Insecticidal effect of registered larvicides against *Culex pipiens* (Diptera: Culicidae) under laboratory and field conditions

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Background

- *Culex pipiens*: Important WNV vector
- Increase of WNV cases in Greece during the last years—numerous deaths
- Insecticidal applications are probably the most important action in managing mosquito populations
- From the available insecticides, larvicides can be used as a tool for area-wide management
- Larvicides are essential where drainage is not available or adequate
- Effective treatment against larvae requires the identification of larval sites: **Larviciding means Surveillance!!**
Floodwater pools
Questions raised- Aims of the present work

- Efficacy of all registered larvicides in Greece against *C. pipiens*
- Efficacy in comparison with temephos (withdrawal in 2007) and non-biocide formulations (Aquatain)
- Influence of abiotic and biotic factors on larvicide efficacy (exposure etc.), including residual effect
- Testing different strains (wild vs lab mosquitoes)
- Testing larvicides under both lab and field conditions
STATUS in Greece

UNTIL 2006

- Temephos (OP)
- Bti (bacterial)
- Diflubenzuron (IGR, only as WP)
- Pyriproxifen (IGR, not used)

2007-2014

- Bti (2 formulations)
- Diflubenzuron (7 form. SC, DT, GR)
- Spinosad (bacterial, 1 form.)
- S-methoprene (IGR, 1 form.)
- Polydimethylsiloxane (PDMS, inert material, 1 form., not a biocide)
The six larvicides used in the tests

- Temephos (OP, neurotoxic)
- S- methoprene (IGR, juvenile hormone analogue, non-neurotoxic)
- Diflubenzuron (IGR, chitin synthesis inhibitor, non-neurotoxic)
The six larvicides used in the tests (2)

- **Spinosad** (bacterial metabolites, neurotoxic)
  - Spinosyn A (major component)
  - Spinosyn D

- **PDMS** (intert material, acts mechanically, NOT A BIOCIDIE)

- **Bti** (bacterial toxins, not neurotoxic)
Formulations that tested for each active ingredient

**Formulation**
- Du-Dim 15 SC
- Vectobac 12 SC
- Biopren BM 20
- Aquatain AMF
- Mozkill 120 SC
- Abate

**Active ingredient**
- Diflubenzuron
- *Bacillus thuringiensis* (H-14)
- S-Methoprene
- PDMS
- Spinosad
- Temephos
Test 1: Lab efficacy assessment

The tests were carried out in walk-in chambers of controlled conditions (3 x 3 x 2.7 m, 24.5±0.5 °C, 75±5% RH, photophase 14L:10D) 

Larvae from F3-F10 of lab strain
The tests were conducted in plastic containers with 150 ml of water and 15 mg of food (daily).

The larvicides were applied at the label rate on the surface (approx. 47 cm²).

The containers were sealed and kept at the same conditions for 3 months.

Bioassays were performed at weekly intervals (20 3rd instar larvae/container) - 6 reps.
Efficacy was recorded according to the number of emerged adults
Test 2: Lab evaluation of larvicides against a wild *C. pipiens* population

- Similar bioassays as in the case of Test 1
- Larvicides used were Du-Dim, Orpah (difiubenzuron), Mozkill, Biopren, Vectobac
- Two dose rates: label rate and 1/3 of the label rate, 14 days of exposure
- Separate bioassays at two larval instars, 3\textsuperscript{rd} and 4\textsuperscript{th}
Test 3: Efficacy assessment in field conditions

- Rearing and bioassays were conducted as in the previous test
- Containers were sealed and placed outside, at the beginning of the months May, June and July 2013 (separate containers for each month)
- Efficacy was recorded at weekly intervals, as in the previous tests
Test 4: “Delayed” effects on adults

In cases of mortality <50 %, the adults emerged were transferred back to walk-in chambers at plexi-glass boxes (20 x 20 x 20 cm) with food (10 % sugar).

Adult survival was assessed 28 d after their emergence (blood-feeding was carried out on the 20th day for 30 min).

Other parameters tested:

- Oviposition
- Number of females that oviposited
- Egg hatching/larval emergence
Results: lab tests

- Treated
- Control (untreated)

**Biopren**

**Du-Dim**

**Vectobac**

**Aquatain**

**Abate**

**Mozkill**

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Mean mortality ± SE</th>
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<tr>
<td>Treated</td>
<td>Control (untreated)</td>
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</table>
Results: lab tests with wild *C. pipiens*

Efficacy of lavicides against 4\textsuperscript{th} (A) and 3\textsuperscript{rd} instars (B) at the label rate
Results: lab tests with wild *C. pipiens* (2)

Efficacy of lavicides against 3\textsuperscript{rd} instars at 1/3 of the label rate (Biopren was not included since there was no mortality)
Results: Field tests

MAY 2013

Full month exposure

Mean mortality ± SE

Days after treatment (from the beginning of May 2013)

- Vectobac, Mozkill and Biopren had no significant differences in comparison with the control containers
- No significant differences in the biological parameters of adults in comparison with control

These three formulations were tested for 1,3,5 and 7 days of exposure in the first week of June
JUNE 2013

1-day exposure

Mean mortality ± SE

Control  Vectobac  Mozkill  Biopren

0  25  50  75  100

a

b

c

c
Biological parameters of the adults that emerged

1-day exposure

- Adult survival (%)
  - Males: Control (a), Vectobac (b), Biopren (b)
  - Females: Control (ab), Vectobac (a), Biopren (b)

- % of females that laid eggs
  - Males: Control (a), Vectobac (b), Biopren (b)
  - Females: Control (a), Vectobac (b), Biopren (b)

- Larval emergence
  - Control, Vectobac, Biopren
JUNE 2013

3-day exposure

Mean mortality ± SE

Control

Mozkill

Mean temperature (ºC)

Days

Mean temperature (ºC)

Days
JUNE 2013

Full month exposure

Days of exposure from the beginning of June 2013
**JULY 2013**

Full month exposure

![Graph showing mean mortality ± SE over days of exposure from the beginning of July 2013.](image)

Days of exposure from the beginning of July 2013

- Control
- Abate
- Du-Dim
- Aquatain
Biological parameters of the adults that emerged

Full month exposure

JUNE 2013

- Adult survival (%)
- % of females that laid eggs
- Larval emergence

Control
Abate
Du-Dim

Bar charts showing differences in adult survival, % females that laid eggs, and larval emergence among different treatments. Differences are indicated by letters above the bars.
SUM of FIELD TESTS for MAY, JUNE, JULY

Mean mortality ± SE

Weeks after treatment:

1. Abate
2. Du-Dim
3. Aquatain

MAY
JUNE
JULY
Summary of efficacy of larvicides

**LAB TESTS**

- Spinosad (Mozkill)
- PDMS (Aquatain)
- Temephos (Abate)
- Diflubenzuron (Du-Dim)

**FIELD TESTS**

- PDMS (Aquatain)
- Temephos (Abate)
- Diflubenzuron (Du-Dim)
- Spinosad (Mozkill)
- Bt-i (Vectobac)
- S-Methoprene (Biopren)

More effective

Less effective
Conclusions

 совершаемые различия в эффективности уровня тестированных ларвикидов

- **Spinosad**: увеличенная эффективность в лаборатории, быстрое рассасывание вне
- **PDMS**: увеличенная эффективность в всех тестах
- **PDMS, abate and diflubenzuron** более стабильны - лучше долгосрочный эффект

Месяц оказал критическое влияние на эффективность: **efficacy is a function of conditions (temperature, sunlight etc.)**

- Adult survival and biological parameters were only marginally affected
  - Adults that had survived laid eggs
  - Egg hatching/larval emergence was not affected
What’s next

- Evaluation of more factors that affect larvicides (more species, more scenarios), incl. novel substances
- Additional studies on PDMS efficacy
- Assessment of efficacy of larvicides in “real-world” conditions (i.e. sampling and bioassays right after field application etc.)
- Screening for resistance to larvicides (for Greece)

THANK YOU!!