Review of Literature
REVIEW OF LITERATURE

Insect growth regulators tend to be more compatible with the biological approaches which include entomopathogenic micro-organisms, parasites and predators. The insect growth regulators (IGRs) are metabolic disruptors, molt inhibitors and behaviors modifiers of insects. Since the target site of action for these chemicals are known and susceptible to disruption only in certain species at certain times during the life cycle, these compounds have fewer serious deleterious effects on non-target species (Retnakaran and Granett, 1985). IGRs may belong to selective insecticides and can be grouped according to their mode of action as: chitin synthesis inhibitors and substances that interfere with the action of insect hormone (i.e. JHa, ecdysteroids) (Tunaz and Uygun, 2004).

Diflubenzuron, a benzoylphenlyurea was the first chitin synthesis inhibitor to be introduced as a novel insecticides. It was found to be effective against Coleopterans and Dipterans (Goktay and Kismali, 1990). Besides Coleopterans and Dipterans, diflubenzuron has also been considered a potent compound against lepidopterous larvae of common cutworm, Spodoptera litura and Cydia pomonella (Miyamoto et al., 1993). The chitin synthesis inhibitor were quite effective against multi-resistant Musca domestica strains, except for one strain with strong resistance against chitin synthesis inhibitors, developed after extensive treatments with benzoyl-phenly-urea for several years (Pospischil et al., 1997). A decrease in egg hatching was observed in the lacewing Cryospa carnea (Stephens) and in the nymph survival of Gaucheries punctipes (Say) due
to diflubenzuron treatment (Apperson et al., 1978; Medina et al., 2002). The effects of diflubenzuron on terrestrial non-target organisms (NTOs) however, tend to be minimal compared to the effects of conventional insecticides.

Earlier, the important factor for toxicity against Chilo suppressalis (Oikawa et al., 1994), Leptinotarsa decemlineata (Nakagawa et al., 1999), and Spodoptera exigua (Smagghe et al., 2003) was the hydrophobic characters (logP) of IGRs and it was related to the structure of the insect cuticle that consists from the outside to the inside of a thin wax and cement layer, a dense epicuticle and a thick lamellated chitin-containing endocuticle. Farinos et al. (1999) confirmed the negative effects of RH-0345 on yolk protein accumulation and egg formation on the adult beetle of Leptinotarsa decemlineata. RH-0345 was considered a potent stimulator of the release of hormone into the culture medium by pupal integument explants and by ovaries of Tenebrio molitor (Soltani et al., 1998; 2002). It has also been shown that RH-0345 interferes with the reproductive events and was able to modify the composition of ecdysteroid amounts in T. molitor (Taibi et al., 2003). In the recent past, it was found that RH-0345 is able to partly reverse the depressive effects on the reproductive events induced by KK-42 in mealworm (Amrani et al., 2004). Chebira et al., (2006) conducted a topical bioassay with the pupae and adults of the mealworm, Tenebrio molitor (Coleoptera: Tenebrionidae) an important stored-product pest with three insect growth inhibitors (IGRs) viz., diflubenzuron, flucycoxuron and halofenozide. Two chitin synthesis inhibitors i.e., diflubenzuron and flucycoxuron and one ecdysone agonist halofenozide (RH-0345). The rate of absorption through the cuticle was highest for flucycoxuron and this concurred with its high toxicity and its
accumulation in the reproductive system of males and females was relatively high. It was also observed that the clearance/excretion of the IGRs takes place at different rate. Diflubenzuron and flucycloxuron were excreted to a similar extent in males and females whereas halofenozide showed low excretion between 2-6 days after topical treatment.

The order of rate of absorption: flucycloxuron > diflubenzuron > halofenozide concurs with a decrease in the hydrophobicity value (logP) of the IGRs. Studies also reported the ovicidal effects of halofenozide (RH-0345) and an anti-ecdysteroid KK-42 on the mealworm *Tenebrio molitor* (Berchighe et al., 2008).

Studies showed that diflubenzuron found to be toxic against the 5th instar larvae of *Galleria mellonella*. Diflubenzuron (DFB) affected the integument via feeding method, the larvae failed to ecdysis, cuticle ruptured, haemolymph lost and blackened. Reports also showed the inhibitory effects of DFB on the 6th and 7th instar of *Galleria mellonella* (Hegazy et al., 1980). The effect of diflubenzuron on the ultrastructure of cuticle, protein and chitin content of the Colorado beetle, *Leptinotarsa decemlineata* was also reported (Hegazy et al., 1989). In the later years, Lee et al., (1990) and Yin-Chang et al., (1990) worked out the mode of action of chitin synthesis inhibitor and stated that the endocuticle lamellae are added to the procuticle during the intermolt period in lepidopterous larvae. Histological evidence revealed that ecdysial failure and mortality were invariably related to the blocking of endocuticular formation and in higher doses to the development of extra layer and globular bodies between endocuticle and epidermis in *Tenebrio molitor* and *M. separate* larvae (Ren et al., 1988). The effect of DFB and OMS-2017 on the reproductive potential of 4th instar larvae of *Aedes aegyptii* was investigated,
diflubenzuron showed higher larval and pupal mortality than OMS-2017 which induced more delayed effects. At 0.001 mg/liter, DFB induced 17% larval and 21% pupal mortalities which is quite higher than 5% larval and pupal mortalities in case of OMS-2017, the females that survived from OMS-2017 laid 30 % less eggs whereas the fecundity of DFB treated females was not affected. The basal follicle number showed wide variability from the effects of both IGRs i.e., diflubenzuron and OMS-2017. Degenerating and non-matured follicles were more abundant in DFB treated females than in OMS-2017 treated ones (Fournet et al., 1993).

Karimzadeh et al., (2007) studied the effects of five CSIs viz., diflubenzuron, cyromazine, lufenuron, hexaflubenzuron and triflumuron on the second instar of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Crysomelidae). Among the five IGRs the most toxic was hexaflumuron (Lc50 0.79 mg ai/L) followed by lufenuron (Lc50 27.3 mg ai/ L) and diflubenzuron (Lc50 58.6 mg ai/L), cyromazine (Lc50 9.6 mg ai/ L) and triflumuron seem to be less toxic. The above mentioned CSIs were more potent as compared to Phosalone (Lc50 48.72-64.12) which is one of the most commonly used insecticides for controlling Colorado potato beetle in Iran. The efficacy of cyzomazine on the survival rate of larvae, pupal formation and survival and emergence of Colorado potato beetle, *Leptinotarsa decemlineata* was also reported (Sirota and Grafius, 1994).

Several workers reported the effect of hexaflumuron against *Ephestia kuehniella* and *Spodoptera littoralis* (Marco and Vinuela, 1994) and against fungus growing termite
*Pseudocanthotermes spiniger* (Isoptera: Macrotermitinae) (Peppuy *et al.*, 1998). Kellouche and Soltani (2006) studied the effect of hexaflumuron, a benzoylphenyl urea derivative on growth, development and reproduction in *Callosobruchus maculates* (Coleoptera: Bruchidae). Four doses of hexaflumuron were tested topically on the adults of *Callosobruchus maculates* and evaluation of fecundity, hatchability and viability of eggs, longevity and morphometric of oocytes was done. The longevity and fecundity was reduced and the growth and development of oocytes was also found to be affected. In addition, the viability rate of eggs laid by F1 females were affected significantly. Toxicity evaluation of Radiant SC 12% and hexaflumuron EC 10% against eggs of *Pectinophora gossypiella* (Saund) and biological effect of these compounds on larvae, pupae and adult emergence resulted from the treated eggs were reported (El-Barkey *et al.*, 2009).

*Spodoptera litura* was found to be susceptible to diflubenzuron under induced hyper hormone condition (Sundaramurthy and Balasubraniam, 1978), they are also treated with nuclear polyhedrosis virus along with diflubenzuron for controlling *Spodoptera litura*. The latent effects of acylurea i.e. chlorfluazuron and diflubenzuron is related to the amount of the substance accumulating at the biochemical site of action in the larvae of *Spodoptera littoralis* and *Heliothis virescens* (Guyer and Neumann, 1988). Both direct and latent effects of Lefenuron and the combination of Lefenuron/Deltanet were evaluated on *Spodoptera littoralis*, different doses of both the compounds were incorporated in the diet and were fed. Lefenuron was found to be more toxic than Lefenuron/deltanet. The deleterious effects includes immediate mortality, mortality during moulting, larvae failed to pupate successfully, survived moths of the treated larvae
showed reduction in the reproductive potential, these effects were concentration-dependent (Rahman et al., 2007). The fate of two acylurea compounds i.e., chlorfluazuron and leufenuron were investigated which reduce the reproductive capability of the *Spodoptera littoralis*. Each sublethal dose showed reduction in the percent pupation, pupal weight and adult emergence, an appreciable reduction in the longevity and mating frequency was observed. Both chlorfluazuron and leufenuron treatment showed significantly low percentage of fecundity (i.e. 33.3 to 53.9), the egg hatchability was also reduced (Sammour et al., 2008). Reduction in egg hatchability could be due to the penetration of acylurea compounds into the eggs which prevents hatching by interfering with the embryonic cuticle synthesis. So, new hatch unable to use its muscles to free itself from egg wall (Marco & Vinuela, 1994; Mass et al., 1980). Another possible reason that reduced hatchability in *Spodoptera littoralis* is caused by the defects in the differentiation of oocytes and sperms (Meola et al., 1980; Horowitz et al., 1992). It has previously been reported (Ishaaya, 1992; Spates and Wright, 1980; Ive and Wright, 1978) that in stable flies and houseflies the secretion of unmetabolized acylurea compounds into the eggs has led to the toxicity to the developing embryos.

There are number of reports concerning the evaluation of different CSIs against other species of insects such as diflubenzuron against *Mamestra brassica* (Grosscurt, 1978) and *Pseudoplusia includens* (Reed and Bass 1980), CGA-112913 against *Heliothis virescens* (Schgeurer et al., 1983), MK-139 (CME-134) against *Plutella xylostella* (Kohyama, 1986) and (ME-13406) against *Leptinotarsa decemlineata* (Tuttle and Ferro, 1988). Several researchers have worked on different chitin synthesis inhibitors in the form of
active ingredients to evaluate the insecticidal activity against different stages of the cotton leafworm, *Spodoptera littoralis* using different application techniques (Ascher and Nemmy, 1976; Radwan *et al.*, 1978; Ascher and Liyahn 1981; Ascher and Nemmy; 1984; El- Sayed, 1984; and Aldebis *et al.*, 1988). The effects of diflubenzuron (DFB) and flucyloxuron (FCX) was also studied on the ciliated protiste cellular model *Paramecium species*. At the concentration of 10μg/ml and 20μg/ml with both DFB and FCX the growth of ciliated protiste inhibited appreciably but the effect is a lot more marked with FCX than with DFB (Rouabhi *et al.*, 2005).

Study were conducted to evaluate the efficacy of an IGR Dimilin (TH-6040) against the susceptible and resistant strains of *Spodoptera littoralis* (El-Guindy *et al.*, 1982). Further, evaluation of the biological activity of various concentrations of Chlorfluazuron 5% EC, Flufenoxuron 5% EC and Teflubenzuron 5% FC against insecticides susceptible laboratory and field strains of 3rd and 5th instar of cotton leafworm *Spodoptera littoralis*. In susceptible lab strains flufenoxuron and chlorfluazuron were almost equal in their toxicity at the Lc50 values against 3rd instar larvae whereas teflubenzuron was almost twice as less toxic than the above mentioned chemicals. Against 5th instar larvae, chlorfluazuron was found to be the most potent compound, followed by teflubenzuron and flufenoxuron being the least toxic. In the field strains the toxicity against 3rd instar was highest for flufenoxuron followed by chlorfluazuron whereas teflubenzuron was the least toxic and against 5th instar larvae the toxicity decreased in the order of chlorfluazuron followed by teflubenzuron then flufenoxuron (Bayoumi *et al.*, 1998).
A novel IGR with high potency having importance in IPM (Integrated Pest Management) programme is Novaluron (Ishaaya et al., 2002; 2003). It has also been observed that 2nd instars of an imidacloprid-resistance Colorado potato beetle strains exhibited reduced susceptibility which is about 2.5 folds to novaluron, the toxicity of novaluron were then enhanced by a synergist S,S,S-tributylphosphorotrithioate (Cutler et al., 2005). It was assessed that the transovarial transport of novaluron via the females results in egg hatch inhibition (Mommaerts et al., 2006). Later, it was described that the penetration of novaluron may be affected through contact or ingestion which was studied on various developmental stages of Tribolium castaneum (Kostyukovsky et al., 2006). The transovarial activity of Novaluron on egg hatch and on larval development of Tribolium castaneum was also reported (Trostanetsky and Kostyukousky, 2008).

Tanani (2001) recorded shortened developmental duration of Rhynchophorus ferrugineus by lufenuron (CGA-184699) and CGA-59205. The morphogenic and developmental responses of lufenuron and diofenolan on the housefly Musca domestica was also conducted (Ghoneim et al., 2004). Partial and complete blocking of lufenuron on the adult emergence have been demonstrated against Lobesia botrana (Saenz-de-Cabezon et al., 2006). The effects of lufenuron (CGA-184699) on the growth, development and metamorphosis of the desert Locusta Schistocerca gregaria (Orthoptera: acrididae) was studied and it was found that the growth of lufenuron-treated nymphs was profoundly inhibited. Inhibitory effects on the adult emergence was also seen, if emerged the adults suffered a morphogenic action of lufenuron because of different deformed females were
produced in increasing percentage, the adult females spent only shortened longevity and then died (Bakr et al., 2008). All the above mentioned effects were dose-dependent.

The efficiency of different IGRs in comparison with the registered product on the development of the heart-shaped scale was reported (Du-Troit and Villiers, 1990). Various concentrations of CGA-211446 (Ciba Geigy), a chitin synthesis inhibitor were tested on the 3rd instar of the heart-shaped scale, Protopuluinaria pyriformis (Cockrell) (Hemiptera: Coccidae) and the comparison of the efficacy with the registered product, buprofezin (FBC) was also done. Effective results were obtained at a concentration of 40 ml/100 of water and higher (Steyn et al., 1993).

Toxicity evaluation of another chitin synthesis inhibitor Triflumuron was also done by various scientists on different insect pests. In the recent past, Amir and Preveling, (2004) verified that triflumuron-exposed Apis mellifera exhibited lower flight activity. Batra et al., (2005) showed the efficacy of triflumuron against mosquito larvae in the clear and polluted water. Triflumuron also reported to diminished the longevity of the triatomine, Rhodnius prolixus when administered by contact, blood ingestion and injection (Mello et al., 2008). Efficiency of triflumuron on the development, adult longevity, locomotory activity, reproduction and viability of eggs of Aedes aegyptii was conducted by Belinato et al., (2009), the treatment interfered with the blood-feeding ability of the surviving females of the treated individuals, both the number of the blood-fed females and the amount of ingested blood were showed alteration.
The similar effects as of chitin synthesis inhibitors was observed by treatment with the quinones for plumbagin. Interruption of moulting by the quinones may be due to interference with hormonal regulation, possibly with the production of ecdysoids. The adverse effects of plumbagin on prothoracic glands of Dysdercus sp. have also been observed by Joshi et al. (1989). The inhibition of production of ecdysoids by juglone and plumbagin have been demonstrated (Mitchell and Smith, 1988). Several workers have shown the inhibition of chitin synthetase, and thus affected chitin formation by plumbagin (Kubo et al., 1983; Gujar and Mehrotra, 1988; Krishnayya and Rao, 1995).

The comparative study of 3 naphthaquinones viz. plumbagin, juglone and menadione and 2 benzoquinones viz 2,6 -dimethylbenzoquinones, 2,3,6- trimethylbenzoquinone and 2,6-dimethylhydroquinone was conducted to show the growth-inhibitory effect on Dysdercus koenigii (Hemiptera: Pyrochocoridae). The data showed that amongst naphthoquinones, plumbagin which has methyl group at 2-position and hydroxyl at 5-position was the least toxic followed by juglone (which has hydroxyl at 5-positioned) & menadione (with methyl group at 2-position). Based on the LD50 values, the following increasing order of toxicity: plumbagin< 2,6dimethylhydroquinone< 2,3,6trimethylbenzoquinone< juglone< menadione< 2,6, dimethylbenzoquinone. In plumbagin, the high doses between 30 & 50µg/nymph showed deformity such as smaller body size, crumpled wings and deformed legs in the emerging adult and they survived for 24-48 hrs only. Same abnormalities were seen when the 5th instar nymph was treated with juglone at much lower doses i.e. 3-10 µg/nymph. In case of menadione with 0.2-1.5 µg/nymph the adults emerged from 5th instar were short-lived. In 2,6-dimethylbenzoquinone the number of deformed adults
from the treated 5\textsuperscript{th} instars increased with increasing doses although abnormal adults emerged from 0.3 \( \mu \text{g} \) treated nymph. The 5\textsuperscript{th} instar nymph treated with 8.15 \( \mu \text{g}/\text{nymph} \) (LD50) or near the LD50 value produced deformed adults and the effects increased with higher doses and such adults lived only for 24-48 hours. The treated 5\textsuperscript{th} instar nymph with 5-10 \( \mu \text{g} \), 2,3,6-trimethyl benzoquinone reduced the life span (Banerjee \textit{et al.}, 2001).

Lectins are proteins or glycoproteins of non-immune origin that agglutinate cells and/or precipitate complex carbohydrates. They are isolated from a variety of sources like plant roots and bark, fungi, bacteria, seaweed and sponges, mollusks, fish eggs, body fluids of invertebrates and lower vertebrates, and from mammalian cell membranes. Most sigma lectins are highly purified by affinity chromatography. WGA was first isolated and purified by Burger and Goldberg, (1967) and later by others using affinity procedures, an extensive studies have led to a detailed characterization of its chemical, physical and biological properties (Allen \textit{et al.}, 1973; Rice and Etzler, 1975) \textit{Triticum vulgaris} lectin is a wheat-germ agglutinin (WGA) i.e. a protein capable of agglutinating erythrocytes and other types of cells (Sharon and Lis, 1972). Wright \textit{et al.}, (1972) have reported a preliminary low-resolution X-ray crystallographic structure of WGA, which indicated that eight 23,000-molecular-weight molecules form asymmetric units in an orthorhombic unit cell and the high half-cystine content made the three dimensional structure of WGA unique among lectins. The amino-acid composition of WGA indicates that it has high content i.e., 20% half-cystine residues and 21% glycine residues (Allen \textit{et al.}, 1973). It exists as a 35,000-molecular-weight dimmer in neutral pH buffers with two-binding sites for inhibitory saccharides; at lower pH the molecule exists as a 17,000-molecular-weight
monomer (Nagata and Burger, 1974). WGA which is N-acetylglucosamine-specific lectin was found to produce several different effects on biological systems, all of which are apparently caused by interaction of a lectin with a membrane components (Goldstein and Hayes, 1978). Peumans et al. (1982) isolated lectins from embryos of Secale cereale (rye) and Hordeum Vulgare (barley) by affinity chromatography on immobilizd N-acetylglucosamine which strongly resembled WGA with respect to their chemical, biological and immunological properties.

It has been shown that WGA has high anti-insect activity in vitro (Czapla and Lang 1990; Murdock et al., 1990; Huesing et al., 1991) and therefore, the transfer of its gene into crop plants has been suggested to increase their insect-resistance. Twenty-six plant lectins were tested for anti-insect activity against neonate European corn borer, Ostrinia nubilalis (Hiibner) and Southern corn rootworm, Diabrotica undecimpunctata howardi (Barber) larvae, lectins from wheat (Triticum vulgaris L.), castor bean and camels foot tree were lethal to neonate O. nubilalis larvae when applied to diet surface as 2% solution whereas lectins from pokeweed and green marine algae were found to be toxic against neonate D. undecimpunctata howardi larvae when applied topically (2%) to the artificial diet (Czapla and Lang, 1990).

The efficacy of plant lectins viz., wheat germ agglutinin (WGA), jacalin lectin (JCA), pea lectin (PL) and soyabean agglutinin (SBA) was evaluated against neonates of tobacco caterpillar, Spodoptera litura following artificial diet surface incorporation technique. Laboratory bioassay revealed that WGA was most toxic with the toxicity in order of
WGA> JCA>PL>SBA, the lectin intoxicated diets adversely affected the growth and development of *Spodoptera litura* (Gupta *et al.*, 2007). The activity of leek lectin (APA) in transgenic tobacco plants against cotton leafworm, *Spodoptera littoralis* was studied by Sadeghi *et al.*, (2009). The inhibitory effect such as retarded development of the larvae and metamorphosis, reduced larval and pupal weight and increased mortality was observed throughout the experiment.

It was found that wheatgerm and snowdrop lectin showed antimetabolic activity against sugarcane whitegrubs, (*Antitrogus parvulus*), these lectins are insect growth-inhibiting proteins whose genes could potentially be manipulated into sugarcane and improve host-plant resistance to whitegrubs (Allosopp and McGhie, 1996). Lectins from different sources shown to have antimetabolic and insecticidal properties towards insects such as lectins from *Galanthus nivalis* agglutinin (GNA), *Narcissus psuedonarcissus* agglutinin (NPA), *Allium sativum* agglutinin (ASA), *Oryza sativum* agglutinin (OSA) and *Urtica dioica* agglutinin (UDA) have antimetabolic affects against rice brown planthopper, *Nilaparvata lugens* (Powell *et al.*, 1995), PF2 lectin from *Olneya tesota* (Palo Fierro) against *Zabrotes subfasciatus* larvae (Irlanda *et al.*, 2009) and lectin from Yam (*Dioscorea batatas*) tubers against *Helicoverpa armigera* (Ohizumi *et al.*, 2009).

Effects of soybean lectin have been reported against O. nubilalis larvae (Czapla and Lang, 1990) and sugarcane borer, *Diatraea saccharalis* (Setamou *et al.*, 2002). The efficacy of soyabean lectin were investigated against rats and turkey poults, it was reported that at higher concentration of lectin in the diet the growth and nutritional
digestibility was reduced (Li et al., 2003; Fasina et al., 2004). Further, it was also reported that lectin from the seeds of soyabean (Glycine max) was extracted and purified by chromatography and studied on freshly laid eggs and on 2nd instar larvae of melon-fly Bactrocera cucurbitae. Lectin failed to influence egg-hatching of the treated eggs though on the 2nd instar larvae lectin showed significant effectiveness on larval period, pupal period, total development period, pupation and emergence (Kuljinder Singh et al., 2006).

Previous reports showed the transgenic tobacco plants expressing trypsin inhibitor gene resulted in increased mortality, reduced insect growth and reduced plant damage by Helicoverpa zea (Hoffman et al., 1992) and Spodoptera litura (McManus et al., 1999; Yeh et al., 1997). Soyabean trypsin inhibitor has earlier been shown to reduce the growth of Helicoverpa armigera (Johnston et al., 1993; Wang et al., 1996). In the recent past, a comparative study of the biological activity of soyabean trypsin inhibitor and seven plant lectins against cotton bollworm/legume pod borer, Helicoverpa armigera was carried out. The seven plant lectins viz., concanavalin A, jacalin, chickpea, lentil, peanut, wheatgerm and snowdrop were bio-assayed by treating the surface of the artificial diet with 1% or 2% solutions of different lectins. Out of the above mentioned lectins soyabean trypsin inhibitor, chickpea and snowdrop showed marked anti-biotic effects in terms of insect survival and development (Shukla et al., 2005).

Lectin from snowdrop were shown to be toxic to pea aphid, Acrythosiphon pisum (Rahbe et al., 1995); tomato moth, Lacanobia oleracea (Fitches et al., 1997); alfalfa beetle (Elden, 2000). Rachel et al., (1996) tested the effects of snowdrop lectin on the Glass
Potato Aphid (*Aulacothum solani*) and found that the presence of *Galanthus nivalis* agglutinin (GNA) in the diet decreased the fecundity of adult aphids and nymphal development. Snowdrop lectin also found to be effective against legume pod borer, *Maruca vitrata*, the efficacy of 25 lectins were evaluated on the larval development of *Maruca vitrata* by feeding bioassay: a total of 16 lectins had detrimental effects such as larval mortality, weight loss, feeding inhibition, reduction in pupation and adult emergence and poor fecundity. Twayblade lectin i.e., *Listera ovata* agglutinin (LOA) and snowdrop lectin i.e., *Galanthus nivalis* agglutinin (GNA) were proved to be effective against the larvae of Maruca pod borer for all the six parameters examined (Machuka *et al.*, 1999). Much later, Arora *et al.*, 2005 reported the biological effects of plant lectins from field bean (*Phaseolus vulgaris*), pigeonpea (*Cajanus cajan*), chickpea (*Cicer arietinum*), and garlic (*Allium sativum*) along with snowdrop (*Galanthus nivalis*) lectin on the growth and development of *H. armigera* so as to identify the candidate genes for development through transgenic plants to control the pest. Experiments also conducted to investigate direct effects of snowdrop lectin (GNA) on larvae of three species of aphid predators that differ in their feeding and digestive physiology and the gut enzyme from none of the three species (*Chrysoperla carnea*, *Adalia bipunctata* and *Coccinella septempunctata*) were able to breakdown GNA (Hogervorst *et al.*, 2006).

Earlier, it was reported that peanut ML (mannose-binding lectin) is not detectable in mature seeds (Law *et al.*, 1990, 1991), but a well-characterized galactose-binding seed lectin is present that has the ability to retard the development of cowpea weevil, *Callosobruchus maculates* F. (Goldstein and Poretz, 1986; Murdock *et al.*, 1990). After
few years, a comparative study of two mannose-binding lectins (ML) were conducted one
from peanut, *Arachis hypogaea* and another from pea, *Pisum sativum* for toxic effects on
larvae of the stem borer, *Chilo partellus*. There were significant decrease in *C. partellus*
larval length and weight and increased mortality on the diet containing peanut ML
(mannose-binding lectin) at concentration of 1.0% and 0.5% whereas lectin from pea did
not have any toxic effect at a dietary concentration upto 1% (Law and Kfir, 1997).

Indirect evidence for a possible deterrent activity of a plant lectin was shown by Guzman-
Maldonado *et al.*, (1996) who investigated the relationship between physical and
chemical characteristics of common bean (*Phaseolus vulgaris* L.) varieties and their
susceptibility to *Zabrotes subfaciatus* (Boh.). Further, much later, it was reported by
Sadeghi *et al.*, (2006) that plant lectins showed inhibitory activity on cowpea weevil
*Callosobruchus maculates* (F.) oviposition. Lectin coated chickpea seeds caused a
significant reduction in egg laying. All of the 14 lectins scored a significant deterrent
activity on the oviposition, the strongest was that of bean lectin PHA (*Phaseolus
vulgaris*) being 78.1% and other lectins showed a deterrent activity ranged between
66.4% and 36.5%. The tulip lectin Tx LC-I exhibited the lowest. Also the deterrent
activity of plant lectins decreased upon enhancing insect density.

Behavioural assays in rats have also been studied by feeding on a food containing Con A.
The food was readily refused by the animals, but this behaviour was not due to neophoby
or to any conditioned taste aversion (Larue-Achagiotis *et al.*, 1992). Later, incorporation
of N-acetylglucosamine specific agglutinins from wheat-germ (*Triticum aestivum*; WGA),
thorn apple (*Datura stramonium*) or nettle (*Urtica dioica*) rhizome in the diet showed anti-nutritive effects which appreciably reduced digestibility, utilization of dietary protein and growth of rats, with WGA being highly toxic (Pusztai et al., 1993). In relation with *Canavalia ensiformi* (Con A), Nicolas et al., (2003) investigated the feeding alterations induced in pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphiidae) with a diet containing the lectin from *Canavalia ensiformi* (Con A). A series of behavioural experiments were carried out to detect potential sensory mediation of lectin activity. An electrical penetration graph technique was adapted to artificial diets and provided short-term continuous analysis on feeding/probing events. At the 400μg ml⁻¹ level, adults were affected and had reduced ingestion durations as early as in the first 4 hrs of contact, but experienced an adaptation to the behavioural alterations induced by lectin feeding. Overall, feeding deterrency following exposure to lectins appeared to be a consequence of intoxication, and not due to a sensory mediated process.

Apart from plants, lectins are also isolated from number of sources such as microorganisms, body fluids of invertebrates and lower vertebrates, and from mammalian cell membranes. Earlier, purification and characterization of lipopolysaccharide-binding protein from the haemolymph of American cockroach, *Periplaneta americana* was done by Jomori et al., (1990) and reported that this lectin specifically binds to bacterial LPS (Jomori et al., 1991). The characterization of a novel C-type lectin, immunolectin, from a lepidopteran insect, *Manduca sexta* (tobacco hornworm) was expressed in response to bacterial challenges and appears to interact with bacterial LPS (lipopolysaccharide) to activate the prophenol oxidase system in the plasma (Xiao-Oiang et al., 1999). Isolation,
purification and characterization of Dorin M lectin which is a sialic-acid binding lectin was found to be abundantly present in the haemolymph of the tick (*Ornithodoros moubat*) (Grubhoffer and Kovar, 1998; Kovar et al., 2000). It was also reported that Dorin M is a fibrinogen-related lectin likely playing a role as a pattern recognition molecule (Rego et al., 2006).