REVIEW OF LITERATURE
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Review of available information relevant to such aspects as feeding, growth and development and control measures with neem in relation to insects control is provided to enable a proper understanding as well as meaningful discussion of these aspects.

In view of the ill effects of the present day organic synthetic pesticides in the long run, including pest resurgence, pest resistance, pesticide residues in foods, environmental pollution, high costs and hazards involved in their usage, safer alternatives of crop protection, such as use of natural plant products as insecticides is gaining importance. The botanical pesticides are easily biodegradable and offer immense scope in integrated pest management (Jacobson, 1986).

Among the plants, Neem (*Azadirachta indica*) has a great potential to save the environment in the tropics, particularly in India. This tree has answers for several environmental problems such as the rehabilitation of degraded ecosystem and wastelands, reduction in the use of agro-chemicals such as fertilizers and pesticides. Use of eco-friendly neem products for plant protection, particularly fruit and vegetable crops, can avert a major environmental crisis, by avoiding pesticidal residues in the food chain (Saxena, 1993).

Neem is bitter in taste. The bitterness is due to the presence of an array of complex compounds called "triterpenes" or more specifically "limonoids". The neem compounds act together on several different behavioural and physiological processes. Their effects include repellency, feeding deterrence, reduced ingestion and digestion of food, poor growth and development, reduced longevity and
fecundity, oligospermia, mating disruption, oviposition deterrence, inhibition of egg hatchability, aposymbiosis, molting failures and direct toxicity (Saxena, 1993). The most important bioactive principle is azadirachtin (AZA). At least 10 other neem limonoids also possess insect growth regulating activity (Saxena, 1989; Schmutterer 1990; Mordue and Blackwell, 1993).

Feeding behaviour is of enormous practical concern in insect nutrition because many species feed poorly or not at all in the absence of specific external stimuli which have no direct relation to the nutritional value of potential food. Dadd (1985) in his comprehensive review of the regulatory mechanisms, suggested that several factors like molting, ovarian development, and temperature besides feeding and deprivation influence the feeding behaviour. Further, Strong (1975) showed that allatectomy in young males of *Locusta migratoria* did not alter the total amount of food consumed and the total quantity of faeces produced, but interestingly, it was observed that the quantity of fresh food eaten was less than that of dry food when compared to the control. Loueaux (1978) has furnished similar information about the feeding mechanism of *L. migratoria*. Studies on the food and feeding habits of grasshoppers and locusts in India were made by Hussian et al. (1949), Muralirangan and Ananthakrishnan (1981).

The antifeedant property of neem kernel was first described by Pradhan et al. (1962) for desert locust, *Schistocerca gregaria* Forsk. Subsequently the interest in neem as a pest control agent has been reviewed all over the world. Ketkar (1976) listed 95 publications on insect repellent and antifeedant effects of neem derivatives. The antifeeding effects of neem leaf powder, neem oil and neem seed kernel extracts were investigated by Abdul Kareem (1980), Sombatsiri and Tigvattanont (1984), Sombatsiri and Temboonkeat (1986) and Murugan et al. (1993) against many species of insects.
The crude alcoholic (ethanolic, methanolic) extracts of dried neem leaves, repelled females of *Crocidolomia binotalis* from treated cabbage leaves. This was a pure olfactory effect (Fagoonee, 1981). In *Helicoverpa armigera*, the volatiles of neem seed kernels and their aqueous distillates offered at a distance prevented contact and repelled the moths.

Azadirachtin (AZA) (C_{35}H_{14}O_{16}) is a highly oxidized limonoid with many reactive functional groups in close proximity to each other, its biosynthesis is thought to involve tirucallol, a tetracyclic triterpenoid, and a series of oxidation and rearrangement reactions which produce finally, amongst others, the tetrnor-triterpenoids, salannin, nimbin and AZA (Ley *et al.*, 1993). AZA is a complex and highly oxygenated compound belonging to tetrnortriterpenoid class and is the most potent antifeedant and growth disruptant to many insects. AZA, the main active principle, was identified in 1968 by Butterworth and Morgan (1968), using *S. gregaria*, the desert locust, as test insect. This voracious insect was repelled from feeding by very low concentrations of AZA (Butterworth and Morgan, 1971). This antifeedant activity has been demonstrated in other insect species (Warthen, 1979; Jacobson, 1986; Isman, 1993).

A large amount of data is available detailing both feeding deterrency and the growth disruptive properties of AZA and neem formulations to numerous species and stages of insects of many orders (Schmutterer *et al.*, 1981; Schmutterer and Ascher, 1984; 1987; Jacobson, 1986; 1988; Schmutterer, 1988; 1990; Arnason *et al.*, 1989; Warthen, 1989; Ascher, 1993; Mordue and Blackwell, 1993).
Laboratory trials have been replicated in the field (Kaethner, 1992), with AZA and neem extracts often showing superiority to certain commercially available pesticides (Kirsch and Schmutterer, 1988) and there have been promising results with some groups of pests which are developing resistance rapidly against conventional pesticides, including Diabrotica spp. beetles (Hummel, 1989).

Within the acridids, there are marked susceptibility differences between Old World species and New World species, the latter often being far less sensitive to AZA than the former (Mulkern and Mongolkiti, 1975; Adler and Uebel, 1984). On systemically treated plants, high concentrations of AZA (100 ppm) are required to produce primary antifeedant effects on aphids (Griffiths et al., 1978; Nisbet et al., 1993).

Meisner et al. (1981) reported on the feeding responses of S. litura and Earias insulana larvae, both of which are much more inhibited by AZA than salannin. Kubo and Klocke (1982) found feeding inhibition by AZA in the four cotton pests Heliothis zea, Heliothis virescens, Spodoptera frugiperda and Pectinophora gossypiella at much lower doses than needed for growth inhibition. Because of its systemic action, the compound can also protect the plant, after it has been taken up by the root, as effectively as after topical application on the leaves. Nasiruddin and Mordue (1994) studied the antifeedancy and growth regulative activities of AZA and its analogues against S. gregaria.

Many investigations have been conducted on the antifeedant effects, growth inhibition and abnormal development in various insects caused by neem seed kernel extracts (NSKE) and AZA (Schmutterer et al., 1981; Schmutterer and Ascher, 1984; Jagannadh and Nair, 1992). Zebitz (1987) reported that NSKE
strongly inhibited pupal development in several species of mosquitoes. In addition, the larval period was rather strikingly prolonged when larvae were kept in contaminated water.

Treatment of insects or their food with AZA causes growth inhibition, malformation and mortality in a dose-dependent manner. Such phenomena have now been described throughout a wide variety of insect taxa including Lepidoptera (Arnason et al., 1985; Schluter et al., 1985; Barnby and Klocke, 1987; Koul et al., 1987; Isman et al., 1990; Koul and Isman, 1991), Orthoptera (Sieber and Rembold, 1983; Mordue (Luntz) et al., 1985; Rao and Subrahmanyam, 1986; Ascher et al., 1989; Champagne et al., 1989), Hemiptera (Redfern et al., 1981; Koul, 1984; Garcia and Rembold, 1984; Dorn et al., 1986), Coleoptera (Ladd et al., 1984; Schluter, 1985 a,b) and Hymenoptera (Rembold et al., 1982, 1984).

AZA interferes strongly with larval growth and development of all the insects that have been studied so far. The morphological effects, like growth retardation, moulting inhibition and induction of malformations, can also be induced by hormone application. Koul et al. (1987) studied the effect of AZA on ecdysone synthesis in vitro. Prothoracic glands were taken from 5th-instar, Bombyx mori larvae and were incubated in the presence or absence of the ecdysone releasing factor PTTH. They found no marked difference in prothoracic glands of controls and those activated with PTTH when exposed to AZA. These results clearly prove that the compound neither exerts a direct competitive effect on prothoracic-gland secretory activity nor blocks any PTTH receptors of the hormone gland in this insect.

Haemolymph is an important medium for carrying xenobiotics to their site(s) of action. The quantity of a compound that finally reaches and binds to a
target tissue or receptor, and not the quantity ingested or absorbed, determines the
degree of intoxication (Koul et al., 1994). The differences in AZA effects between
insect species may be due to more than one sites of action and dependent on time,
mode and stage of treatment (Koul et al., 1987; Koul and Isman, 1990). AZA is
more efficacious via the oral route than through contact with the integument for
many species of lepidopterans (Koul et al., 1994).

A detailed study of the AZA effects on the last-larval instar of *L. migratoria*
showed a typical dose dependence (Sieber and Rembold, 1983). The inter-
moult of AZA injected locust larvae varied between 8 and 60 days. Even more
dramatic effects are induced in *Rhodinus prolixus* after the substance is taken up
with a blood meal (Garcia and Rembold, 1984). There is a pronounced effect of
AZA on ecdysteroid titres, as first demonstrated in 5th instar *L. migratoria*, with
a shift or even complete disappearance of the moulting hormone titre (Sieber and
Rembold, 1983). Injection of ecdysone at physiological concentrations, decreased
the live-weight gain, and all the treated 5th-instar *L. migratoria* failed to moult
(Rao and Rembold, 1983). The AZA effect can be explained by interference of
the compound with the neuroendocrine system of the larvae. The neuroendocrine
system and especially the corpus cardiacum has been identified as a target organ
for AZA (Rembold et al., 1989).

Growth regulatory effect is the most important physiological effect of
neem on insects. It is because of this property that neem has emerged as a potent
source of insecticides. Koul (1985) in a detailed study on the effect of AZA on
the larvae of *S. litura* reported that first instar larvae when fed on castor leaves
treated with 0.5, 1, 2, and 4 ppm concentration of AZA caused 0, 16, 41, and
62% mortality, respectively within 7 days. Surviving larvae when transferred to
untreated food did not show much recovery. Older larvae when fed similarly on treated foliage, showed considerable reduction in mortality.

The growth regulating effects on insect nymphs and larvae by NSKE and AZA were observed for the first time (Leuschner, 1972), and was thought to result either from the action of an ecdysone analog or because AZA as an ecdysone-like compound might act on the hormonal balance of insects (Ruscoe, 1972). AZA was found to interfere with the secretion of the prothoracic gland or with the function of the prothoracic hormone excluded in B. mori (Koul et al., 1987). Several authors demonstrated the AZA influence on the hormonal control of moulting (Redfern et al., 1981; Sieber and Rembold, 1983; Subrahmanyam and Rao, 1986; Dorn et al., 1987; Rembold, 1989). L. migratoria nymphs treated with higher doses of AZA did not moult and showed only small ecdysteroid concentrations. This means that the ecdysteroid titres of the locusts were either altered or abolished by injected AZA, depending on the dose, and this was correlated with morphogenetic defects (Sieber and Rembold, 1983). Similar results were obtained after injection of AZA into last-instar nymphs of the large milkweed bug Oncopeltus fasciatus (Dorn et al., 1987).

IGR effects of AZA manifested as developmental aberrations in immature insects are caused by significant reduction and/or delay in moulting hormone titres of the haemolymph (Redfern et al., 1982; Sieber and Rembold, 1983; Schluter et al., 1985; Garcia et al., 1986; Dorn et al., 1986; Mordue (Luntz) et al., 1986; Barnby and Klocke, 1990). The effects of AZA are both dose and time dependent; prevent both apolysis and ecdysis; can cause death before the moult, during the moult, or delay of the moult, or induce permanent larvae. Injections of 20-hydroxyecdysone into AZA treated insects, however, will not restore AZA treated
insects to normal development (Pener et al., 1988; Barnby and Klocke, 1990), although partial recovery of moulting was seen in *H. virescens* after such treatment (Barnby and Klocke, 1990).

Malczewska et al. (1988) used chilled *Galleria mellonella* larvae to investigate the effects of AZA on juvenile hormone and ecdysteroid titres: chilled larvae undergo supernumerary moults, due to increase in juvenile hormone titre (Bogus and Cymborowski, 1981). AZA inhibits, in a dose-dependent manner, such supernumerary moults of last instar larvae possibly, by affecting the release of allatotropins into the corpora allata and hence blocking the synthesis and release of juvenile hormone. This block causes a rapid decrease in whole body juvenile hormone titres which is maintained for several days. In last instar *Manduca sexta* larvae, AZA injection results in the induction of supernumerary moults (Schluter et al., 1985; Beckage et al., 1988) presumably due to an inhibition and subsequent delay in juvenile hormone titres so that the presence of the hormone extends into the critical period for commitment to larval rather than pupal cuticle. In adult female *L. migratoria* also, AZA treatment causes a rapid decrease in juvenile hormone titres with associated disturbances in oogenesis (Rembold, 1984; Rembold et al., 1987).

Adult locusts treated with AZA become sluggish and show reduced locomotory and flight activity (Wilps et al., 1992). Such a reduced "tendency" to fly results in a significantly reduced elevations of blood lipids after flight activity compared with controls, which cannot be increased by previous injections of adipokinetiс hormone (AKH) (Wilps et al., 1992).

The direct effect of the AZA molecule on the neuroendocrine system of insects is well documented by hormone titre measurements, tracer studies and
autoradiography. AZA in minute quantities disturbs the homeostatic situation of an insect in an irreversible way. One interesting restitution has been demonstrated with the blood sucking bug *R. prolixus*. The drug if given through a blood meal, inhibits moulting of 50% of all treated nymphs at a concentration of $4 \times 10^{-4}$ and of 100% at 1 $\mu$g (ml blood). Some of these bugs although subsequently fed 5 times on AZA free blood, had not moulted for up to 5 months (Garcia and Rembold, 1984).

The females of some lepidopterous insects are repelled by neem products on treated plant parts or other substrates and will not lay eggs on them under laboratory conditions. This has been observed in the cabbage webworm, *C. binotalis*, the Afro-Asian cotton boll worm, *H. armigera* and the fall armyworm, *S. frugiperda*. The dipterous insect, *Lucilia sericata*, was also deterred from egg-laying as were some beetles (*Callosobruchus* spp.) (Schmutterer, 1990).

In the sheep blowfly, *L. sericata*, neem oil and the enriched, formulated neem seed kernel extract AZT-VR-K were powerful antiovipositional agents (Rice *et al.*, 1985). Neem oil in a concentration of 30 mg/10 g of green gram seeds was antiovipositional in the bruchid, *Callosobruchus maculatus*, and of 10 mg/kg in *C. analis* and *C. chinensis*.

The effects of AZA and a neem seed extract (NSE) on tephritid fruit flies and their parasitoids have shown that AZA is selective against fruit flies and prevents them from either emerging to the adult stage, or else reduces the survival of those adults which do emerge. Low doses of NSE, allow the hymenopteran parasitoids to emerge unharmed and able to mate and seek new fruit fly hosts, so enhancing the effectiveness of biological control (Stark *et al.*, 1990 a,b ; 1992).
The effect on neural centres of AZA treated locusts is indicated by changes in behaviour of the treated larvae. Larvae with extended duration of metamorphosis show sexual behaviour like adults (Shalom and Pener, 1984) and flight-muscle activity that resembles the flight motor pattern of young locusts (Kutsch, 1985). Inhibition of reproduction, either by the crude neem extracts or by AZA, has long been known (Steets and Schmutterer, 1975; Rembold and Sieber, 1981; Koul, 1984; Subrahmanyam and Rao, 1986). The main effect of AZA is to cause a change in the ecdysteroid titres (Redfem et al., 1981; Sieber and Rembold, 1983; Dorn et al., 1986; Garcia et al., 1986; Schluter et al., 1985; Mordue et al., 1986; Rembold, 1989).

*L. migratoria* over-aged nymphs do, however, achieve partial adult competence despite the lack of moulting and metamorphosis as demonstrated by male mating behaviour (Pener and Shalom, 1987) and the competence to respond to adipokinetic hormone (AKH) (Pener et al., 1989). It is known that AZA induced over-aged nymphs of *O. fasciatus, L. migratoria* and *S. gregaria* after a period of time show some corpus allatum activity and endogenous juvenile hormone activity (Dorn et al., 1986; Pener and Shalom, 1987). Certainly over-aged female nymphs of *L. migratoria* and *O. fasciatus* produce vitellogenin, which is juvenile hormone dependent process, and showed some egg development (Rembold, 1984; Dorn et al., 1987).

Adverse effects on ovarian development, fecundity and fertility (egg viability) have all been reported (Karnavar, 1987; Babu et al., 1995a). Interference with either the synthesis of vitellogenin or its uptake by oocytes is a linking factor between different insect species. In *L. migratoria* AZA inhibits both oogenesis and ovarian ecdysteroid synthesis, so preventing any oviposition.
Female *Spodoptera exempta* which emerged from larvae topically treated with 0.01 and 0.1 µg AZA, exhibited reduced fecundity but not fertility due to a failure of many oocytes to mature (Tanzubil and McCaffrey, 1990). Through measurements of both fat body and ovarian protein levels of *S. exempta*, it was concluded that AZA treatment caused a lower uptake of fat body proteins by the ovary in the treated insects. Strambi *et al.* (1994) studied that, AZA significantly inhibited the ecdysteroid titre in the haemolymph and correlated with the oocyte and fecundity of *Acheta domesticus*. *O. fasciatus* injected with 4 µg AZA also exhibited reduced fecundity (Dorn, 1986; Dorn *et al.*, 1987) and in adult female *R. prolixus*, ingestion of AZA in the blood meal was associated with reduced vitellogenin levels in both the haemolymph and ovaries with a dose-dependent reduction in oocyte growth. Radioimmuno assay of such AZA treated *R. prolixus* revealed lower ecdysteroid titres in both haemolymph and ovaries (Feder *et al.*., 1988). AZA treatment of larvae or pupae resulted in reduced oogenesis in the surviving adults of stored grain pest, *Trogoderma granarium* (Chellayan and Karnavar, 1990). Reduced longevity and fecundity in the fruit fly *Ceratitis capitata* (Stark *et al.*, 1990b) and the leaf miner *Liriomyza trifolii* (Parkman and Pienkowski, 1990) was also recorded after sand or soil drenches with AZA.

The corpus cardiacum has been suggested to be a target for AZA in terms of PTTH, eclosion hormone, bursicon or general tropic hormone release (Sieber and Rembold, 1983; Mordue (Luntz) *et al.* 1986; Bidmon *et al.*, 1987; Rembold *et al.*, 1989). Staining neurosecretory proteins with paraaldehyde fuchsin in rapidly maturing females of *L. migratoria* compared with similar aged AZA treated females revealed an accumulation of stainable material in the corpora cardiaca and neuropilar storage areas of the brain neurosecretory system in treated insects.
which was associated with a lack of ovarian development (Subrahmanyam et al., 1989). A similar condition occurs in starved insects (Highnam and Mordue (Luntz), 1974) although such AZA treated insects (3 μg/g) ate sufficient food to maintain their body weight. AZA blocks the release of neurosecretory material from the corpora cardiaca with a reduced turnover, seen as a subsequent accumulation of material, within the system. Such a feedback may also affect the rate of synthesis of PTTH by brain neuro secretory cells as has been suggested by Barnby and Klocke (1990) in *H. virescens*.

Injection of AZA in females of *L. m. migratorioides* 2-10 days after the last moult led to an inhibition of follicle growth, which was explained as a possible consequence of the interference of the active principle with vitellogenin synthesis and/or with incorporation into the oocytes either directly or indirectly through endocrine control. In untreated females, juvenile hormone III titre in the haemolymph increased about 8 days after the last molt and induced bio-synthesis of vitellogenin in the fat body and consequently oogenesis. The injection of AZA prevented juvenile hormone production and therefore also prevented vitellogenin synthesis and egg production (Rembold et al., 1984). Untreated females of *L. m. migratorioides* shows a maximum concentration of ecdysteroids in their ovaries towards the end of vitellogenesis. Treated females, on the other hand, show a drastic reduction of ovarian ecdysteroids if AZA is injected after the end of oogenesis on day 10 to day 13 from the last molt. AZA probably reduces the ecdysteroid titre by acting on the neuroendocrine system, which could also explain a loss in body weight and reduction of fecundity (Rembold, 1989). There might also be an involvement of allotropic hormone in the inhibitory activity of AZA.
In *Tenebrio molitor* pupae, the injection of 1 μg AZA induces a delayed and reduced ecdysteroid peak which inhibits the imaginal moult, despite this insect having no prothoracic glands at this stage (Marco *et al.*, 1990). Epidermal cells or oenocytes, as potential sites of ecdysteroid production, may well be the target for AZA action in this case. In adult female *L. migratoria* ecdysteroid synthesis by the ovaries, together with oogenesis, is inhibited by a dose of 10 μg AZA per insect (Rembold and Sieber, 1981).

The majority of investigations into the reproductive effects of AZA in insects have been concentrated on females. Reports involving changes in the male reproductive organs as a result of AZA are scarce and little literature is available. Effects, however, are also apparent in males as seen by over-aged nymphs of locusts which displayed mating behaviour but at a subnormal level (Pener and Shalom, 1987). In an *in vitro* study of spermiogenesis in diapausing *Mamestra brassicae* males, 3 ppm AZA caused spermatocysts to degenerate, even in the presence of 20-hydroxyecdysone, suggesting a direct effect on the testicular membrane, rendering the tissue incapable of developing spermatocysts (Shimizu, 1988).

Rao and Subrahmanyam (1986) studied the effect of AZA on nymphs and adults of desert locust, *S. gregaria* by injection method. General effects of AZA when injected into 5th instar nymphs were extension of nymphal period, reduction of feeding and body weight but no absolute feeding inhibition even at the highest dose.

Investigations (Schluter, 1985 a,b) of the histopathological effects following feeding of AZA to the penultimate larval instars of the Mexican bean beetle, *Epilachna varivestis*, revealed considerable disturbances in epidermal and fat body
tissues that eventually led to a cessation of development. In those studies emphasis was laid on the mode and course of cell death.

Effects on stored product pests include antifeedancy, oviposition, reduced egg hatch and emergence, and direct lethality (Morallo-Rejesus et al., 1990; Ivbijaro, 1990; Naqvi et al., 1990). Lowry and Isman (1994) showed the populations of predacious and parasitic insects were not adversely affected by sprays of neem seed oil or neem seed extract. Spiders and mites play important roles in the suppression of insect pests in certain agroecosystems and there are many examples of neem's lack of toxicity against them after treatment of the crop. These include *Chiracanthium mildei*, a useful predator of citrus fruit pests (Mansour et al., 1986), with one of its prey, *Tetranychus cinnabarinus* being highly susceptible to neem (Mansour and Ascher, 1983). Feng and Isman (1995) demonstrated the resistance mechanism developed by aphids treated with AZA and neem crude extracts.

Lack of feeding associated with a change in gut motility and physiology have been studied after treatment of AZA (Mordue (Luntz) et al., 1985; Timmins and Reynolds, 1992). Digestion in grasshoppers is said to take place mainly in the capacious crop by salivary enzymes ingested together with the food and secretions passed forward from the midgut (Uvarow, 1966; Wigglesworth, 1972). Midgut, the primary absorbing epithelial tissue in phytophagous insects is capable of multiple functions, including nutrient transport from lumen to haemocoel (Chino, 1985; Turunen, 1990), potassium transport from haemocoel to midgut lumen (Dow, 1986), and release of neuroendocrine-like amines and peptides (Nishiitsutsuji-Uwo, 1988; Sehnal and Zitnan, 1990).
The midgut cells do not produce cuticle, but in most species a delicate peritrophic membrane is formed as a lining of the midgut (Chapman, 1985). The cells of the midgut are concerned primarily with the production and secretion of digestive enzymes and with the absorption of the products of digestion. Both functions may be carried out by the same cells, and most cells in the midgut have the same basic structure. The midgut of *S. gregaria* has the "typical" arrangement of circular and longitudinal muscles, but has in addition an inner layer of small groups of longitudinal fibres embedded in connective tissue (Anderson and Cochrane, 1977). The midgut epithelium is a bidirectional sorting area in the insect, providing the enzymes for digestion in the lumen, plus supplying major proteins and amino acids to the haemolymph. The midgut cells responsible for the uptake, synthesis, and release of proteins and amino acids, are the columnar cells (Turunen, 1985).

The midgut is that part of the alimentary canal in which the cells secrete digestive enzymes and absorb nutrients as well as playing an important role in ion transport (Cioffi, 1979; Martoja and Ballan-Dufrancais, 1984). An extensive literature exists on the organisation of the midgut in a wide variety of insects, light and electron microscopy studies having established both histological and cytological features (Smith, 1968; Wigglesworth, 1972; Martoja and Ballan-Dufrancais, 1984; Dow, 1986; Billingsley, 1990). The anatomy, histology and cytology of the alimentary canal of locusts have been reported by Anderson and Cochrane (1977). Studies were undertaken in the midgut of the silkworm, *Bombyx mori* after the introduction of *Bacillus thuringiensis* crystal toxin (Endo and Nishiiitsu-suji-Uwo, 1980; Percy and Fast, 1983; Singh *et al.*, 1986). Histopathological effects of dietary tannin were observed and compared on the midgut epithelium of two swallowtail caterpillar pillars, *Papilio polyxenes* and *P. glaucus* (Steinly and Berenbaum, 1985).
Morphology of the head, wings and thorax have been extensively studied by various authors, but very little attention has been paid to the morphology and structure of reproductive organs especially histology, physiology and biochemical parameters. Uvarov (1966) has reviewed studies on the structure, development and maturation of gonads, studies on the morphology, histology of the reproductive system including spermatophore formation and insemination in *S. gregaria*.

In *Melanoplus sanguinipes* as speculated by Gillott and Friedel (1977) the transferred material forms a significant part of the egg production of the female; this explains the highly promiscuous behaviour of the males. Shepherd *et al.* (1981) reported an increase in the concentration of the free amino acids like alanine, gamma-amino butyric acid and serine during spermatophore formation.

Detailed knowledge on nature and origin of spermatophoric components in this groups of insect is lacking. Most of the secretions required for the formation of spermatophoric complex in insects are derived from a variety of male accessory reproductive glands (ARGs) which are often more conspicuous than the primary reproductive organs. Aspects of morphology, histology and biochemical significance of the male ARG's in insects have been reviewed by Leopold (1976), and Chen (1984).

The accessory reproductive glands of *Melanoplus sanguinipes* consists of a pair of long hyaline glands, 4 pairs of white glands, 10 pairs of short hyaline glands and 1 pair of seminal vesicles (Pickford *et al.*, 1969). In mature animals each gland produces a characteristic pattern of 20 or more electrophoretically separable proteins (Gillott and Venkatesh, 1985). Accessory gland secretions involved in the formation of the spermatophore, contribute to the seminal fluids and modify the female oviposition behaviour (Pickford *et al.*, 1969; Friedel and Gillott, 1976).
The secretions of male accessory glands constitute to the formation of the wall of the spermatophore, and also a part of the sperm liquid. During copulation the spermatophores are introduced into the spermatheca of the females and after mating, some male accessory gland proteins are detected unchanged in the haemolymph of the mated female and these results were based on immunological and radio-active tracer experiments in *M. sanguinipes* (Friedel and Gillot, 1977). The ARG's secretion serve a variety of function in different insect groups (Hinton, 1974; Leopold, 1976). Besides playing a role in spermatophore production, the glands serve other important functions such as the secretion of seminal fluid.

In *S. gregaria*, the male ARG consists of two large masses, each consisting of sixteen glands viz., long hyaline, short hyaline, seminal vesicle and white glands (Even and Pickford, 1975). Pickford *et al.* (1969) showed that egg laying stimulants were produced by the ARGs and these were differentiated into hyaline glands (2 pairs) one of which is considerably larger than the other and white glands (4 pairs). Pickford *et al.* (1969) observed that implantation of the short hyaline glands, into virgins effected the most pronounced increase in fecundity, though implantation of the white glands or spermatheca from mated females stimulated egg production to some degree. Odhiambo (1969) and Pickford *et al.* (1969) have studied in *L. migratoria manileis*, topical application of ARG extract reduced the preoviposition period from 72 to 81 days. Ultrastructure of the seminal vesicle and the isoelectric focussing pattern of its secretion during sexual maturation and after allatectomy has been described (Couch and Gillott, 1988). Blum *et al.* (1962) studied the presence of fructose, glucose and trehalose in the seminal vesicle, the same were carried out in haemolymph and testis also.
The influence of the endocrine system on the reproductive behaviour and development of grasshopper was reported by a number of workers (Hartmann et al., 1972; De Wilde and De Loof, 1973a, b; Gillott and Elliott, 1976). Friedel and Gillott (1976) experimentally proved that the accessory glands of *M. sanguinipes* contained an oviposition stimulant and the extract from mature males induced oviposition in 75 per cent of the virgin females. The work of Leopold (1976) was a mile-stone in the understanding of the role of male accessory glands in insect reproduction.

Many insects use a variety of prepackaging devices in sperm transference. The spermatophore is entirely the product of the male and in most cases it is produced at the time of copulation (Davey, 1985). Engelmann (1970) reported that mating has some important additional effects, including enhancement of fecundity and modification of female receptivity.

Biochemical processes underlying insect growth and development have been studied. It has been shown that glycogen and glucose whose functions are firmly established among almost all other animals play equally important role, in the organisation of metabolic activity of this largest class of Arthropods (Friedman, 1985).

Acid phosphatase is often used as a lysosomal marker both in vertebrate and invertebrate tissues. Previous studies on the activity of acid and alkaline phosphatases on a few species of insects reported a gradual increase in the two phosphatases during the larval stages, followed by an abrupt fall in the pupa (Sridhara and Bhat, 1963; Ludwig et al., 1962). Khoja (1991) studied the activity of ALP from the excretory system of the grasshopper, *Poekilocerus bufonius*. Since, alkaline phosphatase is a highly active and stable enzyme, it is intensively used in immunological assays as an enzyme label (Eruk et al., 1984; Kohn et al.,
Adenosine tri-phosphatases (ATPases) are a group of membrane bound enzymes that take part in the active transport of ions across cell membranes (Cott and Weiner, 1983). Cott and Weiner (1983) stated that Na\(^+\) - K\(^+\) ATPases is predominantly associated with plasma membrane and is concerned with maintaining the ionic balance of the whole cell. It is a lipoprotein plasma membrane component. Changes in membrane lipid content or physical properties of the membrane have been shown to influence Na\(^+\) - K\(^+\) ATPase activity (Fogg et al., 1991).

Lipid reserves are used as energy sources in different processes such as flight, egg development etc. (Sacktor, 1975; Downer, 1985). Triacylglycerol (TAG) forms the major part of the lipid content of insects during all the developmental stages (Chino and Gilbert, 1965). TAG was shown to be an important metabolic reserve in insects (Gilbert, 1967; Sacktor, 1970; 1976; Beenakkers et al., 1981; Downer, 1985) and it was found in the fatbody, ovaries and haemolymph (Downer, 1985). TAG is also the main source of metabolic energy in insects which undergo prolonged periods of metabolic activity without feeding during diapause, migratory flight and non-feeding developmental stages of embryogenesis and pupation (Downer, 1985). The advantages of storing TAG as metabolic reserve are: i) high caloric content, ii) the liberation of metabolic water, in higher quantity as compared to carbohydrate and iii) its capacity for storage in anhydrous form. The sterols are important for growth, development, reproduction, maintenance of the integrity of cell membranes, imaginal moult, as a precursor for ecdysteroid synthesis and other unknown physiological functions.

The insect haemolymph acts as a medium for the transport of a wide variety of proteins and other metabolites. An important feature of many haemo-
lymph proteins is that they are stage specific, appearing and disappearing on a specific time table often in response to hormonal signals (Riddiford and Law, 1983).

Insect ontogeny includes both embryonic and post embryonic development and the developing organism represents a dynamic system which changes continuously in its physiological and biochemical properties as morphogenesis proceeds (Chaubey and Bhatt, 1988). Nucleic acid, protein, carbohydrate, lipid and some hydrolytic enzyme concentrations have been studied at different times of development in several insect species (Painter and Kilgore, 1967; Chaubey and Bhatt, 1988; Shimizu, 1992). The widely distributed phosphatases in animal tissue are thought to be associated with the transport of metabolites, metabolism of phospholipids, phosphoproteins, nucleotides and carbohydrates and synthesis of proteins. Phosphatases are widely distributed and are found to exist in multiple forms in a large number of organisms (Sridhara and Bhatt, 1963; Eguchi, 1975; Fishman, 1990). A common developmental process of insects is the synthesis of storage proteins which are synthesised by the fat body released into the haemolymph during the last larval instar, and selectively taken up by the fat body during non-feeding stages (Wyatt and Pan, 1978; Levenbook, 1985). During metamorphosis, these storage proteins are used as an amino acid pool for protein synthesis.

Amino acid composition of the protein component of the accessory gland secretion of male insects and the available knowledge have been derived mostly from studies on the Diptera (Leopold, 1976; Chen, 1984). Chen and Oechslin (1976) demonstrated a progressive accumulation of amino acids during adult life of Drosophila melanogaster with glutamic acid having the highest concentration in the paragonia.
The role of the corpora cardiaca in controlling the release of stored lipids in the form of diacylglycerol is now well established (Candy, 1981; Goldsworthy, 1983). During flight, the glands release the peptide hormones and adipokinetic hormones into the haemolymph and these act on the fat body to stimulate diacylglycerol release. Phytochemicals are known to interfere with the growth and development of phyto-phagous insects, but the biochemical mechanisms of the toxicology are not well understood.

Reproductive strategies of insects are extraordinary discussed, reflecting the full range of complexities, various neural mechanisms and accessory structures and secretions that are involved in the location of receptive partners and the copulatory behaviour. The role of AZA in the regulation of growth and development has been extensively studied, but many of the features of the "fine tuning" of reproductive mechanisms in relation to biochemical changes during reproductive programming in insects particularly acridids are still poorly understood. Hence, an attempt has been made to investigate the effect of AZA on biochemical and histological changes of reproductive organs of male Atractomorpha crenulata.