Spinosad Effects on Mortality and Reproduction of Culex pipiens (Diptera; Culicidae)

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ABSTRACT

Spinosad, which is an active substance, derived from the bacterium Saccharopolyspora spinosa, is one of the most known insecticides used as a biological control agent against populations of various insect pests nowadays. Within this axis, we tested the formulation (Tracer 480SC) against fourth stage larvae of Culex pipiens where they were exposed to different concentrations of spinosad (20µg/l, 50 µg/l, 100 µg/l) for 48h, 120h, 240h and 360 hours. High larval mortality was recorded; this toxicity is expressed in terms of dose and time. Lethal concentrations LC50 calculated after 48 hours is 33,88µg/l and the LC90 is 131,82µg/l. However, at 360 hours of the treatment, the CL50% and the CL90% does not exceed the 7,76 µg/l and 44,67 µg / L. Spinosad disrupts fecundity and fertility of adults issued from treated larvae, with an average low eggs of 37,70 ± 27,52 induced by the dose of 20 µg/l and low hatching duration of eggs with 1,90 ± 1,663 % and some of them have witnessed malformations in the wings.

INTRODUCTION

Considerable quantities of chemical synthetic insecticides are used world wide to fight against insect pests such as mosquitoes. [1] But the increase of resistance cases to these chemical insecticides and the adverse effects on non-targeted species [2] led to the research for new solutions to fight against these insects pests biologically.

Biological control is the use of living beings (control auxiliaries) or their products (inert biopesticides) to fight pests or disease vectors. Control auxiliaries can be predatory arthropods, parasitoids, nematodes and entomopathogenic microorganisms (viruses, fungi, bacteria, protozoa), while inert biopesticides are generally derived from bacteria or fungi and are mostly quickly biodegradable [3].

Spinosad is one of these inert biopesticides. It is an active ingredient of the plant-protection product which has an insecticidal effect. Is a fermented product secreted by a bacterium living in the soil, Saccharopolyspora spinosa [4]. Spinosad causes in a short time in the insect nervous system excitation leading to involuntary muscle contractions, prostration with tremors and paralysis. The insect stops feeding and paralysis can occur within minutes after ingestion, death. [4].

In general, spinosad is effective against caterpillars, flies and thrips and against some species of beetles and grasshoppers. [5] It has a larvicide effect on different types of mosquito such as Aedes aegypti, Aedes albopictus, Culex quinquefasciatus, Culex pipiens, Anopheles gambiae and Anopheles stephensi ; An. quadrimaculatus say [6; 7; 8 ; 9; 10].

In this study, we are interested in the direct and indirect effects of this product on the most abundant species of Culicidae in Algeria: Culex pipiens. The study aims to investigate the effects of the insecticide on mortality and development of Cx.pipiens.

MATERIAL AND METHODS

Insects: Cx. pipiens it is a cosmopolitan species that acts as a major vector in the transmission of many diseases and considered as an important nuisance to human populations exposed to itsbites in most African
countries [11]. Its development cycle lasts 10 to 14 days and includes four stages: egg, larva, pupa, and adult. Pre-
imaginal stages (egg, larva, and pupa) are adapted to the aquatic lifestyle, while the imaginal stage (imago) is
aerial [12].

Mosquito Rearing:

The larva used in this study provokes a mass livestock of adults collected in urban areas of Annaba, East
Algerian city. Livestock is kept in laboratory cages (20 x 20 x 20 cm) at a temperature of 25 ± 2 °C, humidity of
75 ± 10% and a 12-hour scotophase. A mixture of biscuit and dried yeast insures the nutrition of larvae while
the adults feed on dried raisins.

Spinosad:

The bacterium of this active substance was isolated for the first time in the soil samples in 1988 [13]. It is a
fermented product derived from the mixture of two toxins: spinosyn A and D [14]. Solid and light gray,
spinosad is characterized by a stagnant water smell whose chemical formula is C41H65NO10 (spinosyn A) or
C42H67NO10 (spinosyn D) [15].

Treatment:

Treatment of Culex pipiens was inspired by the technique of standardized sensitivity tests of the World
Health Organization [14]. The tests are carried out in beakers with a capacity of 200 ml each containing 15
fourth-stage larvae of Cx. pipiens (L4) in 200 ml of spring water. After a preliminary test, three concentrations
of the L4 stage larvae (20 µg/l, 50 µg/l, 100 µg/l) were administered. These values are chosen in order to get
the lethal doses 100 (LD100). Each concentration is applied to 3 repetitions, with a preparation of 15 larvae of
Cx. pipiens as a control.

After 24 hours the water of treated groups has been changed including the control group. The variable
measured daily, during 15 days, is the number of dead individuals (L4 larvae, nymphs, and adults).

Lethal concentrations and lethal times (LC50%, LC90%, LT50%, and LT 90%) were calculated using the
Finney’s [16] mathematical method. Data are normalized and processed according to the tables of Bliss and
calculations were performed on XLStat 2009.

Effects of Spinosad on fecundity and female fertility:

Adults from larvae treated with the lowest dose (20 µg/l), having completed their development were
isolated and separated in couples (male and female) in cages (20 x 20 x 20 cm) containing water containers.

After coupling, the number of laid and hatched eggs for each female is counted. The results were subject to
statistical description and comparison of variances.

Results:

Effects of spinosad on the mortality of Cx. pipiens:

The obtained results show that the spinosad affects the mortality of larvae based on the applied
concentration and exposure time. Low larvicidal activity was recorded for the concentration of 20 µg/l.
Regarding the concentration of 50 g/l and 100 g/l, more than 80% of treated individuals die after 15 days
of treatment, and the results show that there are no significant differences between the recorded mortality rate and
the used concentrations (Tab. 1).

Table 1: Mortality rate (%) of C. pipiens treated with spinosad.

<table>
<thead>
<tr>
<th>Concentration (µg/l)</th>
<th>2 Days</th>
<th>5 Days</th>
<th>10 Days</th>
<th>15 Days</th>
<th>F_OBS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>20µg /l</td>
<td>31.09</td>
<td>46.67</td>
<td>62.33</td>
<td>71.11</td>
<td>2.65</td>
<td>0.12</td>
</tr>
<tr>
<td>50µg /l</td>
<td>66.67</td>
<td>80.33</td>
<td>91.00</td>
<td>95.55</td>
<td>2.08</td>
<td>0.18</td>
</tr>
<tr>
<td>100µg /l</td>
<td>84.44</td>
<td>93.33</td>
<td>93.33</td>
<td>95.55</td>
<td>0.11</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>F_OBS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.49</td>
<td>0.005</td>
<td>2.09</td>
<td>1.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.29</td>
<td>0.95</td>
<td>0.20</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(*: Significantly different)

Larvae mortality rates are positively correlated with the used concentrations of spinosad (R² = 0.80 to
1.00) (Tab. 2A). Two days after treatment, the lethal concentration of 50% was 33.88 µg/l and decreased to
21.87 µg/l in 5 days, 11.48 µg/l in 10 days and 5.12 µg/l in day 15. The mortality of 90% of the larvae may be
caused with concentrations ranging between 7.19 µg/l and 131.82 µg/l (Tab. 2A).

There is also a strong correlation between the larval’s exposure time to different
concentrations of spinosad (R² = 0.88 to 0.98) (Tab. 2 B). Calculated lethal times range from 0.06 to 6.06 days
for a 50% mortality (Tab. 2 B) and vary between 3.9 and 112.07 days for LT 90% (Tab. 2 B). Lower limits
range between 5.58 µg/L and 26.26 µg/l while the upper limits are from 10.79 µg/L to 43.71 µg/l (Tab. 2A).
Regarding the time limits, they are of 0.12 to 9.33 days (Tab. 2 B).
Table 2: Toxicological parameters of spinosad A: Based on larvae’s exposure time / B: Based on the used concentration.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Regression line</th>
<th>A (µg/l)</th>
<th>B (µg/l)</th>
<th>C (µg/l)</th>
<th>D (µg/l)</th>
<th>Slope Lower Limit</th>
<th>Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Y=1,69+2,16 X</td>
<td>0,99</td>
<td>11,75</td>
<td>33,88</td>
<td>97,89</td>
<td>2,89</td>
<td>26,26</td>
</tr>
<tr>
<td>5</td>
<td>Y=1,97+2,26 X</td>
<td>1,00</td>
<td>7,91</td>
<td>21,87</td>
<td>60,08</td>
<td>2,76</td>
<td>17,09</td>
</tr>
<tr>
<td>10</td>
<td>Y=3,16+1,72 X</td>
<td>0,89</td>
<td>3,08</td>
<td>11,48</td>
<td>44,19</td>
<td>3,79</td>
<td>8,32</td>
</tr>
<tr>
<td>15</td>
<td>Y=3,51+1,68 X</td>
<td>0,82</td>
<td>1,96</td>
<td>7,76</td>
<td>29,93</td>
<td>3,91</td>
<td>5,58</td>
</tr>
</tbody>
</table>

Table 3: Comparison of eggs from control females of Cx. pipiens and eggs from females treated with spinosad (n = 10).

<table>
<thead>
<tr>
<th>Mean ± S</th>
<th>Min</th>
<th>Max</th>
<th>tobs</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>45,20 ± 9,23</td>
<td>33</td>
<td>59</td>
<td>0,82</td>
</tr>
<tr>
<td>B</td>
<td>37,70 ± 27,52</td>
<td>0,000</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

Effect on fecundity and fertility:

After treating the fourth stage larvae by sublethal concentration of 20 µg/l, we notice a longer larval life and a disturbance of the fecundity and fertility of adults from this treatment.

Effect on the number of eggs:

The results in the table (Tab. 3) show that the females treated with spinosad lay on average 37.70 ± 27.52 eggs with a minimum of zero and a maximum of 75 eggs, however, healthy females lay between 33 and 59 eggs.

Comparison of eggs means shows that there are no significant differences between the fertility of the two lots (tobs = 0,82, p = 0,43) (Table 3).

Table 4: Effects of spinosad on the eggs hatch of Cx. pipiens females.

<table>
<thead>
<tr>
<th>Hatch duration</th>
<th>Hatch rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± s</td>
<td>Min</td>
</tr>
<tr>
<td>A</td>
<td>2,8 ± 0,422</td>
</tr>
<tr>
<td>B</td>
<td>1,90 ± 1,663</td>
</tr>
</tbody>
</table>

A: The eggs of the control females; B: The eggs of females from larvae treated with spinosad

Discussion:

The toxicological study conducted using spinosad on L4 larvae showed good larvicidal activity on Cx. pipiens species explained by the high mortality rates. Spinosad insecticide activity is gradual since an increase in the exposure time to reach a mortality rate of 95% for the highest concentrations.

Several studies like those of Aouati [17] and Merabeti [18] have shown the larvicidal effects of spinosad on Cx. pipiens, An. multicolor.

Other works have shown the delayed impact of this bioinsecticide on several insect groups (Diptera, Dictyoptera, ... etc.) such as the study of Bourbia [19] that revealed the effect of sublethal concentrations of spinosad on the sexual behavior of Drosophila melanogaster, besides the study of Habbachi et al. [20] on the Blattella germanica.

Spinosad acts as a neurotoxin active by contact and ingestion, although ingestion is judged as 5 to 10 times more effective. Its effects are fast, which is quite unusual for an biological product. Insects are paralyzed and stopped feeding [21].
Fig. 1: Malformations of adult wings from treatments with spinosad are observed under a binocular microscope (Gr: x 4).

Fig. 2: Malformations of adult legs from treatments with spinosad observed under a binocular microscope (Gr: x 4).

We also recorded the indirect effects of spinosad on Cx. pipiens. Treatment with low concentrations (20 g / l) allows the appearance of some adults unable to fly. Malformations are mainly localized in legs and wings; this dose of spinosad causes also a disturbance in fecundity and fertility in adults from treated larvae.

The decrease in fecundity and hatch rates in treated females, would be under the effect of spinosad properties which are poisoning the stomach and affects specifically the function of γ-Aminobutyric (GABA) and nicotinic receptors of the target insects acetylcholine [21]. These symptoms were also noted after treatment with neem on An. stephensi [22].

Other insecticides also affect the reproductive capacity and lifespan of surviving mosquitoes. Sub-lethal exposure to organophosphates or pyrethroids can lead to changes in fecundity and immature development times. [23; 24; 25] Similarly, reductions in wing length, fecundity, egg size, glycogen reserves and the longevity and feeding capabilities of adult females have been reported in the survivors of juvenile hormone analogue treatments [23; 26; 27]. Other results showed that spinosad has sublethal doses that affect the brain of Oreochromis niloticus [28].

So its effectiveness at low concentrations on the larvae of mosquitoes sensitive and resistant to insecticides has made it an excellent choice for vector control.

**Conclusion:**

For this work we have determined that spinosad has a larvicidal activity against larvae of Culex pipiens. The observed mortality is strongly correlated with the dose and exposure duration of larvae to spinosad. We also found that treatment with sub-lethal doses of spinosad reduced significantly fecundity and fertility as well as the development and growth of this insect pest.

The obtained results show that spinosad is promising as a larvicide against Culex pipiens; it could be a good alternative to biological pesticides, while preserving the human health and the environment. Further studies such as mode of action and synergism with the biocides under field condition are needed.

**ACKNOWLEDGEMENTS**

The authors would like to thank the Laboratory of Applied Neuro-Endocrinology at Biology Department, Badji Mokhtar University, Algeria, for providing the facilities during mosquito rearing and preparation of concentrations. The comments by two anonymous reviewers towards improving the quality of this paper are acknowledged.

**REFERENCES**


