2

# Chlorfluazuron as Reproductive Inhibitor

Farzana Perveen
*Chairperson, Department of Zoology*
*Hazara University, Garden Campus*
*Mansehra*
*Pakistan*

## 1. Introduction

Benzoyl phenyl ureas (BPUs) inhibit chitin synthesis during growth and development in insects and act as moult disruptors, therefore, they have been called insect growth regulators (Wright and Retnakaran, 1987; Binnington and Retnakaran, 1991). IGRs, such as dimilin, are effective against a considerable range of insect larvae and adults in a variety of situations. The compound disrupts the moulting process by interfering with chitin synthesis. Research on the different aspects of dimilin as a chitin synthesis inhibitor, toxicant, ovicide, disrupting adult emergence, and residual effects have been done with various insect species, e.g., Jakob, 1973; James, 1974; Qureshi et al.,1983; Naqvi and Rub, 1985; Ganiev, 1986; Khan and Naqvi, 1988; Gupta et al., 1991; Tahir et al., 1992; Nizam, 1993. Several modes of action have been reported for these pesticides. For example, phagodeterrents and repellents (Abro et al., 1997), chitin synthesis inhibition (Hajjar and Casida, 1979), growth inhibition and abnormal development (Hashizume, 1988), ovicidal action (Hatakoshi, 1992), insecticidal effects on the reproductive system (Chang and Borkovec, 1980) and neurotoxic effects on insect behaviour (Haynes, 1988).

### 1.1 Chlorfluazuron

Chlorfluazuron (Atabron®) is a benzyl phenyl urea (BPU) chitin-synthesis inhibitor (CSI) and insect growth regulator (IGR) is formed by Ishihara Sangyo Kaisha, Japan. Some important details concerning the insecticide chlorfluazuron are given below (provided by Ishihara Sangyo Kaisha, Japan) (Perveen, 2005):

| | | |
|---|---|---|
| Common name | : | Chlorfluazuron (proposed to ISO) |
| Other names | : | Atabron® or Helix® or Aim® |
| Source | : | Ishihara Sangyo Kaisha Ltd., Tokyo, Japan |
| Code number | : | IKI-7899, CG-112913, pp-145 |
| Formulation type | : | 5% w/w (Emulsifiable concentration: EC) |
| Chemical name | : | [1-{3,5-dichlor-4-(3-chlor-5-trifluoromethyl-2-pyridyloxy) phenyl}-3-(2,6-ifluorobenzoyl) urea] (IUPAC nomenclature) |

#### 1.1.1 Salient physical and chemical properties (Perveen, 2005)

| | | |
|---|---|---|
| Appearance | : | Crystalline solid at 20 °C |
| Odor | : | Odorless |

| | | |
|---|---|---|
| Melting point | : | 222.0 – 223.9 °C (decomposes after melting) |
| Vapor pressure | : | <10 – 8 $p_a$, <10-10 Torr at 20 °C |
| Volatility | : | Relatively non-volatile |
| Specific gravity | : | 1.4977 at 20 °C |
| Stability | : | No detectable decomposition over at least 3 months at 50 °C |

### 1.2 Spodoptera litura

The *S. litura* is found in most of the Caroline and in the South Pacific Island including American Samoa. It also occurs in the northern two thirds of Australia. The moth is also widespread throughout India and recognized as quarantine pest in EU legislation. It is present in Mediterranean Europe and Africa. It is the most commonly intercepted in the UK, on imported ornamentals and their products. *Spodoptera litura* is also a destructive pest of subtropical and tropical agriculture, and has the potential to be a serious pest of glasshouse crops in northern Europe. It was found as feeding on impatience on Victoria Peak on Hong Kong Island and readily switched to (western) lettuce (Etman and Hooper, 1979). In 1974, Etman and Hooper initiated an investigation into the radiobiology of *S. litura*, and reported that it was a significant pest of cotton in the Ord River region, Australia (Etman and Hooper, 1979). Its larvae are a major cosmopolitan pest of a wide range of crops (Skibbe et al., 1995). Matsuura and Naito (1997) reported that *S. litura* causes serious widespread damage to many agricultural crops in the far southern of the Central Japan every year. They hypothesized that adult *S. litura* immigrate into Japan from overseas every year by long-distance migration. The larvae are destroyed many economically important crops such as *Gossypium hirsutum* L., *Brassica oleracea* L., *Spinacea oleracea* L., *Trifolium alexandrinum* L., *Medicago sativa* L., *Arachis hypogaea* L., *Phaseolus aureus* Roxb., *Phaseolus vulgaria* L. and *Nicotiana tabacum* L. during different seasons throughout the year in Pakistan (Younis, 1973). Their larvae eat nearly all types of herbaceous plants. Some examples of plants are: tobacco, *Nicotiana tabacum* L.; tomatoes, *Lycopersicum esculentum* Mill.; cauliflower, *Brassica botrytis* L.; beetroot, *Beta vulgaris conditiva* L.; silver beet (swiss chard) *Beta vulgaris cicla* (L.); peanuts, *Arachis hypogaea* L.; beans, *Phaseolus vulgaris* L.; banana, *Musa* paradisiaca L.; strawberry, *Fragaria* vesca L.; apple, *Malus pumila* Mill.; lettuce, *Lactuca sativa* L.; zinnia, *Zinnia elegans* Jacq.; dahlia, *Dahlia pinnata* Cav.; ape, *Alocasia macrorrhiza* (L.); geranium, *Pelargoniumx zonale*; St. John's lily, *Crinum asiaticum* L.; mangrove lily, *Crinum pedunculatum* (Fragrant); leek, *Allium porrum* (Leek); horsetail she oak, *Casuarina equisetifolia* L.; *Fuchsia* and many other garden plants (Baloch and Abbasi, 1977). Several common names have been used for *S. litura*, for example defoliator cutworm, oriental leafworm, cluster caterpillar and common cutworm. The larvae are quite polyphagous for example eat all types of herbaceous plants and have been reaching the status of international pest. In 1968, a panel convened by the International Atomic Agency listed species of *S. litura* as a pest on which basic and applied research was needed in order to evaluate the potential of the sterile insect release method for control (Anonymous, 1969).

Eggs of *S. litura* are laid in batches, on plants and other surfaces such as pots, benches or glasshouse structures. Eggs are normally laid in the irregular furry masses covered with orange-brown hairs giving them a "felt-like" appearance on the underside of a leaf of a food plant similar to the egg of the brown locust, *Locustana pardalina* (Walk.) (Matthee, 1951). On hatching, larvae (caterpillars) are 2–3 mm long with white bodies and black heads and are very difficult to detect visually. If they emerge from eggs laid on glasshouse structures or

hanging pots, they can reach the plants below by "parachuting" down on silken threads. The overall colour of the later-stages of the larvae can vary from light to dark brown, and the body is strongly speckled with tiny white spots. Initially, when larvae grow become a translucent green with a dark thorax. The young larvae are smooth-skinned with a pattern of red, yellow, and green lines, and with a dark patch on the mesothorax. Larvae initially eat only the flesh of their food leaves, leaving the veins intact. Later, as they grow, they eat whole leaves, and even flowers and fruit (Khuhro et al., 1986).

Many populations are extremely resistant to pesticides and, if they become well established, can be exceptionally difficult to control. In these cases, it is important that a comprehensive treatment programme is implemented, incorporating a range of reliable control methods, including physical destruction of insects. The *S. litura,* as it is the most common to be encountered in a UK nursery, but the larvae and adults of all noctuids are similar in appearance and are difficult to tell apart without laboratory examination (Khuhro et al., 1986). Larvae become brown with three thin yellow lines down the back, one in the middle and one on each side. A row of black dots runs along each side, and a conspicuous row of dark triangles decorates each side of the back. The last-instar larva is very dark, with four prominent yellow triangles on the mesothorax. When disturbed, the larvae curl into a tight spiral with the head protected in the centre. Larvae further develop characteristic markings on their backs. These include: a square of four yellow spots, each on a black patch, located just behind the head; a further pair of black patches just behind these, and another pair of black patches towards the end of the larva; typically, there are three orange-brown lines, punctuated with dashes of black and yellow along the back of the body. Depending on the background colour, these markings may be more evident on some larvae than others; the larvae ultimately grow up to 4.5 cm long; larvae are nocturnal, and during the day can be found at the base of plants or under pots. The feeding activity of young larvae causes "windows" in the leaves, while older larvae can completely defoliate plants if present in large numbers. Stems, buds, flowers and fruits may also be damaged. The larvae burrow into the soil below the plant for several centimeters and pupate there without a cocoon. As they do so, they produce a quantity of fluid, and drown in this if they pupate in captivity in an empty glass jar. They pupate successfully if 0.5 cm of sand is provided in the container. In January in Melbourne, the pupal stage lasts three weeks, but larvae that pupate at the end of summer emerge the following spring. The red-brown pupae are up to 2 cm long. The thoracical, ventral knobs found on covering of pupa (Khuhro et al., 1986). Adult moths with brown colour are up to 2 cm long with a wingspan of approximately 4 cm. The fore-wings are brown, with a large number of pale cream streaks and dashes and, when the adults are newly emerged, there may be a violet tint to the fore-wing. The hind-wings are a translucent white, edged with brown. The hind-wings are silvery white. It has a wingspan of about 4.0 cm. The males but not the females have a blue-grey band from the apex to the inner margin of each forewing. The pheromones of this species (specific sex-attractant scents used by females to attract males) have been elucidated. As the adults are nocturnal, light or pheromone traps should be used for monitoring purposes. Seek assurance from suppliers that plants are free from this pest as part of any commercial contract: carefully inspect new plants and produce on arrival, including any packaging material, to check for eggs and larvae and for signs of damage (Khuhro et al., 1986).

Early notification of the presence of this pest, will allow rapid implementation of a comprehensive treatment programme, and will help eradicate it quickly from nursery. Established outbreaks are very damaging and difficult to eradicate. Various methods of

control of *S. litura* have been investigated. Biologically, it has been controlled by the nematode, *Steinernema carpocapsae* (Weiser) and parasitoid fly, *Exorista japonica* (Townsend). A baculovirus has also been used. Resistant species of plants are also grown to save the crops from this pest. Resistant tomatoes are most commonly cultivated (Khuhro et al., 1986).

## 2. Effects of chlorfluazurn on reproduction of *Spodoptera litura*

Reproductive inhibition induced by BPUs has been reported the most widely when applied to adults or eggs of insect pests rather than to application to larvae or pupae (Fytizas, 1976). When these compounds were applied to females, males or both sexes of insect pests, BPUs induced a variety of effects on reproduction; they caused a decrease in fecundity, fertility and/or hatchability. It has been reported that treatment of adult insect pests with diflubenzuron disrupts the secretion of adult cuticle (Hunter and Vincent 1974; Ker, 1977), and the production of peritrophic membrane in the grasshopper, *Locusta migratoria* (L.) (Clark et al., 1977) and the meal worm, *Tenebrio molitor* L. (Soltani, 1984; Soltani et al., 1987). In addition, topical treatments of male and female adult boll weevils, *Anthonomous grandis* Boheman; stable flies, *Stomoxys calcitrans* (L.) and *M. domestica* with TH-6040 [{N-(4-chlorophenyl)-N-26-difluorobenzoyl}urea] caused significant reduction of egg fertility and hatchability. It also causes inhibition in the fecundity of female adults of several species of insect pests (Holst, 1974; Taft and Hopkins, 1975; Crystal, 1978; Hajjar and Casida, 1979; Otten and Todd, 1979). Diflubenzuron applied to adult females caused a decrease in fecundity in the Mexican bean beetle, *Epilachna varivestis* Mulsant and Colorado potato beetle, *Leptinotarsa decemlineata* Say (Holst, 1974), and adversely affected egg viability in *St. calcitrans* and *M. domestica* (Wright and Spates, 1976). When 2 day-old female adults of the Japanese beetle, *Oryzae japonica* Willemse, were starved for 6 hours, and then allowed to consume 500 µg a.i. of diflubenzuron over another 6 hours, the fecundity of the treated females, in term of number of eggs laid per pod, was significantly decreased from controls. In controls, most pods gave an egg hatch of 82.5% but a hatch of only 8.5% hatched in the treated females (Lim and Lee, 1982). Similarly, treating eggs with diflubenzuron caused reduction in hatching in the mosquitoes, *Culex pipiens* Say, *C. quinquefascialus* Say (Miura et al., 1976), the almond moth, *Ephestia cautella* (Walk.) (Nickle, 1979), the two-spotted lady beetle, *Adalia bipunctata* (L.) and the seven-spotted lady beetle, *Coccinella septempunctata* L. (Olszak, 1994). Reports of inhibition of reproduction when larvae or pupae (instead of adults or eggs) were treated with BPUs are rare and little literature is available (Madore et al., 1983). Brushwein and Granett (1977), working with the spruce budworm, *Choristoneura fumiferana* (Celemens), demonstrated that certain moult-inhibiting IGRs such as EL-494 (Eli Lilly and Co., New York, USA) fed to sixth-instar larvae, caused reproductive failure in adults surviving after the larval treatment. Therefore, in this research newly ecdysed fifth-instar larvae and newly ecdysed pupae of *S. litura* were used as test materials. Chlorfluazuron, a comparatively new IGR and BPU that was discovered by Ishihara Sangyo Kaisha Ltd., Tokyo, Japan, that has been developed and sold commercially as Atabron®, Helix® and Aim® in many countries, including Japan in cooperation with Novelties Co. Ltd, ICI-AGRO and Ciba Geigy. It is a relatively highly active chitin synthesis inhibitor and it is, therefore, an effective treatment for the control of major lepidopteran insect pests in crops such as cotton, fruits, tea, vegetables and where insect resistance to conventional insecticides is becoming a serious problem. Chlorfluazuron exhibits no activity against important beneficial insects (Haga et al., 1992). The highly selective insecticidal activity of

chlorfluazuron is particularly suited to integrated pest management programmes. Although chlorfluazuron has contact toxicity at higher rates, the major route of toxicity to insects is ingestion, and it has no root, systemic or foliar translaminar activity. Like other BPUs, chlorfluazuron is believed to disrupt chitin formation and, thus, kills the insects when they moult. This mode of action necessarily means that it is effective only against immature insects and that it is relatively slow actions. When higher dosages of chlorfluazuron were applied to newly ecdysed fifth-instar larvae, it had a devastating effect on the *S. litura* population by killing them during larval, pupal and adult stages (Hashizume, 1988). In insect pest management, the purpose of research is to maintain the pest population below the economic injury level. The mode of action of chlorfluazuron, as a CSI is known to some extent. However, but the knowledge of its effects on reproduction are rare. Insect structure and physiology may vary considerably during growth and development, with certain stages being more susceptible to insecticides than others. For example, the cuticle varies in its composition during larval development and this has been related to changes in IGR susceptibility. The activity of various insecticides detoxifying enzymes, such as MFO, glutathione S-transferase and epoxide hydrase also fluctuate during the life cycle of an insect (Yu, 1983). For this purpose newly ecdysed fifth-instar larvae and newly ecdysed pupae were selected for the treatments for the present research. The main objective of the present research is to determine the effects of sublethal doses ($LD_{10}$: 1.00 ng larva$^{-1}$; 0.12 ng female pupa$^{-1}$; 1.23 ng male pupa$^{-1}$ or $LD_{30}$: 3.75 ng larva$^{-1}$) of chlorfluazuron on the reproduction (e.g., fecundity, fertility and hatchability) when ha been apply to newly ecdysed fifth-instar larvae and newly ecdysed pupae of *S. litura* (Perveen, 2000a).

### 2.1 Experimental procedures

#### 2.1.1 Insect rearing

Experiments were conducted with *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) taken from a stock that was established from eggs obtained from Aburahi Laboratory of Shionogi Pharmaceutical (Koga-Shiga-Pref., Japan). The larvae of *S. litura* were reared in the laboratory under controlled conditions on the artificial diet Insecta LF® (Nihon Nohsan-kohgyo, Kanagawa, Japan). The rearing temperature was maintained at 25±1 °C, with a L16:D8 hour photoperiod and 50-60% r.h. To facilitate observations, the dark period was set from 06:00 to 14:00 hours. Adults were fed on a 10% sucrose solution soaked in cotton. The eggs, which were laid on Rido® cooking paper (Lion, Tokyo, Japan), were collected every 3$^{rd}$ day and kept in 90 ml plastic cups (4 cm in diameter: 4×4 cm high) for hatching under the same environmental conditions (Perveen, 2000a).

#### 2.1.2 Chlorfluazuron and its application

Sublethal doses, $LD_{10}$ (1.00 ng larva$^{-1}$; 0.12 ng female pupa$^{-1}$; 1.23 ng male pupa$^{-1}$) or $LD_{30}$ (3.75 ng larva$^{-1}$) were applied to newly ecdysed fifth-instar larvae and newly ecdysed pupae. These $LD_{10}$ and $LD_{30}$ values were calculated based on interpret alone of the results of the toxicity data of larval tests at adult emergence. The treated and untreated insects, at all developmental stages including fifth- and sixth-instar larvae, pupae and adults, were weighed separately, on different developmental days, using an analytical balance (Sartorius Analytical AC-2105, Tokyo, Japan) to a precision of 0.001 mg, to determine the effect of chlorfluazuron on the body weight. The duration of each developemental stage was also strictly recorded (Perveen, 2000a).

### 2.1.3 Mating

After both larval and pupal treatments, females and males that emerged between 2 and 8 hour (most adults emerged in the dark photoperiod) on the same day were collected at 0200–1000 hour. These adults were considered as 0 day old and paired just before the dark photoperiod (12 hour old) of the next day. Each female and male pair was kept separately in a plastic cup (430 $cm^3$; height: 8.0 cm; diameter: 9.5 cm) for the whole life-span. The cup was padded with Rido cooking paper on its wall and with a disc of 70 mm filter paper on the bottom. The pairs were fed throughout their life by cotton wool soaked in 10% sugar solution in small plastic cups. All *S. litura* were examined daily (Perveen, 2000a).

To determine the effects of sublethal doses of chlorfluazuron on reproductivity, seven different mating combinations of female and male crosses were established. These were: (1) Untreated female mated with untreated male ($_{U}♀\times_{U}♂$); (2) $LD_{10}$-treated female mated with untreated male ($_{LD10}♀\times_{U}♂$); (3) Untreated female mated with $LD_{10}$-treated male ($_{U}♀\times_{LD10}♂$); (4) $LD_{10}$-treated female mated with $LD_{10}$-treated male ($_{LD10}♀\times_{LD10}♂$); (5) $LD_{30}$-treated female mated with untreated male ($_{LD30}♀\times_{U}♂$); (6) Untreated female mated with $LD_{30}$-treated male ($_{U}♀\times_{LD30}♂$); (7) $LD_{30}$-treated female mated with $LD_{30}$-treated male ($_{LD30}♀\times_{LD30}♂$). For the fecundity, fertility and hatchability experiments, 15–30 pairs were used for each cross. Eggs were laid on the cooking paper after 24 hours and were collected during 0800–1000 hour, cut out and kept in cups (90 $cm^3$) for hatching. Eggs laid were hatched within 84 hours. Observation of oviposition continued until the death of female. Four days after each collection of eggs, the fecundity, fertility and hatchability of the laid eggs were assessed. After the natural death of females, the spermatophores were separated from the bursa copulatrix with a fine forceps in 0.9% NaCl (Saline or Ringer's solution: Barbosa, 1974) under the binocular microscope (10×magnification) (Olympus Co. Ltd., Tokyo, Japan) (Perveen, 2000a).

### 2.1.4 Data analysis

Data for the effects of sublethal doses of chlorfluazuron on reproductivity and viability were analyzed using analysis of variance, one way ANOVA (Concepts, 1989; Minitab, 1997; Walpol and Myers1998) at $P<0.0001$ and Scheffe's F-test (multiple range) (Scheffe, 1953) at 5%. Hatchability percentage values were normalized by arcsin transformation before statistical analysis (Anderson and McLean, 1974).

## 2.2 Results

When the $LD_{10}$ (1.00 ng $larva^{-1}$; 0.12 ng female $pupa^{-1}$; 1.23 ng male $pupa^{-1}$) and the $LD_{30}$ (3.75 ng $larva^{-1}$) of chlorfluazuron were applied to newly ecdysed fifth-instar larvae or newly ecdysed pupae, it was observed that the fecundity of the resulting adults and the fertility and hatchability of their eggs, was significantly reduced {$P<0.0001$ (for larval treatment); $P<0.05$ (for pupal treatment)}, compared with untreated adults, but no significant differences were observed between larval and pupal treatments ($P<0.02$) (Tables 1 and 2). When chlorfluazuron was applied to newly ecdysed fifth-instar larvae at sublethal doses, the number of eggs oviposited by a treated females mated with an untreated male ($_{T}♀\times_{U}♂$) was suppressed to the same degree as an untreated female mated with a treated male ($_{U}♀\times_{T}♂$) or a treated female mated with a treated male ($_{T}♀\times_{T}♂$) (Table 1). The mean female fecundity was 2250±198 eggs when both male and female were untreated (control), i.e., ($_{U}♀\times_{U}♂$), (Table 2). When the female was treated either by the $LD_{10}$ or $LD_{30}$ and mated

with an untreated male, the fecundity was 1462±353 ($_{LD10}♀\times_{U}♂$) and 1266±237 ($_{LD30}♀\times_{U}♂$), respectively. When the male was treated with the $LD_{10}$ and mated with an untreated female, the fecundity was 1407±334 ($_{U}♀\times_{LD10}♂$). However, in the same cross when the male was treated with $LD_{30}$ instead of the $LD_{10}$ ($_{U}♀\times_{LD30}♂$), the fecundity was 1270±215. When both sexes were treated with the $LD_{10}$ ($_{LD10}♀\times_{LD10}♂$), it was 1330±295 and when both sexes were treated with the $LD_{30}$ concentration ($_{LD30}♀\times_{LD30}♂$), it was 1331 ±295. In all the crosses, the fecundity was significantly reduced when compared with the control cross ($_{U}♀\times_{U}♂$) (Table 1) (Perveen, 2000a).

| Mating pairs[a] (female×male) | n[a] | Fecundity[b,c] (mean±SD) | Fertility[b,c] (mean±SD) | Hatchability %[c,d] (mean±SD) |
|---|---|---|---|---|
| $_{U}♀\times_{U}♂$ | 30 | 2250±198a | 1984±208a | 88.4±6.6a |
| $_{LD10}♀\times_{U}♂$ | 30 | 1462±353b | 1010±315b | 68.9±11.8b |
| $_{U}♀\times_{LD10}♂$ | 28 | 1407±334b | 688±317c | 48.3±17.1c |
| $_{LD10}♀\times_{LD10}♂$ | 30 | 1330±295b | 643±265c | 48.6±14.2c |
| $_{LD30}♀\times_{U}♂$ | 29 | 1266±237b | 828±206b | 65.8±11.4b |
| $_{U}♀\times_{LD30}♂$ | 30 | 1270±215b | 36±155d | 28.8±11.2d |
| $_{LD30}♀\times_{LD30}♂$ | 29 | 1331±295b | 33±121d | 27.1±9.1d |

[a]$LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75ng larva$^{-1}$; U: untreated (control); ♀: female adults; ♂: male adults; n: number of pairs used

[b]Number of eggs oviposited during the whole life of female adult were counted (fecundity) and from the eggs number of hatched that larvae were counted (fertility).

[c]Data were analyzed using one way ANOVA (Concepts, 1989) at $P<0.0001$). Means within columns followed by different letters indicate significant differences by Scheffe`s $F$-test (Scheffe, 1953) at 5%.

[d]Hatchability % values were normalized by arcsin transformation before statistical analysis (Anderson and McLean 1974).

Table 1. Effects of sublethal doses of chlorfluazuron on fecundity, fertility and hatchability after topical application of newly ecdysed-fifth instar larvae of *Spodoptera litura* (Source: Perveen, 2000a).

When the $LD_{10}$ of chlorfluazuron was applied to newly ecdysed pupae and the resulting adults were paired, the control fecundity was 2170±175. It was not significantly reduced ($P<0.02$) compared with the larval treatment. The fecundity in the treated pupal cross ($_{LD10}♀\times_{U}♂$) was 640±83. It was not significantly reduced ($P<0.02$) compared with the same cross with treated larvae. In the pupal treatment, the fecundity was suppressed to a similar degree in the crosses $_{LD10}♀\times_{U}♂$, $_{U}♀\times_{LD10}♂$, $_{LD10}♀\times_{LD10}♂$. This reduction was significant ($P<0.0001$) when compared with the control cross (Table 1) (Perveen, 2000a). There was no significant reduction ($P<0.02$) between the larval and pupal treatments with respect to fecundity (Tables 1 and 2) (Perveen, 2005).

The mean fertility of females was 1984±208 larvae when both the male and female were untreated, i.e. the control ($_{U}♀\times_{U}♂$) (Table 1). When the female was treated with either the $LD_{10}$ or $LD_{30}$ and mated with an untreated male, the fertility was ($_{LD10}♀\times_{U}♂$: 1010±315; $_{LD30}♀\times_{U}♂$: 828±206, respectively), i.e., significantly reduced compared with the control cross ($_{U}♀\times_{U}♂$). When the male was treated with the $LD_{10}$ and mated with an untreated female, the fertility ($_{U}♀\times_{LD10}♂$: 688±317) was significantly reduced compared with the crosses, i.e.,

$_{LD10}$♀×$_{U}$♂ and $_{LD30}$♀×$_{U}$♂. However, in the same cross when the male was treated with the $LD_{30}$ instead of $LD_{10}$, the fertility was ($_{U}$♀× $_{LD30}$♂: 368±155) significantly lower than the $_{U}$♀×$_{LD10}$♂ cross. When both sexes were treated with the $LD_{10}$, the fertility was ($_{LD10}$♀×$_{LD10}$♂: 643±265) not significantly different from the $_{U}$♀ ×$_{LD10}$♂ cross. Similarly, when both sexes were treated with the $LD_{30}$, the fertility was ($_{LD30}$♀×$_{LD30}$♂: 333±121) not significantly different from the $_{U}$♀×$_{LD30}$♂ cross (Table 1) (Perveen, 2000a).

| Mating pairs[a] (female×male) | n[a] | Fecundity[b,c] (mean±SD) | Fertility[b,c] (mean±SD) | Hatchability %[c,d] |
|---|---|---|---|---|
| $_{U}$♀×$_{U}$♂ | 15 | 2170±175a | 2123±177a | 97.8a |
| $_{LD10}$♀×$_{U}$♂ | 15 | 1640±83b | 1090±79b | 66.5b |
| $_{U}$♀×$_{LD10}$♂ | 15 | 1580±75b | 827±49c | 52.3c |
| $_{LD10}$♀×$_{LD10}$♂ | 15 | 1524±76b | 751±51c | 49.3c |

[a]$LD_{10}$: 0.12 ng female pupa$^{-1}$; 1.23 ng male pupa$^{-1}$; n: number of pairs used
[b]Number of eggs oviposited (fecundity) the during whole life of female adults were counted and from the number of eggs that hatched larvae were counted (fertility).
[c]Data were analyzed using one-way ANOVA (Concepts, 1989) at $P < 0.001$. Means within a column followed by different letters indicate significant differences according to Scheffe's F-test (Scheffe, 1953) at 5%.
[d]Hatchability% values were normalized by arcsin transformation before statistical analysis (Anderson and McLean 1974).

Table 2. Effects of a sublethal doses of chlorfluazuron on fecundity, fertility and hatchability after topical application of newly ecdysed pupae of *Spodoptera litura* (Source: Perveen, 2005).

When the $LD_{10}$ of chlorfluazuron was applied to newly ecdysed pupae and resulting adults were paired, the fertility of the control cross, $_{U}$♀×$_{U}$♂ was 2123±177, which was not significantly reduced (P<0.02) than that in the larval treatment. In the same way, with the $_{LD10}$♀×$_{U}$♂ cross, the fertility was 1090±79, which was not significantly reduced (P<0.02) than the same cross in the larval treatment. The fertility was reduced 61 –65% in the $_{U}$♀×$_{LD10}$♂ cross, which was significantly reduced (P<0.02) than the $_{LD10}$♀×$_{U}$♂ cross with a fertility reduction of 46–49%. The fertility of the $_{U}$♀×$_{LD10}$♂ cross was not significantly reduced (P<0.02) than the $_{LD10}$♀×$_{LD10}$♂ cross, which was reduced 65–68% for both larval and pupal treatments. There were no significant reductions (P<0.02) between larval and pupal treatments with respect to fertility (Tables 1 and 2). The mean hatchability during a female life-span was 88.4(±6.6)% when both male and female were untreated, i.e. the control ($_{U}$♀×$_{U}$♂) cross (Table 1). When the female was treated, either by the $LD_{10}$ or $LD_{30}$ and mated with an untreated male, the hatchabilities were 68.9 (±11.8)% ($_{LD10}$♀×$_{U}$♂) and 65.8(±11.4)% ($_{LD30}$♀×$_{U}$♂), respectively significantly reduced compared with the control cross. When the male was treated with the $LD_{10}$ and mated with an untreated female, the hatchability, 48.3(±17.1)% ($_{U}$♀×$_{LD10}$♂) was significantly reduced than the $_{LD10}$♀×$_{U}$♂ and $_{LD30}$♀×$_{U}$♂ crosses. However, in the same cross when the male was treated with $LD_{30}$, instead of $LD_{10}$, hatchability, 28.8(±11.2)% ($_{U}$♀×$_{LD30}$♂), significantly reduced than the $_{U}$♀×$_{LD10}$♂ crosses. When both sexes were treated with $LD_{10}$, the hatchability was 48.6(±14.2)% ($_{LD10}$♀×$_{LD10}$♂), not significantly different from the $_{U}$♀×$_{LD10}$♂ cross. Similarly, when both sexes were treated with $LD_{30}$, the hatchability was 27.1(±9.1)% ($_{LD30}$♀×$_{LD30}$♂), which was not significantly different from the $_{U}$♀×$_{LD30}$♂ cross (Table 3.1). The hatchability for the $_{U}$♀×$_{U}$♂ cross was 88.4% and

97.8%, respectively, for the larval and pupal treatments. The larval-treated cross, $_{LD10}♀\times_{U}♂$, was 68.9%, which is not significantly reduced (P<0.02) than the same cross of the pupal treatment in which the hatchability was 66.5%. The hatchability was reduced 48.7% and 52.7% in the $_{U}♀\times_{LD10}♂$ cross, respectively, for the larval and pupal treatments. In the $_{LD10}♀\times_{LD10}♂$ cross, it was reduced to 48.6% and 49.3%, respectively, for these treatments. There was no significant reduction (P<0.02) between larval and pupal treatments with respect to hatchability (Tables 1 and 2) (Perveen, 2000a).

### 2.3 Discussion

When chlorfluazuron was applied to newly ecdysed fifth instars at sublethal doses, $LD_{10}$ (1.00 ng larva$^{-1}$) or $LD_{30}$ (3.75 ng larva$^{-1}$), it was observed that the fecundity of resulting adults as well as the hatching rate of their eggs was suppressed. The hatching rate of eggs oviposited by an untreated female mated with a treated male was suppressed to the same degree as that ofeggs oviposited by a treated female mated with a treated male. However, Madore et al. (1983) studied the effects when different concentrations of sublethal doses of the UC-62644 (chlorfluazuron-25) fed to sixth instar larvae of spruce budworm. Homologous crosses between adults of the 0.01, 0.025 and 0.034 ppm treatments showed 0, 69 and 97% reduction, respectively, in the numbers of eggs laid per 30 pairs of moths when compared with control. Emam et al. (1988) reported the fecundity of *S. littoralis* adults decreased significantly from 977.64 eggs in control to 421.75 eggs, a decrease of about 56%, for adults feeding 10% honey solution containing 0.5 p.p.m. chlorflufluazuron. The corresponding fertility inhibition amounted to 32%. In the present case the fertility was significantly different when only the female was treated or only the male was treated. It is obvious from the results that the fertility and hatchability were affected more when the male was treated in comparison with the female, as also reported by Abro et al. (1997), who found that males were more sensitive to insecticides than females, when five concentrations of cyhalothrin and fluvalinate were tested against fourth instar larvae of *S. litura*.

### 2.4 Conclusion

To clarify the sublethal effects of chlorfiuazuron on reproductivity of common cutworm, *Spodoptera litura*, experiments were conducted under laboratory conditions. Reduction in the body weight was observed in the larvae and pupae when treated with a sublethal dose ($LD_{30}$: 3.75 ng larva$^{-1}$) and in the adults when treated with sublethal doses ($LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$) as newly ecdysed fifth instar larvae of *S. litura*, although the number of matings per female and life span of adult females and males remained unaffected by the same treatments. When sublethal doses were applied only to females or only to males, or both sexes, the average fecundity reduction was up to 35–44%. When only females were treated with sublethal doses, fertility was reduced by 49–58%; when only males were treated fertility was reduced by 65–81% and when both sexes were treated, fertility was reduced by 68±83%. Hatchability was reduced by 22–26% when only females were treated, by 44–66% when only males were treated and by 45–72% when both sexes were treated with $LD_{10}$ or $LD_{30}$ doses as newly ecdysed fifth instars. The results from these observations suggest that the fecundity was reduced to a similar degree when only females or only males or both sexes were treated with $LD_{10}$ or $LD_{30}$ doses as newly ecdysed fifth instars. However the fertility and hatchability were affected more when only males were treated with $LD_{10}$ and much more when treated with $LD_{30}$. Currently, work is in progress to find out the main reasons for the sublethal effects of chlorfluazuron on reproductivity and viability.

## 3. Effects of chlorfluazurn on female reproductive system of *Spodoptera litura*

In many insects oviposition requires the development of the ovary, egg maturation, mating and, in some insects, feeding of the females. Ovarian development, which includes oöcyte growth and vitellogenesis, is under the hormonal control, of either juvenile hormone or ecdysteroid (Engelmann, 1979). In many insects, juvenile hormone (JH) regulates the biosynthesis and uptake of vitellogenin by the oöcytes. Among Lepidoptera, e.g., the tobacco hawkmoth, *Manduca sexta* L. (Sroka and Gilbert, 1971; Nijhout and Riddiford, 1974) and the large white butterfly *Pieris brassica* L. (Karlinsky, 1963 and 1967; Benz, 1969), juvenile hormone is required for full development of the ovaries in adults, whereas in the silkworm *Bombyx mori* L. (Chatani and Ohnishi, 1976), giant silk moth, *Hyalophora cecropia* (L.) (Williams, 1952; Pan, 1977), ailanthus silkmoth, *Samia cynthia* (Drury) (Takahashi and Mizohata, 1975) and ricemoth, *Corcyra cephalonica* (Stainton) (Deb and Chakarvorty, 1981) ovarian development occurs as part of adult development initiated by ecdysteroid. Juvenile hormone or juvenile hormone analogue (JHA) application at a critical period, however, induces abnormal development of the ovary as well as other tissues, although juvenile hormone analogues can replace natural juvenile hormone in regulating oöcyte maturation (Nomura, 1994). In the normal state, the ovary develops during one day before and after eclosion in the presence of juvenile hormone (as described above) and a haemolymph factor stimulates the ovary to start oviposition. When S-71639 was applied to pupae, it inhibited adult emergence when a relatively a high dose was applied. If adults did emerge, they could not oviposit through inhibition of the haemolymph factor, hatchability was also reduced (Hatakoshi and Hirano, 1990). The effects of diflubenzuron on fecundity resulted from treatment of adult females by contact or ingestion (Leuschner, 1974; Fytizas, 1976). When *O. japonica*, adults females were fed diflubenzuron, it retarded the maturation of oöcytes (Lim and Lee, 1982). In *T. molitor*, diflubenzuron reduced mealworm longevity (Soltani et al., 1987), the number of oöcytes per ovary, the duration of the oviposition period and the fecundity (Soltani, 1984). Diflubenzuron, topically applied (0.5 μg insect$^{-1}$) to codling moth, *Cydia pomonella* L. on pupal ecdysis, inhibited the growth and development of oöcytes. It delayed adult emergence and caused a decrease in both the thickness of the follicular epithelium and the size of the basal oöcytes during pupal development. On the other hand, the size of basal oöcytes, the protein content per ovary and the number of oöcytes per ovary recorded in newly emerged adults were significantly reduced by the diflubenzuron treatment. These results, together with observations in several other species, indicated that the reduction in fecundity and egg viability was probably due to interference by diflubenzuron with vitellogenesis (Soltani and Mazouni, 1992). Under laboratory conditions, effects of topical application of sublethal doses of chlorfluazuron ($LD_{10}$: 1.00 ng larva$^{-1}$ or $LD_{30}$: 3.75 ng larva$^{-1}$) on newly ecdysed fifth-instar on fecundity, fertility and hatchability have been investigated. Thus, it is investigated the causes of the decrease in these parameters. To obtain more information, sublethal doses of chlorfluazuron topically have been applied to newly ecdysed fifth-instar larvae of *S. litura* and the effects on female reproductive system during ovarian development and oögenesis have been observed.

### 3.1 Experimental procedure

#### 3.1.1 Ovary measurement

Experimental *S. litura* were rared in the same way as mentioned in Section 2.1.1. Sublethal doses, $LD_{10}$ (1.00 ng larva$^{-1}$; 0.12 ng female pupa$^{-1}$; 1.23 ng male pupa$^{-1}$) or $LD_{30}$ (3.75 ng

larva$^{-1}$) were applied to newly ecdysed fifth-instar larvae same mentioned in Section 2.1.2. To determine the effects of chlorzuazuron on the ovaries, control and treated batches of insects, collected from the fifth day after pupation to the seventh day after adult emergence, were used, depending on the experiment requirements. Ovaries were dissected from these insects in Ringer's solution under a binocular microscope (10×magnifcation: Nikon, Nippon Kogaku, Tokyo, Japan), and the lengths of pedicle, vitellarium, and germarium of each ovariole were measured. The number of mature oöcytes in each of the ovarioles was also counted. The Ringer's solution was removed, and the freshly dissected ovaries were placed in a small covered container that had been preweighed. The dissected ovaries were weighed on an analytical balance (AC-205, Sartorius Analytical, Tokyo, Japan) and kept in the same container in the oven for 24 h at 62±1 °C for evaporation of water. The dried ovaries were reweighed (Perveen and Miyata, 2000).

### 3.1.2 Histology

The procedure to stain the nuclei for cell density from the germarium during female adults required age was adapted from the method described by He (1994). First, the germarium of the female were removed and kept on a microscope slide and carefully crushed with micro forceps until it was extended and roughly evenly distributed over the slide. Second, several drops of 3:1 methanol-acetic acid were introduced to the slide to fix the preparation for 15 min and then the excess fixing solution was absorbed with a filter paper after the fixation. Third, several drops of a 2–5% Giemsa solution dissolved by Sorensen-Gomori buffer solution (monobasic and dibasic sodium phosphate, 0.07 M, pH 6.8) were introduced to the slide for 10–30 min to stain the preparation. Then after the staining, the slide was washed gently and carefully and dried in the air. Finally, the air-dried preparation was checked with a phase-contrast microscope at 20×magnification (Perveen and Miyata, 2000).

The length and width of the basal oöcytes were measured from the 5$^{th}$ d after pupation to 0 day after adult emergence in control and treated insects. Oöcyte measurements were made on three to four basal oöcytes per pair of ovaries that were taken from 9-10 insects. Measurements were made with a graduated slide under the phase contrast microscope at 400×magnification ($BH_2$, Olympus, Tokyo, Japan). The size of basal oöcytes was calculated by the formula used by Loeb et al. (1984) for the size of a prolate spheroid, $4/3\pi(ab^2)$, where $a$ is the radius of the long and $b$ is the short dimension of the same oöcyte (Perveen and Miyata, 2000).

The thickness of the follicular epithelium of basal oöcytes was observed by making a parafilm microtomy conducted according to the method described by Yoshida (1994). Basal oöcytes were fixed in Carnoy's solution for 3 hour, washed in 70% ethanol for 2 hour, and dehydrated in an alcohol series 70, 80, 90, and 95% and twice in 100%, followed by a benzene and ethanol solution (1:1), each for 30 min. Incubation was done three times at 60 °C in benzene and paraffin (1:1), and then in parafin only, each for 30 min. Five-mm microtome sections were cut into a rolling ribbon. It was stained in xylene I (10 min), xylene II (5 min), followed by an ethanol series of 100, 90, 80, and 70%, each for 5 min, Mayer's hematoxylin for 15 min, and washed under running water. Microtome sections were mounted in 1% eosin (10 min), distilled water (2 sec), followed by an alcohol series 50, 70, 80, and 90%, and twice in 100% (each for 1-5 min), xylene and 100% ethanol (1:1; 5 min), xylene I (5 min), xylene II (5 min). Finally, the sections were embedded on microscopic graduated slide in a drop of Canada balsam. The microscopic graduated slide was covered with a glass cover slip. The thickness of follicular epithelium was measured under a phase

contrast microscope ($BH_2$, Olympus, Tokyo, Japan) at 400×magnifcation (Perveen and Miyata, 2000).

### 3.1.3 Data analysis

Data were analyzed using analysis of variance, one way ANOVA (Concepts, 1989) at $P<0.01$ and Scheffe's *F*-test (Scheffe, 1953) at 5%.

## 3.2 Results

The morphology of the adult female reproductive system of *S. litura* is shown in Figure 1.

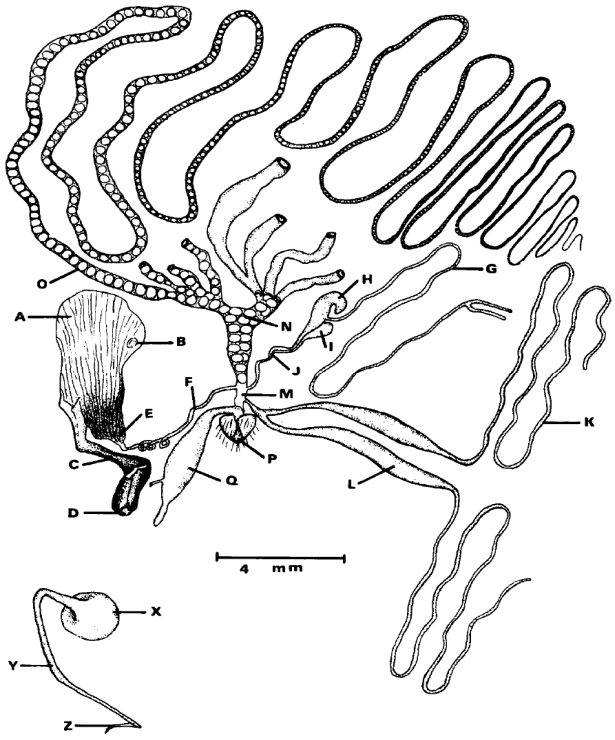



Fig. 1. The morphology of the female reproductive system of *S. litura*: A: corpus bursae; B: signum, C: ductus bursae; D: ostium bursae; and E: diverticulum of bursa copulatrix; F: ductis seminalis; G: spermathecal gland; H: utriculus I: lagena of spermatheca; J: ductus receptaculi; K: accessory gland (paired); L: accessory gland reservoir (paired); M: vestibulum; N: calyx of the unpaired oviductus communis; O: one of four ovarioles of ovary (paired); P: papillae anales; Q: rectum; X: corpus, Y: collum, and Z: frenum of spermatophores; (Source: Etman and Hooper, 1979).

### 3.2.1 Effects on ovarian development

Sublethal doses of chlorfluazuron ($LD_{10}$: 1.00 ng $larva^{-1}$; $LD_{30}$: 3.75 ng $larva^{-1}$), applied to newly ecdysed fifth-instar larvae significantly ($P<0.0001$) reduced the body weight, fresh

ovarian weight and dry ovarian weight in newly emerged adults when compared with the controls (Table 3). Significant reductions were not observed in fresh body weight (P=0.0567), fresh ovarian weight (P=0.7788) and dry ovarian weight (P=0.5757), when the $LD_{10}$ and $LD_{30}$ treatments were compared. Similarly, ratios of fresh ovarian/fresh body weight (31.0%), dry ovarian/fresh ovarian weight (28.0%) and dry ovarian/fresh body weight (9.0%), were not significantly different (Table 3) (Perveen and Miyata, 2000).

| T[a] | n[a] | FBW[a,b] (M±SD) mg | FOW[a,b] (M±SD) mg | DOW[a,b] (M±SD) mg | % R=FOW /FBW[a,b] (M±SD) | % R=DOW /FBW[a,b] (M±SD) | % R=DOW /FOW[a,b] (M±SD) |
|---|---|---|---|---|---|---|---|
| C | 30 | 255±11.6a | 81.1±9.2a | 23.6±1.4a | 31.5±2.9a | 28.9±2.4a | 9.2±0.7a |
| $LD_{10}$ | 30 | 229±3.2b | 72.0±3.4b | 20.0±1.4b | 31.2±1.5a | 28.1±1.2a | 9.0±0.8a |
| $LD_{30}$ | 30 | 224±5.9b | 70.9±4.2b | 19.9±1.3b | 31.3±2.4a | 28.0±0.5a | 9.1±0.8a |

[a]C: control; T: trearment; $LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; n: number of insects used; FBW: fresh body weight; FOW: fresh ovarian weight; DOW: dry ovarian weight; % R: percent ratio
[b]Data were analyzed using one-way ANOVA (Concepts, 1989) at P<0.001. Means within a column followed by different letters indicate significant differences according to Scheffe's F-test (Scheffe, 1953) at 5%.

Table 3. Effects of sublethal doses of chlorfluazuron on the ovarian and body weight of newly emerged adults after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen and Miyata, 2000).

Ovaries are small on the 8th day after pupation. From the 8th day after pupation to the day before adult emergence, ovarian weight slowly increased; after that it increased sharply until the day of adult emergence, and then, increased gradually until the 2nd day after adult emergence, when it reached maximum (120±19.4 mg). Then, in the controls it decreased gradually until the 7th day after adult emergence. The pattern of changes in fresh ovarian weight in the $LD_{10}$- or $LD_{30}$-treated females was similar as observed in the controls during various developmental days of pupae and adults. The fresh ovarian weight was significantly reduced on the 8th day after pupation (P<0.0001); on the 9th day after pupation (P<0.0001); on the 1st day after adult emergence (P<0.0003); on the 2nd day after adult emergence (P<0.0001); on the 3rd day after adult emergence (P<0.0001); on the 4th day after adult emergence (P<0.0001); on the 5th day after adult emergence (P<0.0001); on the 6th day after adult emergence (P<0.0001); on the 7th day after adult emergence (P<0.0001) in the $LD_{10}$- or $LD_{30}$-treated females compared with the controls, but no significant reduction was observed (P=0.0979–0.970) between the $LD_{10}$- or $LD_{30}$-treated females during ovarian development (Figure 2) (Perveen and Miyata, 2000).

In newly emerged the $LD_{10}$- or $LD_{30}$-treated adults, the total length of the ovariole was significantly reduced (P<0.0001) compared with the control, but there were no significant reductions (P=0.0508) between the $LD_{10}$- or $LD_{30}$-treatments. In the $LD_{10}$- or $LD_{30}$-treated insects, the germarium (immature oögonia) was significantly longer (P<0.0001) than that of the pedicle (fully mature ova) and the vitellarium (under developing oöcytes) compared with the controls in which the vitellarium was significantly longer (P<0.0001) than the germarium and pedicle (Figure 3; Table 4) (Perveen and Miyata, 2000; Perveen, 2011).

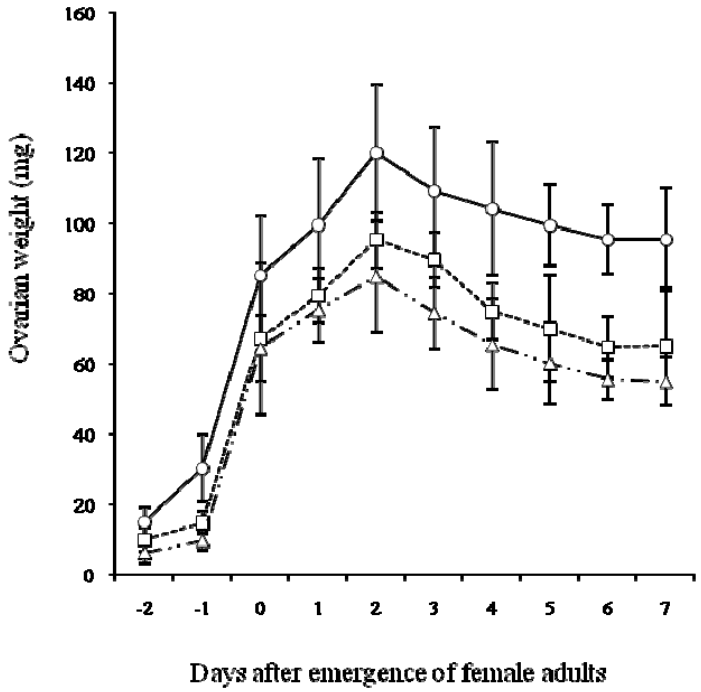



Fig. 2. Effect of sublethal doses of chlorfluazuron on ovarian weight during different developmental days (post pupal and 1 to 7 day after adult emergence). For control (○; n = 10), $LD_{10}$ (1.00 ng larva$^{-1}$) treated (□; n = 9), and $LD_{30}$ (3.75 ng larva$^{-1}$) treated (Δ; n = 9) after topical application to newly ecdysed fifth instars of *Spodoptera litura*. Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0001$ and Scheffe's *F*-test (Concepts, 1989) at 5%. Vertical bars indicate SD; (Source: Perveen and Miyata, 2000).

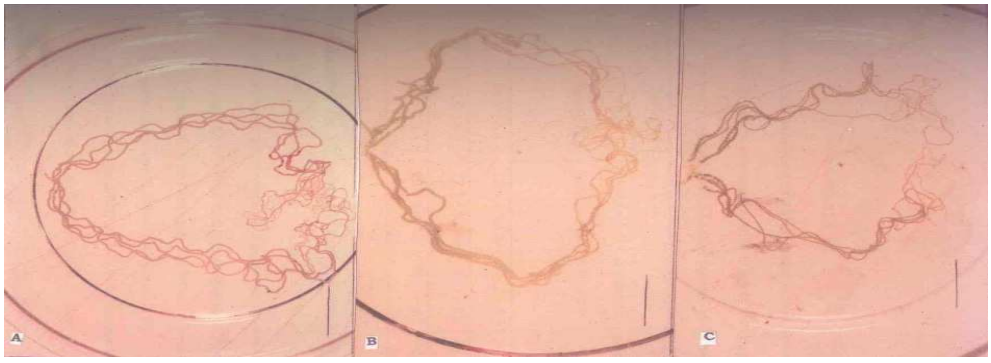



Fig. 3. A comparison of ovarian morphology of newly emerged adult *Spodoptera litura*, in A: untreated (control); B: treated with the $LD_{10}$ dose and C: treated with the $LD_{30}$ dose of chlorfluazuron. Bars in photographs indicate 100 µm (Source: Perveen, 2011).

| Treat-ments[a] | $n_1$ | $n_2$ | TLO[bc] (M±SD)mm | LP[bc] (M±SD)mm | LV[bc] (M±SD)mm | LG [bc] (M±SD)mm | R P:V:G (M±SD)mm |
|---|---|---|---|---|---|---|---|
| Control | 10 | 80 | 104.8±5.1a[3] | 33.5±3.4a | 45.2±4.4a | 26.1±4.2a | 32:43:25 |
| $LD_{10}$ | 9 | 72 | 91.5±5.5b | 22.1±5.0b | 30.5±2.1b | 39.0±2.7b | 24:33:43 |
| $LD_{30}$ | 9 | 72 | 88.7±9.6b | 20.4±4.4 | 28.6±6.7b | 40.0±8.9b | 23:32:45 |

[a]$LD_{10}$, 1.00 ng larva$^{-1}$; $LD_{30}$, 3.75 ng larva$^{-1}$; $n_1$: number of insects used; $n_2$: number of ovariole measured; TLO: total length of ovariols; LP: length of pedicle; LV: length of vitellarium; LG: length of germarium; RP:V:G: Ratio of pedicle:vitellarium:germanium

[b]In Spodoptera litura, the paired ovaries are composed of 8 ovarioles. Four ovarioles are found on each side of the body cavity, forming several loops. Each ovarioles differentiated into 3 parts: (1) pedicle (fully matured eggs), (2) vitellarium (oöcytes and trophocytes), (3) germanium (oögonia) (Etman and Hooper 1979).

[c]Data were analyzed using one-way ANOVA (Concepts, 1989) at P<0.0001. Means within a column followed by different letters indicate significant differences according to Scheffe's F-test (Scheffe, 1953) at 5%.

Table 4. Effects of sublethal doses of chlorfluazuron on ovarian development in newly emerged adults after topical application to newly ecdysed fifth instars of *Spodoptera litura* (Source: Perveen and Miyata, 2000).

When ratios of the length of the pedicle, vitellarium and germarium were compared, they were 32:43:25 for the controls, 24:33:43 for the $LD_{10}$ and 23:32:45 for the $LD_{30}$. There was a significant reduction when the %ratios of the $LD_{10}$ and $LD_{30}$ were compared with the controls, but there was significant reduction between the $LD_{10}$ and $LD_{30}$ treatments (Table 4). When ovarian maturation was observed untreated females had mature ova with an occasional one or two being absorbed (solid ova) in the ovarioles. In the $LD_{10}$-treated females, the spacing in the ovarioles and the absorption of ova different from the control. In $LD_{30}$-treated females, besides the spacing and absorption , sometimes only immature ova (germarium) were found in the ovarioles (data is not presented) (Perveen and Miyata, 2000). Mature ova were not observed in the pupae during the 2nd day before adult emergence, but a few mature ova were found the day before adult emergence. The number of mature ova sharply increased until the 1st day after adult emergence, and then gradually increased until the 2nd day after adult emergence. The maximum number of mature ova 725±2.0 was found on the second day after adult emergence. On the same day, the number of mature eggs was significantly reduced (P<0.0001) in the $LD_{10}$- or $LD_{30}$-treated females as compared with the controls, but no significant reduction was observed (P=0.0984) between the $LD_{10}$ and $LD_{30}$ treatments. From the 2nd day to the 7th day after adult emergence, absorption of mature ova started gradually in the controls, and the $LD_{10}$- or $LD_{30}$-treated females. The pattern of maturation of ova in ovaries was similar in the controls, $LD_{10}$ and $LD_{30}$ treatments (Figure 4) (Perveen and Miyata, 2000).

The cell density, expressed as number of nuclei per mm$^2$, was determined at various days during sexual maturation in the germaria of the controls, $LD_{10}$- or $LD_{30}$-treated females (Table 4.3). In the controls, on the 2nd day of adult emergence, the density was 1636±9.17 nuclei mm$^{2-1}$. The cell density increased until the 3rd day, when it was 1829±8.87 nuclei mm$^{2-1}$ and decreased thereafter. On the 4th day of adult emergence, the cell density was 1323±56.20 nuclei mm$^{2-1}$. In the $LD_{10}$- or $LD_{30}$-treated insects, the patterns of the cell density change in the germarium were the same as in the controls but the values were significantly (P<0.01) lower in the $LD_{10}$-treated females and more were significantly decreased in the $LD_{30}$-treated females compared with the controls, during the days the 2nd, 3rd and 4th of adult female development. There

was also a significant reduction ($P<0.05$) in the cell density between the $LD_{10}$- and $LD_{30}$-treated females during adult development (Table 5) (Perveen, 2011).

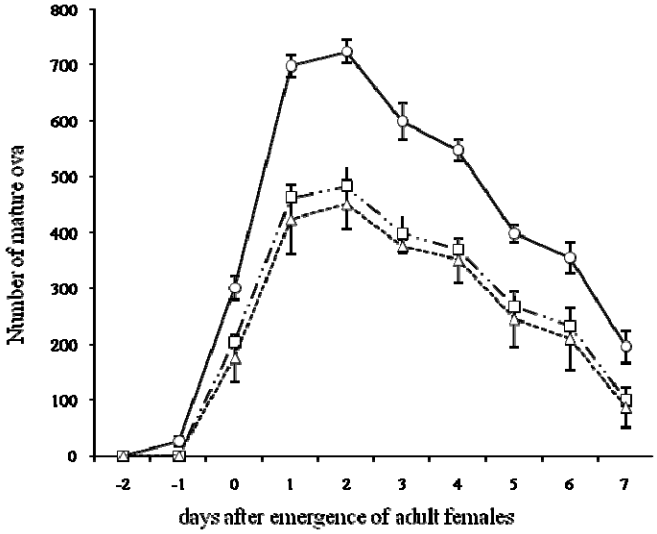



Fig. 4. Effect of sublethal doses of chlorfluazuron on number of mature eggs in the ovaries during different developmental days (post pupal and 1 to 7 day after adult emergence). For control (○; n = 13), $LD_{10}$ (1.00 ng larva$^{-1}$) treated (□; n = 11), and $LD_{30}$ (3.75 ng larva$^{-1}$) treated (Δ; n = 13) after topical application to newly molted fifth instars of *Spodoptera litura*. Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0001$ and Scheffe's *F*-test (Scheffe, 1953) at 5%. Vertical bars indicate SD; (Source: Perveen and Miyata, 2000).

| | | | Cell density number of nuclei (mm$^2$)$^{-1}$ in the germarium during female adults age (M±SD)[b,c] | | |
|---|---|---|---|---|---|
| Treatments[a] | $n_1$ | $n_2$ | 2 day-old | 3 day-old | 4 day-old |
| Control | 5 | 10 | 1636±9.17a | 1829±8.87a | 1323±56.20a |
| $LD_{10}$ | 5 | 10 | 1570±42.50b | 1753±49.91b | 1235±9.50b |
| $LD_{30}$ | 5 | 10 | 1489±8.60c | 1644±7.68c | 1089±61.42c |

[a]$LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; $n_1$: number of insects used; $n_2$: number of ovariole measured

[b]The age of female adults was taken from the day of adult emergence.

[c]Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.01$. Means within a column followed by different letters indicate significant differences according to Scheffe's F-test (Scheffe, 1953) at 5%.

Table 5. Effects of sublethal doses of chlorfluazuron on the cell density in the germarium after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2011).

### 3.2.2 Effects on oöcytes development

In the controls, the basal oöcytes were tiny on the 5th day after pupation, but increased sharply until the 8th day, after which they increased slowly until adult emergence. The maximum size of the basal oöcytes on the day of adult emergence was significantly reduced ($P<0.0002$) in the $LD_{10}$- or $LD_{30}$-treated females, but there was no significant difference ($P=0.9976$) between $LD_{10}$- and $LD_{30}$-treated females (Figure 5) (Perveen and Miyata, 2000).

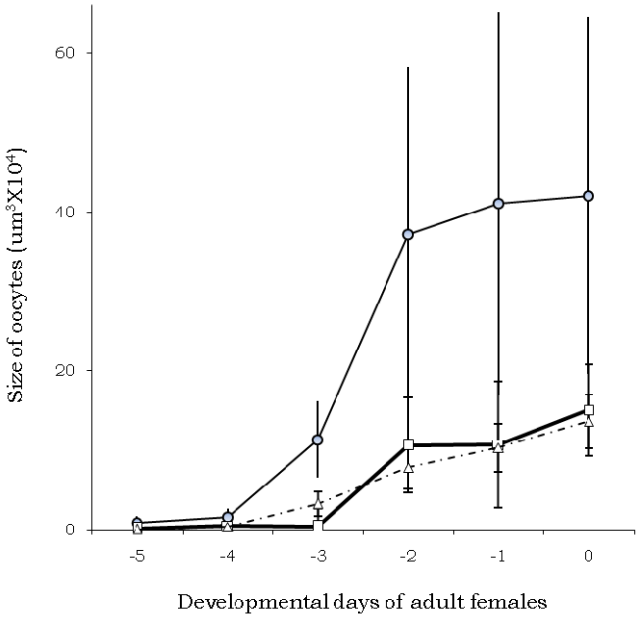



Fig. 5. Effect of sublethal doses of chlorfluazuron on size of basal oöcytes during different developmental days (5 to 9 day after pupation pupae and newly emerged adults). For control (○), $LD_{10}$ (1.00 ng larva$^{-1}$) treated (□), and $LD_{30}$ (3.75 ng larva$^{-1}$) treated (Δ) after topical application to newly ecdysed 5th instars of *Spodoptera litura*. Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0002$ and Scheffe's *F*-test (Scheffe, 1953) at 5%. Verticle bars indicate SD (n = 9-10); (source: Perveen and Miyata, 2000).

The thickness of the follicular epithelium of the basal oöcytes gradually increased and reached a maximum on the 8th day after pupation, after which it sharply declined. On the 8th day after pupation, it was significantly reduced ($P<0.005$) in the $LD_{10}$- or $LD_{30}$-treated females when compared with control females, but there was no significant difference ($P=0.8686$) between the $LD_{10}$ or $LD_{30}$ treatments. The reduction was approximately 34% in the $LD_{10}$- and 39% in the $LD_{30}$-treated females. The patterns of the development of the follicular epithelium of basal oöcytes were not similar in the $LD_{10}$- or $LD_{30}$-treated females compared with the controls. However, pattern was similar between the $LD_{10}$- and $LD_{30}$-treated females. In the $LD_{10}$- or $LD_{30}$-treated females, the follicular epithelium reached maximum on the 9th day after pupation, and then declined. The development of the follicular epithelium was delayed by one day in the $LD_{10}$- or $LD_{30}$-treated females compared with the controls. On the 9th day, it was thicker in the $LD_{30}$-treated females and thickest in

$LD_{10}$-treated females than in control females, but significant differences were not observed (P=0.7611) among these three groups, i.e. controls, $LD_{10}$- and $LD_{30}$-treated females (Figure 6) (Perveen and Miyata, 2000).

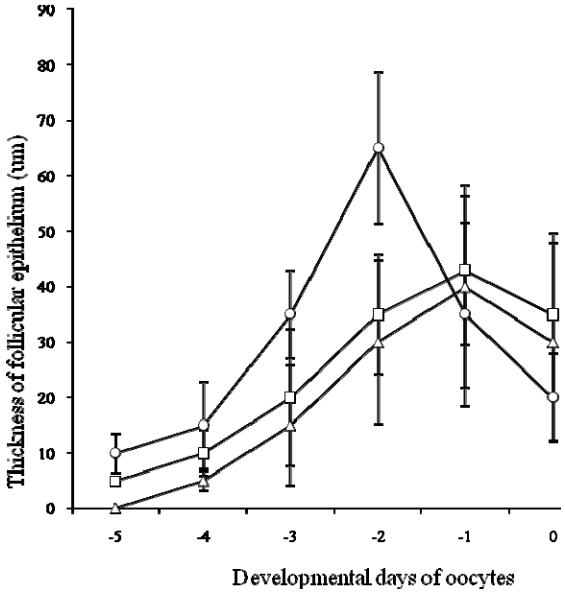



Fig. 6. Effect of sublethal doses of chlorfluazuron on thickness of follicular epithelium of basal oöcytes during different developmental days (5 to 9 days after pupation and newly emerged adults). For control (○), $LD_{10}$ (1.00 ng larva$^{-1}$) treated (□), and $LD_{30}$ (3.75 ng larva$^{-1}$) treated (Δ) after topical application to newly ecdysed 5$^{th}$ instar larvae of *Spodoptera litura*. Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.005$ and Scheffe's $F$-test (Scheffe, 1953) at 5%. Vertical bars indicate SD (n = 9-10); (Source: Perveen and Miyata, 2000).

### 3.3 Discussion

Topical application of sublethal doses of chlorfluazuron ($LD_{10}$: 1.00 ng per larva or $LD_{30}$: 3.75 ng per larva) on newly ecdysed fifth instars had an effect on the reproduction of *S. litura* by reducing fecundity, fertility, and hatchability (Perveen, 2000a). Thus, this study was conducted to establish the causes of the reduction in these parameters. It was found that topical appication of sublethal doses of chlorfuazuron had an affect on ovarian development and oögenesis by decreasing the weight of ovaries during postpupal and adult developmental days of $LD_{10}$ or $LD_{30}$ treated females. The basic factors responsible for the reduction in ovarian weight were reduction in the length of different parts of the ovarioles, decrease in the number of mature ova, reduction in the size of basal oöcytes and thickness of their follicular epithelium, and reduction in protein content of ovarian constituents as compared with the controls. In this study, topical application of sublethal doses of chlorfuazuron signifcantly reduced the ovarian weight to that of the controls in postpupal and adult developmental days (Figure 2; Table 4.3). The ratios of fresh ovarian/fresh body

weight, dry ovarian/fresh ovarian weight, and dry ovarian/fresh body weight were the same among the newly emerged control, $LD_{10}$ or $LD_{30}$ treated females, indicating a reduction in the body, fresh, and dry ovarian weights with the same degree of reduction in treated females and controls. However, Soltani and Pickens and DeMilo (1977) reported that 0.5 mg DFB, when topically applied at pupal ecdysis to *Cydia pomonella* (L.), did not cause the ovarian weight be significantly reduced ($P$<0.05) between control and treated newly emerged adult females. Nor did Hatakoshi (1992) observe any signifcant reduction ($P$<0.05) in the ovarian weight between control and treated last day of pupae to third day after adult emergence of *S. litura,* when 0.3 ng pyriproxyfen was topically applied at pupal ecdysis. The differences in results observed in these experiments may be related to the pesticides, the kind of insect used and their developmental stages. *Spodoptera litura* has paired ovaries that branch into four polytrophic meroistic ovarioles located on the ventral side of the body cavity, making several loops of ovarioles, with all basal oöcytes developing simultaneously each ovariole is differentiated into tree portions according to the developmental stages of the oöcytes: (1) the yellowish green pedicle, where fully matured ova are stored; (2) the reddish orange vitellarium, which contains the developing oöcyte and trophocyte follicles which undergo accumulation of yolk proteins, and choriogenesis; and (3) the whitis germarium, which contains oögoia, from which germ cells proliferate and follicles are formed. Similar observations were reported by Riakhel and Dhadialla (1992) and Etman and Hooper (1979), which were confirmed here.

As in other Lepidopterous species, the ovaries of *S. litura* start to differentiate, and develop at the pupal stage. Indeed, in controls, the thickness of follicular epithelium of basal oöcyte reached its maximum size on the eighth day after pupation. This coincided with the start of follicular epithelium resorption. Histological examination on *S. litura* showed that topical application of sublethal doses of chlorfuazuron to newly ecdysed ffth-instar larvae affected growth and development of oöcytes during pupal and adult stages by affecting size and thickness of follicular epithelium (Figures 5 and 6). However, in *C. pomonella,* (in controls) the basal oöcytes reached their maximum size 7 day after pupation. In this insect, this coincided with the start of follicular epithelium resorption. Hence, a 0.5-mg dose of DFB applied topically to newly ecdysed pupae affected the growth and development of oöcytes by causing a decrease in both the thickness of the follicular epithelium and size of basal oöcytes during the pupal development (Soltani and Mazouni, 1992). Lim and Lee (1982) reported that 2-d-old adult females of *O. japonica,* starved for 6 h and consumed 500 mg (AI) of DFB with two maize discs. The females were found to have retarded ovarian development, caused by a delay of oöcytes development, and an increased percentage of oöcytes resorption. This caused a decrease in fecundity and egg viability of the females. However, significant reduction was not observed either in the number of ovarioles or in the length of basal oöcytes in treated insects. Differences in these results might be a result of the use of different BPUs. Also, the doses used by Lim and Lee (1982) were very high compared with those used in present study or in the Soltani and Mazouni (1992) experiments.

In newly emerged treated adults, the germarium was much longer than the pedicle and vitelarium as compared with the controls in which the vitelarium was longer than the germarium and pedicle (Table 4). This shows that maturation of oöcytes was delayed in treated adult females as compared with the controls. The maximum thickness of the follicular epithelium of basal ooctes was observed on the 9 day after pupation in treated females, whereas it was on the 8 d after pupation in the controls (Figure 6). Subsequent to the 8$^{th}$ or 9$^{th}$ day, resorption of follicular epithelium started in control and treated females,

respectively. When ovarian maturation was scored, as depicted in Figure 4.4, a maximum number of matured oöcytes were found in the second day after adult emergence in the controls. From this day, resorption of mature oöcytes started. The chlorfuazuron-treated females showed the same pattern of mature oöcyte resorption up to the seventh day after adult emergence as in the controls. However, Hatakoshi (1992) reported that when 0-day pupae of female *S. litura* were topically treated with pyriproxyen (0.3 ng per pupa), few or no mature oöcytes were found in newly emerged females, but controls had mature oöcytes with one occasionally being resorbed. The maturation of insect eggs dependent, among other factors, on the materials taken up from the surrounding hemolymph (Telfer et al., 1981), and by materials synthesized by the ovary in situ (Indrasith et al.,1988). These materials include proteins, lipids, and carbohydrates, all of which are required for the embryogenesis (Kunkel and Nordin 1985, *Kanost* et al., 1990). Difubenzuron also caued a decrease in ovarian protein content in *C. pomonella* (Soltani and Mazouni, 1992). Decrease in the ovarian protein content suggests an interference of BPUs with vitellogenesis. It has been reported that DFB could affect ecdysteroid secretion from other organs, such as the epidermis, in *T. molitor* (Soltani, 1984), ovaries in *C. pomonella* (Soltani et al., 1989a; 1989b), and the concentration of hemolymph constituents in *T. molitor* (Soltani, 1990). Future studies should clarify the biochemical mechanism. Moreover, this work dose not clarify why signiÞcant differences were not observed ($P<0.0001$) between effects of $LD_{10}$ (1.00 ng larva$^{-1}$) and $LD_{30}$ (3.75 ng larva$^{-1}$) treated females, although $LD_{30}$ dose was much higher than $LD_{10}$ dose. Further studies are needed to obtain more knowledge about the effects of chlorfuazuron on oögenesis. Currently, the biochemical mechanism involved has been explored.

### 3.4 Conclusion

Sublethal doses of chlorfiuazuron ($LD_{10}$: 1.00 ng larva$^{-1}$ or $LD_{30}$: 3.75 ng larva$^{-1}$) topically applied on newly ecdysed fifth instars of *S. litura* significantly reduced ovarian weight and number of mature eggs in pupae and adults, compared with those of the controls. The ratios of fresh ovarian/fresh body weight, dry ovarian/fresh ovarian weight, and dry ovarian/fresh body weight were the same among controls, $LD_{10}$, and $LD_{30}$ treated newly emerged adults. In treated adults, the germarium was significantly longer than the pedicle and vitelarium compared with those of the controls, whereas in controls the vitelarium was significantly longer than the germarium and pedicle. This indicates a delayed of maturation of ovarioles in treated cutworms. These doses also disrupt growth and development of oöcytes by significantly affecting the size of basal oöcytes and thickness of follicular epithelium. The maximum size of basal oöcytes recorded on the day of adult emergence was significantly reduced in $LD_{10}$ or $LD_{30}$ treated females, compared with those of the controls. The thickness of the follicular epithelium of basal oöcytes reached to a maximum in the controls on the 8$^{th}$ day and in treated females on the ninth day after pupation. The effects of chlorfiuazuron on ovarian development and oögenesis are presumed to be responsible for the reduction in fecundity caused by sublethal exposure to chlorfiuazuron.

## 4. Effects of sublethal doses of chlorfluazurn on male reproductive system of *Spodoptera litura*

The deep yellow-coloured testes of *S. litura* are distinctly paired in larvae and they appear as a single round organ in adults. The testes of *S. litura* resemble those of other lepidopterans,

being enclosed in a common membrane called the scrotum. The testes lie dorsally and appear to be held in place by trachea and strands of basement membrane-like material (Amaldoss, 1989). Although reports of several layers surround the testes of lepidopteran have been made, a single capsule and follicular layers are present in *S. litura* (Amaldoss, 1989). Chase and Gilliland (1972) described the *tunica externa* and *interna* as nothing more than basement membranes over the capsule and follicular layers. The intra-follicular layer is divided into eight incomplete compartments in the tobacco leafminer, *Phthorimaea operculella* (Zeller). This layer is similar to that in the larger canna leafroller, *Calpodes ethlius* (Stoll), and tobacco moth, *Ephestia elutella* (Hübner). It also bears the pigments responsible for the bright yellow-coloured testes. It is clear that spermatogenesis persists in the adult testis.

A characteristic feature of the testicular follicles is the presence of large cells or a nucleated mass of protoplasm in the apex of the germarium. This is known as an apical cell or versonian cell. This is the region where there are successive stages of development of the germ cells occur. The upper part contains the primary spermatogonia and is known as the germarium. This is followed by a region called the zone of growth. The region or zone of growth is where spermatogonia multiply and usually become encysted. The maturation zone, where maturation takes place follows. Finally, is the zone of transformation where the spermatocytes develop into spermatids (spermiogenasis) completing spermatogenesis (Amaldoss, 1989). Two distinct types of spermatozoa are produced in the Lepidoptera: eupyrene (nucleated) spermatozoa which can fertilize the egg; and apyrene (anucleated) which are smaller and completely lacking in nuclear material, and do not appear to play any role in activation of the eggs (Doncaster, 1911; Goldschmidt, 1916). The eupyrene sperm can easily be counted in the male tract because they remain in bundles until they are transferred during mating, but the apyrene sperm are dispersed shortly after they leave the testis. Like eupyrene sperm, the apyrene sperm are produced in large numbers, usually contributing over half the sperm complement, and are transferred to the females with the eupyrene sperm during mating. It was thought that the apyrene sperm did not appear to play any role in activation of the eggs (Friedlander and Gitay, 1972). Their function has remained unclear ever since their discovery by Meves (1902), although several hypotheses concerning the function of the apyrene sperm have been proposed (Silberglied et al., 1984). Holt and North (1970 b) proposed that, in the cabbage looper, *Trichoplusia ni* (Hüebner), apyrene sperm might aid the transport of eupyrene sperm from the male reproductive tract to the female reproductive tract. Katsuno (1977 a) reported that the apyrene sperm in the *B. mori*, might facilitate the migration of eupyrene sperm through the cellular barrier, separating the testis from the efferent ducts. Gage and Cook (1994) reported that nutritional stress seriously affected the number and size of eupyrene and apyrene sperm production in the Indian meal moth, *Plodia interpunctella* (Hübner). However, sperm development in Lepidoptera takes place in the larvae (Munson, 1906; Machida, 1929; Garbini and Imberski, 1977). Usually, spermatogenesis starts in the late larval instars and proceeds on a schedule well correlated with the insect's metamorphosis. Studies *in vitro* and *in vivo* indicated that high titre of juvenile hormone inhibits spermatogenesis, and that sperm mitosis and meiosis require sufficient ecdysteroid titre. During the post-embryonic development of eupyrene and apyrene sperm bundles, when the insect is going to pupation, the juvenile hormone titre declines (Leviatan and Friedlander, 1979). Other factors have also been reported to promote spermatogenesis *in vitro* and *in vivo* (Dumser, 1980 a). In adult males *S. litura*, both apyrene and eupyrene sperm appear in bundles in the testis, but in the vas deferens only the eupyrene sperm were still in bundles, as reported for *B. mori* (Katsuno 1977 b), *T. ni* (Holt

and North, 1970 a) and the army worm, *Pseudaletia separata* (Walk.) (He, 1994). The testes of early larvae contain a large number of spermatogonial cells near the outer border of the follicles. There is a preponderance of spermatocytes containing primary spermatocytes during the penultimate and early last-instar larvae. Secondary spermatocytes are present in the early last-instar larvae, persisting through to the middle of the last-instar larvae. They then begin to differentiate into spermatids. In the process of elongation and maturation of spermatids, the spermatocytes assume an elliptical shape. The sperm bundles formed as a result of maturation of the spermatids are seen abundantly in adults. Spermiogenesis is, however, not synchronous, and spermatozoa in various stages of differentiation can be detected in the testes of freshly emerged adults (Sridevi et al., 1989a). In the pupa, the apyrene sperm bundles emerge from the testicular follicle into the vas efferens earlier than the eupyrene sperm bundles and the bundles separate when they pass through the basement membrane of the testis (Katsuno, 1977b). The apyrene spermatozoa migrate from the vas efferens into the seminal vesicle through the vas deferens during the pupa (Katsuno, 1977c). In the post-pupal period, however, the eupyrene sperm bundles and apyrene spermatozoa migrate simultaneously through the same way (Katsuno, 1977d). The effects of chlorfluazuron have been examined on male reproductive system during testicular development and spermatogenesis when sublethal doses have been topically applied to newly ecdysed fifth-instar larvae of *S. litura*.

### 4.1 Experimental procedure

#### 4.1.1 Histology of testis

Testes from newly molted sixth instar larvae to 5-day-old virgin adult males (treated and control), were dissected in 0.9% of NaCl under a binocular microscope. The length and width of each testis were measured by the same procedure as used for the oöcytes measurement. Testis volume was calculated for larval testes using the formula $4/3\pi$ (length×width$^2$), assuming that the testis is a prolate spheroid (Loeb et al., 1984). For the fused pupal and adult testes, the formula $4/3\pi r^3$ was used, with r as the radius of the globular gonad. The treated and control weight and sheaths thickness of testes were measured by the same procedure used for the ovaries as described above (Perveen, 2000b).

The thickness of treated and control testes sheaths or vas deferenti of untreated and treated relevant stages of insect was observed by making a parafilm microtomy conducted according to the method used by Yoshida (1994) and the procedure to stain the nuclei of sperm was adapted from the method by He (1994) (Perveen, 2000b).

#### 4.1.2 Spermatogenesis

A staining method was used for determining number of the cysts, eupyrene and apyrene sperm for treated and control (He et al., 1995). First, the testis was transferred to a microscopic grid slide (each square= 1mm$^2$) and crushed until it was evenly distributed on the slide. Secondly, several drops of methanol-acetic acid solution (3 : 1; v/v) were added to the slide to fix the reparation for 15 min, and the excess fixing solution was absorbed with filter paper. Third, several drops of 2-5% Giemsa solution dissolved in Sorensen-Gomori buffer solution (monobasic and dibasic sodium phosphate, 0.07 M, pH 6.8) were added to the slide to stain the preparation for 10-30 min. The slide was washed with water and air dried after staining. Finally, the air-dried preparation was observed for counting of bundles and cysts under a phase contrast microscope at 20×mignification. Cysts were classified into the following six developmental stages as described by Chaudhury and Raun (1966): (1)

spermatogonia; (2) primary spermatocytes; (3) secondary spermatocytes; (4) spermatids; (5) elongated cysts with maturing sperm and (6) bundles with fully matured sperm. The length and width of sperm bundles were measured with a calibrated ocular micrometer a phase contrast microscope at 400×magnification (Perveen, 2000b).

### 4.1.3 Data analysis

Data were analyzed using analysis of variance, one way ANOVA (Concepts, 1989) at $P<0.0001$ and Scheffe's $F$-test (Scheffe, 1953) at 5%.

## 4.2 Results

The structural morphogenesis was seen in sixth-instar larvae of *S. litura* during development. When fifth-instar larvae ecdysed into newly sixth-instar larvae (N: 0 day), larvae remained unchanged for upto 2 hour. Then, they changed to a slender surface during 1st day (S). After that, they changed to being puffy during 2nd day (early-last-instar stage: P). Then, they changed to digging stage during 3rd day (mid-last-instar stage: D). Then, they changed to early burrow during 4th day (pre-late-last-instar stage: $B_1$). After that, it changed to late burrow during 5th day (post-late-last-instar stage: $B_2$). The morphogenesis for phase variations used for convenient of observations during experiments is given in Table 6. The morphology of the adult male reproductive system of *S. litura* is shown in Figure 7 (Perveen, 2000b).

| Developmental category (days) | Symbols for phase variations[a] | Name of the phases[a] | Duration (hour day$^{-1}$) |
|---|---|---|---|
| 0 | N | newly ecdysed | 0–2 |
| 1 | S | slender surface | 1st |
| 2 | P | puffy (early last instar stage) | 2nd |
| 3 | D | digging (mid last instar stage) | 3rd |
| 4 | $B_1$ | early burrowed (pre late last instar stage) | 4th |
| 5 | $B_2$ | late burrowed (post late last instar stage) | 5th |

[a]Symbols for phase variations observed during developmental days of sixth-instar larvae were used for the convenience of observations.

Table 6. Structural morphogenesis during five developmental days of sixth-instar larvae of *Spodoptera litura* (Source: Perveen, 2005).

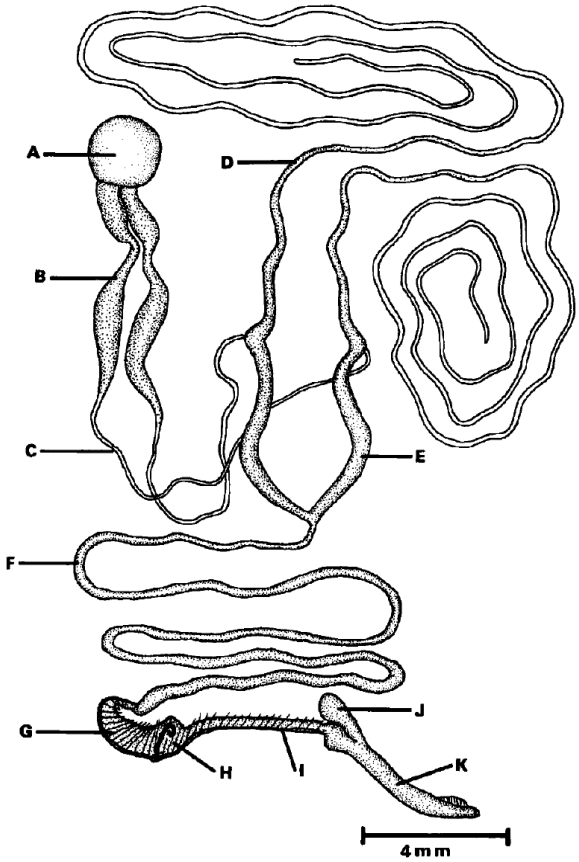



Fig. 7. The morphology of the male reproductive system of *Spodoptera litura*: A: testis; B: seminal vesicle (paired); C: vas deferens (paired); D: accessory glands (paired); E: ductus ejaculatorious duplex; F: primary segment of ductus ejaculatorious simplex; G: muascular area; H: area of frenum formation; and I: area of collum formation of the cuticular secondary segment of the ductus ejaculatorious simplex; J: caecum of aedeagus; K: aedeagus (Source: Etman and Hooper, 1979).

#### 4.2.1 Effects on testicular development

The testes of *S. litura* show the three dimentional measureable structure. Each mature testis consists of four follicles or lobes, each separated by an inner layer of sheath cells. An outer sheath cell layer further surrounds all follicles. In sixth-instar larvae, the volume and weight of the testes gradually then rather sharply increased until the 4$^{th}$ day of sixth-instar larvae. The volume weight decreased when two larval testes fused on the 5$^{th}$ day (last day) after moulting of sixth-instar larvae. They again sharply increased in size, reached a maximum (6.21±1.31 mm$^3$ and 11.94±0.42 mg, n=30, respectively) on the 0 day of pupation and gradually declined until the 5$^{th}$ day after adult emergence. Sublethal doses of chlorfluazuron rapidly disrupted the development of testes by decreasing the volume and weight of testes

compared with the controls. The weight and size of testes were significantly reduced ($P<0.001$) in the $LD_{10}$-treated and more significantly reduced ($P<0.0001$) in $LD_{30}$-treated males compared with the controls from newly ecdysed sixth-instar larvae to the 5th day after adult emergence. Testes reached their maximum size in treated males ($LD_{10}$: 4.16±1.54 $mm^3$ and 8.0±0.83 mg; $LD_{30}$: 2.79±1.00 $mm^3$ and 4.88±1.05 mg; n= 10, respectively) on the same day as the controls. The patterns of development of the testes with respect to the volume and weight were the similar in the controls and the $LD_{10}$- or $LD_{30}$-treated males (Figures 8a and b) (Perveen, 2000b).

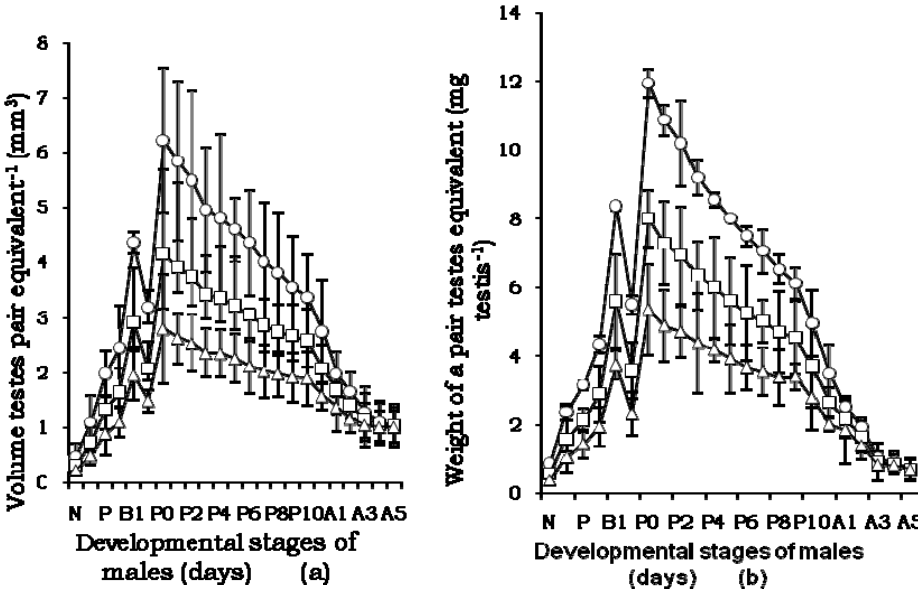



Fig. 8. Effects of sublethal doses of chlorfluazuron ($LD_{10}$: 1.00 ng $larva^{-1}$; $LD_{30}$: 3.75 ng $larva^{-1}$) on the testis volume (a) and weight (b) of *Spodoptera litura* during newly ecdysed sixth-instar larvae to 5th day after adult emergence; controls: O; $LD_{10}$: □; $LD_{30}$: Δ; data analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0001$ and Scheffe`s *F*-test (Scheffe, 1953) at 5%; vertical bars: SD; N, S, P and D: larval (Table 5.1), P: pupal and A: adult developmental days; n = 10 for each point; paired larval testes and fused single pupal or adult testis were considered as testes pair equivalent; (Source: Perveen, 2000b).

The thickness of the testis sheath gradually and then sharply increased until the 4th day of moulting of sixth-instar larvae. It decreased when the two larval testes fused on the 5th day (last-day) of the sixth-instar larvae; it again increased and reached to a maximum [(6±0.51)×$10^{-2}$ mm; n=10] on the 0 day of pupation and gradually declined in the newly emerged adults. The thickness remained constant until the 2nd day after adult emergence. Sublethal doses rapidly disrupted the development of testis by significantly decreasing ($P<0.0001$) the thickness of the testes sheath as compared with that of the controls. This reduction occurred from newly ecdysed sixth-instar larvae to the 0 day of pupation in the $LD_{10}$-treated and the 1st day after pupation in the $LD_{30}$-treated males. The thickness of the

testes sheath in chlorfluazuron-treated males reached a maximum [(5.9±0.67)×10$^{-2}$ mm] in the $LD_{10}$-treated males on the 1st day and [(5.9±1.1)×10$^{-2}$ mm] in the $LD_{30}$-treated males on the 2nd day after pupation whereas, in the controls, it was on the 0 day of pupation. This result shows that attainment of the maximum thickness of the testes sheath was delayed by one day in $LD_{10}$- and by two days in $LD_{30}$-treated males compared with the controls. However, no significant reduction was observed in the maximum thickness of the testes sheath among the control and $LD_{10}$- or $LD_{30}$-treated males. The developmental pattern of the testes sheath in the $LD_{10}$- or $LD_{30}$-treated males was similar to that of the controls (Figure 9) (Perveen, 2000b).

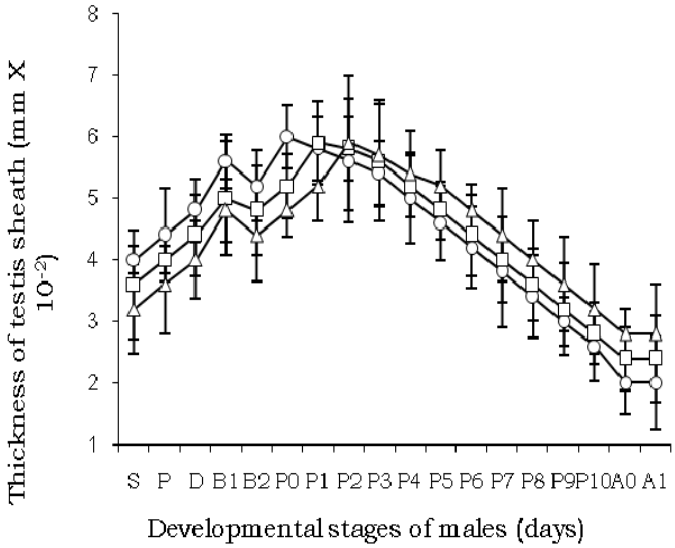



Fig. 9. Effects of sublethal doses of chlorfluazuron ($LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$) on the thickness of testis sheath of *Spodoptera litura* during newly ecdysed sixth-instar larvae to 5th day after adult emergence; controls: O; $LD_{10}$: □; $LD_{30}$: Δ; data analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0001$ and Scheffe`s $F$-test (Scheffe, 1953) at 5%; vertical bars: SD; S, P, D and $B_1$: larval (Table 5.1), P: pupal and A: adult developmental days; n = 10 for each point; paired larval testes and fused single pupal or adult testis were considered as a testes pair equivalent; (Source: Perveen, 2000b).

### 4.2.2 Effects on spermatogenesis

When the different developmental stages of cysts were observed in testes during spermatogenesis on the 1, 3 and 5 day-old sixth-instar larvae, the number of spermatogonia, primary and secondary spermatocytes was significantly reduced ($P<0.001$) in the $LD_{10}$- and even more significantly reduced ($P<0.0001$) in the $LD_{30}$-treated males as compared with those of controls. Spermatids, elongated cysts with mature sperm and eupyrene sperm bundles were not found in the controls and $LD_{10}$- or $LD_{30}$-treated larval testes (Table 7) (Perveen, 2000b).

| TS[a] | T[a] | n[a] | *Sg[b] (M±SD) | *PS[b] (M±SD) | *SS[b] (M±SD) | St (M±SD) | ECMS (M±SD) | ESB (M±SD) |
|---|---|---|---|---|---|---|---|---|
| 1st | C | 13 | 2447±18a | 4893±35a | 1397±18a | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | $LD_{10}$ | 11 | 1960±7b | 3920±7b | 1120±6b | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | $LD_{30}$ | 10 | 1320±6c | 2641±5b | 754±4c | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| 3rd | C | 13 | 1311±8a | 4280±9a | 3146±8a | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | $LD_{10}$ | 11 | 1050±5b | 3430±7b | 2520±7b | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | $LD_{30}$ | 10 | 707±5c | 2311±7c | 1689±7c | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| 5th | C | 13 | 437±8a | 3574±5a | 4717±7a | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | $LD_{10}$ | 11 | 342±5b | 2870±7b | 3780±6b | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| | $LD_{30}$ | 10 | 224±6c | 1933±8c | 2546±9c | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

[a]TS: treatment stages; T: treatments; n: number of males used; C: control; $LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; Sg: spermatogonia; PS: primary spermatocytes; SS: secondary spermatocytes; St: spermatids; ECMS: elongated cysts with mature sperm; ESB: eupyrene sperm bundles; larvae ecdysed usually during 0200 to 0800 hour and collected between 0800 to 1000 hour

[b]Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0001$. Means within columns followed by different letters are significantly different by Scheffe`s F-test (Scheffe, 1953) at 5%.

Table 7. Effect of sublethal doses of chlorfluazuron on spermatogenesis in the testes of sixth-instar larvae during 1st, 3rd and 5th days of development after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2000b).

When different developmental stages of the cysts were observed in testis during spermatogenesis in newly ecdysed pupae, on the 5th and 10th day after pupation, the number of spermatogonia, primary and secondary spermatocytes, were decreased. However, the spermatids and elongated cysts with mature sperm gradually increased in controls. Eupyrene sperm bundles were not present on the 0-5 day-old pupae. However, they were found on the 10th day after pupation (mean: 1002±3.0 numbers). The pattern of spermatogenesis was the same in the controls, $LD_{10}$- and $LD_{30}$-treated male pupae. However, the developmental stages of the sperm were significantly reduced ($P<0.001$) in the $LD_{10}$-treated and even more significantly reduced ($P<0.0001$) in the $LD_{30}$-treated males compared with the controls (Table 8) (Perveen, 2000b).

Different developmental stages of cysts were observed in testes during spermatogenesis in newly emerged, 1 and 2 day-old adults. The spermatogonia were not present in newly emerged and 1 day-old adults. Primary spermatocytes were not found in 1 and 2 day-old adults, but they (mean: 174±4.0 number) were found in newly emerged adults. The secondary spermatocytes, spermatids and elongated cysts with mature sperm were present, but gradually decreased in number in the controls. Eupyrene sperm bundles gradually increased in number in the controls. The pattern of spermatogenesis was the same in the controls, $LD_{10}$- and $LD_{30}$-treated male pupae. However, the stages of sperm development were significantly reduced ($P<0.001$) in the $LD_{10}$- and even more significantly reduced ($P<0.0001$) in $LD_{30}$-treated males as compared with the controls (Table 9) (Perveen, 2000b).

| TS[a] | T[a] | n[a] | Sg[b] (M±SD) | PS[b] (M±SD) | SS[b] (M±SD) | St (M±SD) | ECMS (M±SD) | ESB (M±SD) |
|---|---|---|---|---|---|---|---|---|
| 1st | C | 13 | 262±6a[c] | 1747±5a | 2710±7a | 3670±6a | 349±8a | 0.0±0.0 |
| | $LD_{10}$ | 11 | 205±6b | 1400±4b | 2170±6b | 2940±7b | 280±5b | 0.0±0.0 |
| | $LD_{30}$ | 10 | 142±5c | 943±6b | 1462±5c | 1980±4c | 189± 5c | 0.0±0.0 |
| 3rd | C | 13 | 175±7a | 1047±8a | 2010±4a | 4805±5a | 697±11a | 0.0±0.0 |
| | $LD_{10}$ | 11 | 140±4b | 840±5b | 1610±3b | 3805±5b | 560±5b | 0.0±0.0 |
| | $LD_{30}$ | 10 | 94±5c | 566±6c | 1085±3c | 2593±7c | 377±4c | 0.0±0.0 |
| 5th | C | 13 | 86±4a | 436±5a | 698±3a | 2969±6a | 3581±3a | 0.0±0.0 |
| | $LD_{10}$ | 11 | 70±3b | 350±4b | 560±6b | 2660±4b | 3339±3b | 0.0±0.0 |
| | $LD_{30}$ | 10 | 47±4c | 236±3c | 377±5c | 1792±3c | 2263±4c | 0.0±0.0 |

[a]TS: treatment stages; T: treatments; n: number of males used; C: control; $LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; Sg: spermatogonia; PS: primary spermatocytes; SS: secondary spermatocytes; St: spermatids; ECMS: elongated cysts with mature sperm; ESB: eupyrene sperm bundles; pupation occurred usually during 0200 to 0800 hour and collected between 0800 to 1000 hour.
[b]Data were analyzed using one-way ANOVA (Concepts, 1989) at P<0.0001. Means within columns followed by different letters are significantly different by Scheffe`s F-test (Scheffe, 1953) at 5%.

Table 8. Effect of sublethal doses of chlorfluazuron on spermatogenesis in the testes of pupae during 1st, 5th and 10th days of development after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2000b).

| TS[a] | T[a] | n[a] | Sg[b] (M±SD) | PS[b] (M±SD) | SS[b] (M±SD) | St (M±SD) | ECMS (M±SD) | ESB (M± SD) |
|---|---|---|---|---|---|---|---|---|
| 0 | C | 13 | 0±0a | 174±4a | 524±3a | 1048±4a | 2097±5a | 4893±3a |
| | $LD_{10}$ | 11 | 35±3b | 140±7b | 385±3b | 840±3b | 1680±6b | 3920±4b |
| | $LD_{30}$ | 10 | 24±2c | 94±3b | 259±2c | 566±6c | 1132±3c | 2641±2c |
| 1st | C | 13 | 0±0a | 0±0a | 174±6a | 611±3a | 1047±6a | 6902±5a |
| | $LD_{10}$ | 11 | 14±3b | 28±3b | 98±1b | 490±5b | 840±3b | 5530±4b |
| | $LD_{30}$ | 10 | 9±2c | 19±2c | 66±2c | 330±4c | 566±2c | 3728±5c |
| 2nd | C | 13 | 0.0±0.0 | 0.0±0.0 | 43±3a | 219±4a | 437±6a | 8037±6a |
| | $LD_{10}$ | 11 | 0.0±0.0 | 0.0±0.0 | 35±3b | 175±3b | 350±5b | 6440±5b |
| | $LD_{30}$ | 10 | 0.0±0.0 | 0.0±0.0 | 24±2c | 118±4c | 236±4c | 4338±4c |

[a]TS: treatment stages; T: treatments; n: number of males used; C: control; $LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; M: mean; Sg.: spermatogonia; PS: primary spermatocytes; SS: secondary spermatocytes; St.: spermatids; ECMS: elongated cysts with mature sperm; ESB: eupyrene sperm bundles; adults emerged usually between 2300 to 0200 hour and and between collected 0800 to 1000 hour.
[b]Data were analyzed using 1-way ANOVA (Concepts, 1989) at P<0.0001. Means within columns followed by different letters are significantly different by Scheffe`s F-test (Scheffe, 1953) at 5%.

Table 9. Effect of sublethal doses of chlorfluazuron on spermatogenesis in the testes of adults during 0, 1st and 2nd day of development after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2000b).

In the testis of newly emerged $LD_{10}$- treated adults, the number of eupyrene and apyrene sperm bundles was significantly reduced (P<0.001), and even more significantly reduced

($P<0.0001$) in $LD_{30}$- treated adults compared with the controls. When males were treated with the $LD_{10}$ or $LD_{30}$, the ratio of eupyrene to apyrene sperm bundles was not significantly changed; apyrene sperm bundle comprised about half of the total sperm complement (Table 10).

| T[a] | n[a] | NESB[b] (M±SD) | NASB[b] (M±SD) | Ratios (%)= ESB:ASB |
|---|---|---|---|---|
| Control | 30 | 4893±546a | 4697±520a | 51.1:48.9 |
| $LD_{10}$ | 30 | 3920±426b | 3763±466b | 51.0:48.9 |
| $LD_{30}$ | 30 | 2641±161c | 2386±271c | 52.5:47.5 |

[a]$LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; T: treatments; n: number of males used; NESB: number of eupyrene sperm bundle; NASB: number of apyrene sperm bundle; ESB: eupyrene sperm bundle; ASB: apyrene sperm bundle

[b]Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0001$. Means within columns followed by different letters are significantly different by Scheffe`s F-test (Scheffe, 1953) at 5%.

Table 10. Effect of sublethal doses of chlorfluazuron and comparison of the number of eupyrene and apyrene sperm bundles in the testis of newly emerged unmated adults after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2000b).

| Developmental stages of sperm | T[a] | n[a] | Size (dm in μm) | |
|---|---|---|---|---|
| | | | (M±SD)[b] | ranges (min–max) |
| Spermatogonia (S) | Control | 10 | 7.1±0.7a | 6–8 |
| | $LD_{10}$ | 10 | 5.9±0.8b | 5–7 |
| | $LD_{30}$ | 10 | 4.1±0.9c | 3–5 |
| Primary spermatocytes (D) | Control | 10 | 15.1±0.7a | 14–16 |
| | $LD_{10}$ | 10 | 13.0±1.3b | 11–14 |
| | $LD_{30}$ | 10 | 11.4±1.5c | 10–48 |
| Secondry spermatocyte ($B_2$) | Control | 10 | 31.0±1.8a | 28–33 |
| | $LD_{10}$ | 10 | 29.2±1.6b | 27–31 |
| | $LD_{30}$ | 10 | 25.0±1.2c | 24–27 |
| Spermatids ($P_{10}$) | Control | 10 | 4.1±0.9a | 3–5 |
| | $LD_{10}$ | 10 | 2.9±0.87b | 2–4 |
| | $LD_{30}$ | 10 | 1.9±0.7c | 1–3 |

[a]$LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; T: treatments; n: number of males used; for S, D and $B_2$ refer to Table 5.1; $P_{10}$: ten day old pupae

[b]Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0001$. Means within columns followed by different letters are significantly different by Scheffe`s *F*-test (Scheffe, 1953) at 5%.

Table 11. Effect of sublethal doses of chlorfluazuron on the size of various developmental stages of sperm observed in the testes of sixth-instar larvae and puape after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2000b).

The size of the spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids was significantly reduced (P<0.001) in $LD_{10}$-treated and more significantly reduced (P<0.0001) in $LD_{30}$-treated insects compared with the controls (Table 11) in newly emerged adults (Perveen, 2000b).

In newly emerged adults, the width and length of elongated cysts with mature sperm, eupyrene and apyrene sperm bundles were significantly reduced (P<0.001) in $LD_{10}$- and more significantly reduced (P<0.0001) in $LD_{30}$- treated insects compared with the controls (Table 12) (Perveen, 2000b).

| Developmental stages of sperm | T[a] | n[a] | sizes of various developmental stages | | | |
|---|---|---|---|---|---|---|
| | | | length (μm)[b] | | width (μm)[b] | |
| | | | M±SD | Ranges (min–max) | M±SD | Ranges (min–max) |
| Elongated spermatocytes | Control | 10 | 45.5±2.4a | (42–50) | 40.0±2.0a | (35–42) |
| | $LD_{10}$ | 10 | 42.2±1.7b | (40–44) | 37.2±1.3b | (35–39) |
| | $LD_{30}$ | 10 | 40.0±0.8c | (39–41) | 35.2±1.6c | (34–38) |
| Eupyrene sperm bundles | Control | 10 | 98.0±4.4a | (92–105) | 33.2±1.9a | (30–36) |
| | $LD_{10}$ | 10 | 94.7±1.4b | (93–97) | 31.3±1.4b | (29–33) |
| | $LD_{30}$ | 10 | 91.5±1.0c | (90–93) | 29.1±1.2c | (28–31) |
| Apyrene sperm bundles | Control | 10 | 24.9±1.2a | (23–27) | 16.1±1.7a | (14–19) |
| | $LD_{10}$ | 10 | 94.7±1.4b | (21–25) | 14.2±1.3b | (12–16) |
| | $LD_{30}$ | 10 | 20.3±0.9c | (19–22) | 12.2±1.2c | (11–14) |

[a]$LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; T: treatments; n: number of males used
[b]Data were analyzed using one-way ANOVA (Concepts, 1989) at *P*<0.0001. Means within columns followed by different letters are significantly different by Scheffe`s *F*-test (Scheffe, 1953) at 5%.

Table 12. Effect of sublethal doses of chlorfluazuron on size of various developmental stages of sperm observed in the testes of newly emerged adults after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2000b).

| T[a] | n[a] | Eupyrene sperm bundles in vas deferens | | |
|---|---|---|---|---|
| | | Pre-adult[b] (M±SD) | Newly emerged adult[b] (M±SD) | One day old adult[b] (M±SD) |
| Control | 30 | 102±29a | 1002±116a | 2513±407a |
| $LD_{10}$ | 30 | 0b | 23±4.9b | 1621±159b |
| $LD_{30}$ | 30 | 0b | 0c | 1080±75c |

[a]$LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; T: treatments; n: number of males used
[b]Data were analyzed using one-way ANOVA (Concepts, 1989) at *P*<0.0001. Means within columns followed by different letters are significantly different by Scheffe`s *F*-test (Scheffe, 1953) at 5%.

Table 13. Effect of sublethal doses of chlorfluazuron on the number of eupyrene sperm bundles in the vas deferens during different developmental days of adults after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2000b)

In the vas deferens of male pre-adult controls, the mean number of eupyrene sperm bundles was 102±29, but no sperm bundles observed in the $LD_{10}$- or $LD_{30}$-treated males of the same age (Table 13) (Perveen, 2000b).
In newly emerged control males, the mean number of eupyrene sperm bundles was 1002±116, and in $LD_{10}$-treated adult males, 23 ± 4.9. In $LD_{30}$-treated adults male there was no sperm bundles were observed. Moreover, in 1 day-old $LD_{10}$-treated adult males, the number of eupyrene sperm bundles was significantly ($P<0.001$) reduced and more significantly ($P<0.0001$) reduced in $LD_{30}$-treated males compared with the controls (Table 14) (Perveen, 2000b).
In the testis and vas deferens of newly emerged, $LD_{10}$-treated males, the total number of eupyrene sperm bundles was significantly reduced ($P<0.001$) and more significantly reduced ($P<0.0001$) in $LD_{30}$-treated males had no sperm bundles in the vas deferens compared with the controls (Table 14) (Perveen, 2000b).

| T[a] | n[a] | NESB in testis[a,b] (M±SD) | NASB in vasa deferens[a,b] (M±SD) | TNESB (testis+ vasa deferens)[a,b] (M±SD) |
|---|---|---|---|---|
| Control | 30 | 4893±546a | 1002±116a | 5791±640a |
| $LD_{10}$ | 30 | 3920±426b | 23±4.9b | 3943±425b |
| $LD_{30}$ | 30 | 2641±161c | 0±0c | 2641±161c |

[a]$LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$; T: treatments; n: number of males used; NESB: number of eupyrene sperm bundle; NASB: number of apyrene sperm bundle; ESB: eupyrene sperm bundle; ASB: apyrene sperm bundle; TNESB: total number of eupyrene sperm bundle
[b]Data were analyzed using one-way ANOVA (Concepts, 1989) at $P<0.0001$. Means within columns followed by different letters are significantly different by Scheffe`s *F*-test (Scheffe, 1953) at 5%.

Table 14. Effect of sublethal doses of chlorfluazuron on the total number of eupyrene sperm bundles in the testis and vas deferens of newly emerged adults after topical application to newly ecdysed fifth-instar larvae of *Spodoptera litura* (Source: Perveen, 2000b)

### 4.3 Discussion

Topical application of sublethal doses of chlorfuazuron ($LD_{10}$: 1.00 ng larva$^{-1}$; $LD_{30}$: 3.75 ng larva$^{-1}$) has an effect on reproduction of *S. litura* by reducing the fecundity, fertilityand hatchability. Fecundity was reduced to a similar degree (35±44%) when females, males or both sexes were treated with $LD_{10}$ or $LD_{30}$. Fertility was reduced by 42% or 52% when females were treated with $LD_{10}$ or $LD_{30}$, respectively, and by 60% or 63%, respectively, when males or both sexes were treated with $LD_{10}$. Fertility was reduced by 78% or 80% when males or both sexes were treated with $LD_{30}$. The hatchability was reduced by 20% or 23% when females were treated with $LD_{10}$ or $LD_{30}$, respectively, and by 37% or 39%, respectively, when males or both sexes were treated with $LD_{30}$. Hatchability was reduced by 55% or 56% when males or both sexes were treated with $LD_{30}$ (Perveen, 2000a). Thus, this study was conducted to determine the causes of the reduction in these reproductive parameters. Topical application of sublethal doses of chlorfuazuron has an effect on testis development by decreasing the volume and weight of testes and its sheath thickness, and also on spermatogenesis by decreasing the number of cysts, eupyrene and apyrene bundles during different times in development. Reduction in the testes volume and weight might be caused

by a reduction in the number of cysts, number and size of sperm bundles, thickness of testes sheath, and/or the reduction in protein content of testes constituents. When females are treated with $LD_{10}$ of chlorfuazuron, no signifiant reduction in the inseminated eupyrene sperm number is observed compared with controls. However, $LD_{10}$ and $LD_{30}$ treatment of males significantly reduces (65.8% and 88.6%) the number of inseminated eupyrene sperm. Moreover, no significant ($P<0.0001$) differences in the reduction are observed on the inseminated eupyrene sperm number when males or both sexes are treated either with $LD_{10}$ or $LD_{30}$ (Perveen, 2008). Therefore, the main cause of the reduction in the fecundity, fertility and hatchability is a decrease in inseminated eupyrene sperm numbers. In larval *S. litura*, the bright yellow-coloured testes are distinctly paired, reniform and situated between the 5th and 6$^{th}$ abdominal segments. Each of the lateral testicular lobes is made up of four follicles. The testicular lobes are enclosed within two thick, double-layered peritoneal sheaths. Each of these sheaths comprises two layers of epithelial cells. The external sheath is made up of lightly stained cuboidal cells resting on a basement membrane. This forms a common envelope to all the follicles of the testis and is penetrated by tracheal branches. The inner sheath is made up of more darkly stained elliptical cells, within which muscle fibres are distinguisable. Ingrowths from this sheath in the form of double-walled septa penetrate between the follicles to separate them from one another. The testes sheath reaches its maximum thickness at day 0 of pupation in the controls, whereas in $LD_{10-}$ treated males maximum thickness occurs later at 1 day. In $LD_{30}$-treated males maximum thickness occurs at 2 day after pupation. Difubenzuron affects ecdysteroid secretion from the epidermis in Tenebrio molitor (Soltani, 1984), ovaries of *Cydia pomonella* (Soltani et al., 1987; Soltani et al., 1989b) and also the concentrations of haemolymph constituents in *T. molitor* (Soltani, 1992). Ecdysteroids have been reported to stimulate spermatogenesis in many insect species (Dumser, 1980b; Gelman and Hayes, 1982). In Mamestra brassica (Shimizu et al., 1985), *Heliothis virescens* (Loeb, 1986) and *L. dispar* (Loeb et al., 1988) testes synthesize ecdysteroids. The treatment with chlorfuazuron effects ecdysteroid production by the testis sheet remains to be investigated. Gelman and Hayes (1982) and Gelman et al. (1988) observed in *Ostrinia nubilalis*, the size and weight of separate testes paired. Topical application of the sublethal dose, $LD_{10}$ of chlorfuazuron significantly reduces ($P<0.0001$) the weight and size of testes, and this is even greater in $LD_{30}$-treated males. However, the topical application of similar sublethal doses of chlorfuazuron significantly reduced ($P<0.0001$) ovarian weight in post-pupal and developing pharate adult females as compared with that of the controls (Perveen and Miyata, 2000). However, significant differences are not observed in ovarian weight when adult females are treated with $LD_{10}$ or $LD_{30}$ doses. The maturation of insect testes depends, among other factors, upon the materials that are taken up from the surrounding haemolymph and by materials synthesized by the testes *in situ*. These materials include protein, lipid and carbohydrate, all of which are required for development of the genital tract (Kunkel and Nordin, 1985; Kanost et al., 1990). In newly emerged males from $LD_{10-}$ or $LD_{30}$-treated larvae the eupyrene and apyrene sperm bundles are significantly ($P<0.0001$) smaller in size and number compared with those of controls. Spermatozoa descend regularly from the testis through the vas deferens into the seminal vesicles, which fill with eupyrene sperm bundles with cysts and individual apyrene sperm. Yoshida (1994) also reported that the number of eupyrene sperm bundles in the testis and vas deferens of newly emerged treated ($LD_{10}$) males is reduced by 36%, and the initiation of sperm movement from testis to seminal vesicle was delayed. The present results show that initiation of sperm movement from testis to vas deferens is delayed after chlorofuazuron treatment and this is

caused by the delay of spermatogenesis. When males or both sexes are treated on the first day of pairing, few or none mate. However, seven to nine pairs mate in control or female treated crosses. During the next day, seven to 10 pairs mate in 10±13 pairs of seven combinations of crosses (Perveen, 2008). These results suggest that the delay of the first mating is caused by the delayed spermatogenesis. The effect of chlorfuazuron on testicular development and spermatogenesis is one of the factors responsible for the reduction in fecundity, fertility and hatchability caused by the sublethal doses of chlorfuazuron. More work is in progress to determine the biochemical mechanism of these effects in *S. litura*.

### 4.4 Conclusion

The physiological mechanism of action of chlorfluazuron describes here on testicular development and spermatogenesis when sublethal doses ($LD_{10}$: 1.00 ng larva$^{-1}$ or $LD_{30}$: 3.75 ng larva$^{-1}$) are applied topically to the cuticle of newly ecdysed fifth instars of *Spodoptera litura*. These doses disrupt the growth and development of testes by decreasing the volume and weight of testes and thickness of testes sheath as compared with that of the controls. Additionally, such doses disrupt spermatogenesis by reducing the number and size of eupyrene and apyrene sperm bundles in the testis. Very few or no eupyrene sperm bundles are observed in vas deferens of pre- and newly ecdysed adults compared with controls. This result shows that the transfer of sperm bundles from testes to vas deferens is delayed in treated males. The effects of chlorfluazuron on testicular development and spermatogenesis are thought to be one of the factors responsible for the reduction in fecundity, fertility and hatchability caused by sublethal doses of chlorfluazuron.

## 5. References

Abro, G. H., Memon, A. J. and Syed, T. S. (1997). Sublethal effects of cyhalothrin and fluvalinate on biology of *Spodoptera litura* F. Pakistan J. Zool. 29: 181–184.

Anderson, V. L. and McLean, R. L. (1974). Design of experiments: a realistic approach. Dekker, New York. p. 70.

Amaldoss, G. (1989). Ultrastructure and physiology of the ductus ejaculatorius duplex and seminal vesicle of male *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). Proc. Indian Acad. Sci. (Anim. Sci.) 98 (6): 405–418.

Anonymous, (1969). Summary of panel meeting in "Sterile male technique for eradication or control of harmful insect". Intl. Atom. Ener. Com. Vienna. pp. 80

Baloch, S. H. and Abbasi, Q. D. (1977). Investigation of the ecology and biology of the cutworm (Noctuidae: Lepidoptera) in the Hyderabad region. First Annu. Res. Report, Pakistan Sci. Found. Islamabad. p. 80.

Barbosa, P. (1974). Dissecting fluids and saline solutions. *In*: "Manual of basic techniques in insect' histology". (Mass, A. D. ed.), Oxford Publisher, London. p. 243.

Bargers, A. and Raw, F. (1967). Soil biology. Academic Press. New York. pp. 53. Beeman, R. W. (1982). Advances in mode of action of insecticides. Ann. Rev. Entomol. 27: 253–281.

Benz, G. (1969). Influence of mating, insemination and other factors on oÖgenesis and oviposition in the moth *Zeiraphera diniana*. J. Ins. Physiol. 15: 55–71.

Binnington, K. and Retnakaran, A. (1991). Epidermis: A biologically Active target for metabolic inhibitors. *In*: "Physiology of the insect epidermis" (Binnington, K. and Retnakaran, A. eds.) CSIRO Publications, Australia. p. 307–334.

Brushwein, J. R. and Granett, J. (1977). Laboratory tests of two moulting disruptants, El-494 and dimilin, against the spruce budworm (Lepidoptera: Tortricidae): mortality and reproductive effects. Me. Life Sci. Agr. Exp. St. Mis. Report. p. 192.

Chase, J. A. and Gilliland, F. R. J. (1972). Testicular development in Thet tobacco budworm. Ann. Entomol. Soc. Amer. 65: 901– 906.

Chatani, F. and Ohnishi, E. (1976). Effects of ecdysone on theovarian development of *Bombyx* silkworm. Develop. Gro. Diff. 18: 48–484.

Chaudhury, M. F. B. and Raun, A. S. (1966). Spermatogenesis And testicular development of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyraustidae). Ann. Entomol. Soc. Amer. 59: 1157–1159.

Clark, L., Temple, G. H. R. and Vincent, J. F. V. (1977). The effects of a chitin inhibitor, dimilin on the reproduction of the peritrophic membrane in the locust, *Locusta migratoria*. J. Insect Physiol. 24: 241–246.

Concepts, A. (1989). Super ANOVA. Abacus Concepts, Berkeley, *CA*. pp. 120.

Crystal, M. M. (1978). Diflubenzuron-induced decrease of egg hatch of screw worms (Diptera: Calliphoridae). J. Med. Entomol. 15: 52–56.

Deb, D. C. and Chakaravorty, S. (1981). Effects of a juvenoid on the growth and differentiation of the ovary of *Corcyra cephalonica* (Lepidoptera). J. Insect Physiol. 27: 103–111.

Doncaster, L. (1911). Some stages in the spermatogenesis of Abraxas *grossulariata* and its variety *lacticolor*. J. Gent. 1: 179–184.

Dumser, J. B. (1980 a). The regulation of spermatogenesis in insects. Ann. Rev. Entomol. 25: 341–369.

Dumser, J. B. (1980 b). *In vitro* effects of ecdysterone on the spermatogonial cell in *Locusta*. Intl. J. Invert. Rep. 2: 165–174.

Emam, A. K., El-Refal, S. A. and Degheele, D. (1988). Effect of Sublethal dosages of four chitin synthesis inhibitors on the reproduction potential and F-1 generation of the Egyptian cotton leafworm *Spodoptera litoralis* (boisd.) (Lepidoptera: Noctuidae). Med. Van de Facland. Rij. Gent. 53: 249–254.

Engelman, F. (1979). Insect vitellogenin: identification, bio-synthesis, and role in vitellogenesis. Adv. Insect Physiol. 27: 49–108.

Etman, A. A. M. and Hooper, G. H. S. (1979). Developmental and reproductive biology of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). J. Aus. Entomol. Soc. 18: 363–372.

Friedlander, M. and Gitay, H. (1972). The fate of the normal-a-nucleated spermatozoa in inseminated females of the silkworm, Bombyx mori. J. Morphol. 138: 121–129.

Fytizas, E. (1976). L'action du TH6040 sur la metamorphose de *Dacus oleae* Gemel. (Diptera, Trypetidae). *Z. Angew. Entomol. 31*: 440–444. (Abstract in English read).

Gage, M. J. G. and Cook, P. A. (1994). Sperm size or number? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth, *Plodia interpunctella* (Lepidoptera: Pyralidae). Func. Ecol. 8: 594–599.

Ganiev, S. G. (1986). Residual amount of dimilin in the plantand soil of paistaccio stands in SW Kirgizia. *Lesn. Khoz. 10*: 63–70.

Gelman, D. B. and Hayes, D. K. (1982). Methods and markers for synchronizing the maturation of fifth-stage diapause-bound and non diapause-bound larvae, pupae and pharate adults of the European cornborer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Amer. 75: 485–493.

Gelman, D. B., Woods, C. W. and Borvokovec, A. B. (1988). Ecdysteroid profile for haemolymph and testes from larvae, pupae, and pharate adults of the European cornborer, *Ostrinia nubilalis*. Arch. Ins. Biochem. Physiol. 7: 267–279.

Goldschmidt, R. (1916). The function of the apyrene spermatozoa. Science 44: 544–546.

Gupta, H. C., Bareth, S. S. and Sharma, S. K. (1991). Bioefficacy of edible and non-edible oils against pulse, *Callosobrochus chinensis* L. on stored pulses and their effect on germination. Agri. Biol. Res. 7: 101–107.

Haga, T., Toki, T., Tsujii, Y. and Nishiyama, R. (1992). Development of an insect growth regulator, chlorfluazuron. J. Pestic. Sci.17: S103–S113.

Hajjar, N. P. and Casida, J. E. (1979). Structure-activity relationships of benzoyl phenyl urea as toxicants and chitin synthesis inhibitors in *Oncopeltus fasciatus*. Pestic. Biochem. Physiol. 11: 33–45.

Hashizume, B. (1988). Atabron® 5E, a new IGR insecticide (chlorfluazuron). Jpn. Pestic. Inform. 58: 32–34.

Hatakoshi, M. (1992). An inhibitory mechanism over oviposition in the tobacco cutworm, *Spodoptera litura* by juvenile hormone analogue pyriproxyfen. J. Insect. Physiol. 38: 793–801.

Hatakoshi, M. and Hirano, M. (1990). Effects of S-71639 on the reproduction of *Spodoptera litura.* Adv. Invert. Rep. 5: 429–434.

Haines, L. C. (1981). Changes in colour of a secretion in the reproductive tract of adult males of*Spodoptera littoralis* (Boisduval) (Lepidoptera: Notuidae) with age and mated status. Bull. Entomol. Res. 71: 501–598.

Haynes, K. F. (1988). Sublethal effect of neurotoxic insecticides on insect behaviour. *Ann. Rev. Entomol. 33*: 149–168.

He, Y. (1994). Reproductive strategies in the armyworm, *Pseudaletia separata* (Lepidoptera: Noctuidae): with special reference to rearing density. Ph. D. Thesis. Graduate School of Agricultural Sciences, Nagoya University, Japan. pp. 122.

He, Y., Tanaka, T. and Miyata, T. (1995). Eupyrene and apyrene sperm and their numerical fluctuations inside the female reproductive tract of the armyworm, *Pseudaletia separata.* J. Insect. Physiol. 41: 689–694.

Holst, H. Z. (1974). Die fertility ätsbeeinflussende Wirkung des neuen insektizids P D 60-40 bei *Epilachna varivestis* Muls (Col. occinellidae) und *Leptinotarsa decemlineata* Say. (Col. Chrysomelidae). Z. Pflkranzk. Pflschutz. 81: 1–7. (Abstract in English read)

Holst, H. Z. (1974). Die fertility ätsbeeinflussende Wirkung des neuen insektizids P D 60-40 bei *Epilachna varivestis* Muls (Col. occinellidae) und *Leptinotarsa decemlineata* Say. (Col. Chrysomelidae). Z. Pflkranzk. Pflschutz. 82: 1–7. (Abstract in English read)

Holt, G. G. and North, D. T. (1970a). Spermatogenesis in the cabbage looper, *Trichoplusia ni*. (Lepidoptera: Noctuidae). Ann. Entomol. Soc. Amer. 63: 501–507.

Holt, G. G. and North, D. T. (1970b). Effects of gamma Irradiation on the mechanisms of sperm transfer in *Trichoplusia ni*. J. Insect. Physiol. 16: 2211–2222.

Hunter, E. and Vincent, J. F. V. (1974). The effects of a novel insecticide on insect cuticle. Experientia 4: 484–490.

Indrasith, L. S., Sasaki, T., Yaginuma, T. and Yamashita, O. (1988). The occurrence of a premature form of egg-specific protein in vitellogenic follicles of *Bombyx mori*. J. Comp. Physiol. 158: 1–7.

Jakob, W. L. (1973). Development inhibition of mosquitoes and house fly by urea analogues. *J. Med. Entomol. 10*: 540–543.

James, E. W. (1974). Laboratory and field evaluation of TH-6040, against House fly and stable fly. J. Econ. Entomol. 67: 746–747.

Kanost, M. R., Kawooya, J. K., Law, J. H., Ryan, R. O., Heusden, V. M. C. and Ziegler, R. (1990). Insect haemolymph proteins. *In: "Advances Insect physiology"* (Evans, P. D. and Wigglesworth, V.B. eds.), Academic Press, London. v. 22, p. 299–396.

Karlinsky, A. (1963). Effects de l'ablation des corpora allata imaginaux sur le developement ovarien de *Pieris brassicae* L. (Lepidoptera). *C. R.* Acad. Sci. Paris. *256*: 4101–1103. (Abstract in English read)

Kasuga, H., Osani, M., Yonezawa, Y. and Aigaki, T. (1985). An energy supply device for spermatozoa in the spermatophore of the silkworm. Develop. Gro. Diff. 27: 514–522.

Katsuno, S. (1977a). Studies on eupyrene and apyrene spermatozoa in the silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae). I. The intra testicular behaviour of the spermatozoa at various stages from the 5th instar to the adult. Appl. Entomol. Zool. 12: 142–147.

Katsuno, S. (1977 b). Studies on eupyrene and apyrene spermatozoa in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). II. The intra testicular behaviour of the spermatozoa after emergence. Appl. Entomol. Zool. 12: 148–153.

Katsuno, S. (1977c). Studies on eupyrene and apyrene spermatozoa in the silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae). III. The post testicular behaviour of spermatozoa at various stages from the pupa to adult. Appl. Entomol. Zool. 12: 142–247.

Katsuno, S. (1977d). Studies on eupyrene and apyrene spermatozoa in the silkworm *Bombyx mori* (Lepidoptera: Bombycidae). IV. The behaviour of the spermatozoa in the internal reproductive organs of female moths. Appl. Entomol. Zool. 12: 352–359.

Ker, F. (1977). Investigation of locust cuticle using the insecticide diflubenzuron. *J. Insect Physiol. 23*: 39–48.

Khan, M. F. and Naqvi, S. N. H. (1988). Toxicological studies of diflubenzuron and nicotine cyanurate on house flies larvae. Pakisrtan J. Entomol. 3: 95–106.

Khuhro, R. D., Abbasi, Q. D., Abro, G. H., Baloch, S. H. and Soomor, A. H. (1986). Population dynamics of *Spodoptera litura* (F.) as influenced by ecological conditions and its host plant preference at Tandojam. Pakisrtan J. Entomol. 18: 351–357.

Kunkel, J. G. and Nordin, J. H. (1985). Yolk proteins. *In: "Comprehensive Insect Biochemitry, Physiology and Pharmacology"* (Kerkut, G. A. and Gilbert, L. I. eds.), Pergamon Press, Oxford. v. 1, p. 83–111.

Leuschner, K. (1974). Effects of the new insecticide PDD 60-40 on nymphs and adults of the cabbage bug, *Eurydema oleraceum* L. (Heteroptera, Pentatomidae). Z. Pflkrankh. Pflschutz. 81: 8–12.

Leviatan, R. and Friedlander, M. (1979). The eupyrene-apyrene dichotomous spermatogenesis of Lepidoptera. I. The relationship with post embryonic development and the role of the decline in juvenile hormone titre towards pupation. Develop. Biol. 68: 515–525.

Lim, S. J. and Lee, S. S. (1982). Toxicity of diflubenzuron to the grass hopper, *Oxy japonica*; effect on reproduction. Entomol. Exp. Appl. 31: 154–158.

Loeb, M. J. (1986). Ecdysteroids in testis sheaths of *Heliothis Virescens* larvae: An immunological study. Arch. Insect Biochem. Physiol. 3: 173–180.

Loeb, M., Brand, E. P. and Birnbaum, M. J. (1984). Ecdysteroid production by testes of budworm, *Heliothis virescens*, from last-larval instar to adult. *J. Insect Physiol. 30: 375–381.*

Loeb, M. J., Brandt, E. P., Woods, C. W. and Bell, R. A. (1988). Secretion of ecdysteroids by sheaths of testis of the gypsy moth, *Lymantria dispar*, and its regulation by testis ecdysiotropin. J. Exp. Zool. 248: 94–100.

Machida, J. (1929). Feine experimentelle untersuchung über die apyrene spermatozoen des seidenspinners *Bombyx mori* L. Zur. Beitru. Wissen. 9: 466–510. (Abstract in English read)

Madore, C. D., Drion, G. B. and John, B. D. (1983). Reduction of reproductive potential in spruce budworm (Lepidoptera: Tortricidae) by a chitin-inhibiting insect growth regulator. J. Econ. Entomol. 76: 708–710.

Matsuura, H. and Naito, A. (1997). Studies on the cold-hardiness and overwintering of *Spodoptera litura* F. (Lepidoptera: Noctuidae) VI. possible overwintering areas predicted from meteorological data in Japan. Appl. Entomol. Zool. 32: 167–177.

Matthee, J. J. (1951). The structure and physiology of the egg of *Locustana pardalina* (Walk.). Bull. Develop. Agri. For. Un. S. Afr. no 316: p. 83.

Mellanby, K. (1976). Pesticides, the environment and the balance of nature.*In: "Pesticides and Human Welfare"* (Gunn, D. I. and Steven, J. G. R. eds) Oxford University Press, p. 217–227.

Meves, F. (1902). Über oligpyrene und apyrene spermien und über ihre entstehung, nach beobachtugen an *Paludina* und *Pygaera*. *Arch. Mikro. Anatom. 61*: 1–84. (Abstract in English read)

Minitab, Inc. (1979). Statistics software. Release 12. Minitab Inc., Stat College, USA. p. 16801.

Mitsuhashi, J. (1995). A continuous cell line from pupal ovaries of the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Appl. Entomol. Zool. 30: 75–82.

Munson, J. P. (1906). Spermatogenesis of the butterfly, *Papilio rutulus*. Proc. Boston Soc. Natu. His. 33: 43–124.

Naqvi, S. N. H. and Rub, A. (1985). Effect of dimilin on the activity of melanization enzyme of *Musca domestica* L. (PCSIR strain). J. Sci. Karachi Univ. 13: 171–177.

Nickle, D. A. (1979). Insect growth regulators: New protectants against the almond moth in stored inshell peanuts. J. Econ. Entomol. 72: 816–819.

Nijahout, M. M. and Riddiford, L. M. (1974). The control of egg maturation by juvenile hormone in the tobacco hornworm, *Manduca sexta*. Biol. Bull. 146: 377–392.

Nizam, S. (1993). Effects of allochemicals against 3rd instar larvae of *Musca domestica* L. (Malir strain). Ph. D. Thesis, Department of Zoology, University of Karachi. pp. 250.

Nomura, M. (1994). Physiological effects of pyriproxyfen against the common cutworm, *Spodoptera litura. M. Sc*. Thesis, Laboratory of Applied Entomology, Nagoya University. pp. 125.

Olszak, R. W., Pawlik, B. and Zajac, R. Z. Z. (1994). The influence of some insect growth regulators on mortality and fecundity of the aphidophagous coccinellids, *Adalia bipunctata* L. and *Coccinella septempunctata* L. (Col., Coccinellidae). J. Appl. Entomol. 117: 58–63.

Ottens, R. J. and Todd, J. W. (1979). Effect of diflubenzuron on reproduction and development of *Graphognathus peregrinus* and *G. leucoloma*. J. Econ. Entomol. 72: 743–746.

Pan, M. L. (1977). Juvenile hormone and vitellogenin synthesis in the *Cecropia* silk moth. Biol. Bull. 153: 336–345.

Perveen, F. (2011) Effects of Sublethal Doses of Chlorfluazuron on Ovarioles in the Common Cutworm, *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Journal of Life Science, USA (Accepted).

Perveen, F. (2000a). Sublethal effects of chlorfluazuron on reproductivity and viability of *Spodoptera litura* (F.) (Lep., Noctuidae). J. Appl. Entomol. 124: 223–231.

Perveen, F. (2000b). Effects of sublethal dose of chlorfluazuron on testicular development and spermatogesis in the common cutworm, *Spodoptera litura*. J. Physiol. Entomol. 25: 315–323.

Perveen, F. (2005). Effects of chlorfluazuron on the growth of common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) with special reference to the morphology and histology of the reproductive system. Ph.D. Thesis, Department of Zoology, University of Karachi.

Perveen, F. (2008). Effects of sublethal doses of chlorfluazuron on the number of inseminated sperm in *Spodoptera litura* (Lepidoptera: Nocutidae). J. Entomol. Sci. 11: 111–121.

Perveen, F. and Miyata, T. (2000). Effects of sublethal dose of chlorfluazuron on ovarian development and oögenesis in the common cutworm, *Spodoptera litura* (F.) Lepidoptera: Noctuidae). Ann. Entomol. Soc. Ame. 93: 1131–1137.

Qureshi, R. A., Qadri, S., Anwarullah, M. and Naqvi, S. N. H. (1983). Effect of neopesticides (JHA) on the morphology, emergence and fertility of *Musca domestica* L. (PCSIR strain) and its relation to phosphatases. Zool. Ang. Entomol. 95: 304–309.

Riakhel, A. S. and Dhadialla, T. S. (1992). Accumulation of yolk proteins in insect oÖcytes. Ann. Rev. Entomol. 37: 217–251.

Ross, D. C. and Brown, T. M. (1982). Inhibition of larval growth in *Spodoptera frugipera* by sublethal dietary concentrations of insecticides. J. Agr. Food Chem. 30: 193–196.

Scheffe, H. (1953). A method for judging all contrasts in the analysis of variance. Biometrika 40: 87–104.

Shimizu, T., Moribayashi, A. and Agui, N. (1985). *In vitro* analysis of spermatogenesis and testicular ecdysteroids in the cabbage armyworm, *Mamestra brassicae* L. (Lepidoptera: Noctuidae). Appl. Entomol. Zool. 20: 56–61.

Silberglied, R. E., Shepherd, J. G. and Dickinson, J. L. (1984). Eunuchs: the role of apyrene sperm in Lepidoptera? Amer. Natur. 123: 255–265.

Skibbe, J. T., Challaghan, P. T., Eccles, C. D. and Laing, W. A. (1995).Visulization of pH in the larval midgut of *Spodoptera litura* using $^{31}$P-NMR Microscopy. J. Insect Physiol. 42: 777–790.

Soltani, N. (1984). Effects of ingested diflubenzuron on the longevity and peritrophic membrane of adult mealworms (*Tenebrio molitor* L.). Pestic. Sci. 15: 221–225.

Soltani, N., Quennedey, A., Delbecque, J. P. and Delchambre, J. (1987). Diflubenzuron-induced alterations during *in vitro* development of *Tenebrio molitor* pupal integument. Arch. Insect Biochem. Physiol. 5: 201–209.

Soltani, N., Delachambre, J. and Delbecque, J. P. (1989a).

Stage-specific effects of diflubenzuron on the ecdysteroid titres during the developmental of *Tenebrio molitor*: Evidence for a change in hormonal source. Gen. Comp. Endocrinol. 76: 350–356.

Soltani, N., Mauchamp, B. and Delbeque, J. P. (1989b). Effects of diflubenzuron on the ecdysteroid titres in two insect species. Akad Landwirtsch Wiss. Tag. Ber., DDR, Berlin. 274: 171–177.

Soltani, N. and Mazouni, N. S. (1992). OÖgenesis in mealworms: cell density of germarium, thickness of chorion and ecdysteroid production. Effects of regulators. Med. Fac. Landbouww. Univ. Gent. 62: 565–571.

Sridevi, R., Gupta, A. D. and Ramamurty, P. S. (1989a). Spermatogenesis in *Spodoptera litura* (Lepidoptera: Noctuidae). Entomon 14: 01–09.

Sroka, P. and Gilbert, L. I. (1971). Studies on the endocrine control of post emergence ovarian maturation in *Manduca sexta*. J. Insect Physiol. 71: 2409–2419.

Taft, H. M. and Hopkins, R. A. (1975). Boll weevils: field populations controlled by sterilizing emerging over winter females with a TH-6040 sprayable bait. J. Econ. Entomol. 68: 551–554.

Tahir, S., Anwer, T. and Naqvi, S. N. H. (1992). Toxicity and Residual effects of novel pesticides against rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Pakistan J. Zool. 24: 111–114.

Takahashi, S. and Mizohata, H. (1975). Critical period necessary for the imaginal moulting and egg maturation of the eri-silkworm, *Samia cynthia* Ricini. Zool. Mag. 84: 283–295.

Telfer, W. H., Rubenstein, E. and Pan, M. L. (1981). How the ovary makes yolk in *Hylophora. In: "Regulation of Insect Development and Behaviour"* (Wroclaw, M. K. ed.) Technical University Press, p. 637–654.

Walpole, R. E. and Myers, R. H. (1998). Probability, statistics for engineer scientist, 6$^{th}$ eds. Prentice-Hall, USA.

Williams, C. M. (1952). Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the *Cecropia* silk. Biol. Bull. 103: 120–138.

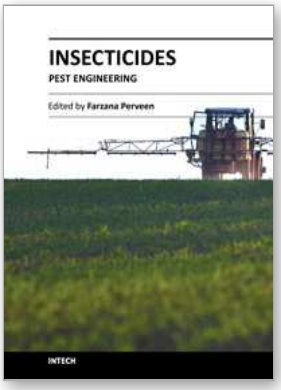
Wright, J. E. and Spates, G. E. (1976). Reproductive inhibition activity of the insect growth regulator TH-6040 against the stable and house fly: Effect on hatchability. J. Econ. Entomol. 69: 365–368.

Wright, J. E. and Retnakaran, A. (1987). "Chitin and Bezoyl phenyl Ureas" (Junk, K. eds.) The Hague. v. 1, pp. 225.

Yoshida, K. (1994). Studies on the physiological effects of chlorfluazuron: with special reference to reproductive effects of chlorfluazuron on *Spodoptera litura*. M. Sc. Thesis, Graduate School of Agricultural Sciences, Nagoya University. p. 69.

Younis, M. (1973). Losses of cotton crop due to insect pests. *Proc. Crop. Produc. Sem. Organ. ESSO (Pak.) Ltd*. p. 110.

Yu, S. J. (1983). Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) larvae. J. Econ. Entomol. 76:




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This book is compiled of 24 Chapters divided into 4 Sections. Section A focuses on toxicity of organic and inorganic insecticides, organophosphorus insecticides, toxicity of fenitrothion and permethrin, and dichlorodiphenyltrichloroethane (DDT). Section B is dedicated to vector control using insecticides, biological control of mosquito larvae by Bacillus thuringiensis, metabolism of pyrethroids by mosquito cytochrome P40 susceptibility status of Aedes aegypti, etc. Section C describes bioactive natural products from sapindacea, management of potato pests, flower thrips, mango mealy bug, pear psylla, grapes pests, small fruit production, boll weevil and tsetse fly using insecticides. Section D provides information on insecticide resistance in natural population of malaria vector, role of Anopheles gambiae P450 cytochrome, genetic toxicological profile of carbofuran and pirimicarp carbamic insecticides, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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Phone: +86-21-62489820
Fax: +86-21-62489821

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