Phytochem Rev (2008) 7:77–88
DOI 10.1007/s11101-006-9048-7

Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuncta*

Y. Akhtar · Y.-R. Yeoung · M. B. Isman

Received: 6 July 2006 / Accepted: 2 November 2006 / Published online: 13 March 2007

Abstract Plant-derived extracts and phytochemicals have long been a subject of research in an effort to develop alternatives to conventional insecticides but with reduced health and environmental impacts. In this review we compare the bioactivities of some plant extracts with those of commercially available botanical insecticides against two important agricultural pests, the cabbage looper, *Trichoplusia ni* and the armyworm, *Pseudaletia unipuncta*. Test materials included extracts of *Azadirachta indica* (neem), *A. excelsa* (sentang), *Melia volkensii, M. azedarach* (Chinaberry) and *Trichilia americana*, (all belonging to the family Meliaceae) along with commercial botanical insecticides ryania, pyrethrum, rotenone and essential oils of rosemary and clove leaf. Most of the extracts and botanicals tested proved to be strong growth inhibitors, contact toxins and significant feeding deterrents to both lepidopteran species. However, there were interspecific differences with *T. ni* generally more susceptible to the botanicals than the armyworm, *P. unipuncta*. All botanicals were more inhibitory to growth and toxic (through feeding) to *T. ni* than to *P. unipuncta*, except for *M. azedarach* which was more toxic to *P. unipuncta* than to *T. ni*. Athough, pyrethrum was the most toxic botanical to both noctuids, *A. indica*, *A. excelsa*, and *M. volkensii* were more toxic than ryania, rotenone, clove oil and rosemary oil for *T. ni*. As feeding deterrents, pyrethrum was the most potent against *T. ni*, whereas *A. indica* was the most potent against the armyworm. Based upon growth inhibition, chronic toxicity, and antifeedant activity, some of these plant extracts have levels of activity that compare favorably to botanical products currently in commercial use and have potential for development as commercial insecticides.

Keywords *Azadirachta indica* · *A. excelsa* · *Melia volkensii* · *M. azedarach* · *Trichilia americana* · Rotenone · Rosemary oil · Clove oil · Ryania · Feeding deterrence

Y. Akhtar · M. B. Isman (✉)
Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, CanadaV6T 1Z4
e-mail: murray.isman@ubc.ca

Y.-R.Yeoung
Department of Applied Plant Science, Kangnung National University, Gangneung, Kangwon-Do 201-702, Korea

Introduction

Biopesticides are an important group of naturally occurring, often slow-acting crop protectants that are usually safer to humans and the environment than conventional pesticides, and with minimal residual effects. Biopesticides can be biochemical or microbial. Biochemical pesticides may include plant-derived pesticides (botanicals) that can interfere with the growth, feeding, or reproduction of pests or insect pheromones applied for mating disruption, monitoring or attract-and-kill strategies (Copping and Menn 2000). Microbial pesticides contain a microorganism such as a bacterium, virus, fungus, protozoan or an alga as an active ingredient to control pests. The most widely used microbial pesticide is the bacterium *Bacillus thuringiensis* or Bt for control of insect pests on various crops including cotton, rice, potatoes and cabbage. More information on microbial pesticides can be found in a review by Copping and Menn (2000). In the present paper we will focus on some botanicals with insecticidal activities.

Plant extracts and phytochemicals have long been a subject of research in an effort to develop alternatives to the conventional insecticides. The plant families Meliaceae and Rutacae (order: Rutales) have received much attention at least partly owing to the presence of limonoid triterpenes (Klocke and Kubo 1982; Connolly 1983; Arnason et al. 1987; Champagne et al. 1992). The Meliaceae (mahogany family) contains approximately 50 genera and over 500 species (Pennington and Styles 1975). Chemically, it is characterized by a diverse variety of limonoids, many of which are known to possess insecticidal properties. This has lead to a systematic investigation of this family for bioactivity against insects (Isman et al. 2002). Most work has been focused on azadirachtin, a limonoid from the seeds of the Indian neem tree, *Azadirachta indica*. Neem seed extracts rich in azadirachtin (10–25%) act both as potent antifeedants and insect growth regulators (Koul 1992; Govindachari et al. 2000; Kraus 2002). The role of other triterpenoids (nimbin, salannin, and derivatives thereof) present in neem seed extracts, as contributors to overall bioactivity, is controversial and most evidence points to azadirachtin as the most important active principle (Isman et al. 1996). Similarly, the limonoids in *Azadirachta excelsa* (sentang) are known to be excellent antifeedants for many pest species with no deleterious effects on humans, animals or beneficial insects (Mordue and Blackwell 1993). A methanolic extract of *A. excelsa* wood inhibited growth, feeding and was toxic to the larvae of *Crocidolomia binotalis* (Ng et al. 2003). *A. excelsa* is native to the Malaysia, Indonesia and the Philippines. The remarkable bioactivity exhibited by azadirachtin from the Indian neem tree (*A. indica*) led to the search for other natural insecticides in the closely related genus *Melia*. *M. azedarach* (syn. *M. toosendan* in China) grows mainly as an ornamental and medicinal plant. As an avenue tree it can be found in Spain, Greece, Cyprus, Israel, Tunisia, Algeria, India, Australia, New Zealand, the Caribbean, Brazil and Argentina (Ascher et al. 2002). *M. azedarach* contains limonoids closely related to *A. indica.* Some of the limonoids isolated from the fruits of *M. azedarach* are meliantriol, melianone, melianol (Lavie and Jain 1967), meliacin (1-cinnamoyl melianone), meliacarpin (Lee et al. 1991) and meliartenin (Carpinella et al. 2002). Meliacarpins were found first in *M. azedarach* extracts (Kraus 1986) and later also in the seeds of *A. indica* (Kraus 2002). Meliantriol showed strong antifeedant properties against the desert locust, *S. gregaria* (Kraus et al. 1981) and meliartenin inhibited larval feeding of *E. panuelata* and *S. eridania* (Carpinella et al. 2002). Seed oil of *M. azedarach* acted as a strong oviposition deterrent for rice gall midge, *Orseolia oryzae*, and a feeding deterrent for oriental armyworm, *Mythimna separata* (Chiu et al. 1984). Fruit extracts of *M. azedarach* and *A. indica* showed feeding deterrent effects against the larvae of *Plutella xylostella* at higher doses (Charleston et al. 2005) and also a variety of insect species belonging to three different orders including Coleoptera, Lepidoptera and Orthoptera (Carpinella et al. 2003). Seed oil sprays were also effective against citrus red mite, *Panonychus citri* and the orange spiny whitefly, *Aleurocanthus spiniferus* but was not harmful to several predatory mites (*Amblyseius* spp.) (Chiu 1989).

Melia volkensii is a tall (15–25 m) woody tree, which grows in semi-arid areas of East Africa between ca. 350 and 1700 m above sea level. Its large, olive-like yellow ripe fruits are 4–5 cm long and ca. 3 cm in diameter and consequently more than four times heavier in weight than the fruits of *A. indica* or *M. azedarach* (Rembold and Mwangi 2002). *M. volkensii* (Meliaceae) seeds also contain several limonoids including volkensin and salannin, the latter also occurring in neem (Rajab and Bentley 1988). Little is known about the insecticidal activities of individual limonoids in the extract. Volkensin, ohchinin-3-acetate, and salannin reduced the feeding of third instar fall armyworm (*Spodoptera frugiperda*) on corn leaf discs with DC_{50} (concentration causing 50% feeding deterrence compared with the control) values ranging from 3.5 μg/cm^2 for volkensin to 1.3 μg/cm^2 for salannin (Rajab et al. 1988). We have found salannin to possess weak growth-inhibiting and antifeedant properties against third instar *T. ni* larvae (Akhtar and Isman, unpublished data) as well as to larvae of *S. litura* and nymphs of *Myzus persicae* [10-day dietary EC_{50} (concentrations inhibiting larval growth by 50% relative to controls) was 0.21 ppm for azadirachtin and 15.7 ppm for salannin in *S. litura;* LC_{50} (concentration causing 50% mortality compared with the control) was 1.3 and 383 ppm for azadirachtin and salannin, respectively in the nymphs of *M. persicae*, Isman et al. 1996]. The fruit extract of *M. volkensii* is toxic to a broad range of insects including dipterans, lepidopterans, and coleopterans (Mwangi and Rembold 1987, 1988) and the seed extract is toxic to Lepidoptera and Coleoptera (Akhtar and Isman 2004a). Fruit extracts were first reported to exert insect growth-inhibiting and antifeedant effects (22.4% deterrence at 1 ppm to 95% deterrence at 10 ppm) on the nymphs and adults of the desert locust, *Schistocerca gregaria* (Mwangi 1982).

The genus *Trichilia* contains a variety of limonoids and has been identified as a potential source of plant-based insecticides. A series of limonoids from *T. roka* (Meliaceae), the trichilins, are antifeedants for the southern armyworm, *Spodoptera eridania* and the Mexican bean beetle, *Epilachna varivestis* (Nakatani et al. 1981, 1985). Limonoids isolated from *T. hirta* inhibited larval growth of *Peridroma saucia* and reduced consumption rates and dietary utilization, indicating behavioural effects and post-ingestive toxicity (Xie et al. 1994). Foliar extracts of *T. hirta* inhibited growth of *P. saucia* (Champagne et al. 1989), and *T. americana* extract from small woody twigs strongly inhibited larval growth of *Spodoptera litura* (Wheeler and Isman 2001). *T. americana* is a tropical deciduous tree found from north-west Mexico through Central America. In addition to *Trichilia*, other members in the family Meliaceae, such as *Aglaia* (containing the benzofuran rocaglamide), *Turraea* and *Cedrela* have shown high levels of bioactivity and warrant further study. Although, numerous plant species in the family Meliaceae exhibit promising bioactivity against a variety of insects, only neem extract is approved for use and sold as a botanical insecticide in the USA (Isman 1997). Table 1 shows the active ingredients and the modes of action of the botanicals used in the present study.

Table 1 Plant extracts and botanical insecticides tested for toxicity, larval growth inhibition and feeding deterrence in the cabbage looper and armyworm

Botanicals	Active ingredient	Mode of action
Azadirachta excelsa	Azadirachtin analogues; content unknown	Antifeedant, growth inhibitor, IGR
Azadirachta indica (neem)	Azadirachtin A (31%); Azadirachtin B (6%)	Antifeedant, growth inhibitor, IGR
Melia azedarach	Toosendanin (3%) and limonoid analogues	Antifeedant, growth inhibitor
Melia volkensii	Mixture of limonoids; content unknown	Antifeedant, growth inhibitor
Trichilia americana	Unknown limonoids	Antifeedant, growth inhibitor
Tanacetum cinerariaefolium	Pyrethrins (20%)	Contact neurotoxin
Lonchocarpus sp. (dust)	Rotenone (1%) and other isoflavonoid analogues	Mitochondrial poison
Ryania speciosa (dust)	Ryanodine (0.05%) and related alkaloids	Neuro-muscular toxin, contact and stomach poison
Syzygium aromaticum (clove) oil	Eugenol (60%)	Contact neurotoxin
Rosmarinus officinale (Rosemary) oil	1,8-Cineole (50%)	Contact neurotoxin

Although, hundreds of plant natural products have demonstrated deleterious effects on insects only a handful of botanical insecticides are currently approved for use in industrialized countries (Isman 1994) for several reasons (outlined in Isman 2006). At present there are four major botanical products (pyrethrum, rotenone, neem, and essential oils) used for insect control along with three others (ryania, nicotine, and sabadilla) in limited use (Isman 2006). Nicotine, owing to its extreme toxicity to mammals (rat oral LD_{50} = 50 mg kg^{-1}) and its rapid dermal absorption in humans has lost its regulatory approval in many countries (Isman 1997). The stem wood of the Caribbean shrub, *Ryania speciosa* (Flacourtiaceae) contains the alkaloid ryanodine, that acts as a muscle poison. Ryania has toxic and growth inhibiting effects against the tobacco budworm, *Heliothis virescens* (Yoshida and Toscano 1994), and has seen limited use by organic apple and pear growers for control of the codling moth, *Cydia pomonella*. Rotenone is an isoflavonoid extracted from the roots of derris plants (*Derris elliptica* and *Lonchocarpus* spp; Leguminoseae). Although, rotenone has been used as an insecticide for over 150 years, its use as a strong fish poison dates back even further (Shepherd 1951). Rotenone is a very toxic compound (rat oral lD_{50} = 132 mg^{-1}) active against a wide range of insects. Rotenone dusts (containing 1–5% active ingredients) and sprays (containing 8% rotenone and 18% total rotenoids) have been used for years to control aphids, beetles and caterpillars on plants, as well as fleas and lice on animals. Rotenone is a mitochondrial poison, which blocks the electron transport chain and prevents energy production (Hollingworth et al. 1994). It acts as a stomach poison and a contact insecticide. As an agricultural insecticide, use of rotenone is limited to organic food production (Isman 2006).

The active ingredients in pyrethrum extract consisting of a mixture of pyrethrin I (40%), pyrethrin II (36%), cinerin I and cinerin II (12%) are obtained from the dried flowers of the pyrethrum daisy (*Tanacetum cinerariaefolium*; Asteraceae). Technical grade pyrethrum, the resin used in formulating commercial insecticides, typically contains 20–25% pyrethrins (Charleston 2004). Pyrethrins I and II account for most of the insecticidal activity, and have been used as insecticides from ancient times. Initial effects include paralysis followed by death. Most flying insects are highly susceptible to pyrethrins, causing them to 'drop' almost immediately upon exposure whereas, hyperactivity and convulsions are common in most insects. The mode of action of pyrethrins relates to their ability to affect sodium channel function in the neuronal membranes. Natural pyrethrins are moderately toxic to mammals (rat oral acute LD_{50} values range from 350–500 mg kg^{-1}) but technical grade pyrethrum is less toxic (~1500 mg kg^{-1}) (Casida and Quistad 1995). Natural pyrethrins are unstable in light compared with the synthetic derivatives (pyrethroids). Pyrethrum is the predominant botanical in use, accounting for 80% of the world botanical insecticide market (Isman 2005).

Essential oils, produced by steam distillation of many aromatic plants, have recently received much attention due to their broad spectrum of action. The oils are generally composed of complex mixtures of monoterpenes, biogenetically related phenols, and sesquiterpenes (Isman 2005). Plants producing essential oils that have been used for insect control include the mint family (Lamiaceae), such as garden thyme (*Thymus vulgaris*), rosemary (*Rosmarinus officinalis*), various species of mint (*Mentha* spp.) (Isman, 1999, 2004), clove (*Syzygium aromaticum*, Myrtaceae), eucalyptus *(Eucalyptus globus*) and cinnamon (*Cinnamomum zealanicum*, Lauraceae). Insecticidal activity of essential oils has been reported against a number of insects including cockroaches (Appel et al. 2001), mosquitoes (Watanabe et al. 1993), houseflies (Singh and Singh 1991), stored product pests (Dales 1996) and termites (Zhu et al. 2001a, b). The insecticidal activities of essential oils result from effects on the nervous system either by interference with GABA-gated sodium channels (Priestley et al. 2003) or antagonism of octopamine receptors (Enan 2001). Essential oils have seen some commercial success as insecticides in the past 7–8 years and most are non-toxic to birds, mammals and fish (Isman 1999; Stroh et al. 1998). Some essential oils used in processed food and beverages are exempt from registration in the United States (Quarles 1996).

In the present study we compare bioactivity of plant extracts of *A. indica, A. excelsa, M. volkensii, M. azedarach* and *T. americana*, (Meliaceae) with commercially available botanical insecticides, ryania, pyrethrum, rotenone and two essential oils, rosemary oil and clove leaf oil against two important agricultural pests, the cabbage looper, *Trichoplusia ni* and the armyworm, *Pseudaletia unipuncta*. These were obtained from established colonies maintained on an artificial diet in the insectary of the University of British Columbia (UBC) at room temperature (19–24°C) with a photoperiod of 16:8 LD. The cabbage looper is a polyphagous pest, best known as an important pest of cruciferous plants, but it can also attack several other crops including lettuce, celery, tomato, certain ornamentals and many weedy plants (Davidson and Lyon 1979). The true armyworm is an oligophagous pest of grain crops with wheat, corn, oats, barley, and rye among its favored food plants (Davidson and Lyon 1979).

Materials and methods

Botanicals

A refined extract of seeds of *M. volkensii* was obtained from the University of Nairobi, Kenya. A refined extract of seeds of *A. indica* was purchased from ITC Ltd. (India) and a crude extract of *A. excelsa* stemwood was prepared in our laboratory (Schmutterer et al. 2002). *Trichilia americana* was collected from Costa Rica and a twig extract was prepared in our lab (Wheeler et al. 2001). A refined bark extract of *M. azedarach* (containing 3% toosendanin) was provided by North West Agricultural University, Yangling, China. Rosemary oil, clove leaf oil, and technical pyrethrum (20%) were provided by EcoSmart Technologies Inc. (Franklin, TN, USA). Rotenone dust was purchased from Later Chemicals Ltd. (Richmond, B.C. Canada) and ryania dust was a gift from Dr. Alan Knight (USDA ARS, Wenatchee, WA, USA).

Growth inhibition and toxicity bioassays

The effects of botanicals on larval growth were investigated by feeding neonate larvae (<24-h-old) on leaf discs treated with 5 µl of a methanolic solution of the test extract (botanical) for 72 h in a 50 × 9 mm Petri dish (Falcon®) transferred thereafter onto normal diet for an additional 96 h as described in Isman (2005). Mortality and fresh weights of surviving larvae were recorded. Mortality was also determined after spraying larvae directly with botanicals at 24 and 168 h (7 days). Third instar *T. ni* larvae were sprayed with botanicals until wet in 90 × 15 mm Petri dishes (Falcon®) lined with Fisher scientific filter paper (90 mm diameter). Small plastic bottles (50 ml capacity) were used for spraying. Larvae were then transferred to Petri dishes (90 × 15 mm) with a small piece of diet. Each Petri dish contained 10 larvae.

Feeding deterrent bioassays

Leaf disc choice bioassays (Akhtar et al. 2003; Akhtar and Isman 2004a) were conducted to determine feeding deterrent effects of the botanicals using freshly moulted third instar larvae starved for 4–5 h prior to each bioassay. Larvae were given the choice of feeding on two leaf discs, one treated with 10 µl of a solution of the test substance painted on each side and the other with carrier solvent alone. Areas of control and treated leaf discs consumed by the larvae were measured using Scion Image software and feeding deterrence was calculated as detailed in Akhtar et al. (2003) using the formula: $\{(C - T)\ (C + T)\}*100$ where C and T are areas consumed of the control and treated leaf discs, respectively.

Comparison of EC_{50} and LC_{50} values of botanicals

The EC_{50} values for each noctuid were plotted against their respective dietary LC_{50} values determined in the chronic growth and toxicity bioassays, respectively, to explore the relationship between the two bioassays using correlation analysis.

Results

Growth inhibition

Most of the botanicals tested inhibited larval growth of neonate *T. ni* and *P. unipuncta* in a dose-dependent manner when applied to leaf discs. EC_{50} values generated by linear regression analyses after 168 h (7 days) of feeding (72 h (3 days) on leaf discs painted with extract and 96 h (4 days) on the normal diet) are shown in Table 2. The botanical most inhibitory to growth of *T. ni* was *A. indica* (EC_{50} = 2.08 ppm). The second most active botanical was *A. excelsa* (EC_{50} = 2.9 ppm) followed by pyrethrum (EC_{50} = 6 ppm), *M. volkensii* (EC_{50} = 9 ppm), *M. azedarach* (EC_{50} = 100 ppm), clove oil (EC_{50} = 400 ppm) and rotenone (EC_{50} = 800 ppm). *T. americana* (EC_{50} = 5200 ppm) and ryania (EC_{50} = 6500 ppm) were of medium range and rosemary oil was the least active (EC_{50} = 12,000 ppm) as shown in Table 2. The most active growth inhibitor to *P. unipuncta* was *A. indica* (EC_{50} = 5 ppm) followed by pyrethrum (EC_{50} = 670 ppm), *M. volkensii* (EC_{50} = 2400 ppm) and *A. excelsa* (EC_{50} = 4000 ppm). Ryania (EC_{50} = 104,000 ppm) is the least active with all others falling in the medium range (Table 2).

Toxicity (feeding)

Oral LC_{50} values for the botanicals are shown in Table 3. For *T. ni* larvae pyrethrum was the most toxic botanical (LC_{50} = 40 ppm), followed by *A. indica* (LC_{50} = 100 ppm), *T. americana* (LC_{50} = 1200 ppm), *M. volkensii* (LC_{50} = 1500 ppm) and *A. excelsa* (LC_{50} = 2100 ppm). Rosemary oil and *M. azedarach* were the least toxic with all others falling in the medium range (Table 3). The most toxic botanical to *P. unipuncta* was pyrethrum (LC_{50} = 100 ppm), followed by clove oil (LC_{50} = 4900 ppm). The LC_{50} values for all other extracts tested exceeded 5000 ppm.

Toxicity (spraying)

LC_{50} values for the botanicals through direct spraying are shown in Table 4. Mortality data (day 7) shows that pyrethrum was the most toxic botanical for *T. ni* larvae (LC_{50} = 0.4 ppm), followed by *A. indica* (LC_{50} = 1700 ppm), rotenone (LC_{50} = 2600 ppm), *A. excelsa* (LC_{50}= 4700 ppm), and *T. americana* (LC_{50}= 6400 ppm). *Melia azedarach* and rosemary oil were the least toxic with *M. volkensii*, ryania and clove oil falling in the medium range (Table 4). Comparison of 24 h toxicity with that at 168 h (7 days) indicates that most of the botanicals are slow acting toxins.

Feeding deterrent effects

Pyrethrum was the most active feeding deterrent (DC_{50} = 0.94 μg/cm^2) (Table 5) for *T. ni*, followed

Table 2 Growth inhibition by selected plant extracts and botanical insecticides in two noctuid caterpillars

Botanicals	*T. ni*		*P. unipuncta*	
	EC_{50} (ppm)[b]	r^2 [c]	EC_{50} (ppm)[b]	r^2 [c]
A. excelsa[a]	2.9	0.75	$4.0*10^3$	0.96
A. indica	2.08	0.63	5.0	0.95
M. azedarach[a]	100	0.85	$2.26*10^4$	0.87
M. volkensii[a]	9.0	0.73	$2.4*10^3$	0.96
T. americana[a]	$5.2*10^3$	0.95	$1.72*10^4$	0.99
Pyrethrum	6.0	0.99	670	0.98
Rotenone	800	0.96	$1.25*10^4$	0.90
Ryania[a]	$6.5*10^3$	0.88	$1.04*10^5$	0.77
Clove oil	400	0.99	$6.9*10^3$	0.98
Rosemary oil	$1.2*10^4$	0.98	$6.29*10^4$	0.97

[a] Previously reported in Isman (2005)

[b] Concentration causing 50% growth relative to controls; extracts were applied to leaf discs fed to neonate caterpillars for 3 days; larvae were transferred to the normal diet; larval weight and survival determined after 7 days altogether; linear regression analysis was applied for all dose-response experimental data in chronic growth bioassays. Twenty neonates were used for each of 4–5 concentrations (ranging from 1250–20,000 ppm) for each botanical. Botanicals with EC_{50} and LC_{50} values greater than 2% were tested at higher concentrations

[c] Coefficient of determination

Table 3 Toxicity by selected plant extracts and botanical insecticides to two noctuid caterpillars through feeding

Botanicals	*T. ni*		*P. unipuncta*	
	LC_{50} (ppm)[b]	r^2 [c]	LC_{50} (ppm)[b]	r^2 [c]
A. excelsa	2.1*10^3	0.99	1.29*10^5	0.96
A. indica[a]	100	0.90	8.3*10^3	0.99
M. azedarach[a]	6.01*10^4	0.89	3.06*10^4	0.88
M. volkensii[a]	1.5*10^3	0.72	6.1*10^3	0.93
T. americana[a]	1.2*10^3	0.98	1.26*10^5	0.95
Pyrethrum	40	0.99	100	0.82
Rotenone	6.1*10^3	0.89	1.28*10^6	0.89
Ryania[a]	3.5*10^3	0.95	8.8*10^3	0.82
Clove oil	3.7*10^3	0.97	4.9*10^3	0.99
Rosemary oil	2.59*10^4	0.99	6.40*10^5	0.96

[a] Previously reported in Isman (2005)

[b] Concentration causing 50% toxicity calculated by PROBIT analysis (Finney 1971); extracts were applied to leaf discs fed to neonate caterpillars for 3 days; larvae were transferred to the normal diet; larval weight and survival determined after 7 days altogether to observe the full extent of toxicity

[c] Coefficient of determination

Table 4 Toxicity by selected plant extracts and botanical insecticides to *Trichoplusia ni* caterpillars through spraying

Botanicals	*T. ni*			
	LC_{50} (ppm)[a] 24 h	r^2 [b]	LC_{50} (ppm)[a] 168 h (7 days)	r^2 [b]
A. excelsa	4.7*10^4	0.99	4.7*10^3	0.96
A. indica	9.8*10^4	0.90	1.7*10^3	0.99
M. azedarach	1.32*10^5	0.89	1.26*10^5	0.89
M. volkensii	2.52*10^5	0.89	9.4*10^4	0.95
T. americana	3.9*10^4	0.98	6.4*10^3	0.97
Pyrethrum	0.7	0.99	0.4	0.99
Rotenone	3.3*10^3	0.89	2.6*10^3	0.89
Ryania	8.9*10^4	0.95	7.2*10^4	0.98
Clove oil	6.3*10^4	0.94	5.4*10^4	0.99
Rosemary oil	2.14*10^5	0.96	1.36*10^5	0.98

[a] Concentration causing 50% toxicity calculated by PROBIT analysis (Finney 1971); extracts were sprayed directly on freshly moulted third instar *T. ni* larvae (n = 25–30). Survival determined after 24 h and 168 h (7 days) of feeding on the normal diet to observe the full extent of toxicity

[b] Coefficient of determination

by *M. volkensii* (DC_{50} = 5.8 μg/cm^2), rotenone (DC_{50} = 9.3 μg/cm^2), *A. indica* (DC_{50} = 21.9 μg/cm^2) and *A. excelsa* (DC_{50} = 36.7 μg/cm^2). Rosemary oil, clove oil, *M. azedarach* and *T. americana* were of medium range and ryania was the least active (DC_{50} = 725.1 μg/cm^2) feeding deterrent for *T. ni*. *A. indica* was the most active feeding deterrent for *P. unipuncta* (D_{50} = 0.6 μg/cm^2), followed by pyrethrum (DC_{50} = 3.8 μg/cm^2), *M. volkensii* (DC_{50} = 10.8 μg/cm^2), *A. excelsa* (DC_{50} = 46.9 μg/cm^2) and rotenone (DC_{50} = 61.5 μg/cm^2). Clove oil and *M. azedarach* were of medium range with ryania (DC_{50} = 400 μg/cm^2) and rosemary oil (DC_{50} = 501 μg/cm^2), the least active feeding deterrents for *P. unipuncta* (Table 5).

Comparison of EC_{50} and LC_{50} values of botanicals

There was no correlation between EC_{50} values and their respective LC_{50} values (dietary) ($P > 0.05$) for either noctuid (r = 0.0357, t 0.05

Table 5 Feeding deterrence by selected plant extracts and botanical insecticides in two noctuid caterpillars

Botanicals	*T. ni*		*P. unipuncta*	
	DC_{50} (μg/cm^2) [b]	r^2 [c]	DC_{50} (μg/cm^2)[b]	r^2 [c]
A. excelsa	36.7	0.99	46.9	0.95
A. indica[a]	21.9	0.99	0.6	0.89
M. azedarach[a]	288.0	0.94	248.9	0.87
M. volkensii[a]	5.8	0.94	10.8	0.92
T. americana	189.6	0.9	–	
Pyrethrum	0.94	0.99	3.8	0.99
Rotenone	9.3	0.98	61.5	1.0
Ryania[a]	725.1	0.92	400.1	0.87
Clove oil[a]	217.4	0.98	206.2	0.92
Rosemary oil	158.2	0.91	501.5	0.97

[a] Previously reported in Isman (2005)

[b] Concentrations causing 50% feeding deterrence; extracts were applied to leaf discs presented to caterpillars in leaf disc choice bioassay; DC_{50}s (concentrations causing 50% feeding deterrence compared with the control) were calculated for each botanical (n = 20) using Excel; linear regression analysis was applied for all dose- response experimental data

[c] Coefficient of determination

– Not tested

[2],8,8 = 2.306 > t for *T. ni* and r = 0.0662, t 0.05 [2],8,8 = 2.306 > t for *P. unipuncta*).

Discussion

Most of the botanical extracts tested proved to be strong growth inhibitors, acutely toxic and active feeding deterrents against both lepidopteran species. However, there were interspecific differences with *T. ni* generally more susceptible to the effects of botanicals than the armyworm, *P. unipuncta*.

Extracts from both *Azadirachta* species and *M. volkensii* strongly inhibited growth in both noctuid species, although, the armyworm (*P. unipuncta*) was much less susceptible to the botanicals (Table 2). The least active botanical, rosemary oil, inhibited growth of *T. ni* with EC_{50} = 12,000 ppm. However, ryania did not inhibit growth of *P. unipuncta*. In terms of insecticidal action through feeding, pyrethrum is the most active against both species athough, *T. ni* is 2.5-fold more susceptible than the armyworm. *T. americana* and *A. excelsa* were ~105 times less toxic to the armyworm than to *T. ni*, while rotenone was ~209-fold and rosemary oil ~24-fold more toxic to *T. ni* than to the armyworm. In contrast, the armyworm was twice as susceptible to *M. azedarach* as *T. ni.* Clove oil was equitoxic to the noctuids. Comparison of *T. ni* toxicity (day 7) through feeding and spraying shows the greater penetrating ability of pyrethrum through the cuticle (LC_{50} = 0.4 ppm) than its absorption from the gut (LC_{50} = 40 ppm). Same was true for rotenone. However, all other botanicals including *A. excelsa, A. indica*, *M. azedarach, M. volkensi, T. americana*, ryania, clove oil and rosemary oil were more active through feeding.

As feeding deterrents, pyrethrum proved to be the most potent against *T. ni*, whereas *A. indica* was the most potent against the armyworm (Table 5). *M. azedarach* and clove leaf oil (*Syzygium*) equally deterred both noctuid species. *Ryania* was relatively ineffective as a feeding deterrent in the noctuids whereas, rosemary oil was ineffective as a feeding deterrent to the armyworm.

Many studies have shown that even closely related species can differ markedly in susceptibility to the same plant extract or pure allelochemical (Isman 1993; Akhtar and Isman 2004a, b). Arnason et al. (1987), demonstrated that gedunin (a limonoid from spanish cedar, *Cedrela odorata*, Meliaceae), is not very active against the noctuids *Peridroma saucia* or *Spodoptera litura*, but is toxic to the European corn borer *Ostrinia nubilalis* (Pyralidae) as well as to aphids and earwigs.

Although, azadirachtin is a potent antifeedant for most phytophagous insects, its potency varies between species (Isman 1993). It has outstanding antifeedant properties against the desert locust *Schistocerca gregaria*, but does not deter feeding in the grasshopper *Melanoplus sanguinipes* (Champagne et al. 1989).

Previous studies have suggested that gustatory sensitivity of insect herbivores to deterrents is greater in specialists than in generalists (Bernays et al. 2000), but this was not the case for all botanicals in our study. Although, *P. unipuncta* was more susceptible to *A. indica* (21-fold) than *T. ni*, it was less susceptible to *M. volkensii* and *A. excelsa* than *T. ni*. Similar findings have been reported in a previous study (Akhtar and Isman, 2004b) in which *P. unipuncta* was less responsive to *Origanum vulgare* (oregano) (DC_{50} = 783.9 μg/cm^2) and thymol (DC_{50} = 462.9 μg/cm^2) than *T. ni* (DC_{50} = 524.3 μg/cm^2 for oregano and 247.2 μg/cm^2 for thymol). Other studies have also reported lesser susceptibility to plant allelochemicals among oligophagous species compared to polyphagous species. Azadirachtin is an extremely active antifeedant for the polyphagous species *Schistocerca gregaria*, *Spodoptera frugiperda, and S. littoralis*, whereas the oligophagous *Locusta migratoria* was less responsive (Mordue et al. 1998).

Our studies confirm that plant species of the family Meliaceae such as *A. indica, A. excelsa, T. americana*, and *M. volkensii* are rich sources of active botanical insecticides. In terms of growth inhibition and toxicity our extract of *A. indica* was far more active than *A. excelsa* to both noctuids. In some other cases *A. excelsa* have been shown to be more active than *A. indica.* For example, the leaf, bark and seeds of *A. excelsa* have been shown to have greater bioactivity than *A. indica* against the Mexican bean beetle, *Epilachna varivestis* (Schmutterer and Doll 1993) and larvae of *Spodoptera litura* on Chinese kale *(Brassica olboglabra)* (Pipithsangchan et al. 2005). Sentang (*A. excelsa*) seed oil was found to be five times more active in reducing the life span and fecundity of adult females of *E. varivestis* than *A. indica* (Doll and Schmutterer 1993).

Based upon growth inhibition, chronic toxicity, and antifeedant activity, these plant extracts have levels of activity that compare favourably to some of the most active botanicals in current use. *A. indica* and *A. excelsa* are more active growth inhibitors for *T. ni* and *P. unipuncta*, respectively, than pyrethrum. Strong bioactivity of *A. excelsa*, *A. indica*, *Melia volkensii*, and *M. azedarach* compared with rotenone, ryania, clove oil and rosemary oil may be attributed to the presence of limonoids. *M. azedarach* and *T. americana* are not active feeding deterrents for *T. ni*, even though they are twice as active as ryania. Although the most potent feeding deterrent for *P. unipuncta* is *A. indica*, *A. excelsa* and *M. volkensii* are more active feeding deterrents than rotenone, ryania, clove oil and rosemary oil. As to their compatibility in IPM programs, natural enemies and pollinators are susceptible to poisoning by pyrethrum, essential oils and to a lesser extent, neem (Isman 1997). Neem has been shown to possess low toxicity to beneficial insects (Schmutterer 1990), but other members in the family Meliaceae need to be tested against non-target species to assess their full impact on them.

In the present study, the insects were exposed to the botanicals only for the first 3 days out of the total 7 days of the experiment, suggesting that short exposure to botanicals may have prolonged effects, contributing to pest management. Since most of the botanicals showed additional bioactivity in the post-exposure period (days 4–7), this might suggest damage to the gut or other organs of test species.

There was a lack of correlation between EC_{50} and dietary LC_{50} values for both noctuids in our study, emphasizing the need for bioassays with different endpoints and more than a single bioassay species with candidate extracts if the goal of the research is discovery and development of an insecticide for management of phytophagous pests of agriculture and forestry (Isman 1997; Akhtar and Isman 2004a). Our results indicate some interspecific differences even in the two noctuid species in regard to susceptibility to the botanicals. Such information regarding species-specific response to each botanical could be very important in designing a pest control program involving a suite of insect species.

Our results suggest both antifeedant activity, causing a reduction in food consumption, and post-ingestive chronic toxicity for most of the botanicals tested. Both actions can reduce growth and increase development time. In the field, prolongation of developmental stages (as a result of growth inhibition) and increased search time (as a result of feeding deterrence) to seek viable food sources, likely expose herbivores to increased mortality as a result of biotic and abiotic factors (Akhtar and Isman 2004a). Based on their comparable efficacy with neem and other commercial insecticides, many members in the family Meliaceae have potential for development as commercial insecticides with broad-spectrum activity and lesser adverse effects on beneficial insects.

Acknowledgements We thank Nancy Brard for insect rearing and technical assistance. We also thank Ikkei Shikano, Veronica Robertson, Sylvia Altius and Sincere Wong for their assistance with bioassays. Supported in part by an International Cooperative Research Grant (F01200400010127) from the Korea Science and Engineering Foundation and the Natural Sciences and Engineering Research Council of Canada (NSERC) to M.B.I. and Y.R.Y.

References

Akhtar Y, Rankin CH, Isman MB (2003) Decreased response to feeding deterrents following prolonged exposure in the larvae of a generalist herbivore, *Trichoplusia ni (*Lepidoptera: Noctuidae). J Insect Behav 16:811–831

Akhtar Y, Isman MB (2004a) Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. J Appl Entomol 128:32–38

Akhtar Y, Isman MB (2004b) Feeding responses of specialist herbivores to plant extracts and pure allelochemicals: effects of prolonged exposure. Entomol Exp Appl 111:201–208

Appel AG, Gehret MJ, Tanley MJ (2001) Repellency and toxicity of essential oils to German cockroaches (Dictyoptera, Blattodea and Blatellidae). J Agric Urban Entomol 18:149–156

Arnason JT, Philogene BJR, Donskov N, Kubo I (1987) Limonoids from Meliaceae and Rutaceae reduce feeding growth and development of *Ostrinia nubilalis*. Entomol Exp Appl 43:221–226

Ascher KRS, Schmutterer H, Mazor M, Zebitz CPW, Naqvi SNH (2002) The Persian lilac or Chinaberry tree: *Melia azedarach* L. In: Schmutterer H (ed) The neem tree *Azadirachta indica* A. Juss. and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purposes, 2 edn. Neem foundation, Mumbai, India, pp 770–820

Bernays EA, Oppenheim S, Chapman RF, Kwon H, Gould F (2000) Taste sensitivity of insect herbivores to deterrents is greater in specialists than in genreralists: a behavioural test of the hypothesis with two closely related caterpillars. J Chem Ecol 26:547–562

Carpinella CM, Ferravoli C, Valladares G, Defago M, Palacios S (2002) Potent limonoid insect antifeedant from *Melia azedarach*. Biosci Biotechnol Biochem 60:1731–1736

Carpinella MC, Defago MT, Valladares G, Palacios SM (2003) Antifeedant and insecticidal properties of a limonoid from *Melia Azedarach* (Meliaceae) with potential use for pest management. J Agric Food Chem 51:369–374

Casida JE, Quistad JB (1995) Pyrethrum flowers: production, chemistry, toxicology and uses. Oxford Univ. Press, Oxford, UK, 356 pp

Champagne DE, Isman MB, Towers GHN (1989) Insecticidal activity of phytochemicals and extracts of the Meliaceae. In: Arnason JT, Philogene BJR, Morand P (eds) Insecticides of plant origin, vol. 387. Amer. Chem. Soc. Symp. Ser. Washington DC, USA, pp 95–109

Champagne DE, Koul O, Isman MB, Scudder GGE, Towers GHN (1992) Biological activities of limonoids from the Rutales. Phytochemistry 31:377–394

Charleston DS (2004) Integrating biological control and botanical pesticides for *Plutella xylostella.* PhD thesis. Wageningen Univ., 176 pp

Charleston DS, Kfir R, Dicke M (2005) Behavior responses of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae) to extracts derived from *Melia azedarach* and *Azadirachta indica*. Bull Entomol Res 95:457–469

Chiu SF (1989) Recent advances in research on botanical insecticides in China. In: Arnason JT, Philogene BJR, Morand P (eds) Insecticides of plant origin, vol. 387. Amer. Chem. Soc. Symp. Ser. Washington DC, USA, pp 69–77

Chiu SF, Huang BQ, Huang ZX, Zu MC, Huang DP, Hu MY (1984) Investigation on extraction of the toxic principles from seed kernels of Meliaceae and their effects on agricultural insects. Res. Bull. No 3, Lab. Toxicol. Dept. Pl. Prot. South China Agric. Univ. 32 pp

Connolly JD (1983) Chemistry of the Meliaceae and Cneoraceae. In: Waterman PG, Grunden MF (eds), Chemistry and chemical taxonomy of the Rutales, Academic Press, London, pp 175–213

Copping LG, Menn JJ (2000) Biopesticides: a review of their action, applications and efficacy. Pest Manag Sci 56:651–676

Dales MJ (1996) A review of plant materials used in controlling insect pests of stored products. Natural Resources Institute, Chatham (UK), 84 pp. NRI Bulletins; No. 65 (08927/J11.D35)

Davidson RH, Lyon WF (1979) Insect pests of farm, garden, and orchard. John Wiley and Sons Inc., USA

Doll M, Schmutterer H (1993) Vergleigh der Wirkung Von SamenkernextraktenUnd Ol Von *Azadirachta excelsa* and *A. indica* beim Mexikanischen Bohnenkafer *Epilachna varivestis*. Mitt Dtsch Ges Allg Angew Entomol 8:775–780

Enan E (2001) Insecticidal activity of essential oils: octopaminergic sites of action. Comp Biochem Physiol 130:325–337

Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, London, UK

Govindachari TR, Suresh G, Gopalakrishnan G, Wesley SD (2000) Insect antifeedant and growth regulating activities of neem seed oil—the role of major triterpenoids. J Appl Entomol 124:287–291

Hollingworth R, Ahmadsahib K, Gedelhak G, McLaughlin J (1994) New inhibitors of complex I of the mitochondrial electron transport chain with activity as pesticides Biochem. Soc Trans 22:230–233

Isman MB (1993) Growth inhibitory and antifeedant effects of azadirachtin on six noctuids of regional economic importance. Pestic Sci 38:57–63

Isman MB (1994) Botanical insecticides and antifeedants: new sources and perspectives. Pestic Research J 6:11–19

Isman MB (1997) Neem and other botanical insecticides: barriers to commercialization. Phytoparasitica 25:339–344

Isman MB (1999) Pesticides based on plant essential oils. Pestic Outlook 10:68–72

Isman MB (2004) Plant essential oils as green pesticides for pest and disease management. In: Nelson WM (ed) Agricultural applications in green chemistry, vol. 887. Amer. Chem. Soc. Symp. Ser. Washington DC, USA, pp 41–51

Isman MB (2005) Problems and opportunities for the commercialization of insecticides. In: Regnault-Roger C, Philogene BJR, Vincent R (eds) Biopesticides of plant origin. Lavoisier, Paris, pp 283–291

Isman MB (2006) The role of botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu Rev Entomol 51:45–66

Isman MB, Arnason JT, Towers GHN (2002) Chemistry and biological activity of ingredients of other species of Meliaceae. In: Schmutterer H (ed) The neem tree *Azadirachta indica* A. Juss. and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purposes, 2nd edn. Neem Foundation, Mumbai India, pp 827–833

Isman MB, Matsuura H, MacKinnon S, Durst T, Towers GHN, Arnason JT (1996) Phytochemistry of the Meliaceae: so many terpenoids, so few insecticides. In: Romeo JT, Saunders JA, Barbosa P (eds) Recent advances in phytochemistry, vol. 30. Phytochemical diversity and redundancy in ecological interactions. Plenum Press, New York, London, pp 155–178

Klocke JA, Kubo I (1982) Citrus limonoid by-products as insect control agents. Entomol Exp Appl 32:299–301

Koul O (1992) Neem allelochemicals and insect control. In: Rizvi SJH, Rizvi B (eds) Allelopathy, basic and applied aspects. Chapman and Hall, London, pp 389–412

Kraus W, Cramer R, Sawitzki G (1981) Tetranortriterpenoids from the seeds of the neem tree *Azadirachta indica*. Phytochemistry 20:117–120

Kraus W (1986) Constituents of neem and related species. A revised structure of azadirachtin. Stud Org Chem 26:237–256

Kraus W (2002) Azadirachtin and other triterpenoids. In: Schmutterer H (ed) *Azadirachta indica* A Juss and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purposes, 2nd edn. Neem Foundation, Mumbai, India, pp 39–110

Lavie D, Jain MK (1967) Tetranortriterpenoids form *Melia azadirachta* L. J Chem Soc Chem Commun 278–280

Lee SM, Klocke JA, Barnby MA, Yamasaki RB, Balandrin MF (1991) Insecticidal constituents of *Azadirachta indica* and *Melia azedarach* (Meliacea). In: Hedin PA (ed) Naturally occurring pest bioregulators, vol. 449. Amer. Chem. Soc. Symp. Ser. Washington DC, USA, pp 293–304

Mordue AJ, Blackwell A (1993) Azadirachtin: an update. J Insect Physiol 39:903–924

Mordue AJ, Simmonds SJM, Ley VS, Blaney MW, Mordue W, Nasiruddin M, Nisbet JA (1998) Actions of azadirachtin, a plant allelochemical against insects. Pestic Sci 54:277–284

Mwangi RW (1982) Locust antifeedant activity in fruits of *Melia volkensii*. Entomol Exp Appl 32:277–280

Mwangi RW, Rembold H (1987) Growth regulating activity of *Melia volkensii* extracts against the larvae of *Aedes aegypti.* In: Schmutterer H, Ascher KRS (eds) Proc. 3rd Int Neem Conf, Nairobi, Kenya (1986), pp 669–681

Mwangi RW, Rembold H (1988) Growth inhibiting and larvicidal effects of *Melia volkensii* extracts on *Aedes aegypti* larvae. Entomol Exp Appl 46:103–108

Nakatani M, Iwashita T, Naoki H, Hase T (1985) Structure of a limonoid antifeedant from *Trichilia roka*. Phytochemistry 24:195–196

Nakatani M, James JC, Nakanishi K (1981) Isolation and structures of trichilins, antifeedants against the southern armyworm. J Am Chem Soc 13:1228–1230

Ng LT, Yuen PM, Loke WH, Kadir AA (2003) Effects of *Azadirachta excelsa* on feeding behaviour, body weight, and mortality of *Crocidolomia binotalis* Zeller (Lepidoptera, Pyralidae). J Sci Food Agric 83:1327–1330

Pennington PD, Styles BT (1975) A generic monograph of the Meliaceae. Blumea 22:419–540

Pipithsangchan S, Subhadhirasakul S, Palintorn P, Yuenyongsawad S (2005) Effects of extracts from Tiam seeds on cotton leafworm. Songklanakarin J Sci Technol 27:511–521

Priestley CM, Williamson EM, Wafford KA, Sattelle DB (2003) Thymol, a constituent of thyme essential oil is a positive allosteric modulator of human GABA receptors and a homo-oligomeric GABA receptor from *Drosophila melanogaster*. Br J Pharmacol 140:1363–1372

Quarles W (1996) EPA exempt least toxic pesticides. IPM Pract 18:16–17

Rajab MS, Bentley MD (1988) Tetranortriterpenes from *Melia volkensii*. J Nat Products 51:840–844

Rajab MS, Bentley MD, Alford AR, Mendel MJ (1988) A new limonoid insect antifeedant from the fruit of *Melia volkensii*. J Nat Prod 51:168–171

Rembold H, Mwangi RW (2002) *Melia volkensii* Gurke. In: Schmutterer H (ed) *Azadirachta indica* A Juss and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purposes, 2nd edn. Neem Foundation, Mumbai, India, pp 770–820

Schmutterer H (1990) Properties and potential of natural insecticides from the neem tree, *Azadirachta indica*. Annu Rev Entomol 35:271–297

Schmutterer H, Doll M (1993) The marrango or Philippine neem tree, *Azadirachta excelsa* (=*A. integrifoliola*): a new source of insecticides with growth regulating properties. Phytoparasitica 21:79–86

Schmutterer H, Ermel K, Isman MB (2002) The Tiam, Sentang, or Marrango tree: *Azadirachta excelsa* (Jack). In: Schmutterer H (ed) *Azadirachta indica* A Juss and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purposes, 2nd edn. Neem Foundation, Mumbai, India, pp 760–779

Shepherd HH (1951) The chemistry and action of insecticides. New York, McGraw Hill, 504 pp

Singh D, Singh Ak (1991) Repellent and insecticidal properties of essential oils against housefly *Musca domestica* L. Insect Sci Appl 12:487–491

Stroh J, Wan MT, Isman MB, Moul DJ (1998) Evaluation of the acute toxicity to juvenile Pacific coho salmon and rainbow trout of some plant essential oils, a formulated product and the carrier. Bull Environ Contam Toxicol 60:923–930

Watanabe AK, Shono Y, kakimiziu A, Matsuo N, Saton A, Nishimura H (1993) New mosquito repellent from *Eucalyptus camaldulensis*. J Agric Food Chem 41:2164–2166

Wheeler DA, Isman MB (2001) Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. Entomol Exp Appl 98:9–16

Wheeler DA, Isman MB, Sanchez-Vindas PE, Arnason JT (2001) Screening of Costa Rican *Trichilia* species for biological activity against the larvae of *Spodoptera litura* (Lepidoptera, Noctuidae). Biochem Syst Ecol 29:347–358

Xie YS, Isman MB, Gunning P, MacKinnon S, Arnason JT, Taylor DR, Sanchez P, Hasbun C, Towers GHN (1994) Biological extracts of *Trichilia* species and the limonoid hirtin against lepidopteran larvae. Biochem Syst Ecol 22:129–136

Yoshida AH, Toscano CN (1994) Comparative effects of selected natural insecticides on *Heliothis virescens* (Lepidoptera, Noctuidae) larvae. J Econ Entomol 87:305–310

Zhu BCR, Henderson G, Chen F, Fei HX, Raine RA (2001a) Evaluation of vetiver oil and six insect-effective essential oils against the Formosan subterranian termite. J Chem Ecol 27:1617–1625

Zhu BCR, Henderson G, Chen F, Maistrallo L, Raine RA (2001b) Nootkatone is a repellent and toxicant for Formosan subterranian termite (*Coptotermes formosamus*). J Chem Ecol 27:523–531