Review of Literature
II. REVIEW OF LITERATURE

Several authors have studied the reproductive biology and the effect of insecticides on the male and female reproductive system, mortality, fecundity, fertility, longevity, histochemistry (Nucleic acid, Protein and Glycogen) and the malformation of different stages of experimental insects. There are certain factors that affect the reproduction of insects. These factors may be endogenous as well as exogenous such as food, chemicals, pheromone temperature, humidity etc. Insecticides affect the normal function of specific cells and tissue of insects' body and make the survival of insect difficult. Sufficient information on the effect of insecticidal application must be available before deciding on the control measures against harmful insects. It is all the more relevant for the selective use of pesticides in view of their harmful effects on the non-target animal and humans. Further, insect pests are developing resistance against many of the commonly used insecticides. Quite many of the lepidopterous insects fall in the group of well-known pests and considerable information is becoming available on the effect of such chemical on these pests besides causing environmental pollution.

Bio-pesticides (neem extracts), cause a series of deleterious effects such as morphological, physiological and biochemical alterations and certain other organ damages. Any toxic substance which induce damage to the living organ may ultimately lead to imbalance in the cell constituents resulting in bio-chemical perturbations, thereby causing malformations of the physiological systems. Histopathological observations are also used as one of the tools in understanding the nature of damage and mode of action of the pesticides. Available information on the reproductive biology and behaviour of the test insect, histopathological changes in the reproductive organs, under the toxic
stress of various pesticides and chemosterilants have been reviewed in the present chapter.

REPRODUCTIVE BIOLOGY AND BEHAVIOUR

_Diacrisia (Spilosoma) obliqua_ Walker is an important pest of various crops in India. The larvae of this moth are polyphagous and voracious feeders of the foliage. It has been reported from all over the country to cause considerable damage to pulse, cotton, vegetable, sorghum, maize, ragi, oil seeds, small millets, sugarcane, paddy, wheat, guineae grass, jute, sannhemp, beet root, potatoes and sweet potatoes (Mathur, 1962; Pant, 1964; Gargav & Katiyar, 1971 and Premchand, 1975). It has been reported as the most destructive pest of soybean (Gangrâde, 1976; Bhardwaj & Bhalla, 1977; Ram & Bhattacharya, 1978; Khaleque, 1983; Haq et al., 1984 and Ali, 1988). It causes significant damage to oil-seed crops like groundnut, castor, mustard and sunflower. Srivastava et al. (1965); Pachori et al. (1980); Islam et al. (1983) and Vora et al. (1985) have reported it on groundnut. _Anomis flava_ has been recorded as a pest of cotton, okra (bhindi), deacon hemp, urd, sunflower, hollyhock, silk cotton tree and ban bhindi (Puttarudriah and Mahesiwariah, 1956 and Patel et. al., 1964). It has been reported on mustard by Bakhetia & Brar (1982) and Bakhetia (1986); on castor by Srivastava et al. (1972); on Soybean by Singh & Gangrade (1974) and on Sunflower by Sethi et al. (1979) and Rohilla et al. (1981) and on jute by Sharif (1962) and Tripathi (1967); on maize by Verma et al. (1977). Among pulses it has been found causing severe damage to mug bean (Lal, 1985) and has been reported on numerous species of forest shrubs and trees including _Butea frondosa, Cedrela toona, Colebrookia oppositifolia, Lantana camara, Morus alba, M. indica, Tectona grandis_ and _Vitex negundo_ by Beeson (1941).

Among ornamental plants, the flowers of Gladiolus and leaves of globe artichoke (_Cymara scalymus_) are very prone to its attack (Krishnah et al., 1975). Mathur (1962) has also recorded its incidence on few medicinal plants. Another
species *S. lubricipeda* has been reported on apple and pear foliage by Sengelevich (1960) and *Spilosoma virginica* was found very common on weeds (Rizzo, 1978 & 1979). The larvae of *D. obliqua* have been deployed to control the cosmopolitan weed *Lantana* (Beeson & Chattargee, 1940). *D. obliqua* being a polyphagous pest consume a variety of foods. These foods have variable affect on their growth, development, nutrition and reproduction (Babu et al., 1978; Gupta et al., 1979; Prasad & Premchand 1980; Gupta, 1982; Srivastava & Pandey, 1983; Goel et al., 1986 and Kabir & Miah, 1987). A number of natural enemies of *D. obliqua* have been reported (Lall, 1960 and Kalra et al., 1967) but the practical utilization of these natural enemies has not been explored.

Morphological studies of the adults of *D. obliqua* have been done by Ahmad & Ahmad (1976) and the larval morphology was studied by Goel & Kumar (1983), while biology and larval morphology of *S. virginica* was studied by Rizzo (1978 & 1979). Banerjee (1971) reported the eclosion and oviposition of the moth *Andraca bipunctata* follow specific rhythmic patterns with fixed time during a 24-hour cycle. Moth emerges throughout the night, but with increased frequency at both dawn and dusk. Eclosion and oviposition follow daily rhythmic patterns irrespective of fluctuation in light and climatic factors in different seasons. It is suggested that these rhythms are responsive to an endogenous biological clock. The model for determining theoretical moth densities appropriately predicts the moth density for any sampling day, provided eclosion is continuous. The record of larvae of the moth *A. bipunctata* are known to cause periodic defoliation of mature tea bushes, *Comellia sinesis* in North-East (Watt and Mann, 1903). An outline of its life history has been given by Das (1965), while Banerjee (1970) demonstrated the presence of a diurnal rhythmic or aggregation and damage caused by the 5th instar larvae.

In most Lepidoptera, diurnal changes in activity patterns and rhythmic behaviour is influenced by the light intensity (Larsen, 1943 and Edwards, 1964). These observations thus suggest that each behaviour pattern reacts differently
to light–dark sequences, though changes in temperature, relative humidity and other environmental factors their interactions can also be confounded with light changes (Corbetl, 1960). Rao and Patel (1973) studied the eggs of Anomis flava F. that hatched in 2 days at 27.21°C but fated to hatch at 38°C. The percent larvae completing their development in 12.7 days was 80.0, while remaining completed it in 13.0 days at of average laboratory temperature of 29.17°C. The pupal period was 6.27 days at 29.17°C and 6.05 days at 32°C. The pupae can be sexed by examining the distance between the genital and anal slits. The adults are dimorphic in type of antennae and wing patters. The females lay an average of 370 eggs when each was caged with a single male and 407 eggs when each was caged with two males. The total life cycle from laying of eggs to death of the adult averaged 31.42 days. The incubation period has been reported from 2 to 6 days in A. flava (Khan, 1956; Puttarudriah and Maheswariah, 1956 and Srivastava and Bhatnagar, 1963).

Deshmukh et al. (1979) have recorded the survival of D. obliqua larvae at 4 days interval on 16 plant species belonging to 12 different families as 27 ± 1°C temperature and 85 ± 5 percent relative humidity (RH) and 12 : 12 hours photoperiod. Observations indicate that on Cardia myxa Linn., Solanum melogena Linn., Ocimum gratissimum Linn., Ficus benghalensis Linn., Acalypha colorata Sprg and Cavabis sativa Linn. larvae were unable to feed because of physico-chemical nature of the plant species. Although on O. gratissimum and C. myxa greater feeding was recorded in preference tests but these two plants failed to support growth and development of test insects. This may perhaps be attributed to the difference in selection behaviour between 1st and 6th instar larvae. The other food plants viz., Carica papaya Linn, Cucurbita maxima Duch, Gossypium arboreum Linn., Vigna mungo Hepper., V. aureus, Ricinus communis Linn. Brassica Juncea (Linn.), Luffa autangula (Linn.), Zea mays Linn. and Glycine max (Linn.) proved to be better as indicated by higher survival of larvae. The type of food plants also had a direct effect on the average weight of the
larvae. It has been emphasized that apart from the presence or absence of phagostimulants / phagodeterrents and attractants / repellents, the basic chemical constitution of plants and efficiency of utilization also plays an important role in accepting a plant by any insect (Kogan, 1972; Barney and Rock, 1975).

Prasad and Premchand (1980) have recorded the effect of nine food plants viz., cow-pea, Vigna unguiculata Linn.; groundnut, Arachis hypogaea Linn.; sunflower, Heliothis annuum Linn.; kalat, Phaseolus mungo Linn.; cotton, Gossypium sp.; lucerne, Medicago sativa Linn.; jawar, Sorghum vulgare Pers.; velvet bean on the development of Diacrisia obliqua Walker. None of the larvae survived on jowar, velvet bean and lantana. On the basis of larval period, larval survival, larval weight, pupal period, pupal weight and sex-ratio of D. obliqua, sunflower was found to be the most favorable food and cowpea was the least. On the basis of growth-index, six of the remaining food plants could be arranged in order of decreasing suitability as sunflower> cotton> kalai> lucerne> cow-pea> groundnut. Thus the food plants which were favorable for larval survival were suitable for rapid development of the insect. In general, food plants, which were the best for the development of the insect, produced more females and vice-versa. The growth of D. obliqua on sunflower (20.9 days), cotton (21.0 days) and kalai (21.4 days) were quicker, while longest larval period was recorded on groundnut (24.5 days) a view supported by Pandey et al. (1968) and Lal & Mukharji (1978). Snyder (1954) and Kapil (1967) also observed marked effect of food plants on the larval period of Peridroma margaritosa and Philosamia ricini. Pandey and Srivastava (1967) obtained similar results with Prodenia litura. Role of food plants on survival and maturity of insect had been emphasized by Smith (1959) who worked on grasshopper. Snyder (1954) on cutworm obtained similar results, P. margaritosa and Lal & Mukharji (1978) on D. obliqua.

Binder (1996) studied the adults of Agrotis ipsilon, which consumed sucrose solution and water just after eclosion. The percentage daily feeding and
the mean daily consumption for females and male fed sucrose solution declined with time, whereas the percentage daily feeding and the mean daily consumption of those fed with water increased with time. Adults lived longer by feeding on carbohydrate and their life expectancy was similar to that of the corn earworm, *H. zea* (Adler, 1989). Long-duration flight behaviour in *A. ipsilon* was significantly affected on the third day following eclosion if supplemental carbohydrate was not in the adult died (Sappington & Showers, 1993). Consumption of carbohydrate solution during the adult stage significantly extended *A. ipsilon* longevity, which has been reported for other moths (Gunn & Gatehouse, 1985 and Willers et al., 1987). Shalam & Pener (1984) recorded sexual behaviour in azadirachtin induced 'over-aged' last instar male larvae of *Locusta migratoria*. Also in this case the corpora allata may have been (partially) activated at the 'appropriate' time.

The moths of *A. bipunctata* mated immediately after emergence (Banerjee, 1971) whereas the moths of *H. armigera* (Singh & Singh, 1975), *Phyllocnistis citrella* (Pandey & Pandey, 1964) and *Lamprosema indicata* (Kapoor et al., 1972) respectively matured for mating in about 36 minutes to 32 hours, 14 to 24 hours and one day after emergence respectively. In moths generally the mating starts at late night as reported in *P. citrella* (Pandey & Pandey, 1964), *D. obliqua* (Siddiqi, 1985), *Cnaphalocrosis medicinalis* (Velusany & Subramaniam, 1974), *A. bipunctata* (Banerjee, 1971), *L. indicata* (Kapoor et al., 1972), *Polytela gloriosae* (Sachan & Srivastava, 1965) and *Hemithea tritonaria* (Mehra & Shah, 1966). However, in *Parnara mathias* (Teotia & Nand, 1966) and *Chilo zonellus* (Trehan & Bhutani, 1949) mating occurs in the morning. In the moths of *Polymatus boeticus* (Pandey et al., 1978) and *Amrasca biguttula biguttula* (Singh, 1978) the mating was accomplished during the daytime. On the other hand in *Thiacidas postica* it can occur at any time during the day or night (Mehra & Shah, 1970).
The male of *D. obliqua* showed fast activity and excitement before mating as was also observed in *L. indicata* (Kapoor *et al.*, 1972). Mating took place in tail-to-tail position in *D. obliqua* like that of *P. gloriosae* (Sachan & Srivastava, 1965) and *A. biguttula biguttula* (Singh, 1978). Mating in *D. obliqua* was observed (Siddiqi, 1985) only once in its life which occurred before laying of first batch of eggs. Single mating during the whole life was also recorded in *P. citerella* (Pandey & Pandey, 1964) and *H. armigera* (Singh & Singh, 1975), but the moths of *L. indicata* mated more than once during their life (Kapoor *et al.*, 1972).

Siddiqi (1985) observed mating period of *D. obliqua* which was 11:15 ± 1.35 hours, in *Thiacidas postica* varied from 10-12 hours (Mehra & Shah, 1970), 15-30 minutes in *P. citerella* (Pandey & Pandey, 1964), 20-25 minutes in *P. gloriosae* (Sachan & Srivastava, 1965), 5-10 minutes in *P. mathias* (Teotia & Nand, 1966), 15 minutes to 3 hours in *L. indicata* (Kapoor *et al.*, 1972), 5 minutes in *C. medinalis* (Velusany & Subramaniam, 1974) and 5-25 minutes in *A. biguttula biguttula* (Singh, 1978).

The growth disrupting effect of azadirachtin obtained from neem trees, *Azadirachta indica* A. Juss, in now well recorded but its exact mode of action is still not very well understood (Rembold *et al.*, 1980, 1982). Azadirachtin has juvenilizing effects on the last instar larvae of *Spodoptera litura* (Gujar and Mehrotra, 1983). Application of azadirachtin, resulting in poor larval growth due to inability of larvae to moult into next instar, has been reported in *Lymantria dispar* (Skatulla and Meisner, 1975), *Plutella xylostella* (Ruscoe, 1972) and *Spodoptera frugiperda* and *Oncopeltus fasciatus* (Redfern *et al.*, 1981). Gujar and Mehrotra (1983a) observed the effect of azadirachtin and other neem products like mathanolic neem kernel extract and neem oil on the growth and development of the tobacco caterpillar, *S. litura*. Neem products showed varying degree of deleterious effects on growth and development. These are presumed to be due to interference with neuro-endocrine system of insect.
Neem product treated adults were deformed and have shorter life span than control adult moth in *Ephistia kuehniella* (Rembold *et al.*, 1980, 1982 and Sharma *et al.*, 1980). The findings suggest that azadirachtin and other neem products affect larval moulting, longevity of adult, fecundity and egg hatchability. These results are in conformity with similar effects of azadirachtin on larval-pupal moulting in *E. kuehniella* (Rembold *et al.*, 1981).


Srivastava *et al.* (1997) observed the alcoholic and hexane extracts of 17 neem ecotypes and found it toxic to the egg, larval and pupal stages of the cosmopolitan, external gregarious larval parasitoid *Bracon brevicornis* (Wesm). In general, the hexane extracts showed higher toxicity against the egg and pupal stages whereas the alcoholic extracts were more toxic against the larvae. Azadirachtin content of the neem ecotypes revealed no apparent correlation with the observed toxicity against different stages of the parasitoid. The bio-activity of the test materials bears no apparent correlation with their azadirachtin content, as also reported against *S. litura* (Kumar, 1994) and against *Chetosiphon fragaefolii* (Quariess, 1994).
Pandey et al. (1968) found highest survival rate of *D. obliqua* on *Sesamum indicum* followed by *G. hirsulum*, *P. mungo* and *Vigna sineris* and least on *Zea mays* and *Crotolaria juncea*. Katiyar et al. (1975) reported that *Solanum melongena* is a suitable host for the development of *D. obliqua*. Marked variation in the larval development of *D. obliqua* on different food plants was also reported by Ratan and Nayak (1963); Pandey et al. (1968) and Prasad and Bhattacharya (1975) on *S. littoralis*. Pandey et al. (1968) reported shortest larval period of *D. obliqua* on *R. communis* and longest on *Crotolaria juncea*. Ishley (1936) has established this fact in cotton boll-worm, *Heliothis zea*. Such differences in selection behaviour of early and later instars have also been reported in case of *Pyrausta nubilalis* Huber (Guthrie et al., 1960), and *Papilio machaon* Linn. (Wilkund, 1973). The growth may be inhibited due to the presence of toxic chemicals, nutritional deficiencies or nutritional imbalances, (House, 1969; Barney & Rock, 1975). Shapiro (1968) also found that older larvae of *Isia isabella* (Smith) were able to tolerate *Petunia volaceo* Sudanerika while the same plant was toxic to young larvae. Hard texture of leaf or presence of hairs and spines in certain plant species have been reported to be possible deterrent (SooHoo & Fraenkel, 1966 and Smith et al., 1975). Prasad & Bhattacharya (1975) also postulated that certain plant species fed to *S. littoralis* larvae could not support the development due to physical structure of leaves. Zwodfer & Harris (1971) mentioned that the ability to support the larval development is usually restricted to a smaller range of plants. Such behaviour has been reported in *Gastrophysa cynea* (Malsh) (Force, 1966), *Altica caerulea* Olivier (Sankaran et al., 1967), *Uroplata gerardi* Pic (Bennett & Maraj, 1967), *Agasicles* sp. (Simons et al., 1968) and *Octotoma scabripennis* Gurin (Harley, 1969).

Singh and Sandhu (1981) observed oviposition behaviour of *C. partellus* from cotyledonary to fourth leaf of the plants, but 94.9 percent of the egg masses were found to be laid on cotyledonary. Singh & Sandhu (1978) reported the
relationship of oviposition with plant age and stage of growth, plant height, plant condition, dates of sowing, different cultivars and spacing. Singh (1963) reported 93.33 percent of the egg masses near base of the leaf and 6.67 percent away from it.

Pandey et al. (1968) studied the rearing of larvae of *D. obliqua* on 12 natural food plants and their effect on the larval and post-larval development. On the basis of survival percentage of larvae, growth index, gain in weight of larvae, pupal weight, percentage of adult emergence, size and fecundity of moths, *till* was found to be the most preferred food for *D. obliqua*. Sannhemp was the least preferred of the food plants tested and was found to have a distinct retarding effect on the growth rate of *D. obliqua* resulting in a prolonged larval period and a slightly longer pupal period than in the case of other food plants. Similarly, the larvae completely failed to survive when reared on jowar and bajra. Shaw et al., (1988) studied the biology of the arctiid *D. obliqua* on *Dalichos lablab*, *Sesamum indicum*, *Phaseolus mungo*, *Cynodon dactylon*, *Azadirachta indica*, sweet potatoes, groundnuts, okra, maize and rice in the laboratory.

Jeyabalan and Murugan (1997) have reported the limonoid compounds deacetylnimbin, 17-hydroxy-azadiradione, gedunin, salannin and deactylgedunin. Constituents of neem tree (*A. indica*) and their effects on development, feeding and reproduction in a polyphagous insect *H. armigera*. Murugan et al. (1993) studied the effect of neem products (neem oil and neem kernel extracts) on the nutritive and reproductive physiology of *H. armigera*. Jeyabalan & Murugan (1995) investigated the neem timonoids on its antifeedant effect, growth inhibition and toxicity in *H. armigera*. Murugan & Kumar (1996) studied the amount of food consumed, total egg output and adult longevity were maximum on young leaves reared *H. puera* than reared on aged leaves. Insects are known to alter their feeding behaviour in response to the changes in both dietary composition and their nutritional requirements (Mattson, 1980; Scriber & Slansky, 1981 and Murugan et al., 1992). The growth rates of herbivorous
lepidoptera larvae are strongly dependent upon water content of their food (Timmins et al., 1988 and Murugan & AncyGeorge, 1992).

Singh and Jakhmola (1980) reported the larval-pupal intermediates (deformed pupae) which were observed in 8.5% larvae during the mass rearing of D. obliqua. The studies on endocrine organs indicate that the juvenile hormone secreted by the corpora allata is responsible for these deformities resulting in the formation of larval-pupal intermediate. As a correlation of neurosecretory change with growth and development in D. obliqua (Singh, 1977) some larval-pupal intermediates (deformed pupae) were observed. Novak (1966) observed that the juvenile hormone of the corpora allata is responsible for the cocoon spinning in the last larval instar of lepidopterous larvae. Piepho (1967) also observed that implantation of active corpora allata in the spinning larvae of Galleria mellonella, does not influence the spinning behaviour but actually leads to restoration of feeding instincts.

Sethi et al. (1979) studied the incidence of Bihar hairy caterpillar, D. obliqua on sunflower, Helianthus annus. Unseasonable rains during February followed by gradual rise in temperature, under fairly high humidity and longer sunshine period provided conducive conditions for the population build up of this polyphagous insect-pest, attaining its peak with 1350 caterpillars per plant by the third week of March. The damage to sunflower by hairy caterpillar, Pericallia ricini has been reported by Ayyanna et al. (1978). D. obliqua was first noted by Sethi et al. (1976) causing serious damage to the crop.

Pathak and Krishna (1991) reported detrimental effects of oil volatile on post-embryonic development and adult emergence of Corcyra cephalonica in varying degrees when individuals were reared for the 2 weeks of larval life or for a similar duration from the sixteenth day of their lives in an environment of eucalyptus oil volatile. But a marked decline in the reproductive potential, in terms of egg output and egg hatchability of the moth was observed when the
larvae were reared for the first 15 days in the presence of eucalyptus oil volatile. Tripathi et al. (1987) studied the antifeedant activity of 26 plant extract against S. obliqua 3rd instar larvae in laboratory and reported that the extracts of Lindenbergia grandiflora has highest antifeedant activity (82.75%) followed by Passiflora melliissima (71.84%), Schima khasiana and Ehvetia canareensis which showed moderate activity (61.1 & 60.7) respectively. Ghatak and Bhusan (1995) on the basis of involution on the ovicidal activity of the some indigenous 8 plant extracts on eggs of S. obliqua revealed that none of the eggs could hatch when treated with 1.0% methanol and petroleum ether extract of Piper nigrum. Similarly, petroleum ether extracts of A. indica and Erythrina indica at 1.5% and methanol extract of E. indica and Annona squamosa at 2.0% and A. indica at 1.5% produced the same result.

**BIOCIDES**

Jhansi and Singh (1993) tried the neem seed Kernel extracts viz., aqueous, ethanolic, hexane, ethanol soluble part of hexane extract, ethanol insoluble part of hexane extract, chloroform extract of deviled kernel and various combinations screened for their effects on egg hatching, oviposition, feeding, growth and development of the Helicoverpa armigera. The observations showed that except EtoHIHE all other extracts significantly reduced egg hatching. EtoHSHE and CHEKD caused considerable inhibition of egg hatching. The high activity of EtoHSHE is due to azadirachtin and of CHEFDK is due to both salanin and azadirachtin (Singh et al., 1988). But susceptibility of eggs is influenced by several factors such as permeability of chorion and solvent of active substances (Smith and Wagenknecht, 1950). Thus, pure compounds such as Salanin, Salannol, Salannol Acetate present in seed oil have low antifeedant activity than azadirachtin when combined with CHEDK (Krauns et al., 1987). The significant higher larval mortality with 36.6 to 100 percent was recorded in different treatments of the extracts in comparison to the control (6.6-13.3%). CHEDK,
CHEDK + EtoHSHE and CHEDK + HE caused 100% mortality at 4% concentration, with extended larval period (Rembold et al., 1981; Ladd et al., 1984 and Schluter et al., 1985).

Raman et al. (1993) observed the physiological effects of an enriched neem formulation (achook), containing azadirachtin, azadiradione, nimbocinol and epinimbocinol (≥ 2800 ppm) under laboratory condition on lepidopteran pests: *H. armigera* and *Earias vitella* and recorded strong antifeedant effects. It also affected the larval and pupal survival and adult emergence in a dose-dependent manner in both the species. It reduced the larval growth and total developmental growth, prolonged the time for pupation, and lowered pupal weights, resulting in the emergence of deformed individuals. Studies on the effect of achook on pests of cotton, okra, brinjal, cabbage, jute and tea testified significant reduction in pest population and crop damage (Kogan, 1988 and Saxena, 1987). Dose dependent effects leading to reduction in larval growth rate has been observed in *Spilosoma maculatus* Walk (Umeh, 1988) and *Spodoptera frugiperda* (Mikalajczak et al., 1989). Formulations based on neem are also reported to be environment friendly due to their low toxicity to natural enemies of insects (Saxena, 1987).

Murugan et al. (1993) reported that neem kernel extract and neem oil interfere with the food utilization and biochemical and enzyme profiles related to nutritive and reproductive physiology of *Heliothis armigera*. The amount of food consumed and the efficiency of ingestion and digestion of food were markedly inhibited by the neem products. Moths obtained from treated larvae failed to produce mature oocytes, probably as a result of interference of azadirachtin with vitellogenin synthesis and its uptake by oocytes. Neem products in male and female reproductive tissues suppressed protein level as well as fat body development in the larvae along with the enzymes involved in lipogenesis. The choice of the food plant is intricately associated with diverse aspects of the feeding behaviour with considerable effect on the reproductive potential of the
species (Slansky & Scriber, 1985; Slansky, 1992; Murugan & Ancy-George, 1992). Where the decreased fecundity and oocyte development is a consequence have impaired vitellogenin synthesis and its uptake by the developing oocytes (Ludlum & Sieber, 1988). Decrease in the activities of ACP and ALP in the testes and seminal visicle may be due to the inhibitory role of NSKE and neem oil on spermatogenesis and sperm survival (Schluter and Schulz, 1983).

Saradamma (1993) reported that the juvenomimetic activity of benzene extracts of neem against red cotton bug, Dysdercus cingulatus on fifth instar nymphs showed variation of nymphal stage significantly. Extracts of Azadirachta indica and Eupatorium odoratum could completely inhibit the normal adult emergence. Egg laying was suppressed with the use of extracts of Clerodendron infortunatum, Vitex megundo, Solanum indicum and Ageratum conyzoides. Nerium oleander, Codiaeum veriegatum and Adathoda visica extracts caused the production of non-viable eggs only. Maximum number of malformed adults was observed in insects treated with A. indica with longevity varying from 0.87-7.44 days. However, the oil of marigold containing tagetone with juvenile hormone mimicking activity was not found effective on D. koenigii (Saxena and Srivastava, 1973).

Rao and Tilak (1993) observed in a laboratory experiment conducted for two generations with nine botanical pesticides. Neemguard, Vepanic, Morgosal and Necknool (neem products), Alletin (garlic synthetic extract), Annmet and Stipple (Annona), Karrich (Pongamia) and RD 9 Replin for the control of first, second and fourth instar larvae of citrus butterfly, Papilio demoleus Linn. Allitin gave 94.55%, 95.82% and 93.22% protection over control with highest larval mortality (79.44) among all botanicals (Solunke and Deshpande, 1991). The superiority of Allitin was reported by Chitra et al. (1990) in the control of Spodoptera litura and RD 9 Repelin case of P. demoleus (Skatulla and Meisner, 1975). Aphids on rapeseed and mustard, are controlled mainly by the use of
insecticides viz., methyldemetone 0.02 % or dimethoate 0.03% or phosphamidon 0.03% (Reddy & Reddy, 1980). Earlier pyrathrins, rotenone, nicotine, lantana, senhund and sukhadarshan also were found to be effective against pests and also safer to mammals and other higher animals (Pandey et al., 1977; Tikku and Kout, 1978). The fecundity was also significantly lowered at 1.0 and 1.5% concentrations of A. indica, L. camera and l. cornea (Pandey et al., 1987).

Azadirachtin, a tetranoctiterpenoid from the neem tree (Azadirachta indica A. Juss) is a strong antifeedant and growth disrupter to several insect species and thus a potential candidate for use in plant protection (Ruscoe, 1972; Steets & Schmutterer, 1975; Ladd et al., 1978; Warthen, 1979 and Schmutterer & Rembold, 1980). Growth disruption by azadirachtin has been shown to be primarily due to its effect on neuroendocrine centers leading to change in pool size of technical morphogenetic hormones (Sieber & Rembold, 1983).

Schluter et al. (1985) administered azadirachtin injection to freshly emerged last instar larvae of Manduca sexta and elicited different reaction according to the dose administered. At low doses, pupation occurred in most of the cases, but the resulting pupae were defective for the most part. Individuals complete development, moulting to supernumerary larvae or death of larva (Kauser & Koolman 1984). The action of azadirachtin on Manduca larvae on endocrine mechanism regulate moulting and metamorphosis on weight gain in Locusta migratoria and Epilachna varivertis (Sieber and Rembold, 1983). Nymphs treated with higher dose in Locusta with azadirachtin show incomplete or no moulting (Cymborowski et al., 1982; Sieber & Rembold, 1983).

Goyal et al. (1971) studied the non-edible character of various minor oil seeds viz., neem (A. indica), mahua (Bassia latifolia) and Karanj (Pangamia glabra) which was probably due to the presence of some odoriferous, toxic and bitter constituents. Sinha (1960) initiated systematic investigation of better utilization of non-edible cakes with a view to improve the economy of non-edible oils. Pradhan & Jotwani (1968) reported that the aqueous neem seed
suspension showed absolute antifeeding property up-to 0.05 percent against the desert locust, *Schistocerca gregaria* Forsk and the migratory locust, *L. migratoria*. The antifeeding properties of alcohol extract of neem seed cake was quite significant against *S. gregaria* (Sinha, 1964). Seed extracts of neem has feeding deterrence effects on insects like *Apogona blanchardi* Retsema, *Maladera insannabilis* Brenske, *Adoretus bicolor* Brenske and *Adoretus* sp. (Pradhan et al., 1962; Narayanan et al., 1980). Doharey & Singh (1989) expend their view that the neem seed kernel extracts being cheap, easily obtainable and environmentally safe could be utilized in the management of chaffer beetles.

The leaves, seeds and fruits of chinaberry, *Melia azadirach* are known to display feeding inhibition growth retardation and insecticidal activity in some insects (McMillian et al., 1969; Morgan and Thornton, 1973; Schmutterer, 1989; Oroumechi and Lorra, 1993). The plants treated with neem oil have been demonstrated to reduce oviposition in the dried fruit beetle, *Corpophilus hemipterous* (L), *Nilaparvata lugens* and *Dacus cucurbitae* (Saxena et al., 1981 and Singh & Srivastava, 1983). Chen et al. (1996) reported that the oviposition of the diamond back moth, *Plutella xylostella* got deterred by extracts from fruits of the chinaberry, *M. azadarach* in Taiwan. Hough-Goldstein and Hahn (1992) reported that an aqueous extract of tansy, *Tanacetum vulgare* L was detrimental to oviposition by the imported cabbage-worm and the diamond back moth. Javer et al. (1987) found that pine oil was an ovipositional deterrent for the onion maggot, *Delia aniqua* (Meigen), under laboratory conditions.

Sharma (1996) investigated the effect of three neem-based formulations viz., Nimbecidine, Achook and Nethrin as protestant against *Rhyzopertha dominica* (F) and showed that Nimbecidine (10, 20 ppm.) and Nethrin (1:30, 2:30 v/w) were most effective. These products had a three-fold effect, suppressing the adult emergence, reducing grain damage as demonstrated by Jilani and Su (1983) and further it did not adversely affect germination of stored maize as shown by Jotwani and Sirear (1965). Agarwal & Mall (1988) found the
insecticidal and antifeedant activity of extracts of neem (fraction c and thionemone) and of seeds of *Calophyllum inophyllum* against 3rd instar larvae of *D. obliqua* at concentrations of 1, 2.5 and 5% by contact as well as oral application and obtained a positive correlation between concentration and mortality with the contact method (33, 33-100% mortality) than with the feeding method (2.66-80%).

Martinez and Emden (1999) have reported sub-lethal concentrations of azadirachtin incorporated into artificial diet and when offered to 3rd instar larvae of *Spodoptera littoralis* (Boisduval) prolonged larval instar and reduced food intake. These effects were observed after the treated larvae had been transferred to plain diet; the reduced food intake was therefore a secondary antifeedant effect. This effect declined with time once the treatment with azadirachtin was stopped. Growth was more severely reduced than food intake, and the reduction in growth also occurred during periods when food intake was not affected, possibly due to post-ingestive effects. The secondary antifeedant effect by azadirachtin Mordue (Luntz) *et al.* (1985) and Rembold (1989) was seen to vary with species. It was not observed in *Manduca sexta* (Timmins & Reynolds, 1992) and in *Pridroma saucia* (Koul & Isman, 1991). Also, such effects seemed to be greater at the higher than the lower dose, as reported in *Spodoptera exempta* (Walker) after topical application of azadirachta (Tanzubil & McCaffery, 1990). Agarwal (1993) reported that the four plant extracts viz., Thionemone (*Azadirachta indica* A. Juss preparation), heartwood extract (*A. indica*) extract of *Swietenia macrophylla* king seed, and *C. inophyllum* at three different concentrations i.e., 1.0, 2.5 and 5.0% were found to be equally effective. Certain plants extracts possess the ovicidal activity (Nakajina & Kazuyoshi, 1980; Osmani & Sighamony, 1980 and Katiyar and Srivastava, 1984) and azadirachtin, an isolate of neem seed, has been reported to be one of them (Koul, 1984 and Dorn, 1986).
Jagannadh and Nair (1996) observed the effect of 5 μg and 20 μg of azadirachtin on the eggs of *Spodoptera mauritia* and the hatchability and post-embryonic development. Although the treatments have no effect on egg hatchability, the larvae showed high rate of mortality during post-embryonic development. No effect on the embryonic development and hatchability with topical treatment of AZA to the eggs of *Dysdercus koenigii* (Koul, 1984), *Tribolium castaneum* (Mukherjee & Ramachandran, 1989), *Oncopeltus fasciatus* (Dorn, 1986) and *S. exampta* (Tanzubil & McCaffery, 1990) has been observed. Several orthopteran and lepidopteran species show that ecdysteroids accumulate in the eggs mainly as conjugates (Hoffman & Laguex, 1985). From these reserves the future embryo will elaborate its own ecdysteroid peaks necessary for the control of embryonic molts and cuticulogenesis (Hoffman & Laguex, 1985; Delbecque *et al.*, 1990). Such delayed effects of neem seed derivatives on larval mortality have been reported in several insect species (Webb *et al.*, 1983; Arnason *et al.*, 1985 and Simmonds *et al.*, 1990).

Barnby and Klocke (1987) observed that plant chemicals could be administered, either through artificial diet or by oral injection, to fifth instar larvae of the tobacco bud-worm, *Heliothis virescens*. At a dietary concentration of 0.03125 ppm azadirachtin significantly reduced the amount of diet consumed and the weight gained by the larvae. Higher dietary concentrations (0.25 and 0.5 ppm) were necessary to reduce efficiency of larval conversion of digested and ingested food respectively.

Azadirachtin however did not reduce pupal weight of lepidopteran pests of rice (Schmutterer *et al.*, 1983). Reduced and delayed maximal liters of the hormones associated with molting have resulted from azadirachtin test (Rembold, 1984 and Dorn, 1986). Thus, azadirachtin effects *H. virescens* in a manner similar to other tested species of insects. Neem extracts including azadirachtin has deterrent antifeedant effect in *S. gregaria* (Pradhan *et al.*, 1962; Butterworth and Morgan, 1968 & 1971) and Saxena *et al.* (1981) also showed
feeding deterrent activity of neem oil to Brown Plant Hopper (BPH), *Nilaparvata lugens*. Neem seed kernel suspension has been used for protection of tobacco nurseries against *Spodoptera litura* (Joshi and Ramprasad, 1975; Joshi et al., 1978), systemic studies on repellency and ovipositional deterrence of the extracts against larvae and adult are lacking (Pawar & Ramakrishnan, 1971 and Fagoonee, 1981).

Bhathal *et al.* (1994) evaluated the feeding deterrent activity of Neemark (80% extract of *A. indica* water miscible formulation containing azadirachtin) against 3rd instar larvae of *Spilosoma obliqua* by the leaf-disc dipping method, concluded that the antifeedant activity over control ranged between 6-7% at the lowest concentration (0.313%), and 86% at the highest concentration (5%) being significantly reduced over control. Tripathi and Jain (1993) isolated the excelsin by bioassay directed fractionation of the MeOH extract of stem bark and found that excelsin (0.3%) exhibited an antifeedant activity against *S. obliqua* at 80% with effective dose (ED-50) as 0.563% excelsin.

**MORPHOLOGY, HISTOLOGY AND HISTOPATHOLOGY**

Raju *et al.* (1990) investigated the effect of the flower extract of *Thevitia neriifolia* on the reproductive organs of male. Fifth instar nymphs of *Dysdercus similis* and reported small testis lobe with incomplete development of testis follicles, thin and filamentous vasa deferentia and small sized accessory glands. Raju and Thakur (1993) noticed morphological and gonadotrophic effects in fifth instar nymph of *D. similis* when applied topically with the extracts of flowers of *Prosopis specigera* (Leguminosae) in petroleum either, chloroform and methanol. The treated insects moulted into adults, adultoids and super-nymphs and also noticed retention of first batch of oocytes in the ovarioles. These mutagenic effects could be due to flavones present in the flower’s extract. Of particular interest is that a single dose of injected azadirachtin (10 μg / female)
inhibits ovarian development in *Locusta migratoria* (Rembold, 1984). Since ovarian development and associated metabolic changes are under endocrine control (Gilbert *et al*., 1980 and Raabe, 1982), it would be worthwhile to study azadirachtin action from this angle. With the changes in oocyte, it appears that the somatic growth precedes initiation of ovarian cycle.

The studies on the hickory shuck-worm, *Laspeyresia caryana* showed that the sperm leave the spermatophore, travel through the ductus seminals across the common oviduct up to the ductus spermatheca (Callahan & Cascio, 1963). It has been observed that the reproductive system of the hickory shuck-worm is similar to that of the pink bollworm, *Pectinophora gossypiella* as described by Wellso and Adkisson (1962). The paired testes enclosed in a common scrotum is seen easily through abdominal wall of male pupae as is also the case in several species in moths (Callahan, 1958; Callahan & Chapin, 1960; Callahan & Casio, 1963).

Mathur (1967) reported in *Syntomis cyssea*, the testes as a median mass, with four follicles. Each follicle is separated by the deep in folding of the inner coat and bears numerous tracheoles. The vasa deferentia originates from deep folds on the ventral side of the testis. Each seminal vesicle is oval in shape and opens into the ejaculatory duct. The common ejaculatory duct recourse at the terminal end to from a chitinous bulbous ejaculatorius. It is different from the primitive forms where each follicle is digitate and is enclosed in a separate scrotum (Imms, 1964). Tracheoles are, however, reported absent in *Leucinodes orbonalis* (Srivastava, 1960) and *Utetheisa pulchella* (Mathur, 1966). Demokidoff (1902) also regarded that there are no tracheoles in the follicular epithelium but Ruckes (1919) observed them in a number of lepidopterous insect. The epithelial cells are secretory in nature (Eltringham, 1925; Omura, 1938 and Callahan & Cascio, 1963). The lumen of the gland is filled with secretion which helps in the formation of spermatophore (Khalifa, 1950). Hewer (1934) reported the ductus receptacle as a spiral duct in *Ephestia*. Norris (1933
& 1934) and Raichoudhury (1936) discussed the occurrence of a brush border in the bursa deferentia. Musgrave (1937) studied the receptaculum seminalis and associated structures and suggested that the accessory glands in the male secrete materials for the nourishment of the sperm.

Ahi (1988a) observed the histopathological changes brought about by sub-lethal dose of aldrin injected into the gonads of adult Poekilocerus pictus. In males, the germ cells and differentiating germ cells showed pycnosis. In female, vitellogenesis was arrested in most of the oocytes. Abnormal fragmentation of oocytes was evident and follicular epithelial cells showed degeneration. Thus chlorinated hydrocarbon, aldrin, appeared to make tissue hyperactive and probably there was a stress causing cellular deformation.

Jain and Bhide (1990 & 1991) observed that adult female cockroaches when treated with sub-lethal concentrations of BHC & DDT arrested ovulation and vitellogenesis with severe histopathological changes. The treated adults exhibited prolonged duration of mating and nymphs failed to emerge from ootheca. It was further reported that when adult male and female of Poecilocerus pictus were injected with sub-lethal concentrations of BHC into haemolymph, severely damaged fat bodies and adversely affected gonads with marked histopathological changes were observed.

Paul et al. (1991) reported that larvae of Diacrisia obliqua when topically treated with endosulfan and quinalphos at both lethal and sub-lethal doses resulted in severe abnormalities in the testicular structures. Reduction of testis size, complete inhibition of spermatogenesis, relative decrease in the number of testicular cysts and presence of deformed spermatogonial, spermatocytic and spermatid cysts were recorded after lethal treatment. Sub-lethal dose also induced more or less similar effects at histological levels but to a lesser extent than in the lethal doses. Further, malformed and reduced size of sperm-bundles was observed in the testes of imagines from pupae resulting out of larvae
receiving sub-lethal dose of insecticides. Ahi (1988b) recorded the histopathological changes of HCH on the testes of *P. pictus*. Bhuya and Dash (1976) also report the effect of systemic insecticide dimethion on cyst formation and size reduction to a lesser extent with inhibition of nucleus. Thus, insecticides exert both nucleotoxic and cytotoxic effects on spermatocytic chromosomes as suggested by Davis (1968). Sub-lethal treatment of insecticides accounts for the reproductive incapability besides adverse physiological activities of *D. obliqua*. Loosening of the germ cells, pycnosis of the spermatogonia, spermatocytes, hypertrophied spermatids hypertrophied and dissociated spermatozoa, formation of 'brown coloured body' between the testes is comparable with the effect of apholate on the reproductive organs of locusta as reported by Vishwanath *et al.* (1978).

Gangrade and Pant (1970) studied the freshly emerged males and females of *Cadra cautella* (Walker) which were separately administered 2.0, 3.0 and 5.0% apholate in 10% sucrose solution for 48, 96 and 144 hours. Ovarian development was inhibited at 48 hours in all concentrations of apholate and was more severe in some flies at 144 hours. Testes were however; not affected either in their form or spermatogenesis at 48 or 96 hours though at 144 hours spermatogonial cells appeared reduced in numbers. The sections of ovaries of treated females showed that the fully developed chorionated eggs remained unaffected, while the undifferentiated anterior region of the ovarioles was severely affected so much so that oocytes and nurse cells failed to become distinct. While nurse cells and oocytes were clearly separated in the middle region of the ovarioles, the effect of apholate was indicated by clumping of the chromatin material in the nurse cells and the nuclei of the oocytes.

Inhibition of ovarian growth was noted in the vinegar fly (Goldsmith & Frank, 1952) and in the housefly, *Musca domestica* with mitotic and tumor inhibiting substances (Mitlin *et al.*, 1957 and Mitlin & Baroody, 1958). Alkylating agents sike tepa, metapa, and apholate were found to retard ovarian growth in
M. domestica (Morgan and LaBreeque, 1962 & 1964), in the screw-worm fly, Cochliomyia hominivorax (Chamberlain, 1962 and Crystal & LaChance, 1963), in D. melanogaster (Cantwell & Henneberry, 1963), and in the boll worm, Heliothis zea and the tobacco bud-worm, H. virescens (Sotto & Graves, 1967). These studies have apparently failed to examine the histological basis of the retarded development except perhaps those of Cantwell and Henneberry (1963) and Morgan and LaBreeque (1962 & 1964) who attributed the decreased ovarian size to clumping of chromatin in nurse cells. Rai (1964) presented evidence of degeneration of follicular epithelium and of indistinction between nurse cells and oocytes in the mosquito Aedes aegypti.

In male reproductive organs, Smittle et al. (1966) and Hedin et al. (1967) observed clumping of chromatin in the germarium of tepa - injected German Cockroach, Blattella germanica and boll weevils, Anthonomus grandis respectively. Henneberry & Kishaba (1966) observed no effect of tepa, metepa and apholate on the testes and sperm packets of cabbage looper, Trichoplusia ni. LaChance & Riemann (1964) presumed that in the screwworm fly, the chromatin material is affected soon after application of tretamine in oocytes. A complete breakdown of follicles of the ovarioles of Cadra conformed to the effects of apholate in A. aegypti (Rai, 1964) and D. melanogaster (Cantwell & Henneberry, 1963).

Venugopalan and Nair (1995) studied the testes of fifth and sixth instar larvae of S. mauritia which were implanted into female larvae of the same age group. Testes were reclaimed in day 1 pupa and the effects on testicular development and spermatogenesis were studied. Reclaimed testes showed an increase in volume eupyrene and apyrene spermatogenesis. However, the number of different types of spermatocysts was considerably less when compared to testes kept as control. Regulatory factors involved in lepidopteran spermatogenesis is promoted by two blood borne factors: eupyrene spermatogenesis inducing macromolecular factor (Kiss & Williams, 1976) and
apyrene spermatogenesis inducing factor (Jans et al., 1984). In many insect species the role of ecdysteroids in promoting spermatogenesis has been well documented in vivo (Takeuchi, 1969 and Dumser & Davey, 1975) and in vitro (Schmidt & Williams, 1953; Kambysellis & Williams, 1972 and Fukushima & Yagi, 1975).

Ramanathan et al. (1997) reported the biological activity of the leaf extract of *Pongamia glabra* on male accessory reproductive gland (MARG) of *Periplaneta americana*. The LD-50 value was 0.05 ml of 5% concentration of leaf extract of *P. glabra* for adult male *P. americana*. The insects were injected with 0.05 ml of 5% concentration of leaf extract whereas the control insects were injected with same quantity of distilled water. In the leaf extract treated insects, the mushroom shaped gland showed disintegration of the epithelial layer and the lumen consist of transparent secretary substances with many vacuoles and highly apycnthetic nucleus. The lumen had fewer amounts of secretory substances.

Rao et al. (1993) studied the effect of neem (*A. indica*) on *S. litura*, which reveals that Hexane and Methanol extracts of neem seed kernel proved to be larval repellents and oviposition deterrents. A single dose of azadirachtin (1 μg/g body weight) on sixth instars larvae affected food consumption and utilization, midgut enzymes, haemolymph constituents, secretions of the median neurosecretory cells of the brain, corpora allata size, and ecdysteroid litters (Toja et al., 1985). It reduced nucleic acid and protein concentrations of larval fat body, reduced pupal weight and disrupted ovarian development. Histological studies on ovary development revealed that azadirachtin drastically affected the follicular epithelium, greater vacuolization and sparse yolk granules in the ooplasm of treated insects (Gupta, 1988). Histological disturbances in developing ovary of *Epilachna varivestis* was reported by Schulz (1981) and Schluter (1984) and on maturation of oocytes of lepidopteran by Englemann (1971).
**TOXICOLOGY**

Ayyanagar and Rao (1989) worked with the methanol and hexane extracts of neem seed kernel, which were tested for their repellent and ovipositional deterrent protein against the larvae and adults, respectively, of *Spodoptera litura*. Methanol extract was superior to hexane extract as it elicited greater repellency to different larval stages. Both methanol and hexane extracts also exhibited ovipositional deterrenncy, the former being more deterrent at a lower concentration.

Subramanyam and Rao (1986) observed the effect of azadirachtin (2 μg/g body weight) on the fresh weight of body and ovary, haemolymph proteins, amino acids and activity of the median neurosecretory cells of the desert locust, *S. gregaria* up to 12 days after treatment. Though there was a marginal increase in body weight, ovarian development was completely inhibited. Haemolymph protein decreased both quantitatively and qualitatively. It delayed the synthesis and release of neurosecretion from the A-type median neurosecretory cells of brain thereby affecting the ovarian development. *Agrotis ipsilon* larval feeding was determined by changes in the nutrient content in the carbohydrate digestive enzymes in the haemolymph at a concentration of 5% (Bell *et al.*, 1990; Shapiro *et al.*, 1994). Whereas feeding of larvae from on petroleum extracts from *Ammi majus* and *Apium graveolens* and *Melia azadarach* and *Vinca rosea* resulted in considerable reduction in the total nutrient content and biochemical changes as demonstrated by Qadri and Narsaiah (1978).

Lim and Lee (1982) found out that diflubenzuron retarded the ovarian development *Oxya japonica* causing an increase in the percentage of terminal oocyte resorption. The fecundity of the female and egg hatchability were significantly reduced, in addition to treated female showing characteristic tendency for the hind legs to break off the body. Ingestion of the compound decreased the life span of the adult female. Nath *et al.* (1997) reported that
feeding of mulberry leaves treated with lethal and sub-lethal doses of fenitrothion and ethion by the silkworm larvae resulted in reduced growth. Single cocoon weight, single shell weight, silk index and filament length in comparison to control. The reduction was more during lethal intoxication. Ethion seemed to be more effective than fenitrothion. Satyanarayana and Sukumar (1991) investigated embryonic effect of radiolabelled diflubenzuron which was maximum in the 2nd batch of eggs, while the subsequent batches of eggs exhibited a steady decline and found penfluron 1.5 times more potent than diflubenzuron against D. cingulatus.

Agrawal (1991 & 1993) observed insecticidal and antifeedant activity of four plant extracts namely thionemone (Azadirachta indica A. Juss seed extract), heartwood extract (A. indica), Sweietenia macrophylla seed and Calophyllum sp. seed. Application at three different concentrations on 3rd and 5th instar of D. koenegii and found that all the treatments were significantly superior to control. The oral feeding treatments were found to be insignificant in respect to the nymphal mortality as some of the extracts showed antifeedant property. Agarwal also reported that three different concentrations of these extracts were very effective as ovicidal against early stages of the eggs.

Thomas and Hiradhar (1993a, 1993b & 1994) evaluated the influence of ethanolic extract of neem seed kernel and neem leaf (A. indica) on the reproductive potential of D. cingulatus and reported them as oviposition suppressors. They also made an attempt to evaluate the impact of Annona reticulata (seeds) and Ipomoea fistulosa (leaves) on the reproductive potential of D. cingulatus and observed that Annona seeds extract interfered with the reproductive performance as it is reported to have growth regulatory properties in insects. The adults treated with Annona seeds exhibited greater fecundity reduction and sterilization but Ipomoea leaf extract did not exhibit similar degree of effects. They also studied the morphogenetic effects at the nymphal (5th instar)-adult transformation by tropical application in D. cingulatus and recorded
varying degree of morphological observations mediated by these extracts in terms of insectostatic activity, moulting defects and viability.

Topical application of different plant extracts on ovicidal activity of *S. litura* and *D. koenegii* was studied by Suryakala & Rao (1995) which resulted in complete inhibition of hatching of eggs thus acting as good ovicides. Wilps *et al.* (1993) reported the effects of various neem products on nymphs of *Schistocerca gregaria* tested in the field trials in the Southern and Western Sahara desert. The neem product application differed with regard to both formulation and concentration of ingredients. All products were tested either on locusts that hatched from eggs of laboratory cultures or nymphs caught in the field. The products supplied by Trifelia caused 100 percent mortality in both test populations at the rate of 1 Lit./hac. compared with mortality rates of 60-70 percent for the products from Giessen @ 10 Lit./hac. This difference could be due to varying contents of active constituents in the neem seeds. The laboratory reared nymphs, proved to be more sensitive and exhibited higher rates of morphogenetic defects after application of sub-lethal dosages which differ with hypothesis given by Nicol and Schmutterer (1991) regarding abiotic conditions. The duration of nymphal stage and phase shifts are greatly influenced with the relation between the concentrations of ecdysteroids and juvenile hormones being directly or indirectly affected by azadirachtin and its analogous as postulated in good agreement with Mordue *et al.* (1986) and Rembold *et al.* (1987).

Bhathal and Singh (1993) reported that the commercial products and neem oil proved highly toxic to third and fourth instar (presumptive alate) nymphs. On the other hand AZT-VR-K was more toxic to fourth-instar nymph while in third instar treated nymphs considerably delayed mortality and abnormality in adult's form were observed. The extent of adultoid formation was 26.7 to 50 percent when treated with doses of 0.5 to 5 μg / nymph. The reproductive period, fecundity and longevity of adultoids were also greatly
reduced as compared to control. Likewise rapturous adult treated with AZT-VR-K exhibited reduced rate of reproduction and longevity (Chiu & Zeng, 1986). Schauer (1984) reported the toxic effects of neem seed extracts against *Acyrthosiphon pisium* and *Aphis fabae*. The crude extract (1.5%) of neem provided 86.7% control of *L. erysimi* after 72 hours of treatment (Pandey *et al.*, 1987).

Subramanyam and Rao (1993) investigated the aromatic oils and terpene fractions of plant origin and evaluated against growth regulatory activity of desert locust (*Schistocerca gregaria*), castor semilooper, (*Achacea janata*) and Bihar hairy caterpillar, *Spilosoma obliqua*. Terpene fraction of the mint oil (*Menta arvensis*) has the highest biological activity causing deformation at the nymphal-adult moult. The result was similar to Artemesia oil and Himalayan cedar wood oil, though to a lesser extent. Terpene fraction of mint oil is active on *A. janata* causing deformities at larval-pupal moult (Saxena and Srivastava, 1972; Banerjee *et al.*, 1971). The plants yielded insect morphogenetic hormones viz., juvenoids and ecdysone disrupt growth by antagonizing juvenile hormone action (Slama & Williams, 1966).

Dorn *et al.* (1986) recorded the effect of azadirachtin, injected into newly moulted last instar larvae of *Oncopeltus faciatus*, inducing a variety of effects, which are also dose-dependent. Low dose of azadirachtin prolongs the intermoult stage, apparently due to a delayed ecdysteroid peak. Medium doses however prevent ecdysis, apolysis and secretion of adult cuticle. The ecdysteroid peak is further delayed in the larvae and is somewhat lower than in control. Sieber and Rambold, (1983); Shalam and Pener, (1984) reported the reduction in ecdysteroid peak or interference with releases of eclosion hormone as in *Locusta migratoria*. Permanent larvae induced by high azadirachtin doses show maturing cycle in which hormone can be extracted (Kelly and Hunt, 1982). The last larval ecdysteroid peak sets a clock for activation of the corpora allata i.e. its
gonadotropic function regardless of whether the adult moult takes place or not (Sharma et al., 1980).

Ali-Niazee et al. (1997) observed that neem insecticide Margosa-o, caused 100 percent mortality of first instar larvae of *Archips rosanus* at 1% and higher concentrations within 48 hours after feeding on treated diet. At higher concentration it caused 100% mortality of larvae by 0.001% diet within 24 hours (Zehnder and Warthen, 1988). Larew (1988) and Blommers (1994) observed larvae exposed to higher concentration of micro-deterrence activity with regulatory effects. In addition to azadirachtin, other related compounds in the seeds may be insecticidal or deterrent to insect feeding (Schmutterer, 1990). Neem seed extract have been tested against a number of insect (Ladd et al., 1978; Larew et al., 1985; Saxena & Khan, 1985; Prabhaker et al., 1986; Jilani et al., 1988; Larew, 1988; Zehnder & Warthen, 1988; Stark et al., 1990 and Naumann et al., 1994).

The insecticidal control of diamond black moth has become difficult due to development of resistance against most categories of insecticide (Cheng, 1981; Hama, 1987; Doichuanngam and Thornhill, 1989; Motoyama et al., 1992) and to microbial insecticides based on *Bacillus thuringiensis* (Tabashnik et al., 1992). McMillian et al. (1969) reported that the leaf extract affects feeding behaviour, developmental rates and causes mortality in the larval stages of *Heliothis zea* and *Spodoptera frugiperda*. Oroumechi and Lorra (1993) found that an aqueous leaf extract applied to alfalfa leaves in the laboratory caused high mortality and exhibited strong growth-disturbing effects on larval stages of *Hypera positica*. The antifeedant activity has also been demonstrated in insects resulting from neem extracts (Isman et al., 1990). Azadirachtin applying to sand or soil may also reduce adult emergence and longevity in three tephritid fruit flies, *C. capitata*, *Dacus dorsalis* and *D. cucurbitae*, and the leaf minor, *Liriomyza trifolii* (Parkman and Pienkowski, 1990; Stark et al., 1990). Tanzubil and McCaffery (1990) found reduced fecundity when the larvae of *Spodoptera exempta* were
topically treated with 0.01 and 0.1 μg azadirachtin. Chen et al. (1996) studied the effects of Chinaberry extracts on larval mortality, feeding inhibition, and reproduction of the *Plutella xylostella*, storage condition is however entirely non-toxic (Oelrichs et al., 1983). Dorn (1986) recorded similar effect of neem on DBH control in Chinaberry extract.

The physico-chemical characteristics, shelf life and relative efficiency of 3 neem formulations were compared (Parmar et al., 1986). A water-dispersible powder (25% w/w) and a dust preparation (10% w/w) both based on crushed neem seed kernels and an emulsifier concentration (25% w/w) based on neem seed oil, were evaluated in laboratory bioassays at 28°C and 75% RH with 2nd to 5th instar larvae of the arctiid, *S. obliqua*. It was concluded that neem formulations gave a level of control which was comparable to that of cypermethrin (0.0075%), flucythrinate (0.0075%), fenvalerate (0.01%), guinalphos (0.05%), malathion (0.05%), neem oil-synergized malathion (oil 1%, malathion 0.05%) and endosulfan (0.07%). Agarwal and Mall (1988) reported insecticidal activity of extract of *Calophyllum inophyllum* seeds, fraction C and thionemone of neem (*A. indica*) seeds under laboratory conditions on 3rd instar larvae of *D. obliqua* at concentration levels of 1, 2.5 and 5% by contact and oral feeding methods. There was a positive correlation between applied concentration and mortality. Contact methods gave more promising results (mortality rate was from 33.33% to 100%) in comparison to oral feeding (mortality rate 2.66 to 80%). Certain plants have been reported to possess insecticidal properties (Morallo-Rezesus and Eroles, 1978; Deb-Kiranya et al., 1980a, b. Mohamed and Fred 1984; Arnason et al., 1985; Das, 1986). Antifeedant property of different plants have been reported (Butterworth and Morgan, 1968, 1971; Meisner et al., 1981; Abivardi and Benz, 1984; Tripathi and Rizvi, 1985). Neem seed kernel extracts act as an antifeedant for many insect species (Ladd et al., 1978).

Sharma et al. (1980) reported that NN 18-701 C fraction of neem seed is without antifeedant activity but another fraction NN 18-704 was slightly
antifeedant against three insect (Epilachna varivestis; *Ephestia kuebniella* and *Apis mellifera*. Azadirachtin, a neem seed extract in pure form showed no feeding inhibition on the above mentioned 3 insect species (Rembold *et al.*, 1982).

Tanzubil and McCaffery (1990) have reported that the treatment of larvae of the *Spodoptera exempta* with azadirachtin and aqueous neem seed extracts produced a range of adverse effects that were dose dependent. High dose of upto 10 |µ|g per larva of azadirachtin resulted in 100% larval mortality, but this effect was delayed and prolonged. At lower doses of azadirachtin, however, inhibition and disruption of moulting was observed and larval-pupal intermediates or abnormal pupae were commonly found. Similar, results were obtained with the aqueous extracts of neem seeds. The few pupae obtained from larvae treated with lower doses of the extract (0.01 and 0.1 |µ|g/larva) either failed to develop further or developed into adults that died during eclosion, or had frizzled and curled wings. Seeds and leave of the neem tree are known to contain many compounds that possess biological activity against insects (Butterworth & Morgan, 1968; Zanno *et al.*, 1975 and Kraus *et al.*, 1981). The antifeedant properties of azadirachtin and other neem derivatives have been extensively studied in many insects (Butterworth and Morgan, 1968; Haskell & Mordueluntz, 1969 and Schmutterer & Ascher, 1984). Neem treatments decreased the efficiency with which larvae converted ingested and digested food into body matter (Tanzubil, 1989), possibly because of a diversion of energy by such larvae from production of biomass. Similar, causes of a reduction in growth without feeding inhibition have been reported by other workers (Sieber & Rembold, 1983; Gaaboub & Hayes, 1984).

**HISTOCHEMISTRY**

Aggarwal (1962) studied the polytrophic ovary of the silkworm *Bombyx mori* and found three types of lipid bodies namely L1, L2 & L3 present in which L1
bodies were in the form of granules consisting of pure phospholipids, L₂ bodies in the form of vesicles and crescents of phospholipid sheath and triglyceride core. The L₃ bodies in the form of spheres made of triglycerides. The lipids in the oocytes are contributed by the trophocytes and follicular epithelium contributes the lipids in the oocytes. The lipids in the trophocytes are present as L₁ and L₂ bodies mainly in three concentrations at the periphery. The L₃ bodies are central in distribution, while L₁ and L₂ are cortical. The mitochondria in the form of granules are filaments having lipoproteins and RNA. King (1960) has also described ‘streams’ of mitochondria passing from the trophocytes into the ooplasm. In Drosophila, Hsu (1952) describes a similar contribution of trophocytes towards the lipid production in ooplasm.

Beament (1946) and Das-Gupta & Ray (1955) also studied the chorion and complex series of proteinaceous layers and lipoproteins in Rhodnius prolixus and Cimex. Ascorbic acid granules are also associated with vitamin in Golgi apparatus, which is important for vitellogenesis, oocyte formation and further, it prevents oxidation of dictyosomes as reported by Moussa & Banhawy (1960).

The silk gland enzymes could possibly involve to life span the finished protein (Bradfield, 1951) in which the glycogen is distributed throughout the fully mature oocyte contributed by trophocyte (Kugler, 1956). In the simple polytrophic ovary of Anisolabis and Labidura (Bonhag, 1956; Nath et al., 1959), the lipid and protein yolk along with the nucleoproteins are directly contributed by trophocyte to the oocyte through the pores in the intra-follicular septum. The distribution of DNA and RNA in the silkworm ovary is similar to that described by Colombo (1956). As pointed out by Nath et al. (1959) there is sufficient proof of the RNA being contributed by the trophocytes to the oocytes in polytrophic ovaries. Morgenthaler (1952), Bier (1954) and Colombo (1956) have also advocated this but the exact form, in which it may be contributed, varies.
King (1960) who worked on *Drosophila* is of the opinion that the RNA which initially accumulates in the ooplasm arises from the trophocyte nuclei, and according to him the subsequent build-up of the ooplasmic RNA is independent of the DNA of the trophocytes and the oocyte. The formation of spermatophore is well known amongst certain groups of insects (Davey, 1959 and Cantacuzene, 1967). Cantacuzene (1967) opined that secretion of these glands is a mixture of protein and mucopolysaccharide. Davey (1959) reported absence of lipid in both the glands and spermatophore, but demonstrated polysaccharide in both. Anderson (1950) showed in the Japanese beetle, that the accessory glands secrete a mucous like substance which is a protein-polysaccharide compound containing droplets of lipoidal substances. The cytochemical analysis of seminal vesicle is reactive to protein and nucleic acid in proportion to DNA synthesis as opined by Chefurka (1965). Banerjee and Raychaudhuri (1972) have studied the cytochemistry of gland and demonstrated the DNA and RNA structure of hyaline gland. Thus, the hyaline glands are perhaps most actively concerned with spermatophore production. Seminal fluid is also rich in carbohydrate, protein and lipid.

Subramaniam *et al.* (1990) have studied the testes of *Gryilloides sigillatus* and reported that it contains spheroidal translucent basophilic secretory granules, ranging in size from 3 to 15 μm in diameter. Histochemical characterizations of these granules reveal that they contain proteins, acid mucopolysaccharides and glycogen. The secretory granules in the testes of G. *sigillatus* fixed in Carnoy's fluid and 10% neutral buffered formalin appear distinct, although Lusis *et al.* (1970) reported that the secretary granules have not been observed in Carnoy fixed tissues. These secretory materials contain spermatids and spermatozoa in a role of sperm maturation and maintenance (Gillott, 1988).

Tiwari (1989) observed the effect of diflubenzuron on the 5th instar larvae of *D. obliqua*. The topical application of diflubenzuron gave LD-50 value 0.0307
μg e.i. per larva and also produced larval-pupal intermediates. There was reduction in the total haemolymph protein and a few new proteins were produced due to diflubenzuron intoxication (Wyatt et al., 1956; Chen & Levenbook, 1966). Kulkarni & Mehrotra (1973) observed an initial decrease and late stage increase in HP level in *Schistocerca gregaria* due to sumithion and dieldrin treatment.

Mall and Pal (1980) reported twenty free amino acids in the haemolymph of fully-grown fifth instar, normal fed larvae of insect. Aspartic acid, glutamic acid, histidine and ornithine were predominant. All amino acids except aspartagine were also present in the haemolymph of starved larvae but with reduced concentration. Sixteen amino acids have been identified as the principal and almost permanent constituents of the amino acid pool of the insect haemolymph (Florkin & Jeuniaux, 1974). Histidine, lysine, glutamine and serine were the predominant amino acid in the haemolymph of *Bombyx mori* Linn (Wyatt et al., 1956), in most of the lepidopteran larvae as reported by Duchateau & Florkin (1958) and in the haemolymph of *Calpodes ethlin* larvae (Barett & Lai-Fook, 1976). Almost all insects have high concentration of glutamine and protein and of one or more of basic amino acids, arginine, lysine and histidine (Wyatt, 1961).

Pratt (1950) identified 21 free amino acid in the haemolymph of larvae of *Prodenia eridania* and *Galleria mellonella*. Nineteen amino acids have been recorded in larval haemolymph of *Attacus ricini* (Pant & Agarwal, 1963). Bactor & Salem (1973) detected 19 free amino acids in the haemolymph of larvae of *Spodoptera littoralis* Boisd. They also recorded that only three amino acids occurred in higher concentration in all instars of *S. littoralis*.

Gupta et al. (1960a) studied the spermatogenesis of *Chrotonus* and revealed some interesting morphological characteristics of the mitochondria in the spermatocytes which do not seem to have been recorded earlier. The results of the various histochemical tests, revealed that in the spermatogonia and the earliest spermatocytes the mitochondria are present in the form of a juxta-nuclear mass of ill defined granules and filaments. During the leptotene and
zygotene stage the mitochondrial filaments get shortened and thickened, thus appearing as rods. However, in the late diplotene and early diakinesis stages a number of these laminated mitochondria always appear closely applied to the nuclear membrane as nucleo-mitochondrial interactions (Barer & Jaseph, 1957). Such laminated mitochondria have also been observed in red cotton bug, *Dysdercus cingulatus* (Gupta et al., 1960b). The dictyosomes and the acroblast possess a typical duplex nature having a phospholipid-protein sheath enclosing a lipoprotein medulla. This is in conformity with the observation of Clayton et al. (1958) on *Acheta domesticus*. The minute acrosome reacts histochemically like a neutral polysaccharide protein complex as usual (Schrader & Leuchtenberger, 1951; Moriber, 1956; Clayton et al., 1958).

Mitlin & Cohen (1961) studied the composition of ribonucleic acid in the developing housefly ovary and showed two-and-a-half fold increase from eclosion to 96 hours after emergence. Berry et al. (1967) reported that regulation of nucleic acid metabolism might depend more on the position of the oocyte and associated cells than on the hormonal control. Burr and Hunter (1969) in *Drosophila melanogaster* found that difference in the RNA content between male and female insects are related to egg production. In saturniid moths, the rate of RNA synthesis differed not only between tissues of one developmental stage, but also within a given tissue at different developmental stages (Berry et al., 1967). Pemrick and Butz (1970) stated that the pattern of fat body RNA synthesis for unmated and mated females of *Tenebrio molitor* followed the cyclic pattern of protein synthesis and ovarian development (Mordue, 1965). Chinzei and Toja (1972) determined the levels of nucleic acids in the whole body and several organs of silkworm, *B. mori* and found that during the phorate adult development the ovary gained DNA and RNA. This is interpreted as reflecting active synthesis of protein. The RNA / DNA ratio has extremely high values in the eggs, indicating the greater of synthetic activity that takes place during embryogenesis (Ring, 1973). Davenport (1975) assumed that there is no
transport of newly synthesized RNA out of the follicle cells into the oocytes. The synthesis of ribonucleic acid is one of the prominent features of the nurse cells in polytrophic, meroistic ovarioles (Bier, 1970 and Telfer, 1975). Much of the product is destined for storage in the developing oocyte and support of synthetic processes in the embryo (Muhach & Schwalm, 1977). Bownes & Blair (1986) stated that the nutritional state of the females clearly affects the amount of yolk protein RNA present in fat bodies as well as ovaries.

Nettles & Betz (1965) observed the glycogen content of the boll weevil with respect to age and diet and showed that the glycogen concentration was highest in the eggs and also in the boll fed than in square fed weevils. The changes in the glycogen level are related to several physiological events in reproductive cycle (Wiense and Gilbert, 1967). Norden and Paterson (1969) estimated the glycogen content of blowflies and tsetse flies and showed considerable amounts of glycogen in the former. D’costa and Rice (1973) and D’costa et al. (1973) observed approximately 16-20% glycogen in the tsetse fly and blow-flies are known to rely mainly on carbohydrates as a metabolic fuel (Sacktor, 1965). Blood trehalose and glycogen reserves have been shown to be present in the blowfly (Clegg and Evans, 1961 and Chidress et al., 1970). Hoglund (1976) reported that the glycogen is the sole neutral polysaccharide. In Calliphora its principal use is for the formation of chitin, only minor amounts being used as an energy source (Crompton and Birt, 1967 and Stafford, 1973).

Nagabhushanam et al. (1983) have reported the changes in lipids, glycogen and proteins in Hyalomma anatolicum anatolicum during the reproductive cycle. These workers found a definite correlation between these chemical constituents and various reproductive stages of the animal.