THE EFFECT OF TOLYPIN IN TOLYPoclADiUM NIV~UM CRUDE EXTRACT AGAINST MOSQUITO AND BLACKFLY LARVAE IN THE LABORATORY

V. MATHA, J. WEISER and J. OLEJNIČEK

Institute of Entomology and 1 Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice

Abstract. The efficacy of tolypin against mosquito and blackfly larvae was studied under laboratory conditions. It was tested against Culex molestus, Aedes aegypti, Anopheles maculipennis, Simulium nebuli and Oidigna orcutti. A concentration 0.1 mg/ml caused 100 % mortality in all species tested and a concentration 0.001 mg/ml caused 100 % mortality only in the two species of blackflies used within 24 hours.

Members of the genus Tolypocladium, T. cylindrosporum and T. niveum are known as natural pathogens of mosquito larvae (Weiser and Pillai 1981, Soares et al. 1979, Weiser 1986), infecting them with conidia and blastospores. The fungi grow in the larval hemocoel and produce a surface growth of hyphal bodies and conidiophores. These fungi may be specific to members of Culicidae and Simuliidae. Recently a new insecticidal water soluble, thermostable metabolite was discovered in the cultivation medium of these fungi (Weiser and Matha 1988a).

In an earlier paper we had described the appearance of rapid tetany and mortality in Galleria mellonella larvae after intrahemocoenic injection and for a rapid knock down, accompanied by melanization of midgut epithelium resulting in high mortality when fed to larvae of Culex molestus Forskii and other Diptera (Weiser and Matha 1988a). The insecticidal activity of tolypin seems to be quite different from the activity of the cyclosporines, the immunosuppressive metabolites of T. niveum described in an earlier paper (Weiser and Matha 1988b). In our studies, we used a crude extract of the toxin against some mosquitoes and blackfly larvae under laboratory conditions.

MATERIALS AND METHODS

A chloroform extract of the fermentation broth or the acetate extract of Sabouraud agar slants of T. niveum were evaporated and the dry substance obtained was used as the crude extract of tolypin. This was diluted in tap water to concentrations ranging from 0.1 to 0.0001 mg/ml for testing.

For bioassays 4th instar larvae of laboratory reared Culex molestus Forskii and Aedes aegypti L., and field collected larvae of Anopheles maculipennis s. l. were used. The method used was the same as described by Weiser and Matha (1988a), i.e. L4 larvae were used in lots of 50 in plastic cups with 100 ml of tap water. Powdered of dry rye bread was used for feeding larvae after 24 hours. The blackfly bioassay was performed as described by Olejnichek et al. (1985). Larvae of Oidigna orcutti (Meigen) and Simulium nebuli Friederichi were collected in the field and brought in thermos flasks to the laboratory where they were kept in groups of 50 specimens each in cylinders with 100 ml of stream water. Aeration and water current were constantly provided by bubbling air from an aquarium diaphragm pump. During the 24 hours of the experiment the larvae were not fed and the untreated (control) larvae were maintained under identical conditions. Experiments with mosquito larvae were evaluated after 144 hours, and blackflies after 24 hours. Dead larvae were removed at 12 hour intervals. Each treatment was repeated at least three times.
RESULTS AND DISCUSSION

The activity of the tolypin crude extract against species of mosquitoes from three genera and two of blackflies is summarized in Table 1. It is evident that all tested species were susceptible to the toxin. It is also evident that there is a difference in susceptibility of the three mosquitoes species, which is not attributable to differing feeding habits. *Culex molestus* is most susceptible (LD₅₀ = 0.004 mg. ml⁻¹/144 hours) the other two species are more resistant. The crude tolypin extract showed high insecticidal activity against both blackfly larvae with an LD₅₀ of 0.00007 mg. ml⁻¹ during the first 24 hours. Stress factor can be ruled out as a contributory cause of mortality, as the larvae were maintained in near perfect conditions during the first 24 hours. Other studies of the host specificity and host range, general toxicity for non-target species and of the mode of action of tolypin are now needed before its potential use for vector control.

Acknowledgements. We are grateful to Professor J. S. Pillai, University of Otago, New Zealand for language supervision and Mrs. V. V. Smirnov for technical assistance. This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

Table 1. The susceptibility of three species of mosquito and two species of blackfly larvae to Tolypin crude extract (% mortality/time of dead)

<table>
<thead>
<tr>
<th>Conc. of Tolypin crude extract (mg. ml⁻¹)</th>
<th><em>Culex molestus</em></th>
<th><em>Aedes aegypti</em></th>
<th><em>Anopheles maculipennis</em></th>
<th><em>Simulium reederi</em></th>
<th><em>Odagmia ornata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>100/24</td>
<td>100/24</td>
<td>100/24</td>
<td>100/24</td>
<td>100/24</td>
</tr>
<tr>
<td>0.01</td>
<td>100/144</td>
<td>80/144</td>
<td>90/144</td>
<td>100/24</td>
<td>100/24</td>
</tr>
<tr>
<td>0.001</td>
<td>40/144</td>
<td>30/144</td>
<td>30/144</td>
<td>100/24</td>
<td>100/24</td>
</tr>
<tr>
<td>0.0001</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>100/24</td>
<td>100/24</td>
</tr>
</tbody>
</table>

The treatment of mosquito larvae by the highest concentration is accompanied by rapid KD.

ВОЗДЕЙСТВИЕ ТОЛИПИНА В ОБЩЕМ ЭКСТРАКТЕ TOLYPOCLADIUM NIVEUM ПРОТИВ ЛИЧИНКОВ КОМАРОВ И МОШЕК В ЛАБОРАТОРИИ

В. Матья, Я. Вайзер и Й. Олейничек

Режим. Использовали воздействие толипина против личинок комаров и мошек в лабораторных условиях. В экспериментах использовали следующие виды насекомых: *Culex molestus*, *Aedes aegypti*, *Anopheles maculipennis*, *Simulium argeratum* и *Odagmia ornata*. Концентрация 0,1 мг/мл вызывала 100% смертность у всех изучаемых видов насекомых, тогда как концентрация 0,001 мг/мл вызывала 100% смертность, только у двух изучаемых видов мошек в течение суток.

REFERENCES

OLEJNICZEK J., MATHA V., WEISER J., 1985: The efficacy of *Bacillus thuringiensis* var. *israelensis* against larvae of the blackfly *Odagmia ornata* (Meig.) (Simuliidae, Diptera) at low temperatures. Folia parasitol. 32: 271—277.


WEISER J., 1986: Mosquitoicidal activity in Toypoladum strains. Fundamental and supplemental aspects of invertebrate patho-


tebr., Pathol., 1986.)

MATHA V., 1988a: Toypolin, a new insectici-

dal metabolite of entomopathogenic fungi of the genus Toypoladum Gams. J. Invert-

etebr. Pathol. 51: 94—96.

MATHA V., 1988b: The insecticidal activity of
ciclosporine on mosquito larvae. J. Invert-

tebr. Pathol. 51: 92—93.

PILLAI J. S., 1981: Toypoladum cipindro-

sporum (Dentemorpecia, Moullacoeae) a new pathogen of mosquito larvae. Entomo-

plaga 26: 357—361.

Received 9 July 1987

V. M., Entomologický ústav ČSAV,

Braníkovská 31,

370 05 České Budějovice, ČSSR