The experimental analyses carried out during the course of this study had been summarized in the previous chapters, providing an overview of the various morpho-anatomical and physico-chemical responses of the selected plant apple (*Malus pumila* Mill) cultivar Red Delicious under the influence of different systemic pesticides with varying concentrations.

The morphological and anatomical characteristics of plants greatly influence the distribution, retention and uptake of pesticides into their tissues. Plants differ greatly in habit, size, morphological and anatomical features. The leaves of different species of plants differ greatly in wettability and, within a given species, immature leaves tend to be more difficult to wet than the mature ones. Thus, specific plant differences considerably effect the uptake and persistence of pesticides. Not only do different species of plants take up pesticides at different rates, but also do different varieties. Pesticides that remain on the surface of plant organs can disappear rapidly through various forms of weathering, and the speed at which they penetrate into plants considerably effect their persistence. Most non-ionic organic pesticides are soluble in plant oils and waxes, they can penetrate into cuticular and subcuticular tissues of the treated parts of plants.

Modern techniques of farming have led to an increased dependence on pesticides for enhanced food production. In fact doubling of global food production in the last 35 years was accompanied by large increase in global pesticide use (Tilman *et al.*, 2001). In order to achieve the desired results
pesticides are often applied repeatedly (Gevao et al., 2000). Early studies have indicated an increase in residue formation with repeated pesticide application Katan et al. (1976), Fuherman and Lichteinstein, (1978), Khan and Hamilton, (1980), Zhang et al. (1984). The intake of pesticides with food is a major concern of people now a day’s but only a few studies have been focused on the uptake of pesticides by plants (Kaufman, 1983). Most important factors effecting the pesticide uptake by plants are the species, growth stage and intended use (Finalyson and McCarthy, 1970). Pesticide uptake by plants is also influenced by the pH, temperature, moisture content, method of application and mode of action.

According to Kirkwood, (1983) mode of action is sequence of events which cause injury or death to plant. For systemic pesticides, uptake must be followed by metabolism of the taken up material in which case the compound is lost. The structural features that influence the distribution, retention and uptake of pesticides are leaf shape, leaf thickness, leaf surface, stomata, cuticle, epicuticular wax, epidermis and mesophyll tissue. Pesticides can be taken up from the sprays applied to the foliage, stem, seeds and fruits. This probably occurs most easily through the absorptive areas of roots, although it also occurs quite easily through the leaves, stems and fruits (Van overbeek, 1959). Once a pesticide has penetrated the leaf cuticle, the absorptive area of the roots or some other sites of uptake, it can be carried through the plant tissue via the continuous protoplasmic phase (symplast) or across the mesophyll into the phloem and then translocated to the assimilation stream.

Although it has been widely accepted that pesticides applied at realistic concentrations do not impair plant functioning significantly and permanently (Domesch, 1972), but higher pesticide concentrations repeatedly can effect the plant’s morphological, anatomical, biochemical and physiological characteristics. The greatly increased use of pesticides has enabled the land
already under cultivation to be farmed much more intensively. Increased use of pesticides lead to depletion of global agricultural productivity. There would be least environmental contamination, if these fall exactly on the target and get degraded to harmless levels. But the non-target effects of pesticides are more harmful and lead to biomagnification and alter the whole food chain. The unregulated and excessive use of pesticides has become a major bottleneck in our fight against insect pests. More than 55% of the land used, for agricultural production in developing countries uses about 26% of the total pesticides produced in the world (Dollacker, 1991). Pesticides, however, are double-edged weapons although essential for controlling pests, and protect crops from losses that may amount to about 45% of the total production world wide (Pimentel and Levitan, 1986), their indiscriminate use leads to adverse effects on plants.

Excessive applications of systemic pesticides cause regular or sporadic damage to established vegetation within the vicinity of agricultural lands. Botanical diversity in field buffer vegetation has declined considerably in recent decades (Bunca et al., 1994). Some of these changes may be attributed to pesticide spray-drifts and drift losses (Mars et al., 1989). Large scale use of pesticides to control the pests resulted in the development of resistance which is more serious in the successful use of pesticides, 137 species of insects and mites have already become resistant to pesticides. Today the number of resistant species has risen to about 500 (Georghion, 1990). At global level insect pests have developed resistance to all the major classes of pesticides and will develop resistance to future pesticides as well (Brattsten et al., 1968), (Brown, 1987).

Found in the epidermis, stomata are the microscopic pores that regulate gas exchange between leaves and the air (Vavasseur and Raghavend, 2005), (Camoni et al., 2000). Surrounding each stoma is a pair of guard cells that
regulates stomatal aperture (and rates of transpiration and photosynthesis) by
turgor pressure driven cell movements (Tallman, 2004).

Stomata have been claimed to be the primary sites of entry for
pesticides, Norman et al. (1957), Van overbeek, (1954), Turrell, (1936),
(1954), Vanoverbeek, (1956), Volk et al. (1954), Wallihan et al. (1956),
Weaver et al. (1946), Knight et al. (1929), Minshall et al. (1949),
Rohrbaugh, (1934) and to varying degrees of aqueous solutions containing
surfactants, Leonard et al. (1956). Stomata regulate the flux of both water
vapors (transpiration) and carbon dioxide into and out of the leaf. There can
be direct or indirect effects of pesticides on stomatal aperture (guard cells).
Direct action (foliar spray) involves attack on the sensitive sites in the guard
cells, indirect action might occur due to metabolic disturbances elsewhere in
the leaf causing changes that eventually have an impact on stomatal
functioning.

Present study revealed that systemic pesticides effect the stomatal
dimensions applied at different treatment concentrations at different stages of
plant growth and development in comparison to control. The stomatal length,
width, stomatal pore length, pore width, epidermal cell length, width was
maximum at TICI (0.02%) and minimum at T1C4 (0.08%) at first bloom
(Table 1).

At petal fall stage maximum value was found at T2C1 (0.01%) and
minimum at T2C4 (0.07%) (Table 6).

In fruit development and pre-harvest stages maximum values are found
at T3C1 (0.03%), T4C1 (0.05%), and minimum values at T3C4 (0.03%),
T4C4 (0.20%) (Tables 11 and 17).

The systemic pesticides fenarimol (EC), hexaconazole (EC),
triadimefon (WP) and propiconazole (EC) applied at the first bloom, petal
fall, fruit development and pre-harvest stages, at the higher treatment
concentrations, might cause partial closure of stomata and reduce the stomatal dimensions in comparison to control, similar results are reported by Santakumari et al. (1987) in *Commelina benghalensis* L, Bora et al. (1990) in *Pisum sativum* L and Gopi et al. (2005) in *Amorphophallus campanulatus* Blume.

At the lower treatment concentration stomata are fully open with maximum dimensions in comparison to control, this might be due to less toxic and growth enhancing effects, similar results are reported in uniconazole treated *Phaseolus vulgaris* L by Mackay et al. (1990), in mulberry plants Asare – Boamah et al. (1986), Sreedhar, (1991), Davis et al. (1987), Fletcher and Hofstra, (1988).

The partially open stomata in the treated plants reduce the rate of transpiration in comparison to control with fully open stomata, similar results are reported by Abdul et al. (2007) by hexaconazole and triadimefon treatments in white yam *Dioscorea rotundata* L, in pea, wheat and soybean by Fletcher and Nath, (1984).

The decrease in dimensions of epidermal cells at the higher treatment concentration in comparison to control might be due to the penetration of systemic fungicides, which act as the preferential sites of pesticides. Epidermal cells are covered with a cuticle which is generally thinner (Turrell, 1947) and more easily penetrated by systemic pesticides (Holly, 1964), (Hamilton, 1967).

Leaf anatomy, an important feature for the process of photosynthesis and water balance of the plants. The anatomical characteristics of leaves, upper cuticle, lower cuticle, upper epidermis, lower epidermis, palisade thickness, spongy thickness, palisade cells per unit area, spongy cells per unit area, and leaf thickness change under the different treatment concentrations in comparison to control (Tables, 2, 7, 12 and 18). The increase in thickness of
upper epidermis, lower epidermis, upper cuticle, lower cuticle at first bloom, petal fall, fruit development and pre-harvest, in comparison to control was in conformity with the findings of Burrows et al. (1992) in Chrysanthemum, Heather et al. (1991) in wheat seedlings.

In the present study, the number of spongy and palisade cells per unit area increased in the treated plants in comparison to control (Tables, 2, 7, 12 and 18) the results are in conformity with the findings of Neelam et al. (1979) in Brassica juncea L and Liu-ChengLian et al. (1988).


The increase in palisade, spongy thickness and increase in number of spongy and palisade cells per unit area might be due to chlorophyll differentiation that accelerates the integrity of chlorophyll molecule (Fletcher et al., 2000). The increase in leaf thickness in the treated plants might increase the mesophyll surface per unit area.

Photosynthesis, one of the most important processes is the only mechanism of energy input into living plant world. The raw materials required for photosynthesis are carbon dioxide, sunlight, chlorophyll pigments and water. The plants treated with the fenarimol (EC) at first bloom, show maximum value of chlorophyll a, b, total chlorophyll and caroteniods at T1C1 (0.02%) and minimum value at T1C4 (0.08%) (Table 3).
At petal fall stage hexaconazole (EC), show maximum value at T2C1 (0.01%) and minimum value at T2C4 (0.07%) (Table 9).

In fruit development and pre-harvest stages triadimefon and propiconazole show maximum values at T3C1 (0.03%) and T4C1 (0.05%), and minimum values at T3C4 (0.09%) and T4C4 (0.02%), (Tables 14 and 20).


The higher treatment concentration show minimum chlorophyll value in comparison to control, this might be due to the fact that triazole systemic pesticides prevent the oxidation of ent-kaurenoic acid through inactivating cytochrome P-450 dependent oxygenase. The triazole systemic pesticides prevent the senescence of leaves and keep the leaves darker green at lower treatment concentration in comparison to control (Davis and Curry, 1991), (Binns, 1994) as observed in the present study. The increase in leaf area at the lower treatment concentration also supports the findings.

The increase in chlorophyll content at the lower treatment concentration was in conformity with findings of Ganapathy Murugan et al. (2007) in Plectranthus forskholi Briq, Gopi et al. (2007) in Daucus carota L, Muthian et al. (2007) in Manihot esculenta L, Kisorekumar et al. (2007) in Solenostemon rotundifolius Poir, Saroj et al. (1982), Tekalign et al. (2005) in potato. The increase in soluble sugar content at the lower treatment concentration by different systemic pesticides at different stages in comparison to control plants also support the results.
The higher treatment concentration decreases the soluble sugar content at first bloom, petal fall, fruit development and pre-harvest stages (Tables 3, 9, 14 and 20), this might be due to formation of toxic conjugate products by photooxidation. Systemic pesticides often form the conjugate products with sugar, which may be the reason for their denaturation or breakdown, thereby decrease the soluble sugar content in the treated plants in comparison to control. It might also be due to the decrease in chlorophyll content with the increase in concentration of different pesticides applied at different stages in comparison to control plants as observed in the present study.

The leaf area was maximum at the lower treatment concentration at different stages by different systemic pesticide applications in comparison to control. At first bloom the maximum leaf area was recorded at T1C1 (0.02%) and minimum at T1C4 (0.08%) (Table 1).

At petal fall maximum value was found at T2C1 (0.01%) and minimum at T2C4 (0.07%) (Table 6).

In fruit development and pre-harvest stage maximum values are found at (T3C1) (0.03%) and T4C1 (0.05%) and minimum values at T3C4 (0.09%) and T4C4 (0.20%) (Tables 11 and 17).


Anatomically the leaves are formed by a meristematic tissue containing layers of cells dividing anticlinically called plate meristem (Easu, 1977).
divisions in this meristem constitute major part of the intercalary growth by means of which the leaf reaches its mature size. The triazole systemic pesticides might reduce the leaf area at higher treatment concentration in comparison to control plants by diminishing the multiplication of plate meristem.

The moisture content increased in the treated plants under the influence of different treatment concentrations in comparison to control (Tables 1, 6, 11 and 17). The increase in moisture content was more pronounced at higher treatment concentration in comparison to control, similar results are reported by Fletcher and Arnold, (1984) in cucumber, Fletcher and Nath, (1988) in radish, pea and soybean by Muthukumarasamy and Pannerselvam, (2000) in *Raphanus sativus* L.


The increase in thickness of cuticle and epidermis at the higher treatment concentration in comparison to control plants, might increase the moisture content, similar results are reported by Gilley and Fletcher, (1997) in wheat. The decrease in the number of stomata at the higher treatment concentration in comparison to control plants as observed in the present study, might reduce the rate of transpiration and increase the moisture content.

The foliar applications of systemic pesticides differ according to the physico-chemical properties of the active ingredient and the leaf surface characteristics of the plant species (Stevens and Bukovac, 1987). In the present study the number of areoles, number of free vein endings per areole at the apical, marginal and middle region show great variation at different
treatment concentrations, by the application of different systemic pesticides in comparison to control. The maximum number of areoles and free vein-endings per areole was found at the lower treatment concentration at first bloom, petal fall, fruit development and pre-harvest stage and minimum number was recorded at the higher treatment concentrations in comparison to control plants (Tables 4, 8, 13 and 19). The increase in number might be due to the growth enhancing effects of systemic pesticides in comparison to control plants. It might also be due to the increase in leaf area as observed in present work in comparison to control.

However at the higher treatment concentration, the tracheidal accumulation and reduction in number of areoles and number of free vein endings per areole was found in comparison to control, similar results are reported by Rohrbaugh, (1934) in citrus and Briquet, et al. (1968) in bean, Foy, (1962) in maize, Kamimura and Goodman, (1964) in apple leaves. It may probably be due to lateral and longitudinal movements of applied systemic pesticides within the leaf lamina of the foliage, which probably occurs by three mechanisms:

i. Intercellularly in the mesophyll via the interconnected living symplast.

ii. Intercellularly in the mesophyll, but via the apoplast, i.e. within the cell wall or along the interface between cell wall and intercellular spaces and

iii. Within the conductive tissue of the veins either by symplast movement in the phloem or apoplastic movement in the xylem. Combinations of the above types of movements are also possible.

The anatomical characteristics of stem, vessel length, vessel diameter, vessel frequency, transectional area of vessel element, vulnerability and
mesomorphy were found to get changed by different treatment concentrations at different stages in comparison to control plants (Tables 5, 10, 15 and 22). The reduction in length and width of vessel element and decrease in vessel frequency at higher treatment concentration in comparison to control plants were found at first bloom, petal fall, fruit development and pre-harvest stages of plant growth and development.

The secondary xylem formation was stimulated at the lower treatment concentration and increase in length, width and frequency of vessel element were found in comparison to control plants, similar results are reported by Neelam Setia et al. (1979) in Brassica juncea L, Murti et al. (2001) in mango Magnifera indica L cv Alphanso.

The reduction in dimensions of vessel elements at the higher treatment concentration might be due to reduced cell proliferation that might lead to the inhibition in growth and reduce stem elongation, due to restricted water and nutrient uptake, the results are in conformity with the findings of Wang and Grogg, (1988). The stimulation in secondary xylem differentiation at lower treatment concentration might increase the transectional area of vessel element in comparison to control plants, similar results are reported by Ganapathy Murkugan et al. (2007) in Plectranthus forskholi Briq, Buchenaver et al. (1981), Steffens, (1988) in apple cultivar Gala, Eric et al. (1989) in apple.

Fruit quality in relation to fruit length, fruit diameter, pedicle length, total soluble solids, flesh firmness, fruit yield and yield efficiency were analyzed at fruit development and pre-harvest stages (Tables 16 and 21). The influence of systemic pesticides on fruit qualitative and quantative parameters was attributed to two factors. In addition to their primary fungicidal effects they often have physiological advantageous and anatomical adverse side effects. The decrease in fruit length, pedicle length, fruit diameter and length:
diameter ratio was found at the higher treatment concentrations in comparison to control plants, the results are in conformity with the findings of Jones et al. (1991) in Hi– Early Red Delicious, Steffens et al. (1992) in apple cultivar Gala and Triple Red Delicious, Huang, (1995) in apple, Roversi et al. (1991) in cultivar Red Delicious, Golden Delicious and Strydom et al. (1985) in Starking Delicious by triadimefon treatment.

The fruit firmness was found to increase at the lower treatment concentration at fruit development and pre harvest stages in comparison to control plants (Tables 16 and 21), similar results are reported by Calvo, (2000) in Red Delicious, Bound, (2001), LuoYoung et al. (1989), Magnitsky et al. (2006) in cucumber Cucumis sativus L and tomato.

The increase in average yield and yield efficiency at the lower dose treatment concentration was found in comparison to control at fruit development and pre-harvest stages, by the application of triadimefon and propiconazole (Tables 16 and 21). This might be due to growth enhancement in the treated plants compared to control, the results are in agreement with the reports of Stevens and Palmer, (1980), Singh and Dhillion, (1986), Harminder et al. (2002), Rosenberg et al. (2003), Martin et al. (1987). It might also be due to assimilate partitioning of the plant, as the demand is unidirectional to the developing fruit because of enhanced vegetative growth, like increase in leaf area, chlorophyll and sugar content, similar results are reported by Vijayalakshmi and Sirivasan, (2002). However at the higher treatment concentrations, decrease in physico-chemical parameters, yield efficiency and average yield was found in comparison to control plants. The decrease in leaf area, chlorophyll and soluble sugar content at the higher treatment concentrations, might inhibit the yield of plant in comparison to control.

The effects of systemic pesticides on anatomical features of apple fruits cultivar Red Delicious are found at fruit development and pre – harvest
Discussion

stages in comparison to control (Plate, 1, III). The reduced cuticular thickness found in the treated plants at the higher treatment concentration might be due to the penetration of systemic pesticides, which might increase the wettability of the chemical, the results are in conformity with the reports of Wortman, (1965). Differences were found in the epidermis, vascular bundle and outer cortex regions in the treated fruits compared to control, might be due to effect of systemic pesticides on thin walled cells get easily effected at the higher treatment concentration exceeding the recommended dosage in comparison to control, the decrease in physico-chemical characteristics at the higher concentration in comparison to control as observed in the present study also support the results.

The scanning electron microscopic studies (SEM) (Plate, II, IV) show changes in the epicuticular wax layers in fruits treated at higher concentrations in comparison to control plants. The wax surface of fruits in control fruits consists of overlapping platelets, the inhibition in epicuticular wax production was found, resulting in greater penetration of chemical at the higher treatment concentration, similar results are reported by Gentner, (1966) in Brassica oleracea L, Fog, (1947), Martin and Somers, (1967), Burkovac and Norris, (1967). This might increase spray retention which might increase the contact angle of spray droplets of applied systemic pesticides.

The studies reveal that despite variable results it is not possible to predict effects of different systemic pesticides on anatomical features of apple (Malus pumila Mill), because same chemical may show varying effects on anatomical, morphological and physiological characteristics. In spite of the inhibitory effects of systemic fungicides at the higher treatment concentrations greatly exceeding recommended dosage, at lower concentrations growth enhancing effects were found in comparison to control plants.