

# Possibilities of Botanical Insecticide Exploitation in Plant Protection

Roman Pavela

Research Institute of Crop Production, Drnovská 507, 161 06 Praha 6-Ruzyně, Czech Republic

Correspondence: pavela@vurv.cz

## ABSTRACT

In this article three types of botanical insecticides are shown: products of neem oil from seeds of *Azadirachta indica* Juss., pongam oil from *Pongamia pinnata* L. and essential oils from some aromatic plants. Their effective use against common greenhouse pests such as whitefly (*Trialeurodes vaporariorum* West, *Bemisia tabaci* Gennad.), two-spotted spider mite (*Tetranychus urticae* Koch), aphids and caterpillars is demonstrated. The capacity to use botanical insecticides as effective pest-controlling agents, as well as some of their limitations, are also discussed.

**Keywords:** azadirachtin, essential oils, greenhouse pests, plant extracts, pongam oil

**Abbreviations:** AzaA, azadirachtin; EO, essential oil

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## INTRODUCTION

The use of plants as traditional protectants of plant products is an old practice used all over the world. Our ancestors were quite successful in exploring and exploiting this natural treasure. The documented use of plant extracts and powdered plant parts as insecticides goes back at least as far as the Roman Empire. There are reports of the use of pyrethrum (*Tanacetum cinerariaefolium*, Asteraceae) already in 400 B.C. The first pure botanical insecticide, used as such, dates back to the XVII<sup>th</sup> Century when it was shown that nicotine, obtained from tobacco leaves, would kill plum beetles. Around 1850 a new plant insecticide known as rotenone was introduced. Rotenone is a flavonoid derivative extracted from the roots of two different *Derris* spp. and *Lonchocarpus* spp., all Fabaceae. The ground seeds of *Sabadilla*, a plant of South American origin known as *Schoenocaulon officinale* (Liliaceae), are one of the plant insecticides with the lowest mammal toxicity (Ador 1995). These traditions were largely neglected by farmers after the Second World War.

When synthetic insecticides appeared in the 1940's some people thought that botanical insecticides would disappear gradually. However, problems like environmental contamination, residues in food and feed, and pest resistance has led to a renewed interest in nature as a source of novel crop protection compounds. The fact that plants during evolution have developed an effective defense system against most insects makes plants the richest natural source for biocidal compounds. There is thus no doubt that plants are of great interest for novel botanical insecticides

for insect pest control. In fact, only very few of the more than 250,000 plant species on our planet have been properly evaluated for this purpose. Therefore, there is a huge potential for developing novel products from plants for Integrated Pest Management Programs and Organic Farming (Pedigo 1999).

Recently research has emphasized finding alternative insecticides from plants as a solution to control insect pests in agricultural and ornamental plants in cultivated and urban areas, and is currently influenced by four concerns: 1) the banning of synthetic insecticide use in the harvested period; 2) public perception that natural compounds are better; 3) these are products that are generally regarded as being safe; 4) complex mixtures are also likely to be more durable with respect to insects evolving resistance and developing behavioral desensitization. Research is again focusing on the plant kingdom for solutions since the interaction between plants and insects has led to the production of a myriad of secondary compounds that include properties such as toxicity (Hiremath *et al.* 1997), growth retardation (Breuer and Schmidt 1995), feeding inhibition (Klepzig and Schlyter 1999; Wheeler and Isman 2001), oviposition deterrence (Dimock and Renwick 1991; Hermawan *et al.* 1994; Zhao *et al.* 1998), suppression of calling behaviour (Khan and Saxena 1986) and reduction of fecundity and fertility (El-Ibrashy 1974; Muthukrishnan and Pushpalatha 2001). Such a wide variety of effects provide potential natural alternatives for the use of synthetic chemical insecticides. Although certain plant families, particularly Malvaceae, Asteraceae, Rutaceae, Labiaceae, Annonaceae and Canellaceae, are viewed as exceptionally promising sources of plant-

based insecticides (Jacobson 1989; Schmutterer 1990), entomotoxic properties of extracts from plants belonging to several other families have been frequently reported (Hermawan *et al.* 1994; Pavela 2004).

It is not possible to deal with all plant extracts and potential botanical insecticides in this article, which briefly covers three main types of botanical insecticides used against common greenhouse pests such as whitefly (*Trialeurodes vaporariorum* West., *Bemisia tabaci* Gennad.), two-spotted spider mite (*Tetranychus urticae* Koch), aphids and caterpillars.

## Neem oils

*Azadirachta indica* Juss. (syn. *Antelaea azadirachta* L., *Melia indica* (Juss) Brand.; Meliaceae; **Fig. 1**) is a unique plant with numerous medicinal and pesticidal properties. Its original name in Sanskrit was nimba, which is equivalent to neem in Hindi.



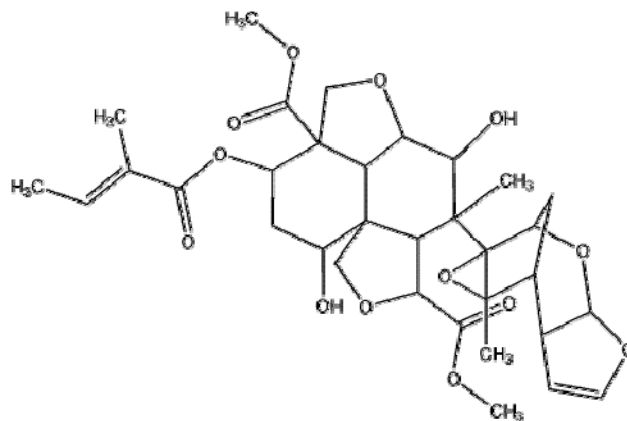
**Fig. 1** *Azadirachta indica*. (A) Tree and (B) flowers.

*A. indica* is indigenous to the Indian subcontinent but neem is found in a belt extending southwards from Delhi to Cape Comorin and is also found in Bangladesh, upper Myanmar and the dry areas of Sri Lanka is also widespread in SE Asia, primarily scattered in Thailand, Malaysia and Indonesia. *A. indica* also grows in the Southern plains of Yemen and Saudi Arabia; in Africa it is found in Nigeria, Sudan, Ethiopia, Kenya, among others. It grows in Europe; Croatia, Northern Italy and Southern France. In the New world it is found in Haiti and Surinam and was recently introduced into Cuba, Jamaica and some of the Southern States of the USA.

Many biologically active constituents have been isolated from the crude extracts of neem plants. From the neem seed extract and seed oils, a number of chemical components have been isolated and identified, from which azadirachtin, deacetyl-salannin, salannin, nimbin, epinimbin and meliantrinol possessed biological activity. During their evaluation process insect repellent, antifeedant, growth inhibitor/regulator and insecticidal activities were found when tested against a wide range of insect pests. The most well known and extensively examined is azadirachtin.

Azadirachtin, also termed AzaA (Rembold 1989; **Fig. 2**) is a tetranortriterpenoid plant limonoid with potent insect antifeedant and growth disrupting properties. It was isolated from the seeds of *A. indica* by Butterworth and Morgan (1968), of which it is the main component, and its full structural determination was completed some 17 years later. Nakanishi and co-workers presented the first structure proposal (Zanno *et al.* 1975), the correct structure appeared in papers submitted in 1985 and 1986 (Kraus *et al.* 1985, Broughton *et al.* 1986), and full details were finally described by all groups in 1987 (Bilton *et al.* 1987; Kraus *et al.*

1987; Turner *et al.* 1987). AzaA is a highly oxidized limonoid with many reactive functional groups in close proximity to each other. Its biosynthesis is thought to involve tirucallol, a tetracyclic triterpenoid, and a series of oxidation and rearrangement reactions which produce finally, amongst others, the tetranortriterpenoids salannin, nimbin and AzaA (Ley *et al.* 1993). 3-Tigloylazadirachtol (AzaB) is present at 20% higher concentrations than AzaA, other azadirachtins (AzaC-I) occur at much lower concentrations. For a comprehensive review of the chemistry of AzaA see Ley *et al.* (1993).



**Fig. 2** Chemical structure of Azadirachtin, or AzaA.

The first commercial product of neem, Margosan-O (W. R. Grace & Co., Cambridge, MA, U.S.A.) was registered in the U.S.A. in 1985 (Mordue and Blackwell 1993). Several commercial and semi-commercial preparations are now available including Azatin (Agridyne Technologies, Salt Lake City, UT, U.S.A.); Bioneem and Neemesis (Ringer Corp., Minneapolis, MN, U.S.A.); Safer's EN1 (Safer Ltd, Victoria, B.C., Canada, now incorporated into Ringem Corp.); Wellgro and RD-Repelin (ITC Ltd, Andora Pradesh, India); Neemguard (Gharda Chemicals, Bombay, India); Neemark (West Coast Herbochem, Bombay, India) and Neemazal (Trifolio M GmBH, D-6335 Lahnau 2, Germany). Neem seed oil is often a starting material for such insecticides and its biological activity is closely related to its AzaA content (Isman *et al.* 1990).

After several years of development AzaA-based insecticides are now available for use on food and non-food crops. However, all AzaA-based insecticides do not have the same performance (toxicity or insect control properties) due to differences in the production and formulation of these materials.

For example Stark and Walter (1995) demonstrated that three different AzaA-containing formulations, when applied at the same rate of AzaA, showed very different abilities to control the pea aphid, *Acyrtosiphon pisum* Harris. In these tests we examined the effects of Neemix™ (Margosan-O), Azatin™ and an experimental formulation RH-9999 on the pea aphid on broad beans. Trials conducted on mixed age populations as well as first instar nymphs showed that Neemix™ was significantly more effective than Azatin™ and RH-9999, the latter having the lowest activity (**Tables 1, 2**). Similar results were reported by Eckberg *et al.* (1995) who

**Table 1** Final population density of aphids (*Acyrtosiphon pisum*) exposed to broad bean treated with several neem insecticides at the equivalent rate of 100 mg of azadirachtin/L.

Treatment	No. of aphids ± SD
Control	1392.25 ± 58.66 <sup>a</sup>
Azatin™	654.75 ± 41.33 <sup>b</sup>
Neemix™	232.00 ± 39.7 <sup>c</sup>
RH-9999™	1378.50 ± 56.51 <sup>a</sup>

ANOVA followed by LSD test. Means followed by the same letter are not significantly different.  
Based on four replicates.



noted that Neemix™ used at a low rate killed forest caterpillars, *Malacosoma disstria*, more quickly than Azatin™ at a much higher rate (Table 3).

**Table 2** Toxicity of neem insecticides to 1<sup>st</sup> instar nymphs of *Acyrtosiphon pisum* (100 mg of azadirachtin/L).

Treatment	% mortality ± SD
Control	2.0 ± 4.47 <sup>c</sup>
Azatin™	68.00 ± 10.95 <sup>b</sup>
Neemix™	90.00 ± 7.07 <sup>a</sup>
RH-9999™	8.0 ± 13.04 <sup>c</sup>

ANOVA followed by LSD test. Means followed by the same letter are not significantly different.

Based on five replicates.

**Table 3** The effect of Azatin EC and Neemix (Margosan-o) on the mortality of forest tent caterpillars. Fourth instar larvae were placed in a Petri dish with ash leaves treated with the products for six days. The treated leaves were replaced with untreated leaves.

Treatment	Rate	7 DAT	11 DAT	15 DAT	19 DAT	24 DAT
Control		0.0 <sup>a</sup>	0.0 <sup>b</sup>	4.0 <sup>b</sup>	8.0 <sup>b</sup>	38.0 <sup>b</sup>
Azatin™	50 ppm	0.0 <sup>a</sup>	10.5 <sup>b</sup>	30.0 <sup>b</sup>	70.0 <sup>a</sup>	86.0 <sup>a</sup>
Neemix™	12.5 ppm	16.0 <sup>a</sup>	61.3 <sup>a</sup>	68.0 <sup>a</sup>	82.0 <sup>a</sup>	90.0 <sup>a</sup>

Number within a column followed by the same letter are not significantly different ( $P=0.05$  by SNK).

Based on five replicates.

DAT = days after treatment.

The analysis conducted by Stark and Walter (1995) suggests that the presence of other limonoids, other than AzaA, present in Neemix™ including nimbadiol, deacetyl-salannin, deacetylnimbin, nimbin and salannin and the oil components are responsible for the enhanced activity of Neemix™. These limonoids have no insecticidal property of their own at the levels present but appear to stimulate the activity of AzaA.

The AzaA-containing formulation of botanical insecticides is effective in the protection of greenhouse plants. Good preparative formulations contain effective UV protectants, which enlarge the persistence period on plants and thus also their efficiency. Nowadays emulsive oils are appearing on the market. These are not enriched with AzaA, and thus serve as effective preventive sprays having repellent, antioviposition and antifeedant effects.

The effectiveness of the systemic effects of AzaA-enriched preparations is enhanced by the use of bioactivators, which are as enhancers and surface activators, improving penetration, translocation and effectiveness of crop protection products such as herbicides, fungicides (including biological fungicides), insecticides (including biological insecticides) as well as fertilizers e.g. commercial Greemax® (Pavela *et al.* 2005). Of interest nowadays is the application of AzaA through the plant root system, which, if possible, would act as a systemic insecticide and repellent. One of the first studies to indicate this following the purification of AzaA showed that the terpenoid was taken up by the roots of bean plants, and acted as an antifeedant to locusts (Gill and Lewis 1971). Subsequently, these observations were confirmed for a variety of plants and pests, mainly aphids (Sundaram 1996; Pavela *et al.* 2004). It was



**Fig. 3** *Aesculus hippocastaneum*. (A) Tree infected with *Cameraria ohridella* and (B) rejuvenated leaves after treatment with AzaA.

successfully demonstrated that AzaA acts systemically against insect pests in mature trees after injection into the trunks of chestnut trees (Pavela and Barnett 2005; Fig. 3). Low dosages applied systemically throughout conducting tissues could effectively protect plants against some pests.

## Pongam oil

*Pongamia* is a monospecific genus i.e. *Pongamia pinnata* L. (syn. *P. glabra* Vent.; *Derris indica* Lamk.) which belongs to the Leguminosae family (subfamily Papilionaceae; Meera *et al.* 2003). *P. pinnata* (common names: puna oil tree, Pongamia, Kharanja, or Karanja oil) is a rich source of flavonoids, the B-ring linked either to a furan or a pyran ring, some of which possess biological activity.

Quercetin has been found to have antiulcer activity (Khanna and Seshardi 1965). 3-Methoxy and 5-hydroxy-flavones are necessary for antiviral activity against rhinovirus and the activity is modified by various groups at other position (Tsuchiya *et al.* 1985). Flavonoids with multiple methoxy and ethoxy groups are effective inhibitors of blood cell aggregation (Meera *et al.* 2003).

The secondary metabolites (flavonoids, chalcones, steroids and terpenoids) in pongam oil serve as defensive agents against insect pests. Numerous defensive chemicals belonging to various chemical categories with cause behavioral and physiological effects on insect pests have already been identified from different medicinal plants like *Azadirachta indica* and *Melia* sp., *Leuzea carthamoides*, aromatic plants from genus *Lamiaceae* (Fig. 4), and others (Breuer and Schmidt 1995; Pavela 2004; Pavela *et al.* 2005a, 2005b).

Parmar and Gulati (1969) reported ethanolic extracts of de-oiled karanja cake, its defatted seed and pure karanjin to show insecticidal activity against mustard aphid (*Lipaphis erysimi*). Rao and Niranjana (1982) reported an active component, karanjin, isolated from karanja seed oil to show juveno-mimetic activity against *Tribolium castaneum* larvae, whose final instar larvae, when fed treated (0.5 to 2 mg karanjin/ml/g) flour, resulted in the moulting of pupae by the end of the 4<sup>th</sup> week. For plant protection the use of oil preparations is recommended. The active components of the karanjin group, extracted in water medium from its oil, were toxic to *Spodoptera litura* larvae (Meera *et al.* 2003). In practice a combination of Neem and Pongam oils is a good prevention against pests and diseases.

Antifeedant activities of various *P. pinnata* extracts were observed against many insect pests of different crops. Under laboratory conditions, 0.1% water emulsion of oil showed antifeedant activity against *Amsacta moorei* Butler (Verma and Singh 1985). *P. pinnata* oil, so-called karanj oil, is known to possess a strong egg-laying repellent activity in many insect pests when applied at 1.0% (v/v), e.g. by *Callosobruchus chinensis* L. on *Cajanus cajan* (L.) Millsp. (Khaire *et al.* 1993). Seed and aqueous plant extracts are known to possess ovicidal action against *Phthorimae operculella* Zell and *Helopeltis theivora* Waterh. (Shelke *et al.* 1987; Deka *et al.* 1998).

The use of pongam oils against greenhouse pests is possible: pongam oil-treated potato (*Lycopersicon esculentum*) and chrysanthemum (*Chrysanthemum indicum*) plants showed a strong repellent effect on the adults of greenhouse whitefly and deterred oviposition (Pavela, unpublished results). Our experiments showed that plants treated with



**Fig. 4** *Solanum tuberosum*. (A) Plant infected with *Leptinotarsa decemlineata* and (B) healthy plant after treatment with pongam oil.

**Table 4** Average number (means ± SE) of adults on treated plants.

Concentration (%)	Choice test			No-choice test		
	4 days	8 days	12 days	4 days	8 days	12 days
control	163.3 ± 51.7 <sup>b</sup>	156.8 ± 38.3 <sup>c</sup>	180.0 ± 35.0 <sup>c</sup>	74.7 ± 7.4 <sup>c</sup>	81.3 ± 8.3 <sup>b</sup>	79.0 ± 15.8
0.5	16.3 ± 4.3 <sup>a</sup>	22.8 ± 5.4 <sup>b</sup>	20.8 ± 2.2 <sup>b</sup>	34.8 ± 6.8 <sup>b</sup>	53.3 ± 18.4 <sup>b</sup>	38.2 ± 6.8
1.0	3.0 ± 0.9 <sup>a</sup>	2.3 ± 0.4 <sup>a</sup>	4.3 ± 3.3 <sup>a</sup>	11.5 ± 3.3 <sup>a</sup>	7.0 ± 0.5 <sup>a</sup>	63.0 ± 23.5
2.0	1.5 ± 1.0 <sup>a</sup>	2.5 ± 1.7 <sup>a</sup>	6.8 ± 4.3 <sup>ab</sup>	0.1 ± 0.1 <sup>a</sup>	4.3 ± 0.8 <sup>a</sup>	31.0 ± 18.6
F-value	18.23	23.18	34.63	19.89	5.11	NS

Values per row followed by the same letter are not significantly different at  $P \leq 0.05$  (Turkey's test). 12<sup>th</sup> day of no-choice test was not significant (NS).

**Table 5** Average eggs number (means ± SE) per plant leaf.

Concentration (%)	Choice test			No-choice test			Caged		
	4 days	8 days	12 days	4 days	8 days	12 days	4 days	8 days	12 days
control	28.5 ± 5.7 <sup>b</sup>	18.1 ± 3.6 <sup>b</sup>	49.6 ± 18.9 <sup>b</sup>	19.5 ± 1.1 <sup>b</sup>	16.1 ± 3.2 <sup>b</sup>	22.4 ± 2.6 <sup>b</sup>	17.5 ± 1.1 <sup>c</sup>	17.1 ± 3.2 <sup>b</sup>	20.4 ± 2.6 <sup>b</sup>
0.2	ND	ND	ND	ND	ND	ND	3.4 ± 1.7 <sup>b</sup>	22.3 ± 7.5 <sup>b</sup>	21.1 ± 6.9 <sup>b</sup>
0.5	4.6 ± 4.0 <sup>a</sup>	1.9 ± 1.0 <sup>a</sup>	3.5 ± 2.4 <sup>a</sup>	0.4 ± 0.1 <sup>a</sup>	2.6 ± 1.2 <sup>a</sup>	2.2 ± 1.3 <sup>a</sup>	0.9 ± 0.2 <sup>ab</sup>	3.8 ± 1.3 <sup>a</sup>	8.1 ± 2.8 <sup>a</sup>
1.0	0.3 ± 0.4 <sup>a</sup>	0.3 ± 0.2 <sup>a</sup>	1.1 ± 0.5 <sup>a</sup>	1.0 ± 0.5 <sup>a</sup>	0.8 ± 0.7 <sup>a</sup>	5.5 ± 2.2 <sup>a</sup>	0.5 ± 0.3 <sup>a</sup>	0.9 ± 0.1 <sup>a</sup>	8.5 ± 1.7 <sup>a</sup>
2.0	0.0 ± 0.1 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>	1.4 ± 0.8 <sup>a</sup>	0.0 ± 0.1 <sup>a</sup>	0.1 ± 0.1 <sup>a</sup>	2.4 ± 0.7 <sup>a</sup>	0.1 ± 0.1 <sup>a</sup>	1.2 ± 0.5 <sup>a</sup>	2.3 ± 0.9 <sup>a</sup>
F-value	89.77	37.9	10.79	29.1	23.06	32.21	123.55	13.37	13.33

Values per row followed by the same letter are not significantly different at  $P \leq 0.05$  (Turkey's test).

ND – not determined

different concentrations of pongam oil suspended in water (0.2-2.0% (v/v)) show a relatively long-lasting repellent (Table 4) and anti-oviposition effect (Table 5) on the adults of greenhouse whitefly. The repellent effect declined independent of time and concentration. A strong effect on the reduction of oviposition was also found, but this expires depending on the concentration – at least 12 days after application.

The persistence of pongam oil is greater than other tested botanical insecticides. Dosages at 1-2% better control insect pests compared to lower concentrations. Pongam oil and karanjin have shown greater biological activity than other extracts from Pongamia plants. Pongam oil also shows a good synergistic effect with a number of chemical insecticides, increasing its potential as a biopesticide due to its antifeedant, oviposition deterrent, ovicidal, roachicidal, juvenile hormone activity and insecticidal properties against a wide range of pests (Kumar and Singh 2002). Moreover, the tree is widely distributed throughout the world and effective *in vitro* protocols are available for mass production (Sharry and Teixeira da Silva 2006). Insecticidal formulations commercially available on the market such as Plexin, Karrich, Salotrap, RD-Repelin™ and RD-9 Repelelin™ include extracts from Pongamia as one of the components for the control of various insect pests. More emphasis is likely to be given to botanical insecticides because of their compatibility with other methods of control in IPM programmes.

## Essential oils

Against this backdrop, natural pesticides based on essential oils may represent the latest alternative crop protectants. Essential oils, obtained by steam distillation of plant foliage, and even the foliage itself, have traditionally been used to protect stored grain and legumes, and to repel flying insects in the home.

Contact and fumigant insecticidal actions of plant essential oils (EOs) have been well demonstrated against stored product pests. Among 22 EOs tested as fumigants against the bean weevil *Acanthoscelides obtectus* (Bruchidae), those of *Thymus serpyllum* (rich in the phenols thymol and carvacrol) and *Origanum majorana* (rich in terpinen-4-ol) were the most toxic (Regnault-Roger *et al.* 1993). In a more detailed study, Shaaya *et al.* (1991) evaluated the fumigant toxicity of 28 EOs and 10 of their major constituents against four different species of stored product coleopterans.

Recent studies have also indicated efficacy of several EOs against pests on plants. EOs of cumin (*Cuminum cyminum*), anise (*Pimpinella anisum*), oregano (*Origanum syriacum* var. *bevanii*) and eucalyptus (*Eucalyptus camaldu-*

*lensis*) were effective fumigants against cotton aphid (*Aphis gossypii*) and the carmine spider mite (*Tetranychus cinnabarinus*), two greenhouse pests (Tuni and Sahinkaya 1998). Efficacy of basil (*Ocimum* spp.) against garden pests has recently been reviewed (Quarles 1999).

Lee *et al.* (1997) reported on the toxicity of a range of EO constituents on western corn rootworm (*Diabrotica virgifera*), on two-spotted spider mite (*Tetranychus urticae*) and on the common housefly (*Musca domestica*). Dietary effects of a number of monoterpenoids against the European corn borer (*Ostrinia nubilalis*) have also been reported (Lee *et al.* 1999).

Çalmaşur *et al.* (2006) reported on the toxicity of EO vapours from *Micromeria fruticosa*, *Nepeta racemosa* and *Origanum vulgare* on the nymphs and/or adults *Tetranychus urticae* and *Bremisia tabaci*, suggesting that for reasonable efficacy an estimated 2.5-3.5 L of *M. fruticosa*, *N. racemosa* and *O. vulgare* EOs would be necessary for a 1,000 m<sup>2</sup> area of greenhouse and with active fumigation for a period of 24 hours.

Perhaps the most attractive aspect of using EOs and/or their constituents as crop protectants (and in other contexts for pest management) is their favourable mammalian toxicity. Some of the pure EO compounds are slightly toxic, with rat acute oral LD<sub>50</sub> values of 2-3 g.kg<sup>-1</sup> (viz. carvacrol, pulegone), but an EO insecticide consisting of a proprietary mixture of essential oil constituents resulted in no mortality when fed to rats at 2 g.kg<sup>-1</sup>, the upper limit required for acute toxicity tests by most pesticide regulatory agencies including the EPA (US). Since many EOs and their constituents are commonly used as culinary herbs and spices, should pesticide products contain these, they are actually exempt from toxicity data requirements by the EPA.

Static water toxicity tests using juvenile rainbow trout (*Oncorhynchus mykiss*) indicated that based on 96 h-LC<sub>50</sub> values, eugenol is approximately 1,500 times less toxic than the botanical insecticide pyrethrum, and 15,000 times less toxic than the organophosphate insecticide azinphosmethyl (Stroh *et al.* 1998). What pointed to this fact was that the essential oils had high biological affectivity against insects but their toxicity against animals was lower.

In a review paper on neem and other botanical insecticides, three barriers to the commercialization of new products of this type were identified: (a) the scarcity of the natural resource; (b) the need for chemical standardization and quality control; and (c) difficulties in registration (Isman 2000). As the EOs and their purified constituents have a long history of global use by the food and fragrance industries, and most recently in the field of aromatherapy, many of the oils and/or constituents that are pesticide are readily available at low to moderate cost in quantity (USD 7-30 kg<sup>-1</sup>). A number of constituents are available commercially

in reasonable purity (95%), and EO producers and suppliers can often provide chemical specifications for even the most complex oils.

Of greatest importance, some of these materials are exempt from the usual data requirements for registration, if not exempt from registration altogether (at least in the USA.). Some US companies have recently taken advantage of this situation and have been able to bring EO-based pesticides to market in a far shorter time period than would normally be required for a conventional pesticide. Myco-tech Corp., for example produces Cinnamite™, an aphidicide/miticide/fungicide for glasshouse and horticultural crops, and Valero™, a miticide/fungicide for use in grapes, berry crops, citrus and nuts. Both products are based on cinnamon oil, with cinnamaldehyde (30% in EC formulations) as the active ingredient (Isman 2000).

With over a dozen registered products by the end of 1999, EcoSMART Technologies is aiming to become a world leader in EO-based pesticides. They currently produce aerosol and dust formulations containing proprietary mixtures of EO compounds, including eugenol and 2-phenethyl propionate aimed at controlling domestic pests (cockroaches, wasps, ants, fleas etc.). These are marketed to pest control professionals under the brand name EcoPCO®, with less concentrated formulations for sale to the consumer under the name Bioganic™. Insecticides and miticides for horticultural crops and for glasshouse and nursery crops will be released shortly. Commercial success with these products based on well-known chemistry will likely provide an impetus for the development and commercialization of future pesticides based on more exotic essential oils with even greater potency (Shaaya and Kostjukovsky 1998).

## CONCLUSIONS

The main market for insecticides and other biopesticides from plants are organic agriculture, greenhouses, parks, gardens and households. Organic agriculture is a market with high demand for natural insecticides as organic growers cannot use conventional agricultural chemicals. At the moment this market is expanding based on consumers' demands for improved Food Safety and pressures placed on reducing environmental problems associated with the use of synthetic pesticides. With an annual average growth of 30%, organic farming in the EU is one of the most dynamic agricultural sectors. After the enforcing of the Community Legislation regulating organic production (Council Regulation No 2092/91/EEC of 24 June 1991), many farmers have joined. One of the main objectives of the Common Agricultural Policy (CAP) is the achievement of a sustainable agricultural production in Europe which requires environmentally-friendly pest control measures.

Natural pest control methods are needed in parks and gardens due to their low environmental persistence so that people will be less exposed to the toxic compounds. It is expected that within 10-15 years, these compounds will increase by 25% in their insecticide market share and they will not be limited to garden areas but there may be a massive growth towards urban and agricultural uses. Botanical pesticides also have a great advantage by being compatible with other low risk options which are acceptable for insect management, such as pheromones, oils, detergents, entomopathogenic fungi, predators and parasitoids, among others, which greatly increase the probability of being integrated in IPM programs.

To fill in this growing market new products need to be developed. For this purpose a systematic approach to find new products from plants should be developed. Different sources can be considered, such as traditionally used plants, readily available plants or agricultural waste products. Extracts of these plants are screened for activity, followed by the isolation, identification and testing of active molecules. The successful development of biocides from discarded citrus peels in the US is an excellent example of the chances

of success of such an approach (Isman 2000).

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## REFERENCES

- Addor RW** (1995) Insecticides. In: Godfrey CRA (Ed) *Agrochemicals from Natural Products*, Marcel Dekker, Inc. pp 1-62
- Bidmon HJ, Huser G, Miibus P, Koolman J** (1987) Effect of azadirachtin on blowfly larvae and pupae. In: Schmutterer H Ascher KRS (Ed) *Proceeding of 3<sup>rd</sup> Intl. Neem Conf.* GTZ, Eschborn, Germany, pp 232-272
- Bilton JN, Broughton HB, Jones PS, Ley SV, Lidert Z, Morgan ED, Rzepa HS, Sheppard RN, Slawin AMZ, Williams DJ** (1987) An X-ray crystallographic, mass spectroscopic, and NMR study of the limonoid insect antifeedant azadirachtin and related derivatives. *Tetrahedron* **43**, 2805-2815
- Breuer M, Schmidt GH** (1995) Influence of a short period treatment with *Melia azedarach* extract on food intake and growth of the larvae of *Spodoptera frugiperda* (J.E. Smith) (Lep., Noctuidae). *Journal of Plant Disease Protection* **10**, 633-654
- Broughton HB, Ley SV, Slawin AMZ, Williams DJ, Morgan ED** (1986) X-Ray crystallographic structure determination of detigloyldihydro-azadirachtin and reassignment of the structure of the limonoid insect antifeedant azadirachtin. *Journal of the Chemical Society, Chemical Communications* 46-47
- Butterworth JH, Morgan ED** (1968) Isolation of a substance that suppresses feeding in locusts. *Journal of the Chemical Society, Chemical Communications* 23-24
- Çalmaşur Ö, Aslan İ, Şahin F** (2006) Insecticidal and acaricidal effect of three Lamiaceae plant Essentials oils against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Industrial Crops and Products* **23**, 140-146
- Deka MK, Singly K, Handique R** (1998) Antifeedant and repellent effect of pongam (*Pongamia pinnata*) and wild sage (*Lantana camara*) on tea mosquito bug (*Helopeltis theivora*). *Indian Journal of Agricultural Science* **68**, 274-276
- Dimock MB, Renwick JAA** (1991) Oviposition by field populations of *Pieris rapae* (Lepidoptera: Pieridae) deterred by an extract of a wild crucifer. *Environmental Entomology* **20**, 802-806
- Eckberk TB, Granshaw W, Sclar DC** (1995) Evaluation of neem insecticides and persistence for control of forest ten caterpillars. Ft. Collins, Co, USA. *Arthropod Management Test* **20**, 327-338
- El-Ibrashy MT** (1974) Sterilization of the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) with a foliage extract of *Podocarpus gracilior* P. *Journal of Applied Entomology* **75**, 107-109
- Gill, JS, Lewis, CT** (1971) Systemic action of an insect feeding deterrent. *Nature* **232**, 402-403
- Hermawan W, Kalama S, Tsukuda R, Fujisaki K, Koboyashi A, Nakasuji F** (1994) Antifeedant and antioviposition activities of the fraction of extract from tropical plant, *Andrographis paniculata* (Acanthaceae), against the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). *Applied Entomology and Zoology* **29**, 533-538
- Hiremanth IG, Ahn YJ, Kim SI** (1997) Insecticidal activity of Indian plant extracts against *Nilaparvata lugens* (Homoptera: Delphacidae). *Applied Entomology and Zoology* **32**, 159-166
- Isman MB, Koul O, Luczynski A, Kaminski J** (1990) Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. *Journal of Agriculture and Food Chemistry* **38**, 1406-1411
- Isman MB** (2000) Plant essential oils for pest and disease management. *Crop Protection* **19**, 603-608
- Jacobson M** (1989) Botanical insecticides. Past, present and future. In: Armason JT, Philogene BJR, Morand P (Eds) *Insecticides of Plant Origin*. American Chemical Society Symposium series 387, Washington, DC, American Chemical Society, pp 1-10
- Khanna RN, Seshardi TR** (1965) Chemical components of *Pongamia* species. *Beitrag Biochemikal Physiologisch Naturstoffen Festschreiben*, pp 251-265
- Khan ZR, Saxena RC** (1986) Effect of steam distillate extracts of resistant and susceptible rice cultivars on behavior of *Sogatella furcifera* (Homoptera: Delphacidae). *Journal of Economic Entomology* **79**, 928-935
- Khaire VM, Kachare BV, Mote UN** (1993) Effect of vegetable oils on mortality of pulse beetle in pigeon pea seeds. *Seed Research* **21**, 78-81
- Klepzig KD, Schlyter F** (1999) Laboratory evaluation of plant derived antifeedants against the pine weevil *Hyllobius abietis* (Coleoptera: Curculionidae). *Journal of Economic Entomology* **92**, 644-650
- Kraus W, Bokel M, Klenk A, Piihl H** (1985) The structure of azadirachtin and 22, 23-dihydro-23-β-methoxyazadirachtin. *Tetrahedron Letters* **26**, 6435-6438
- Kraus W, Bokel M, Bruhn A, Cramer R, Klaiber I, Klenk A, Nagl G, Piihl H, Sadlo H, Vogler B** (1987) Structure determination by NMR of azadirachtin and related compounds from *Azadirachta indica* (Meliaceae). *Tetrahedron* **43**, 2817-2830

- Kumar M, Singh R** (2002) Potential of *Pongamia glabra* Vent. as an insecticide of plant origin. *Biological Agriculture and Horticulture* **20**, 29-50
- Lee S, Tsao R., Peterson CJ, Coats JR** (1997) Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: Chrysomelidae), twospotted spider mite (Acari: Tetranychidae), and house fly (Diptera: Muscidae). *Journal of Economic Entomology* **90**, 883-892
- Lee S, Tsao R, Coats JR** (1999) Influence of dietary applied monoterpenes and derivatives on survival and growth of the European corn borer (Lepidoptera: Pyralidae). *Journal of Economic Entomology* **92**, 56-67
- Ley SV, Denholm AA, Wood A** (1993) The chemistry of azadirachtin. *Natural Products Reports*, 109-157
- Meera B, Kumar S, Kalidhar SB** (2003) A review of the chemistry and biological activity of *Pongamia pinnata*. *Journal of Medicinal and Aromatic Plants Science* **25**, 441-465
- Mordue (Luntz) AJ, Blackwell A** (1993) Azadirachtin: an update. *Journal of Insect Physiology* **39**, 903-924
- Muthukrishnan J, Pushpalantha E** (2001) Effects of plant extracts on fecundity and fertility of mosquitoes. *Journal of Applied Entomology* **125**, 31-35
- Parmar BS, Gulati KC** (1969) Synergist for pyrethrins-Karanja. *Indian Journal of Entomology* **31**, 239-243
- Pavela R** (2004) Insecticidal activity of certain medical plants. *Fitoterapia* **75**, 745-749
- Pavela R, Barnet M, Kocourek F** (2004) Effect of Azadirachtin applied systemically through roots of plants on the mortality, development and fecundity of the Cabbage Aphid (*Brevicoryne brassicae*). *Phytoparasitica* **32**, 286-294
- Pavela R, Kazda J, Herda G** (2005a) Field evaluation of azadirachtin against brassica pod midge (*Dasineura brassicae* Winn.) on oilseed rape (*Brassica napus* L.). *Test of Agrochemicals and Cultivars* **26**, 8-9
- Pavela R, Barnet M** (2005) PROFF: Systemic applications of Neem in control of *Cameraria ohridella*, a pest of horse chestnut (*Aesculus hippocastanum*). *Phytoparasitica* **33**, 49-56
- Pavela R, Harmatha J, Bárnet M, Vokáč K** (2005b) Systemic effects of phytoecdysteroids on the cabbage aphid *Brevicoryne brassicae* (Sternorrhyncha: Aphididae). *European Journal of Entomology* **102**, 647-653
- Pavela R, Teixeira da Silva JA** (2006) New control technologies against pests based on Azadirachtin. In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1<sup>st</sup> Edn), Volume III, Global Science Books, UK, pp 564-566
- Pedigo LP** (1999) *Entomology and Pest Management*, Prentice Hall, New Jersey, 742 pp
- Quarles W** (1999) Grow pesto for your pests! *Common Sense Pest Control* **15**, 13-19
- Rao GR, Dhingra S** (1997) Synergistic activity of some vegetable oils in mixed formulations with cypermethrin against different instars of *Spodoptera litura* Fabricius. *Journal of Entomology Research* **21**, 153-160
- Regnault-Roger C, Hamraoui A, Holeman M, Theron E, Pinel R** (1993) Insecticidal effect of essential oils from mediterranean plants upon *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae), a pest of kidney bean (*Phaseolus vulgaris* L.). *Journal of Chemical Ecology* **19**, 1233-1244
- Rembold H** (1989) Azadirachtins: their structure and mode of action. In: Armason JT, Philogene BJR, Morand P (Eds) *Insecticides of Plant Origin*, ACS Symp. Ser. 387, American Chemical Society, Washington, DC, pp 150-163
- Schutterer H** (1990) Properties and potential of natural pesticides from the neem tree. *Annual Reviews of Entomology* **35**, 271-297
- Shaaya E, Kostjukovsky M** (1998) Efficacy of phyto-oils as contact insecticides and fumigants for the control of stored-product insects. In: Ishaaya I, Degheele D (Eds) *Insecticides With Novel Modes of Action. Mechanisms and Application*, Springer, Berlin, pp 171-187
- Shaaya E, Ravid U, Paster N, Juven B, Zisman U, Pissarev V** (1991) Fumigant toxicity of essential oils against four major stored product insects. *Journal of Chemical Ecology* **17**, 499-504
- Sharry S, Teixeira da Silva JA** (2006) Effective organogenesis, somatic embryogenesis and salt tolerance induction *in vitro* in the Persian Lilac tree (*Melia azedarach* L.). In: Teixeira da Silva JA (Ed) *Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues* (1<sup>st</sup> Edn), Volume II, Global Science Books, UK, pp 317-324
- Shelke SS, Jadhav LD, Salunkhe GN** (1987) Storageability of seed potatoes treated with vegetable oils/extracts against *Phthorimaea operculella* Zell. *Current Research Reporter* **3**, 33-38
- Stark JD, Walter JF** (1995) Neem oil and neem oil components affect the efficacy of commercial neem insecticides. *Journal of Agriculture and Food Chemistry* **43**, 507-512
- 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. *Bulletin of Environmental Contamination and Toxicology* **60**, 923-930
- Sundaram KMS** (1996) Root uptake, translocation, accumulation and dissipation of the botanical insecticide azadirachtin in young spruce trees. *Journal of Environmental Science and Health, Part B* **31**, 1289-1306
- Tsuchiya Y, Shimizu M, Hiyama Y, Itoh K, Hashimoto Y, Nakayama M, Horie T, Morita N** (1985) Antiviral activity of naturally occurring flavonoids *in vitro*. *Chemistry and Pharmacology Bulletin* **33**, 3881-3886
- Tuni I, Sahinkaya S** (1998) Sensitivity of two greenhouse pests to vapours of essential oils. *Entomology Experimentalis et Applicata* **86**, 183-187
- Turner CJ, Tempesta MS, Taylor RB, Zagorski MG, Termini JC, Schroeder DR, Nakanishi K** (1987) An NMR spectroscopic study of azadirachtin and its trimethylether. *Tetrahedron* **43**, 2789-2803
- Verma SK, Singh MP** (1985) Antifeedant effects of some plant extracts on *Amsacta moorei* Butler. *Indian Journal of Agriculture Science* **55**, 298-299
- Wheeler DA, Isman MB** (2001) Antifeedant and toxic activity of *Trichilia americana* extract against the larvae of *Spodoptera litura*. *Entomology Experimentalis et Applicata* **98**, 9-16
- Zanno PR, Miura I, Nakanishi K, Elder DL** (1975) Structure of the insect phagorepellent azadirachtin. Application of PRFTJCWD carbon-13 nuclear magnetic resonance. *Journal of the American Chemical Society* **97**, 1975-1977
- Zhao B, Grant GG, Langevin D, MacDonald L** (1998) Detering and inhibiting effects of quinolizidine alkaloids on spruce budworm (Lepidoptera: Tortricidae) oviposition. *Environmental Entomology* **27**, 984-992