CHAPTER II

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 LD$_{50}$ value of cypermethrin to the tune of 0.036 µg bee$^{-1}$.

The LD$_{50}$ (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 (LD$_{50}$ 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 LD$_{50}$ 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 (LD$_{50}$ values 0.6 ± 0.03 and 0.27 ± 0.08 µg bee$^{-1}$ for cypermethrin and fenitrothion, respectively) compared to adult bees (LD$_{50}$ 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 LC$_{50}$ value of endosulfan to the tune of 0.4503 per
A. c. indica was recorded by Singh et al. (1974). Arzone (1975a) also reported the toxic nature of endosulfan to honey bees. The LD$_{50}$ 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 LD$_{50}$ 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 LC$_{50}$ 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 (LD$_{50}$ 2.0-3.6 µg bee$^{-1}$), photo beta endosulfan and photo alpha endosulfan had intermediate toxicity (LD$_{50}$ 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 LD$_{50}$ 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 LD$_{50}$ 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 LD$_{50}$ values of imidacloprid to A. mellifera between 0.040 and 0.104 µg bee$^{-1}$. While determining the LD$_{50}$ 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 LD$_{50}$ values of 0.018 µg bee$^{-1}$ for imidacloprid, 0.022 µg bee$^{-1}$ for clothianidin, 0.030 µg bee$^{-1}$ for thiamethoxam and 0.138 µg bee$^{-1}$ for nitenpyram.
compared to cyano substituted neonicotinoids (LD$_{50}$ values of 7.1 µg bee$^{-1}$ for acetamiprid and 14.6 µg 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 µg kg$^{-1}$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 µg 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 µg bee$^{-1}$ (Pilling and Jepson, 1993). The contact toxicity of cyhalothrin was found to be least (LD$_{50}$ 0.036 µg bee$^{-1}$) to alkali bee, Nomia melanderi, intermediate (LD$_{50}$ 0.022 µg bee$^{-1}$) to the honey bees, A. mellifera and highest (0.002 µg 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 tetronic 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 LD$_{50}$ 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 LD$_{50}$ 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 (at 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 µg 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 µg larvae^{-1}. A dose of 196 µg 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 µg bee^{-1}. On the basis of LD_{50}, the honey bee (LD_{50} 0.612µg
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 LD$_{50}$ (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 LC$_{50}$ value of 1178 mg kg$^{-1}$, which was less compared to fenitrothion, fenthion and trichlorfon (Thakur et al., 1981). Akratanakul 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 Akratanakul (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 µg L\(^{-1}\) (Ambolet et al., 1999). LD\(_{50}\) value of imidacloprid to the tune of 0.005 µg bee\(^{-1}\) was reported after 48 hours of exposure by Suchail et al. (2000) and between 0.048 to 0.081 µg bee\(^{-1}\) by Nauen et al. (2001). It was also reported that sugar syrup spiked with imidacloprid up to 20 µg 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 µg bee\(^{-1}\)). Decourtye et al. (2003) worked out the oral LD\(_{50}\) value against A. mellifera as 0.153 µg bee\(^{-1}\). The lowest observed effect concentration was less to summer bees (12 µg kg\(^{-1}\)) compared to winter bees (48 µg kg\(^{-1}\)). Oral LD\(_{50}\) values of imidacloprid to A. mellifera varied from 0.040 to 0.104 µg bee\(^{-1}\) (Schmuck et al., 2003a) and 0.004 to 0.021 µg 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), Shivran and Jain (1994) and Mayer et al. (1998).

Oral LD\(_{50}\) value of spiromesifen was reported to be 200 µg 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 g a.i. ha\(^{-1}\) (Calypso\(^\text{®}\) 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 LD\(_{50}\) values to the tune of 37 and 61 µg g\(^{-1}\) body weights for first and third instar larvae, respectively. Ladurner \textit{et al.} (2005) found azadirachtin dose as high as 196 µg a.i. did not produce any toxic effects to the adults of \textit{A. mellifera} and \textit{O. lignaria}.

Mortalities were not observed in the honey bees when workers were infected in the laboratory with 2 x 10\(^7\), 2 x 10\(^8\) or 4 x 10\(^8\) spores of \textit{B. thuringiensis}, or the corresponding amounts of exotoxin was fed in sugar solution or sugar candy. The multiplication of spores of \textit{Bacillus} was inhibited by acidity and the presence of honey inhibine in the gut (Marletto \textit{et al.}, 1972). Similarly, no adverse effects to caged honey bees were observed when \textit{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 $9 \times 10^5$ 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 $5 \times 10^8$ 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 µg 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 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 up to 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 up to 2 days of application.
It was found that alphamethrin (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 µg kg\(^{-1}\) imidacloprid was found not to cause any objectionable adverse effects under field relevant exposure conditions (Schmuck, 1999). Similarly, Confidor\(^\circledast\) application (50 ml ha\(^{-1}\)) on apple and seed dressing of sunflower did not cause any adverse effect to bees (Cantoni \textit{et al.}, 2001). However, daily consumption of imidacloprid 0.1 µl L\(^{-1}\) for ten days proved toxic to honey bees (Suchail \textit{et al.}, 2001).


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 \textit{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 \textit{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 (\textit{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 mg a.i. L\(^{-1}\) did not repel foragers from the blossoming B. campestris because individual bee acquired only small quantity < 30 pg of azadirachtin (Naumann \textit{et al.}, 1994). Schmutterer (1990), Mordue and Blackwell (1993) and Thapa \textit{et al.} (1997) did not observed any toxic effect of azadirachtin to adult honey bees.

In two different experiments, bees were released on caged cotton (\textit{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 5µg 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, monocrotrophos 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\(^{-1}\)dimethoate 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 annus* 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. annus*) 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 µg 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 imidaclorpid in honey or pollen collected by honey bees from treated sunflower did not exceed
>10 µg kg\(^{-1}\). Whereas, Maus et al. (2003) found imidacloprid residues below 5 µg 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 µg kg\(^{-1}\) in the pollens of sunflower. Highest residue levels of clothianidin in field studies on pollen were observed to be 6 µg 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 organophosphorous group of pesticides. Maximum residues to the tune of 215 µg kg\(^{-1}\) and 358 µg 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 µg 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 0.01 mg 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 \(\mu g\) 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 \(\mu g\) kg\(^{-1}\)). Renvall (1977) reported Swedish 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 \(\mu g\) 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 – 0.01 mg kg\(^{-1}\)). Ritter (1980) reported kelthane residues to the tune of 0.2-0.7 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 (0.0-0.2 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 µg 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 mg kg\(^{-1}\)). However, the levels were below the previously reported levels in honey that caused poisoning (30-90 and
70-170 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 µg kg\(^{-1}\) and 5-85 µg 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 \textit{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 \(\alpha\) and \(\beta\) endosulfan to the tune of 0.001-0.100 mg kg\(^{-1}\), whereas, \(\gamma\)- HCH was found in few samples (Karak \textit{et al.}, 1999). Bogdanov \textit{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 \textit{et al.} (2004).

Khan \textit{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 determine 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 38°C. 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, organophosphorous 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 H₂SO₄ (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).


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* Kirkardly 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). Uoglauh 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, Hawlett Packard model 5890 series II system, equipped with splitless inlet, an ECD and ultra 2 column (25 x 0.5 mm id x 0.1µm 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 235°C), injector temperature (270°C) and detector temperature (280°C). 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 µm). Other GLC parameters were carrier gas (nitrogen) flow rate 1.3 ml min⁻¹, make up gas (nitrogen)
flow rate 30 ml min\(^{-1}\), H\(_2\) 3.3 ml min\(^{-1}\) and air 110 ml min\(^{-1}\). Oven temperature was programmed to vary from 70 to 268\(^{0}\)C, injector temperature 250\(^{0}\)C and detector temperature 280\(^{0}\)C. 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 (Hawlett Packerd 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 µm film thickness). Column temperature was programmed to vary from 150 to 280\(^{0}\)C, injection port and detector temperatures were 280 and 300\(^{0}\)C. 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 m id x 2.65µm film thickness, column temperature was programmed to vary from 100 to 260\(^{0}\)C. Temperature of injection port was 250\(^{0}\)C whereas, detector temperature was 275\(^{0}\)C. Flow rate of different gases were maintained as 18 ml min\(^{-1}\) for N\(_2\), 1.5 ml min\(^{-1}\) for H\(_2\) and 135 ml min\(^{-1}\) for zero gas. It was reported that limit of detection for organochlorine was 0.005- 0.01 µg kg\(^{-1}\) and for organophosphorus was 15-50 µg kg\(^{-1}\).

Karak et al. (1999) carried out pesticide residue analysis in honey through GLC (Hawlett Packard 5890A) equipped with Ni \(^{63}\) (ECD) coupled with 3392 A integrator for the analysis of organochlorine and synthetic pyrethroid pesticides. Whereas, for the determination of organophosphorus pesticides GLC (Hawlett Packerd 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 1.5µm. For organochlorine pesticides oven, injector and detector temperatures were 200, 200 and 300\(^{0}\)C with flow rate of carrier gas 30 ml min\(^{-1}\). For organophosphorus
pesticides, oven, injector and detector temperatures were 220, 200 and 280°C. The flow rate of different gases *viz.* 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 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 residue 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. It was also concluded that both GLC and HPLC methods were effective in assay, however, GC methods with MS were the 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\(^\circledR\) 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 DT\(_{50}\) of 1.3 weeks and a DT\(_{90}\) 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 (RL_{50}) 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**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Special</td>
</tr>
<tr>
<td>Specific gravity at 27°C, min.</td>
<td>1.41</td>
</tr>
<tr>
<td>Moisture per cent by mass, max.</td>
<td>20.0</td>
</tr>
<tr>
<td>Total reducing sugars, per cent by mass, min.</td>
<td>70.00</td>
</tr>
<tr>
<td>Sucrose, per cent by mass, max.</td>
<td>5.00</td>
</tr>
<tr>
<td>Fructose: glucose ratio, per cent by mass, min.</td>
<td>1.00</td>
</tr>
<tr>
<td>Ash, per cent by mass, max.</td>
<td>0.50</td>
</tr>
<tr>
<td>Acidity expressed as formic acid, per cent by mass, max.</td>
<td>0.20</td>
</tr>
<tr>
<td>Fiehe’s Test</td>
<td>-ve</td>
</tr>
</tbody>
</table>

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 was found to be 0.138, 9.020 minutes, 16.92 per cent, 1.495, 1.624 g cm\(^{-3}\), 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 was 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.