin it was 85.61 ± 0.60 per cent and 91.02 ± 0.72 per cent, respectively. Spiromesifen fortified samples resulted in least average recovery of 82.85 ± 0.81 and 91.31 ± 0.78 per cent by bioassay and GLC, respectively.

4.3.3.2 Pollen

The highest recoveries, 86.44 ± 0.56 and 91.31 ± 0.46 per cent were recorded for imidacloprid with bioassay and chromatographic methods of estimation, respectively (Table 4.47 and 4.48). Samples fortified with endosulfan resulted in 85.17 ± 0.88 and 91.15 ± 0.37 per cent, whereas lambda cyhalothrin had 83.02 ± 0.71 and 88.50 ± 0.51 per cent recoveries by bioassay and GLC methods, respectively. It was least 81.44 ± 0.40 and 88.59 ± 0.32 per cent in respect of spiromesifen by bioassay and GLC, respectively.

Table 4.45 Recovery of pesticides through bioassay method from nectar samples

Pesticides	Amount of samples (gm)	Amount of pesticide added (ppm)	Amount recovered* (ppm)	Recovery (%)	Average recovery (%)
Endosulfan	0.17	0.00037 (2 ppm)	1.690	84.51	86.54 $\pm$ 0.73
		0.00074 (4 ppm)	3.461	86.52	
		0.00148 (8 ppm)	6.980	87.25	
		0.00296 (16 ppm)	14.061	87.88	
Imidacloprid	0.15	0.00032 (2 ppm)	1.757	87.85	88.90 $\pm$ 0.54
		0.00063 (4 ppm)	3.530	88.26	
		0.00126 (8 ppm)	7.140	89.25	
		0.00253 (16 ppm)	14.440	90.25	
Lambda cyhalothrin	0.15	0.00032 (2 ppm)	1.683	84.15	85.61 $\pm$ 0.60
		0.00063 (4 ppm)	3.411	85.27	
		0.00126 (8 ppm)	6.879	85.99	
		0.00253 (16 ppm)	13.923	87.02	
Spiromesifen	0.18	0.00036 (2 ppm)	1.618	80.89	82.85 $\pm$ 0.81
		0.00073 (4 ppm)	3.290	82.25	
		0.00146 (8 ppm)	6.694	83.68	
		0.00291 (16 ppm)	13.533	84.58	

Table 4.46 Recovery of pesticides through GLC method from nectar samples

Pesticides	Amount of samples (gm)	Amount of pesticide added (ppm)	Amount recovered* (ppm)	Recovery (%)	Average recovery (%)
Endosulfan	0.17	0.00037 (2 ppm)	1.824	91.22	93.01 $\pm$ 0.81
		0.00074 (4 ppm)	3.690	92.25	
		0.00148 (8 ppm)	7.489	93.62	
		0.00296 (16 ppm)	15.194	94.96	
Imidacloprid**	0.15	0.00032 (2 ppm)	1.843	92.17	93.55 $\pm$ 0.53
		0.00063 (4 ppm)	3.729	93.24	
		0.00126 (8 ppm)	7.540	94.25	
		0.00253 (16 ppm)	15.123	94.52	
Lambda cyhalothrin	0.15	0.00032 (2 ppm)	1.784	89.21	91.20 $\pm$ 0.72
		0.00063 (4 ppm)	3.648	91.21	
		0.00126 (8 ppm)	7.348	91.85	
		0.00253 (16 ppm)	14.806	92.54	
Spiromesifen	0.18	0.00036 (2 ppm)	1.799	89.98	91.31 $\pm$ 0.78
		0.00073 (4 ppm)	3.599	89.99	
		0.00146 (8 ppm)	7.379	92.24	
		0.00291 (16 ppm)	14.882	93.01	

* Average of three replications; ** HPLC used for estimation of residue

Table 4.47 Recovery of pesticides through bioassay method from pollen samples

Pesticides	Amount of samples (gm)	Amount of pesticide added (ppm)	Amount recovered* (ppm)	Recovery (%)	Average recovery (%)
Endosulfan	0.075	0.00016 (2 ppm)	1.651	82.56	85.17 $\pm$ 0.88
		0.00033 (4 ppm)	3.396	84.90	
		0.00065 (8 ppm)	6.880	86.01	
		0.00130 (16 ppm)	13.954	87.21	
Imidacloprid	0.082	0.00017 (2 ppm)	1.697	84.84	86.44 $\pm$ 0.56
		0.00035 (4 ppm)	3.430	85.75	
		0.00069 (8 ppm)	6.964	87.05	
		0.00143 (16 ppm)	14.099	88.12	
Lambda cyhalothrin	0.065	0.00013 (2 ppm)	1.631	81.57	83.02 $\pm$ 0.71
		0.00027 (4 ppm)	3.305	82.62	
		0.00055 (8 ppm)	6.635	82.94	
		0.00109 (16 ppm)	13.592	84.95	
Spiromesifen	0.075	0.00015 (2 ppm)	1.598	79.88	81.44 $\pm$ 0.40
		0.00030 (4 ppm)	3.294	81.23	
		0.00060 (8 ppm)	6.503	81.29	
		0.00121 (16 ppm)	13.336	83.35	

Table 4.48 Recovery of pesticides through GLC method from pollen samples

Pesticides	Amount of samples (gm)	Amount of pesticide added (ppm)	Amount recovered* (ppm)	Recovery (%)	Average recovery (%)
Endosulfan	0.075	0.00016 (2 ppm)	1.811	90.56	91.15 $\pm$ 0.37
		0.00033 (4 ppm)	3.620	90.50	
		0.00065 (8 ppm)	7.320	91.50	
		0.00130 (16 ppm)	14.723	92.02	
Imidacloprid**	0.082	0.00017 (2 ppm)	1.811	90.54	91.31 $\pm$ 0.46
		0.00035 (4 ppm)	3.634	90.85	
		0.00069 (8 ppm)	7.300	91.25	
		0.00143 (16 ppm)	14.818	92.61	
Lambda cyhalothrin	0.065	0.00013 (2 ppm)	1.745	87.25	88.50 $\pm$ 0.51
		0.00027 (4 ppm)	3.525	88.12	
		0.00055 (8 ppm)	7.129	89.12	
		0.00109 (16 ppm)	14.320	89.50	
Spiromesifen	0.075	0.00015 (2 ppm)	1.758	87.88	88.59 $\pm$ 0.32
		0.00030 (4 ppm)	3.529	88.23	
		0.00060 (8 ppm)	7.119	88.99	
		0.00121 (16 ppm)	14.280	89.25	

* Average of three replications; ** HPLC used for estimation of residue

4.3.4 Sensitivity and Detection Limits of Residue Estimations

4.3.4.1 Bioassay

Results of sensitivity of bioassay method to predict residues of four pesticides are given in Table 4.49 and fig. 4.11. Perusal of data show that bioassay method was highly sensitive for imidacloprid detecting a deposit/ residue of 0.0003 ppm followed by endosulfan (0.0012 ppm), lambda cyhalothrin (0.0078 ppm) and spiromesifen (0.0597 ppm).

4.3.4.2 Chromatography

4.3.4.2.1 Gas Liquid Chromatography

Residues of endosulfan, lambda cyhalothrin and spiromesifen were determined by gas liquid chromatography (Fig. 4.14, 4.15 and 4.16). The sensitivities were found to be 5.00, 5.00, 2.00 and 5.00 pg for α -endosulfan, β -endosulfan, lambda cyhalothrin and spiromesifen, respectively, the corresponding detection limits were 0.10, 0.10, 0.5 and 0.1 $\mu\text{g kg}^{-1}$, respectively.

4.3.4.2.2 High Performance Liquid Chromatography

The residues of imidacloprid were determined through high performance liquid chromatography (Fig. 4.17). The sensitivities and detection limits were 2.00 pg and 0.01 mg kg^{-1} , respectively.

4.4 Persistence of pesticide residues in/on bee foraged nectar and pollen

Details of layout have been given in section 3.1.1.3. Residues (ppm) of four pesticides in bee foraged nectar and pollen were extracted as per 3.2.5.1. Extracts were first analyzed by bioassay method and then results were verified by Gas liquid chromatography (GLC). The results obtained were subjected to analysis of variance from which 95 per cent confidence limit of the respective mean values were obtained.

Table 4.49 Dosage- mortality response of pesticides to *D. melanogaster*

Pesticides	Regression equation	Heterogeneity* ($\chi^2 = 3df$)	LC ₅₀ (ppm)	Fiducial limits	LC ₂₀ (ppm)	LC ₈₀ (ppm)	Relative susceptibility	F= LC ₂₀ / LC ₈₀	Sensitivity (ppm)
Endosulfan	Y = 3.1953 + 1.4300 x	1.0674	0.0183	0.0143 0.0234	0.0047	0.0709	1.000	0.0664	0.0012
Imidacloprid	Y = 4.1017 + 1.5451 x	0.6710	0.0038	0.0030 0.0048	0.0011	0.0134	4.8158	0.0814	0.0003
Lambda cyhalothrin	Y = 3.5102 + 1.8173 x	0.6559	0.0660	0.0541 0.0805	0.0227	0.1918	0.2773	0.1185	0.0078
Spiromesifen	Y = 1.7603 + 2.0052 x	0.4444	0.4127	0.3435 0.4959	0.1570	1.0848	0.0443	0.1447	0.0597

Y = Probit kill

x = log of concentration x 10³, 10², 10², 10², for endosulfan, imidacloprid, lambda cyhalothrin and spiromesifen, respectively
 LC₂₀, LC₅₀ and LC₈₀ = lethal concentrations (ppm) which gave 20, 50 and 80 per cent mortality of *D. melanogaster*, respectively
 *Significantly homogenous at p = 0.05

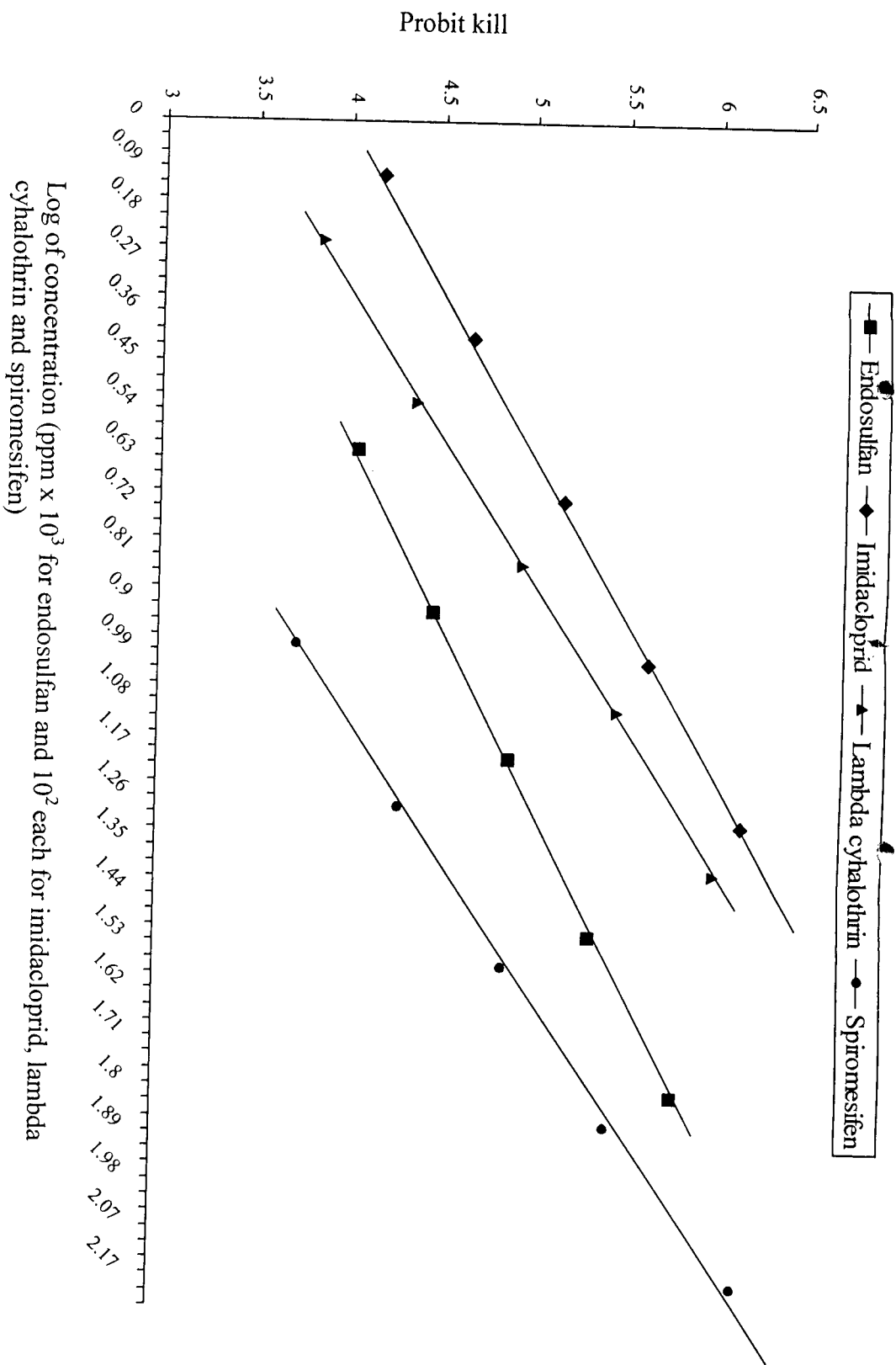
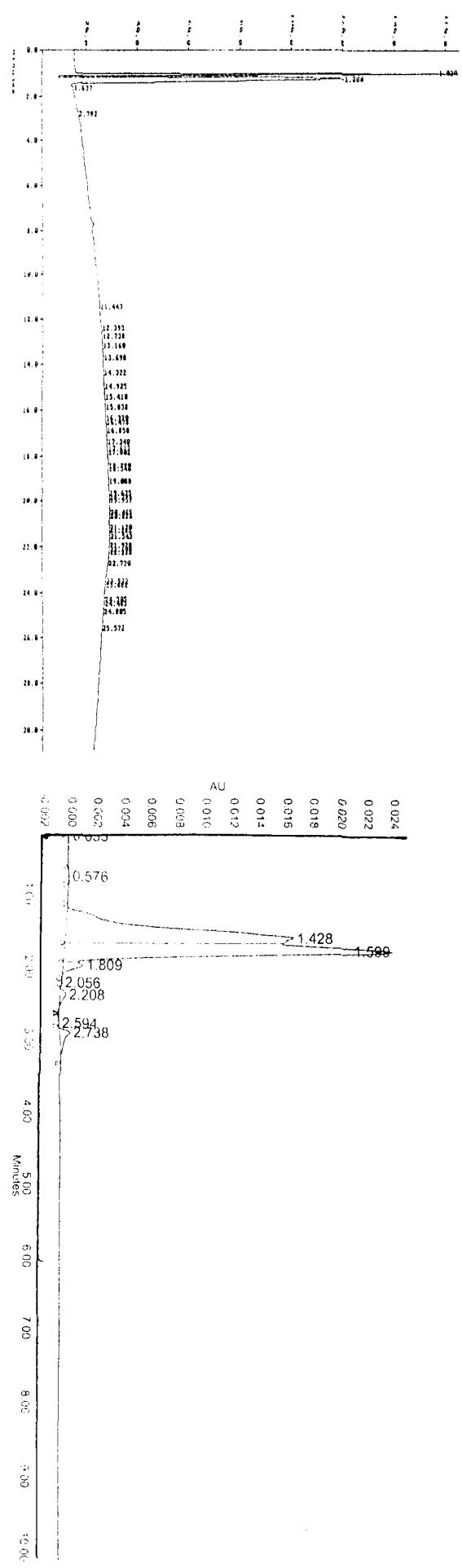


Fig. 4.11 Log dose probit mortality lines of pesticides to *D. melanogaster*

Fig. 4.12 Gas liquid and high performance liquid chromatogram for the blank mustard nectar sample



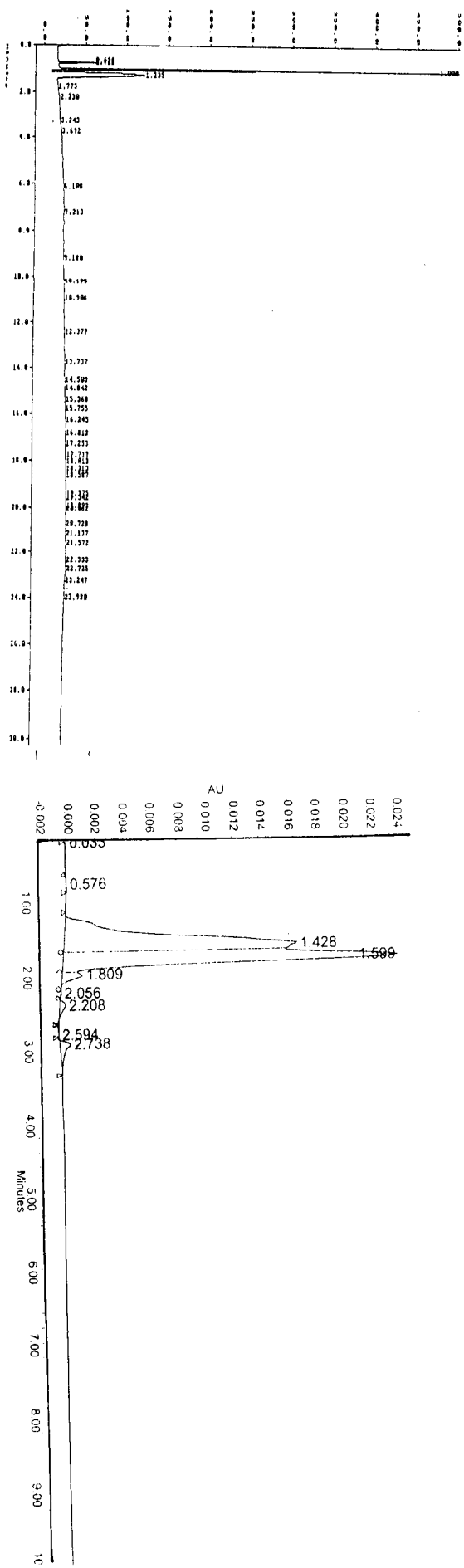


Fig 4.13 Gas liquid and high performance liquid chromatogram for the blank mustard pollen sample

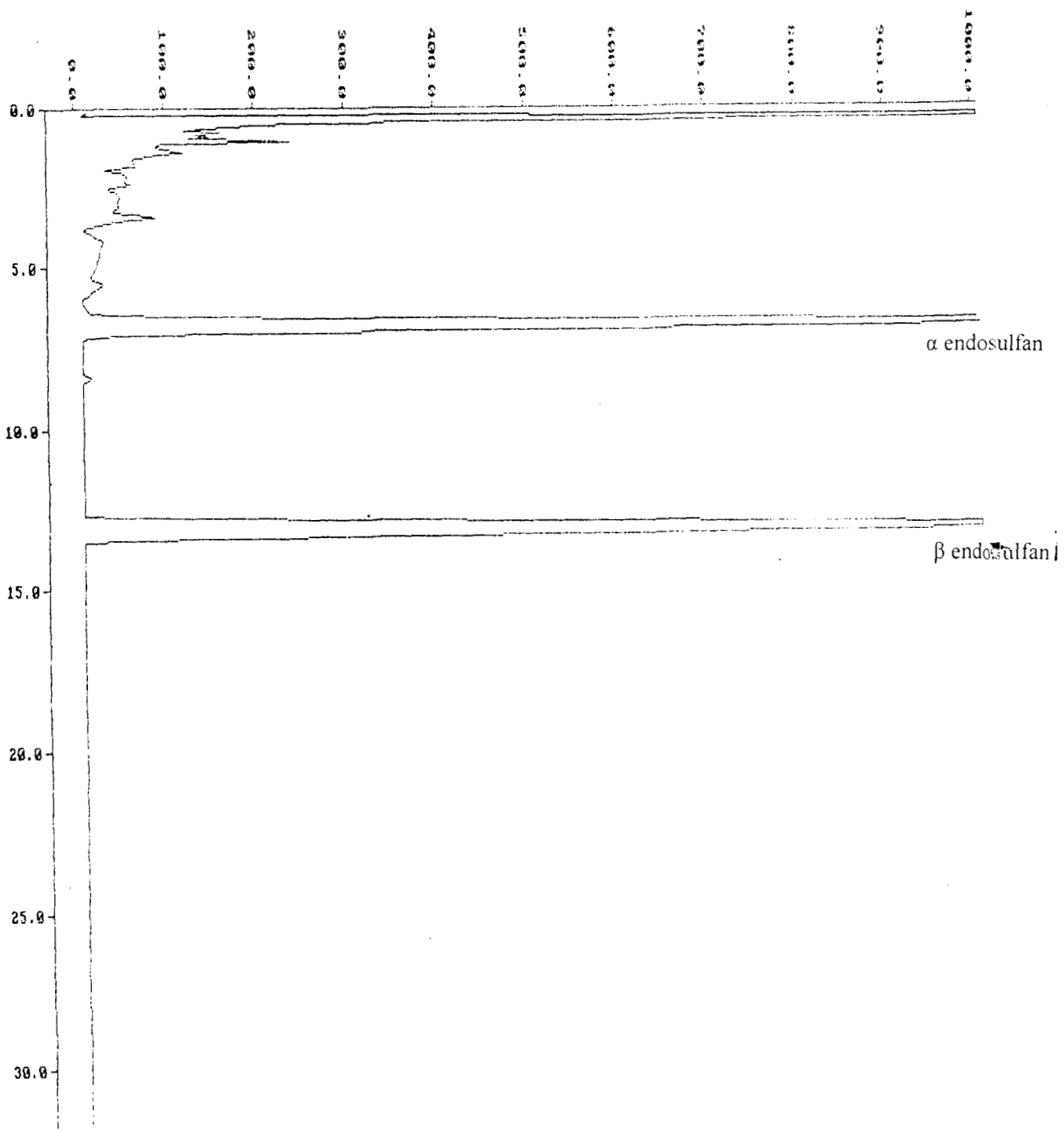


Fig. 4.14 Gas liquid chromatogram for the analysis of endosulfan

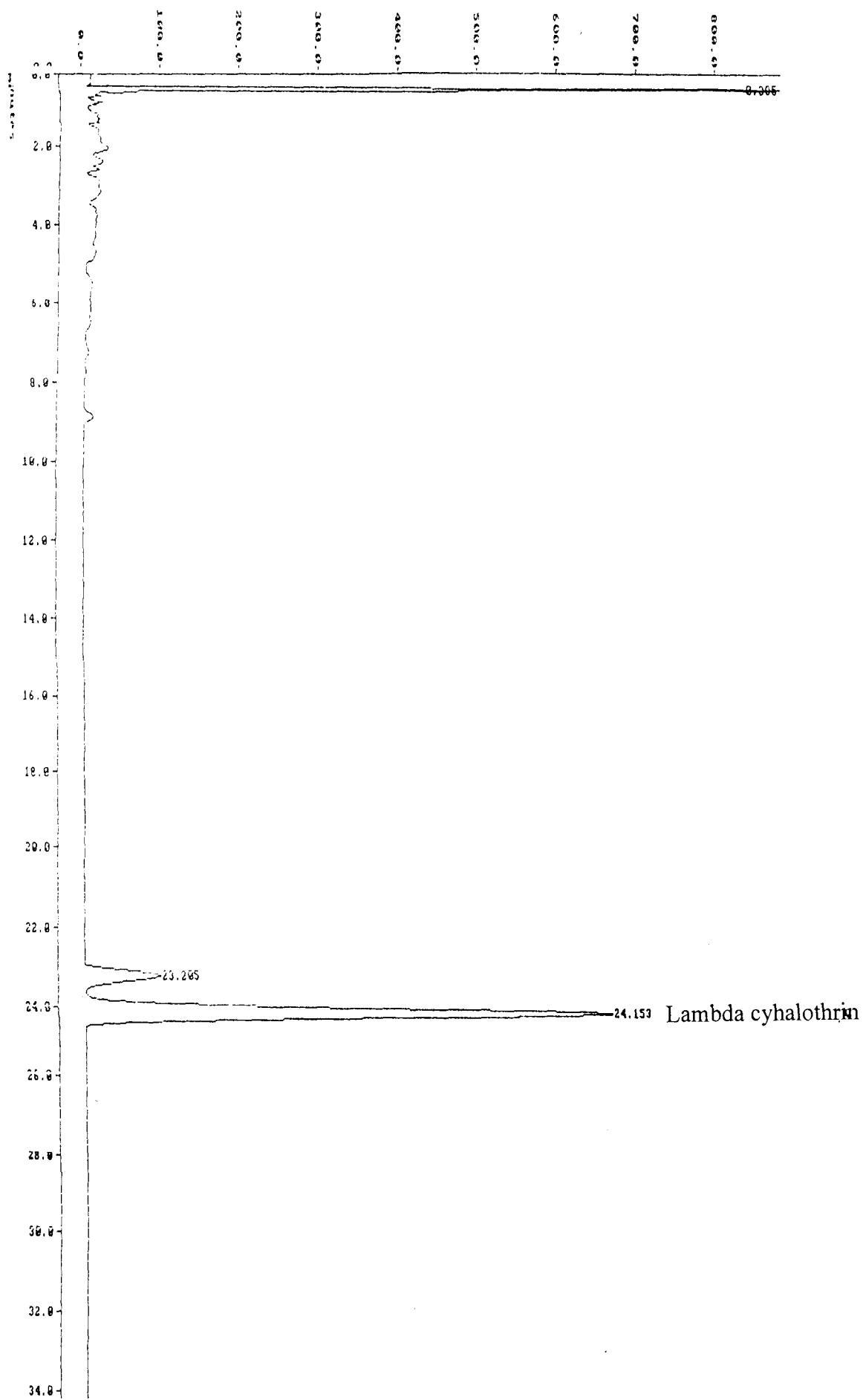
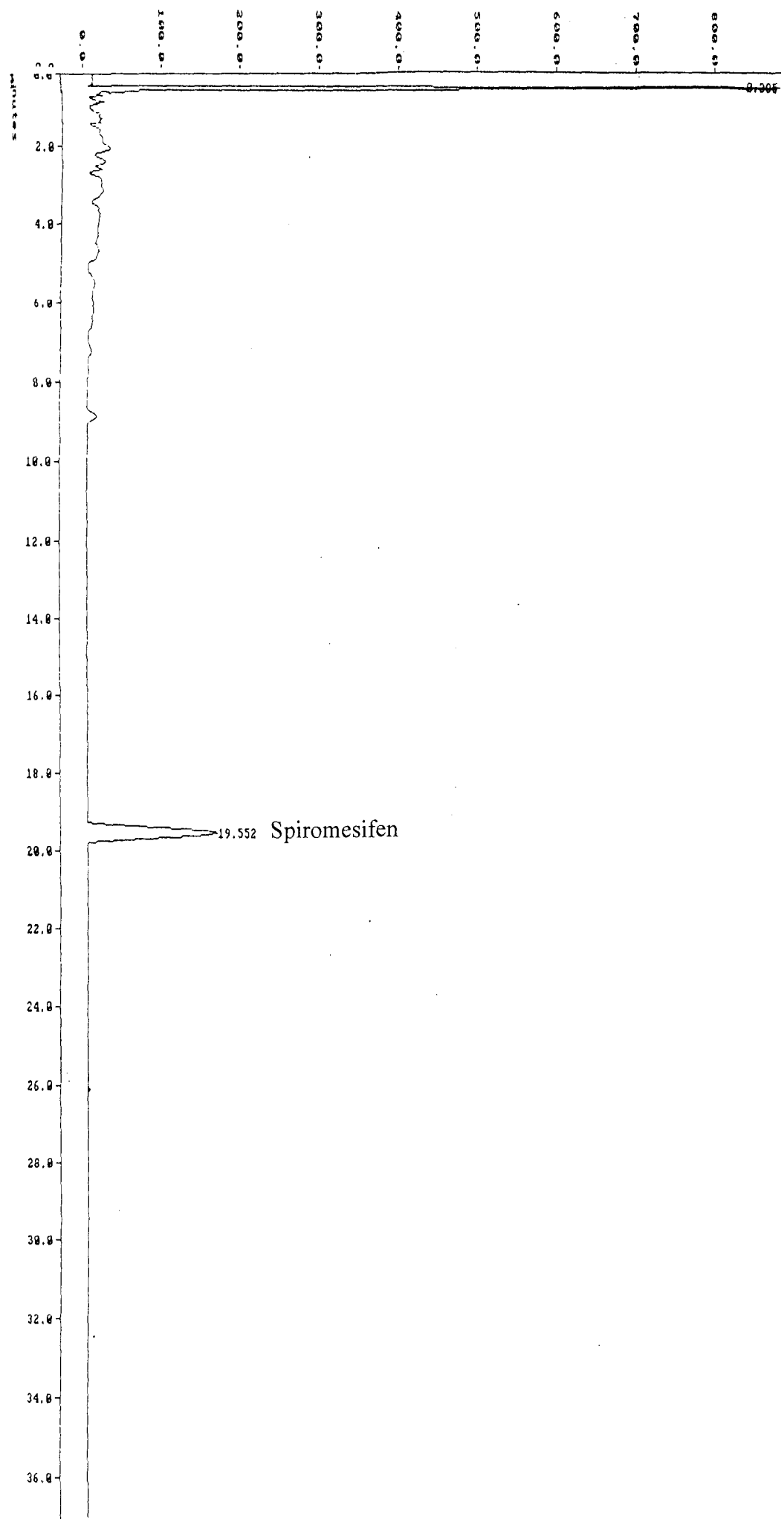


Fig. 4.15 Gas liquid chromatogram for the analysis of lambda cyhalothrin

Fig. 4.16 Gas liquid chromatogram for the analysis of spiromesifen



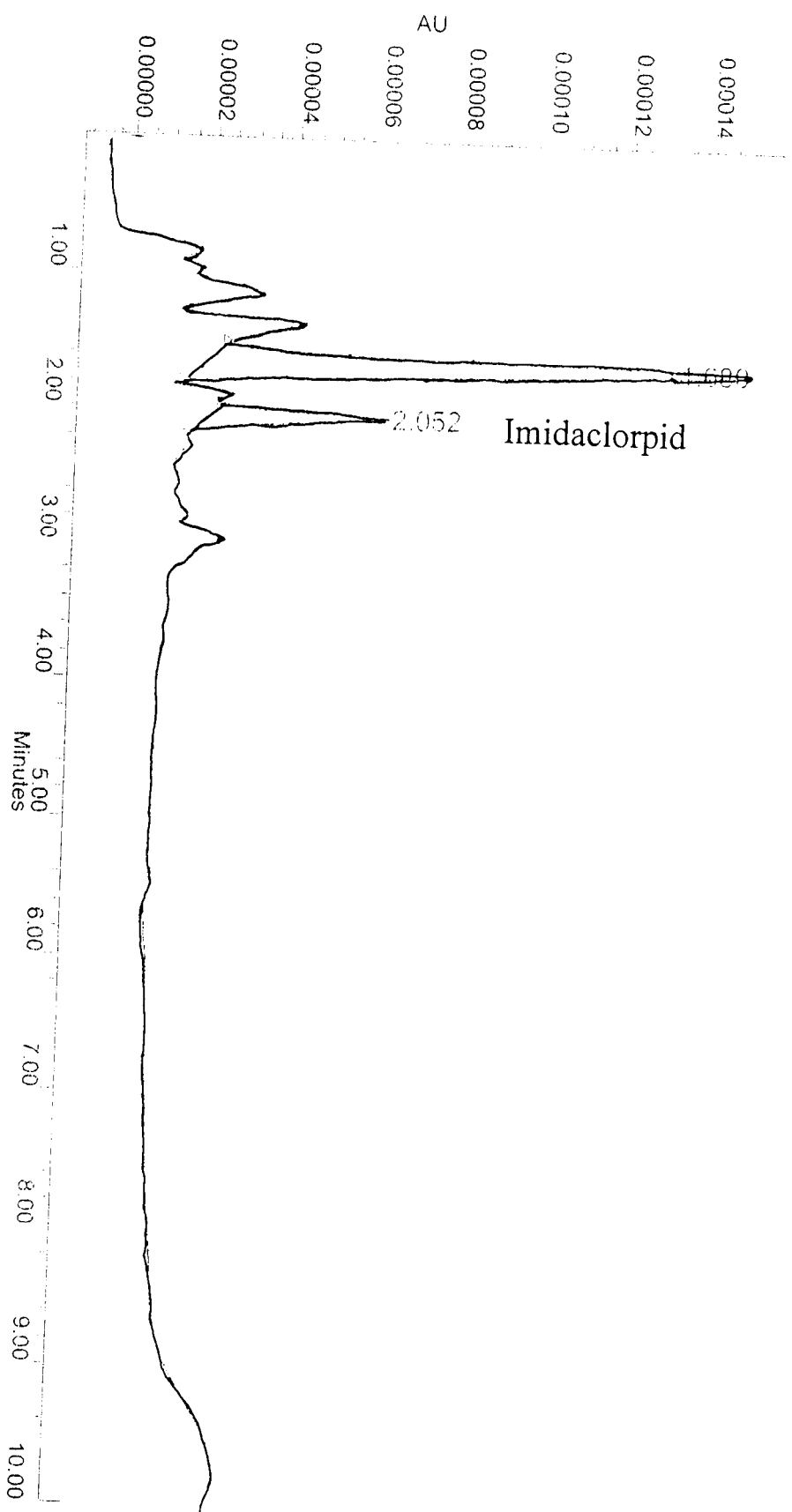


Fig 4.17 High performance liquid Chromatogram for the analysis of imidacloprid

The results of the pesticide residues in bee foraged nectar and pollen during first year (2003-04) are presented in tables 4.50 and 4.52, respectively and are further illustrated graphically in fig. 4.18 and 4.20. Similarly, for the second year (2004-05) data are presented in tables 4.51 and 4.53 and illustrated graphically in fig. 4.19 and 4.21. The regression equations were worked out by taking X as hours and Y as log deposits (ppm) for the pesticides and the half life values were calculated (Table 4.54). The correlation between two methods of residue estimation was judged by computing coefficient of correlation (r). Similarly, correlation between residues and per cent mortality of both the species of honey bees due to residues were also calculated (Table 4.55).

4.4.1 Pesticide residue in bee foraged mustard nectar during *rabi*, 2003-04

The data on the persistence of residues are presented in Table 4.50 and also depicted in fig. 4.16a and 4.16b.

4.4.1.1 Endosulfan

The initial deposits of endosulfan were found to be 1.725 ± 0.031 ppm. which declined to 1.526 ± 0.020 , 1.223 ± 0.095 , 1.053 ± 0.077 , 0.907 ± 0.011 , 0.667 ± 0.022 , 0.304 ± 0.005 and 0.029 ± 0.0001 ppm on 1, 4, 8, 24, 48, 72 and 120 hours after spray, respectively, thus the initial deposits degraded to the tune of 11.54, 29.10, 38.95, 47.43, 61.36, 82.41 and 99.46 per cent. The half life value was 22.97 hours (Table 4.54).

4.4.1.2 Imidacloprid

Mustard nectar samples were collected after 75 days after sowing of imidacloprid treated seeds. Samples were extracted and analyzed through HPLC. It was observed that residues were below detection limit after 75 days of sowing.

4.4.1.3 Lambda cyhalothrin

The average initial deposits of lambda cyhalothrin in bee foraged mustard nectar were 0.858 ± 0.038 ppm and after 1, 4, 8, 24, 48 and 72 hours, the residues declined to 0.655 ± 0.001 , 0.536 ± 0.002 , 0.428 ± 0.015 , 0.240 ± 0.004 , 0.139 ± 0.004 and 0.013 ± 0.001 ppm, respectively. The initial deposits degraded to the tune of 23.63, 37.50, 50.16, 72.06, 83.79 and 98.46 per cent, respectively. The half life value was 13.80 hours (Table 4.54). Residue estimation by both the techniques was highly correlated as showed by high value of r (0.989).

4.4.1.4 Spiromesifen

The residues of spiromesifen persisted for 3 days. The initial deposit (1.541 ± 0.078 ppm) was degraded gradually to negligible amount and were 1.176 ± 0.055 , 0.897 ± 0.050 , 0.708 ± 0.041 , 0.569 ± 0.046 , 0.291 ± 0.027 and 0.088 ± 0.003 ppm after 1, 4, 8, 24, 48 and 72 hours of spray in nectar, respectively the corresponding per cent reduction in residues was 23.09, 42.46, 54.76, 64.25, 82.86 and 93.72 per cent after 1, 4, 8, 24, 48, 72 and 120 hours, respectively. The half life of spiromesifen was calculated as 20.06 hours (Table 4.54).

4.4.2 Pesticide residue in bee foraged mustard nectar during *rabi*, 2004-05

The extent of pesticide residues in nectar collected by honey bees (*A. mellifera*) at different sampling intervals are given in Table 4.51 and also depicted in fig. 4.20. The results are detailed below:

4.4.2.1 Endosulfan

The total initial deposit 1.583 ± 0.006 ppm of both the isomers was recorded, which degraded to 1.454 ± 0.019 , 1.195 ± 0.069 , 0.907 ± 0.021 , 0.816 ± 0.130 , 0.577 ± 0.012 ,

Table 4.50 Pesticide residue (ppm)* in *A. mellifera* foraged mustard nectar during *rabi*, 2003-04

Pesticide / g a.i. ha ⁻¹	Method of estimation	Hours after spray									
		0	1	4	8	24	48	72	120	240	
Endosulfan 525.00	Bioassay	1.625±0.019	1.501±0.012	1.224±0.002	1.088±0.059	0.895±0.053	0.618±0.006	0.294±0.021	0.009±0.001	BDL	
	GLC	1.095±0.003	0.929±0.022	0.824±0.009	0.709±0.007	0.576±0.014	0.448±0.022	0.207±0.007	0.006±0.0001	BDL	
	Endo- I										
	Endo- II	0.730±0.041	0.622±0.009	0.509±0.085	0.90±0.104	0.342±0.046	0.267±0.034	0.106±0.025	0.003±0.0001	BDL	
	Endo I+II	1.825±0.043	1.551±0.028	1.333±0.088	1.098±0.101	0.919±0.035	0.715±0.041	0.313±0.031	0.010±0.0001	BDL	
r = Imidacloprid [#] 21.00	Average*	1.725±0.031	1.526±0.020	1.223±0.095	1.053±0.077	0.907±0.011	0.667±0.022	0.304±0.005	0.029±0.0001	BDL	
	0.988	(11.54)	(29.10)	(38.95)	(47.43)	(61.36)	(82.41)	(99.46)			
	Bioassay	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	HPLC	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	Average*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
r = Lambda cyhalothrin 75.00	---	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	Bioassay	0.806±0.040	0.619±0.005	0.505±0.004	0.402±0.030	0.208±0.004	0.115±0.005	0.014±0.001	BDL	BDL	
	GLC	0.909±0.064	0.691±0.002	0.567±0.003	0.453±0.003	0.271±0.008	0.163±0.003	0.012±0.003	BDL	BDL	
	Average*	0.858±0.038	0.655±0.001	0.536±0.002	0.428±0.015	0.240±0.004	0.139±0.004	0.013±0.001	BDL	BDL	
	0.989	(23.63)	(37.50)	(50.16)	(72.06)	(83.79)	(98.46)				
r = Spiromesifen 225.00	Bioassay	1.283±0.042	1.011±0.024	0.784±0.002	0.594±0.0103	0.491±0.031	0.224±0.001	0.08390.009	BDL	BDL	
	GLC	1.452±0.029	1.121±0.006	0.801±0.049	0.705±0.074	0.508±0.009	0.257±0.021	0.094±0.003	BDL	BDL	
	Average*	1.541±0.078	1.176±0.055	0.897±0.050	0.708±0.041	0.569±0.046	0.291±0.027	0.088±0.003	BDL	BDL	
	0.989	(23.09)	(42.46)	(54.76)	(64.25)	(82.86)	(93.72)				

*Based on three replications; [#] 75 days after sowing of pesticide treated seeds
 Figures following ± signs are 95 per cent confidence limits.
 Figures in the parentheses are the values of the per cent reduction of initial deposits
 r = Correlation coefficient between bioassay and chromatographic methods of residue estimation
 BDL= Below detectable limit
 *≡ Average of bioassay and GLC method of residue estimation

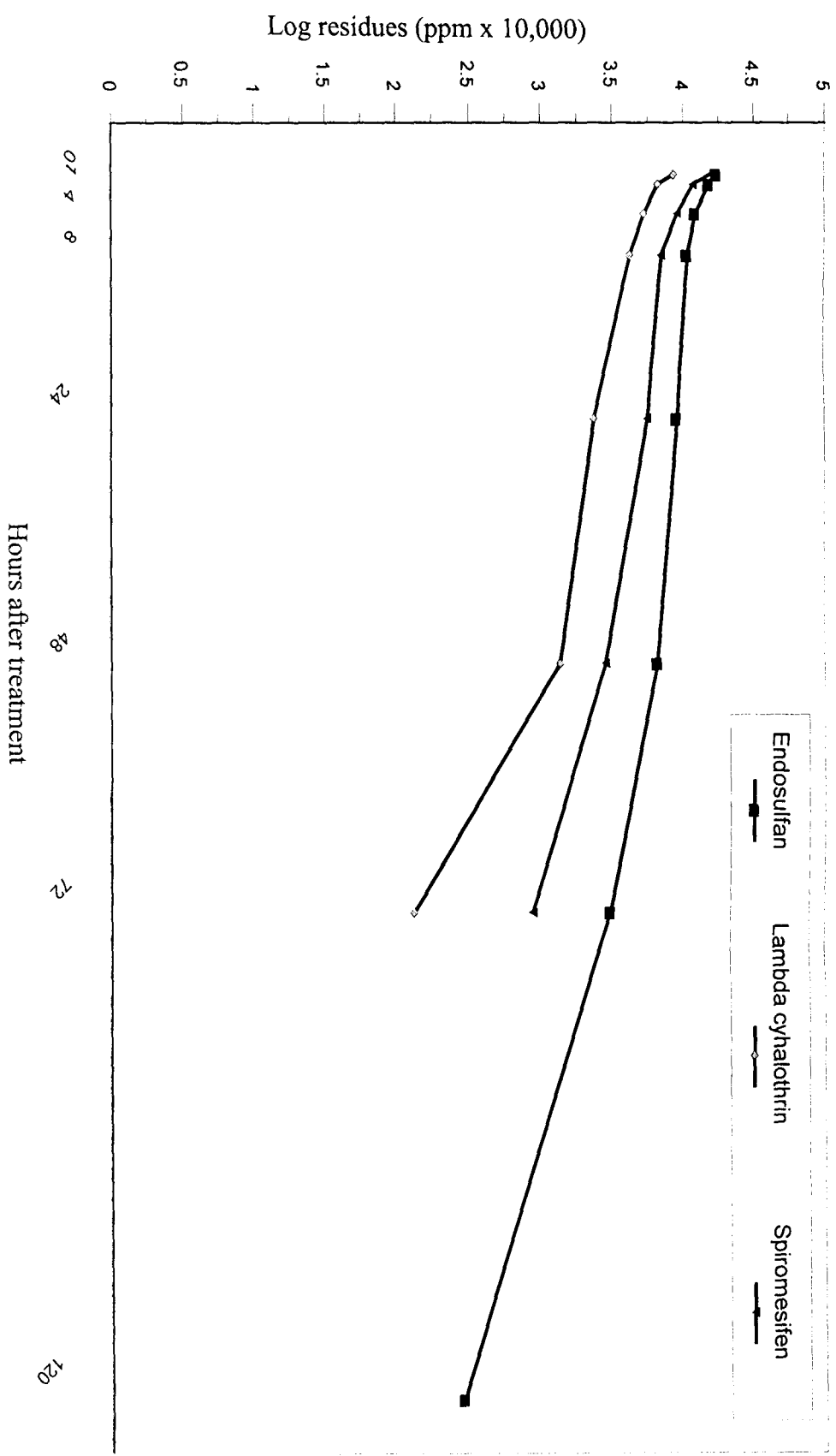


Fig. 4.18 Pesticide residues in *A. mellifera* foraged mustard nectar during *rabi*, 2003-04

0.269 $\pm$ 0.014 and 0.009 $\pm$ 0.001 ppm after 1, 4, 8, 24, 48, 72 and 120 hours after spray. The degradation rate was 8.17 per cent after one hour of spray, followed by 24.51, 42.69, 48.47, 63.59, 83.01 and 99.44 per cent after 4, 8, 24, 48, 72 and 120 hours of spray, respectively. The half life was 32.02 hours (Table 4.54).

4.4.2.2 Imidacloprid

Residues in mustard nectar were below detectable limit after 75 days of seed sowing.

4.4.2.3 Lambda cyhalothrin

From the Table 4.51, it is seen that both the methods of estimation had high correlation ($r = 0.981$). Initial deposit of lambda cyhalothrin 0.728 $\pm$ 0.022 ppm degraded gradually to 0.626 $\pm$ 0.009 (19.94 %), 0.515 $\pm$ 0.006 (34.09 %), 0.419 $\pm$ 0.029 (44.46 %), 0.248 $\pm$ 0.022 (68.25 %), 0.126 $\pm$ 0.013 (83.86 %) and 0.004 $\pm$ 0.001 (99.53 %) ppm on 1, 4, 8, 24, 48 and 72 hours of spray, respectively. The half life was worked out to be 11.11 hours (Table 4.54).

4.4.2.4 Spiromesifen

The initial deposit of spiromesifen was 1.401 $\pm$ 0.016 ppm in mustard nectar, which dwindled to 0.983 $\pm$ 0.051, 0.734 $\pm$ 0.005, 0.560 $\pm$ 0.053, 0.473 $\pm$ 0.019, 0.213 $\pm$ 0.0001 and 0.077 $\pm$ 0.004 ppm showing the per cent reduction of 29.85, 47.58, 60.00, 66.27, 84.81 and 94.50 after 1, 4, 8, 24, 48 and 72 hours of spray, respectively. The half life was 19.93 hours (Table 4.54).

4.4.3 Pesticide Residue in Bee Foraged Mustard Pollen during *Rabi*, 2003-04

The data on persistence of residues in bee foraged mustard pollen during first year are presented in Table 4.52 and fig 4.19.

Table 4. 51 Pesticide residue (ppm)* in *A. mellifera* foraged mustard nectar during *rabi*, 2004-05

Pesticide / Conc.	Method of estimation	Hours after spray									
		0	1	4	8	24	48	72	120	240	
Endosulfan 525.00	Bioassay	1.553±0.005	1.415±0.031	1.177±0.052	0.817±0.009	0.767±0.009	0.553±0.024	0.252±0.019	0.007±0.003	BDL	
	GLC	0.959±0.011	0.927±0.004	0.757±0.008	0.620±0.001	0.538±0.008	0.363±0.026	0.174±0.002	0.007±0.0001	BDL	
	Endo- I										
	Endo- II	0.655±0.023	0.566±0.008	0.457±0.084	0.378±0.040	0.327±0.029	0.236±0.031	0.112±0.034	0.004±0.001	BDL	
	Endo I+II	1.614±0.013	1.493±0.009	1.214±0.091	0.998±0.039	0.865±0.030	0.600±0.005	0.286±0.032	0.011±0.001	BDL	
	Average*	1.583±0.006	1.454±0.019	1.195±0.069	0.907±0.021	0.816±0.13	0.577±0.012	0.269±0.014	0.009±0.001	BDL	
	0.992	(8.17)	(24.51)	(42.69)	(48.47)	(63.59)	(83.01)	(99.44)		BDL	
	Bioassay	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	HPLC	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	Average*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
r =	---	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
Lambda cyhalothrin 75.00	Bioassay	0.727±0.006	0.592±0.003	0.490±0.023	0.387±0.031	0.243±0.010	0.118±0.004	0.003±0.002	BDL	BDL	
	GLC	0.836±0.046	0.660±0.016	0.540±0.010	0.450±0.029	0.253±0.054	0.134±0.029	0.004±0.003	BDL	BDL	
	Average*	0.728±0.022	0.626±0.009	0.515±0.006	0.419±0.029	0.248±0.022	0.126±0.013	0.004±0.001	BDL	BDL	
	0.981	(19.94)	(34.09)	(44.46)	(68.25)	(83.86)	(99.53)		BDL	BDL	
	Bioassay	1.389±0.001	0.954±0.086	0.685±0.009	0.527±0.012	0.454±0.009	0.202±0.001	0.071±0.002	BDL	BDL	
	GLC	1.413±0.033	1.011±0.024	0.784±0.002	0.594±0.103	0.491±0.031	0.224±0.001	0.083±0.009	BDL	BDL	
	Average*	1.401±0.016	0.983±0.051	0.734±0.005	0.560±0.053	0.473±0.019	0.213±0.0001	0.077±0.004	BDL	BDL	
	0.989	(29.85)	(47.58)	(60.00)	(66.27)	(84.81)	(94.50)		BDL	BDL	

*Based on three replications; [#] 75 days after sowing of pesticide treated seeds
 Figures following ± signs are 95 per cent confidence limits.
 Figures in the parentheses are the values of the per cent reduction of initial deposits
 r = Correlation coefficient between bioassay and chromatographic methods of residue estimation
 BDL= Below detectable limit
 *= Average of bioassay and GLC method of residue estimation

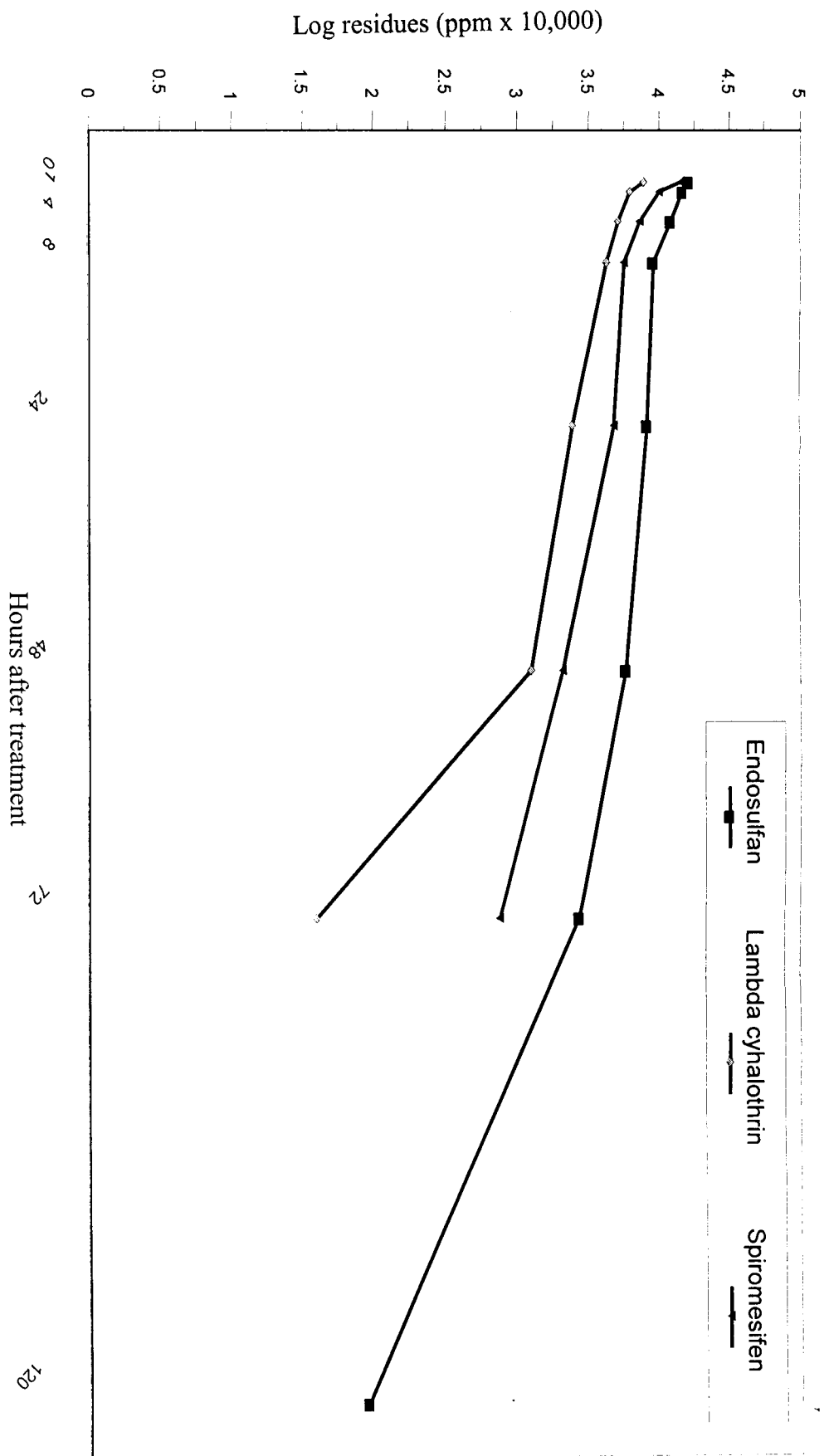


Fig. 4.19 Pesticide residues in *A. mellifera* foraged mustard nectar during *rabi*, 2004-05

4.4.3.1 Endosulfan

Endosulfan residues in mustard pollen though persisted for 72 hours of spray (0.104 ± 0.009 ppm) but 95.11 per cent of initial deposit got degraded. The initial deposit of 2.126 ± 0.088 ppm degraded to 1.391 ± 0.031 , 1.138 ± 0.013 , 0.828 ± 0.0001 , 0.674 ± 0.013 , 0.350 ± 0.004 and 0.104 ± 0.009 ppm after 1, 4, 8, 24, 48 and 72 hours of spray, respectively. The per cent reduction was 34.60, 46.50, 61.05, 68.32, 83.56 and 95.11 after 1, 4, 8, 24, 48 and 72 hours of spray, respectively. The half life of endosulfan residues was calculated to be 19.67 hours (Table 4.54). The coefficient of correlation (r) between bioassay and GLC methods was 0.987.

4.4.3.2 Imidacloprid

The residues in bee foraged pollen on 75 days after sowing of imidacloprid treated seeds were below detectable limit.

4.4.3.3 Lambda cyhalothrin

The residues of lambda cyhalothrin persisted for 72 hours. The average initial deposit of 1.607 ± 0.004 ppm degraded to 1.226 ± 0.063 , 1.001 ± 0.019 , 0.786 ± 0.023 , 0.431 ± 0.006 , 0.189 ± 0.003 and 0.031 ± 0.006 ppm after 1, 4, 8, 24, 48 and 72 hours of spray, respectively. The per cent reduction was 23.69, 37.70, 51.09, 73.17, 88.26 and 98.06 per cent on 1, 4, 8, 24, 48 and 72 hours of spray, respectively. The half life was 14.07 hours (Table 4.54). Coefficient of correlation (r) for bioassay and GLC method was 0.990.

4.4.3.4 Spiromesifen

Initial deposit of spiromesifen was recorded to be 2.052 ± 0.023 ppm in pollen of mustard. Initial deposits degraded to 1.401 ± 0.007 , 1.123 ± 0.004 , 0.813 ± 0.008 , 0.697 ± 0.022 , 0.265 ± 0.030 and 0.001 ± 0.0001 ppm after 1, 4, 8, 24, 48 and 72 hours of

Table 4.52 Pesticide residue (ppm)* in *A. mellifera* foraged mustard pollen during *rabi*, 2003-04

Pesticide / Conc.	Method of estimation	Hours after spray									
		0	1	4	8	24	48	72	120	240	
Endosulfan 525.00	Bioassay	2.028±0.057	1.228±0.042	1.040±0.037	0.764±0.002	0.602±0.021	0.301±0.014	0.083±0.001	BDL	BDL	
	GLC	1.383±0.009	0.973±0.017	0.770±0.012	0.555±0.005	0.466±0.006	0.247±0.004	0.083±0.006	BDL	BDL	
	Endo- I										
	Endo-II	0.841±0.011	0.580±0.004	0.456±0.003	0.337±0.004	0.279±0.001	0.151±0.003	0.042±0.006	BDL	BDL	
	Endo I+II	2.224±0.020	1.553±0.020	1.235±0.013	0.892±0.003	0.745±0.005	0.398±0.006	0.125±0.001	BDL	BDL	
r =	Average *	2.126±0.088	1.391±0.031	1.138±0.013	0.828±0.0001	0.674±0.013	0.350±0.004	0.104±0.009	BDL	BDL	
	0.987	(34.60)	(46.50)	(61.05)	(68.32)	(83.56)	(95.11)		BDL	BDL	
	Bioassay	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	HPLC	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	Average*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
r =	---	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
Lambda cyhalothrin 75.00	Bioassay	1.542±0.011	1.189±0.001	0.979±0.006	0.766±0.006	0.425±0.004	0.180±0.003	0.019±0.014	BDL	BDL	
	GLC	1.672±0.004	1.264±0.125	1.023±0.033	0.806±0.047	0.437±0.015	0.197±0.003	0.043±0.003	BDL	BDL	
	Average*	1.607±0.004	1.226±0.063	1.001±0.019	0.786±0.023	0.431±0.006	0.189±0.003	0.031±0.006	BDL	BDL	
	0.990	(23.69)	(37.70)	(51.09)	(73.17)	(88.26)	(98.06)		BDL	BDL	
	Bioassay	2.003±0.040	1.365±0.007	1.096±0.007	0.876±0.008	0.674±0.013	0.213±0.010	BDL	BDL	BDL	
Spiromesifen 225.00	GLC	2.101±0.007	1.437±0.019	1.151±0.009	0.950±0.007	0.719±0.031	0.317±0.067	0.002±0.0001	BDL	BDL	
	Average*	2.052±0.023	1.401±0.007	1.123±0.004	0.813±0.008	0.697±0.022	0.265±0.030	0.001±0.0001	BDL	BDL	
	0.996	(31.73)	(45.26)	(55.51)	(66.06)	(87.09)	(99.95)		BDL	BDL	

* Based on three replications; # 75 days after sowing of pesticide treated seeds
Figures following ± signs are 95 per cent confidence limits.
Figures in the parentheses are the values of the per cent reduction of initial deposits
r = Correlation coefficient between bioassay and chromatographic methods of residue estimation
BDL= Below detectable limit
*= Average of bioassay and GLC method of residue estimation

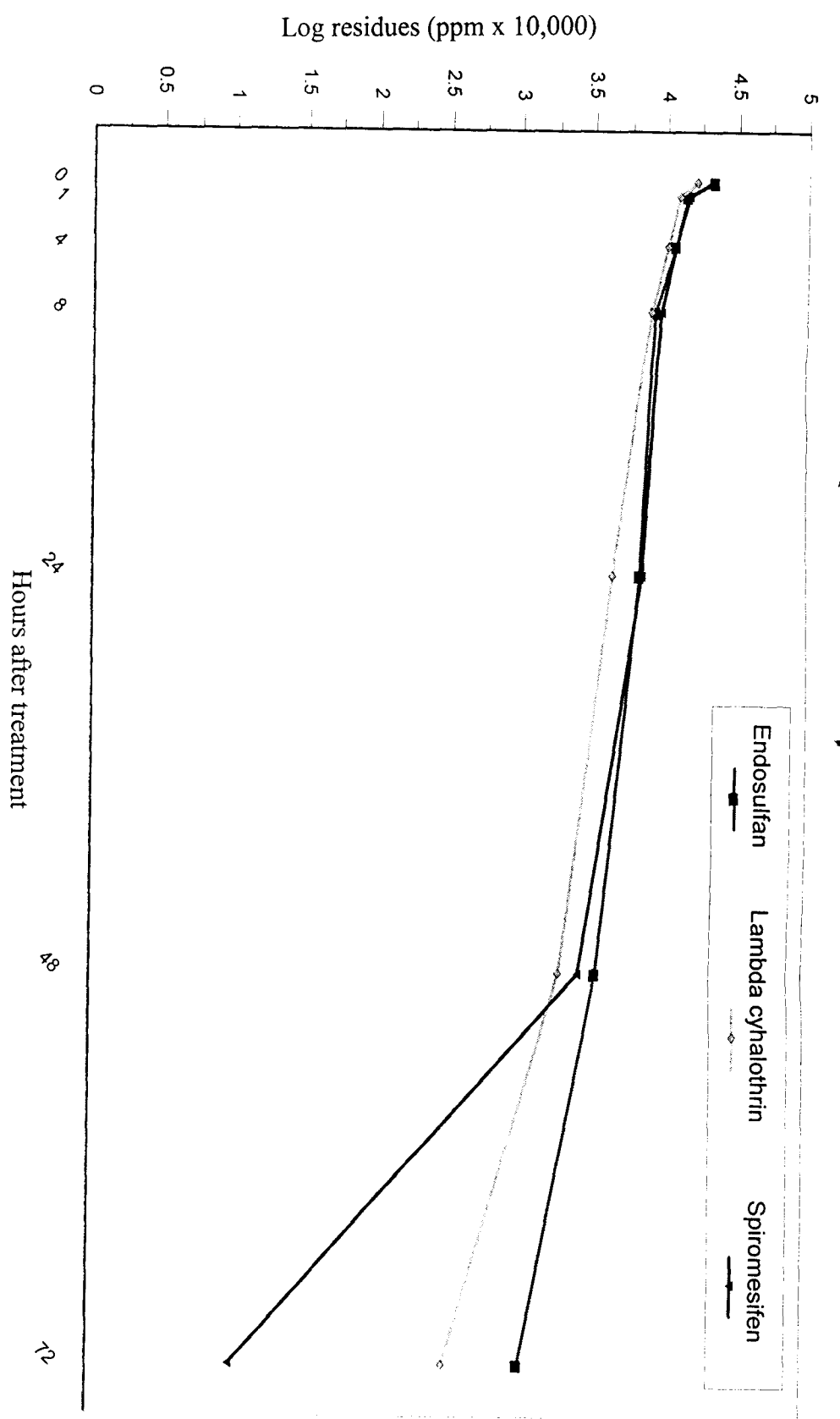


Fig. 4.20 Pesticide residues in *A. mellifera* foraged mustard pollen during *rabi*, 2003-04

spray, respectively with corresponding per cent degradation of 31.73, 45.26, 55.51, 66.06, 87.09 and 99.95 per cent. The half life was calculated to be 7.98 hours (Table 4.54).

4.4.4 Pesticide Residue in Bee Foraged Mustard Pollen during *Rabi*, 2004-05

The results are presented in Table 4.53 and illustrated in fig 4.20.

4.4.4.1 Endosulfan

The initial deposit of 2.068 ± 0.048 ppm degraded to 1.311 ± 0.011 (36.59 %), 1.028 ± 0.043 (50.30 %), 0.745 ± 0.002 (63.99 %), 0.542 ± 0.0001 (73.78 %), 0.275 ± 0.002 (86.72 %) and 0.051 ± 0.003 (97.56 %) ppm after 1, 4, 8, 24, 48 and 72 hours of spray, respectively. The half life was calculated to be 16.01 hours (Table 4.54).

4.4.4.2 Imidacloprid

Imidacloprid residues were below detectable limits in mustard pollen after 75 days of imidacloprid treated seed sowing.

4.4.4.3 Lambda cyhalothrin

Lambda cyhalothrin residues were estimated by bioassay and GLC method. There was a high degree of correlation between these two methods ($r = 0.998$). Initial deposit of active ingredient in pollen during second year trial was found to be 1.577 ± 0.018 ppm. The initial deposit after 1, 4, 8, 24, 48 and 72 hours of spray degraded to 1.111 ± 0.003 , 0.914 ± 0.015 , 0.729 ± 0.005 , 0.399 ± 0.004 , 0.157 ± 0.001 and 0.008 ± 0.002 ppm with per cent reduction of 29.56, 42.01, 53.77, 74.73, 90.08 and 99.50 per cent, respectively. The half life was 10.87 hours (Table 4.54).

4.4.4.4 Spiromesifen

Spiromesifen residues persisted for 72 hours after spray thereafter residues dwindled to the level below detectable limit. The initial deposit of spiromesifen was recorded to be 1.813 ± 0.005 ppm, which reduced to 1.218 ± 0.002 , 1.030 ± 0.036 ,

Table 4.53 Pesticide residue (ppm)* in *A. mellifera* foraged mustard pollen during *rabi*, 2004-05

Pesticide / Conc.	Method of estimation	Hours after spray									
		0	1	4	8	24	48	72	120	240	
Endosulfan 525.00	Bioassay	2.008±0.053	1.210±0.023	0.953±0.085	0.697±0.001	0.492±0.003	0.241±0.003	0.004±0.001	BDL	BDL	
	GLC	1.341±0.047	0.875±0.014	0.683±0.001	0.491±0.002	0.369±0.001	0.195±0.001	0.059±0.003	BDL	BDL	
	Endo- I										
	Endo- II	0.786±0.012	0.537±0.008	0.419±0.001	0.301±0.001	0.223±0.003	0.113±0.001	0.038±0.003	BDL	BDL	
	Endo I+II	2.127±0.073	1.412±0.022	1.102±0.002	0.792±0.003	0.592±0.003	0.308±0.0001	0.097±0.006	BDL	BDL	
r = Imidacloprid [#] 21.00	Average*	2.068±0.048	1.311±0.011	1.028±0.043	0.745±0.002	0.542±0.0001	0.275±0.002	0.051±0.003	BDL	BDL	
	0.995		(36.59)	(50.30)	(63.99)	(73.78)	(86.72)	(97.56)	BDL	BDL	
	Bioassay	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	HPLC	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	Average*	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
r = Lambda cyhalothrin 75.00	---	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	
	Bioassay	1.542±0.009	1.060±0.004	0.878±0.014	0.687±0.007	0.355±0.006	0.150±0.003	0.005±0.004	BDL	BDL	
	GLC	1.612±0.029	1.161±0.002	0.951±0.019	0.771±0.010	0.442±0.010	0.163±0.004	0.011±0.001	BDL	BDL	
	Average*	1.577±0.018	1.111±0.003	0.914±0.015	0.729±0.005	0.399±0.004	0.157±0.001	0.008±0.002	BDL	BDL	
	0.998		(29.56)	(42.01)	(53.77)	(74.73)	(90.08)	(99.50)	BDL	BDL	
r = Spiromesifen 225.00	Bioassay	1.799±0.033	1.193±0.006	1.023±0.062	0.836±0.117	0.572±0.048	0.321±0.054	BDL	BDL	BDL	
	GLC	1.827±0.042	1.242±0.008	1.037±0.014	0.900±0.048	0.592±0.064	0.296±0.031	0.004±0.002	BDL	BDL	
	Average*	1.813±0.005	1.218±0.002	1.030±0.036	0.881±0.053	0.582±0.009	0.309±0.012	0.002±0.0007	BDL	BDL	
	0.977		(32.83)	(43.18)	(51.38)	(67.90)	(82.97)	(99.72)	BDL	BDL	

* Based on three replications; [#] 75 days after sowing of pesticide treated seeds

Figures following ± signs are 95 per cent confidence limits.

Figures in the parentheses are the values of the per cent reduction of initial deposits

r = Correlation coefficient between bioassay and chromatographic methods of residue estimation

BDL= Below detectable limit

*= Average of bioassay and GLC method of residue estimation

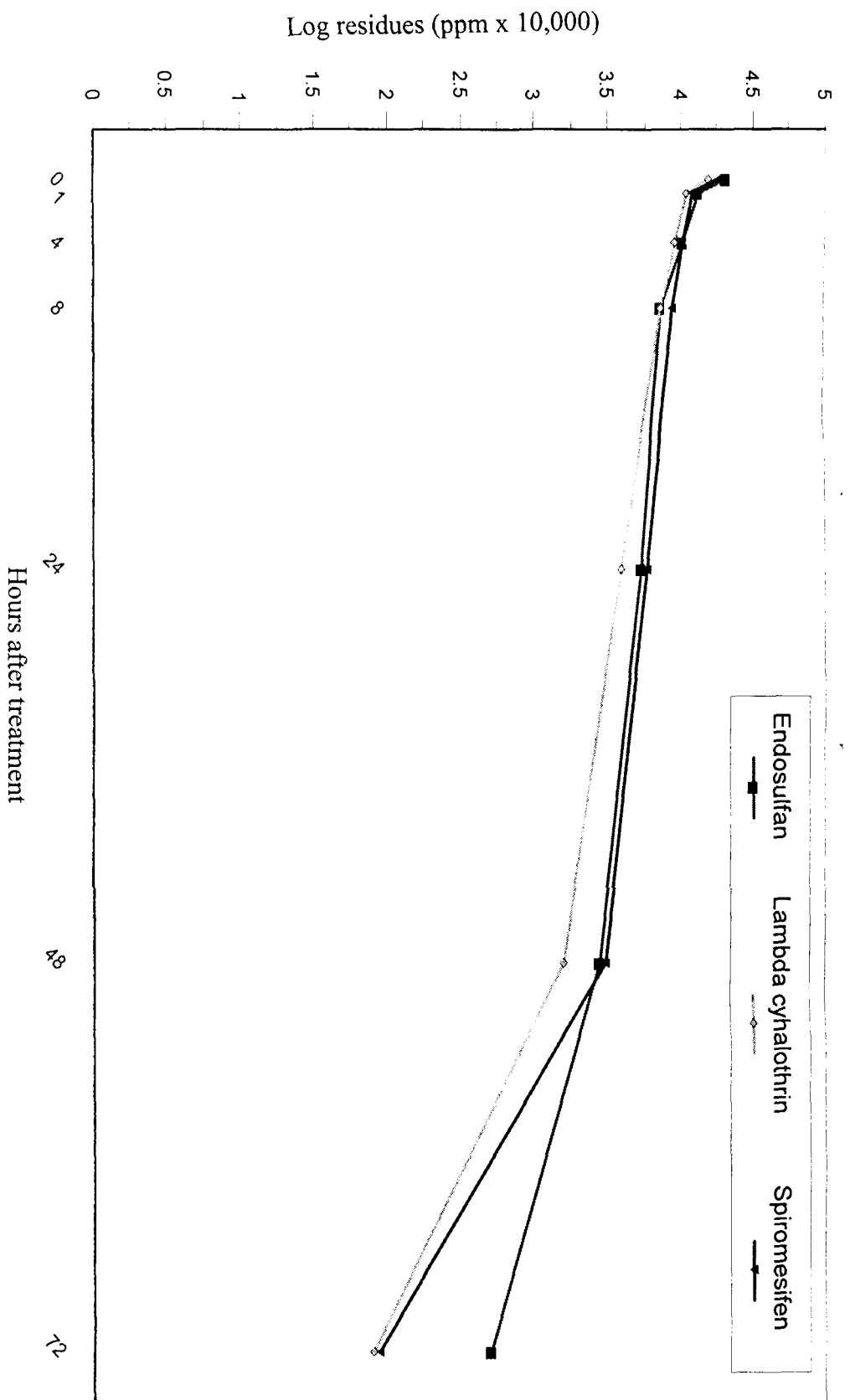


Fig. 4.21 Pesticide residues in *A. mellifera* foraged mustard pollen during *rabi*, 2004-05

0.881 $\pm$ 0.053, 0.582 $\pm$ 0.009, 0.309 $\pm$ 0.012 and 0.002 $\pm$ 0.0007 ppm after 1, 4, 8, 24, 48 and 72 hours of spray with per cent reduction of 32.83, 43.18, 51.38, 67.90, 82.97 and 99.72 per cent, respectively. The half life value was calculated to be 11.40 hours (Table 4.54).

4.5 CORRELATION BETWEEN BEE MORTALITY AND RESIDUES OF PESTICIDES

It is seen from the Table 4.55 that spiromesifen had low value of correlation coefficient between bee mortality and residues (0.51-0.52). However, it was highest for lambda cyhalothrin (0.88-0.97). The correlation coefficient for endosulfan varied from 0.61-0.72.

4.6 HONEY CHARACTERISTICS

During the research period (2002-2005) fifty one samples of honey of *A. cerana indica*/ *A. c. cerana* and *A. mellifera* were collected from different localities of Himachal Pradesh (Table 4.56) with some basic information regarding source of collection, date of collection, floral characteristics, method of honey extraction and any other information. 500 g samples were collected and stored at room temperature for further analysis work. Honey samples were first analyzed for their physical and chemical properties and data were tabulated in Tables 4.57-4.59.

4.6.1 Physical Characters

It is seen that optical density of the honey samples at 560 nm varied from 0.055 $\pm$ 0.001 to 0.670 $\pm$ 0.001 (Table 4.57), which correspond to the colour grades of water white and light amber. The average O.D. of honey samples was 0.219 $\pm$ 0.022. The 95 per cent confidence interval was 0.175 – 0.263.

The refractive index varied from 1.4882 $\pm$ 0.0002 to 1.4985 $\pm$ 0.0002 with a mean of 1.4930 $\pm$ 0.0004. The 95 per cent confidence interval was 1.4922 - 1.4931. Moisture is

Table 4. 54 Regression equations and half life values of pesticides in nectar and pollen of mustard

Pesticides (conc. %)	Years	Regression equations		Half life (hrs.)		Half life (days)	
		Nectar	Pollen	Nectar	Pollen	Nectar	Pollen
Endosulfan (0.07%)	1 st year	Y= 4.237- 0.0131 X	Y= 4.177- 0.0153 X	22.97	19.67	0.96	0.82
	2 nd year	Y= 4.139- 0.0094 X	Y= 4.164- 0.0188 X	32.02	16.01	1.33	0.67
Lambda cyhalothrin (0.01%)	1 st year	Y= 3.880- 0.0218 X	Y= 4.135- 0.0214 X	13.80	14.07	0.58	0.59
	2 nd year	Y= 3.911- 0.0271 X	Y= 4.157- 0.0277 X	11.11	10.87	0.46	0.45
Spiromesifen (0.03%)	1 st year	Y= 4.082 - 0.0150 X	Y= 4.378- 0.0377 X	20.06	7.98	0.84	0.33
	2 nd year	Y= 4.002- 0.0151 X	Y= 4.236- 0.0264 X	19.93	11.40	0.83	0.48

Y = Log (residues x 10⁴)
X = Time elapsed (hrs.)

Table 4.55 Correlation between residues and bee mortality

Pesticides	<i>A. c. cerana</i> (1 st year)		<i>A. c. cerana</i> (2 nd year)		<i>A. mellifera</i> (1 st year)		<i>A. mellifera</i> (2 nd year)	
	Nectar	Pollen	Nectar	Pollen	Nectar	Pollen	Nectar	Pollen
Endosulfan	0.64	0.71	0.65	0.72	0.67	0.67	0.61	0.61
Lambda cyhalothrin	0.93	0.91	0.97	0.96	0.90	0.88	0.93	0.92
Spiromesifen	0.52	0.52	0.51	0.51	0.52	0.52	0.51	0.51

Data were used from tables 4.35-4.36 and 4.38-4.39 for persistent toxicity and 4.50-4.54 for pesticide residue

Table 4.56 List of honey samples collected from different localities of Himachal Pradesh

Sample No	Source of collection	Date of collection	Honey bee species	Floral characteristics	Method of honey extraction	Any other information
1.	Regional Research Station (HAREC) Bajaura District Kullu	2 nd week, June 2003	<i>A. mellifera</i>	Rapeseed & mustard	Honey extractor	---
2.	Regional Research Station (HAREC) Dhaura Kaun District Sirmour	2 nd week, May 2003	<i>A. mellifera</i>	Eucalyptus	Honey extractor	---
3.	Regional Research Station (HAREC) Dhaura Kaun District Sirmour	2 nd week, May 2003	<i>A. mellifera</i>	Sarson	Honey extractor	---
4.	Regional Research Station (HAREC) Dhaura Kaun District Sirmour	2 nd week, May 2003	<i>A. mellifera</i>	Berseem	Honey extractor	---
5.	Sh Gopal Chand Village & Post office Ichhi, Distt Kangra	1 st week, May 2003	<i>A. mellifera</i>	Multiflora	Honey extractor	---
6.	Sh Ashwani Kumar Mehla Chamba	1 st week, June 2003	<i>A. mellifera</i>	Multiflora	Hand extraction	---
7.	Regional Research Station (HAREC) Bajaura District Kullu	2 nd week, May 2004	<i>A. mellifera</i>	Multiflora	Honey extractor	---
8.	Sh Manoj Negi Rekong peo District Kinnaur	4 th week, May 2004	<i>A. mellifera</i>	Plectranthus	Hand extraction	---
9.	Sh Beli Ram S/o Sh Daulat Ram Devidar PO Balkhol Kotkhai District Shimla	3 rd week, May 2004	<i>A. mellifera</i>	Plectranthus	Hand extraction	---
10.	Sh Prem Chauhan S/o Sh Mast Ram. Bukhneol Post office Pudak Kotkhai District Shimla.	3 rd week, May 2004	<i>A. mellifera</i>	Plectranthus	Honey extractor	---
11.	Dept. of Entomology, CSK HPKV Palampur	3 rd week, Apr. 2004	<i>A. c. indica</i>	Rape seed & mustard	Hand Extraction	---
12.	Sh Pratap Chand Bajinath District Kangra	1 st week, May 2004	<i>A. dorsata</i>	Multiflora	Hand Extraction	---
13.	Sh Nek Raj S/o Sh Ram Chand Palampur District Kangra	1 st week, May 2004	<i>A. c. indica</i>	Multiflora	Hand Extraction	---
14.	Sh Nek Raj S/o Sh Ram Chand Palampur District Kangra	1 st week, May 2004	<i>A. c. indica</i>	Multiflora	Honey extractor	---
15.	Sh Ram Singh Malan District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Acacia	Honey extractor	Market problem due to low price
16.	Sh Deva Singh Negi Ashrang Teh. Moorang District Kinnaur	4 th week, May 2004	<i>A. mellifera</i>	Plectranthus	Hand Extraction	---
17.	Sh Bsldev Singh Uncha Gaon District Solan	1 st week, June 2004	<i>A. mellifera</i>	Multiflora	Hand Extraction	---
18.	Sh Parkash Kumar Nauni District Solan	1 st week, June 2004	<i>A. mellifera</i>	Multiflora	Honey extractor	Market problem due to low price
19.	The Kangra Valley Beekeepers Co-op Industrial Society Ltd. Ichhi District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Multiflora	Honey extractor	---
20.	The Kangra Valley Beekeepers Co-op Industrial Society Ltd. Ichhi District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Berseem+Sunflower	Honey extractor	Market problem due to low price
21.	The Kangra Valley Beekeepers Co-op Industrial Society Ltd. Ichhi District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Honey dew (mango)	Honey extractor	Market problem due to low price
22.	RHRS, YSPUHF, Jacch District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Multiflora	Honey extractor	---
23.	Thakur Jeet Singh Village & Post office Jonta District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Multiflora	Honey extractor	Market problem due to low price
24.	Sh Shakti Singh Village & Post office Khajjian	1 st week, May 2004	<i>A. mellifera</i>	Rapeseed &	Honey extractor	Market problem due to low price

	District Kangra					Mustard		low price
25.	Sh Dalkhu Minhas Village & Post office Jaunta District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Eucalyptus	Honey extractor	Market problem due to low price		
26.	Sh Dalkhu Minhas Village & Post office Jaunta District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Rapeseed & Mustard	Honey extractor	Market problem due to low price		
27.	Golden Drops Sh S.K. Gupta Village & Post office Kangra District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Litchi	Honey extractor	---		
28.	Sh Madan Lal Village & Post office Ichhi Distt Kangra	1 st week, May 2004	<i>A. mellifera</i>	Rapeseed & Mustard	Honey extractor	High price of nucleus		
29.	Golden Drops Sh S.K. Gupta Village & Post office Kangra District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Rapeseed & Mustard	Honey extractor	---		
30.	Sh Ram Krishan Village & Post office Malan District Kangra	1 st week, May 2004	<i>A. mellifera</i>	Acacia	Honey extractor	---		
31.	Sh Ram Krishan Village & Post office Malan District Kangra	1 st week, June 2004	<i>A. mellifera</i>	Rapeseed & Mustard	Honey extractor	---		
32.	Dept. of Entomology CSKHPKV, Palampur	1 st week, June 2004	<i>A.c. indica</i>	Rapeseed & Mustard	Honey extractor	---		
33.	Sh Inshwar Singh Karl District Kangra	1 st week, June 2004	<i>A. mellifera</i>	Multiflora	Honey extractor	Varroa mite infestation		
34.	Market sample Palampur	1 st week, June 2004	<i>A. dorsata</i>	Multiflora	---	---		
35.	Sh. Thakur Singh Post office Sakri District Kangra	1 st week, May 2005	<i>A.c. indica</i>	Multiflora	Honey extractor	---		
36.	Sh Pradhan Singh Tehsil Jaisinghpur District Kangra	1 st week, May 2005	<i>A. mellifera</i>	Multiflora	Hand Extraction	Varroa mite infestation		
37.	Sh Gian Singh Tehsil Jaisinghpur District Kangra	1 st week, May 2005	<i>A.c. indica</i>	Multiflora	Hand Extraction	---		
38.	Mahila evm Paryavaran Simiti Changer Project Thakurdwara District Kangra	1 st week, May 2005	<i>A. mellifera</i>	---	---	---		
39.	The Kangra Pickle unit, Nagrota Bagwan District Kangra	1 st week, May 2005	<i>A. mellifera</i>	---	---	---		
40.	Regional Research Station (HAREC) Dhaula Kuan District Sirmour	3 rd week, May 2005	<i>A. mellifera</i>	Eucalyptus	Honey extractor	---		
41.	Regional Research Station (HAREC) Dhaula Kuan District Sirmour	3 rd week, May 2005	<i>A. mellifera</i>	Sarson	Honey extractor	---		
42.	Regional Research Station (HAREC) Dhaula Kuan District Sirmour	3 rd week, May 2005	<i>A. mellifera</i>	Berseem	Honey extractor	---		
43.	Dabur India Limited	3 rd week, May 2005	---	---	---	---		
44.	Sh. Pir Singh Bherewala District Sirmour	3 rd week, May 2005	<i>A. mellifera</i>	Rapeseed & Mustard	Honey extractor	Varroa mite infestation		
45.	Hazel Chamba	1 st week, Apr 2005	---	---	---	---		
46.	Market sample Bilaspur	1 st week, June 2005	<i>A.c. indica</i>	---	---	---		
47.	Market sample Bilaspur	1 st week, June 2005	<i>A.c. indica</i>	---	---	---		
48.	Market sample Una	1 st week, June 2005	<i>A. mellifera</i>	---	---	---		
49.	Market sample Una	1 st week, June 2005	<i>A. mellifera</i>	---	---	---		
50.	Market sample Hamirpur	1 st week, June 2005	<i>A. mellifera</i>	---	---	---		
51.	Sh Ravi Dutt Sharma Jassur District Kangra	1 st week, May 2005	<i>A. mellifera</i>	Multiflora	Hand extraction	Varroa mite infestation		

From the Table 4.57, it is observed that viscosity of the honey samples varied from 2.114 ± 0.001 to 9.123 ± 0.001 minutes. Average viscosity was calculated to be 5.772 ± 0.170 minutes with 95 per cent confidence interval of $5.431 - 6.114$. Density was measured as per method given in section 3.2.5.4.1.1.3. It was found that a minimum density of $1.551 \pm 0.001 \text{ gcm}^{-3}$ and a maximum of $1.707 \pm 0.007 \text{ gcm}^{-3}$ were observed in sample numbers 40 and 38, respectively. Average density was recorded to be $1.617 \pm 0.005 \text{ gcm}^{-3}$ with 95 per cent confidence interval of $1.608 - 1.627$.

It is observed that pollen counts had a great variation in honey samples collected from different localities of Himachal Pradesh with different floral resources. It was recorded that sample number 26 had maximum pollen count of 140000 ± 2887 pollens 10g^{-1} , whereas a minimum of 13667 ± 1667 pollens 10g^{-1} were counted in sample number 40. The mean pollen count of 64141.17 ± 4231.60 pollens 10g^{-1} honey was calculated with 95 per cent confidence interval of $55635.37 - 72646.69$.

4.6.2 Chemical Characters

The TSS value varied between a maximum value of 84.73 ± 0.07 per cent and a minimum of 80.67 ± 0.01 per cent (Table 4.58). An average of 82.40 ± 0.16 per cent was calculated with 95 per cent confidence interval of $82.08 - 82.73$.

Fructose content of the various samples collected had variation of 30.21 ± 0.02 to 44.25 ± 0.01 per cent. The average fructose content was 35.98 ± 0.51 per cent with 95 per cent confidence limit of $34.95 - 37.01$. Similarly, glucose content was found to vary from a minimum of 23.76 ± 0.08 to maximum of 40.73 ± 0.08 per cent. The average glucose content was 33.05 ± 0.54 per cent with 95 per cent confidence interval of $31.95 - 34.14$ per cent. The fructose glucose ratio was also worked out. It varied from 0.899 ± 0.003 to

Table 4.57 Physical properties of honey samples collected from Himachal Pradesh

Sample No.	O.D. (at 560 nm)	Colour specification (USDA)	Refractive index at 20°C	Moisture (%)	Viscosity (mins.) at 40°C	Density (gcm ⁻³) at 27°C	Pollens per 10g
1	0.122 ±0.001	Extra white	1.4932±0.0002	17.33±0.06	6.241±0.001	1.621±0.001	46333±1856
2	0.211 ±0.001	White	1.4908±0.0002	18.27±0.06	5.653±0.001	1.581±0.001	115667±7881
3	0.194 ±0.002	White	1.4908±0.0002	18.27±0.06	5.421±0.001	1.620±0.001	115333±8213
4	0.182 ±0.001	Extra white	1.4920±0.0000	17.80±0.00	6.532±0.001	1.642±0.002	87667±1856
5	0.065 ±0.001	Water white	1.4964±0.0002	16.07±0.06	7.235±0.001	1.601±0.001	55000±2887
6	0.072 ±0.001	Water white	1.4910±0.0000	18.20±0.00	5.307±0.007	1.622±0.001	61333±1764
7	0.072 ±0.001	Water white	1.4907±0.0002	18.33±0.06	4.253±0.002	1.663±0.002	90667±1764
8	0.071 ±0.001	Water white	1.4956±0.0000	16.40±0.00	6.421±0.001	1.621±0.001	29333±667
9	0.072 ±0.001	Water white	1.4982±0.0000	15.40±0.00	7.533±0.001	1.570±0.001	31333±1453
10	0.085 ±0.001	Water white	1.4963±0.0002	16.13±0.06	6.488±0.001	1.620±0.001	29667±1453
11	0.185 ±0.001	Extra white	1.4905±0.0000	18.40±0.00	5.601±0.001	1.611±0.001	99000±2082
12	0.101 ±0.001	Extra white	1.4883±0.0002	19.27±0.06	4.231±0.001	1.611±0.001	40000±1155
13	0.070 ±0.001	Water white	1.4951±0.0000	16.60±0.00	6.509±0.008	1.692±0.001	46667±667
14	0.083 ±0.001	Water white	1.4882±0.0002	19.33±0.06	3.605±0.004	1.611±0.001	51333±2404
15	0.454 ±0.001	Extra light amber	1.4961±0.0000	16.20±0.00	6.894±0.003	1.661±0.001	87667±1453
16	0.065 ±0.001	Water white	1.4935±0.0000	17.20±0.00	6.902±0.001	1.620±0.000	26667±882
17	0.175 ±0.001	Extra white	1.4912±0.0002	18.13±0.06	5.232±0.001	1.653±0.001	46667±2404
18	0.254 ±0.002	White	1.4949±0.0002	16.67±0.06	6.225±0.007	1.602±0.001	60333±1453
19	0.081 ±0.001	Water white	1.4969±0.0002	15.87±0.06	9.123±0.001	1.642±0.001	52667±667
20	0.185 ±0.001	Extra white	1.4912±0.0002	18.13±0.06	5.873±0.001	1.621±0.001	82667±1202
21	0.461 ±0.001	Extra light amber	1.4910±0.0000	18.20±0.00	4.122±0.001	1.620±0.000	78333±1667
22	0.101 ±0.001	Extra white	1.4966±0.0000	16.00±0.00	6.213±0.001	1.601±0.001	42333±1202
23	0.074 ±0.002	Water white	1.4985±0.0002	15.27±0.06	7.312±0.001	1.601±0.001	40667±882
24	0.182 ±0.001	Extra white	1.4890±0.0000	19.00±0.00	4.561±0.001	1.562±0.002	92000±1155
25	0.225 ±0.001	White	1.4895±0.0000	18.80±0.00	5.622±0.001	1.661±0.001	127000±3606
26	0.212 ±0.001	White	1.4922±0.0002	17.73±0.06	5.801±0.001	1.611±0.001	140000±2887
27	0.212 ±0.001	White	1.4935±0.0000	17.20±0.00	6.883±0.001	1.690±0.001	53533±22984
28	0.112 ±0.001	Extra white	1.4946±0.0000	16.80±0.00	6.542±0.001	1.642±0.001	42000±1155
29	0.055 ±0.001	Water white	1.4920±0.0000	17.80±0.00	6.102±0.001	1.620±0.001	21667±2028
30	0.321 ±0.001	White	1.4954±0.0002	16.47±0.06	6.552±0.001	1.661±0.001	83333±1202

31	0.142	±0.001	Extra white	1.4973±0.0002	15.87±0.06	5.225±0.001	1.571±0.001	46333±1856
32	0.150	±0.001	Extra white	1.4946±0.0000	16.80±0.00	6.536±0.006	1.621±0.001	53667±1333
33	0.193	±0.001	White	1.4905±0.0000	18.40±0.00	6.241±0.001	1.641±0.001	37000±577
34	0.154	±0.002	Extra white	1.4932±0.0002	17.33±0.06	6.232±0.001	1.601±0.001	47333±1202
35	0.111	±0.001	Extra white	1.4910±0.0000	18.20±0.00	6.202±0.001	1.621±0.001	57667±1453
36	0.083	±0.001	Water white	1.4961±0.0000	16.20±0.00	7.233±0.002	1.663±0.002	44000±2309
37	0.176	±0.004	Extra white	1.4905±0.0000	18.40±0.00	5.122±0.001	1.583±0.003	64333±1202
38	0.423	±0.001	Extra light amber	1.4973±0.0002	15.87±0.06	7.703±0.002	1.707±0.007	83667±882
39	0.451	±0.001	Extra light amber	1.4910±0.0000	18.20±0.00	4.122±0.001	1.621±0.001	88667±1856
40	0.261	±0.001	White	1.4898±0.0002	18.67±0.06	5.302±0.001	1.551±0.001	13667±1667
41	0.670	±0.001	Light amber	1.4883±0.0002	19.27±0.06	4.231±0.001	1.611±0.001	4000±577
42	0.191	±0.001	White	1.4915±0.0000	18.00±0.00	5.662±0.001	1.611±0.001	81667±333
43	0.542	±0.001	Extra light amber	1.4888±0.0002	19.07±0.06	2.114±0.001	1.521±0.001	107000±6507
44	0.156	±0.001	Extra white	1.4918±0.0002	17.87±0.06	5.825±0.001	1.622±0.001	45000±2517
45	0.561	±0.001	Extra light amber	1.4900±0.0000	18.60±0.00	5.422±0.001	1.620±0.001	95000±2309
46	0.381	±0.001	Extra light amber	1.4905±0.0000	18.40±0.00	5.488±0.001	1.620±0.001	83000±2517
47	0.501	±0.001	Extra light amber	1.4888±0.0002	19.07±0.06	4.316±0.002	1.600±0.000	93667±882
48	0.422	±0.001	Extra light amber	1.4900±0.0000	18.60±0.00	5.424±0.002	1.621±0.001	76000±1155
49	0.443	±0.001	Extra light amber	1.4907±0.0002	18.33±0.06	5.982±0.002	1.621±0.001	83333±1202
50	0.262	±0.001	White	1.4966±0.0000	16.00±0.00	5.002±0.001	1.621±0.001	25667±1202
51	0.164	±0.002	Extra white	1.4888±0.0002	19.07±0.06	4.006±0.004	1.563±0.002	63667±882
Mean	0.219		White	1.4930	17.59	5.772	1.617	64141.17
S.E.	0.022			0.0004	0.16	0.170	0.005	4231.60
C.I.	0.175-0.263			1.4922-1.4931	17.27-17.29	5.431-6.114	1.608-1.627	55635.37-72646.69
C.V.	9.965			0.026	0.93	2.943	0.301	6.60

Table 4.58 Chemical properties of honey samples collected from Himachal Pradesh

Sample No.	TSS (%)	Fructose (%)	Glucose (%)	f/g ratio
1	82.67 ± 0.01	36.55 ± 0.03	36.52 ± 0.40	1.001 ± 0.007
2	81.73 ± 0.01	35.62 ± 0.07	24.95 ± 0.54	1.427 ± 0.003
3	81.73 ± 0.01	37.54 ± 0.06	37.44 ± 0.02	1.003 ± 0.012
4	82.20 ± 0.01	35.56 ± 0.02	29.31 ± 0.08	1.213 ± 0.012
5	83.93 ± 0.01	35.55 ± 0.07	35.54 ± 0.18	1.000 ± 0.002
6	81.80 ± 0.01	37.77 ± 0.01	32.00 ± 0.16	1.180 ± 0.001
7	81.67 ± 0.01	41.19 ± 0.01	35.81 ± 0.04	1.150 ± 0.000
8	83.60 ± 0.01	38.28 ± 0.01	38.03 ± 0.23	1.007 ± 0.009
9	84.60 ± 0.01	37.28 ± 0.01	37.32 ± 0.59	0.999 ± 0.015
10	83.87 ± 0.02	34.25 ± 0.03	33.67 ± 0.03	1.017 ± 0.042
11	81.60 ± 0.01	34.25 ± 0.03	33.86 ± 0.01	1.012 ± 0.042
12	80.73 ± 0.01	33.72 ± 0.02	34.41 ± 0.14	0.980 ± 0.000
13	83.40 ± 0.00	30.21 ± 0.02	30.06 ± 0.26	1.005 ± 0.000
14	80.67 ± 0.01	41.19 ± 0.02	35.81 ± 0.06	1.150 ± 0.001
15	83.80 ± 0.02	36.56 ± 0.03	30.95 ± 0.59	1.181 ± 0.006
16	82.80 ± 0.01	37.26 ± 0.01	36.92 ± 0.13	1.009 ± 0.003
17	81.87 ± 0.02	43.13 ± 0.01	34.50 ± 0.12	1.250 ± 0.000
18	83.33 ± 0.02	43.56 ± 0.02	36.00 ± 0.23	1.210 ± 0.001
19	84.13 ± 0.01	43.19 ± 0.02	35.40 ± 0.19	1.220 ± 0.000
20	81.87 ± 0.03	32.12 ± 0.01	26.72 ± 0.26	1.202 ± 0.010
21	81.80 ± 0.01	30.24 ± 0.01	25.74 ± 0.07	1.175 ± 0.010
22	84.00 ± 0.01	35.71 ± 0.04	34.01 ± 0.06	1.050 ± 0.000
23	84.73 ± 0.07	32.34 ± 0.03	31.10 ± 0.05	1.040 ± 0.001
24	81.00 ± 0.02	38.12 ± 0.01	38.59 ± 0.03	0.988 ± 0.019
25	81.20 ± 0.01	32.21 ± 0.01	23.76 ± 0.08	1.355 ± 0.007
26	82.27 ± 0.04	32.10 ± 0.04	33.67 ± 0.06	0.953 ± 0.018
27	82.80 ± 0.04	33.31 ± 0.02	29.73 ± 0.00	1.120 ± 0.003
28	83.20 ± 0.01	37.12 ± 0.02	40.73 ± 0.08	0.911 ± 0.007
29	82.20 ± 0.01	34.10 ± 0.01	34.05 ± 0.04	1.001 ± 0.018
30	83.53 ± 0.01	32.25 ± 0.06	25.70 ± 0.09	1.255 ± 0.007
31	84.13 ± 0.01	35.12 ± 0.02	35.12 ± 0.07	1.000 ± 0.000

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32	83.20 ± 0.02	34.22 ± 0.01	34.09 ± 0.03	1.004 ± 0.009
33	81.60 ± 0.04	33.25 ± 0.01	33.08 ± 0.07	1.005 ± 0.000
34	82.67 ± 0.05	34.15 ± 0.02	34.15 ± 0.02	1.000 ± 0.000
35	81.80 ± 0.00	32.14 ± 0.04	30.91 ± 0.08	1.040 ± 0.002
36	83.80 ± 0.01	43.56 ± 0.02	34.85 ± 0.09	1.250 ± 0.003
37	81.60 ± 0.01	44.25 ± 0.01	33.52 ± 0.03	1.320 ± 0.000
38	84.13 ± 0.01	34.25 ± 0.01	34.07 ± 0.01	1.005 ± 0.003
39	81.80 ± 0.03	32.12 ± 0.06	28.65 ± 0.02	1.121 ± 0.003
40	81.33 ± 0.04	34.12 ± 0.03	26.54 ± 0.05	1.286 ± 0.024
41	80.73 ± 0.02	35.14 ± 0.01	29.21 ± 0.05	1.203 ± 0.021
42	82.00 ± 0.01	37.26 ± 0.01	32.22 ± 0.09	1.156 ± 0.003
43	80.93 ± 0.07	36.65 ± 0.02	37.12 ± 0.01	0.987 ± 0.003
44	82.13 ± 0.01	36.54 ± 0.01	35.07 ± 0.03	1.042 ± 0.012
45	81.40 ± 0.02	35.52 ± 0.01	39.50 ± 0.09	0.899 ± 0.003
46	81.60 ± 0.01	38.23 ± 0.03	34.72 ± 0.05	1.101 ± 0.004
47	80.93 ± 0.01	33.25 ± 0.01	33.21 ± 0.01	1.001 ± 0.003
48	81.40 ± 0.00	32.21 ± 0.01	31.54 ± 0.01	1.021 ± 0.003
49	81.67 ± 0.07	35.55 ± 0.00	28.41 ± 0.02	1.251 ± 0.003
50	84.00 ± 0.04	32.01 ± 0.02	31.76 ± 0.01	1.008 ± 0.003
51	80.93 ± 0.02	43.09 ± 0.02	35.32 ± 0.01	1.220 ± 0.000
Mean	82.40	35.98	33.05	1.095
S.E.	0.16	0.51	0.54	0.017
C.I.	82.08 – 82.73	34.95 – 37.01	31.95 – 34.14	1.060 - 1.130
C.V.	0.20	1.42	1.65	1.584

1.427 ± 0.003 in sample number 2. The average fructose: glucose ratio was calculated to be 1.095 ± 0.017 with 95 per cent confidence interval of 1.060 – 1.130.

Honey is acidic in nature which is well proven by the values in the Table 4.59. The maximum pH value (5.037 ± 0.022) was recorded in sample number 28, however it was least (3.530 ± 0.015) in sample number 22. This showed that sample number 22 was highly acidic whereas, 28 was least acidic. The 95 per cent confidence interval was 4.304 – 4.524. Total reducing sugar content varied from a minimum of 55.979 ± 0.097 per cent in sample number 25 with a maximum of 79.569 ± 0.047 per cent in sample number 18. An average TRS content was 69.034 ± 0.919 per cent. The 95 per cent confidence limit was calculated to be 67.187 – 70.880 per cent.

Average sucrose content was found to be 4.883 ± 0.160 per cent. The 95 per cent confidence limit for sucrose content was 4.561 – 5.205 per cent. Sucrose content was minimum (1.233 ± 0.031 %) in sample number 21. However, it was maximum (7.213 ± 0.007 %) in sample number 45.

Ash content was calculated and results are given in Table 4.59. From the table it is seen that variation in ash content in fifty one samples analyzed was from a minimum of 0.086 ± 0.006 per cent in sample number 6 to a maximum of 0.703 ± 0.033 per cent in sample number 21. The average content of ash was 0.139 ± 0.012 per cent with 95 per cent confidence interval of 0.115 – 0.162 per cent.

At the time of sample analysis, it was found that honey samples had very low value of HMF content. It was observed that HMF varied from a minimum of 1.255 ± 0.061 to 25.164 ± 0.015 ppm in sample number 27 and 33, respectively. Average HMF was 9.027 ± 0.634 ppm. The 95 per cent confidence limit was calculated to be 7.752 – 10.302 ppm.

Table 4.59 Chemical properties of honey samples collected from Himachal Pradesh

Sample No.	pH	TRS (%)	Sucrose (%)	Ash (%)	HMF (ppm)
1	4.950± 0.006	73.082 + 0.037	5.136 + 0.061	0.144 + 0.003	4.257 + 0.007
2	4.370± 0.006	60.580 + 0.047	5.249 + 0.019	0.145 + 0.003	9.213 + 0.006
3	4.023± 0.007	74.984 + 0.057	5.224 + 0.015	0.154 + 0.012	5.265 + 0.007
4	4.443± 0.003	64.877 + 0.009	4.215 + 0.007	0.122 + 0.009	10.124 + 0.003
5	4.313± 0.009	71.093 + 0.075	4.116 + 0.079	0.168 + 0.006	7.841 + 0.297
6	4.517± 0.007	69.781 + 0.006	5.245 + 0.048	0.086 + 0.006	5.612 + 0.012
7	3.670± 0.012	77.009 + 0.008	3.213 + 0.015	0.111 + 0.006	3.413 + 0.007
8	4.337± 0.003	76.319 + 0.014	5.228 + 0.002	0.148 + 0.012	15.053 + 0.018
9	4.217± 0.003	74.612 + 0.049	5.693 + 0.019	0.127 + 0.006	18.265 + 0.010
10	4.337± 0.007	67.931 + 0.179	4.367 + 0.012	0.128 + 0.002	10.012 + 0.001
11	4.450± 0.006	68.115 + 0.100	4.321 + 0.000	0.099 + 0.007	6.246 + 0.042
12	4.533± 0.033	68.136 + 0.020	4.254 + 0.012	0.141 + 0.006	12.521 + 0.003
13	3.777± 0.005	60.280 + 0.049	3.281 + 0.006	0.161 + 0.006	14.413 + 0.009
14	3.787± 0.019	77.012 + 0.028	4.123 + 0.024	0.120 + 0.003	3.165 + 0.015
15	4.573± 0.007	67.521 + 0.067	6.122 + 0.009	0.122 + 0.003	4.238 + 0.030
16	4.650± 0.006	74.189 + 0.005	6.254 + 0.000	0.127 + 0.000	9.257 + 0.013
17	4.537± 0.019	77.639 + 0.029	5.372 + 0.093	0.088 + 0.006	5.183 + 0.280
18	4.517± 0.038	79.569 + 0.047	4.624 + 0.023	0.103 + 0.007	10.227 + 0.115
19	3.850± 0.035	78.599 + 0.039	5.478 + 0.049	0.121 + 0.006	12.225 + 0.017
20	4.563± 0.003	58.851 + 0.032	3.297 + 0.006	0.128 + 0.006	14.104 + 0.012
21	4.600± 0.012	55.983 + 0.022	1.233 + 0.031	0.703 + 0.033	4.250 + 0.001
22	3.530± 0.015	69.730 + 0.022	6.115 + 0.077	0.102 + 0.003	13.214 + 0.780
23	3.890± 0.010	63.450 + 0.050	3.212 + 0.012	0.143 + 0.010	2.158 + 0.003
24	4.037± 0.007	76.722 + 0.039	6.124 + 0.000	0.133 + 0.006	10.212 + 0.012
25	4.376± 0.002	55.979 + 0.097	5.214 + 0.012	0.150 + 0.003	10.232 + 0.009
26	4.323± 0.003	65.782 + 0.016	5.245 + 0.060	0.128 + 0.006	8.257 + 0.013
27	4.562± 0.002	63.050 + 0.070	6.222 + 0.006	0.145 + 0.007	1.255 + 0.061
28	5.037± 0.022	77.862 + 0.098	4.323 + 0.010	0.125 + 0.012	6.127 + 0.001
29	4.997± 0.003	68.162 + 0.028	4.445 + 0.018	0.116 + 0.020	7.126 + 0.151
30	4.587± 0.003	57.962 + 0.043	5.255 + 0.010	0.145 + 0.015	5.264 + 0.001

Contd....

31	4.977± 0.009	70.249 ± 0.013	6.545 ± 0.018	0.123 + 0.013	8.236 + 0.009
32	4.640± 0.015	68.323 ± 0.020	6.125 ± 0.006	0.127 ± 0.015	9.114 ± 0.015
33	4.037± 0.007	66.337 ± 0.016	2.115 ± 0.018	0.150 ± 0.003	25.164 ± 0.015
34	4.970± 0.012	68.309 ± 0.044	5.134 ± 0.023	0.121 ± 0.009	10.124 ± 0.013
35	5.017± 0.003	63.056 ± 0.064	6.716 ± 0.028	0.098 ± 0.003	8.167 ± 0.102
36	4.990± 0.006	78.421 ± 0.014	4.134 ± 0.020	0.097 ± 0.006	9.323 ± 0.007
37	3.760± 0.012	77.776 ± 0.015	4.113 ± 0.024	0.102 ± 0.003	4.384 ± 0.032
38	4.560± 0.006	68.330 ± 0.020	5.265 ± 0.007	0.132 ± 0.003	6.213 ± 0.005
39	4.373± 0.009	60.780 ± 0.078	6.253 ± 0.010	0.124 ± 0.013	8.236 ± 0.012
40	5.020± 0.035	60.671 ± 0.011	4.701 ± 0.020	0.122 ± 0.012	8.255 ± 0.007
41	4.597± 0.009	64.356 ± 0.027	4.216 ± 0.011	0.124 ± 0.003	6.223 ± 0.009
42	4.567± 0.009	69.488 ± 0.085	5.125 ± 0.007	0.142 ± 0.003	9.213 ± 0.007
43	4.240± 0.010	73.777 ± 0.019	6.234 ± 0.006	0.145 ± 0.003	10.235 ± 0.019
44	4.297± 0.009	71.613 ± 0.077	4.226 ± 0.012	0.145 ± 0.003	17.014 ± 0.042
45	4.124± 0.001	75.030 ± 0.032	7.213 ± 0.007	0.125 ± 0.007	18.023 ± 0.008
46	4.145± 0.003	72.953 ± 0.031	4.234 ± 0.006	0.134 ± 0.007	12.013 ± 0.007
47	4.150± 0.006	66.464 ± 0.004	4.880 ± 0.333	0.128 ± 0.003	10.986 ± 0.009
48	4.663± 0.003	63.755 ± 0.005	5.212 ± 0.007	0.098 ± 0.003	10.000 ± 0.010
49	4.663± 0.003	63.961 ± 0.082	4.235 ± 0.007	0.138 ± 0.003	9.234 ± 0.003
50	4.993± 0.012	63.780 ± 0.015	5.045 ± 0.007	0.122 ± 0.009	6.256 ± 0.012
51	4.003± 0.007	78.418 ± 0.047	5.125 ± 0.025	0.143 ± 0.010	5.225 ± 0.091
Mean	4.414	69.034	4.883	0.139	9.027
S.E.	0.055	0.919	0.160	0.012	0.634
C.I.	4.304 – 4.524	67.187 – 70.880	4.561 – 5.205	0.115 – 0.162	7.752 – 10.302
C.V.	1.240	1.331	3.281	8.354	7.026

4.7 PESTICIDE RESIDUES IN HONEY

Pesticides (organochlorine, cyclodiene, synthetic pyrethroids and organophosphorus) were determined in honey samples collected from different parts of Himachal Pradesh by GLC.

4.7.1 Standard curves for Gas liquid Chromatography

Technical pesticides dissolved in acetone were injected in GLC, which was made ideal on the operational parameters given in section 3.2.4.2.1.2. The retention time and areas were calculated from standard curves (Fig. 4.24 & 4.25) for each pesticide.

4.7.2 Recovery of Pesticides

A honey sample (floral source: *Plectranthus* sp.) was fortified with pesticides standard solutions. The residues were extracted and clean up as per section 3.2.5.4.2. Analysis was made on GLC. It was observed from the Table 4.60 that per cent recoveries for organochlorine and cyclodiene pesticides varied from 83.66 ± 0.15 per cent for dicofol to 91.33 ± 0.10 for β -endosulfan. Per cent recoveries for the synthetic pyrethroid pesticides varied from 87.83 ± 0.11 to 90.33 ± 0.23 for deltamethrin and fenvalerate, respectively.

Organophosphorus pesticides were estimated on NPD. It was found that per cent recoveries varied from 76.33 ± 0.15 to 83.66 ± 0.03 for malathion and dimethoate, respectively (Table 4.61).

4.7.3 Pesticide Residues in Honey Samples

4.9.3.1 Organochlorine Pesticides

All fifty one honey samples were analyzed for the simultaneous detection of residues of 10 different organochlorine pesticides. It was found that honey samples collected from different parts of the state had residues of organochlorine pesticides.

Fig 4.22 Gas liquid chromatogram for the blank honey sample on ECD

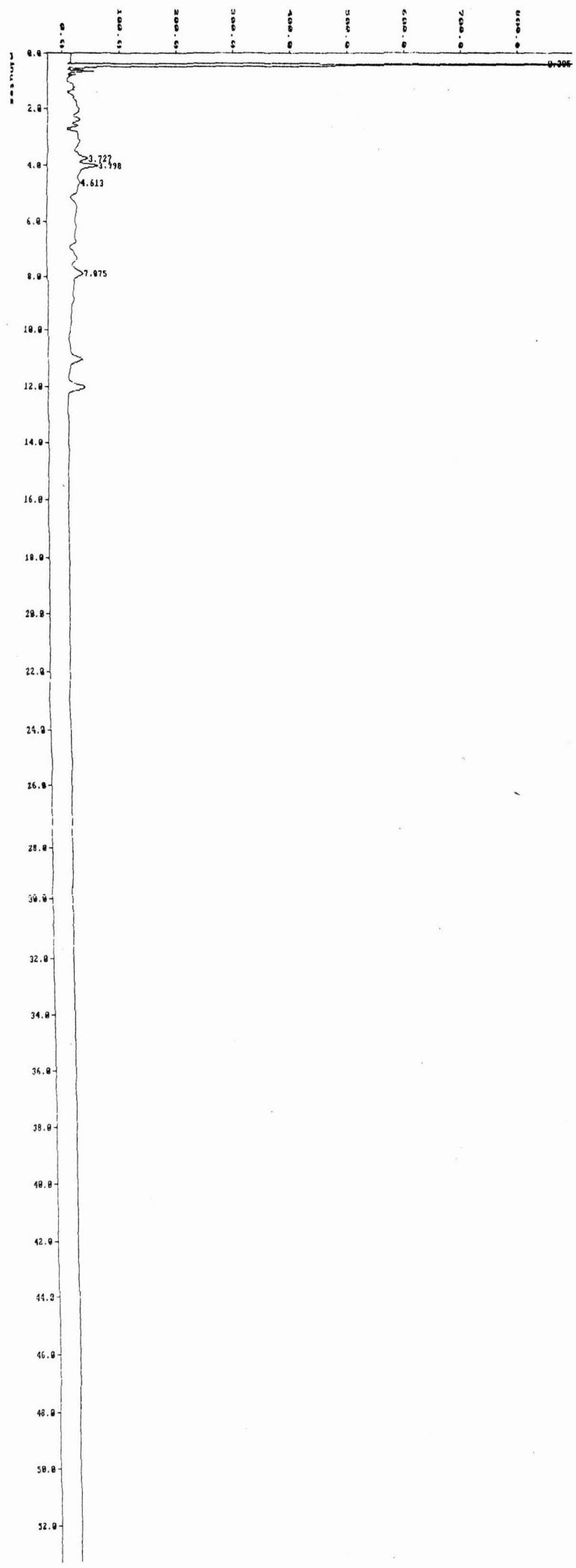


Fig 4.23 Gas liquid chromatogram for the blank honey sample on NPD

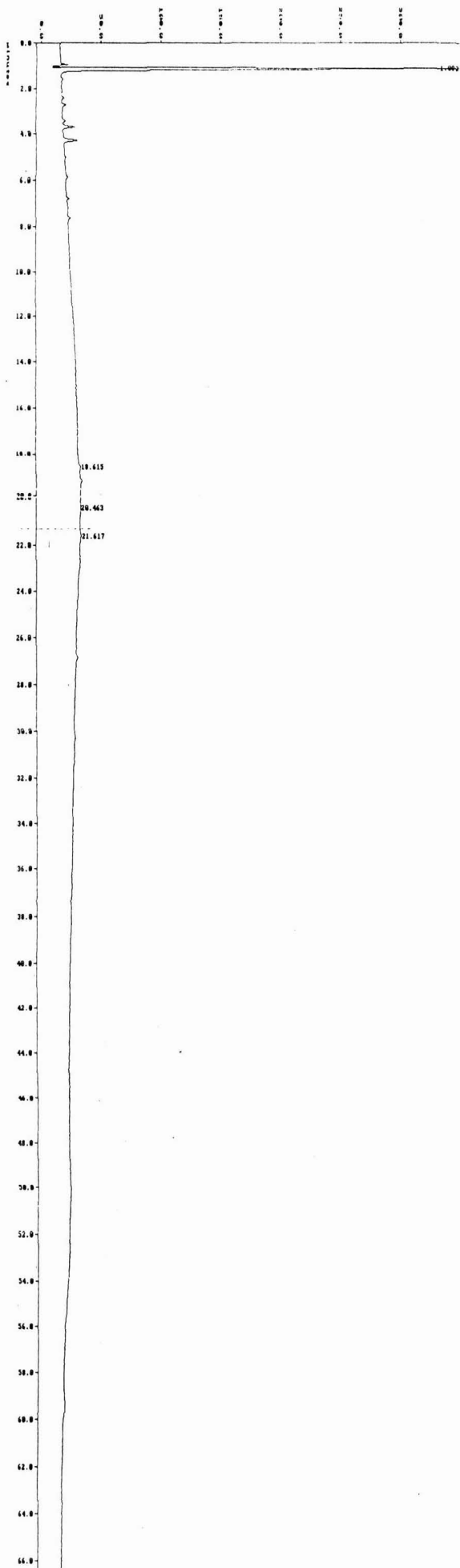


Fig. 4.24 Gas liquid chromatogram for multiresidue analysis of organochlorine, cyclodiene and synthetic pyrethroid pesticides in honey

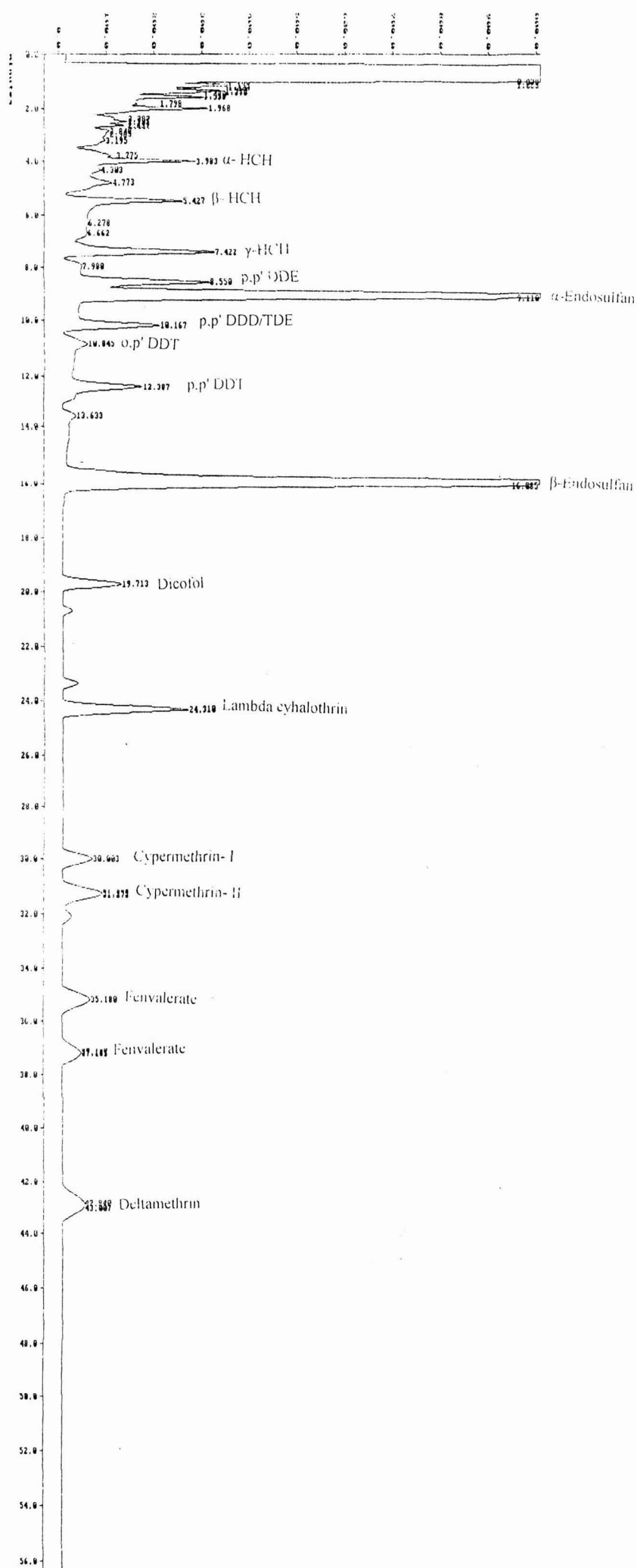


Fig. 4.25 Gas liquid chromatogram for multiresidue analysis of organophosphorus pesticides in honey

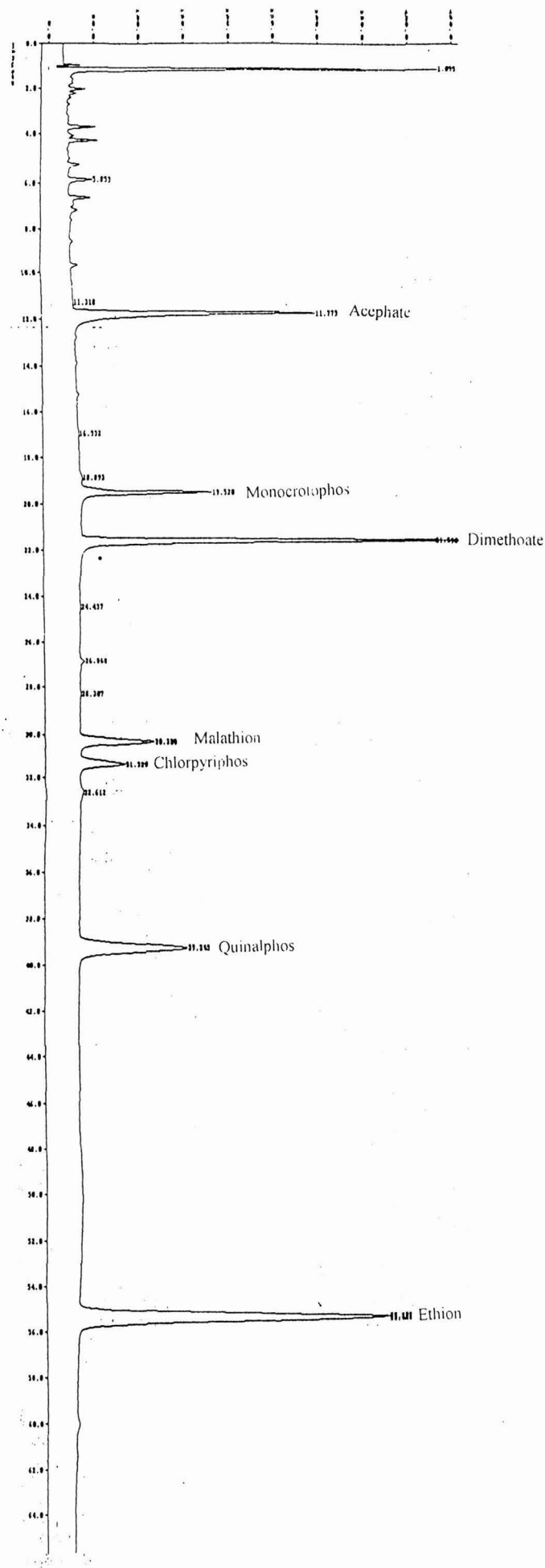


Table 4.60 Recoveries of different pesticides (organochlorine, cyclodiene and synthetic pyrethroids) through GLC in honey samples

Pesticides	Amount added (mg) in 20 g honey sample	Amount recovered (ppm)*	Recovery per cent	Average per cent recovery
p,p' DDE	0.0020 (0.100 ppm)	0.00184	92.00	90.66±0.04
	0.0010 (0.050 ppm)	0.00090	90.00	
	0.0005 (0.025 ppm)	0.00045	90.00	
p,p' DDD/TDE	0.0020 (0.100 ppm)	0.00183	91.50	89.50±0.21
	0.0010 (0.050 ppm)	0.00089	89.00	
	0.0005 (0.025 ppm)	0.00044	88.00	
o,p' DDT	0.0020 (0.100 ppm)	0.00175	87.50	85.50±0.03
	0.0010 (0.050 ppm)	0.00085	85.00	
	0.0005 (0.025 ppm)	0.00042	84.00	
p, p' DDT	0.0020 (0.100 ppm)	0.00176	88.00	87.00±0.01
	0.0010 (0.050 ppm)	0.00087	87.00	
	0.0005 (0.025 ppm)	0.00043	86.00	
Dicofol	0.0020 (0.100 ppm)	0.00170	85.00	83.66±0.15
	0.0010 (0.050 ppm)	0.00084	84.00	
	0.0005 (0.025 ppm)	0.00041	82.00	
α-Endosulfan	0.0020 (0.100 ppm)	0.00185	92.50	90.83±0.03
	0.0010 (0.050 ppm)	0.00090	90.00	
	0.0005 (0.025 ppm)	0.00045	90.00	
β-Endosulfan	0.0020 (0.100 ppm)	0.00186	93.00	91.33±0.10
	0.0010 (0.050 ppm)	0.00091	91.00	
	0.0005 (0.025 ppm)	0.00045	90.00	
α- HCH	0.0020 (0.100 ppm)	0.00180	90.00	88.00±0.02
	0.0010 (0.050 ppm)	0.00090	90.00	
	0.0005 (0.025 ppm)	0.00042	84.00	
β- HCH	0.0020 (0.100 ppm)	0.00184	92.20	88.40±0.01
	0.0010 (0.050 ppm)	0.00087	87.00	
	0.0005 (0.025 ppm)	0.00043	86.00	
γ-HCH	0.0020 (0.100 ppm)	0.00185	91.50	88.17±0.02
	0.0010 (0.050 ppm)	0.00089	89.00	
	0.0005 (0.025 ppm)	0.00042	84.00	
Cypermethrin I+II	0.0020 (0.100 ppm)	0.00184	92.00	88.66±0.12
	0.0010 (0.050 ppm)	0.00088	88.00	
	0.0005 (0.025 ppm)	0.00044	88.00	
Deltamethrin	0.0020 (0.100 ppm)	0.00181	90.50	87.83±0.11
	0.0010 (0.050 ppm)	0.00087	87.00	
	0.0005 (0.025 ppm)	0.00043	86.00	
Fenvalerate I+II	0.0020 (0.100 ppm)	0.00186	93.00	90.33±0.23
	0.0010 (0.050 ppm)	0.00090	90.00	
	0.0005 (0.025 ppm)	0.00044	88.00	
Lambda cyhalothrin I+II	0.0020 (0.100 ppm)	0.00184	92.00	89.33±0.02
	0.0010 (0.050 ppm)	0.00090	90.00	
	0.0005 (0.025 ppm)	0.00043	86.00	

*Average of three replications

Table 4.61 Recoveries of different pesticides (organophosphorus) through GLC in honey samples

Pesticides	Amount added (mg) in 20 g honey sample	Amount recovered (ppm)*	Recovery per cent	Average per cent recovery
Acephate	0.0020 (0.100 ppm)	0.00164	82.00	79.66±0.03
	0.0010 (0.050 ppm)	0.00079	79.00	
	0.0005 (0.025 ppm)	0.00039	78.00	
Chlorpyriphos	0.0020 (0.100 ppm)	0.00157	78.50	76.83±0.21
	0.0010 (0.050 ppm)	0.00076	76.00	
	0.0005 (0.025 ppm)	0.00038	76.00	
Dimethoate	0.0020 (0.100 ppm)	0.00170	85.00	83.66±0.03
	0.0010 (0.050 ppm)	0.00084	84.00	
	0.0005 (0.025 ppm)	0.00041	82.00	
Ethion	0.0020 (0.100 ppm)	0.00161	80.50	79.16±0.11
	0.0010 (0.050 ppm)	0.00079	79.00	
	0.0005 (0.025 ppm)	0.00039	78.00	
Malathion	0.0020 (0.100 ppm)	0.00158	79.00	76.33±0.15
	0.0010 (0.050 ppm)	0.00076	76.00	
	0.0005 (0.025 ppm)	0.00037	74.00	
Monocrotophos	0.0020 (0.100 ppm)	0.00167	83.50	81.50±0.04
	0.0010 (0.050 ppm)	0.00081	81.00	
	0.0005 (0.025 ppm)	0.00040	80.00	
Quinalphos	0.0020 (0.100 ppm)	0.00158	79.00	77.66±0.19
	0.0010 (0.050 ppm)	0.00078	78.00	
	0.0005 (0.025 ppm)	0.00038	76.00	

*Average of three replications

The residues of p,p' isomer of DDE were recorded in 5.88 per cent samples. The residue content varied from a minimum of 0.290±0.034 µg kg⁻¹ to a maximum of 0.639±0.007 µg kg⁻¹ in sample numbers 26 and 41, respectively. Only 7.84 per cent honey samples had the residues of p,p' isomer of DDD. It was found that residue varied from a minimum of 0.380±0.040 µg kg⁻¹ to a maximum of 3.701±0.403 µg kg⁻¹ in sample numbers 13 and 39, respectively. A maximum residue level to the tune of 1.891±0.171 µg kg⁻¹ was found in the sample number 41, however, sample number 33 contained least amount of o,p' DDT residue (0.377±0.030 µg kg⁻¹). Residues were present in 3 samples each for o,p' DDT and p,p' DDT. A minimum amount of p,p' DDT residue (0.271±0.025

$\mu\text{g kg}^{-1}$) was found in sample number 26 whereas, sample number 33 had the maximum amount ($3.052 \pm 0.014 \mu\text{g kg}^{-1}$).

It was observed that out of total 51 samples analyzed 11.76 per cent samples were contaminated with the residues of α isomer of HCH. The minimum quantity of α -HCH was found to be $0.095 \pm 0.005 \mu\text{g kg}^{-1}$. However, maximum residue to the tune of $1.401 \pm 0.309 \mu\text{g kg}^{-1}$ was found in the sample number 1. In contrary to this, the residues of β isomer of HCH were recovered from 5.88 per cent samples. The quantity of residues varied from a minimum of $0.139 \pm 0.008 \mu\text{g kg}^{-1}$ to the maximum of $1.650 \pm 0.047 \mu\text{g kg}^{-1}$ in sample numbers 29 and 3, respectively.

Gamma isomer of HCH was recovered from 17.65 per cent samples. It was found that maximum residues ($2.970 \pm 0.058 \mu\text{g kg}^{-1}$) were found in sample number 1. The lowest quantity of residues of gamma isomer of HCH ($0.126 \pm 0.017 \mu\text{g kg}^{-1}$) was observed in sample number 20.

The residues of cyclodiene pesticide (isomers of endosulfan), dicofol and synthetic pyrethroid pesticides (cypermethrin, deltamethrin, fenvalerate and lambda cyhalothrin) were analyzed in all the 51 samples collected from the different parts of the state. Results are given in Table 4.63.

From the Table 4.63, it is seen that 9.80 per cent samples contained residues of α isomer of endosulfan. The highest value of residue was found to the tune of $2.731 \pm 0.396 \mu\text{g kg}^{-1}$ in sample number 20 whereas, lowest value of residue to the tune of $0.258 \pm 0.002 \mu\text{g kg}^{-1}$ was found in sample number 45. In comparison to this, β - endosulfan was found only in 3.92 per cent of total honey samples analyzed. The highest residue level of β -endosulfan ($2.502 \pm 0.141 \mu\text{g kg}^{-1}$) was found in sample number 51, whereas, it was lowest

($2.038 \pm 0.313 \mu\text{g kg}^{-1}$) in sample number 20. Only one sample (sample number 27) contained dicofol residue to the tune of $2.226 \pm 0.024 \mu\text{g kg}^{-1}$.

A high value of cypermethrin residue ($10.234 \pm 0.010 \mu\text{g kg}^{-1}$) was recorded from sample number 1 whereas, residue was found minimum ($2.856 \pm 0.685 \mu\text{g kg}^{-1}$) in sample number 29. The deltamethrin residue to the tune of $6.663 \pm 1.020 \mu\text{g kg}^{-1}$ was found only in sample number 38. Sample number 50 and 41 had lambda cyhalothrin residue to the tune of 0.636 ± 0.034 and $0.765 \pm 0.004 \mu\text{g kg}^{-1}$, respectively. Similarly, fenvalerate (I and II) residue was found only in one sample to the tune of $0.696 \pm 0.137 \mu\text{g kg}^{-1}$. Over all, it was found that synthetic pyrethroid pesticides found to contaminate 5.88, 1.96, 1.96 and 3.92 per cent of honey samples, respectively.

4.7.3.2 Organophosphorus pesticides

Residues were determined on GLC equipped with NPD. It was found (Table 4.64) that none of the samples were contaminated with either of acephate, chlorpyrifos, ethion or monocrotophos residues above detection limit.

Both, dimethoate and malathion, contribute towards 5.88 per cent contamination of total honey samples analyzed. Sample numbers 28, 35 and 44 contained dimethoate residues to the tune of 8.667 ± 0.882 , 2.448 ± 0.221 , $3.265 \pm 0.002 \mu\text{g kg}^{-1}$, respectively whereas, malathion residues to the tune of 11.077 ± 1.157 , 9.187 ± 1.412 and $6.663 \pm 1.020 \mu\text{g kg}^{-1}$ were observed in sample numbers 11, 23 and 26, respectively.

Residues of quinalphos were found in 3.92 per cent samples. Sample number 24 contained residue to the tune of $10.270 \pm 0.319 \mu\text{g kg}^{-1}$ whereas, $8.907 \pm 0.689 \mu\text{g kg}^{-1}$ residues were recovered in sample number 31.

Table 4.63 Level of cyclodienes and synthetic pyrethroids pesticide residues (ppb) in different samples of honey

Sample no*	α -Endosulfan	β -Endosulfan	Dicofol	Cypermethrin	Deltamethrin	Fenvalerate	Lambda cyhalothrin
1.	BDL	BDL	BDL	10.234 $\pm$ 0.010	BDL	BDL	BDL
11.	BDL	BDL	BDL	5.550 $\pm$ 0.887	BDL	BDL	BDL
20.	2.731 $\pm$ 0.396	2.038 $\pm$ 0.313	BDL	BDL	BDL	BDL	BDL
27.	BDL	BDL	2.226 $\pm$ 0.024	BDL	BDL	BDL	BDL
28.	0.645 $\pm$ 0.057	BDL	BDL	BDL	BDL	BDL	BDL
29.	BDL	BDL	BDL	2.856 $\pm$ 0.685	BDL	BDL	BDL
31.	2.122 $\pm$ 0.107	BDL	BDL	BDL	BDL	BDL	BDL
38.	BDL	BDL	BDL	BDL	6.663 $\pm$ 1.020	0.696 $\pm$ 0.137	BDL
41.	BDL	BDL	BDL	BDL	BDL	BDL	0.765 $\pm$ 0.004
45.	0.258 $\pm$ 0.002	BDL	BDL	BDL	BDL	BDL	BDL
50.	BDL	BDL	BDL	BDL	BDL	BDL	0.636 $\pm$ 0.034
51.	1.990 $\pm$ 0.031	2.502 $\pm$ 0.141	BDL	BDL	BDL	BDL	BDL
Range	BDL-2.731	BDL-2.502	BDL-2.226	BDL-10.234	BDL-663	BDL-0.696	BDL-0.765

ppb Parts per billion

BDL Below detectable limit

Values following $\pm$ are S.E. of mean

*Samples no's 2-10, 12-19, 21-26, 30, 32-37, 39-40, 42-44, 46-49 had residues BDL

Table 4.64 Level of organophosphorus pesticide residues (ppb)* in different samples of honey

Sample no**	Dimethoate	Malathion	Quinalphos
11.	BDL	11.077±1.157	BDL
23.	BDL	9.187±1.412	BDL
24.	BDL	BDL	10.270±0.319
26.	BDL	6.663±1.020	BDL
28.	8.667±0.882	BDL	BDL
31.	BDL	BDL	8.907±0.689
35.	2.448±0.221	BDL	BDL
44.	3.265±0.002	BDL	BDL
Range	BDL-8.667	BDL-11.077	BDL-10.270

ppb Parts per billion; BDL Below detection limit; Values following ± are S.E. of mean

*Residues of acephate, chlorpyrihos, ethion and monocrotophos were BDL

**Sample no's 1-10, 12-22, 25, 27, 29-30, 32-34, 36-43 and 45-51 had residues BDL

Form the Table 4.65, it was found that a total of 25 samples (49.02 %) were contaminated with the residues of either one or other pesticide. But if we compare the amount of residues with the MRL's as proposed by the Ministry of Commerce, Government of India for the Honey Export (Anonymous, 2002), it was observed that only 5.88 per cent samples (containing the residues of malathion above MRL 5ppb) had residue above the prescribed limits.

4.8 Effect of pesticide residue on physico- chemical properties of honey

In honey samples, collected from the state, there were differences in physico-chemical properties of contaminated and non contaminated honeys of following type: multifloral, market samples and rapeseed and mustard samples (Table 4.66). However, when a control experiment by adding known amount of pesticide in pesticide free honey sample was carried out, no such differences were observed (Table 4.67).

Table 4.65 Per cent honey samples contamination due to pesticides

Pesticides residues detected	Mean	Range	MRL* (ppb)	Frequency	Per cent contamination	Per cent above MRL
p,p' DDE	0.484±0.102	BDL-0.639	50.0	3	5.88	0.00
p,p' DDD/TDE	1.492±0.864	BDL-3.701	50.0	4	7.84	0.00
o,p' DDT	0.894±0.498	BDL-1.891	50.0	3	5.88	0.00
p,p' DDT	1.804±0.815	BDL-3.052	50.0	3	5.88	0.00
α- HCH	0.530±0.286	BDL-1.401	5.0	6	11.76	0.00
β- HCH	0.674±0.489	BDL-1.650	5.0	3	5.88	0.00
γ-HCH	0.985±0.543	BDL-2.970	5.0	9	17.65	0.00
α-Endosulfan	1.549±0.605	BDL-2.731	100.0	5	9.80	0.00
β-Endosulfan	2.270±0.189	BDL-2.502	---	2	3.92	---
Dicofol	2.226±0.024	BDL-2.226	50.0	1	1.96	0.00
Cypermethrin	6.213±2.155	BDL-10.234	50.0	3	5.88	0.00
Deltamethrin	6.663±1.020	BDL-6.663	50.0	1	1.96	0.00
Fenvalerate	0.696±0.137	BDL-0.696	20.0	1	1.96	0.00
Lambda cyhalothrin	0.701±0.052	BDL-0.765	---	2	3.92	---
Acephate	---	BDL	---	0	0	---
Chlorpyrifos	---	BDL	5.0	0	0	---
Dimethoate	4.793±1.951	BDL-8.667	---	3	5.88	---
Ethion	---	BDL	5.0	0	0	---
Malathion	8.976±1.278	BDL-11.077	5.0	3	5.88	5.88
Monocrotophos	---	BDL	---	0	0	---
OVERALL MEAN	--	BDL-11.077		25	49.02	3.00

* Anonymous, 2002

Table 4.66 Differences in physico-chemical properties of contaminated and non contaminated honey samples

Physico- chemical properties	Multiflora			Rapeseed and mustard			Market sample	
	Contaminated	Uncontaminated	Contaminated	Uncontaminated	Contaminated	Uncontaminated	Contaminated	Uncontaminated
O.D. At 560 nm	0.119±0.017	0.119±0.022	0.203±0.054	0.150±0.002	0.457±0.020	0.416±0.087		
Refractive index	1.4932±0.0011	1.4913±0.0002	1.4922±0.0002	1.4942±0.0001	1.4900±0.0001	1.4950±0.0002		
Moisture (%)	17.50±0.38	17.45±0.68	17.83±0.32	16.80±0.10	18.55±0.12	16.88±0.11		
Viscosity (mins.)	5.918±0.445	5.852±0.571	5.555±0.230	6.536±0.007	4.510±0.488	6.042±0.840		
Density (gcm ⁻³)	1.620±0.008	1.626±0.022	1.609±0.008	1.621±0.001	1.603±0.014	1.649±0.029		
Pollen grains per 10g honey	53861±4197	51066±4189	65166±13861	53667±3333	87142±3995	68111±21498		
Total soluble solids (%)	82.49±0.37	82.57±0.68	82.17±0.32	83.20±0.02	81.44±0.12	83.17±0.81		
Total reducing sugars (%)	73.48±1.13	68.58±3.93	71.03±1.44	68.32±0.03	65.38±2.42	69.04±3.21		
Fructose (%)	38.92±1.28	36.17±2.80	35.62±0.58	34.22±0.02	34.03±1.05	33.93±1.09		
Glucose (%)	34.55±0.32	32.32±1.00	35.41±0.99	34.09±0.01	34.34±1.56	35.11±2.25		
f:g ratio	1.126±0.033	1.111±0.051	0.990±0.012	1.004±0.001	1.081±0.038	0.971±0.036		
Sucrose (%)	4.382±0.300	4.741±0.661	4.981±0.262	6.122±0.011	4.612±0.641	5.842±0.602		
pH	4.206±0.145	4.245±0.233	4.569±0.127	4.640±0.015	4.405±0.089	4.559±0.251		
Ash (%)	0.191±0.071	0.121±0.004	0.122±0.051	0.121±0.002	0.212±0.002	0.121±0.031		
HMF (ppm)	9.761±1.076	7.022±2.002	7.860±1.104	9.112±0.015	9.272±0.195	10.161±3.903		

Table 4.67 Effect of pesticide residues on physico- chemical properties of honey

Physico- chemical properties	After 1 month	
	Blank sample	Contaminated sample
O.D. At 560 nm	0.119±0.017	0.119±0.022*
Refractive index	1.4880± 0.00011	1.4880± 0.00011*
Moisture	19.4	19.4*
Viscosity	5.487± 0.0001	5.488± 0.0002*
Density	1.620±0.0001	1.622±0.0001*
Specific gravity	1.407±0.0002	1.406±0.0001*
TSS	80.93±0.012	80.92±0.011*
Fructose	33.254±0.190	33.250±0.0001*
Glucose	33.210±0.201	33.220±0.110*
pH	4.150±0.002	4.140±0.102*
TRS	66.464±0.325	66.462±0.315*
Sucrose	4.880±0.021	4.882±0.121*
Ash	0.128±0.0001	0.126±0.001*
HMF	10.986±0.102	10.984±0.120*

* Non significant

DISCUSSION

DISCUSSION

In the past two decades, “Green Revolution Technologies” have doubled the yield potential of cereals and oilseed crops, especially in Asia. These high- input production systems, requiring massive quantities of pesticides, cause several environmental hazards including toxicity to bees and residues in honey and other hive products. Institution of WTO agreements on trade, concomitant changes in patent regime and the related requirements have forced open the gates of the National systems to International requirements. Therefore, it was felt essential to study the relative and persistent toxicity of pesticides against the foragers of two most common hive species of honey bee viz. *Apis cerana cerana* and *Apis mellifera*. Keeping in view the international requirements of pesticide free honey, the residue level of various pesticides with their effects on its physico- chemical properties were also worked out to avoid rejection of any export consignment on the basis of presence of hazardous pesticide residues and deteriorated quality.

5.1 TOXICITY OF PESTICIDES TO HONEY BEES

5.1.1 Contact Toxicity

The LD₅₀ (contact toxicity) values of cypermethrin to *A. c. cerana* and *A. mellifera* were found to be 0.0171 and 0.0438 µg bee⁻¹. These findings are close to the findings of Kasamatsu and Kawachi (1986). Similarly, Bendahou *et al.* (1997) reported cypermethrin slightly safer to the foragers of honey bee (LD₅₀ 0.16 µg bee⁻¹) compared to present studies. In contrary to these, Saleh *et al.* (1989) reported cypermethrin more toxic to honey bees (LD₅₀ 0.008 µg bee⁻¹). The higher toxicity of cypermethrin against foragers of honey bees was also reported by Gerig (1979), Gromiszowa (1982) and Wael and Laere

(1989). The variation might be due to the difference in the method of testing, age of the insect and exposure time, in the present studies pesticide was applied with microapplicator and mortalities were recorded after 24 hours of treatment.

The results on toxicity of endosulfan to *A. c. cerana* (LD_{50} 7.9871 $\mu\text{g bee}^{-1}$) and *A. mellifera* (LD_{50} 9.0614 $\mu\text{g bee}^{-1}$), are almost similar to those reported by Nath *et al.* (1984). A higher LD_{50} to the tune of 14.87 $\mu\text{g bee}^{-1}$ was reported by Batista *et al.* (1975) against *A. mellifera ligustica* x *A. mellifera adansonii* hybrid. The lower contact toxicity level of endosulfan against honey bee foragers was also advocated by Arzone (1975a) and Thakur *et al.* (1981). The possible reason for low toxicity may be the difference in the honey bee race and technique of testing employed.

Imidacloprid had LD_{50} values to the tune of 0.0317 $\mu\text{g bee}^{-1}$ against *A. c. cerana* and 0.1157 $\mu\text{g bee}^{-1}$ against *A. mellifera*. These findings support the findings of Nauen *et al.* (2001) and Schmuck *et al.* (2003a). However, Suchail *et al.* (2000 & 2001) and Iwasa *et al.* (2004) found imidacloprid slightly more toxic to honey bees. The difference might be due to difference in the method of testing, strain of honey bee species, climatic variation of testing locations and method employed for testing.

LD_{50} values of lambda cyhalothrin against *A. c. cerana* and *A. mellifera* were observed to be 0.0124 and 0.0241 $\mu\text{g bee}^{-1}$, respectively after 24 hours exposure. Similarly, highly toxic nature of lambda cyhalothrin was also reported by Smart and Stevenson (1982), Gough and Wilkinson (1984) and Mayer *et al.* (1998).

In the present study, spiromesifen was found to be relatively non toxic to *A. c. cerana* (LD_{50} 141.5198 $\mu\text{g bee}^{-1}$) and *A. mellifera* (LD_{50} 159.7212 $\mu\text{g bee}^{-1}$). Spiromesifen reported to have almost same level of safety to *A. mellifera* (Anonymous, 2005).

Thiacloprid was observed to have low contact toxicity to *A. c. cerana* (LD_{50}

22.6433 $\mu\text{g bee}^{-1}$) and *A. mellifera* (LD_{50} 26.6903 $\mu\text{g bee}^{-1}$). These values lie well within the range reported by Elbert *et al.* (2000), Jeschke *et al.* (2001) and Soloveva (2002).

Both the species of honey bees were found tolerant to benzoylphenyl urea, which is evident from the negligible mortalities of forager bees recorded even at 20.00 $\mu\text{g bee}^{-1}$. Gupta and Chandel (1995) and Tasei (2001) also reported the tolerance of *A. mellifera* to benzoylphenyl urea, thus support the present findings.

Biopesticides viz. azadirachtin and *B. thuringiensis* subsp. *kurstaki* were also found harmless to both the honey bee species. Safety of azadirachtin to honey bee foragers was also reported by Rembold and Czoppelt (1981) and Ladurner *et al.* (2005). Whereas, Davidson *et al.* (1977) and Vandenberg (1990) reported the safety of *B. sphaericus* and *B. thuringiensis* subsp. *tenebrionis* to the foragers of *A. mellifera*. The reason for the safety of azadirachtin to honey bee foragers could be its repellency and antifeedent activity coupled with low contact toxicity. Whereas, *B. thuringiensis* subsp. *kurstaki* is non selective to honey bees.

In contrary to above biopesticides, spinosad was observed to have high contact toxicity against both the species of honey bees (LD_{50} 0.0590 and 0.0851 $\mu\text{g bee}^{-1}$ for *A. c. cerana* and *A. mellifera*, respectively). The present findings corroborate the findings of Mayer *et al.* (2001) who reported LD_{50} of spinosad to *A. mellifera* as 0.078 $\mu\text{g bee}^{-1}$.

5.1.2 Stomach Toxicity

LD_{50} value (stomach toxicity) of cypermethrin to *A. c. cerana* and *A. mellifera* were observed to be as low as 0.0047 and 0.0095 $\mu\text{g bee}^{-1}$, respectively, which support the findings of Gromiszowa (1982). Contrary to this, high LD_{50} value (0.056 $\mu\text{g bee}^{-1}$) was reported by Wael and Laere (1989). The difference, in the toxicity levels, observed in the

present studies to that of earlier reports might be due to the result of different insecticide formulations tested i.e. technical in the present and formulated in the former studies.

Endosulfan was found to have moderate stomach toxicity to *A. c. cerana* (LD_{50} 7.0041 $\mu\text{g bee}^{-1}$) and *A. mellifera* (LD_{50} 7.1657 $\mu\text{g bee}^{-1}$). These findings are supported by the findings of Stevenson and Walker (1975), Nath *et al.* (1984) and Nath and Sharma (1985) who also reported the moderate toxic nature of endosulfan to the foragers of *A. mellifera* and *A. c. indica*, respectively. Non toxic nature of endosulfan against *A. florea* and *A. dorsata* was also advocated by Jain and Guman (1992)

LD_{50} values for imidacloprid were 0.0107 and 0.0161 $\mu\text{g bee}^{-1}$ for *A. c. cerana* and *A. mellifera*, respectively. These values are close to the toxicity values reported by Decourtye *et al.* (2003) and Gregore and Bozi (2004), however different toxicity values of imidacloprid against *A. mellifera* were reported by Suchail *et al.* (2000), Nauen *et al.* (2001) and Schmuck *et al.* (2001 & 2003a) which might be due to the difference in test conditions.

LD_{50} 's of lambda cyhalothrin were 0.0035 and 0.0061 $\mu\text{g bee}^{-1}$ for *A. c. cerana* and *A. mellifera*, respectively. High toxicity of lambda cyhalothrin was also reported by Prakash and Kumaraswami (1984), Arzone and Patetta (1986), Rieth and Kevin (1987), Shivrana and Jain (1994) and Mayer *et al.* (1998).

Oral administration of spiromesifen was observed to be highly safe to foragers of *A. c. cerana* (LD_{50} 111.7038 $\mu\text{g bee}^{-1}$) and *A. mellifera* (LD_{50} 130.3387 $\mu\text{g bee}^{-1}$). This might be due to lower insecticidal and higher acaricidal action of this compound (Liu, 2004; Anonymous, 2005).

The LD₅₀ values of thiacloprid 3.8416 and 6.2934 µg bee⁻¹ for *A. c. cerana* and *A. mellifera*, respectively fit well within the range of 3.20 to 7.20 µg bee⁻¹ as reported by Jeschke *et al.* (2001) against *A. mellifera*.

Benzoylphenyl urea, azadirachtin and *B. thuringiensis* subsp. *kurstaki* were found highly safe to both the species of honey bees at the tested doses. The present findings for the safety towards honey bee foragers corroborate the findings of Ladurner *et al.* (2005) for azadirachtin and Marletto *et al.* (1972), Celli (1974), Krieg *et al.* (1980) for *B. thuringiensis* subsp. *kurstaki*.

High stomach toxicity of spinosad was recorded against both the species of honey bees (0.3661 and 0.0604 µg bee⁻¹ for *A. c. cerana* and *A. mellifera*, respectively). The present findings support the findings of Mayer *et al.* (2001).

In general, *A. c. cerana* foragers were found more susceptible compared to *A. mellifera* foragers to all the pesticides on topical application and when offered in bee diet. This might be due to higher surface area to mass ratio (Mayer *et al.*, 1998) or difference in metabolic and excretion rates (Attri and Sharma, 1969; Hameed *et al.*, 1973; Kapil and Lamba, 1974; Kasturi Bai and Reddy, 1977; Mishra and Verma, 1982).

The contact toxicity was found less compared to the stomach toxicity. The reason for low contact toxicities might be due to low amount of pesticides available at target site on topical application probably due to cuticular barrier which impart inhibitory action to a certain extent during the penetration process of pesticide.

On the basis of LD₅₀ (stomach and contact toxicities) the order of toxicity was lambda cyhalothrin > cypermethrin > spinosad > imidacloprid > endosulfan > thiacloprid > spiromesifen > azadirachtin = *B. thuringiensis* subsp. *kurstaki* = benzoylphenyl urea for both the species of honey bees.

Interestingly, spinosad (a biopesticide) proved more toxic to honey bees than imidacloprid and endosulfan. This might be due to more pronounced neurological effects of spinosyn A and D on honey bees as described by Mayes *et al.* (2003).

Synthetic pyrethroids; cypermethrin and lambda cyhalothrin showed a higher toxicity compared to other group of pesticides tested in the present studies. This might be due to the more sensitivity of foragers to these pesticides. Among these two synthetic pyrethroids, lambda cyhalothrin was more toxic probably due to the difference in the structure activity relationship. Lambda cyhalothrin contained fluorine substitution which showed high toxicity compared to chlorine atom substitution in cypermethrin. Smart and Stevenson (1982) and Cassano *et al.* (2003) also reported similar pattern of toxicity.

Among two neonicotinoids *viz.* imidacloprid and thiacloprid, former was comparatively more toxic than the latter. Difference in level of toxicity could be due to the difference of structure activity relationship as reported by Iwasa *et al.* (2004). Neonicotinoid compounds, having nitro-substitution group (imidacloprid and thiamethoxam), were reported more toxic to honey bees compared to cyano-substituted compounds (thiacloprid and acetamiprid).

Spiromesifen (a tetrionic acid derivative) posed little threat to honey bees because of its non selectivity to honey bees. Their unique mode of action affecting the protein biosynthesis enables them harmless to honey bee foragers (Liu, 2004). In the present study, this compound was found non toxic to both the species of honey bees ($LD_{50} > 110\mu\text{g bee}^{-1}$).

Chitin synthesis inhibitor along with azadirachtin and *B. thuringiensis* subsp. *kurstaki* was recorded safe to the honey bee foragers. The safety of benzoylphenyl urea to honey bee foragers might be due to its mode of action i.e. it acts on the chitin synthesis by

inhibiting the polycondensation of the chitin which make it lethal for the early stages of insects (larvae and pupae) and do not affect the adults (Post and Mulder, 1974). Similarly, azadirachtin reported to have detrimental effects on younger stages of bees which resulted in the formation of malformed pupae and adults (Naumann and Isman, 1996).

Honey bees did not show any contact or stomach toxicity symptoms when exposed to *B. thuringiensis* subsp. *kurstaki*. Marletto *et al.* (1972) reported that honey bee's gut conditions are not suitable for the growth of the spores of this bacterium and have negligible toxicity. What so ever little mortalities were recorded, those could be due to the other constituents of the formulation.

On the basis of toxicity to honey bees, the categorization of pesticides proposed by Abrol (1997), the pesticides tested in the present studies can be classified as follows: cypermethrin, imidacloprid, lambda cyhalothrin and spinosad as highly toxic (LD_{50} 0.001-1.99 $\mu\text{g bee}^{-1}$), endosulfan as moderately toxic (LD_{50} 2.0 – 10.0 $\mu\text{g bee}^{-1}$) and azadirachtin, *B. thuringiensis* subsp. *kurstaki*, benzoylphenyl urea, spiromesifen and thiacloprid as relatively non toxic ($LD_{50} > 11.0 \mu\text{g bee}^{-1}$).

5.2 FIELD WEATERED TOXICITY OF PESTICIDES TO HONEY BEES

The pesticidal toxicity can be determined by suitable laboratory tests, but the hazards from the formulated pesticide are associated with specific circumstances in the field which must be considered while estimating the potential danger to the honey bees and other non target organisms. This hazard is a function of intrinsic toxicity of the pesticide, the field application rate (g a.i. ha^{-1}), the proportion of dose which is available for the transfer to the bees and the behaviour of the bee itself. Important factors include weather conditions, stage of flowering of the crop and its attractiveness to bees. It may

further be related to the formulation type, mode of action, residual toxicity of the pesticide in particular repellency (Johansen, 1979; Smart and Stevenson, 1982).

The descending order of field weathered persistence toxicity (PT values) of different pesticidal treatments given in the present studies to both the species is lambda cyhalothrin > cypermethrin > lambda cyhalothrin + azadirachtin > cypermethrin + *B. thuringiensis* subsp. *kurstaki* > imidacloprid (foliar application) > endosulfan > *B. thuringiensis* subsp. *kurstaki* > spiromesifen > imidacloprid (seed treatment) for *A. c. cerana* and lambda cyhalothrin > cypermethrin > cypermethrin + *B. thuringiensis* subsp. *kurstaki* > lambda cyhalothrin + azadirachtin > imidacloprid (foliar application) > endosulfan > spiromesifen > *B. thuringiensis* subsp. *kurstaki* > imidacloprid (seed treatment) for *A. mellifera*.

Several workers (Smart and Stevenson, 1982; Shires and Debray, 1982; Thapa *et al.*, 1997) have reported that synthetic pyrethroids were safe to honey bees in the fields. The high safety of synthetic pyrethroids in the field trials may be due to the repellent action of these pesticides. Repellency was also advocated as a major reason for the safety of synthetic pyrethroids to honey bees under field conditions by Gerig (1979), Atkins (1981), Smart and Stevenson (1982), Wolmarans *et al.* (1991) and Thapa *et al.* (1997). However in present studies synthetic pyrethroids were found highly toxic because honey bees were forced to forage on nectar and pollen of treated blooms. Lambda cyhalothrin was more toxic than cypermethrin, which was in turn, more toxic than endosulfan. The results are in much agreement with the findings of Smart and Stevenson (1982) and Thakur *et al.* (1985).

The higher susceptibility of *A. c. cerana* compared to *A. mellifera* have contributed towards longer duration toxic action of synthetic pyrethroids against the

former species compared to the latter one even at the same level of pesticidal residue on different persistent toxicity study intervals. The higher susceptibility of *A. cerana* compared to *A. mellifera* was documented by several workers (Attri and Sharma, 1969; Hameed *et al.*, 1973; Kapil and Lamba, 1974; Kasturi Bai and Reddy, 1977; Mishra and Verma, 1982). Imidacloprid foliar application has high toxicity and comparatively low LT_{50} values for both the species of honey bees. Seed treatment of imidacloprid was found harmless to forager bees at the stage of crop blooming and considered as safest treatment. The safety of seed treatment could be attributed to non availability of its residues in crop blooms after 75 days of sowing of treated seeds. The present studies find the support from the findings of Ambolet *et al.* (1999), Wallner (2001) and Maus *et al.* (2003) who were unable to detect imidacloprid residues in nectar and pollen after seed treatment with imidacloprid.

5.3 STANDARDIZATION OF TECHNIQUES FOR THE ESTIMATION OF DEPOSITS/ RESIDUES

5.3.1 Response of Standard Doses of Pesticides

Different dosages of four pesticides *viz.* endosulfan, imidacloprid, lambda cyhalothrin and spiromesifen gave the linear response with respect to log dosages and probit mortality with *D. melanogaster* in petridishes. These studies are similar to the findings of Kashyap and Hameed (1982), Sharma (1993), Vir (1996), Chand (2000) and Sood (2006).

Bioassay method was highly sensitive for imidacloprid (0.0003 ppm) followed by endosulfan (0.0012 ppm) and lambda cyhalothrin (0.0078 ppm). These findings are similar to Sharma (1993), Vir (1996), Chand (2000) and Sood (2006) who also reported almost similar sensitivity for endosulfan and lambda cyhalothrin. The highest sensitivity

of bioassay method was found with imidacloprid, whereas least sensitivity was found with spiromesifen (0.0597 ppm). The higher sensitivity of imidacloprid might be due to its high contact toxicity against the test organism, whereas spiromesifen has least toxicity against *D. melanogaster* so the lower sensitivity (Liu, 2004).

5.3.2 Chromatographic Method of Assay

5.3.2.1 Gas Liquid Chromatography

When 0.01- 0.10 µg of pesticides were injected in 1.0µl of solvent in GLC equipped with ECD (for endosulfan, lambda cyhalothrin and spiromesifen), a linear response was obtained with respect to area (PE units) and quantity injected. The sensitivity in the present investigations was more than that reported by Gopal and Mukherjee (1993) for endosulfan. Sensitivities of lambda cyhalothrin and spiromesifen were almost similar to the findings of the other workers (Sharma and Awasthi, 2002; Sood, 2006) for lambda cyhalothrin and Anonymous (2003) for spiromesifen.

5.3.2.2 High Performance Liquid Chromatography

The sensitivity of HPLC for the imidacloprid was lower (0.01 µg) than that reported by Placke and Weber (1993) and Schmuck *et al.* (2001). The differences might be due to the equipment used (GCMS and HPTLC).

In general, in the present investigation chromatographic methods gave lower values of detection limit and higher sensitivities than bioassay.

5.3.3 Recovery

The recoveries of pesticides vary with the type of substrate, extraction and clean up methods. The average recovery of all the four pesticides was comparatively higher with chromatographic methods than by bioassay method in pollen and nectar. This could be due to the difference in the sensitivity of the assay method.

Higher per cent of pesticide residues were recovered in nectar samples compared to pollen, which might be due to the difference in chemical composition of two substrates. Nectar contained high water content in comparison to pollens, which in turn contained more lipids. Differences in chemical composition and physical status lead to adoption of different extraction and clean up procedure for pesticide residue extraction. Similar findings were also reported by Loper and Ross (1982) and Sharma (1997).

5.4 PERSISTENCE AND DISSIPATION OF PESTICIDES IN/ON MUSTARD NECTAR AND POLLEN

5.4.1 Nectar

There is small difference in the initial deposits of endosulfan observed during two years (1.725 and 1.583 ppm). The ratio between the initial deposits of two years comes out to be 1: 1.08 which indicate that initial deposits of two years were at par with each other. The initial deposits were comparatively less than those recorded by Sharma (1997), which might be due to the age of the crop as in the latter studies sprays were done little late. The residues dissipated rapidly and more than 99.00 per cent get dissipated within 120 hours of application. During both the years, the initial dissipation rate was faster which resulted in dissipation of nearly half of the residues in around 24 hours of spray, which was followed by a comparatively slower rate of dissipation. The slight differences in half life of residues (22.97 and 32.02 hours) might be due to the slight differences in weather factors.

Imidacloprid residues were below detectable limit on 75th day after sowing of treated seeds. The present findings are supported by the findings of Dikshit *et al.* (2002) and Helal *et al.* (2005) who were also unable to detect residues of this pesticide on 60 and

80 days of sowing of okra and mustard seeds in fruits and foliage, respectively. Residues in soil samples were also not detected.

In the present investigations, initial deposits of lambda cyhalothrin were at par (ratio of 1:1.10) during both the years. The residues decreased to around 99.00 per cent after 72 hours of spray. The order of degradation was observed as first order (half life 12.46 hours). Slow photo- degradation coupled with the formation of conjugates with the sugars of nectar, resulted in longer persistence of endosulfan than lambda cyhalothrin in nectar. These results are in very much agreement with the observations of Fries and Wibran (1987), Dalabie *et al.* (1988) and Waller *et al.* (1988).

The initial deposits of spiromesifen for the two years were at par with each other (1.541 and 1.401 ppm). The residues could not be detected at 72 hours of spray. This could be due to the fast metabolism of parent spiromesifen compound into its enol metabolite with in plant system (Anonymous, 2005) and it possessed low toxicity profile against insects of order Hymenoptera.

In general, it was observed that lambda cyhalothrin has lowest initial deposit compared to spiromesifen and endosulfan which might be due to lower application rate of lambda cyhalothrin. The longer persistence of endosulfan may be due to higher initial deposits, slow rate of photo-degradation compared to synthetic pyrethroids and its capability of forming conjugates with sugar molecules in nectar (Nath and Sharma, 1985; Sharma, 1987).

5.4.2 Pollen

In pollens, the initial deposits of endosulfan were found to be at par during both years i.e. 2.126 and 2.068 ppm. Initial rate of dissipation was fast as indicated by fifty per cent dissipation of endosulfan within four hours of spray. Thereafter, the rate of dissipation

was very slow as indicated by the time taken to dissipate 95.0 per cent of its residues as 72 hours. During both the years, dissipation pattern of endosulfan was observed almost similar, which could be due to the prevalence of almost similar climatic conditions during experiment period in both the years (Fig. 3.1 & 3.2). These findings are different to the findings of Sharma (1997), where endosulfan residues in pollen were reported to persist longer. The differences might be due to the difference in the initial deposits and weather parameters, prevailing during the present studies.

Imidacloprid residues were below detectable limit on 75th day of sowing of treated seeds i.e. at 50 per cent flowering. The present findings are well in line with the findings of Tasei *et al.* (2000), Cure *et al.* (2001) and Schmuck *et al.* (2001).

Lambda cyhalothrin recorded initial deposits of 1.607 and 1.577 ppm in bee foraged mustard pollen, which degraded to below detection limit after 72 hours of spray. These results are contrary to the findings of Tasei *et al.* (1994) who found comparatively low initial deposits of deltamethrin in anthers of mustard probably due to the difference in method and rate of application and in substrate also, in the present study whole of anthers were not detached for the residue estimation as were taken in the former investigation.

Initial deposits of spiromesifen for the two years of study were statistically at par (ratio of 1: 1.13). After 72 hours of spray, residues were below detectable limit in pollen samples. This could be due to non lipophilic and environmentally unstable nature of spiromesifen.

In general, initial deposits of pesticides were higher in pollen samples compared to nectar samples, the corresponding dissipation was also faster in pollen compared to nectar samples. These results corroborate the findings of Sharma (1997) where it was documented that the pollens are exposed to weather factors including sunshine and

rainfall which caused fast photo-degradation of chemicals. However, residue dissipation in nectar samples was comparatively slow because the nectaries ~~were~~ embedded deep in the flowers and there ~~were~~ chances of formation of conjugates of pesticides with the sugars present in the nectar. Celli and Porrini (1988) also reported that placement of nectar contributed towards a slow rate of dissipation of residues.

5.5 MONITORING OF PESTICIDE RESIDUES IN HONEY SAMPLES

5.5.1 Standardization of Technique

Pesticide residues estimation of different group of pesticides was carried out by gas liquid chromatography. The retention time of different pesticides (organochlorine and synthetic pyrethroids) were determined individually on BPX5 column with electron capture detector. Then the mixture of each pesticide was prepared and known amount of pesticide solution was injected in GLC. The order of elution (ascending) of various pesticides on the basis of retention time (RT) was observed as follows: α HCH < β HCH < p, p' DDT < endosulfan I < γ HCH < p, p' DDD < o, p' DDT < endosulfan II < dicofol < lambda cyhalothrin I < lambda cyhalothrin II < cypermethrin I < cypermethrin II < fenvalerate I < fenvalerate II < deltamethrin. The order of elution of different pesticides observed in the present studies was slightly different to those reported by Tsipi *et al.* (1999) and Babu *et al.* (2001) this might be due to the difference in the columns and flow rate of gases used.

The ascending order of elution of seven different organophosphorus pesticides on GLC equipped with nitrogen phosphorus detector was as follows: acephate < monocrotophos < dimethoate < malathion < chlorpyrifos < quinalphos < ethion.

5.5.2 Recovery

Recoveries of different pesticides (organochlorine, synthetic pyrethroids and organophosphorus) were carried out in triplicate at various fortification levels (0.10, 0.05 and 0.025 ppm) by adding known volumes of pesticide standards. The recovery values were calculated from calibration curves. It was found that methods gave recoveries to the range of 83.66 - 91.33 per cent for organochlorine, 87.83- 90.33 per cent for synthetic pyrethroids and 76.33-83.66 per cent for organophosphorus pesticides. A wider range of recoveries (72-125 %) were reported by Tsipi *et al.* (1999), which could be due to the difference in the material used in column chromatography. In the present study, material used was Florisil: silica gel mixture. However, in the later study RP-18 was used. The present findings on per cent recoveries of organochlorine and synthetic pyrethroids are well in line with the findings of Rathi *et al.* (1997) and Khan *et al.* (2004). However, organophosphorus pesticide's recoveries recorded in the present studies slightly differ with the earlier findings of Al-Rifai and Akeel (1997), Rathi *et al.* (1997), Blasco *et al.* (2004) and Khan *et al.* (2004). This might be due to the slightly different clean up technique employed in the present studies.

Detection limits were found to vary from 0.05 – 0.50 $\mu\text{g kg}^{-1}$ for the organochlorine, 0.50- 2.00 $\mu\text{g kg}^{-1}$ for synthetic pyrethroids and 2.00- 5.00 $\mu\text{g kg}^{-1}$ for organophosphorus pesticides. However, Al- Rifai and Akeel (1997) reported slightly low sensitivity. The lower detection limits found may be due to the high sensitivity of the detectors used in the present studies.

5.5.3 Pesticides Residues in Honey Samples

Pesticide contamination of honey samples will be hazardous to the consumer health and enforce the rejection of export consignments. Keeping this in view, honey

samples collected from different localities of Himachal Pradesh were analyzed for the presence of pesticide residues. It was observed that 49.02 per cent samples contained residues of one or other pesticide. The prominent groups of pesticides detected were organochlorine (35.29%), synthetic pyrethroids (11.76%) and organophosphorus (13.73%). Contrary to this, Rathi *et al.* (1997) and Karak *et al.* (1999) reported cent per cent contamination of honey samples with above group of pesticide collected from Haryana and West Bengal, respectively. The variations in the honey samples contamination might be due to the collection of nectar and pollen by the bees from the field crops in Haryana and West Bengal those were sprayed heavily with the pesticides. In contrary to this, floral source of large number of honey samples in the present studies were wild bee flora. The other probable reason could be that ground water of these states reported to contain pesticide residues which get translocated into nectar and pollen of the flowers, thereby contaminate the honey.

Interestingly, the organochlorine pesticide residues were recovered in honey samples though their use in agriculture was banned and restricted to public health only. These pesticides came into raw water to be used for the treatment and supply as potable and irrigation water through runoff from the houses, through sewage disposal or through the effluents of industries using these pesticides. These pesticides also have high stability and long persistence in soil and water. Hence, once these pesticides were used, these persisted for years together from where these were picked up by the plants, translocated to flowers and expressed in pollens and nectar and ultimately became the source of honey contamination (Bindra, 1971; Khan *et al.*, 2004). In comparison to organochlorine pesticides contamination, synthetic pyrethroids and organophosphorus pesticide were found in less number of samples which could be attributed to their typically faster

degradation and lesser persistence (Ettø, 1974). However, malathion residues were found above MRL (5ppb) which might be due to high use of malathion sprays in certain areas

Overall, it was found that only 5.88 percent samples contained residues above MRL proposed by Ministry of Commerce, GOI. Hence, the honey from the Himachal Pradesh posed minimal health hazard risk to the consumer.

5.6 EFFECT ON PHYSICO-CHEMICAL PROPERTIES OF HONEY

Studies were also conducted to assess the impact of pesticide residues on physico-chemical properties of honey samples. It was observed that there were no significant differences among the physico- chemical properties of honey samples contaminated with pesticide residues compared to uncontaminated samples. This might be due to low level of pesticide contamination of honey samples.

PRACTICAL APPLICATION OF THE PRESENT INVESTIGATION

In the laboratory evaluation, biopesticides (azadirachtin, *B. thuringiensis* subsp. *kurstaki*), CSI (benzoylphenyl urea) and spiromesifen showed higher safety as compared to cypermethrin, imidacloprid, thiacloprid, lambda-cyhalothrin, spinosad and endosulfan. Simultaneously these pesticides are also reported to have significant effects against various pests infesting cruciferous crops. So, to develop IPM modules for various pests of cruciferous crops, where honey bees contribute major insect fauna, these pesticides should be incorporated to avoid any hazard to the pollinators.

In the field experiments, imidacloprid seed treatment was found to be harmless to the foragers of honey bees. This treatment must be used to manage soil and other sucking pests at the stage just after emergence of cruciferous crops without any harm to the foragers in the lateral stages.

The synthetic pyrethroids (cypermethrin and lambda cyhalothrin) showed a higher degree of contact and stomach toxicity against both the species of honey bees in laboratory bioassay. Besides this, the field weathered toxicity was also recorded up to 5th day of spray. Residue studies in mustard nectar and pollen also revealed that lambda cyhalothrin residues were below detectable limit on 5th day of spray. So keeping the contact and stomach toxicity, field weathered toxicity and residue studies in view, a minimum period of 5 days should be observed to shift the colonies to treated fields so as to avoid any toxic hazard to honey bees.

It was found that only 5.88 per cent honey samples had residues above MRL. Keeping in view the low per hectare use of pesticide and abundance of wild bee flora in the state, poor hill farmers must be encouraged to use these conditions to harvest pesticide free honey and market it in the international market where such honey can fetch higher price and add to the economic stability of the hill farmers.

In general, present investigations will be helpful to select pesticide with minimum hazard to bees in place of already existing toxic pesticides and to maintain the international standards of Himachal honey for the export purpose as per WTO requirements so as to provide maximum economic return to bee keepers of the state.

SUMMARY

SUMMARY

In order to determine the toxicity of some newer pesticides to honey bees and study the dissipation of some pesticides on *Brassica* blooms, experiments were carried out in the Toxicology laboratory and at Experimental Farm of Department of Entomology, CSK HPKV, Palampur during 2003-05. Monitoring of pesticide residues in honey samples collected from Himachal Pradesh along with their effects on physico- chemical properties was also carried out. The salient findings are reported here as under:

- The order of safety of pesticides on the basis of LD₅₀ (contact and stomach) against *Apis cerana cerana* foragers was benzoylphenyl urea = azadirachtin = *B. thuringiensis* subsp. *kurstaki* > spiromesifen > thiacloprid > endosulfan > spinosad > imidacloprid > cypermethrin > lambda cyhalothrin.
- The order of safety against the foragers of *A. mellifera* was; benzoylphenyl urea = azadirachtin = *B. thuringiensis* subsp. *kurstaki* > spiromesifen > thiacloprid > endosulfan > imidacloprid > spinosad > cypermethrin > lambda cyhalothrin.
- On the basis of LD₅₀'s; cypermethrin, imidacloprid, lambda cyhalothrin and spinosad can be classified as highly toxic (LD₅₀ 0.001- 1.99 µg bee⁻¹), endosulfan as moderately toxic (LD₅₀ 2.0 – 10.0 µg bee⁻¹) and azadirachtin, *B. thuringiensis* subsp. *kurstaki*, benzoylphenyl urea, spiromesifen and thiacloprid as relatively non toxic (LD₅₀ > 11.0 µg bee⁻¹).
- *A. c. cerana* foragers were found comparatively more susceptible than *A. mellifera* foragers to all the tested pesticides.
- On the basis of PT values it was observed that imidacloprid (seed treatment) @ 21 g a.i. ha⁻¹ seed found harmless to the foragers of *A. c. cerana* and *A. mellifera*. The

order of safety of spray treatments was spiromesifen (225.00 g a.i. ha⁻¹) > *B. thuringiensis* subsp. *kurstaki* (78.75 g a.i. ha⁻¹) > endosulfan (525.00 g a.i. ha⁻¹) > imidacloprid foliar application (25.00 g a.i. ha⁻¹) > cypermethrin + *B. thuringiensis* subsp. *kurstaki* (37.5 + 39.37 g a.i. ha⁻¹) > lambda cyhalothrin + azadirachtin (37.5 + 1.687 g a.i. ha⁻¹) > cypermethrin (75.00 g a.i. ha⁻¹) > lambda cyhalothrin (75.00 g a.i. ha⁻¹).

- Residues of endosulfan (525.00 g a.i. ha⁻¹), imidacloprid seed treatment (21 g a.i. kg⁻¹), lambda cyhalothrin (75.00 g a.i. ha⁻¹) and spiromesifen (225.00 g a.i. ha⁻¹) were determined through bioassay and chromatographic methods.
- Bioassay method using *D. melanogaster* was more sensitive for the estimation of imidacloprid (0.0003 ppm) followed by endosulfan (0.0012 ppm), lambda cyhalothrin (0.0078 ppm) and spiromesifen (0.0597 ppm).
- In general, chromatographic methods (GLC and HPLC) were more sensitive for the estimation of residues in nectar and pollen samples of mustard than bioassay method. Average recoveries in nectar samples varied between 82.85 and 88.90 per cent by bioassay and 91.20 and 93.55 per cent by chromatographic techniques. In pollen samples, recoveries varied between 81.44 and 86.44 per cent by bioassay and 88.50 and 91.30 per cent by chromatographic methods.
- Average initial deposits of 1.654, 1.793 and 1.471 ppm were recorded in respect of endosulfan, lambda cyhalothrin and spiromesifen, respectively in nectar samples.
- In pollen samples, average initial deposits of endosulfan, lambda cyhalothrin and spiromesifen were found to be 2.097, 1.592 and 1.932 ppm, respectively. Imidacloprid residues were detected neither in nectar nor in pollen samples of mustard.

- In nectar samples, the order of average half life of residues was lambda cyhalothrin (12.45 hours) < spiromesifen (19.99 hours) < endosulfan (27.49 hours). The order of average half life of residues in pollen samples was spiromesifen (9.69 hours) < lambda cyhalothrin (12.44 hours) < endosulfan (17.84 hours).
- Fifty one honey samples collected from the different localities of Himachal Pradesh, were analyzed for the physico- chemical properties and pesticide residues.
- The average physico- chemical properties were as follows: optical density 0.219 at 560 nm, colour white, refractive index 1.4930 at 20⁰C, moisture 17.59 per cent, viscosity 5.772 minutes at 40⁰C, density 1.617 g cm⁻³ at 27⁰C, pollen grains 64141.17 per 10 gram of honey, total soluble solids 82.40 per cent, fructose 35.95 per cent, glucose 33.05 per cent, f/g ratio 1.095, pH 4.414, total reducing sugars 69.034 per cent, sucrose 4.883 per cent, ash 0.139 per cent and HMF 9.027 ppm.
- Pesticide residues in honey samples were estimated through multiresidue analysis on GLC. The average recoveries of organochlorine and cyclodiene pesticides varied between 83.66 and 91.33 per cent. However, the range was 87.83 to 90.33 per cent for synthetic pyrethroids. The average recoveries for the seven organophosphorus pesticides varied between 76.33 and 83.66 per cent.
- Out of total 51 samples, 49.02 per cent samples were found contaminated with the residues of one or other pesticide. However, only 5.88 per cent samples have residues of only one pesticide viz. malathion above MRL, proposed by the Ministry of Commerce, Government of India.
- There was no effect of micro quantities of pesticides on the physico- chemical properties of the honey.

On the basis of above studies, it can be concluded that biopesticides (azadirachtin and *B. thuringiensis* subsp. *kurstaki*) and CSI (benzoylphenyl urea) were harmless to honey bee foragers. The synthetic pyrethroids (cypermethrin and lambda cyhalothrin) were highly toxic both in contact and stomach toxicity and persistent toxicity tests. Imidacloprid (seed treatment), azadirachtin and *B. thuringiensis* subsp. *kurstaki* (foliar applications) showed practically non significant persistent toxicity against *A. c. cerana* and *A. mellifera*. At the same time, residues of imidacloprid seed treatment in nectar and pollen could not be recorded, whereas the residues of other tested pesticides viz. endosulfan, lambda cyhalothrin and spiromesifen dissipated to more than 95 per cent in average of five days. The studies further revealed that a large proportion of honey samples analyzed from different localities of Himachal Pradesh, contained residues below MRL, which did not alter the physico-chemical properties. Thus honey produced in Himachal Pradesh is likely to pose low risk to consumer health and can be exploited for export so that farmers can fetch high economic returns.

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APPENDICES

Appendix 1 Meteorological data for the experiment period during 2003-2004

Months	Standard weeks	Temperature (⁰ C)		Relative humidity (%)	Rainfall (mm)	Rainy days
		Maximum	Minimum			
Oct.	40	26.8	13.2	57.5	0.0	0.0
	41	26.1	12.2	56.0	0.0	0.0
	42	26.5	13.0	48.0	0.0	0.0
	43	25.3	12.1	53.5	0.0	0.0
Nov.	44	24.9	11.8	59.0	0.0	0.0
	45	24.4	11.0	54.5	0.0	0.0
	46	20.1	8.8	65.0	19.5	2.0
	47	21.2	7.0	50.5	11.4	1.0
Dec.	48	20.2	6.1	54.6	0.0	0.0
	49	21.0	8.2	55.7	0.0	0.0
	50	19.5	8.9	61.0	20.6	4.0
	51	17.7	5.8	58.4	0.0	0.0
	52	15.9	4.8	56.5	0.0	0.0
Jan.	1	15.8	4.4	59.95	0.0	0.0
	2	18.2	6.6	60.20	0.0	0.0
	3	18.2	7.0	61.30	22.7	2.0
	4	14.6	3.3	69.85	116.9	3.0
Feb.	5	13.2	3.3	72.00	107.3	3.0
	6	16.8	4.9	56.20	18.3	1.0
	7	20.1	8.7	54.55	2.1	1.0
	8	21.4	8.6	47.65	15.2	1.0
Mar.	9	24.0	9.9	41.30	0.3	1.0
	10	23.4	11.0	46.770	0.0	0.0
	11	27.4	13.5	43.10	0.0	0.0
	12	29.0	14.2	36.85	0.0	0.0
Apr.	13	27.1	13.0	35.25	0.0	0.0
	14	29.2	16.5	36.10	0.0	0.0
	15	30.7	15.7	39.05	1.0	1.0
	16	31.3	18.0	44.70	2.0	2.0
	17	26.0	14.4	53.00	4.0	4.0

Appendix 2 Meteorological data for the experiment period during 2004-2005

Months	Standard weeks	Temperature (⁰ C)		Relative humidity (%)	Rainfall (mm)	Rainy days
		Maximum	Minimum			
Oct.	40	23.50	15.60	77.75	108.30	3.0
	41	23.80	12.80	68.45	73.40	2.0
	42	24.40	11.70	55.30	0.0	0.0
	43	23.60	11.10	56.20	0.0	0.0
Nov.	44	21.90	8.80	57.35	0.0	0.0
	45	22.20	9.80	55.45	0.0	0.0
	46	21.10	9.80	56.90	0.0	0.0
	47	23.40	9.80	48.85	0.0	0.0
Dec.	48	19.80	7.80	62.10	0.0	2.0
	49	20.20	6.40	54.10	0.0	0.0
	50	21.90	8.10	60.15	0.0	0.0
	51	19.60	6.50	80.35	51.7	2.0
	52	16.30	4.70	73.10	0.0	0.0
Jan.	1	17.83	5.70	76.36	3.6	0.4
	2	16.76	4.86	77.00	0.0	0.0
	3	14.16	3.46	86.07	0.6	0.2
	4	12.77	2.99	81.43	13.9	0.4
Feb.	5	16.07	5.56	73.86	0.9	0.7
	6	12.71	5.86	84.57	16.7	0.7
	7	15.27	6.29	73.23	12.5	0.2
	8	14.71	6.03	76.00	7.3	0.4
Mar.	9	19.00	10.07	78.36	1.1	0.2
	10	21.99	10.14	77.50	5.3	0.1
	11	22.54	12.19	75.93	2.3	0.8
	12	19.79	9.61	74.21	12.8	0.1
Apr.	13	22.89	9.93	68.14	0.5	0.0
	14	26.90	14.79	61.21	0.0	0.0
	15	24.56	13.00	56.71	0.0	0.0
	16	23.52	12.25	55.28	0.0	0.0
	17	22.58	13.29	56.84	0.0	0.0