CHAPTER 2
REVIEW OF LITERATURE
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The research work available on the various aspects of present studies was reviewed at length. For the sake of brevity, the review is presented under the following specific headings and subheadings.

2.1 History and Taxonomy

According to Imms (1967), *Papilio demoleus* belongs to

Phylum: Arthropoda
Class: Insecta
Sub class: Pterygota
Division: Endopterygota
Order: Lepidoptera
Sub order: Ditrysia
Super family: Papilionoidea
Family: Papilionidae (Swallow tails)

Lepidoptera is one of the largest and economically important insect order having more than 1,05,000 known species (Nayar et al., 1979). Snodgrass (1935) observed a tail like extension behind the hind wings of the butterflies belonging to the family Papilionidae, hence they are commonly known as a swallow - tailed butterflies. This family includes more than 600 species of diurnal, heliophilous (sun loving) butterflies of medium to large size (Imms, 1967). However, Holloway et al. (1987) reported 550 species whereas,
Shield (1989), Heppner (1991), Scriber (1995) reported nearly 570 species from the world. According to Novak and Severa (1996), about 700 spp. of Papilionidae have been identified worldwide.

Evans (1932), Talbot (1939) and Wynter-Blyth (1957) did comprehensive studies, on the butterfly fauna of Indian region. Chaddha and Singh (1990) listed 120 species from India where as Gay et al. (1992) reported that India is represented by 107 species. Fourteen species of Papilionidae have been reported from Pakistan (Collins and Morris, 1985; Hasan, 1997). Varshney (1993) reported synonym, common name, type species, food plant and geographical distribution of Papilionid butterflies of Southeast Asian countries.

2.2 Distribution and Host plants of *P. demoleus*

The genus *Papilio* is distributed all over the world. The caterpillar of this pest was found in Africa, Japan, USA, Canada, Europe, U.S.S.R and various part of Asia to Formosa (Atwal, 1964). *P. demoleus* is distributed in China, Malaya and Burma where it was considered to be an important pest (Koli et al., 1981). Nair (1986) reported that *P. demoleus* was a widely distributed pest of citrus in tropical and sub-tropical countries of the world. Dadmal (2004) reported *P. demoleus* to be widely distributed in Asia, Africa and Australia.

Hayes (1966) reported that it occurs throughout India in all the states wherever citrus is grown. Butani (1973) and Prem Chand (1984) recorded the distribution of this serious pest in India and the neighbouring
countries including Burma, Bangladesh, Sri Lanka and Pakistan and considered it to be a serious pest particularly in nurseries in Vidarbha region of Maharashtra state.

Butani (1979) observed *P. demoleus* on almost all citrus varieties, though Malta was its preferred host. The pest had alternate host known as Bheria plant, Bawachi, Bel, Wood apple and Karilimb (Wadhi and Batra, 1964). Garg (1978) recorded the larvae of *P. demoleus* feeding on Acronychia iaurifolia, Aegle marmelos, Chierxylon swietenia, Feronia elephantum, Glycosmis pentaphylla, Murraya koenigii, Psoralia corylofolia, Ruta auqustifolia, R. graveolens, Triphasia trifoliata and Zizyphus jujuba from forests of India. It can feed on all cultivated and wild citrus and various other species of the family Rutaceae (Dorge et al., 1968 and Chen Xing Yong et al., 2004).

2.3 Life Cycle and Morphology

2.3.1 Eggs

The female started egg laying after 24 hours from mating (Radke, 1995). Tripathi et al. (1998) has studied the behaviour of egg laying of female butterfly. The fertilized female visits citrus trees or its alternate host plant and lay egg on underside of leaves by bending the abdomen so that the tip touches the leaf surface.

Atwal (1978) found that the eggs were laid on tender shoots and fresh leaves, mostly on the undersurface. Some times the eggs were also
noticed on thorns of citrus plants. The eggs were mostly placed singly (Garg, 1978; Yadav and Rizvi, 1995; Narayanamma and Savithri, 2002) or in groups of 2 - 5 (Koli et al., 1981). Oviposition period ranged from 2.1 ± 0.71 days in June and 5.08 ± 1.99 days in March (Brar and Rataul, 1973).

Kuchanvar et al. (1984), Nair (1986) and Radke (1995) reported 15 – 22 eggs per female under caged laboratory conditions. However Yadav and Rizvi (1994) reported that a single female oviposits 75 to 120 eggs during her life period.

Pruthi et al. (1945) and Atwal (1976) reported that the eggs were smooth and spherical in outline with a diameter of 1.04 mm. Brar and Rataul (1973) reported that the eggs were somewhat spherical, flat based, globular in shape and measures 1.01 mm in diameter. The eggs were glued firmly on to the surface of the leaf and were pale or greenish yellow, when freshly laid, but turn brown latter on, becoming dark grey just before hatching (Ganguli and Ghosh, 1967; Radke and Kandalkar, 1988).

The incubation period varies from 3 to 7 days depending upon temperature (Hayes, 1957; Radke and Kandalkar, 1989). In Punjab, Bindra (1968) observed egg stage for 2 - 7 days in June, 4 days during March - April and 5 days in the month of October - November. Ganguli and Ghosh (1967) observed the incubation period to vary between 3 to 4 days. The incubation period of 3 days was reported during spring by Pruthi et al. (1969). Brar and Rataul (1973) reported that the egg stage lasted for an average of 2.67 ± 0.46 days in June and 5.54 ± 0.41 days in November.
The hatching was observed between 54.5 - 60 percent with an average of 57 per cent on the caged plant (Yadav and Rizvi, 1995).

2.3.2 Larval stage

Atwal (1976) reported that the young larva emerges out by cutting a round hole through the egg - shell and consume the egg - shell as their first meal and later on started feeding on tender leaves. For feeding the larvae show preference for young and shiny leaves of citrus. After making a full meal, they remained motionless, usually near the midrib.

Radke (1995) observed that the larvae ate their own exuviae after each moulting. The caterpillars passed through five instars (Tiwari et al., 1991; Chen Xing Yong et al., 2004) but occasionally four instars were also found (Radke and Kandalkar, 1989) while Asokan (1997) reported an additional sixth instar.

Brar and Rataul (1973) reported that the caterpillars after hatching were smoky black in appearance with white stripes on the dorsum and resembled like bird droppings. Garg (1978) observed that in second and third stage the larva becomes less spiny and turns darker in colour. The dirty white lines were seen obliquely along the pleurite with a break on dorsal surface. They become yellowish-green after the fourth moult and resembled the green leaves of the host plant and becomes spineless. They have a horn-like structure on the dorsal side of the last abdominal segment. The thoracic region of the larva is thicker than rest of the body.
Atwal (1964) observed that the fifth instar larvae were 40 mm long and 6.5 mm wide. Brar and Rataul (1973) reported that the full-grown caterpillar measured about 39.35 ± 1.43 mm.

Ganguli and Ghosh (1967) observed that during April the total larval period was of 10 to 18 days. It lasted for an average of 11 days during summer and 28 – 40 days during cold weather (Pruthi et al., 1969). Butani (1979) reported total larval duration of 8.53 ± 0.69 days in June and 28.60 ± 9.29 days in November - December. Konar and Ghosh (1981) observed it of 8 - 16 days during summer and about 40 days during November - December. Deshpande et al. (1984) reported the total larval duration of 17.3 days during rainy season.

Singh and Singh (1999) observed that besides their protective colouring they were provided with a forked organ termed osmeteria, which was brought out when the larvae were disturbed. It secretes a repelling secretion and thus helps the caterpillars to escape in danger from their natural enemies.

2.3.3 Pupal stage

Ganguli and Ghosh (1967) observed that pupation took place on the branches of the host plant. Brar and Rataul (1973) reported that in rearing cages the pupation took place on wire gauze or on wooden frames etc. While, Atwal (1976) observed that full-grown larvae have a tendency to crawl away from the host plant and pupate on other plant, it is rare that they pupate on the same plant on which they had been feeding.
After selection of suitable site for pupation, the caterpillar attaches itself to the plant by the tip of the abdomen (anal attachment) and supported by a girdle of silk thread around the middle of its body. Initially the colour of the pupa was green but before adult emergence the wing colour can be visible through the pupal case. The thorax gets compressed and the wing covers unite to form a sharp keel, the latter extending up to the anterior ventral margin of the abdominal segments. The abdominal segments were observed to be round (Ramzan and Singh, 1979).

Brar and Rataul (1973) found that the prepupal stage lasted for 16 hrs and 38 hrs in summer and winter, respectively. The pupal stage lasted for $8.42 \pm 0.49$ days in April and $123 \pm 18.06$ days in November to March. In Punjab it hibernated in pupal stage. Butani (1979) found the pupal stage of about 8 days during the summer and 9 to 11 days during the spring and autumn. Radke and Kandalkar (1988) reported that the duration of pupal stage vary between 8 -10 days in the month of April. Chen Xing Yong et al. (2004) reported that the larva over-winters as pupae on host plant.

2.3.4 Adult butterfly

The butterflies usually emerged in the early morning hours (Brar and Rataul, 1973). The adult emerged from the pupal case by breaking it along the antennal suture. The newly emerged butterfly rested for a while and expands the wings. When the wings get fully expanded and are dried they start flying (Narayanamma and Savithri, 2002).
Copulation took place usually on the same day and occasionally on the next day of emergence. Mating took place end to end in opposite direction (Ganguli and Ghosh, 1967).

The adult is a large beautiful butterfly 28 mm in length and 94 mm in wing expanse (Konar and Ghosh, 1981). Head and thorax of adult butterfly were black, with a creamy – yellow coloration on the underside of the abdomen. Wings were dull black ornamented with yellow markings. The general coloration on the underside of the wings was slightly paler and the markings were also larger (Garg, 1978). Röcke (1995) reported a brick red oval patch near the anal margin of hindwings. The antennae were black and have club like structure at their tip (Atwal and Dhaliwal, 1997).

A female of *P. demoleus* usually mate once (Pathak and Pizvi, 2003a). It lived for about a week where as a male lived for three or four days (Tripathi et al., 1998). The average life span of the female varied from $4.21 \pm 1.07$ days to $6.92 \pm 1.32$ days and that of the male from $2.91 \pm 0.48$ to $4.04 \pm 0.22$ days (Pathak and Rao, 1999).

Chatterjee et al. (2000) observed that infestation of *P. demoleus* increased significantly with the increase in maximum temperature and relative humidity. Pathak and Pizvi (2003b) observed that 30°C was most suitable temperature and lemon was the most preferred food for their overall development.

Narayanamma and Savithri (2002) reported that the incidence of *P. demoleus* initiated from August - September and the peak activity periods
synchronized with the emergence of new foliage. Arya (2003) also reported that the population of *P. demoleus* was higher from February to November and peaked during August when the rainfall was in between 311.4 and 473.8 mm and the temperature was 32.8 °C to 31.1 °C.

Hayes (1957) reported that butterflies appeared in March and reached its peak during last week of April. Bindra (1969) observed the peak in April and later on between July and October. Garg (1978) reported that the diapause breaks in March and the cycle again moves normally. Kuchanwar *et al.* (1984) and Nair (1986) found that several thrushes of lemon butterfly were mostly noticed during June-July. Chen Xing Yong *et al.* (2004) reported that there were over five generations each year in Guangzhou, China and adults appeared in the beginning of March.

### 2.3.5 Nature and extent of damage

The pest was particularly devastating in nurseries and its damage to foliage seems to synchronize with the fresh growth of citrus plants in April and August (Bindra, 1966). In heavily attacked plants, growth is checked and the fruit yield also decreased and in severe cases plant bears no fruits (Pruthi *et al.*, 1969). Butani (1973) found that the caterpillar feed voraciously on leaves leaving behind only midribs and sometimes defoliate the entire seedling or the tree.

As a habit, they feed from the margin inwards, reaching the midrib. In the later stages, they feed even on mature leaves defoliating the entire plant (Butani and Jotwani, 1975). Atwal (1976) and Radke and
Kandalkar (1986) reported that the young larvae feed only on the fresh leaves and terminal shoots.

*P. demoleus* broke out in epidemic form during 1970 in Vidarbha region when due to heavy infestation, most of the flowering gardens were defoliated along with 'Bheria', a forest plant from near by areas. The larval population first defoliated Bheria plantation in forest to become full-grown and upon the adult emergence they formed a swarm. These butterflies migrated to the adjoining citrus gardens where 100% defoliation was recorded due to this pest. The larval population per tree was estimated to the extent of about 5000 larvae per tree. Such infected trees were rendered fruitless, where the economic loss was speculated of several crores of rupees (Thakare and Borle, 1974).

Brar and Rataul (1973) reported that a single caterpillar consumed from 16.22 to 25.83 sq. inch of leaf area with an average of 19.16 sq. inch during its larval life. The average consumption per caterpillar per day was 2.03 sq. inch of leaf area. The maximum damage was recorded during the months of April - May and August - October during new vegetative growth. The extent of damage to the orchards in terms of leaves eaten varied from 5.55% to 100%.

2.3.6 Control measures

Sontakay (1943), Atwal (1964) and Pruthi *et al.* (1969) suggested shaking of the plants and killing the larvae by drowning them in kerosinised water. It should be repeated after every 5 - 7 days as the
caterpillars in the first and second stages do not fall quite easily. However shaking of trees should not be practiced in Ambia bahar in the month of July when the trees bear fruits, as this treatment will result into fruit drop.

Bnar and Rataul (1973) advised picking of larvae with hands or forceps and destroying mechanically, but according to Dadmal (2004) it was expensive and impracticable on large scale and applicable only where plant height and girth is reasonably small.

Radke and Ghususkar (1984) and Rao (1988) suggested the use of entomophagous fungi, Bacillus thuringiensis or nematode DD 136 strain in controlling these caterpillars. Narayanamma and Savithri (2003) also reported 100% control of the pest population by spraying B. thuringiensis.

Spraying with BHC 0.1 - 0.16% (Wadhi and Batra, 1964) was found to be very effective against this pest. Ganguli and Ghosh (1967) reported spraying of DDT at 0.25% suspension to be highly effective. Also endrin 0.1% emulsion has been found to be very effective in controlling the larvae, but its application on plants bearing fruits should be avoided. Besides, endrin 0.02% (Dorge et al., 1968) or 0.04% (Thakare et al., 1984) was also recommended but with the ban put on its use and sale by Government of India it is no longer advisable.

Singh and Rao (1978) suggested spraying of 0.4% dimethoate, endosulphan, phosphamidon, monocrotophos, quinalphos etc. The early larval stages were controlled by taking up the field spray application of endosulphan at 0.05% and carbaryl 0.1% concentration.
Konar and Ghosh (1981) suggested use of dimethoate 0.2% for effective control but reinestation was reported on the 14th day after spraying insecticide. Malathion 0.03% emulsion gave 92% mortality when applied at fourth and fifth instar larvae. Malathion 0.05%, methyl parathion 0.02%, diazinon 0.04% were found effective in management of this pest in Punjab state (Deshpande et al., 1984). Prem chand (1984) reported 86.08% mortality with fenvalerate.

Dohrrey and Butani (1985) and Radke and Kandalkar (1986) observed cent percent control with cypermethrin followed by decamethrin and dimethoate but reinestation was observed on the 10th day after spraying insecticide. Later on Ahlawat (1997) recommended diazinon 0.04%, 0.3% dimethoate, parathion 0.02% or malathion 0.03%, though malathion 0.02% was also effective. Shivankar (1999) suggested spraying of fenitrothion 0.025% or diazinon 0.02% or monocrotophos 0.04% for control of this pest.

Various workers advised spray of arsenicals (Vyas, 1994) in combination with gur or molasses (Shivankar and Singh, 1999) or in form of lead arsenate at 0.25% (Chatterjee et al., 2000).

2.4 Biochemical Aspects

2.4.1 Estimation of sugar

In insects, as in other animals, sugars have a central place in metabolism. Kilby (1965) discussed carbohydrate metabolism in insect fat body. In the majority of species, the amount of free glucose was quite small (Wyatt and Kalf, 1967). Chefurka (1965) recorded high sugar levels in insects.
as compared with those found in vertebrates. Usually in insects more than 0.5 percent sugar in blood was recorded however there were several records of more than 5 percent. In the aphid *Megoura vicieae*, the larval haemolymph contained 13 - 20 mg / ml glucose that was greater than the adult (10 – 11 mg / ml) haemolymph. In solitary bees *C. japonica* still greater (i.e. 30 - 48 mg / gm glucose was recorded (Kilby, 1965). The haemolymph of honeybee (*Apis mellifera*) contained 11 - 14 mg / ml of glucose (Gilmour, 1961). *Periplaneta americana* contained 7.2 mg / ml and in locust blood around 24 mg / 100 ml glucose was recorded (Chefurka, 1965). The glucose content decreased in the adult (24 mg / 100 ml) than in the larvae (200 mg / 100 ml) of *Schistocerca gregaria* (Wyatt and Kalf, 1967). The larva of *Phormia regina* showed low glucose level (2 mg / ml) than the adult (8 mg / ml) (Chefurka, 1965).

The levels of reducing sugar in blood ranged in different species of insect between zero and 300 mg / 100ml, which, as Wyatt and Kalf (1967) remarked, appeared remarkably low for such metabolically active animals.

A typical carbohydrate content in haemolymph appeared to reflect specialized diet. Hensen (1964) presented evidence that the blood of locusts fed on fructose rich food contains fructose but the blood of locusts fed on wheat does not contain fructose.

2.4.2 Estimation of lipid

One of the prime impetus to study the lipid content in *P. damoeus* has been the finding that many insect growth hormones, pheromones and sex attractant are liposomal (Gilbert, 1967). Lipids are having vital importance in many insects as substrates for embryogenesis,
metamorphosis and for flight. Several reviews on aspects of insect lipid chemistry and biochemistry have been appeared (Gilby, 1965) while other reviews considered the metabolism of lipids along with other biochemicals (Gilmour, 1961).

According to Gilbert (1967) there was wide variation in lipid content of insects belonging to different orders and even within a single family. Lipid content per individual larva was observed to increase with age and size. The newly hatched larva contained an appreciable amount of lipid, which increased as the larva grows. The prepupa loses water rapidly and lipid content increases, so that at pupation lipids constitute between 5 - 7 percent of the wet weight of the insect. At this time a sexual dimorphism in lipid content is evident, the male pupa contains 50% more lipid than the female (Niemiarko, 1959).

In most insects the female contains more lipid than the male, as lipid is a most efficient substrate for egg development (Fiesl, 1964). However, the reverse may be true for many species as in the Lepidoptera. Gilbert and Schneiderman (1961) observed a sexual dimorphism in lipid content in the adult stage of Hyalophora cecropia. During the pupal-adult transformation this dimorphism increased markedly until in the adult moth the tissues of males contain about 5 times as much lipid per gram fresh weight as the tissues of the females (Dormaese and Gilbert, 1964). There was 50% decrease in lipid content per individual between egg and first instar larva indicating lipid utilization during embryogenesis (Niemiarko et al., 1956 and Rothstein, 1952).
2.5 Enzymological Aspects

2.5.1 Introduction

Glutathione S-transferase (GST; E.C. 2.5.1.18) is a family of multifunctional enzymes, which catalyze the conjugation reaction of the xenobiotics (insecticides) with an endogenous factor viz reduced glutathione (GSH). The conjugates were further metabolized to mercapturic acids and excreted (Yang, 1976; Chasseaud, 1979; Motyama and Dauterman, 1980). Fukami (1980) and Meister and Anderson (1983) reported that GSTs act by catalyzing the conjugation of large variety of compounds bearing an electrophilic site, with reduced glutathione. The conjugates were then eliminated from the cell via the glutathione S-conjugate export pump. Hayes and Wolf (1988) and Scott (1996) reported that the GSTs in insects were primarily of interest because of their role in insecticide resistance.

Habig et al. (1974) separated and purified several glutathione transferases from rat liver and showed that each of the enzymes had overlapping substrate specificities.

GSTs were classified on the basis of the transfer group from the substrate molecule to the sulfur atom of reduced glutathione as glutathione S - aryl (Brooth et al., 1961; Grover and Sims, 1964), aralkyl (Cohen et al., 1964), alkyl (Johnson, 1966), alkeno (Boyland and Chasseaud, 1969), epoxide (Boyland and Williams, 1965; Fjellstedt et al., 1973) and phosphoric acid triester alkyl transferase (Hutson et al., 1972). On the basis of structural homologies and substrate specificities, they have been divided into three
classes designated as α, μ and π (Mannervik et al., 1985). Based on their structure and biochemical properties GSTs have been divided into the cytosolic α, μ, π and θ classes, as well as a microsomal enzyme (Hayes and Pulford, 1995).

GST is ubiquitous and its presence has been demonstrated in almost all animal species, lower and higher, as well as in higher plants. GST have been characterized in many insects, including cockroaches (Usui et al., 1977; Wu et al., 1998), houseflies (Clark et al., 1984; Kerkut and Gilbert, 1985; Bull, 1992), grass grubs (Clark et al., 1985), caterpillars from five phytophagous lepidopterous species (Yu, 1989), bacteria (Lau et al., 1980), mammals (Jakoby, 1978; Awasthi and Singh, 1985; Mannervik and Danielson, 1988) and plants (Lamoureux and Rusness, 1989).

Induction of GST by various xenobiotics and host plants has been reported for a number of insect species. These reports have demonstrated induction by phenobarbital and pesticides in houseflies (Ottea and Plapp, 1981; Hayaoka and Dauterman, 1982 and 1983; Ottea and Plapp, 1984), host plants and allelochemicals in fall armyworm larvae (Yu, 1982; Yu, 1983; Yu, 1984). This is a non-inheritable phenomenon induced in direct response to chemical stimulus (Terriere, 1984; Suckling et al., 1990).

2.5.2 Physiological role of GST

2.5.2.1 Detoxification of Insecticides: Clark et al. (1966); Tanaka et al. (1976) and Tanaka et al. (1981) reported that GST is involved in the detoxification of a wide range of insecticides. Fukami (1980) and Dauterman (1983) also suggested that Glutathione - dependent conjugation is
an important detoxification mechanism. It detoxify many insecticides including organophosphate pesticides and halogenated hydrocarbons such as lindane (Oppenooth et al., 1979; Motoyama et al., 1980; Cheng et al., 1983; Kao et al., 1989) and acaricides (Wu, 2003).

Motoyama and Dauterman (1980), Dauterman (1985) and Armstrong and Suckling (1988) have noted that increase in both glutathione content and GST activities were associated with organophosphate resistance. Oppenooth (1985) also found that glutathione S – transferases were commonly implicated as primary factor in resistance to certain OP (organophosphorus) compounds and may play a secondary role in resistance to other insecticides.

GST enzyme was characterized in premethrin and in DDT resistant and susceptible strains of *Aedes aegypti*. Throughout development enzyme activity was substantially higher (approximately eight fold) in resistant strain compared to susceptible strain (Grant and Matsumura, 1989).

Bull and Pryor (1990) studied the comparative activity of selected enzyme systems in homogenate preparations of susceptible (S) and resistant (R) houseflies and they found that, in cytosol preparations of abdomens, the activity of GST against both substrates (CDNB and DCNB) was about two fold higher in resistant (R) houseflies. Cochrane et al. (1992) analyzed the GSTs in a malathion-resistant strain of *Drosophila* and observed an approximately three-fold elevation in total activity, that is associated with elevated levels of two GST isoforms. High levels of GST are important in
resistance to organophosphorus insecticides in a number of insects including houseflies, mites and diamondback moth (Kao and Sun, 1991).

Although GSTs have not been implicated directly in pyrethroid resistance, there were reports of elevated GSTs in laboratory-selected insects reported by Grant and Matsumura (1983) or pyrethroid resistant wild insects by Pospischil et al. (1996). Insecticide resistance patterns among 16 Brazilian populations of maize weevil Sitophilus zeamais were recognized by surveying resistance to three organophosphorus and three pyrethroids by Fragoso et al. (2003). The results of biochemical in-vitro studies measuring the activity of detoxification enzymes suggested major involvement of enhanced conjugation by GST in pyrethroid resistance.

Dinamarca et al. (1971) studied physical properties on a purified preparation of DDT-dehydrochlorinase and demonstrated no similarity in physical properties with any GST. Similarly, Motoyama and Dauwerman (1975,1977) and Chang et al. (1981) studied the relationship between GST and DDT-dehydrochlorinase activity with DCNB and showed that there was no significant correlation between the two activities.

However, Clark and Shamaan (1984) purified DDT-dehydrochlorinase by a factor of 600 fold. Purified form of DDT-dehydrochlorinase possessed substantial GST activity with both CDNB and DCNB. This hypothesis was supported by a recent study of Hemingway et al. (1991). The enzyme appeared to be dimeric with subunits of molecular weight of 23000 and 25000.
2.5.2.2 GST as antioxidant defence: GSTs also play vital role in protecting tissues against oxidative damage and oxidative stress and helps in antioxidant defence, with the principle function of reducing organic hydroperoxides within membranes and lipoproteins (Fournier et al., 1992; Parkes et al., 1993 and Vontas et al., 2001).

2.5.3 Level of GST in different developmental stages

Saleh et al. (1978) measured GST activity in several stages during the life span of housefly and they inferred that activity increased during the larval stage, however, while susceptible adults had similar or lower activity than the larvae, adults of an insecticide- resistant strain had higher activity than the larvae. The pattern of GST activity during the life span of the mosquito has been reported by Hazelton and Lang (1983) and they found that activity increased during the larval and pupal stages, peaked in the newly emerged adult and then decreased sharply. Kotze and Rose (1987) reported that the conjugation activities for DCNB and CDNB increased throughout larval development to reach a peak early in the pupal stage.

Pattern of GST activity were different between larvae and adult of *H. virescens* suggesting that the regulation and expression of these enzymes activities is dependent on developmental stages (Kirby et al., 1994).

GST activity towards CDNB was detected in various developmental stages of *Drosophila melanogaster*. The specific activity of the enzyme was 100, 35, 25, and 15 μmol / min / mg protein in crude extracts
prepared from eggs, larvae, pupae and adult stages, respectively. Herbicide oxadiazolone caused 4 and 2.5 fold increase in the enzyme activity in pupal and adult stages, respectively (Hunaiti et al., 1995).

Rose et al. (1995) studied metabolic enzymes of multi resistant population of *H. virescens* in comparison with susceptible population. Resistance associated increase in glutathione S-transferase activity were found in fifth instar larvae. Zhang et al. (1996) studied glutathione S-transferase in different larval stages of an insecticide resistant (HJ-R) and a susceptible (HD-S) *H. armigera* strains. They studied GST activity in two days old first to fifth instar larvae. As larval development progressed the enzyme activity increased sharply and the ratio of GST between HJ-R and HD-S strains also increased. Further they reported that the fifth instar larvae possessed highest GST activity.

Khan and Rajurkar (2001) reported the levels of detoxifying enzymes in *Helicoverpa armigera* and detected GST activity towards CDNB in various stages of life cycle of *H. armigera* and found highest activity in the 2nd day of fifth instar larva.

Leonova and Slynko (2004) also reported higher GST activities in fifth instar larvae than in adults and lowest in eggs. Jovanovic-Galovic et al. (2004) determined antioxidant enzymes in whole body homogenates and found that pupae had higher GST activity compared to larvae.
2.5.4 Level of GST in different tissues

According to Motoyama et al. (1980) a common feature is that the fat body was major site of metabolism of many insecticides, especially the organophosphate class, while the gut, cuticle & haemolymph also plays an important role in the metabolism of xenobiotics. Low enzyme activity in whole body extract was probably due to the effect of endogenous inhibitors such as polyphenols & quinines released during homogenization.

Kotze and Rose (1987) reported that 50% of the GST - CNDB conjugation activity of larva was localized in the fat body with the remainder in the cuticle (25%), gut (15%) and blood (10%). Similarly, Rajurkar (2002) also found that GST activity was highest in fat bodies of Helicoverpa armigera indicating the major site of metabolism of organophosphate insecticide.

2.5.5 Purification of GST

The soluble fraction of five phytophagous lepidoptera was first fractionated with ammonium sulfate to obtain a protein fraction, which corresponded to 45 - 70 % saturation. It yielded 54 - 80 % of the initial enzyme activity towards CDNB and purification of 1.5 – 3.0 fold (Yu, 1989).

In H. zea the highest specific activity for CDNB-conjugation was found in the 65 - 70 % ammonium sulfate precipitation (Chien and Dauterman, 1991). Purification by ammonium sulfate precipitation showed a maximum GST activity in 70 % ammonium sulfate fraction in H. armigera (Khan and Rajurkar, 2001).
Clark and Dauterman (1982) purified GST by affinity chromatography from five different strains of housefly of defined resistance to insecticides. Apparent purification factors of 1.8 - 25 were obtained. Clark et al. (1983) purified GST about 10 – 100 fold by affinity chromatography. Clark et al., (1986) proposed that the various strains of houseflies might possess a multiplicity of GST, common to all, individual proteins being present to varying extents in the different strains.

2.5.6 Characterization of GST

Clark et al. (1984) studied multiple forms of GST from many insect species, such as Musca domestica and Moth, Wiseana cervinata and reported the existence of three different sized subunits of M, of 20000, 22000 and 23500.

Yu (1989) purified GSTs from five phytophagous lepidopteran insects using a two-step procedure involving ammonium sulfate fractionation and affinity chromatography on a glutathione – agarose column. The fall armyworm and corn earworm possessed multiple glutathione transferase containing six and four isozymes, respectively. On the other hand tobacco budworm, cabbage looper and velvet bean caterpillar had a single form of the enzyme. These isozymes consisted of two to four subunits with molecular weights of 27000 to 32000 depending on the species.

The heterodimeric nature of GST with molecular weight of 23.7 kDa and 26 kDa was reported from the third instar larvae of Costelytra zealandica (Clark et al., 1985). In Drosophila melanogaster, GST enzyme showed two protein bands in 12 % SDS gel electrophoresis of purified
enzyme from larvae, pupae and adult. The molecular weights of these bands were 23.5 kDa and 27.2 kDa in larvae and pupae. Protein bands with molecular weight of 23 kDa and 35 kDa were observed in the purified adult enzyme. These enzymes were heterodimeric protein (Hunault et al., 1995).

Partial purification of beetle cytosols revealed increased CDNB activity in resistant cytosols eluting as the unbound fraction following DE-52 chromatography when compared with cytosols isolated from susceptible insects. When electrophoresed on an equal protein basis, SDS-PAGE analysis revealed increased staining of two protein bands in the GST molecular weight region (24,000 - 30,000 dalton) in cytosols isolated from resistant beetles (Reidy et al., 1990).

The enzyme responsible for CDNB - conjugation was purified 48 fold by GSH - agarose affinity chromatography in H. zea. SDS gel electrophoresis and chromatofocusing resolved only one isozyme, which was a homodimer, consisting of two equal size subunits having molecular weight 23,900 (Chien and Dauterman, 1991). Tiwari et al. (1991) showed that the GST isozyme was heterodimer with subunits having M, values of 25,000 and 27,000 in D. saccharalis and M, values of 26,000 and 27,000 in E. loftini.

GST was purified from the larval cattle tick Boophilus microplus, by glutathione affinity chromatography. The purified enzyme appeared as a single band on SDS-PAGE and had a molecular mass of 25.8 kDa determined by mass spectrometry (Haiqi et al., 1999).
Khan and Rajurkar (2001) reported that SDS – PAGE showed a single band of GST enzyme after coomassie blue staining that had apparent molecular weight of 30 kDa in *H. armigera* while Yu (2002) purified a single GST isozyme by affinity to the extent of 22 fold from midgut microsomes, which had a sub unit molecular weight of 27,000 dalton.

Rauch and Nauen (2004) reported that the glutathione – affinity chromatography purified GST enzymes of two different *B. tabaci* strains appeared as two bands on SDS - PAGE & had a molecular mass of 23.5 kDa and 27.8 kDa determined by MALDI mass spectrometry.

### 2.5.7 Properties and kinetic study of GST

Kotze and Rose (1987) studied GST in the Australian sheep blowfly, *Lucilia cuprina*, using CDNB as substrates. They found that, the rate of CDNB conjugation increased linearly upto an incubation temperature of 35°C and then decreased. Optimum pH for GST activity was 7.5 - 8.0 and 6.7 - 7.4 for DCNB and CDNB conjugations, respectively. Schroeder *et al.* (1990) reported that the pH optimum was between 7.6 and 8.0 and also reported much higher optimum temperature (40 – 45°C) for GST in Spruce Needles.

Grant and Matsumura (1988) found no difference in enzyme kinetic constants (*K_m* and *V_max*) between resistant and susceptible strains of *Aedes aegypti*. Kotze and Rose (1987) and Reidy *et al.* (1989) reported 4 to 5 fold increase in *V_max* in resistant *Lucilia cuprina* and *Tribolium castaneum*, respectively. The low *K_m* and high *V_max* values of GST isolated from *D. saccharalis* and *E. loftini* indicate that these enzymes have high affinity for electrophilic substrates (Tiwari *et al.*, 1991).