

## Effects of the Insect Growth Regulators Methoxyfenozide and Pyriproxyfen on Adult Diapause in Sunn Pest *Eurygaster integriceps* (Hemiptera: Scutelleridae)

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### ABSTRACT

Sunn pest is a serious pest of cereals causing severe damage to the crop especially wheat. Its life cycle is comprised of two different phases, one is the growth and development phase and the other is diapause phase which takes place at the adult stage. In this study the effects of juvenile hormone analogue Pyriproxyfen, ecdysone analogue methoxyfenozide and a mixture of Pyriproxyfen plus methoxyfenozide on diapause termination of < 24-h-old-, 45day old-, and 90 day old adult Sunn pests were examined using topical application. Pyriproxyfen and a mixture of Pyriproxyfen plus methoxyfenozide induced the growth of the female reproductive organ whilst methoxyfenozide had no effect on adult diapause termination. Also, Pyriproxyfen failed to induce egg deposition in sexually immature adults of Sunn pest. Pyriproxyfen, methoxyfenozide and a combination of the two exerted no effect on the termination of diapause in < 24-h-old treated adult Sunn pests. However, Pyriproxyfen alone and a mixture of Pyriproxyfen plus methoxyfenozide successfully terminated reproductive diapause of 45, and 90 day old adult Sunn pests. Treatment of female only with Pyriproxyfen (10,000 ppm) induced termination of diapause and made egg-laying activity appear. However, egg number and percent of hatchability ( $27.6 \pm 7.5$  and  $9.77 \pm 4.89\%$  respectively) were significantly lower than the treatment of both males as well as females with Pyriproxyfen ( $53.5 \pm 4.8$  and  $33.18 \pm 2.7\%$  respectively) ( $P < 0.01$ ). Treatment of the insect with these Insect Growth Regulators (IGRs) i.e. Pyriproxyfen and a combination of Pyriproxyfen plus methoxyfenozide produced significant differences in the egg and haemolymph protein concentrations, too. Thus, it is shown that these treatments affect vitellogenesis in Sunn pest.

**Keywords:** Diapause, *Eurygaster integriceps*, Methoxyfenozide, Pyriproxyfen.

### INTRODUCTION

Sunn pest (*Eurygaster integriceps* Puton) (Hemiptera: Scutelleridae), is a serious pest of cereals in a wide area of the globe from Near and Middle East to East and South Europe and as well to North Africa. The insect causes severe quantitative and qualitative damage (destruction of gluten protein) to crops (sometimes up to 100%) by feeding on the crop's leaves, stems as well as grains (Radjabi, 2000).

*E. integriceps* possesses a monovoltine life cycle, with obligatorily adult diapause

(reproductive diapause) in each generation regardless of the environmental conditions it lives in. Sunn pest diapause includes arrested development of female's reproductive organ and inhibition of egg deposition. Males undergo weak diapause with diapause causing reduction in maturity of their reproductive organ and sperm production up to almost 45 days post emergence (Unpublished data). The life cycle goes on in two different phases, such that growth and development take place in wheat, whereas diapause (aestivation and hibernation) occurs in such different habitats as forest litters or bushes

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(Critchley, 1998). The long diapause period is divided into two distinct phases, namely aestivation and hibernation (Radjabi, 2000). Thus, it can be estimated that the insect is in the diapausing state during most of the year and is active only in spring for about three months.

Diapause is defined as physiologically controlled suppression of growth, development and/or reproduction. Diapause and non-diapause individuals differ in developmental, physiological, morphological and behavioral characteristics (Denlinger et al., 2005). One of the most important features of adult diapause is a cessation of reproduction. Thus, when an insect undergoes adult diapause, vitellogenesis does not take place following emergence. For this reason immature ovaries have been judged as the criterion of adult diapause, and in males which experience the diapause the testes are reduced in size, while in other cases the testes remain well developed (Denlinger, 2000).

Juvenile Hormone (JH) is known to play an important role in the growth, development, reproduction, diapause, behaviour and caste differentiation of insects. Adult diapause in female insects is characterized by suppression of ovarian development and the cessation of secretion of Juvenile Hormone (JH) by the Corpora Allata (CA). It has been reported that juvenile hormone is required for ovary maturation (Denlinger et al., 2005).

There is evidence that JH plays a major role in diapause regulation in many insects including, *E. integriceps* (Burov et al., 1972), pear psylla *Cacopsylla pyricola* (Foerster) (Krysan, 1990), and the Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Yi and Adams, 2000). Ecdysteroids are capable of breaking larval or pupal diapauses in a wide range of species, while the juvenile hormones are capable of breaking most adult diapauses (Denlinger et al., 2005).

Such gonadotropic hormones as juvenile hormone (JH), ecdysteroids, and peptide hormones released by neurosecretory cells in adults regulate insect vitellogenesis (Belles, 1998). Vitellogenin (Vg) is synthesized in the fat body during vitellogenesis, released into haemolymph and uptaken by the growing oocytes to serve as a nutrient reserve for the developing embryo (Denlinger et al., 2005). Initiation of Vg synthesis is juvenile hormone dependent (Wyatt and Davey, 1996; Sappington

and Raikhel, 1998; Adams, 1999) in most insect species like *Heliothis virescens* (F.) (Zeng et al., 1997), *Helicoverpa zea* (Boddie) (Satyanarayana et al., 1992), *Pseudaletia unipuncta* (Haworth) (Cusson et al., 1994), and *Spodoptera frugiperda* (Smith) (Sorge et al., 2000), cockroach *Blattella germanica* (L.) (Comas et al., 2001). However, in some insects ecdysone also plays a role in Vg synthesis (Kim et al., 2004). It has been shown that ecdysteroids inhibit vitellogenesis in some such insects as cockroaches, beetles and flies (Friedel et al., 1980; Stay et al., 1980; Lanzrein et al., 1981). Also, in some insects like *Bombyx mori* (L.) and *Locusta migratoria* (L.) ecdysone (even exogenous application) is able to terminate diapause (Makka et al., 2002; Kidokoro et al., 2006).

The aim of the current study was to evaluate the effects of a juvenile hormone analogue, Pyriproxyfen, an ecdysone analogue, methoxyfenozide, along with a mixture of Pyriproxyfen and methoxyfenozide on diapause termination in the Sunn pest. Thus, the adult insects were treated at one, 45, and 90 days post emergence along with a number of reproductive parameters evaluated. The evaluated parameters included reproductive organ size, the number of laid eggs, percentage of hatched eggs, the protein content of eggs, the electrophoretic pattern of egg protein bands, the total protein concentration of haemolymph, and the electrophoretic band pattern of haemolymph proteins.

The knowledge thus gained is essential for an understanding of the regulatory mechanism of Sunn pest diapause. Such information will be helpful in developing effective pest management strategies in the control of this species.

## MATERIALS AND METHODS

### Insects

A stock colony of *E. integriceps* was maintained in the laboratory under 16L:8D of photoperiodism at  $26\pm1^{\circ}\text{C}$  and  $55\pm5\%$  RH on soaked wheat kernels as described by Bandani et al. (2009) and Allahyari et al. (2010). Newly emerged adults (< 24-h-old adults) were considered as pre-diapause adults while  $\approx 45$ -day-old and  $\approx 90$ -day-old adults considered as diapause ones in subsequent experiments.

### IGR Treatments

Fresh solutions of Pyriproxyfen (Admiral® Sumitomo Chemical Co., Ltd. Japan) and methoxyfenozide (Runner 2F® Dow AgroSciences Limited) were prepared. Acetone (Merck) and dimethyl sulfoxide (DMSO) (Merck) were used as solvents for Pyriproxyfen and methoxyfenozide, respectively. Preliminary tests were done to choose IGR concentrations as based on their effects on diapause termination. Adult males and females were treated with Pyriproxyfen (5,000; 10,000; 20,000 and 40,000 ppm), methoxyfenozide (30,000; 45,000; 60,000 and 90,000 ppm) and a mixture of Pyriproxyfen (10,000 ppm) plus methoxyfenozide (60,000 ppm). One microliter of the compounds was applied topically to the ventral abdominal segments of adult females and males [<24-h-old, ≈45-day-old (August) and ≈90-day-old (October) adults] using a microapplicator as described by Allahyari *et al.* (2010). Controls were treated with solvents (Acetone or DMSO) alone. Each treatment involved three replicates with each replicate containing ten insects (five females and five males).

### Measurement of Reproductive Organ Size

Testis and ovary sizes of treated adults (<24 hour-old adults), which received Pyriproxyfen, methoxyfenozide, and a combination of Pyriproxyfen and methoxyfenozide, and of control adults were recorded on 7-, 14-, and 21 days post-treatment using a microscope equipped with a drawing tube. Each treatment was comprised of three replicates, with each replicate containing two insects (one female and one male).

### Determination of Egg Number and Their Hatchability

The numbers of eggs laid by 45, and 90 day old adults, following treatment with pyriproxyfen, methoxyfenozide, and a combination of pyriproxyfen plus methoxyfenozide, were recorded during a period of 30 days following the first eggs laid. To do

this, adult males and females were treated with Acetone (C), Pyriproxyfen 5,000 ppm (5P), Pyriproxyfen 10,000 ppm (10P), Pyriproxyfen 20,000 ppm (20P), Pyriproxyfen 40,000 ppm (40P), a mixture of Pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm) (10P+60M). Each treatment was comprised of three replicates, each replicate containing ten insects (five females and five males).

### Determination of Haemolymph Protein Concentration

Haemolymph protein concentrations of Sunn pest females and males were determined in control and IGR treated adults (< 24-h-old and 90 day old) on days 1, 7, 14 and 21 post treatment. The treatments were: Pyriproxyfen 10,000 ppm, methoxyfenozide 60,000 ppm, and, a mixture of Pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm). Control was treated with Acetone.

Protein concentration was measured according to the method of Bradford (1976), using bovine serum albumin (Bio-Rad, Munchen, Germany) as a standard. For haemolymph protein determination, haemolymph from control and from the treated adults (females and males) was collected in a chilled micro capillary pipette through amputated forelegs and diluted (1:1) with anticoagulant buffer (41 mM citric acid, 1.7 mM EDTA, 98 mM NaOH and 186 mM NaCl; pH 4.5) (Strand and Pech, 1995). Each treatment contained three replicates, with each replicate containing 20 insects (ten females and ten males).

The samples were centrifuged at 10,000g for 10 minutes at 4°C to remove haemocytes and other tissue fragments. The resulting supernatants were stored at -20°C for further analyses.

### Determination of Egg Protein Concentration

For egg protein concentration, 1-, 2-, 5- and 6 day old eggs (40 eggs) were collected from treated insects. They were homogenized in 0.02 M phosphate buffer (pH 6.8), centrifugation being applied as described above.



Adult females and males were treated with Pyriproxyfen 10,000 ppm, Pyriproxyfen 40,000 ppm, a mixture of Pyriproxyfen (10,000 ppm) plus methoxyfenozide (60,000 ppm) with the laid egg proteins examined for over 6 days.

### Electrophoresis

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was conducted on 10% slab gels according to the Laemmli (1970). Samples were diluted (1:1) in sample buffer (0.5 M Tris-HCl, pH 6.8, 10% SDS, Glycerol, 2-mercaptoethanol), boiled for 5 minutes and loaded into the gel along with bromophenol blue as tracking dye. Gels were run in Tris-glycine buffer (Tris base, SDS, glycine, pH 8.3). Following electrophoresis, gels were stained in 0.1% coomassie brilliant blue R-250 in 40% methanol and 10% acetic acid at room temperature. Gels were then destained in 40% methanol and 10% acetic acid until bands appeared.

### Statistical Analysis

Data were compared through one-way and two-way Analysis Of Variance (ANOVA), followed by LSD multiple range test. Differences between means were considered to be significant at  $P \leq 0.05$ . SAS Software (SAS, 1997) was employed in all statistical analyses.

## RESULTS

### Effects of IGRs on Reproductive Organ Size

Topical application of Pyriproxyfen and a mixture of Pyriproxyfen plus methoxyfenozide to newly emerged adult Sunn pests induced ovarian maturation in female insects so that the ovaries of the treated insects were significantly larger than those of the controls at every post treatment day (Table 1). By contrast, no ovarian development (i.e. the ovaries were not enlarged) was observed among either control or methoxyfenozide treated insects on any of the days selected for measurement. For example, the width and length of the ovary in methoxyfenozide treated insects at day 7 were 1.58 and 2.04 mm, respectively; whilst width and length of ovary in control insects were 1.40 and 1.67 mm, respectively. As seen in Table 1, there is no significant difference observed between control and methoxyfenozide treated insects. Treatment with Pyriproxyfen caused the ovary to become larger over time as compared with the case for the control and the methoxyfenozide treatment. As an example, the widths and lengths of ovary in Pyriproxyfen treated insects at day 21 were 2.17 and 2.17 mm, whilst these figures in control insects were recorded as 1.62 and 1.67 mm, respectively (Table 1).

A mixture of pyriproxyfen and methoxyfenozide accelerated development of the ovary as compared to controls and insects treated with methoxyfenozide alone (Table 1). The width of the ovary was significantly different for all of the different post treatment days ( $P < 0.01$ ).

There was no significant difference observed in testis width and length between control and different treatments (Table 2). However, testis width and length increased significantly with

**Table 1.** Effects of Pyriproxyfen, methoxyfenozide and mixture a combination of their on the size of the ovary of Sunn pest adults ( $\leq 24$ -h-old), in different post treatment days.

Treatments	Day 7		Day 14		Day 21	
	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)
C	1.40 $\pm$ 0.12d	1.67 $\pm$ 0.17bc	1.46 $\pm$ 0.07c	1.62 $\pm$ 0.06c	1.62 $\pm$ 0.14cd	1.67 $\pm$ 0.10bc
P	1.89 $\pm$ 0.13bc	1.96 $\pm$ 0.08b	2.69 $\pm$ 0.19a	2.29 $\pm$ 0.08a	2.17 $\pm$ 0.17b	2.17 $\pm$ 0.15ab
M	1.58 $\pm$ 0.15cd	2.04 $\pm$ 0.09ab	1.77 $\pm$ 0.10c	1.79 $\pm$ 0.15bc	1.71 $\pm$ 0.09cd	2.08 $\pm$ 0.02ab
P+M	1.67 $\pm$ 0.05cd	1.85 $\pm$ 0.05bc	2.10 $\pm$ 0.07bc	1.69 $\pm$ 0.00bc	2.33 $\pm$ 0.09ab	1.98 $\pm$ 0.17ab

Adults ( $\leq 24$ -h-old) were treated with either C: Acetone, P: Pyriproxyfen (10,000 ppm), M: Methoxyfenozide (60,000 ppm) or a combination of the two P+M. Three replicates, each containing one female were adopted. Means that are followed by different letters are significantly different at  $P < 0.01$  applying LSD tests.

**Table 2.** Effects of Pyriproxyfen, methoxyfenozide and mixture a combination of their on the size of the testis of Sunn pest adults ( $\leq 24$ -h-old), in different post treatment days.

Treatments	Day 7		Day 14		Day 21	
	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)
C	0.83 $\pm$ 0.05c	1.12 $\pm$ 0.07c	1.15 $\pm$ 0.05ab	1.21 $\pm$ 0.08c	1.35 $\pm$ 0.10a	1.69 $\pm$ 0.00ab
P	0.94 $\pm$ 0.09bc	1.08 $\pm$ 0.07c	1.10 $\pm$ 0.07b	1.5 $\pm$ 0.09b	1.12 $\pm$ 0.07b	1.71 $\pm$ 0.09a
M	1.00 $\pm$ 0.04bc	1.23 $\pm$ 0.02c	1.08 $\pm$ 0.02b	1.12 $\pm$ 0.09c	1.21 $\pm$ 0.02ab	1.83 $\pm$ 0.07a
P+M	0.77 $\pm$ 0.07c	1.04 $\pm$ 0.05c	1.21 $\pm$ 0.07ab	1.48 $\pm$ 0.05b	1.15 $\pm$ 0.10ab	1.81 $\pm$ 0.16a

Adults ( $\leq 24$ -h-old) were treated with either C: Acetone, P: Pyriproxyfen (10,000 ppm), M: Methoxyfenozide (60,000 ppm) or a combination of the two P+M. Three replicates, each containing one male were adopted. Means that are followed by different letters are significantly different at  $P < 0.01$  applying LSD tests.

time in both controls and the different IGR treatments ( $P < 0.01$ ).

### Effects of IGRs on Diapause Termination

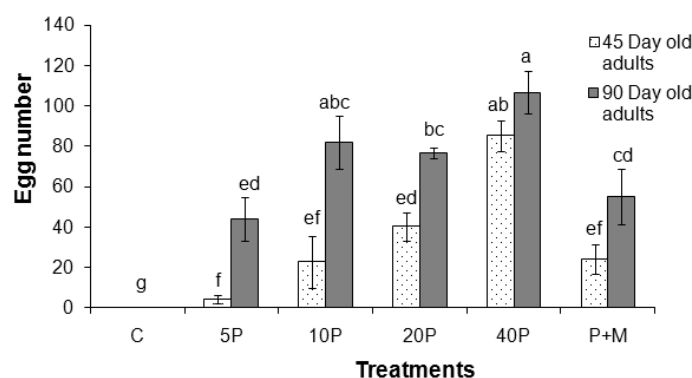
Topical application of pyriproxyfen, methoxyfenozide and a mixture of pyriproxyfen and methoxyfenozide had no effect on termination of diapause in Sunn pest that were treated with IGRs at less than 24 hours following adult emergence ( $< 24$ -h-old). No egg deposition was observed in these treatments. However, when treatment with IGRs was delayed until 45 or 90 days after eclosion, (here designated as 45- and 90 day old treated adults) reproductive

diapause was terminated successfully by application of pyriproxyfen alone and as well by a mixture of pyriproxyfen plus methoxyfenozide. Thus, insects treated in this way started to lay eggs following treatments.

Also, 90 day old treated adults initiated egg laying activity sooner and deposited more eggs (8 day post treatment) than the 45 day old treated adults (13 days post treatment) (Figure 1).

On the other hand, the control and methoxyfenozide treated insects did not terminate diapause with no eggs being observed in either of these treatments, regardless of the age of the insects at the time of treatment (i.e.  $< 24$ h old, 45- and 90 day old treatments).

Diapause termination occurred and egg-laying



**Figure 1.** Total egg number per female (45- and 90 day old treated adults) of Sunn pests over 30 days. Adult males and females were treated as follows: C: Acetone; P5: Pyriproxyfen 5,000 ppm; P10: Pyriproxyfen 10,000 ppm; P20: Pyriproxyfen 20,000 ppm; P40: Pyriproxyfen 40,000 ppm, P+M: A mixture of Pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm). Three replicates, each containing five females and five males were adopted. Means that are followed by different letters are significantly different at  $P < 0.01$  applying LSD tests.

**Table 3.** Effects of treating Sunn pest adults with pyriproxyfen (10,000 ppm) on the number of eggs laid, and their hatchability.

Treatments	Egg number	Hatchability (%)
(F+M)+T	53.47±4.82a	33.18±2.68a
F+T,M-T	27.67±8.57b	9.77±4.89b
F+T	35.53±5.96b	0.00±0.00c
F-T	0.00±0.00c	-
F-T,M+T	0.00±0.00c	-
(F+M)-T	0.00±0.00c	-

(F+M)+T: Females and males, treated with Pyriproxyfen. Three replicates, each containing five females and five males were adopted; F+T,M-T: Females and males were treated with Pyriproxyfen and Acetone respectively; F+T: There was not any males present in the experiment box, females were treated with Pyriproxyfen; F-T: No male was present in the experiment box, females were treated with Acetone; F-T,M+T: Females and males were treated with Acetone and Pyriproxyfen respectively, (F+M)-T: Females and males were treated with Acetone. Means that are followed by different letters are significantly different at  $P < 0.01$  applying LSD tests.

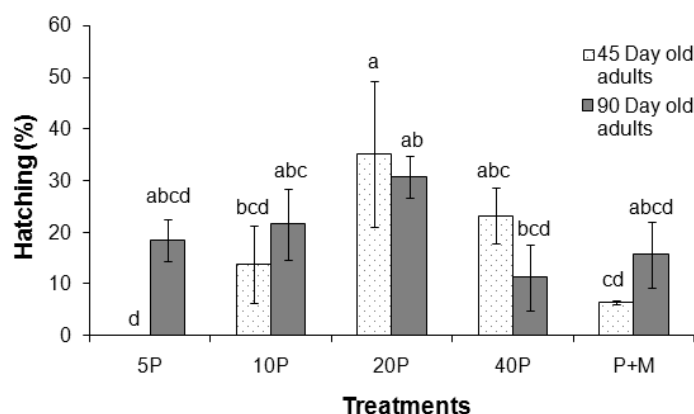
activity appeared when only female insects were treated with just one pyriproxyfen dose (10,000 ppm). However, the number of laid eggs ( $27.6 \pm 7.5$ ) and percentage of egg hatchability ( $9.77 \pm 4.89\%$ ) were significantly lower than where both males and females were treated with pyriproxyfen ( $P < 0.01$ ) (Table 3). IGR treatment of just males had no effect on diapause termination and egg deposition.

#### Effects of IGRs on Egg Number and Its Hatchability

Hatching success (measured as the percentage

of eggs laid that hatched) was dose dependent in eggs of 45 day old females treated Sunn pest except for the at the highest dose. Thus, when pyriproxyfen concentration was increased to 20,000 ppm, egg hatching percent increased, too. However, when the highest dose (40,000 ppm) was employed, egg hatching percent decreased (Figure 2). Thus, the lowest (0%) and the highest (14.06%) egg hatching success was observed when insects were treated with 5,000 and 20,000 ppm Pyriproxyfen. The effect of pyriproxifen on the hatching success of eggs laid by 90 day old treated Sunn pests did not vary significantly for different doses (Figure 2).

Some of the eggs laid by treated females from



**Figure 2.** Hatching success (% of eggs laid) in eggs laid by of 45- and 90 day old treated Sunn pests. Adult males and females were treated as follows: P5: Pyriproxyfen 5,000 ppm; P10: Pyriproxyfen 10,000 ppm; P20: Pyriproxyfen 20,000 ppm; P40: Pyriproxyfen 40,000 ppm, P+M: A mixture of Pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm). Three replicates, each containing five females and five males were adopted. Means that are followed by different letters are significantly different at  $P < 0.05$ , applying LSD tests.

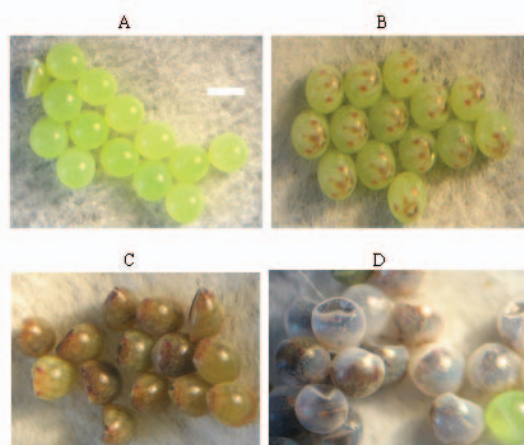
both experimental categories (45 day old adults and 90 day old adults) were abnormal. Germless eggs remained green. Sunn pests that received the mixture of pyriproxyfen and methoxyfenozide often produced such eggs. Some abnormal eggs had germs, but their growth was disordered, so that as they developed they were deformed becoming black and wrinkled (Figure 3).

In 45 day old treated insects, there were no significant differences in the number of eggs laid by each female during the whole period of oviposition activity (30 days) among most pyriproxyfen doses and mixtures of pyriproxyfen and methoxyfenozide. However, the application of 40,000 ppm pyriproxyfen caused treated females to lay more eggs than the other pyriproxyfen doses ( $P < 0.01$ ). The number of eggs in different treatments showed significant differences in 90 day old females ( $P < 0.01$ ). For

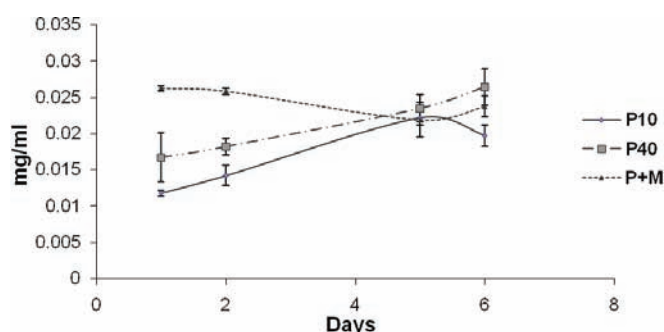
example, application of 5,000 and 40,000 ppm pyriproxyfen caused the lowest (44.12 eggs) vs. the highest (106.9 eggs) egg number, respectively (Figure 1).

### Effects of IGRs on Egg Protein Concentration

There was a significant difference observed in egg protein concentration between different treatments ( $P < 0.01$ ). Eggs of treated females with 10,000 ppm pyriproxyfen vs. a mixture of pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm) showed the lowest ( $0.17 \pm 0.001$ ) and the highest ( $0.24 \pm 0.001$ ) protein concentrations. In all treatments, egg protein concentration increased on days 5 and 6 post oviposition ( $P < 0.05$ ) (Figure 4). Some protein bands in the eggs of



**Figure 3.** Normal vs. abnormal eggs of IGR treated Sunn pest: (A) Eggs without germ; (B) Eggs with normal growing germs, (C and D): Eggs with abnormal growing germs. Scale bar in A (0.7 mm).



**Figure 4.** Changes in the egg protein concentration of IGR treated Sunn pest. Adult females and males were treated as follows: P10: Pyriproxyfen 10,000 ppm; P40: Pyriproxyfen 40,000 ppm, P+M: A mixture of Pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm) and the laid egg proteins, examined over 6 days. Each treatment containing 40 eggs.



females treated with 10,000 ppm pyriproxyfen were not observed in eggs coming from other treatments on days 1 and 2 post oviposition (Figure 5).

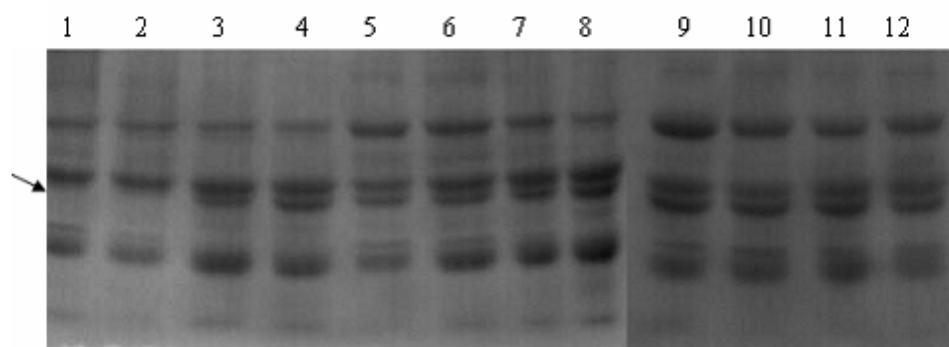
### Effect of IGRs on Haemolymph Protein Concentration

Haemolymph protein concentration was recorded in < 24-h-old and 90 day old IGR treated females and males. There was no significant difference observed in female's haemolymph protein concentration among different treatments at 1 day post treatment. However, protein levels were significantly different in insects from other post treatment days (7, 14 and 21 days post treatment) ( $P < 0.05$ ). Haemolymph protein concentration in control female was reduced 7 days post treatment, but was then elevated throughout the experimental period up to 21 days post treatment (Figure 6). Methoxyfenozide treated females carried significantly lower haemolymph protein concentration than pyriproxyfen- and a mixture of pyriproxyfen plus methoxyfenozide- treated females ( $P < 0.05$ ) (Figure 6). Haemolymph of pyriproxyfen-treated males reached a maximum on day 14 ( $0.73 \pm 0.01$  mg ml<sup>-1</sup>) and then declined.

Treated Sunn pest and control ( $\approx$ 90-day-old

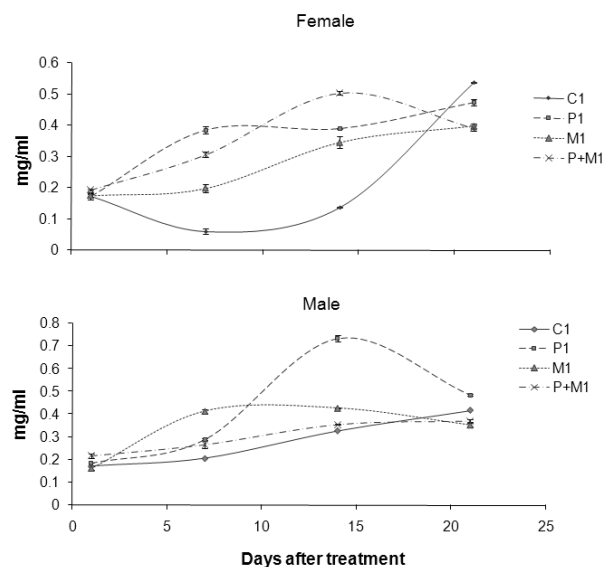
adults) carried lower haemolymph protein concentrations than < 24-h-old treated adults ( $P < 0.05$ ). Also, there was a significant difference in protein content between males and females in control and in all 90 day-old treatments ( $P < 0.05$ ) (Figure 7). In most treatments, the female haemolymph protein concentration was lower than that in the corresponding male treatment. However, treatment with the mixture of pyriproxyfen and methoxyfenozide caused higher haemolymph protein concentrations in females than in males.

A significant increase in haemolymph protein concentration was observed in females that had been exposed to pyriproxyfen ( $P < 0.05$ ). This increase was seen on day 7 post treatment ( $0.21 \pm 0.03$  mg ml<sup>-1</sup>), a time near to the oviposition outset (8 days post treatment). Subsequently, protein level was lowered again on day 21 ( $0.13 \pm 0.01$  mg ml<sup>-1</sup>) and reached a level similar to that of the initial content, i.e. day 1 post treatment ( $0.15 \pm 0.02$  mg ml<sup>-1</sup>) ( $P < 0.05$ ). Haemolymph protein concentrations in females receiving treatment with methoxyfenozide or the mixture of pyriproxyfen and methoxyfenozide were both elevated on day 14 ( $0.19 \pm 0.02$  and  $0.27 \pm 0.01$  mg ml<sup>-1</sup> respectively) and then were lowered on day 21 ( $0.14 \pm 0.02$  and  $0.23 \pm 0.02$  mg ml<sup>-1</sup> respectively) eventually reaching a level similar to the initial content on day 1 post treatment ( $0.12 \pm 0.02$  and  $0.21 \pm 0.02$  mg ml<sup>-1</sup>

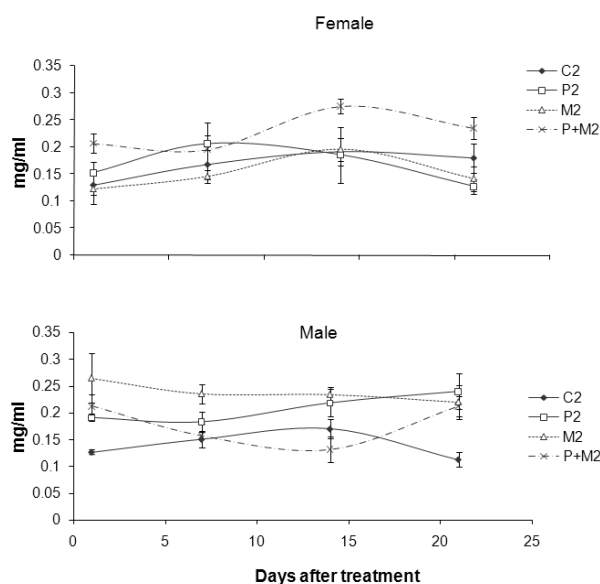


**Figure 5.** SDS-PAGE pattern of Sunn pest egg homogenate proteins following treatment with IGRs. Adult females and males were treated as follows: (1) Pyriproxyfen 10,000 ppm, 1-day- old eggs; (2) Pyriproxyfen 10,000 ppm, 2-day- old eggs; (3) Pyriproxyfen 10,000 ppm, 5-day- old eggs; (4) Pyriproxyfen 10,000 ppm, 6-day- old eggs; (5) Pyriproxyfen 40,000 ppm, 1-day- old eggs; (6) Pyriproxyfen 40,000 ppm, 2-day- old eggs; (7) Pyriproxyfen 40,000 ppm, 5-day- old eggs; (8) Pyriproxyfen 40,000 ppm, 6-day- old eggs; (9) A mixture of Pyriproxyfen and methoxyfenozide, 1-day- old eggs; (10) A mixture of Pyriproxyfen and methoxyfenozide, 2-day- old eggs; (11) A mixture of Pyriproxyfen and methoxyfenozide, 5-day- old eggs; (12) A mixture of Pyriproxyfen and methoxyfenozide, 6-day- old eggs. Each treatment containing 40 eggs.





**Figure 6.** Changes in the haemolymph protein concentrations of females and males of Sunn pest (< 24-h-old) over 21 days. Adult females and males were treated as follows: C1: Acetone; P1: Pyriproxyfen 10,000 ppm; M1: Methoxyfenozide 60,000 ppm, P+M1: A mixture of Pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm). Three replicates, each containing five females and five males were adopted.



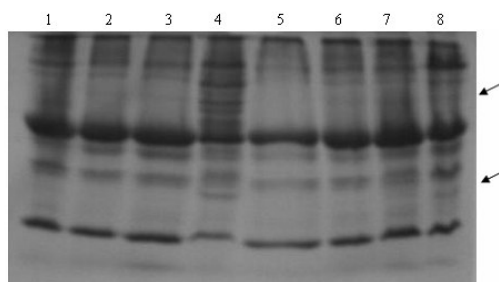
**Figure 7.** Changes in the haemolymph protein concentrations of adult Sunn pest females and males over 21 days in 90 day old control vs. IGR treated insects.

Adult females and males were treated as follows: C2: Acetone; P2: Pyriproxyfen 10,000 ppm; M2: Methoxyfenozide 60,000 ppm, P+M2: A mixture of Pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm). Three replicates, each containing five females and five males were adopted.

respectively) ( $P < 0.01$ ) (Figure 7).

In methoxyfenozide-treated females and males, the protein pattern was similar to that found in the control. In other words,

methoxyfenozide had no visible effect on the haemolymph protein pattern of 90 day old treated males and females (Figure 8). Arrows show visible new bands in Pyriproxyfen alone



**Figure 8.** SDS-PAGE pattern of haemolymph protein of Sunn pest adult females in 90 day old control vs. IGR treated in days 1 and 7 post treatment.

Adult females were treated with Pyriproxyfen 10,000 ppm; methoxyfenozide 60,000 ppm; and a mixture of Pyriproxyfen (10,000 ppm) and methoxyfenozide (60,000 ppm). Control was treated with Acetone. Three replicates, each containing five females and five males were adopted.

(1) Control, day 1; (2) Control, day 7; (3) Pyriproxyfen, day 1; (4) Pyriproxyfen, day 7; (5) Methoxyfenozide, day 1; (6) Methoxyfenozide, day 7; (7) A mixture of Pyriproxyfen and methoxyfenozide day 1, (8) A mixture of Pyriproxyfen and methoxyfenozide day 7.

Arrows show visible new bands in Pyriproxyfen alone vs. a mixture of methoxyfenozide and Pyriproxyfen treatment, at 7 days post treatment.

and a mixture of methoxyfenozide plus Pyriproxyfen treatment, 7 days post treatment (Figure 8).

## DISCUSSION

In most insects, JH or its analogues can induce vitellogenesis and promote ovarian development in sexually immature or in diapause adults (Denlinger *et al.*, 2005). The results obtained from the present investigation showed that topical treatment of adult Sunn pest with pyriproxyfen (a juvenile hormone analogue) successfully terminated reproductive diapause. As a result, and in contrast with controls, the treated insects started oviposition. Also, pyriproxyfen accelerated the development (enlargement) of the ovary, as has been previously observed to occur in prediapause *Conotrachelus nenuphar* (Herbst) (Hoffmann *et al.*, 2007). Both the width and length of the ovary increased in the pyriproxyfen treated Sunn pest females, whereas there was no such enlargement observed in controls.

It has been shown that topical application of fenoxycarb (a juvenile hormone analogue) terminated reproductive diapause of the black rice bug, *Scotinophara lurida* (Burmeister) (Hemiptera: Pentatomidae) and accelerated the development of both ovaries and male accessory glands. These adults developed in their ovaries sooner than the untreated ones (Cho, 2004; Cho *et al.*, 2007). Also fenoxycarb prompted ovarian development in diapausing pear psylla, *Cacopsylla pyricola* (Foerster) (Horton and Lewis, 1996). Agrahari and Gadagkar (2003) reported that juvenile hormone (JH) prompted ovarian development in the *Ropalidia marginata* (Lepeletier) (Hymenoptera: Vespidae). JH III and a JH analogue, methoprene, promoted ovarian maturation and terminated the reproductive arrest in *L. migratoria* (Tanaka, 1994; Tawfik *et al.*, 2002). Diapause termination by topical application of JH or JH analogues was also reported in other such diapausing adult insects as *Aulacophora nigripennis* Motschulsky (Watanabe and Tanaka, 2000), *L. decemlineata* (de Kort *et al.*, 1997). On the other hand these hormones and hormone analogues did not induce diapause or influence its termination in some such other insects as *Aphidius ervi* Haliday (Christiansen-Weniger and Hardie, 1999).

Methoxyfenozide, an ecdysteroid analogue, had no effect on Sunn pest ovary development in terms of its size (width and length). Lagueux *et al* (1976) reported that ecdysteroids did not play a role in vitellogenesis and ovarian maturation in the *L. migratoria*. Similar results have been reported regarding *Modicogryllus confirmatus* (Walker) (Tanaka, 1994; 1999). However, in another cricket *Gryllus bimaculatus* De Geer (Behrens and Hoffmann, 1983) exogenous 20-hydroxyecdysone induced ovarian growth and egg deposition. Also 20-hydroxyecdysone accelerated diapause termination in *Drosophila melanogaster* Meigen (Richard *et al.*, 1998). Ecdysone or 20-hydroxyecdysone induced follicular growth and deposition of yolk in sugar-fed female *Aedes aegypti* (L.) (Lea, 1982).

JH III and a JH analogue, methoprene could not induce ovarian maturation and the reproductive diapause termination in sexually immature females of *Nomadacris succincta* (L.) and *N. japonica* (Bolivar) (Okuda *et al.*, 1996). Also the present results show that pyriproxyfen failed to induce diapause termination and egg

deposition in sexually immature adult Sunn pest. Similarly, it has been shown that JH application terminated diapause in *L. decemlineata* which had spent 2 months in diapause, whereas in pre-diapausing beetles it could postpone only diapause induction (Schooneveld *et al.*, 1977).

The difference in the developmental rate of the ovarioles in summer and autumn (the inert reproductive period) results in differences in susceptibility of the Sunn pest females to juvenile hormone analogues at this time (Reutskaya, 1976).

Adults of *Eurygaster integriceps* had the highest juvenile hormone content during the period between emergence and diapause and the lowest during the 2nd half of winter diapause. In females, a higher content of juvenile hormone was recorded just before diapause and a lower one during diapause. The sensitivity of adults to juvenile hormone analogues was inversely related to the endogenous hormone content (Novozhilov *et al.*, 1984).

Sperm do not mature in adult Sunn pest until 2 months post emergence. We found that applied doses of pyriproxyfen could not terminate Sunn pest reproductive diapause in immature adults. However, the same doses were able to accelerate sperm maturation from almost 60 days to about 40 days, and also caused earlier mating (Zarnegar, 1995). Treatment of Sunn pest males with juvenoids during the first 35 days of adult life (the period of prediapause and diapause initiation) accelerated spermatogenesis but did not result in its completion, so that no sperm was transferred during pairing (Shinyaeva, 1981).

In the present study, the increase of protein concentration and appearance of new band in the SDS-PAGE pattern of haemolymph of treated Sunn pest may be due to the enhancement of vitellogenin in haemolymph because it has been reported that vitellogenin, the precursor of yolk protein, is the major protein in female's haemolymph. Pyriproxyfen increased protein concentration in *Tenebrio molitor* (L.) haemolymph, but, it did not affect protein band pattern (Aribi *et al.*, 2006). In *Perillus bioculatus* (F.) JH III increased vitellogenin levels of diapausing females over 120-fold (Adams *et al.*, 2002). Pyriproxyfen and methoprene induced the appearance of vitellogenin in the *L. migratoria* haemolymph whereas adult males produced no vitellogenin (Edwards *et al.*, 1993). Also in *L.*

*decemlineata*, pyriproxyfen caused induction of vitellogenin synthesis (Yi and Adams, 2000). The vitellogenin concentration in the blood of diapausing hemipteran *Oncopeltus fasciatus* females was not significantly affected by juvenile hormone analogue, apparently because synthesis kept pace with ovarian uptake in this case (Kelly and Davenport, 1976).

In conclusion, it could be said that the juvenile hormone analogue Pyriproxyfen, can successfully terminate Sunn pest reproductive diapause if it is applied to sexually mature adults. However, the ecdysone analogue methoxyfenozide had no effect on Sunn pest reproductive diapause termination. It is assumed that ecdysone does not play a major role in the reproductive diapause in Sunn pest.

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## اثرات تنظیم کننده های رشد متوکسی فنوزید و پایرپروکسی فن بر دیابوز حشره کامل سن گندم

*Eurygaster integriceps* (Hemiptera: Scutelleridae)

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### چکیده

سن گندم یکی از آفات کلیدی غلات است که سبب خسارت شدید به غلات مخصوصاً گندم میشود. چرخه زندگی آن دو مرحله مختلف دارد. یکی مرحله رشد و نمو بوده و دیگری مرحله دیابوز است که در حشره کامل رخ میدهد. در این مطالعه، اثرات شبه هورمون جوانی پایرپروکسی فن، شبه هورمون پوست اندازی متوکسی فنوزید و مخلوط پایرپروکسی فن و متوکسی فنوزید بر پایان دیابوز حشرات کامل سن گندم با ۲۴ ساعت عمر، ۴۵ و ۹۰ روز عمر به روش کاربرد تماسی آزمایش شد. پایرپروکسی فن و مخلوط پایرپروکسی فن و متوکسی فنوزید سبب القاء رشد در اندام تولید مثلی حشره ماده شد، در حالی که متوکسی فنوزید هیچ تاثیری بر پایان دیابوز حشره کامل نداشت. همچنین پایرپروکسی فن نتوانست در حشرات کامل سن گندم که از نظر جنسی نابالغ بودند، تخمگذاری را القاء کند. پایرپروکسی فن، متوکسی فنوزید و مخلوط پایرپروکسی فن و متوکسی فنوزید، هیچ تاثیری در پایان دیابوز در حشرات کامل سن گندمی که در ۲۴ ساعت اول عمرشان تیمار شده بودند، نداشت. اما پایرپروکسی فن به تنهایی و مخلوط آن با متوکسی فنوزید به طور موفقیت آمیزی دیابوز تولید مثلی را در حشرات کامل ۴۵ روزه و ۹۰ روزه تیمار شده سن گندم پایان بخشید. تیمار فقط حشرات ماده با پایرپروکسی فن (۱۰۰۰۰ پی پی ام) پایان دیابوز را القاء کرده و فعالیت تخمگذاری آشکار شد. اما تعداد تخم و درصد تفریخ (به ترتیب ۷/۵ ± ۲۷/۶ و ۹/۷۷ ± ۴/۸۹ درصد) بطور معنی داری نسبت به زمانی که هم نرها و هم ماده ها با پایرپروکسی فن تیمار شده بودند (به ترتیب ۴/۸ ± ۵۳/۵ و ۲/۷ ± ۳۳/۱۸ درصد) کمتر بود ( $P < 0.01$ ). تیمار حشرات با این تنظیم کننده های رشد حشرات (IGRs) یعنی پایرپروکسی فن و مخلوط پایرپروکسی فن و متوکسی فنوزید در غلظت پروتئین تخم و همولف نیز تغییرات معنی داری ایجاد کرد. بنابراین نشان داده شد که این تیمارها در ویلوزنر در سن گندم تاثیر میگذارد.