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# Evaluation of the IGRs Alsystin and Pyriproxyfen as Well as the Plant Extract Jojoba Oil against the Mosquito *Aedes aegypti*

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**The biological activity of two insect growth regulators alsystin and pyriproxyfen as well as the plant extract jojoba oil on larval stages and pupae until adult emergence of *Aedes aegypti* was evaluated. According to $IC_{50}$ values obtained (concentration which to inhibit the emergence of 50% of mosquito adults), alsystin (0.00048ppm) proved to be highly effective against *A. aegypti* than pyriproxyfen (0.005 ppm) and jojoba oil (85 ppm). On the other hand, larval treatments with the present compounds led to a decrease in egg production and hatchability of eggs produced by mosquito adults which survived from larval treatments.**

**Key words:** *Aedes aegypti*, insect growth regulators, plant extract, Mosquito larvae, reproductive potential

The success of insecticide-based control programmes in reducing the prevalence of insect vector-borne diseases has been accompanied by growing interest regarding the harmful effects of wide scale and prolonged used of insecticides on human health and the environment (WHO, 1995). Furthermore, many mosquito vectors have developed resistance to a number of conventional chemical insecticides (Xu *et al*., 2006; Pridgeon *et al*., 2008), creating an urgent need to seek and identify new effective insecticides to control these important disease vectors. Therefore, more attention has been recently paid to the use of non-conventional insecticides such as insect growth regulators (IGRs) and plant extracts for mosquito control in different parts of the world (Mohsen and Mehdi, 1989; Koumu *et al*., 2007; Siddigui *et al*., 2009).

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The present study was planned in part to evaluate the biological activity of two IGRs alsystin and pyriproxyfen as well as the plant extract mosquito oil against mosquito larvae of *Aedes aegypti*, the primary vector of dengue fever in Jeddah governorate, Saudi Arabia. Additional trials were also conducted to study the possible delayed effects of larval treatments with the tested compounds on the reproductive potential of mosquito adult survivors.

## MATERIALS AND METHODS

### Mosquito strain

A field strain of *A. aegypti* was used in the present study. The parental strain was raised from wild larvae collected from Jeddah governorate, Saudi Arabia. This stock colony was maintained under laboratory conditions of 27±1°C and 70±5% R.H. with 14:10 (L:D) photoperiod throughout this study. The larvae were reared until pupation and adult emergence took place for maintaining the stock culture.

### The tested compounds

The following compounds were used:

1. The IGR pyriproxyfen (Sumilarv 0.5% G), 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine, kindly provided by Sumitomo Chem. Co., Japan.
2. The IGR alsystin (tiflumuron 84%EC), 2-Chloro-N- (4-trifluoromethoxy phenyl) amino carbonyl benzamide, kindly supplied by Bayer Ltd., Germany.
3. The plant extract jojoba oil (*Simmondsia chinensis,* Fam. Simmondsiaceae), kindly provided prof. Dr. Moustafa S. Saleh, Dept., of Applied Entomology, Fac. Of Agric., Alexandria Univ. Eygpt. The stock solution of the plant extract was prepared by adding 1 ml of it to 99 ml of distilled water containing 0.5% triton X-100 as an emulsifier to ensure complete solubility of the extract in water. Series of concentrations were prepared in distilled water.

**Test Experiments**

The larval susceptibility test was conducted according to the method of WHO (2005). Treatments were carried out by exposing early 4$^{th}$ instar larvae to various concentrations of the test compounds in groups of glass beakers containing 100 ml tap water. Five replicates of 20 larvae each per concentration, and so for control trails were set up. The larvae were given the usual larval food during these tests. Data on larval mortality, pupation and adult emergence were recorded daily, and compared with control trails. The biological effects of the tested compounds was expressed as the percentage of larvae that do not develop into successfully emerging adults or the inhibition of adult emergence (% IE) according to the formula (WHO, 2005):

$$\%IE = \frac{C - T}{C} \times 100$$

Where C represents the percentage emergence in the control and T represents the percentage emergence in the treatments. The inhibition of adult emergence - concentration-probability line ( IC-p line) was drawn for each compound using the method of Litchfield and Wilcoxon (1949).

Values of $IC_{50}$ (concentrations which to give approximately 50% inhibition of adult emergence) were obtained from the IC-p lines of alsystin, pyriproxyfen and jojoba oil. The concentrations corresponding to these values were prepared and used for treating the early fourth instar larvae of *A. aegypti*. Fifteen replicates of 20 larvae each were conducted for each concentration. Mosquito adults which survived from the above larval treatments were isolated in clean cages. Seventy two hours later, emerged mosquito females were fed on a pigeon for a blood meal. Each engorged female was kept with a male in a small glass-cup, half-field with tap water and covered with muslin cloth. They were fed on a 10% sugar solution soaked on cotton pads and put on top of the covered cups. Number of eggs laid per female and hatchability of eggs were recorded for the 1$^{st}$ gonotrophic cycle.

## RESULTS AND DISCUSSION

Table 1 shows susceptibility levels of *A. aegypti* larvae following treatments with different concentrations of the IGRs alsystin and pyriproxyfen as well as the plant extract jojoba oil. The effective concentrations of the above compounds against 4$^{th}$ larval instar ranged from 0.0002–0.004 ppm, 0.002–0.02 ppm and 30–250 ppm, respectively. The corresponding larval mortalities for these compounds were 3–25%, 8–17% and 12–51%. The records showed that the test compounds did not appear to give high percentage of mortality against larval stages. Moreover, most pupae were often died before the adult emergence or as albino pupae (Bridges *et al*., 1977). Also, some mosquito adults were emerged incompletely or left their antennae, mouth parts and legs attached in the pupal exuvia Fig(1). Therefore, in the present work cumulative mortalities during larval development to pupae and adults have been taken as a criterion for evaluating the biological effects of the tested compounds as they have more juvenilizing effects than toxic mode of action.

Generally, larval treatments with the effective concentrations of the IGRs alsystin and pyriproxyfen as well as the plant extract jojoba oil caused 20.4–89.2%, 16.1–91.4% and 18.3–90.3% inhibition of adult emergence, respectively. Taking $IC_{50}$ values (concentration which to inhibit the emergence of 50% of adults) into consideration, the records indicated that alsystin (0.00048 ppm) proved to be highly effective against *A. aegypti* than pyriproxyfen (0.005 ppm) and the jojoba oil

extract (85 ppm). However, variations in the susceptibility status of the present *A. aegypti* larvae may be attributed to the differential mode of action of the compounds tested and its effective concentrations. The fluctuations in the percentage inhibition of adult emergence obtained for the different concentrations of the test compounds against the present mosquito strain support this conclusion. Laboratory and field trials in this respect were carried out by several authors to determine the susceptibility status of different mosquito species to IGRs and plant extracts (Saleh and Wright, 1990; Sharma *et al*., 2005; Bai *et al*., 2007; Pridgeon *et al*., 2008). However, it can be concluded that determination of susceptibility levels of mosquito larvae in any area will provide baseline data for planning control programmes and making decisions about insecticides usage in these areas (Paeporn *et al*., 2005).

The possible delayed effects of larval treatments with $IC_{50}$ values of alsystin (0.00048 ppm), pyriproxyfen (0.005 ppm) and jojoba oil (85 ppm) on the reproductive potential of mosquito adult survivors are shown in Table 2. The results indicated that larval treatments with the test compounds led to a marked decrease in egg production and hatching levels of eggs laid by mosquito female survivors. The mean number of

**Table 1.** The biological effects of the IGRs alsystin and pyriproxyfen as well as the plant extract jojoba oil on the developmental stages of *A. aegypti*

| Compounds | Effective concentrations (ppm) | Larval mortaility[a] (%) | Pupae produced (%) | Adult emergency (%) | (%IE)[b] | $IC_{50}$ [c] (ppm) |
|---|---|---|---|---|---|---|
| alsystin | 0.0002 | 3 | 97 | 74 | 20.4 | |
| | 0.0004 | 15 | 85 | 57 | 38.7 | |
| | 0.0007 | 18 | 82 | 32 | 65.6 | 0.00048 |
| | 0.001 | 11 | 89 | 20 | 78.5 | |
| | 0.004 | 25 | 75 | 10 | 89.2 | |
| Pyriproxyfen | 0.002 | 8 | 92 | 78 | 16.1 | |
| | 0.004 | 11 | 89 | 58 | 37.6 | |
| | 0.007 | 19 | 81 | 29 | 68.8 | 0.005 |
| | 0.001 | 20 | 80 | 18 | 80.6 | |
| | 0.02 | 17 | 83 | 8 | 91.4 | |
| jojoba oil | 30 | 12 | 88 | 76 | 18.3 | |
| | 70 | 16 | 84 | 53 | 43 | |
| | 120 | 25 | 75 | 36 | 61.3 | 85 |
| | 200 | 36 | 64 | 18 | 80.6 | |
| | 250 | 51 | 49 | 9 | 90.3 | |
| Control | | 4 | 96 | 93 | | |

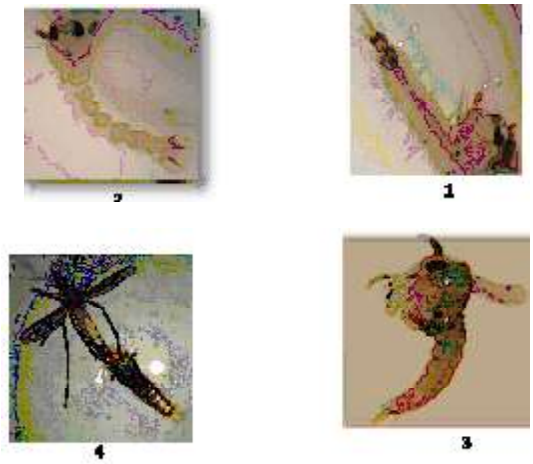
[a] Five replicates, 20 larvae each. [b] WHO(2005) [c] Litchfield and Wilcoxon (1949)

**Table 2.** The delayed effects of larval treatment with $IC_{50}$ values of alsystin, pyriproxyfen and jojoba oil on the reproductive potential of mosquito adult survivors

| Compounds | $IC_{50}$ (ppm) | Total number of eggs* | Mean number of eggs /female | Total number of larvae hatched | Hatchability (%) |
|---|---|---|---|---|---|
| alsystin | 0.00048 | 1271 | 50.8 | 796 | 62.6 |
| Pyriproxyfen | 0.005 | 1448 | 57.9 | 851 | 58.8 |
| Jojoba oil | 66 | 1206 | 48.2 | 665 | 55.1 |
| Control | | 1693 | 67.7 | 1546 | 91.3 |

* Mean of 25 engorged mosquito females.

eggs per female during the 1st gonotrophic cycle was in respect 50.8, 57.9 and 48.2 eggs when compared with the control which was 67.7 eggs (Table 2). This means that larval treatments caused a reduction in egg-laying capacity of female survivors by about 24.9, 14.5 and 28.8%, respectively. On the other hand, the percentage of hatchability of eggs produced by mosquito females that emerged from larval treatments with $IC_{50}$ levels of alsystin, pyriproxyfen and jojoba oil was in respect 62.6, 58.8 and 55.1% when compared with the control (91.3%). The results thus indicate that egg hatchability was decreased by about 31.4, 35.6 and 39.6% after larval treatment with the above compounds. A possible explanation is that larval treatments with sub-lethal doses of the test IGRs and the plant extract may be affect the larval gonads and accordingly the reproductive capacity of mosquito adult survivors (Saleh *et al*., 2003). Generally, studies in this respect were carried out by several investigators using different IGRs and plant extracts against many species of mosquito vectors (Vasuki, 1999; Chowdhury *et al*., 2007; Howard *et al*., 2009; Saleh *et al*., 2013). However, much additional trails are needed to evaluate the possible delayed effects of such IGRs and plant extracts on some biological and behavioural aspects of mosquito adults which survived from larval treatments.



1. Intermediate stage between larvae and pupa
   **a-** Terminal segments of larvae siphon.
   **b-** cephalothorax of pupa respiratory trumpets.
2. Pupa laked the hardening and darkening of the cuticle (albino pupa).
3. Intermediate stage between pupae and adult.
4. Adult completely emerged but they are unable to fly, legs attached in the pupal exuvia.

**Fig. 1.** Abnormalities in the developmental stages of against *A. aegypti* after larval treatments with the compounds test

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