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Reduction of desert locust *Schistocerca gregaria* (Orthoptera: Acrididae) fecundity after pyriproxyfen treatment

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Abstract :

Desert locust *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) 5th nymphal instar, were feed on Egyptian clover treated with five pyriproxyfen concentrations (1000, 500, 250, 125 and 62.5 ppm). Such treatments caused mortality ranged between 36.67 and 93.33 % after 7 days, while the mortality in control treatment was 3.33%. Also, the treatments caused different degrees of molting failure. The percentages of adult emergence ranged between 6.67 and 63.33%. The maturation period of treated adult significantly prolonged in compare with untreated ones. Moreover, pyriproxyfen caused significant reduction in the egg production, also different degrees of destruction into oocytes of treated females when examined under light microscope.

Introduction

Desert locusts *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) can increase their population rapidly when suitable conditions are available e.g., rainfall, and vegetation (Showler, 2002). Such increase in population could lead to swarms formation which migrate to another area carried by wind, causing damage to pasture and crops (Magor *et al.* , 2008). The most successful control strategy for desert locust is preventive control method which mean early intervening, by controlling hopper bands before reaching adult stage and forming swarms (Uvarov, 1937). Pyriproxyfen is JH mimics which showed strong activity against many pests include hormonal imbalance and suppress embryogenesis, metamorphosis, and adult formation, while it considered harmless to non-target organisms (Dorn *et al.*, 1981).

Hence the aim of the present study is to investigate the effect of pyriproxfen on the mortality of 5th nymphal instars under laboratory condition as well as it is influence on the reproduction of resulted adults, moreover it's histopathological effect on the adult female ovary.

Martials and methods

1. Tested *Schistocerca gregaria* nymphs:

Newly molted 5th nymphal instar (1day after molting) of *S. gregaria* were used in the present study, these nymphs were obtained from the stock colony of Locust and Grasshoppers Research Dep., which conserved under crowded conditions as described by Hunter-Jones (1961) for several years.

2. Pyriproxyfen:

The insecticide pyriproxyfen, 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy]pyridine under the trade name Admiral 10% WE, were used in the present study.

3. Bioassay and treatments:

Five concentrations of pyriproxyfen (1000, 500, 250, 125 and 62.5 ppm) were prepared, about 75 g of fresh clover (Berseem**)** *Trifolium alexandrinum* were dipped in 100 ml of each concentration for 5 minutes, left for air dray under room temperature, then each treated clover introduced to 30 newly molted *5th* instar nymphs (1-2 days after molting) of *S. gregaria*, each treated group were left with treated clover under rearing room conditions to feed for 24 hours then separated into 3 replicates 10 nymphs each. Another 30 nymphs were feed same way on clover dipped in 100 ml of tape water as control treatment and separated into 3 replicates. Mortality and malformation were observed, and clean food were introduced, daily. The days before final molt, before adult maturation, and each egg pod laid by mature adult female were recorded. Survived mature adults were paired and provided with cups filled with clean moistened sand for egg deposition. Sand cups were changed daily and tested for presence of egg pods.

4. Histological studies:

Samples of treated and untreated survived mature adult females, were dissected in saline solution, ovaries were removed and fixed in Bouin's solution for 24 hours, washed several times with 70% alcohol, dehydrated in ascending series of ethyl alcohol, cleared in xylene, then embedded in three changes of pure paraffin wax (Drury and Wallington, 1980). Serial section of 6 micron thick were cut, mounted on glass slides, and stained with haematoxylin and eosin. The slides were photographed using light microscope.

5. Statistical analysis:

Mortality data were subjected to correction according to Schneider-Orelli's formula (Püntener, 1981). For each used concentration the LT_{25}, $LT_{50,}$ and LT_{90} were calculated using Ldp Line software (http://www.ehabsoft.com/ldpline) according to Finney (1971). Data of 5^{th} nymphal instar longevity, adult maturation period and period between each egg pod laying were subjected to analyses of variance.

Results and discussion

1. Bioassay experiment :

In the present study accumulative mortality were recorded daily for 7 days post treatment due to pyriproxyfen mode of action which act by the time of molting. Table (1) demonstrate the LT_{25}, $LT_{50,}$ and LT_{90} values as well as the slopes of days-mortality relationship for each treatment, also data illustrated in Figure (1) show the actual daily accumulative mortality of *S. gregaria* 5^{th} nymphal instar feed on deferent concentrations of pyriproxyfen after 7days post treatment, it's clear that mortality was dose and time dependent, the LT_{50} values were 4.46, 4.95, 6.11, 7.36 and 9.83 day after treatment with 1000, 500, 250, 125 and 62.5 ppm of pyriproxyfen, respectively. On LT_{50} basis there were no significant differences between lethal time of the higher two concentrations, where both of those values were significantly lower than other concentrations, also lethal time of 250 ppm concentration was significantly lower than of 62.5 ppm. The actual mortality percentages were 93.33, 80.00, 63.33, 53.33 and 36.67 % in treated nymphs with concentrations 1000, 500, 250, 125 and 62.5 ppm, respectively after 7 days post treatment. While in control treatment the mortality was 3.33 % in the same period (Figure 1).

Table (1): :Lethal time required for 25, 50 and 90 % of treated *Schistocerca gregaria* 5th nymphal instar to died and the slope of days- mortality regression.

Concentrations	LT_{25}	LT_{50} *	LT_{90}	Slope
1000 ppm	3.00	4.46 a	9.47	3.92
500 ppm	3.42	4.95 a	9.98	4.21
250 ppm	4.07	6.11 b	13.20	3.83
125 ppm	4.75	7.36 bc	16.88	3.55
62.5 ppm	5.97	9.83 c	25.32	3.12

***Values with same letter did not significantly differ.**

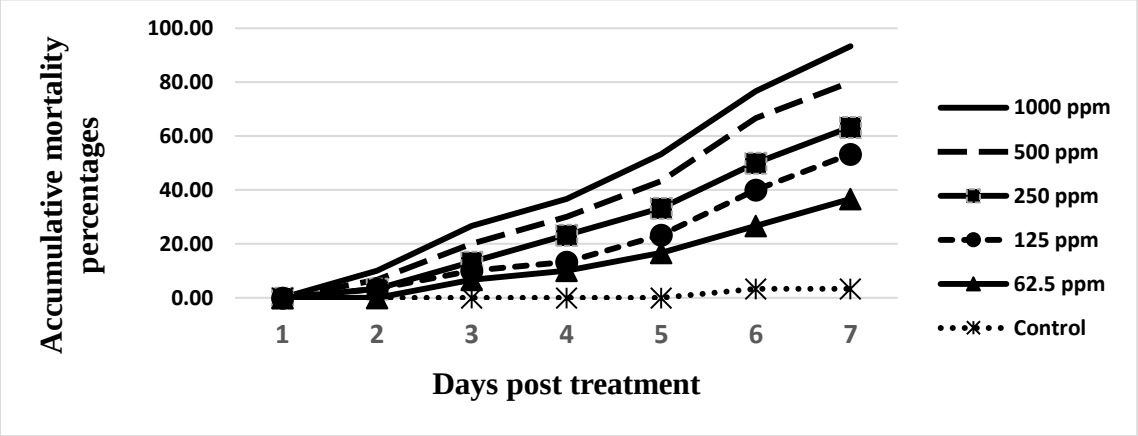

Figure (1): Daily accumulative mortality percentages of 5th nymphal instar of *Schistocerca gregaria* after 7 days post treatments.

On other hand all died treated nymphs by the 5th day to the end of experiment showed molting disruption, the degree of such molting failure varied according to pyriproxyfen concentration, this molting failure illustrated in Figure (2), where the two higher concentrations showed sever damage which caused total molting failure (Figure 2 D and E), while emerged adults from treated nymphs with concentrations 125 and 250 ppm (Figure 2 B and C, respectively) were live malformed adults, where 250 ppm treatment caused emerged adults with old cuticle remained attached to different positions or body appendages. While emerged adults from treated nymphs with 62.5 ppm (Not shown in Figure 2), showed little curled wings.

Furthermore, percentages of emerged adults from treated and untreated nymphs were negatively corresponded with used concentrations, it could be noticed that control treatment showed 96.67% emerged adults, while 62.5, 125, 250, 500 and 1000 ppm concentrations resulted in 63.33, 46.67, 36.66, 20.00 and 6.67 % of emerged adults, respectively (Figure 3). It was notable that all emerged adults from the two-high concentration (1000 and 500 ppm) died within few days after molting before maturation. While in case of untreated and treated nymphs with 250, 125 and 62.5 ppm the percentages of survived immature adults to maturation stage were 69.66, 27.27, 35.71 and 47.37 %, respectively (Figure 4).

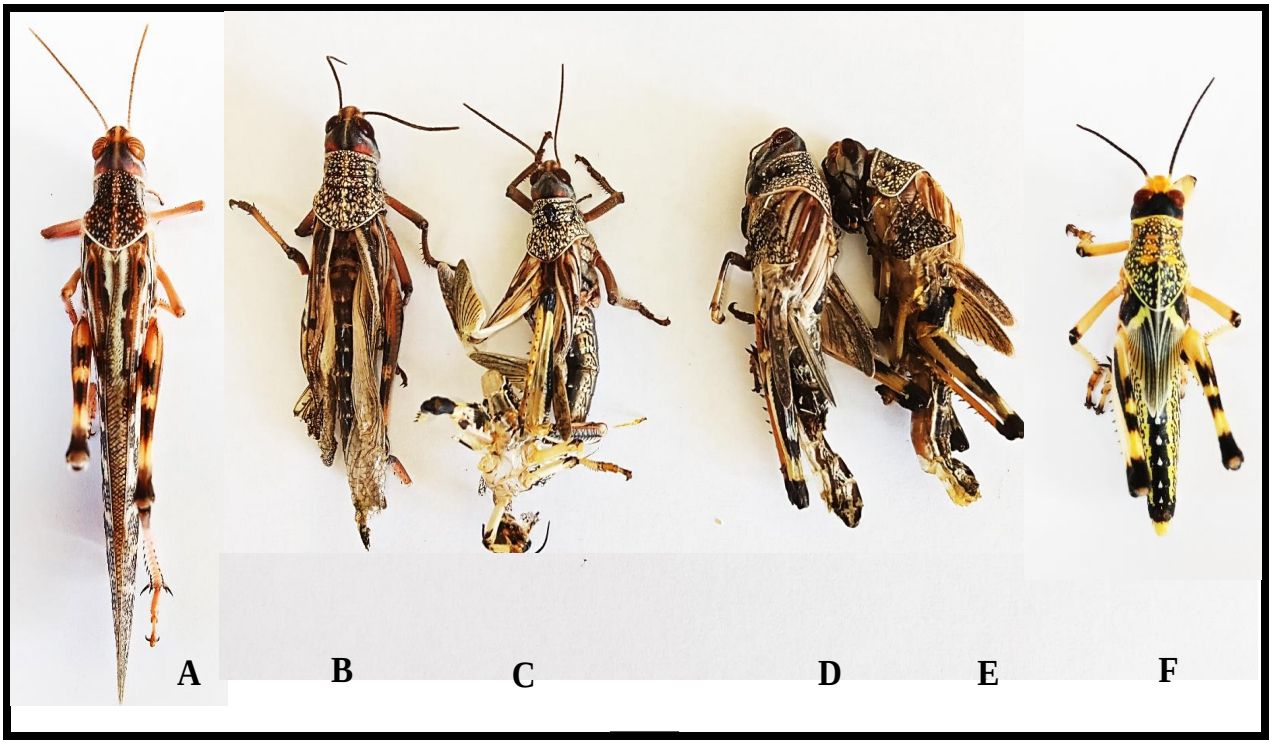

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Figure (2): Molting failure of treated *Schistocerca gregaria* 5th nymphal instars with Several concentrations of pyriproxyfen (dash= 1Cm., A and F untreated emerged adult and 5th nymphal instar, respectively, and B, C, D and E emerged adults from 125, 250, 500 and 1000 ppm treatments, respectively).

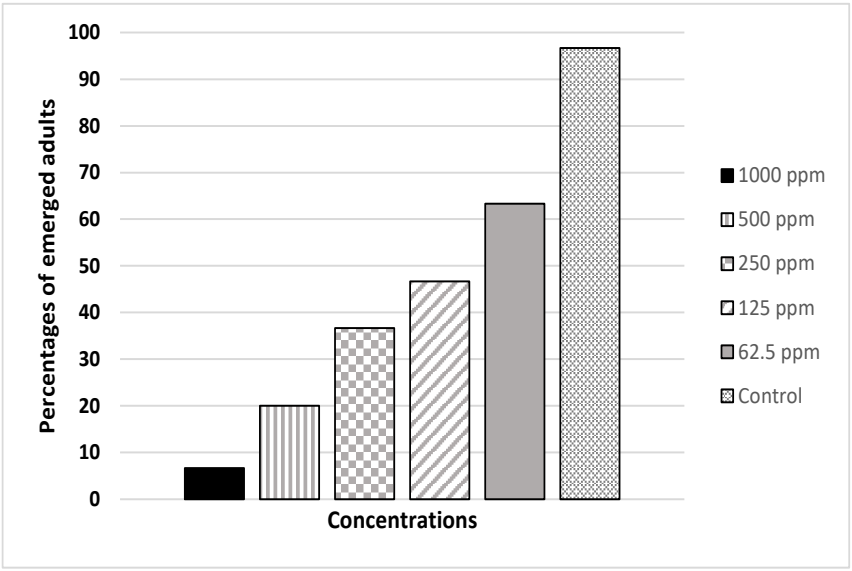

Figure (3): Percent of emerged adults from treated and untreated 5th nymphal instar of *Schistocerca gregaria*.

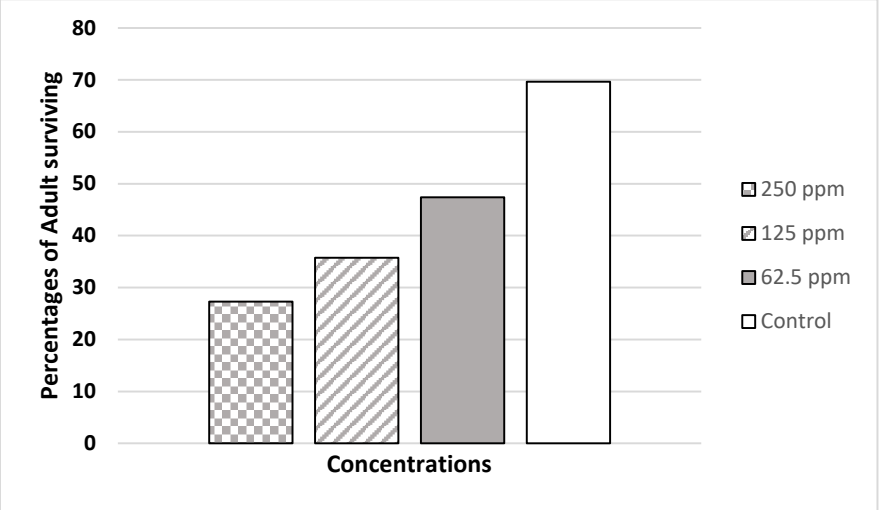

Figure (4): Percent of survived immature adults to maturation stage from treated and untreated 5th nymphal instar of *Schistocerca gregaria* .

2. Longevity of adult maturation:

Maturation in *S. gregaria* is distinguished by transforming to bright yellow color in males, while the females hind wing turned into lite yellow color. Table 2 demonstrates the maturation period in days for emerged adults from treated and untreated nymphs with different concentration of pyriproxyfen, the treatments caused significant prolongation when compared with untreated immature adults. There were no significant differences between the maturation period of the subjected males and females in the present study. The mean of maturation period of normal males and females were 13.67 and 13.21 day, respectively. While in case of emerged adults from treated nymphs with 63.5, 125 and 250 that period were 19.20 and 19.50, 21.00 and 23.00 and 24.00 and 22.50, respectively.

3. Female's fecundity:

In the present study the effect of pyriproxyfen on the female's fecundity was determined on base of number of egg pod deposited per female and the period between each egg pod deposition. Number of egg pods per female were significantly reduced in case of treated females when compared with untreated ones, where the mean number of deposited egg pods per normal female was 3.29 and those for treated females with 62.5, 125 and 250 ppm were 1.80, 1.60 and 1.50 egg pod/female, respectively as shown in Table (3). On other hand the treatment caused significant prolongation in the period between each egg pod deposition when compared with untreated females, also the higher concentration (250 ppm) caused significant prolongation than the other two concentration (125 and 62.5 ppm). It should be noticed that in case of treated females with 250 ppm there were only 2 females survived till maturation one of them deposited only one egg pod the other one deposited two egg pods so it resulted in only one value for calculation the period between each egg pod, same in case of treated female with 125 ppm 3 females survived but two of them deposited 2 egg pods and one female deposited one egg pod. The mean value of the period between each egg pod in case of untreated females was 3.34 day and those for treated females with 63.5, 125 and 250 ppm were 7.25, 7.50, 9.00 day, respectively (Table 3).

Table (2): Duration of maturation period in days of *Schistocerca gregaria* emerged adults from untreated and treated nymphs with different concentrations of pyriproxyfen.

Sex / Treatment	Females		Males	
	N	Mean ± Sd *	n	Mean ± Sd *
Control	14	13.21 ± 1.85 c	12	13.67 ± 1.61 c
62.5 ppm	4	19.50 ± 1.29 b	5	19.20 ± 2.28 b
125 ppm	2	23.00 ± 1.42 a	3	21.00 ± 2.00 ab
250 ppm	2	22.50 ± 0.71 ab	2	24.00 ± 1.42 a
L.S.D.	3.57			

Table (3): The effect of pyriproxyfen treatment on the number of egg pods per female and the period between each egg pod in days of *Schistocerca gregaria*.

Treatment	No of Egg pod/ female		Period between each egg pod in days	
	N	**Mean ± Sd ***	**N**	**Mean ± Sd ***
Control	14	3.29 ± 0.08 a	32	3.34 ± 0.07 c
62.5 ppm	5	1.80 ± 0.04 b	4	7.25 ± 0.03 b
125 ppm	3	1.60 ± 0.06 b	2	7.50 ± 0.02 b
250 ppm	2	1.50 ± 0.01 b	1	9.00 ± 0.00 a
L.S.D.	1.53		1.41	

***Values with same letter did not significantly differ.**

4. Histopathological effects of pyriproxyfen on the females ovary:

No survived adult mature females from treated *S. gregaria* 5th nymphal instars with 1000 and 500 ppm of pyriproxyfen were used for histopathological studies, since all treated nymphs with those two concentrations were died before maturation. Figure 5 shows different deterioration effects in immature oocyte of treated females, in compare to control (Figure 5 A), the normal oocyte is surrounded by regular follicular epithelium cells. While in case of oocytes from treated females with 62.5 ppm (Figure 5 B) there was slight destruction where there was disengagement between the cytoplasm and the irregular follicular epithelium cells which showed disintegration. The destruction increased in oocytes of treated females with 125 ppm (Figure 5 C), it showed cytoplasm disappear and follicular epithelium cells disintegrated. Much more most of oocytes from treated females with 250 ppm (Figure 5 D) were destroyed where the follicular epithelium cells were deteriorated and crashed allowing the oocytes contents to leak out.

In the present study pyriproxyfen caused reduction in the fecundity of desert locust adults emerged from treated nymphs in several ways such as: molting failure, produce malformed adults which soon die after molting, prolongation of maturation period, decrease number of deposited egg pod, prolongation of the period between deposition of each egg pod and destruction of oocytes in the ovary of treated females. The treatments with high concentrations of pyriproxyfen caused high mortality while the lower doses caused high survival percentages, but survived adults of all treatment were suffering from malformation damage that affect it is movements and flaying and reproduction ability.Pyriproxyfen is JH mimic, it is principal action is that nymphs retained in nymphal stage by the time they molting to adults, which lead to the nymphs were unable to molt successfully to adults (Pener *et al.*, 1997; Vennard *et al.*,1998 and Bi-Zhen *et al.*, 2012). Maturation of treated adults were prolonged significantly in conjunction with reduction of egg production, such effect may be due to the mimic effect of pyriproxyfen to JH, which cause hormonal disturbance

therefore inhibiting the egg production finally leading to egg reduction, also maturation prolongation (Ishaaya *et al.*, 1994; Schneider *et al.*, 2008 and Sullivan and Goh 2008). On the other hand, malformed adults may be suffering from hunger due to the inability of movements and malformation of mouth parts such suggestion may need further examination.The oocytes of treated females were corrupted, similar finding was reported by many authors in different insects (E.g. Fathpour *et al.* (2007) in *Blattella germanica*, Tay and Lee (2014) in *Monomorium pharaonis*, Xu *et al.* (2015) in *Spodoptera litura* and *Qian et al.* (2020) in *Bombyx mori*) such corruption may be due to reduction in vitellin synthesis, leading to the decrease of nutrients and energy in the development of ovarian oocytes.

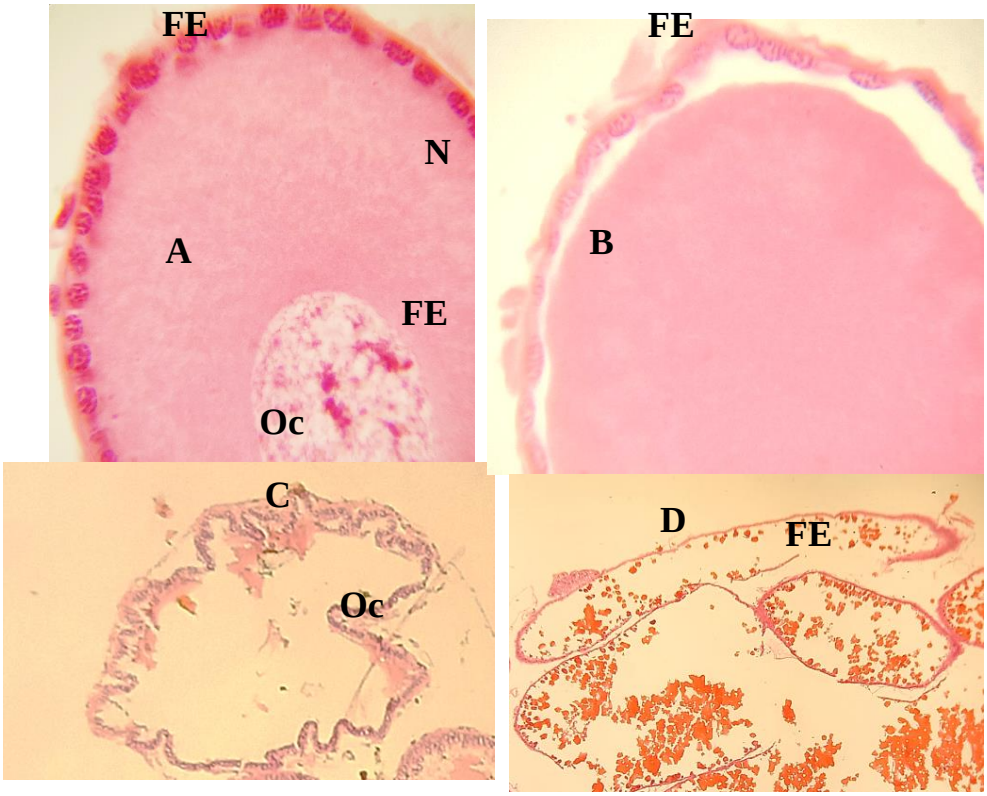

Figure (5): Light micrograph of cross section of treated and untreated *Schistocerca gregaria* femal's ovary showing immature oocytes (A normal adult and B, C and D adults from treated nymphs with 62.5, 125 and 250 ppm pyriproxyfen. FE follicular epithelial cells, N nucleus, Oc oocyte).

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