Research Note

Larvicidal effects of neem (*Azadirachta indica*) seed kernel extracts against *Paratanytarsus grimmii* (Diptera: Chironomidae) and *Aedes albopictus* (Diptera: Culicidae)

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Abstract: Larvicidal effects of neem (*Azadirachta indica* A. Juss.: Meliaceae) seed kernel extracts against *Paratanytarsus grimmii* (Schneider) (Diptera: Chironomidae) and *Aedes albopictus* (Skuse) (Diptera: Culicidae) were examined by the immersion method. The hexane extract of neem clearly showed lethal effects against mature larvae of both species at a concentration of 1,000 ppm.

Key words: neem, larvicidal activity, *Azadirachta indica*, *Paratanytarsus grimmii*, *Aedes albopictus*

INTRODUCTION

The neem tree (*Azadirachta indica*; Meliaceae) is well known in Asia and Africa, and almost every part of the plant is used in indigenous agriculture and medicines (Siddiqui et al., 2002). The seed kernels and leaves of the neem tree contain triterpenoids such as azadirachtin, salannin, gedunin and deacetylnimbim which are known to exhibit insecticidal and multiple biological effects such as antifeedancy and growth regulation. (Jhansi and Singh, 1993; Mulla and Tianyun, 1999; Siddiqui et al., 2002). The most prominent phytochemical pesticides studied in recent years are those based on neem products (Mulla and Tianyun, 1999). Neem insecticides (e.g. Neemgard and Neemmark produced in India and Margosan-O produced in U.S.A.) containing azadirachtin have also shown lethal activities and other biological effects against arthropods of medical and agricultural pests (Murugan and Jeyabalan; 1995; Murugan et al., 1998; Mulla and Tianyun, 1999; Li et al., 2003). However, the biological activity of neem against chironomids has been little known. The Chironomidae contains economically and medically important species that are a serious nuisance and produce allergies upon their outbreak (Ali, 1995; Cranston, 1995). In the present study, the biological effects of neem seed kernel extracts against chironomids were examined using *Paratanytarsus grimmii* (Diptera: Chironomidae) as a test organism (Kondo, 2002). The biological activity of neem seed kernel extracts were compared between chironomid and mosquito larvae.

MATERIALS AND METHODS

Extraction of neem seed kernel

The dried seed kernel of *Azadirachta indica* from India was finely cut and extracted three times with methanol (1,000
ml) by supersonic apparatus for 30 min. Evaporation of the solvent under reduced pressure provided a methanol extract. The methanol extract was suspended in distilled water (800 ml) and partitioned with n-hexane (300 ml × 3).

Test organisms

Larvae of Paratanytarsus grimmii (Diptera: Chironomidae) and Aedes albopictus (Diptera: Culicidae) were used as the test organisms. The chironomid colony was initiated with individuals collected in 1992 from the Yamazaki River of Nagoya, Japan. The mosquito colony “Toyama” was collected in 1992 at Sugitani of Toyama, Japan. These colonies were maintained at the Department of Parasitology, Aichi Medical University.

Toxicity test

The neem extracts were dissolved in 0.3 ml DMSO, and were suspended in 9.7 ml tap water to make a 10 ml test solution. Test solutions in concentrations of 1,000 to 5,000 ppm were prepared. Immersion tests were done in 12-well tissue culture plates (3.0 ml/well) (Corning, Inc.). The 3rd-4th instar larvae of chironomids and mosquitoes were introduced into the wells and kept unfed at 20°C under a photoperiod condition of 12L:12D. Ten chironomid larvae and six to ten mosquito larvae were used for each test and control (3.0% DMSO aqua) solutions. The mortality rates were examined daily for three days. The larvae were counted as dead when they showed no movement on stimulation with a needle. In each experiment, the tests were repeated three times and the mean mortality rates were obtained in each experiment.

RESULTS AND DISCUSSION

The biological effects of neem (Azadirachta indica) against mosquito larvae have been well studied (Mulla and Tianyun, 1999). Recently, Vahiha et al. (2002) reported the lethal effects of methanol extracts (LC50: 2,000–5,000 ppm) of neem and related plants (Pavonia zeylanica L. and Acacia ferruginea D.C.) against Culex quinquefasciatus Say larvae (Diptera: Culicidae). Zebitz (1984) found that the effectiveness of the extracts increased with decreasing polarity of the solvents used for extraction, and the effects were similar to those of a synthetic insect growth regulator (IGR). In this study, the methanol extract showed rapid and high larvicidal activities at 5,000 ppm within 2 days against both species. However, the lethal effects were slower and low at the concentration of less than 2,500 ppm and half of the larvae were still alive within 3 days at

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<tr>
<td>Methanol</td>
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<td>5,000 ppm</td>
<td>87(5)</td>
<td>61(8)</td>
<td>90(5)</td>
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<td>2,500 ppm</td>
<td>43(5)</td>
<td>50(6)</td>
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<td>1,000 ppm</td>
<td>40(3)</td>
<td>33(5)</td>
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<td>Hexane</td>
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<td>2,000 ppm</td>
<td>100(0)</td>
<td>47(6)</td>
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<tr>
<td>1,000 ppm</td>
<td>97(2)</td>
<td>37(2)</td>
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<td>Control (3% DMSO)</td>
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P. g, P. grimmii; A. a, A. albopictus.
1,000 ppm. The hexane extract containing lipids showed more rapid and higher activities than the methanol extract against both species within 3 days at 1,000 ppm (Table 1).

Only a few investigations have examined the biological activity of neem against aquatic organisms other than mosquitoes (Scott and Kaushik, 1998, 2000; El-Shazly and El-Sharnoubi, 2000; Goktepe and Phak, 2002). They studied the effect of neem-based insecticides used for food crop protection on nontarget aquatic invertebrates. The results showed that the toxic effects of Margosan-O (containing 14–20% neem oil, 10% polyethylene, 69–75% ethanol and 0.3% azadirachtin) against mosquitoes (Culex spp.; EC₅₀ = 105 mg l⁻¹) were significantly greater than against chironomids (Chironomus riparius) (Meigen); EC₅₀ = 281 mg l⁻¹) within 48 h (Scott and Kaushik, 1998). In the present study, the acute toxicity for chironomids was greater than that for mosquitoes (Table 1). These differences might be affected by pH, temperature, light and other environmental factors associated with the bioassay system (Scott and Kaushik, 1998, Goktepe and Phak, 2002) as well as by biological factors, such as the differences in susceptibility among the insects.

Neem products at low concentration have many activities such as antifeedancy, growth regulation, fecundity suppression, sterilization and oviposition repellency without acute toxicity against many species of insects (Mulla and Tianyun, 1999; Murugan et al., 1998; Li et al., 2003). Further investigations as well as field trials are required in order to determine other biological activities of neem against chironomids.

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REFERENCES


