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**Original Article**

# Antifeeding Potency of Neem (*Azadirachta indica*) Extractives and Limonoids against Termite (*Reticulitermes speratus*)

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Neem oil (HN) deterred feeding by *Reticulitermes speratus* in a no-choice bioassay. A methanol extract of HN (HN-01) was 4-fold more active than HN. Twelve other methanol extracts were subsequently evaluated, of which six were potent ($PC_{95} \leq 1.0\%$ w/w), three moderate ($PC_{95} = 1$–$3\%$ w/w), and the remaining three inactive ($PC_{95}$ beyond bioassay limits). Eleven main limonoids were purified from the active chromatographic fractions of HN-01, which accounted for 81.5% of its activity. No acute toxicity was found, although *R. speratus* given doses higher than estimated $PC_{95}$ tended to die faster than unfed ones. This suggests a possible use of potent neem extractives for termite control.

## INTRODUCTION

Virtual reliance on chlorinated hydrocarbons for control of subterranean termites has aroused public awareness of deleterious effects of these broad-spectrum toxicants on the environment,[1] and alternative control methods have been sought after. Search for effective and environmentally acceptable methods includes a phase to identify plants naturally resistant to termites. Whether biologically active phytochemicals can be used to protect susceptible woods or as models for new classes of synthetic termiticides were studied[2] and reviewed by Logan[3] and Scheffrahn.[4]

Amongst potential phytochemicals, antifeedants are currently attracting attention of researchers. The chemicals are highly recommended because they do not pollute the environment, nor poison food, nor harm non-pest species.[5] We have identified two limonoids (obacunone and nomilin) from Rutaceae plants (*Phellodendron amurense* and *Citru natsudaidai*) that are antifeeding to termite, *Reticulitermes speratus* KOLBE.[6,7] Subsequent efforts to develop a botanical pesticide from these limonoids have been seriously hampered because of poor availability of the raw materials, high extraction cost, or poor solubility of the Rutaceae limonoids in most commercial organic solvents. The present study is part of the ongoing search for a more suitable source of antifeedant limonoids, based on reports that neem products could deter termites.[8] The results showed that several neem oils and their methanol extracts (collectively termed "neem extractives") have antifeeding activity to *R. speratus*.

The most important constituent of neem is azadirachtin,[8,9] which is known to have adverse effects on almost 200 insect species.[10-15] However, azadirachtin was not a potent antifeedant to termites ($PC_{95} = 6.53\%$ w/w), and apparently had no acute toxicity effect.[16]

Co-chromatography also revealed that azadirachtin was either absent or present at a very low concentration in the active extractives, indicating that the antifeeding compound is not azadirachtin. Further study is necessary to identify the active principles. The practicality of neem extractives for termite control is also discussed in this paper.

## MATERIALS AND METHODS

### 1. *Instruments*

The following spectroscopic and analytical instruments were used: JMS-DX-303 (EI and FAB-MS), JEOL GSX-400 (400 MHz for $^1H$ NMR and 100.6 MHz for $^{13}C$ NMR, using TMS as the internal standard), JAI LC-908 (recycling preparative HPLC), and JASCO 880-PU equipped with a JASCO 875-UV detector (analytical HPLC).

### 2. *Termite and Bioassay*

Termites (*R. speratus* pseudogates) and no-choice bioassay (including data analysis for determination of protection concentrations, $PC_{50}$ and $PC_{95}$) were the same as described in the previous paper.[16]

### 3. *Neem Oils*

Neem oils were first extracted from the kernels with an expeller (expeller oils). Then, the undeoiled cake was steeped in *n*-hexane for further extraction (hexane oils). The two oils are often mixed for sale.

### 4. *Activity of Neem Oil Methanol Extracts*

Hexane oil (HN) was extracted with methanol (10 ml/g oil), filtered (Advantec Toyo No. 2, Toyo Roshi Kaisha Ltd., Japan) and evaporated *in vacuo* (methanol extract, HN-01). Effects of HN, HN-01 and the extraction residue (HN-11) on the survival of *R. speratus* were compared. The other 12 neem oil methanol extracts (Table 2) were assayed in order to determine protection concentrations and to compare their effects on survival at a 0.68% dose level.

### 5. *Purification*

HN-01 was loaded to flash chromatography (Cosmosil 140$C_{18}$OPN; $CH_3CN/MeOH/H_2O=$ 35/20/45) to prepare fractions A (25.0%), B (15.0%), and C (methanol flash, 59.0%). The fractions and fraction mixtures were compared for the effects on the survival of *R. speratus*. Eleven main compounds were purified from active fractions A and B by recycling preparative HPLC following the method of

Table 1 Survival of *Reticulitermes speratus* fed on paper discs containing neem oil, methanol extract, or its extract fractions.

| Samples[b] | Doses (% w/w) | % Survival on various days[a] | | | | | |
|---|---|---|---|---|---|---|---|
| | | 5 | 10 | 15 | 20 | 25 | 30 |
| Unfed termites | No Disc | 98 | 84 | 76 | 58 | 28 | 0 |
| Blank | No sample | 98 | 96 | 96 | 96 | 96 | 94 |
| Neem oil (HN) | 0.680 | 96 | 94 | 94 | 94 | 94 | 84 |
| | 1.360 | 92 | 92 | 90 | 90 | 88 | 80 |
| | 2.720 | 92 | 84 | 74 | 58 | 52 | 18 |
| | 5.440 | 92 | 80 | 68 | 34 | 22 | 4 |
| Methanol extract (HN-01) | 0.170 | 98 | 98 | 98 | 94 | 94 | 86 |
| | 0.340 | 96 | 90 | 86 | 78 | 68 | 36 |
| | 0.680 | 92 | 70 | 48 | 36 | 26 | 6 |
| | 1.360 | 94 | 70 | 62 | 44 | 18 | 0 |
| Residue (HN-11) | 1.360 | 98 | 98 | 96 | 94 | 94 | 94 |
| | 2.720 | 98 | 96 | 96 | 94 | 94 | 90 |
| | 5.440 | 98 | 94 | 94 | 94 | 94 | 92 |
| Fraction A | 0.115 | 96 | 96 | 96 | 96 | 94 | 92 |
| | 0.230 | 94 | 94 | 94 | 90 | 86 | 82 |
| | 0.345 | 100 | 98 | 96 | 68 | 42 | 12 |
| Fraction B | 0.082 | 98 | 98 | 96 | 96 | 90 | 86 |
| | 0.164 | 96 | 92 | 88 | 84 | 74 | 74 |
| | 0.246 | 100 | 92 | 86 | 82 | 72 | 70 |
| Fraction C | 0.456 | 100 | 96 | 92 | 88 | 80 | 80 |
| | 0.912 | 100 | 98 | 98 | 96 | 94 | 92 |
| | 1.368 | 100 | 100 | 96 | 90 | 90 | 90 |
| Fractions A+B | 0.197 | 96 | 96 | 96 | 94 | 92 | 80 |
| | 0.394 | 100 | 98 | 92 | 80 | 62 | 34 |
| | 0.591 | 100 | 98 | 98 | 80 | 62 | 8 |
| Fractions A+B+C | 0.680 | 100 | 96 | 92 | 88 | 80 | 70 |
| | 1.360 | 100 | 96 | 86 | 60 | 28 | 4 |
| | 2.040 | 100 | 88 | 82 | 58 | 38 | 0 |

[a] 10 replicates conducted in two sequential runs.

[b] Neem oil was extracted with methanol yielding a 27.0% methanol extract and 71.9% residue. The methanol extract was chromatographed on Cosmosil 140 $C_{18}$-OPN ($CH_3CN/MeOH/H_2O=35/25/40$) yielding fractions A (25.0%), B (15.0%) and C (methanol flash, 59.0%).

Ishida *et al.*[17] A standard curve was constructed for each compound by analytical HPLC (Waters Nova-Pak $C_{18}$; MeOH/$CH_3CN$/$H_2O$=25/35/45; UV-detector, 217 nm) in order to estimate the contents in HN-01. The purified compounds, except **5** that was very minor (<0.1% w/w), were mixed in proportion to their contents in HN-01 for bioassay.

## RESULTS AND DISCUSSION

Extractives of neem are known to have adverse effects on many insects, mites and nematodes with activities ranging from repellence, deterrence, growth inhibition and reduction of survival rates, fitness, reproduction, mating, oviposition and egg hatchability;[10,11,14,18] their antitermitic activity is shown in Table 1. All starved *R. speratus* (unfed termites) died within 30 days, compared with 94% survival in the blank. Addition of HN increased the mortality (5.44% HN, 4% survival on day 30), but no acute toxicity was observed on *R. speratus*, with a low mortality (normally less than 20%) within the initial 10 days of exposure. In another no-choice bioassay using a feeding indicator,[7] colored termites were markedly reduced by HN, almost all being uncolored at a 5.44% dose, suggesting an antifeeding effect. However, at doses higher than the estimated $PC_{95}$, *R.*

Table 2 Protection concentrations (PC) of various neem oil methanol extracts against *Reticulitermes speratus*.

| Neem oils | Oil types[b] | % Methanol extract contents[c] | Methanol extract activity[a] | | |
|---|---|---|---|---|---|
| | | | Probit lines[d] | $PC_{50}$ (% w/w)[e] | $PC_{95}$ (% w/w)[e] |
| 1. Potent antifeeding activity | | | | | |
| AE[f] | Hexane | 91.1 | $Y=2.47X+7.20$ | 0.13 (0.10–0.17) | 0.58 (0.44–0.77) |
| AD | Hexane | 31.6 | $Y=4.50X+7.50$ | 0.28 (0.25–0.31) | 0.64 (0.58–0.71) |
| G | Expeller | 30.0 | $Y=3.51X+7.06$ | 0.26 (0.23–0.30) | 0.75 (0.67–0.84) |
| I | Expeller | 37.2 | $Y=3.77X+6.75$ | 0.34 (0.30–0.41) | 0.91 (0.69–1.19) |
| AA | Hexane | 33.6 | $Y=5.13X+6.58$ | 0.50 (0.45–0.54) | 1.01 (0.88–1.16) |
| A | Mixed | 25.0 | $Y=2.23X+6.55$ | 0.20 (0.16–0.26) | 1.08 (0.74–1.58) |
| 2. Moderate antifeeding activity | | | | | |
| AB | Hexane | 27.1 | $Y=2.46X+6.24$ | 0.32 (0.28–0.36) | 1.40 (1.13–1.73) |
| H | Expeller | 31.8 | $Y=3.48X+6.07$ | 0.50 (0.47–0.52) | 1.45 (1.32–1.60) |
| D | Hexane | 29.9 | $Y=2.35X+5.67$ | 0.53 (0.46–0.60) | 2.29 (1.51–3.46) |
| 3. Not active | | | | | |
| P | Expeller | 30.0 | $Y=1.46X+5.09$ | 0.87 (0.75–1.02) | XXXX |
| AC | Hexane | 42.5 | $Y=2.78X+4.30$ | 1.77 (1.65–1.90) | XXXX |
| J | Mixed | 17.6 | $Y=1.87X+3.86$ | 4.03 (3.01–5.39) | XXXX |

a) 15 replicates conducted in three sequential runs.
b) Based on neem oil extraction methods.
c) Each oil was extracted with 95% methanol (10 ml/g oil).
d) $Y$=% feeding reduction (probit scale), $X$=log dosages (%).
e) XXXX=Estimated PC beyond bioassay limits; 95% fiducial limits in the parentheses.
f) Ethanol extract and re-extracted with methanol.

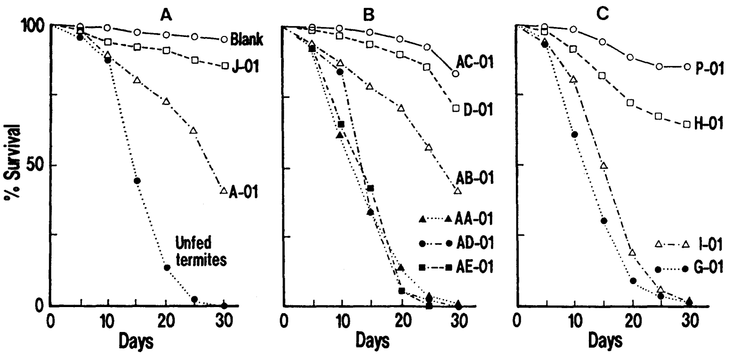


Fig. 1 Effects of various neem oil methanol extracts at a 0.68% dose on the survival of *Reticulitermes speratus*.

Methanol extracts of mixed (A), hexane (B), and expeller (C) neem oils.

*speratus* tended to die faster than unfed termites. This effect was similar to that of obacunone and nomilin, which showed an appreciable effect on the survival of essential hindgut protozoa.[7,16] Chlorpyrifos (Dursban®), a potent toxicant, caused a 100% mortality within two days of exposure to a 0.01% dose (data not shown).

Extracting HN with methanol could pool the activity to the methanol extract (1.36% HN-01, 0% survival on day 30), which was 4-fold more active than HN (Table 1). A check on the other 12 neem oils showed that their methanol extract contents were normally higher than 25% (Table 2), except for J (17.6%).

The antifeeding activity of the methanol extracts varied widely, even amongst those extracted from neem oils of the same type. Furthermore, not all the extracts were active (Table 2). It is therefore necessary to evaluate the extract activity before making any decision. Based on the estimated $PC_{95}$, the most potent extract was AE-01 ($PC_{95}=0.58\%$), followed by AD-01, G-01, I-01, AA-01, A-01, AB-01, H-01 and D-01 with estimated $PC_{95}$ of 0.64, 0.75, 0.91, 1.01, 1.08, 1.40, 1.45 and 2.29%, respectively (Table 2). P-01, AC-01 and J-01 were not active (or $PC_{95}$ beyond bioassay limits). In comparison of Table 2 and Fig. 1, extracts with an estimated $PC_{95}$ of less than or equal to 1% also caused a 100% mortality within 30 days at a 0.68% dose, except for A-01. These extracts are potential for control of termites. In a large scale trial using a Japanese red pine tree (*Pinus densiflora* Sieb.), 10 g/m² of "high activity" extracts ($PC_{95}\leq 1.0\%$) was sufficient to cause a 100% mortality (within 30 days of exposure), with more than 95% feeding reduction (no-choice bioassay).

Further separation of HN-01 by chromatography showed that fraction A was the most active (0.345% dose, 12% survival on day 30), followed by fraction B (0.246% dose, 70% survival), but fraction C had no effect at doses up to 1.368% (Table 1). A mixture of fractions A and B at a 0.591% dose level (or 0.345% A+0.246% B) could further reduce the survival to 8%, indicating that the activity was mainly present in fractions A and B.

Eleven compounds (all limonoids, Fig. 2) purified from fractions A and B were identified as nimbandiol (**1**), 17-hydroxyazadiradione (**2**), deacetylnimbin (**3**), 17-epiazadiradione (**4**), deacetylsalannin (**5**), azadiradione (**6**), nimbin (**7**), deacetylgedunin (**8**), gedunin (**9**), salannin (**10**) and 14-epoxyazadiradione (**11**), based on their spectroscopies ($^1$H NMR, $^{13}$C NMR and FAB-MS) in comparison with those in literature;[19-30] their antifeeding activity is reported elsewhere.[17] The limonoid contents in HN-01 were estimated to be 0.468, 1.348, 0.564, 1.717, <0.1, 6.857, 1.003, 1.077, 1.358, 1.927 and 9.676% w/w, respectively. A mixture of ten limonoids (*i.e.*, all except compound **5**) at a

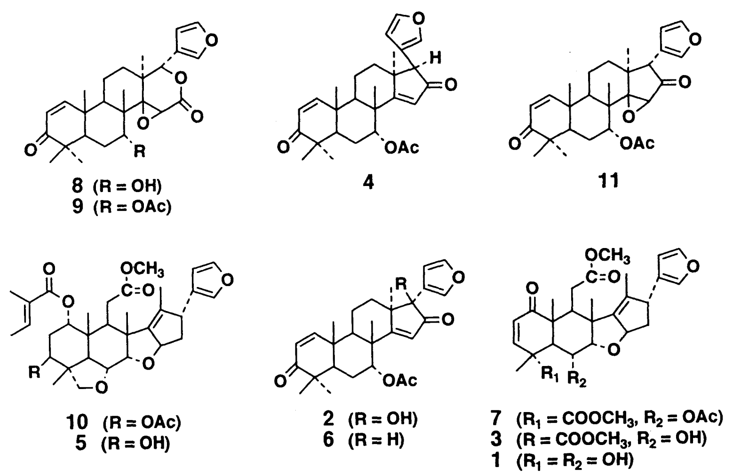


Fig. 2 Main limonoids purified from *Azadirachta indica*.

Nimbandiol (**1**), 17-hydroxyazadiradione (**2**), deacetylnimbin (**3**), 17-epiazadiradione (**4**), deacetylsalannin (**5**), azadiradione (**6**), nimbin (**7**), deacetylgedunin (**8**), gedunin (**9**), salannin (**10**) and 14-epoxyazadiradione (**11**).

Table 3 Comparative effects of a neem oil methanol extract (HN-01) and its limonoid mixture on *Reticulitermes speratus* survival and feeding.[a]

| Treatments | Doses (% w/w) | % Survival on various days | | | | $PC_{50}$ (% w/w) | $PC_{95}$ (% w/w) |
|---|---|---|---|---|---|---|---|
| | | 5 | 10 | 20 | 30 | | |
| Unfed termites | No disc | 98.2 | 88.4 | 13.8 | 1.8 | | |
| Blank | No sample | 98.0 | 94.7 | 92.7 | 90.7 | | |
| Methanol extract | 0.34 | 100 | 96.0 | 92.7 | 88.7 | 0.37 | 1.10 |
| (HN-01) | 0.68 | 96.7 | 90.0 | 72.7 | 57.3 | (0.30–0.48) | (0.82–1.46) |
| | 1.02 | 88.0 | 62.7 | 30.4 | 4.0 | | |
| | 1.36 | 95.3 | 78.0 | 41.3 | 2.0 | | |
| Limonoid mixture[b] | 0.09 | 98.7 | 96.7 | 94.0 | 90.7 | 0.19 | 0.35 |
| | 0.18 | 98.0 | 90.7 | 80.7 | 50.0 | (0.16–0.22) | (0.27–0.46) |
| | 0.27 | 98.0 | 88.7 | 60.0 | 18.0 | | |
| | 0.36 | 96.0 | 83.3 | 56.0 | 2.7 | | |

[a] 15 replicates in three sequential runs.

[b] 10 limonoids (*i.e.*, all except compound **5**) were mixed in proportion to their content in HN-01 for bioassay.

0.36% dose level reduced the termite survival to 2.7% on day 30, which was similar to the mortality with 1.02 or 1.36% HN-01 (Table 3). Estimated $PC_{95}$ for HN-01 and the limonoid mixture (LM) were 1.10 and 0.35%, respectively. Since LM required for $PC_{95}$ is equivalent to the content of 1.35% HN-01, LM is estimated to account for $(1.10/1.35)\times 100\%$ or 81.5% of the HN-01 activity. The "high activity" methanol extracts were also found to contain termite antifeedant limonoids at high concentrations (26.02% in HN-01, Table 3), and it seems possible to use them without processing.

The insecticidal properties of neem are governed by its volatile and non-volatile components, the former playing a major role in determining the repellency (generally at a distance, without contact) of neem extractives towards many insects.[11,31,32] Comparing the estimated $PC_{95}$ of HN-01 and LM, the volatile component, under no-choice bioassay, seems to have little to do with the extract activity (<20%, if any).

Studies on natural antitermitics are still very rare. Many new antitermitics remain to be identified since more than 95% of hundreds of plants already identified to have resistance to termites have not been subjected to chemical identification.[4] More than 300 limonoids have been isolated to date,[33] but their antitermitic activity has remained largely unknown,[4,33] except for some recently evaluated.[6,7,16] There are also some simple insect antifeedants that can be easily synthesized, such as terpenoid drimanes and diterpenoid clerodanes,[5] which have yet to be investigated.

Limonoids are too complicated to be synthesized; their applicability depends much on the availability from natural sources. Neem oil is probably the cheapest among known sources of termite antifeedant limonoids, with India having an annual production potential of 100,000 metric tons. Reviews on the safety of neem products/extractives are voluminous.[13-15] The results have all shown that neem is safe to humans, warm-blooded animals, birds and beneficial insects such as honeybees, spiders, earwigs and ants, except for the parasitic wasps which were slightly affected. There seem to be no resistance problems arising from the use of neem. For instance, *Plutella xylostella*, which is resistant to numerous synthetic pesticides, failed to develop resistance to neem extractives after 42 generations.[34] Such safety data further enhance the potentiality of using neem extractives as botanical pesticides against termites.

### REFERENCES


1) Anonymous: *Pest Cont.* **54**, 22 (1986)
2) F. L. Carter, S. C. Jones, J. K. Mauldin & C. R. R. de Camargo: *Z. Angew. Entomol.* **95**, 5 (1983)
3) J. W. M. Logan, R. H. Cowie & T. G. Wood: *Bull. Entomol. Res.* **80**, 309 (1990)
4) R. H. Scheffrahn: *Sociobiology* **19**, 257 (1991)
5) S. V. Ley & P. L. Toogood: *Chem. Br.* **26**, 31 (1990)
6) H. Kawaguchi, M. Kim, M. Ishida, Y. J. Ahn, T. Yamamoto, R. Yamaoka, M. Kozuka, K. Goto & S. Takahashi: *Agric. Biol. Chem.* **53**, 2635 (1989)
7) M. Serit, M. Ishida, M. Kim, T. Yamamoto & S. Takahashi: *Agric. Biol. Chem.* **55**, 2381 (1991)
8) J. H. Butterworth & E. D. Morgan: *Chem. Commun.* **1169**, 23 (1971)
9) J. H. Butterworth & E. D. Morgan: *J. Insect Physiol.* **17**, 969 (1971)
10) M. Jacobson: "Natural Resistance of Plants to Pest: Roles of Allelochemicals," eds. by M. B. Green & P. A. Hedin, ACS Symp. Ser. 296, American Chem. Soc., Washington, DC, pp. 220–232, 1986
11) R. C. Saxena: "Insecticides of Plant Origin," eds. by J. T. Arnason, B. J. R. Philogene & P. Morand, ACS Symp. Ser. 387, American Chem. Soc., Washington, DC, pp. 111–135, 1989
12) H. Rembold: "Insecticides of Plant Origin," eds. by J. T. Arnason, B. J. R. Philogene & P. Morand, ACS Symp. Ser. 387, American Chem. Soc., Washington, DC, pp. 150–163, 1989
13) H. Schmutterer: *J. Insect Physiol.* **34**, 713 (1988)
14) H. Schmutterer: *Annu. Rev. Entomol.* **35**, 271 (1990)
15) O. Koule, B. I. Murray & C. M. Ketkar: *Can. J. Bot.* **68**, 1 (1990)
16) M. Serit, M. Ishida, N. Hagiwara, M. Kim, T. Yamamoto & S. Takahashi: *J. Chem. Ecol.* **18**, 593 (1992)
17) M. Ishida, M. Serit, K. Nakata, R. L. Juneja, M. Kim & S. Takahashi: *Biosci. Biotech. Biochem.* **56**, 1835 (1992)
18) J. D. Warthen: *Proc. Entomol. Soc. Wash.* **91**, 367 (1989)
19) A. Bruhn, M. Bokel & W. Kraus: *Tetrahedron Lett.* **25**, 3691 (1984)
20) W. Kraus & R. Cramer: *Chem. Ber.* **114**, 2375 (1981)
21) S. Siddiqui, S. Fuchs, J. Lücke & W. Voelter: *Tetrahedron Lett.* **7**, 611 (1978)
22) W. Kraus & R. Cramer: *Tetrahedron Lett.* **27**, 2395 (1978)
23) S. Siddiqui, T. Mahmood, B. S. Siddiqui & S. Faizi: *J. Nat. Prod.* **49**, 1068 (1986)
24) W. Kraus & R. Cramer: *Liebigs Ann. Chem.* **1981**, 181 (1981)
25) I. Kubo, A. Matsumoto, T. Matsumoto & J. A. Klocke: *Tetrahedron* **42**, 489 (1986)
26) D. Lavie, E. C. Levy & M. K. Jain: *Tetrahedron* **27**, 3927 (1971)
27) S. R. Rojatkar, V. S. Bhat, M. M. Kulkarni, V. S. Joshi & B. A. Nagasampagi: *Phytochemistry* **28**, 203 (1989)
28) M. Harris, R. Henderson, R. McCrindle, K. H.

Overtone & D. W. Turner: *Tetrahedron* **24**, 1517 (1968)
29) S. A. Khalid, H. Duddeck & M. Gonzales: *J. Nat. Prod.* **52**, 922 (1989)
30) C. R. Narayanan, R. V. Pachapurkar & B. M. Sawant: *Tetrahedron Lett.* **37**, 3563 (1967)
31) S. M. Lee, J. A. Klocke, M. A. Barnby, R. B. Yamasaki & M. F. Balandrin: "Naturally Occurring Pest Bioregulators," ed. by P. H. Hedin, ACS Symp. Ser. 449, American Chem. Soc., Washington, DC, pp. 291–304, 1990
32) M. F. Balandrin, S. M. Lee & J. A. Klocke: *J. Agric. Food Chem.* **36**, 1048 (1988)
33) D. E. Champagne, O. Koul, M. B. Isman, G. G. E. Scudder & G. H. N. Towers: *Phytochemistry* **32**, 377 (1992)
34) M. Vollinger: "Natural Pesticides from Neem Tree and Other Tropical Plants," ed. by H. Schmutterer & K. R. S. Ascher, Proc. of 3rd Int. Neem Conf., Nairobi, pp. 543–554, 1986

## 要　約

### ニーム抽出物およびリモノイドのヤマトシロアリに対する摂食阻害活性

Muney Serit, 石田宗孝, 中田勝康
金　武祚, 高橋正三

ニームオイル (HN) は，非選択条件下でヤマトシロアリの摂食を阻害した．HN のメタノール抽出物 (HN-01) は HN の 4 倍の活性を有した．次いで，他の 12 種類のメタノール抽出物を評価したところ，6 種類は強い ($PC_{95}$ < 1.0% w/w)，3 種類は中程度の ($PC_{95}$ = 1〜3% w/w) 活性を示し，残りの 3 種類は無活性 ($PC_{95}$ 検定域外) であった．HN-01 の活性画分より精製された 11 種類の主要なリモノイドによって，HN-01 の活性の 81.5% が明らかとなった．$PC_{95}$ より高い供試量のとき，無供餌のものよりも若干はやく死亡する傾向がみられたが，急性毒性効果は認められなかった．本研究によりニーム抽出物のシロアリ防除への有用性が示唆された．